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ABBREVIATIONS

ng	Nannogram
μg	Microgram
mg	Milligram
kg	Kilogram
mg%	Milligram percentage
nmole	Nannomole
μmole	Micromole
mmole	Millimole
nmole	Nannomole
M	Mole
mm	Millimeter
cm	Centimeter
nm	Nannometer
v/v	Volume/volume
min	Minute
h	Hour
rpm	Revolutions per minute
HDL	High density lipoprotein.
LDL	Low density lipoprotein.
VLDL	Very low density lipoprotein
APO-B	Apolipoprotein-B
APO-E	Apolipoprotein-E
APO-A1	Apolipoprotein-A1
TBA	Thiobarbituric acid
TLC	Thin layer chromatography
HPLC	High performance liquid chromatography
ODS	Octadecyl silane

UV	Ultraviolet
SEM	Standard error of mean
HCD	High cholesterol diet
L	Litre
dl	Deci litre
ml	Milli litre
P	Probability
NIH	National institute of health
wt	Weight
HFD	High fat diet
NADPH	α-Nicotinamide adenine dinucleotide phosphate (reduced).
BSA	Bovine serum albumin.
SDS	Sodium dodecyl sulphate.
CDNB	Mono chlorodinitro benzene.
GSH	Glutathione (reduced).
GSSG	Glutathione (oxidised).
SOD	Superoxide desmutase.
Gpx	Glutathione peroxidase.
TBARS	Thiobarbituric acid reactive substances.
Fe^{2+}	Ferrous ion
KCl	Potassium chloride
i,p	Intra peritoneal
AsAT	Aspartate aminotransferase
AlAT	Alanine aminotransferase
NADH	α-Nicotinamide adenine dinucleotide (reduced)
λ	Lambda
EDTA	Ethylene diamine tetracetic acid
LDH	Lactate dehydrogenase

PMNL'S	Polymorphonuclear leucocytes
Sec	Second
kHz	Kilohertz
$M^{-1}cm^{-1}$	Per mole per centimeter
5-HETE	5-hydroperoxy eicosatetraenoic acid
$CaCl_2$	Calcium chloride
ATP	Adenine tri phosphate
TCA	Trichloroacetic acid
NaCl	Sodium chloride
NaOH	Sodium hydroxide
HCl	Hydrochloric acid
$FeSO_4$	Ferrous sulphate
PAGE	Polyacryl amide gel electrophoresis
PBS	Phosphate buffer saline
MDA	Malondialdehyde
$CuSO_4$	Copper sulphate
Cu^{2+}	Cuprous ion
^{o}C	Degree centigrade
pH	Hydrogen ion concentration

CHAPTER - I

GENERAL INTRODUCTION

LIPID METABOLISM

Lipids are important dietary constituents because of essential fatty acids and the the fat soluble vitamins they provide. Combinations of fat and protein are important cellular constituents and also serve as the means of transporting lipids in the blood. Lipids play an indispensable role in cell structure, function and metabolism. Among them triacyl-glycerols are the major storage form of energy in adipose tissue and cholesterol is a vital constituent of cell membranes and also a precursor of bile acids and steroid hormones.

Cholesterol metabolism

Cholesterol is an alicyclic compound having a perhydrocyclopentano phenanthrene nucleus with its four fused rings and a hydroxyl group at C-3 postion. Cholesterol is a lipid with very low solubility in water, the limit of solubility being 4.7 mM. Solubility of cholesterol in blood is almost twice compared to that of glucose solubility.due to the plasma lipoproteins that have the ability to bind and thereby solubilize large amounts of chlesterol.

Absorption: Both dietary (exogenous) and endogenous (provided through bile secretion) cholesterol are absorbed from the intestinal lumen, but exogenous cholesterol absorption depends on the amount of triglycerides in the meal, because it is partitioned in oily emulsions. The intestinal cholesterol pool is heterogenous and biliary cholesterol is absorbed more efficiently than dietary cholesterol (Boyd, 1975). Increased intake of dietary cholesterol results in an increase in concentrations of blood and tissue cholesterol, absorption aided by bile salts and triglyceride and blood cholesterol increases

approximately 15% in response to high (0.5%) cholesterol diet. High cholesterol diet are usually low in fibers and are associated with long transit time and reduced feacal bulk, these factors contribute to the association between high saturated fat, high cholesterol and colon cancer (Shils *et al*, 1994).

Biosynthesis: Virtually almost all tissues are capable of synthesizing cholesterol and their microsomal and cytosol portions are responsible for cholesterol synthesis. Acetyl coenzyme-A is the source of all carbon atoms in cholesterol. Synthesis takes place in several steps. The first is the formation of 6-carbon mevalonate; the next major step is the formation of isoprenoid units from mevalonate by loss of carbon dioxide. Six isoprenoid units combine to form the intermediate squalene (30-carbon), which in turn gives rise to the parent steroid, lanosterol. The formation of cholesterol from lanosterol takes place in the membrane of endoplasmic reticulum and involves changes to the steroid nucleus and side chain. Hydroxy methyl glutaryl coenzyme-A reductase plays an important role in the control of cholesterol biosynthesis through feed back mechanism. Cholesterol may act either by repression of the synthesis of new reductase or by inducing the synthesis of enzymes that degrade existing reductase. It has been proposed that LDL-cholesterol can inhibit cholesterol synthesis at the squalene synthesis step. In humans, extrahepatic synthesis, mainly in the intestine, is more important, whereas in dogs and rats the liver is responsible for most cholesterol synthesis.

Catabolism: Approximately 50% of the cholesterol eliminated from the body is excreted in the feces after conversion to to bile salts. Much of the cholesterol secreted in the bile is reabsorbed and the cholesterol that serves as the precursor for the fecal sterols is derived

from the intestinal mucosa. Thus, cholesterol is catabolized mainly into bile salts, steroid hormones, Vitamin D and a small fraction is converted into chlestanol and coprostanol in the small intestine. The catabolism of cholesterol to bile acids account for 30 to 60% of the eliminated cholesterol from the human body, but it would be 80 to 87% lost as bile acids in rats (Myant, 1981). Bile acid synthesis increases by the addition of cholesterol to the diet and by the amount of certain types of fiber added to the food, which inhibits reabsorption of bile salts from the small intestine (Miettinen, 1981).

Cholesteryl ester: In plasma and adrenals, cholesterol would be predominantly in the form of cholesteryl ester. This is due to the transfer of the PUFA linoleic acid from 2-position of triglyceride to 3-hydroxyl group of cholesterol. Rats and dogs are more resistant to developing atherosclerosis, have higher percentage of 20:4 n-6 all cis PUFA. But rabbits which are known to be even more prone to development of atherosclerosis than man, who have low percentage of 20:4 n-6 PUFA. Man, who regularly consumes diets rich in saturated fats and low in linoleic acid has lower (35 - 50%) cholesteryl linoleate concentrations with correspondingly higher cholesteryl oleate percentages (Holman & Johnson, 1981). This condition appears to correlate with a progressively increased risk to develop peripheral vascular disease such as ischemic heart disease.

Lipoprotein metabolism

Table-1. Physical properties, lipid and apolipoprotein composition of human plasma lipoproteins

	Exogenous lipids	Endogenous lipids			
	Chylomicrons	VLDL	IDL	LDL	HDL
Density (g/ml)	<0.96	0.96-1.006	1.006-1.019	1.019-1.063	1.063-1.21
Diameter (nm)	75-1200	30-80	25-35	22-28	5-12
Electrophoretic mobility	0	pre-β	broad β	β	α pre-β
Composition (%)					
Triglycerides	86	55	23	6	5
Phospholipids	7	18	19	22	33
Cholesteryl ester	4	12	29	42	17
Free cholesterol	2	7	9	8	5
Protein	2	8	19	22	40-55
Main apolipoproteins	A-1, A-11 B-48, C, E	B-100 C, E	B-100 C, E	B-100	A-1, A-11 C, E
Source	Intestine	Liver	Lipolysis of VLDL	Lipolysis of VLDL via IDL	Liver, Intestine Lipolysis of CM & VLDL

The major lipids in the plasma are fatty acids, triglycerides (TG), cholesterol (free and esterified cholesterol) and phospholipids (PL). They are important in maintaining the structure of cell membrane (cholesterol, phospholipids), steroid hormone synthesis (cholesterol), and energy metabolism (TG and fatty acid). Since lipids are water-insoluble, they are transported in the plasma in association with proteins as lipoproteins. Lipoprotein consists of a hydrophobic core of TG and cholesterol esters (CE) surrounded

by a hydrophilic surface of free cholesterol (FC), phospholipids and apolipoproteins. Plasma lipoproteins are classified into five major sub-classes on the basis of their densities. Chylomicrons (CM), Very low-density lipoproteins (VLDL), Intermediate-density lipoproteins (IDL), Low-density lipoproteins (LDL) and High-density lipoproteins (HDL). Some lipoprotein sub-classes can be further separated by particle density or size, electrical charge, or apolipoprotein content.

Chylomicrons: Chylomicrons which are assembled by the intestinal mucosa, functon to keep the exogenous triacyglycerols and cholesterol suspended in aqueous solution. These lipoproteins are transported through the lyphatic vessels beforew draining into the large body veins via the thoracic duct. Chylomicrons adhere to binding sites on the inner surface of the capillaries in skeletal muscle and adipose tissue. Within minutes after entering the blood stream, the chylomicrons component triacylglycerols are hydrolysed through the action of apo C-11. The tissues then take up the liberated monoacyglycerol and fatty acids. The chylomicrons shrink as their triacylglycerols are progressively hydrolysed until they are reduced to cholesterol-enriched chylomicron remnants. The chylomicron remnants reenter the circulation by dissociating from the capillary endothelium and are subsequently taken up by the liver through the mediation of a remnant receptor that specifically binds apo-E. Chylomicrons therefore function to deliver dietary triacyglycerols to muscle and adipose tissue and dietary cholesterol to the liver.

Very low density lipoprotein: VLDL consists of about 8 % protein, 20 % cholesterol and a large amount of other fatty substances. Most of the dietary cholesterol makes its first

appearance in the form VLDL, which is also synthesized in the liver as lipid transport vehicles, and are degraded by lipoprotein lipase. The VLDL remnants appear in the circulation, first as IDL and then as LDL. In the transformation of VLDL to LDL, all their proteins but apo B-100 are removed and much of their cholesterol is esterified by the HDL-associated enzyme lecithin-cholesterol acyl transferase (LCAT). The enzyme transfers a fatty acid residue from C-2 of lecithin to cholesterol with the concomitant formation of lysolecithin.

High density lipoprotein: HDL is synthesized and secreted from both liver and intestine. Nascent HDL consists of discoid phospholipid bilayers containing apoprotein and free cholesterol. Catalysis by LCAT converts surface phospholipid and free cholesterol into cholesteryl esters and lysolecithin. The cholesteryl esters move into the hydrophobic core of the bilayer, whereas lysolecithin is transferred on to plasma albumin. The reaction continues generating a nonpolar core that pushes the bilayer apart until a spherical, pseudomicellar HDL is formed, covered by a surface film of polar lipids and apoproteins. The esterified cholesterol can be transferred from HDL to the lower density lipoproteins by means of cholesteryl ester transfer protein. Thus, this protein allows transfer of cholesteryl ester of HDL to be transported to the liver via remnants of chylomicrons and VLDL or via hepatic uptake of LDL.

Interconversion of lipoproteins: Triglycerides are the major constituents of the chylomicron particles. Apo-B-48, a specific apolipoprotein marker of chylomicron, is synthesized in the gut. TG in chylomicron is hydrolyzed by the enzyme lipoprotein lipase, resulting in the formation of chylomicron remnants which are taken up by the

hepatic cells (Beisiegel, 1995). VLDL particles are synthesized in the liver. VLDL consists mainly of TG, and also some cholesterol, one apo-B-100 molecule, apo-Cs and apo-E. Apo-B-100 is specific for VLDL and is synthesized in the liver. In the circulation, VLDL shares the catabolic pathway with chylomicrons and competes for LDL (Beisiegel, 1995). As VLDL particles become smaller, phospholipids, free cholesterol and apolipoproteins are released from their surfaces and transferred to HDL. During lipolysis, VLDL is converted to the denser particles, IDL, and finally transformed via hepatic lipase to LDL (Eisenberg, 1984). In this cascade, apolipoprotein-B (apo-B) remains tightly associated with the particle. HDL is synthesized primarily in the liver and small intestine. HDL precursor is a discoidal particle that comprises phospholipid, cholesterol, apo-E and apo-A, but is devoid of cholesterol ester (Patsch, 1994). It becomes mature spherical HDL by trapping cholesterol ester into the core of the particle with the help of the enzyme, lecithin:cholesterol acyltransferase (LCAT), which catalyzes the transfer of free fatty acyl group from phosphatidylcholine to free cholesterol with the formation of lyso-phosphatidylcholine and cholesterol ester (Santamarina-Fojo *et al*. 2000). HDL consists of a heterogeneous group of particles with varying density and size.

LDL metabolism: LDL particles are the main carriers of cholesterol in circulation and play the key roles in the cholesterol transfer and metabolism. Most LDL particles originate from the metabolism of TRLs. In VLDL-IDL-LDL cascade, the particle is depleted of TG by the action of lipoprotein lipase (LPL) and hepatic lipase (HL). In addition, the particle loses most of the associated apolipoproteins. Only apo-B-100, the essential structural protein remains in the particle. HL is important in the conversion of IDL into LDL (Beisiegel, 1998). In addition, LDL can also be synthesized directly by the

liver. LDL can pass through the junctions between capillary endothelial cells and bind to LDL receptor on cell membranes that recognize apo-B-100 (Marshall, 1995). The subsequent uptake of LDL into the cells is followed by lysosomal degradation with release of free cholesterol into the cytosol (Beisiegel, 1998). LDL receptors are saturable and subject to down regulation by an increase in intracellular cholesterol. The LDL receptor expression regulates the plasma level of cholesterol (Beisiegel, 1998). The defects of LDL receptor and its function cause familial hypercholesterolemia, a genetic disorder in which the LDL receptor activity is reduced either because of a reduced number of LDL receptors, or formation of structurally altered LDL receptors (Brown & Goldstein, 1975). LDL particles are cleared from the circulation either by hepatic or extrahepatic pathway. Liver takes up 75% of LDL; about 75% of this removal are receptor mediated, and 25% nonreceptor-mediated (Bilheimer *et al*. 1984). Two-third of the extraphepatic uptake is receptor-mediated, and one-third is nonreceptor-mediated. In addition to classical LDL receptors, macrophages derived from circulating monocytes can take up LDL via scavenger receptors. Scavenger receptors recognize chemically and biologically modified lipoproteins, typically acetylated LDL (Brown & Goldstein, 1983) and oxidized LDL. Some data have shown that nonoxidative modification of LDL, like the formation of heparin proteoglycan-LDL complex, can also induce uptake of LDL by macrophage through scavenger receptor-mediated endocytosis or phagocytosis (Tabas, 1999). Unlike LDL receptor, the expression of scavenger receptor is not down regulated by cellular cholesterol content, and therefore results in the intracellular lipid accumulation and consequently induce foam cell formation (Goldstein *et al*. 1979).

Structure and biochemical composition of LDL: LDL is a spherical particle with a diameter of 22-28 nm and density of 1.019-1.063 g/ml. The central hydrophobic core of LDL particles contains approximately 170 molecules of TG, and 600 molecules of free cholesterol and 1600 molecules of cholesteryl ester. It is surrounded by a monolayer of about 700 phospholipid molecules, consisting primarily of phosphotidylcholine, small amounts of sphingomyelin, lyso-phosphotidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol, and one apo-B-100 molecule. LDL consists of about 20% protein and 45% cholesterol. ApoB-100 plays a crucial role in LDL metabolism. About half of the fatty acids in LDL are polyunsaturated fatty acids, mainly linoleic acid with minor amounts of arachidonic acid and docosahexaenoic acid. These PUFAs are protected against free radical attack and oxidation by antioxidants, primarily α-tocopherol (6 moles / LDL particle), with minor amounts of γ-tocopherol, carotenoids, cryptoxanthin and ubiquinol (Esterbauer *et al*. 1992).

LDL - Implications in pathology: Elevated serum low-density lipoprotein cholesterol is the crucial factor for the initiation and progression of atherosclerosis, and lowering LDL cholesterol can reduce the incidence and mortality of cardio- and cerebro-vascular diseases. A number of cardiovascular risk factors other than traditional risk factors may contribute to the development of atherosclerosis (Oparil *et al*. 1999). Among factors, LDL oxidation and LDL particle size have received extensive attention for their atherogenic potentials. Evidence indicates that oxidized LDL is present *in vivo* within the atherosclerotic lesion but not in the normal arterial wall (Witztum & Steinberg, 2001). Oxidized LDL can be recognized by the scavenger receptors of the macrophages and induce sub-endothelial lipid accumulation and foam cell formation, which are the earliest

hallmarks of atherosclerosis (Steinberg *et al.* 1989). Studies demonstrate that higher intakes of antioxidant vitamins are associated with a reduced risk for CAD or stroke.

In animal models, administration of lipid-soluble antioxidants can inhibit athero-genesis during hypercholesterolemia (Heinecke, 2001). LDL particles are heterogeneous with varying density, size, charge, and composition. Small dense LDL particles are especially atherogenic and have been linked to increased risk for CAD. These particles penetrate the arterial wall more easily, and readily bind with intimal proteoglycans, which may further increase the susceptibility of the lipoprotein to oxidation (Hurt-Camejo *et al*. 2000). The following table depicts the possible mechanism of LDL oxidation:

Table-2 Possible mechanisms of lipoprotein oxidative modification

System	Species generated	Mechanism of lipid peroxidation
Reactive oxygen species.	Superoxide, hydroxyl radical.	Electron abstraction
Reactive nitrogen species.	Peroxynitrite	Electron abstraction
Cuprous ions.	Decomposition of pre-existing hydroperoxides.	Electron abstraction.
Ferric ions + reductant (O2-,GSH)	Decomposition of pre-existing hydroperoxides.	Electron abstraction.
Haem proteins.	Superoxide.	Electron abstraction.
Myeloperoxidase.	Hypochlorous acid, tyrosine radical.	Electron abstraction.
Cytochrome P450.	Superoxide, H_2O_2	Electron abstraction.
5- / 15-Lipoxygenase.	Direct hydroperoxide generation	Direct hydroperoxide generation

Oxidized LDL - atherosclerotic lesions: The oxidation of polyunsaturated fatty acids can lead to the formation of aldehydes that modify lysine residues in apolipoprotein B-100. The antibodies avidly stain atherosclerotic lesions in LDL receptor-deficient rabbits, apolipoprotein E-deficient mice (Palinski *et al*. 1994), and humans (Palinski *et al*. 1989). LDL isolated from atherosclerotic lesions possesses properties that resemble those of oxidized LDL formed in vitro. Furthermore, LDL isolated from human atherosclerotic lesions contains elevated levels of chlorotyrosine (Leeuwenburgh *et al*. 1997) and o,o'-dityrosine (Hazen & Heinecke, 1997). These findings demonstrate that lesion LDL is oxidatively modified, and they suggest that HOCl is a likely oxidant participating in these modification reactions.

Oxidized LDL in circulation: LDL circulates in plasma, and a portion traverses the subendothelial space to arrive back in the circulation (Schwenke *et al*. 1989). Indeed, chemical analysis of circulating LDL has been reported to yield a minor fraction, termed LDL− that exhibits an enhanced content of oxidized lipid (Sevanian *et al*. 1996). Human plasma contains immunoreactivity towards epitopes generated from oxidized LDL. However, the existence of oxidized LDL in circulation remains controversial on the basis of potential artifacts that may occur during the *ex vivo* handling of plasma and isolation of LDL. Several studies have shown that circulating levels of oxidized LDL epitopes can be used to distinguish between patients with and without atherosclerosis. Using immunologic methods that detect oxidized phosphatidylcholine and their protein adducts, it has been reported (Ehara *et al*. 2002) that acute coronary syndromes are characterized by increased circulating levels of oxidized LDL. Relatively small amounts of LDL

containing different types of oxidation-specific epitopes can be detected in blood and may reflect atherosclerosis.

LDL Oxidation: Oxidatively modified LDL exists in atherosclerotic lesions. Proteoglycans not only trap LDL in the extracellular matrix, but also change the conformation of apolipoprotein B-100, and such proteoglycan-exposed LDL shows greater sensitivity than native LDL to subsequent *in vitro* oxidative modification by Cu^{2+} (Hurt-Camejo *et al*. 1992). LDL oxidation must occur in the arterial wall rather than the circulation, as lipoprotein lipids in plasma are well protected from oxidation due to antioxidant defenses (Stocker *et al*. 1991). LDL contains α-tocopherol and in fact it is the major transport vehicle for most of the plasma α-tocopherol. Oxidized LDL that may form in plasma is diluted rapidly by either hepatic clearance or accumulation and subsequent degradation in the arterial wall (Juul *et al*. 1996). Composition of native and oxidized LDL is shown in Table-3.

Table-3 Composition of native and oxidized low density lipoprotein

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Native LDL constituents	% by mass	Change following oxidation
Protein	22	Fragmentation, lysine and histidine residue.
Phospholipids	22	Decrease in PC and increase LysoPC.
Triglycerols	6	No change.
Free cholesterol	9	Decreased, with increase in oxysterols.
Cholesteryl ester	42	Decreased, with increase in oxysterols.
Hydroxy and hydroxy peroxy fatty acids	ND	Large increase.
Conjugated dienes	ND	Large increase.
Vitamin E	<1	Decreased, with formation of tocophenyl quinone, 2,3 and 5,6 – epoxytocophenylqinone
Ubiquinone.	<1	Decrease in ubiquinol and increased ubiquinone
Carotenoids.	<1	Decreased, with formation of carotenoid or peroxyl radicals or non-peroxyl radicals.

Source: Ferns G.A.A. & Lamb (2004) What does the lipoprotein oxidation phenomenon

   mean? Biochemical Transactions Society. Vol.32, Part-1

Disturbances of hyperlipaemia

*a. Hypercholesterolemia***:** Hypercholesterolemia is characterized by abnormal level of cholesterol in low-density lipoprotein. It has been reported that feeding saturated fat, animal protein, and sucrose can produce hypercholesterolemia and arteriosclerosis in rats and rabbits.

b. Familial hypercholesterolemia (type 2): Characterized by high LDL cholesterol with lipid deposition in tissues being common. The disease is attributed to be associated with reduced clearance of LDL from circulation due to defective LDL receptors and is associated with increased risk of atherosclerosis.

c. Cholesterol gall stones: Gall stone formation is related to the duration of the existence of lithogenic bile in the gall bladder and the presence of the nucleating protein. People in western countries have high incidence of cholesterol gall stone formation than Oriental and African populations. Cholesterol gall stones in female American Indian are related to genetic disorder, in which biliary output of bile salts and phospholipid is low and that of cholesterol is high due to a traditional diet high in cholesterol and animal fat.

d. Familial hypertriglycerdemia (type 4)**:** Characterized by high levels of endogenously produced VLDL triacylglycerol. Cholesterol levels rise in proportion to the hypertriglycerolemia, and glucose intolerance is frequently present. Both HDL and LDL are subnormal in quantity. This lipoprotein pattern is also commonly associated with coronary heart disease.

f. Atherosclerosis: Hypercholesterolemia, age, gender, hypertension, smoking and diabetes are major risk factors for atherosclerosis. Atherosclerosis is directly related to plasma concentration of LDL cholesterol and inversely related to that of HDL and is characterized by progressive growth of plaques on the luminal surface of arteries. Plaques begin with the recruitment of inflammatory monocytes into the arterial cell wall (Newby & Zoltsmann, 1999). The monocytes differentiate into tissue macrophages and on ingestion of oxidized LDL, become foam cells. The lesion, known as a fatty streak at this

stage, develops into an intimal thickening as smooth muscle cells accumulate within the intima of the artery. This process is further accompanied by the recruitment of inflammatory cells (T-cells, macrophages and mast cells), degradation and deposition of basement membrane. The resulting plaque can grow and eventually occlude flow through the affected artery. The clinical manifestations are myocardial infarction, stroke, abdominal aneurysms, lower limb ischemia and thrombosis.

g. Cancer: The relationship between dietary fat intake and cancer incidence has been the subject of several epidemiological studies. The strongest association is between dietary fat and colon, breast and prostate cancer. Both the amount and type of fat consumed are important determinants of cancer risk. Early investigations using chemically induced breast cancer suggested that dietary fat strongly influenced cancer promotion. Studies of colon cancer also suggested that dietary fat influenced cancer promotion. Initiation of cancer by dietary fat indicate that mammary cancer was enhanced in rats fed diets enriched in lards and skin tumorigenesis was inhibited in mice fed corn oil. However recent studies suggested that higher intake of calorie may cause observed enhancement of cancer. In particular restriction of intake effectively inhibited carcinogenesis at many sites. However, dietary fat contributes more than its high calorie density to the development of cancer.

Treatment of hyperlipemia:

a. Dietary fibers: The health benefits of dietary fiber in reducing the risk of chronc diseases have been hypothesized which relates many chronic disease of the developed world to low intakes of dietary fiber. Important features of dietary fiber include the

bulking effect of dietary fiber in the diet that increases fecal volume, limits calorie intake, slows stomach emptying and along with the water it holds, dilutes the contents of the intestine. Dietary fiber also has the capacity to bind or absorb other organic compounds. This capacity appears to delay or reduce absorption of some compounds and reduce interaction of potentially carcinogenic compounds with intestinal mucosa. Many of the polysaccharides are fermented by the bacteria in the colon, producing short-chain fatty acids that may be utilized by the mucosaal cells or absorbed and transported to other tissues where they regulate several metabolic processes. One of the most likely mechanisms involves the binding of bile acids in the intestine, thus increasing fecal loss and limiting the recycling of the cholesterol pool (Story & Kritchevsky, 1976).

b. Statins and Streptolipin drugs: Lovastatin is an inhibitor of HMG-CoA reductase. Since this enzyme is limiting for cholesterol synthesis, lovastatins decrease endogenous synthesis of cholesterol and stimulates uptake of LDL via LDL receptor. Sometimes combination of lovastatins and cholestyramine is used for severe hyperlipidemia. Statin class of drugs reduce plasma levels of apo B-100 in all lipoprotein density classes. The paradigm shift for this has been the fact that inhibition of cholesterol synthesis lowers hepatic cholesterol levels and upregulates the LDL receptors, causing increased LDL clearance. It has been reported that a decrease in hepatic cholesterol levels by statins could lead to decrease in cholesteryl ester synthesis, which may be an imporant determinant of VLDL secretion (Cuchel *et al*. 1997).

c. Cholestyramine and colestipol: These are bile salt binding drugs that promote excretion of bile salts in the stool. This in turn increases the rate of hepatic bile salt synthesis and of LDL uptake by the liver.

d. Phytic acid: Phytic acid present in legumes, cereal bran is known to lower LDL and total cholesterol, but interferes with the absorption of nutrients and micronutrients.

e. Policosanols: They are a mixture of high molecular weight aliphatic alochols, derived from purified sugar canewax (Uriberri *et al*. 2002). They contain several fatty alcohols under the commercial name Ateromixol. Policosanols when administered at 5 - 20 mg / day have been shown to decrease the risk of atheroma formation by reducing the platelet aggregation, endothelial damage and foam cell formation in animals and also have been shown to reduce total and LDL cholesterol levels (Janikula *et al*. 2002). When compared with statins, policosanols exhibit comparable cholesterol lowering effects at much smaller doses.

f. Guggulipids: Compounds responsible for the hypolipidemic acitvity of gum guggul are the isomers of E and Z-guggulsterone (Satyavati, 1988). Gugulipids have beneficial effects on obesity, inflammation and acne and stimulatory effects on the thyroid gland and drug metabolism. Gum gugulipid displayed a significant anti-inflammatory activity in normal and adrenalectomized rats with formaldehyde induced arthritis (Gujral *et al*. 1960), in rats with Freunds adjuant arthritis (Sharma, 1977) and in rats with paw edema induced by carrageean (Satyavati *et al*. 1969). In rats, administration of guggulsterone at a dose of 10 mg / kg body weight increased thyroid function and also significantly

increased cytochrome P-450 gene expression, which are responsible for metabolizing drugs

g. *Yucca saponins: Yucca schidigera* contains a number of phytochemicals, especially the steroidal saponins. Saponins contain a lipophilic nucleus and one or more side chains of hydrophilic carbohydrate. So the intact saponin molecule is a surfactant due to fat-soluble and water-soluble moities. Hence it forms stable foams. The hydrophobic portion of the saponin (sapongenin) associates (lipophilic bonding) with the hydrophobic sterol nucleus of cholesterol in a stacked micellar aggregation (Oakenfull & Sidhu, 1989). It was demonstrated that dietary saponin reduces blood cholesterol levels (Newman *et al*. 1957). This effect of the saponins is a result of binding to cholesterol excreted in bile, thus inhibiting entero-hepatic cholesterol recycling. Interactions of saponins with cholesterol and other sterols account for many of their biological effects, particularly those involving membrane activity. Dietary yucca extracts lower total and LDL cholesterol levels in hypercholesterolemic humans (Kim *et al*. 2003). Saponins affect the permeability of intestinal cells by forming complexes with cholesterol in mucosal cell membranes (Johnson *et al*. 1986).

OXIDATIVE STRESS

Oxidative stress can be defined as an increased exposure to oxidants and/or decreased antioxidant capacities and is widely recognized as a central feature of many diseases (Halliwell, 1996). Oxidative stress is known to be involved in the pathogenesis of atherosclerosis and other vascular injuries. Recent epidemiological studies have shown that hyperlipidemia and hyperglycemia represent risk factors for cardiovasular disease.

The chemistry of oxygen radicals and antioxidants is well described. Superoxide anion (O_2) formation from oxygen is the first step. O_2 is generated primarily by mitochondrial metabolism, molybdenum hydroxylase (xanthine, sulfite, and aldehyde oxidases) reactions, arachidonic acid metabolism, and NADPH oxidase-dependent processes in phagocytic cells. Reaction of O and hydrogen peroxide (H_2O_2) in the presence of transition metal, usually ferrous iron (Fe^{++}), produces the hydroxyl radical ($^\cdot OH$). When catalyzed by neutrophil myeloperoxidase (MPO), H_2O_2 and a chloride form hypochlorous acid (HOCl). $^\cdot OH$ and HOCl are emphasized because both are extremely potent oxidants. H_2O_2 gains significance as a central precursor to both $^\cdot OH$ and HOCl (Bast *et al*. 1991). Oxidants can not only damage DNA, lipids, and proteins (Heffner & Repine, 1989, but also mediate a variety of processes that could foster the development of COPD. The reactive oxygen species (ROS) such as $O_2^{\tilde{}}$, $OH^{\tilde{}}$ and H_2O_2, together with unstable intermediates in the peroxidation of lipids are well known inducers of cellular and tissue pathogenesis leading to numerous disease states including cardiovascular disease (Witztum, 1993) and age-related degenerative conditions (Finkel & Holbrook, 2000). Oxidants also promote epithelial permeability (Li *et al*. 1994). Oxidants in cigarette smoke even reduce O_2 generation by PMN in vitro. Treatment of endothelial cells with plasma exposed to cigarette smoke activates the pentose phosphate pathway metabolism, increases GSH extrusion, decreases ATP levels, and releases angiotensin-converting enzyme (ACE).

Many forms of cancer are thought to be the result of reactions between free radicals and DNA, resulting in mutations that can adversely affect the cell cycle and potentially

lead to malignancy. Some of the symptoms of ageing such as atherosclerosis are also attributed to free radical induced oxidation of many of the chemicals making up the body. In addition, free radicals contribute to alcohol-induced liver damage, perhaps more than alcohol itself. Radicals in cigarette smoke have been implicated in inactivation of α-antitrypsin in the lung. This process promotes the development of emphysema.

Free radicals may also be involved in Parkinson's disease, senile and drug-induced deafness, schizophrenia and Alzheimer's disease. The classic free radical syndrome, the iron-storage disease haemochromatosis typically associated with a constellation of free radical related symptoms including movement disorder, psychosis, skin pigmentary melanin abnormalities, deafness, arthritis, and diabetes. The free radical theory of ageing proposes that free radicals underly the ageing process itself and also they are very important intermediates in natural processes involved in cytotoxicity, control of vascular tone, and neuro-transmission.

Lipid peroxidation: Free radical damage to cellular target sites includes oxidative damage to proteins, lipids membranes and to DNA. Free radicals trigger lipid peroxidation chain reactions by abstracting a hydrogen atom from a side-chain methylene carbon. The resulting carbon-centered lipid radical then reacts with O_2 in aerobic cells to give a peroxyl radical that subsequently propagates a chain reaction which transforms polyunsaturated fatty acids (either as free acids or as part of lipids) into lipid hydroperoxides. Lipid peroxidation (LPO) can impair membrane function, inactivate membrane-bound receptors and enzymes, disturb membrane fluidity, and increase permeability (Halliwell & Chirico, 1993.). Lipid hydroperoxides can also interact with

antioxidants (such as tocopherol) or decompose after reacting with metal ions (such as iron or copper) or iron proteins (such as hemoglobin), leaving hydrocarbon gases (ethane, pentane) and unsaturated aldehydes (malondialdehyde) as by-products. Methods for detecting and quantifying LPO in vitro and in vivo examine lipid peroxides or derived radicals directly or else detect lipid peroxide conjugates or decomposition products indirectly (Hageman *et al*. 1992).

LPO is free radical mediated chain reaction, which can be initiated by the hydroxyl radical and attacks on PUFA in membranes and plasma lipoprotein particles resulting in oxidative damage (Halliwell, 1994). Perhydroxy radical partitions into lipids in the membranes, at which time unsaturated fatty acid and transmembrane proteins with oxidizable amino acids are attacked, subsequent reactions may proceed into the interior of the cell. (Peck, 1994). In addition, lipid hydroperoxides are readily decomposed by traces of transition metal ions to produce the free radical intermediates of LPO capable of propagating the chain reaction (Wisemann, 1996).

Carbonyl Proteins: Oxygen radicals can modify amino acid side chains, form protein aggregates, cleave peptide bonds, and make proteins more susceptible to proteolytic degradation (Stadtman, 1990). In the process, some amino acid residues are converted to carbonyl derivatives.

Protein oxidation: Amino acids in many reactions can form carbonyls, such as the oxo acids and aldehydes with the same or less number of carbon atom than the parent amino acid; e.g. glycine giving rise to glyoxsal and glyoxylic acid, formaldehyde and formic acid; alanine giving rise to acetaldehyde and acetic acid, etc. During the oxidation of

aliphatic amino acids by hydroxyl, hydroxylated derivates, notably of the side chains, are formed. During the oxidation of aromatic residues, the formation of phenoxyl radicals from tyrosine, and their conversion into dityrosine and further products, can occur. Hydroxylation of phenylalanine, tyrosine and tryptophan is also a characteristic reaction of hydroxyl radicals, and similar reactions of histidine (giving 2-oxohistidine) are important. Histidine can form imidazole decay products or in some cases aspartic acid and can form some histidine derivatives. Fenton reaction can generate both the aliphatic and aromatic products.

It is well known that lipid radicals may damage proteins. Such reactions show differences from those observed with radiolytic systems. End-products of lipid peroxidation, such as malondialdehyde and 4-hydroxynonenal are inactivating agents, through Schiff base formation. Schiff bases are short lived species formed by the reaction of carbonyl groups with amines, and can be formed during exposure of proteins to lipid derivated aldehydes, autoxing sugars and amino acid derivated aldehydes. In systems that contain lipid hydroperoxides and soluble proteins, metal ion catalysed reactions appear to be central. Protein inactivation by peroxidizing lipid is often associated with the binding of lipid components of protein. Thus, in membranes, competition and interactions between protein and lipid oxidation are expected. Metal ion catalysed damage to unirradiated membranes seems to affect lipid and protein in parallel, and is restricted by *a*-tocopherol.

Lipid oxidation products, notably aldehydes, can modify the lysine residues. During metal ion and radiolytic attack, lipid and protein oxidation are often concurrent, and

occur even while much vitamin E remains. In contrast, hypochlorite selectively attacks the protein, consuming mainly lysine, tryptofan, cysteine and methionine residues, and giving rise to chloramines.

Lipid oxidation products: Studies have established the presence of oxidized lipids in human atherosclerotic lesions (Gilbert *et al.* 1969). It is suggested that lipid peroxidation was either secondary to the deposition of lipids or played an active role in the pathogenesis of atherosclerosis.

Fatty acid oxidation products: Several different types of fatty acid oxidation products are present in human atherosclerotic lesions. Of these, hydroxy products of linoleic acid or hydroxyoctadecaenoic acids (HODEs) were the first to be detected. These, together with hydroxyeicosatetraenoic acids (HETEs or hydroxy fatty acid oxidation products of arachidonic acid), are the most abundant type of oxidized lipid in atherosclerotic lesions. Most of these products are present as cholesterol esters. Cholesteryl linoleate and cholesteryl arachidonate are the major, readily oxidizable lipid in LDL (Esterbauer *et al*. 1992), and LDL is the major source of lipids that accumulate during atherosclerosis. Most esterified and nonesterified HODEs are derived from nonenzymatic oxidation reactions (Upston *et al*. 2002). This is consistent with the observation that during lipoxygenase-mediated LDL oxidation, enzymatic oxidation reactions are quickly superceded and dominated by nonenzymatic oxidation reactions (Heydeck *et al*. 2001). Human atherosclerotic lesions also contain oxo-octadecaenoic acids and F_2-isoprostanes, the latter represent the secondary radical oxidation products of arachidonic acid.

Oxysterol: Cholesterol can undergo enzymatic and nonenzymatic oxidation to a range of oxysterols, and a number of studies reported the presence of oxysterols in organic extracts of human aortas (Brown & Jessup, 1999). The product of mitochondrial 27-hydroxylase, 27-hydroxycholesterol is the most abundant oxysterol in atherosclerotic lesions (Smith & van Lier, 1970) and human macrophage foam cells. Its concentration increases in parallel with that of cholesterol and increasing severity of atherosclerosis (Upston *et al.* 2002), suggesting that 27-hydroxycholesterol could be produced by cells in atherosclerotic lesions in response to cholesterol accumulation (Brown & Jessup, 1999). After 27-hydroxycholesterol, 7-ketocholesterol is the next most abundant oxysterol in advanced human atherosclerotic lesions, followed by 7β- and 7α -hydroxycholesterol. The latter three oxysterols are generally considered to be nonenzymatic oxidation products derived from dietary sources or generated *in vivo*. Other oxysterols present in human lesions include 7α-hydroperoxycholesterol that may represent a major cytotoxin. Oxysterols appear to concentrate in foam cells (Hultein *et al.* 1996), although with the exception of 27-hydroxycholesterol, their tissue concentrations do not generally parallel disease severity. Oxysterols are predominantly present in esterified form, as mono- and diesters.

Antioxidant defense system:

To help keep the reactions in check, antioxidants to mop up unwanted free radicals have evolved, e.g., vitamin C, vitamin E. Glutathione, a natural thiol commonly involved in coupling reactions to help eliminate unwanted chemicals by renal excretion. Antioxidant may quench free radicals, change their redox state, be targeted for destruction, regulate oxidative processes involved in signal transduction, affect gene

expression pathways of cell proliferation, differentiation and death. This is being achieved at various subcellular and molecular levels including antioxidants that interact with the redox antioxidant network such as ascorbic acid and a-tocopherol, thiols, bioflavonoides, carotenoides and induction of phase two enzymes and immune cell stimulation.

Antioxidants:

Oxidative damage is involved in the pathogenesis of many diseases, such as cardiovascular disease, cancer and diabetics. The antioxidant defense system plays an important role in protecting body from oxidative damage. Numerous studies have been shown that a single vitamin or mineral supplementation has the beneficial effect on the antioxidant defense system. Cellular free radicals damage, as with radiation damage, can be repaired by natural antioxidants. The most important are vitamin C (ascorbate), vitamin E (à-tocopherol), and glutathione (a thiol, GSH). These can donate either an electron or a hydrogen atom to cellular molecules oxidized by free radicals, including those generated by ionizing radiation. They can thus influence damage to all cellular constituents, including DNA, proteins and lipids (membranes). Lipid peroxidation can be effectively inhibited.

Radical repair by glutathione in the presence of oxygen itself inevitably produces some cellular oxidative stress. There is much recent speculation as to the role of antioxidants in diverse diseases and the ageing process itself. The levels of oxidized DNA bases excreted in the urine are being correlated with lifespan. Other correlations involve

the extent of mitochondrial generation of superoxide radicals. Smokers have a decreased antioxidant status compared to non-smokers, perhaps from taking in 10^{17} free radicals per puff. These are mainly nitric oxide, taken in with 500 ppm isoprene to generate $NO_2^{\cdot}$, which starts off a nasty chain reaction just like photochemical smog in their lungs.

Aqueous antioxidants: During normal process, transfer of aqueous nonproteinaceous antioxidants to the intima, such as ascorbate and urate, occurs via simple diffusion. Thus the concentration of these antioxidants in the extracellular space of the vascular wall may approximate that of the lumen. Indeed, the concentration of ascorbate in interstitial fluid and lymph is similar to that in plasma.

Ascorbic acid: Oxidized LDL has been implicated in the initiation of arterial plaques and antioxidants including vitamin C have been shown in animal studies to prevent that process. In addition, cross sectional data also suggest a role for vitamin C in lowering blood pressure and in improving serum lipid profiles, two of the major risk factors for heart disease. Studies that examined the effects of vitamin C on cultured chondrocytes indicated that this vitamin induces complex changes in cell differentiation (Wu *et al*. 1989). The best characterized funtion of ascorbic acid is the cofactor for prolyl and lysyl hydroxylase enzymes present in all connective tissues. The post-translational modification of these amino acid residues of procollagen is necessary for folding into a triple helix structure that can be secreted by cells. Normal arteries contain approximately one-third the concentration of ascorbate than normal human plasma. Elevated levels of ascorbate in advanced human atherosclerotic plaque compared with normal arteries (Suarna *et al*. 1995). Furthermore, only small amounts of vitamin C were present as

dehydroascorbic acid (the 2-electron oxidation product of vitamin C) in atherosclerotic plaque.

Low blood levels of ascorbic acid are detrimental to health and vitamin C is correlated with overall good health and cancer prevention (Lee *et al.* 2003). There is some evidence that large doses of vitamin C, either in multiple divided oral doses or intravenously, have beneficial effects in cancer therapy (Riordan *et al.* 2003). Oral doses, even in multiple doses, are not as effective as intravenous administration. Vitamin C at a dose of 1.25 g administered orally produced mean peak plasma concentrations of 135 ± 21 μmol/L compared with 885 ± 201 μmol/L for intravenous administration (Padaayatty *et al.* 2004).

Flavonoids: Flavonoids belong to vast group of polyphenolic compounds that are widely distributed in all foods of plant origin. Flavonoids have been of interest owing to their observed biological effects *in vitro* such as free radical scavenging, antioxidant property, modulation of enzymatic activity, inhibition of cellular proliferation, as well as potential utility as antibiotic, anti-allergic, antidiarrheal, antiulcer and anti-inflammatory (Bravo, 1998). Catechins in green tea and isoflavones in soya beans, both of which have potential health benefits have been identified as anti-angiogenic (Joussen *et al.* 2000). They may also have the potential to reduce adipose tissue growth through the inhibition of angiogenesis.

Urate: Reaction of urate with $ONOO^-$ results in formation of a compound that can relax blood vessels, apparently via release of $NO^\cdot$. However, some studies have reported a

direct association between uric acid concentrations and atherosclerosis, hypertension, and cardiovascular mortality.

Bilirubin: There is increasing evidence supporting an inverse association between cardiovascular disease and plasma levels of bilirubin and heme-oxygenase. But a protective role has been attributed to the heme oxygenase product carbon monoxide. However, recent studies indicate that bilirubin may be protective by inhibiting the proliferation of vascular smooth muscle cells.

Glutathione: Glutathione - made from the combination of three amino acids cysteine, glutamate, and glycine, is a powerful antioxidant found within every cell. Glutathione plays a role in nutrient metabolism, and regulation of cellular events (including gene expression, DNA and protein synthesis, cell growth, and immune response). Forms part of the powerful natural antioxidant enzyme glutathione peroxidase which is found in our cells. However, glutathione is predominantly known as an antioxidant protecting our cells from damage caused by the free radical hydrogen peroxide. Glutathione also helps the other antioxidants in cells stay in their active form. Animal and human studies demonstrate that adequate protein nutrition is crucial for the maintenance of glutathione homeostasis. Brain glutathione levels have been found to be lower in patients with Parkinson's disease. Experimental studies have demonstrated that reduced glutathione (glutathione) is involved in cellular protection from deleterious effects of oxygen free radicals in ischaemia and reperfusion (Low blood glutathione levels in acute myocardial infarction). There will be a feedback inhibition in glutathione synthesis. This means that

if glutathione levels are excessively increased with the help of nutrients, the body may decrease its natural production.

Glutathione deficiency contributes to oxidative stress, which plays a key role in aging and the worsening of many diseases including Alzheimer's disease, Parkinson's disease, liver disease, cystic fibrosis, sickle cell anemia, HIV, AIDS, cancer, heart attack, and diabetes. The concentration of glutathione declines with age and in some age-related diseases. Polyphenols in food plants are a versatile group of phytochemicals with many potentially beneficial activities in terms of disease prevention and these phytochemiccals may enhance gamma-glutamylcysteine synthetase. This enzyme is rate limiting in the synthesis of Gluathione. Age-related depletion of glutathione levels deleterious to metabolically active tissues, such as the heart and brain. Treating old rats with lipoic acid (40 mg/kg body wt; by i.p.) markedly increased tissue cysteine levels by 54% 12 h following the treatment and subsequently restored the cerebral glutathione levels. There is an improvement in glutathione status in young adult patients with cystic fibrosis supplemented with whey protein. The lung disease of cystic fibrosis is associated with a chronic inflammatory reaction and an over abundance of oxidants relative to antioxidants. The secretion of a peptide called glutathione by lung cells is impaired in cystic fibrosis, and there is good evidence to suggest that the lack of glutathione in lung fluid plays a key role in the chronic inflammation and infection that occurs.

Protein thiols: In cells, the most abundant low-molecular-weight thiol is GSH with concentrations in the range of 1–5 mM (Bray & Taylor, 1993). This abundance of GSH facilitates the formation of its nitrated ($GSNO_2$) and nitrosated (GSNO) forms that may

serve, as tissue "buffers" of ·NO (Wink & Mitchell, 1998). These GSH adducts possess biologic activity that is comparable to ·NO, including vasodilation and inhibition of platelet aggregation. Indeed, low-molecular-weight thiol adducts of ·NO have been demonstrated with a variety of species such as cysteine, homocysteine, and synthetic species including N-acetyl cysteine and penicillamine (Hogg, 2000). Thiols represent a prominent biologic target for reactive nitrogrn species (RNS) and protein cysteine residues are also subject to modification mediated by RNS. S-Nitrosation of thioredoxin is thought to activate apoptosis signal-regulating kinase 1 (Sambayev, 2003). Indeed, ·NO synthesis by many cells results in S-nitrosation of numerous proteins (Gow *et al.* 2002). This widespread utilization of S-nitrosation throughout nature has prompted considerable speculation that it represents a conserved method of signaling throughout biologic systems. Exposure of cells to toxic levels of oxidants results in the formation of S-glutathiolated proteins that spanned a broad range of cellular functions including metabolism, the cytoskeleton and antioxidant protection (Dafre *et al.* 1996). S-Glutathiolation of protein cysteine residues protects against higher oxidation states of the protein thiol, thereby preserving the reversibility of this type of modification (Barrett *et al* 1999). Reduced protein thiols can be regenerated from their S-glutathiolated forms enzymatically through the action of protein disulfide isomerase, mitochondrial glutaredoxin, or thioredoxin (Jung & Thomas, 1996). Glutathiolation of glyceraldehyde-3-phosphate dehydrogenase preserves its function under conditions of high oxidative stress and sarcoplasmic Ca^{2+}-ATPase activity .

Lipid-soluble antioxidants: In the healthy blood vessel the concentrations of lipophilic nonproteinaceous antioxidants resemble the concentration of lipoprotein lipid present in

interstitial fluid. They are considerably lower than their respective plasma levels or the blood vessel concentration of albumin (Smith EB, 1974). Usually, lipophilic antioxidants are associated with lipoproteins, the concentration of which is low in healthy blood vessels. By implication, lipid-soluble antioxidants detected in healthy arteries are probably located within cells. However, as atherosclerotic lesions develop, lipoproteins including LDL, with their full complement of antioxidants, transfer to the vessel wall from plasma. Thus it is reasonable to assume that, as lesions develop, an increasing proportion of the lipid-soluble antioxidants detected is localized extracellularly within lipoproteins and lipid depositions derived from them.

Vitamin E: α-Tocopherol (α-TOH) oxidation observed at the earliest stage of atherogenesis, at a time when the extent of fatty acid oxidation is minimal and less than that of the vitamin. α-Tocopheryl quinone is the single major oxidation product. As the severity of the disease increases, the ratio of oxidized lipid to oxidized α-TOH also increases. However, at all stages of atherosclerosis, the observed relative abundance of α-tocopheryl quinone over 2,3- and 5,6-epoxy-α-tocopherylquinones resembles the situation when LDL is oxidized *in vitro* by 2e- rather than 1e-oxidants (Terentis *et al*. 2002). These findings are consistent with and further support the notion that 2e-oxidants are primarily involved in the oxidative events taking place in the artery wall and involving vitamin E.

Briefly, vitamin E can enhance the bioactivity of ·NO, inhibits smooth muscle proliferation and limits platelet aggregation (Freedman *et al*. 1996). One common mechanism to account for these effects of vitamin E is the inhibition of protein kinase C

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stimulation. In the setting of atherosclerosis, inhibition of protein kinase C by vitamin E would maintain normal vascular homeostasis and thus reduce the clinical incidence of cardiovascular disease.

Long-term supplementation with α-tocopherol substantially reduced prostate cancer incidence and mortality in male smokers. In a recent study with laboratory mice, It has been found that peroxynitrite, a free radical built around an oxygen and nitrogen molecule, increased activity of cyclooxygenase-2, an enzyme involved in making inflammatory prostaglandins. Giving the mice extra vitamin E reduced this enzyme and proinflammatory prostaglandin E2 levels. Short-term vitamin E supplementation improves immune responsiveness in healthy elderly individuals; this effect appears to be mediated by a decrease in PGE_2 and / or other lipid-peroxidation products.

Carotenoids: Carotenoids are natural pigments synthesized by plants and micro-organisms. The natural functions of carotenoides are to serve as light absorbing pigments during photosynthesis and the protection of cells against photosensitization. Carotenoid is a complex molecule and leads to an array of products. Several carotenoids are precursors of retinoid (Olson, 1994). A wealth of information has been reported from *in vitro* chemical studies, cell culture studies, *in vivo* studies in animals suggesting that β-carotene provides significant protection against a number of disease processes, including cancer (Mayne *et al*. 1994). It has been reported that high intakes of β-carotenoid rich vegetables and fruits, as well as higher blood concentrations of β-carotene, are associated with decreased risk for many cancers (van Poppel*, 1993*). Among the cancers studied, the most consistent evidence is for stomach and lung cancers (Flag *et al*. 1995). β-carotene

supplementation prevents oral leukoplakia, a condition considered an indicator of precancerous lesions (Kaugers *et al*. 1994). In addition to their antioxidant properties, carotenoids exhibit a wide range of biologic activities. They can affect intercellular communication, immune response, neoplastic transformations and growth control, and cellular levels of the enzymes that detoxify carcinogens.

Antioxidant enzymes:

The activities of cellular antioxidant enzymes are modulated in various disease states by the abundance of free radical species (Halliwell & Gutteridge, 1998). The cellular radical-scavenging systems include the enzymes such as superoxide dismutase (SOD), which scavenges the superoxide ion by speeding up its dismutation, catalase (CAT), a haeme enzyme, which removes hydrogen peroxide and glutathione peroxidase (GPX), a selenium-containing enzyme, which scavenges other peroxides as well as hydrogen peroxide (Blake *et al*. 1987). The discovery of the SOD activity of erythrocuprein together with the finding that almost all mammalian cells contain SOD suggests the physiological importance of this enzyme in abstracting the superoxide ion (McCord & Fridovich, 1969).

Maintaining the balance between the rate of generation of radicals and scavenging of radicals is an essential part of biological homeostasis. It is of particular interest that SOD catalyzes the breakdown of $O_2^{\cdot}$ to O_2 and H_2O_2, prevents formation of $O\bar{H}$ and has hence been implicated as an essential defense against the potential toxicity of oxygen. The ROS scavenging activity of SOD is effective only when it is followed by the actions of CAT

and GPX, because the dismutase activity of SOD generates H_2O_2, which needs to be further scavenged by CAT and GPX.

Superoxide dismutase: The discovery of superoxide dismutase (SOD) by McCord and Fridovich (1969) strongly suggested that all aerobically metabolizing cells are capable of producing superoxide ions, which could play a role in normal metabolic processes. Three forms of SOD are important: manganese SOD, which is located in mitochondria, Cu-Zn SOD, which resides in the cytoplasm, and extracellular SOD, which lines blood vessels. Manganese superoxide dismutase (MnSOD) is the primary antioxidant in the mitochondria. This enzyme converts reactive oxygen species to oxygen and hydrogen peroxide. MnSOD as an endogenous antioxidant in mitochondria may play a role in preventing prostate cancer and over-expression of MnSOD in the prostate inhibits cancer cell growth *in vivo* (Li *et al*. 1998). In mitochondria, the superoxide anion is dismutated by MnSOD into oxygen and hydrogen peroxide, which is further detoxified by mitochondrial glutathione peroxidase, an enzyme requiring selenium, or catalase into water. Thus, high levels of MnSOD expression may lead to enzyme imbalance and induce toxicity if glutathione peroxidase activity is low due to inadequate selenium intake or if antioxidants are in high demand due to physiologic conditions or lifestyle factors, such as smoking (Kinnula & Crapo, 2004).

Catalase: Catalase, a 240,000 dalton molecular weight protein tetramer, is located in cytosolic peroxisomes. It detoxifys H_2O_2 to oxygen and water in the reaction:

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$
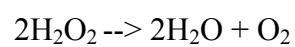
Catalase substrates generally consist of small molecules including H_2O_2 and the methyl or ethyl hydroperoxides. Catalase exhibits high reaction capacity, but has a high K_m value $(10^7/M \cdot sec)$ for H_2O_2

Glutathione Peroxidase: Glutathione peroxidase (GPX), a tetramer, is an 85,000 dalton protein containing selenium, and uses glutathione as a co-substrate. GPX is a cytosolic enzyme and also eliminates H_2O_2; but, in comparison to catalase, has a wider range of substrates including lipid peroxides. Glutathione peroxidase plays a variety of roles in cells, including DNA synthesis and repair, metabolism of toxins and carcinogens, enhancement of the immune system, and prevention of fat oxidation. The kinetics of this enzyme is very complex, but it is having greater affinity for H_2O_2 than catalase. Glutathione peroxidase primarily functions to detoxify low levels of H_2O_2 in the cell.

GPX play an important role in cellular antioxidant defense by reducing H_2O_2 and various hydroperoxides using glutathione as a reducing agent to form water and alcohols, respectively.

H_2O_2 + 2GSH $\rightarrow$ H_2O + GSSG or

ROOH + 2GSH $\rightarrow$ ROH + GSSG + H_2O
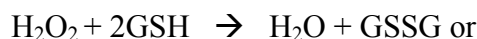
There are at least four isoforms of GPX found in mammals. The major cellular GPX (GPX-1) is expressed in all tissues and contributes to most of the GPX activity present in erythrocytes, kidney, and liver. The phospholipid-hydroperoxide GPX, which is capable of reducing hydroperoxides of phospholipids and cholesterol, is found mainly in the

testis. The plasma GPX is detected in milk, plasma (0.149 U/ml plasma), and lung alveolar fluid (3.6 mU/ml of 25-fold concentrated lavage fluid) of humans.

Antioxidant enzymes may act in a coordinate manner to prevent living tissue from oxidant damage. Two of these mentioned enzymes catabolize H_2O_2 and other peroxides. Since the enzymes which reduce are inactivated by H_2O_2, and the enzymes catabolizing H_2O_2 are inactivated by, one enzyme system complements the other by detoxifying its corresponding inactivating oxidant. And, the catabolism of H_2O_2 by GPX and catalase inhibits the formation of OH. This defensive interplay is critical for cell survival under oxidant stress.

The enhanced Cu-Zn-SOD activity protects animals against tissue injury induced by a variety of pathogenic conditions that include cold-induced brain edema and infarction (Chan *et al*. 1991), alloxan- or streptozotocin- induced diabetes (Kubisch *et al*. 1994) and myocardial ischemia-reperfusion injury, because mitochondrion is one of the major subcellular sites capable of generating superoxide anion radicals.

Inflammation

Inflammation is considered to a primary event of host defense system during cell stress and local tissue changes, promoted by an increasing concentration of oxidants and osmotic pressure. Inflammation can be defined as a change of the morphological equilibrium in a specific area of the tissue caused by different kinds of agents: physical, chemical, or biological. It can be represented by capillary dilatation with fluid accumulation (oedema) and by phagocyte emigration and accumulation (neutrophils,

monocytes, macrophages), which contribute to hyperalgesia generation and loss of tissue function. Other characteristics, such as erithema and fever, can also be observed during inflammatory events. The last feature occurs after cytokine release by activated macrophages, leading to a vessel dilatation resulting from smooth muscular relaxation and followed by an increase in local blood flow (Sigal & Ron, 1994). The increased hematocrit leads to an erythrocyte aggregation, and leukocytes move from the central axial of the vessel to the periphery (Chien, 1982).

The fibrinolytic system, kinins, complement, vasoactive amines and nitric oxide may lead to inflammation when physiologically altered (Sies *et al*. 1991). Specific-immune events, such as hypersensitive reactions (types I, II, III, and IV) can also lead to inflammation (Roitt *et al*. 1997; Terr, 1992). Inflammatory events involve micro-vascular changes with increased vascular permeability, flow exudation, including plasmatic protein and amplification of endogenous chemical mediators (Cirino, 1998). Neuropeptides of the skin nerves may interact with target-cells in the skin, releasing more skin neuropeptides, such as P substance, vase intestinal peptides, and peptides regulator of calcitonin gene, which modulates not only the function of inflammatory and immunocompetent cells but also endothelial and epithelial cells (Luger & Lotti, 1998).

Similarly, many intra- and extracellular phospholipases are activated from the cytoplasmic membrane phospholipids and activate other enzymes, such as cyclooxygenase (COX) and lipoxygenase (LOX), which act on arachidonic acid and eicosanoid metabolism (Samuelsson, 1983). Excessive quantities of free radicals trigger neutrophil NADPH oxidase and dissociate a variety of redox systems, including xanthine

dehydrogenase of endothelial cell in inflamed areas (Winrow *et al*. 1992). Low-density lipoprotein oxidative changes, the restraint inactivation of protease, DNA damage, and heat-shock protein synthesis are also affected by free radicals excess (Lara, 1982). Collagen and hyaluronic acid changes may also occur, interfering with synovial liquid viscosity, forming carbon radicals that react against themselves, decreasing collagen molecule flexibility (Freeman & Crapo, 1982).

Reactive oxygen species may participate in inflammation events, such as: (a) polymorphonuclear leukocyte (PMN) and monocyte/macrophage chemotaxis; (b) specific stimulus related to respiratory burst, especially in inflammatory cells with greater free radical production; (c) low concentration of scavenger enzymes in interstitial spaces; and (d) formation of chelant metal immune complexes which can also produce hydroxyl radical (Delmaestro, 1980).

Several etiological and metabolic pathways are involved in the inflammatory response (Pearson, 1964). Inflammation is an important causative agent of human morbidity and mortality, such as systemic inflammatory response syndrome, multiple organ dysfunction syndrome, and multiple organ failure (Baue *et al*. 1998). In this way, inflammation events permit molecule identification and allow the development of drugs capable of acting on a variety of related metabolic pathways.

Treatment of Inflammation: Corticosteroid drugs have been used in anti-inflammatory therapy in clinical and pre-clinical analysis (Faucheron & Parc, 1996). Corticosteroids act on many different tissues and body systems. At physiological concentrations, they maintain normal blood pressure, heart function, respond to inflammatory prostaglandin

action, and maintain blood volume, diminishing vascular endothelium permeability. However, these steroids effects are accentuated at high pharmacological concentrations due to toxicity, leading to target-cell dysfunction (mast cells, macrophages, vascular smooth muscles, and mucous glands (Guyton & Hall, 1996).

Glucocorticoids are used due to their general anti-inflammatory activity, The activity of these compounds depends on the presence of a hydroxyl group in carbon-11 (Robbers *et al*. 1996), which ranges from clinical suppression of rheumatoid arthritis to palliative treatment of some allergic manifestations, such as bronchitis, asthma, and anaphylatic reactions (Barnes, 1995). They also act upon cytokines involved in eosinophil, basophil, and lymphocyte recruitment (Schwiebert *et al*. 1996). Anti-inflammatory effect of glucocorticoids may involve action on arachidonic acid metabolites, phospholipase A2 inactivation, and COX and LOX (Mygind, 1993).

The effects of non-steroid anti-inflammatory drugs (NSAID) on the synthesis of inflammatory prostaglandins, especially the PGE_2, are widely known (Samuelsson, 1983). The pharmacological aim of most NSAID is the COX-enzyme inhibition (PGHS or PGH2) (Kurumbail *et al*. 1996). The mechanism of all NSAID is based on their involvement with the hydrophobic region of COX-1 and COX-2 isoforms. (Wu, 1998). Many other classes of COX-2 selective inhibitors are: 1) sulfonamides 2) tricyclic methylsulfone derivatives (Volpert R., Elstner EF. 1993). In relation to LOX inhibitors, the monomethylamine analogs, such as LY269415 and LY-221068, are not only antioxidants but also strong inhibitors of iron-dependent lipid peroxidation (Panetta *et al*. 1991). Inhibition of free radicals production has been prevented by endogenous

antioxidant and exogenous antioxidants (Percário & Baptista, 1996). Natural antioxidant mechanisms have been stimulated. Small molecules and chelants of metallic ion (selenium, zinc, copper, manganese, vitamins A, C, and E, cysteine, and reduced glutathione, and some plasma compounds) participate in inflammatory events and act on oxygen toxic reactive species (Bisby, 1990).

Spices : Nutraceuticals with health beneficial properties

Spices are a group of esoteric food adjuncts, which have been in use for thousands of years to enhance sensory quality of foods, the quantity and variety consumed in tropical countries being particularly extensive. These spice ingredients impart characteristic flavour, aroma or piquancy and colour to foods. Some spices like fenugreek can also modify the texture of food. It is a common experience that their distinct aroma stimulates the appetite. Not only are these spices used as flavourings and seasonings but they are also used in perfumery, cosmetics, and toiletries. In addition, several spices have long been recognized to possess a few medicinal properties such as tonic, carminative, stomachic antispasmodic, and antihelmenthic as listed in Table-4 (Nadkarni & Nadkarni, 1976). Although these observations are largely empirical, these undoubtedly efficacious attributes have earned them pharmacological applications in the indigenous system of medicine not only in India, but in other countries also.

Spices are not only used individually, but also in the form of spice mixtures known as `curry powders' to suit different tastes and dishes. Although spices have never been considered to be contributing anything to human nutrition, this group of food adjuncts is in use in human diets for centuries as flavour modifiers to make food more palatable.

Extensive animal studies carried out to evaluate the safety aspect of spices have indicated that even at much higher dietary levels (up to 100 times the normal intake), red pepper, black pepper and turmeric have no adverse effects on growth, organ weights, Feed Efficiency Ratio, nitrogen balance and blood constituents. In the past 3 decades, many more beneficial physiological effects of spices have been experimentally documented (Table-5) which suggests that the use of these food adjuncts extend beyond taste and flavour (Srinivasan, 2005). Among the health problems that affect mankind, diabetes, cardiovascular disease, inflammatory disorders including arthritis, and cancer have received considerable attention. During recent years, spices and their active principles have been studied as possible ameliorative or preventive agents. The salient features of a variety of health beneficial physiological effects of common spices or their active principles so far documented are summarized below.

Table 4. Medicinal properties of spices recognized for long time.

Spice	Medicinal Properties recognised for a long time

Coriander (*Coriandrum sativum*)	Anti-dyspeptic, flavourant
Cumin (*Cuminum cymminum*)	Antispasmodic, carminative, digestive stimulant
Fenugreek (*Trigonella foenumgraecum*)	Diuretic, emmenagogue, emollient, useful in heart diseases
Garlic (*Allium sativum*)	Anti-dyspeptic, anti-flatulent, for ear infection, duodenal ulcers, as rubefacient in skin diseases
Ginger (*Zingiber officinale*)	Sialogogue, Useful in diseases of heart and blood
Onion (*Allium cepa*)	Diuretic, emmenagogue, expectorant, for bleeding piles
Pepper (*Piper nigrum*)	Antipiretic; Rebefacient
Red pepper (*Capsicum annuum*)	Anti-inflammatory, for pain relief (Rheumatism /neuralgia) Useful in indigestion, rubefacient
Turmeric (*Curcuma longa*)	Anti-inflammatory, diuretic, laxative, good for affections of the liver, jaundice, diseases of blood

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Table 5. Experimentally documented health beneficial effects of spices.

Health beneficial effect	Spices observed to exert
Lowering of Blood cholesterol	Garlic, Onion, Fenugreek, Turmeric / Curcumin, Red pepper / Capsaicin
Prevention and dissolution of cholesterol gallstones	Curcumin, Capsaicin
Protection of erythrocyte integrity in hypercholesterolemic condition	Curcumin, Capsaicin, Garlic
Hypoglycaemic potential	Fenugreek, Garlic, Onion, Turmeric, Cumin
Amelioration of diabetic nephropathy	Curcumin, Onion
Antioxidant effect	Turmeric / Curcumin, Capsaicin, Eugenol
Anti-inflammatory and anti-arthritic	Turmeric / Curcumin, Capsaicin, Eugenol
Anti-mutagenic / cancer preventive	Turmeric / Curcumin, Garlic, Mustard
Digestive stimulant action	Curcumin, Capsaicin, Piperine, Ginger, Cumin, Ajowan, Fennel, Coriander, Onion, Mint
Antimicrobial	Turmeric / Curcumin, Garlic, Asafoetida

Hypocholesterolemic effect: Consumption of high fat diet may lead to an increase in serum cholesterol and plasma fibrinogen levels. These in turn may result in decreased fibrinolytic activity and blood coagulation time. These changes may increase the risk of heart disease and atherosclerosis. Some of the commonly used spices have been evaluated for a possible hypocholesterolemic action in a variety of experimental situations in both animals and humans (Srinivasan et al., 2004). The spices - fenugreek, red pepper, turmeric, garlic, onion and ginger are found to be effective as hypocholesterlemics under various conditions of experimentally induced hypercholesterolemia / hyperlipemia. The hypolipidemic effectiveness of turmeric / curcumin (Srimal, 1997), red pepper / capsaicin (Govindarajan & Satyanarayana, 1991) and of onion and garlic (Carson, 1987; Jain & Apitz-Castro, 1994) has been periodically reviewed in recent years by different authors. Further, fenugreek, onion and garlic are effective in humans with hyperlipemic condition. Fenugreek seeds were hypocholesterolemic in rats with hyperlipidemia induced by high cholesterol diet (Sharma, 1984). Apart from the hypocholesterolemic effect of capsaicin, its beneficial effect on overall lipid metabolism under different conditions of lipemia have also been reported (Srinivasan & Satyanaraya, 1987).

Anti-lithogenic effect: Studies on experimental induction of cholesterol gallstones in mice and hamsters by feeding a lithogenic diet have revealed that the incidence of gallstones is 40-50% lower when the animals are maintained on 0.5% curcumin or 5mg% capsaicin containing diet (Hussain & Chandrasekhara, 1992, 1993). Animal studies have also revealed significant regression of preformed cholesterol gallstones by these spice principles in a 10 week feeding trial (Hussain & Chandrashekara, 1994). The anti-

lithogenicity of curcumin and capsaicin was considered to be due not merely to their ability to lower cholesterol saturation index, but also to their influence on biliary proteins (Hussain & Chandrashekara, 1994a).

Anti-diabetic potential: As part of the dietary treatment of diabetes, there has been a continuous search for novel anti-diabetic drugs from plant sources. Spices - the natural and common food adjuncts have also been examined in this direction and reviewed recently (Srinivasan, 2005a). Considerable number of human experiments has also been carried out on this aspect, in addition to experimentally induced animal diabetic models. Fenugreek, garlic, onion, turmeric and cumin have been studied for their antidiabetic potential, but human trials are very limited except with fenugreek. Fenugreek, turmeric or its active principle curcumin, onion or its active principle allyl propyl disulfide, garlic and cumin have been observed to improve glycemic status in diabetic animals / NIDDM patients.

Studies shave demonstrated the antidiabetic potential of fenugreek in both type-1 and type-2 diabetes. Addition of fenugreek seeds to the diets of diabetic patients or animals results in a fall in blood glucose and improvement in glucose tolerance (Sharma, 1986; Sharma *et al*. 1996). The hypoglycemic effect is attributed to the fibre and gum which constitute as much as 52% of the seeds. Garlic and onion are two other spices which have been widely used for their antidiabetic potential. Both these spices have been shown to be hypoglycemic in different animal models and in limited human trials. The hypoglycemic potency of garlic and onion has been attributed to the sulphur compounds present in them (Augusti & Sheela, 1996). Turmeric is another spice claimed to possess

beneficial hypoglycemic effect and to improve glucose tolerance in a limited number of studies. (Tank *et al.* 1990). Dietary curcumin and onion have been found to have a promising ameliorating influence on the severity of renal lesions in streptozotocin induced diabetic rats. (Babu & Srinivasan, 1998, 1999). Capsaicin has been shown to be useful in diabetic neuropathy (The Cpasaicin Study group, 1992).

Digestive stimulant action: Spices have been generally believed to intensify salivary flow, gastric juice and hence aid in digestion (Glatzel, 1968). Turmeric has the property of increasing the mucin content of the gastric juice. Spices such as ginger, mint, ajowan, cumin, fennel, coriander and garlic are used as ingradients of commercial digestive stimulants as well as of home remedies for digestive disorders – flatulence, indigestion and intestinal disorders. Animal studies have confirmed that a good number of spices bring about the enhanced secretion of bile acid content, which play a vital role in fat digestion and absorption (Bhat *et al*, 1984, 1985; Sambaiah & Srinivasan, 1991; Platel & Srinivasan, 2000). Spices - curcumn, (turmeric), capsaicin (red pepper), ginger, cumin, coriander, ajowan, fenugreek, mustard, onion and tamarind stimulate bile acid production by the liver and its secretion into bile. Spice principles / spices such as curcumin, capsaicin, ginger, piperine and mint have been shown to stimulate pancreatic digestive enzymes – lipase, amylase, trypsin and chymotrypsin, which play a crucial role in food digestion (Platel & Srinivasan, 2000a). A few spices have beneficial effect on terminal digestive enzymes of small intestinal mucosa (Platel & Srinivasan, 1996).

Antioxidant property: Spices have been investigated for their antioxidant potency in food systems about 50 years ago (Ramawamy & Banerjee, 1948). Oxidative damage at the cellular, subcellular is now considered to be an important event in disease event processes like CVD, inflammatory, carcinogenesis and ageing. The antioxidant property of curcumin and related compounds was reported by Sharma (1976). Lipid peroxidation in human erythrocyte membrane was inhibited by capsaicin and curucmin (Salimath *et al.* 1986). The antioxidant activity of spice principles – capsaicin, curcumin and eugenol are documented in animal studies by Reddy and Lokesh (1994). These compounds inhibited lipid peroxidation by enhancing antioxidant enzyme activities and antioxidant molecules. Thus antioxidant spices / spice principles have gained more importance for their possible role in the prevention of atherogenesis.

Anti-inflammatory and Anti-arthritic property: Animal studies have revealed that curcumin, capsaicin and eugenol lower the incidence and severity of arthritis and also delay the onset of arthritis. In carrageenan induced edema and cotton pellet granuloma models of inflammation in rats, the order of efficacy of analogs of curcumin was sodium curcuminate > tetrhydrcurcumin > curcumin > phenylbutazone (Mukhopadhyay *et al.* 1982). The anti-inflammatory property of spice principles – capsaicin and curcumin were investigated in rats by Joe and Lokesh (1997). These compounds reduced the incidence of carrageenan induced paw edema, reduced the severity of paw inflammation in arthritic rats, and delayed the onset of arthritis. These spice principles also inhibited the formation of arachidonate metabolites and lysosomal enzymes.

Antimutagenic and anticarcinogenic property: Curcumin possesses antimutagenic property in several experimental systems. Turmeric and curcumin were effective against benzopyrene and DMBA in the Ames test (Nagabhushan & Bhide, 1986). *In vivo* studies on animals suggest that turmeric and curcumin inihibit the formation of mutagens. Mice and rats maintained on turmeric and curcumin containing diet excreted lower levels of mutagenic metabolites as well as carcinogens than the controls (Usha, 1994). Turmric and curcumin also inhibited the mutagenicity of cigarette and beedi smoke condensates as also that of a tobacco based dentrifice (Nagabhushan *et al*. 1987). Shalini and Srinivas (1987) reported that turmeric protected DNA against lipid peroxide induced damage and against fuel smoke condensate induced damage. Like curcumin, the other principles eugenol, sesamolinol and flavourings like anisaldehyde, ethyl vanillin produce anti-mutagenic effect by protecting the cell from damage to DNA. Phenolic strucutre being present in the molecule would help in detoxification process.

Researches during the past few decades, human studies and some epidemiological data have indicated that cancer is preventable by dietary intervention. Extensive reviews have been published in recent years on this aspect (Ho & Huang, 1994). Curcumin has been demonstrated to have anti-tumour effect in animals treated with potent carcinogens. Vegetable extracts like mustard, cabbage, broccoli, cauliflower have the property of inactivating the mutagnicity of of food mutagens like tryptophan pyrolysate. The active principle of mustard family, the dithiolthiones is also used as anti-schistosomal drug and also protective against liver toxicity induced by some chemicals and aflatoxin. The anti-mutagenic effects of mustard seed powder have been assessed in experimental animals treated with potent.carcinogens. These experiments suggested that mustard like turmeric

has excellent antimutagenic properties. Hence protective factors in spices like turmeric, mustard and onion may act in more than one way to confer their beneficial effect.

Anti-microbial activity: Curcumin is a known bacteriostatic agent, whereas the essential oil of turmeric is anti-bacterial and fungistatic. An Oleoresin gum exuded from the root or rhizome of asafoetida is used as an anti-microbial agent in traditional medicine. Its use in chronic bronchitis and whooping cough have been well documented. The anti-bacterial properties of onion and garlic were well described by Louis Pasteur. They are effective against both gram positive and gram negative bacteria. The extracts of onion and garlic are known to inhibit pathogenic fungi belonging to *Aspergillus*, *Candida* and other species (Carson, 1987). Other spices like saffron, nutmeg, cumin, thyme etc, also have anti-microbial potential.

SCOPE OF PRESENT INVESTIGATION

Spices are a group of esoteric food adjuncts, which have been in use as flavour enhancers for thousands of years. In addition to their organoleptic properties, few spices are also known to possess several medicinal properties and are effectively used in the indigenous systems of medicine. In the past three decades, it has been experimentally documented that several common spices can also exert health beneficial physiological effects. These physiological effects of spices in most instances have been attributed to the main spice active principles present in them. Among these physiological influences spices are documented to exhibit, their hypolipidemic and antioxidant properties have far-reaching health implication. The active principles of the spices - turmeric (*Curcuma longa*) and red pepper (*Capsicum frutescens*) have been evidenced in several animal studies to exert hypolipidemic and antioxidant properties.

It is recently evidenced that heat processing of spices – turmeric and red pepper results in a significant loss of their active principles. Hence, it is desirable to understand the nature of altered compounds formed from spice active principles during heat processing of parent spices, and also to ascertain the extent to which these spices retain their health beneficial potency in spite of significant chemical alteration of active principles. Especially the hypolipidemic potency and the antioxidant potency exerted by turmeric and red pepper by virtue of their respective active compounds – curcumin and capsaicin need to be evaluated in heat-processed spices.

Spice principles – curcumin and capsaicin have been understood to exert the health beneficial hypocholesterolemic influence in experimental animal models. The antioxidant potential of curcumin and capsaicin has been experimentally documented in *in vitro* systems and in a few *in vivo* studies. Since spices are generally used in combination (as in spice mixes / curry powders), it would be interesting to know if these two spice principles have any additive or synergic action, with regard to hypolipidemic and antioxidant influences.

TURMERIC RHIZOME
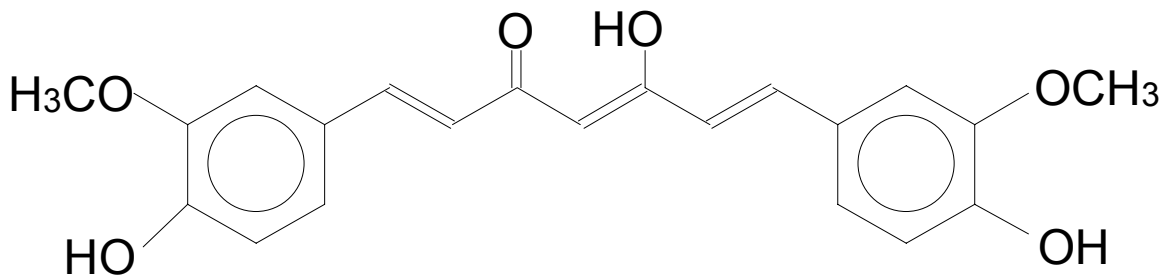
ACTIVE PRINCIPLE OF TURMERIC - CURCUMIN
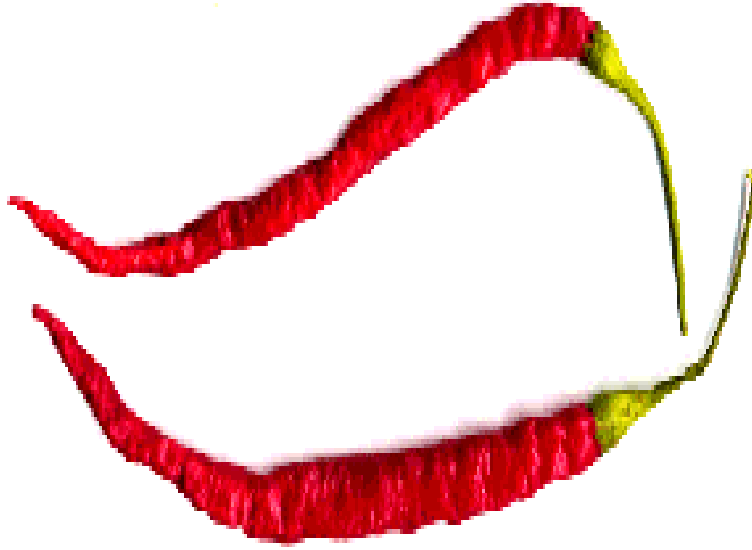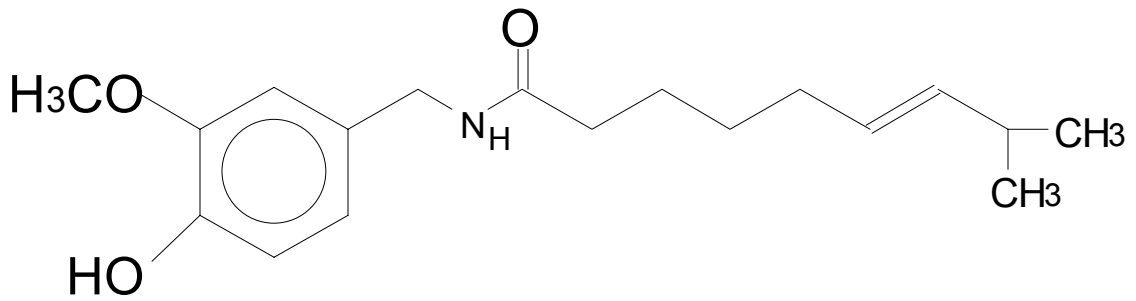
RED PEPPER

ACTIVE PRINCIPLE OF RED PEPPER - CAPSAICIN

The present investigation thus addresses the following objectives:

(1) In this investigation, elaborate animal studies were made to quantitate the relative potency of cooked turmeric and red pepper with regard to their hypolipidemic and antioxidant properties and compared with those of raw spices. The beneficial hypolipidemic and antioxidant potency of dehydrated (heat processed) onion is also evaluated in experimental rats.

(2) The present investigation examines the hypolipidemic influence of dietary curcumin and capsaicin individually and in combination in induced hypercholesterolemic rats and in high fat fed rats. The study has also examined the influence of these spice principles on antioxidant molecules and a few antioxidant enzymes in blood and liver in hypercholesterolemic condition and in high fat fed condition.

(3) The current study examines the beneficial antioxidant influence, if any, of dietary curcumin, capsaicin, and their combination in terms of protecting the damage caused to liver by iron overloading measured in terms of lipid peroxidation and elevation of plasma non-specific enzymes indicative of liver injury. The present study also investigates the anti-inflammatory property of curcumin and capsaicin when fed in combination on carrageenan-induced inflammatory responses in rats.

(4) In the absence of any report on the *in vivo* effects of spice principles on LDL oxidation, the current study examines (a) the beneficial antioxidant influence, if any, of dietary curcumin, capsaicin, and their combination in terms of protecting the low-density lipoprotein from iron and copper induced oxidation in experimental rats. This investigation also makes (b) a study of the protective effect of dietary capsaicin on induced oxidation of low-density lipoprotein in hypercholesterolemic rats.

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CHAPTER-II

HYPOLIPIDEMIC AND ANTIOXIDANT POTENTIAL OF HEAT PROCESSED SPICES

Section-A: Hypolipidemic and antioxidant potency of heat processed turmeric and red pepper in experimental rats

Section-B: Hypolipidemic and antioxidant efficacy of dehydrated onion in experimental rats

Hypolipidemic and antioxidant potency of heat processed turmeric and red pepper in experimental rats

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Summary

The hypocholesterolemic and antioxidant potency of both raw and pressure-cooked turmeric and red pepper were evaluated in experimental rats rendered hypercholesterolemic by feeding 0.5% cholesterol enriched diet and maintained for 8 weeks on 5 % spice diet. Dietary turmeric and red pepper, either raw or heat processed significantly countered the extent of hypercholesterolemia. Serum total cholesterol was 31 and 32% lower as a result of feeding raw and heat processed turmeric. The same was lower by 16 and 23% in animal groups fed raw and heat processed red pepper. The reduction in blood cholesterol brought about by these two dietary spices was predominantly in the LDL-cholesterol fraction. Dietary red pepper, both raw and heat processed fully countered the increase in serum triglyceride content of hypercholesterolemic rats. Increase in hepatic cholesterol in hypercholesterolemic animals was moderately countered by dietary red pepper, either raw or heat processed. Both dietary turmeric as well as red pepper significantly countered the increase in hepatic triglyceride level in hypercholesterolemic rats. Total thiols in serum were slightly but significantly increased by raw turmeric and raw red pepper both in basal and in hypercholesterolemic rats, but not by heat processed spices. Serum α-tocopherol was significantly enhanced (81-113%) by both dietary turmeric and red pepper in hypercholesterolemic animals. Hepatic lipid peroxides were significantly lower (9-15%) as a result of dietary turmeric and red pepper in hypercholesterolemic situation. Thus, the

results of this animal study suggested that although heat processing of turmeric and red pepper by pressure cooking resulted in a considerable loss of the active principles – curcumin and capsaicin, the hypolipidemic potency or the antioxidant potency of the parent spices were not significantly compromised.

Introduction

Spices are a group of esoteric food adjuncts, which have been in use for thousands of years. By virtue of their pleasing colour, flavour or pungency, they can transform our food into attractive and appetizing meal. In addition to these organoleptic properties, few spices are also known to possess several medicinal properties (Nadkarni & Nadkarni, 1976) and are effectively used in the indigenous systems of medicine. In the past three decades, it has been experimentally documented that several common spices can also exert health beneficial physiological effects (Srinivasan, 2005). These physiological effects of spices in most instances have been attributed to the main spice active principles present in them. Among these physiological influences spices are documented to exhibit, their hypolipidemic and antioxidant properties have far-reaching health implication. The active principles of the spices - turmeric (*Curcuma longa*) and red pepper (*Capsicum annuum*) have been evidenced in several animal studies to exert hypolipidemic and antioxidant properties (Srinivasan, 2005).

It is also evidenced that heat processing of spices – turmeric, red pepper and black pepper results in a significant loss of their active principles (Srinivasan *et al*, 1992). Hence, it is desirable to characterize the altered compounds formed from spice active

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principles during heat processing of parent spices, and to ascertain the extent to which these spices retain their health beneficial potency in spite of significant chemical alteration of active principles. Especially the hypolipidemic potency and the antioxidant potency exerted by turmeric and red pepper by virtue of their respective active compounds – curcumin and capsaicin need to be evaluated in heat-processed spices. In this investigation, elaborate animal studies were made to quantitate the relative potency of cooked turmeric and red pepper with regard to their hypolipidemic and antioxidant properties.

Methods and Materials

Heat processing of spices:

Spices – turmeric (*Curcuma longa*) and red pepper (*Capsicum annuum*) were locally purchased and powdered to pass through No.50 mesh sieve. These spice powders were suspended in distilled water (100 g / L) and subjected to pressure cooking for 10 min at 15 p.s.i. At the end of heat treatment, the samples were cooled to room temperature. Appropriate controls were also included wherein the samples did not undergo any treatment. These samples were lyophilized and stored at 4°C.

Quantitation of spice principles by TLC:

Spice samples in the lyophilized food samples were extracted with ethyl acetate in a Soxhlet apparatus for 4 h. The extracts were concentrated in a flash evaporator to a known volume (2 ml) and stored in dark at -20ºC until further analysis. The individual spice principles were quantitated after separation by appropriate TLC procedures as

described below (Ravindranath *et al*. 1981; Srinivasan *et al*. 1981). Care was taken to minimize the exposure to light during the extraction procedure and TLC separation.

Curcumin: Aliquots of the ethyl acetate extracts (quadruplicate) of the lyophilized food material and reference curcumin (6 µg) were spotted on silica gel-G coated plates (20 X 20 cm). The plates were developed with the upper phase of the solvent system: benzene-ethanol-water-acetic acid (100 : 27.5 : 7.5 : 0.5 v/v/v/v) in a chamber pre-equilibrated with the above solvent system for 2 h. The yellow curcumin bands were scraped off and quantitatively transferred to centrifuge tubes. Curcumin in the scrapings was extracted with 4 ml acetone, centrifuged at 2000 rpm for 5 min and 2 ml of the clear supernatant was used for the rubrocurcumin reaction. Two ml of acetonic extract was transferred to another test tube into which were successively added, 1 ml 7.5 mg% boric acid (in acetone) and 1 ml 5 mg% boric acid (in acetone). The contents of the tube were evaporated to dryness over a hot water bath. The residue was redissolved in 2 ml ethanol and absorption of the purple colour was measured at 550 nm.

Capsaicin: Aliquots of ethyl acetate extracts (quadruplicate) were spotted on silica gel-G coated plates (20 X 20 cm) along with reference capsaicin (40 µg). Plates were developed with petroleum ether (60-80ºC) - acetone (65 : 35 v/v) in a chamber pre-equilibrated with the same solvent for 90 min. The developed plates were air-dried and then sprayed uniformly with fresh Gibb`s reagent (0.1% 2,6-dichloroquinone-4-chlorimide in methanol). The plates, when dry, were exposed to ammonia vapours in a closed chamber for exactly 1 min. The blue-coloured zones of capsaicin thus visualized were scraped off and quantitatively transferred to centrifuge tubes containing 2 ml water.

The tubes were vortexed for 10 min to extract the colour and centrifuged at 2000 rpm for 2 min. Absorption of the clear blue supernatants was read at 610 nm.

Animal treatment:

Female Wistar rats (8 per group) weighing 110-120 g and housed in individual stainless steel cages were maintained on various experimental diets *ad libitum* for 8 weeks. The basal diet consisted of (%): casein, 21; cane sugar, 10; corn starch, 54; NRC vitamin mixture, 1; Bernhardt-Tommarelli modified NRC salt mixture, 4, and refined peanut oil, 10. The hypercholesterolemic diet consisted of 0.5% cholesterol and 0.125% bile salts at the expense of an equivalent amount of corn starch in the basal diet wherein the peanut oil is also replaced with hydrogenated vegetable fat. The test material was incorporated into the basal diet / high cholesterol diet at 5 g / 100 g replacing an equivalent amount of corn starch. At the end of the experimental duration, overnight fasted animals were sacrificed under light ether anesthesia. Blood was collected by heart puncture and serum separated by centrifugation. Liver was quickly excised, weighed and stored frozen till lipid extraction.

Lipid profile:

Total lipids were extracted according to Folch *et al*. (1957) and estimated gravimetrically. Cholesterol (Searcy & Bergquist, 1960), triglycerides (Fletcher, 1968) and phospholipids (Charles & Stewart, 1980) were determined in the lipid extracts of serum and liver by using standard procedures. Serum cholesterol and triglyceride associated with HDL fraction were determined after precipitation of apolipoprotein-B containing lipoproteins with heparin-manganese reagent according to the method of

Warnick and Albers (1978). LDL-VLDL precipitate was extracted with chloroform : methanol (2:1 v/v) and used for cholesterol and triglyceride determination.

Lipid peroxides:

Plasma lipid peroxides were estimated by the fluorimetric measurement of thiobarbituric acid complex by the method of Yagi (1984). The fluorimetric measurement was carried out at an excitation wavelength of 515 nm and emission wavelength of 553 nm and compared with the standards prepared by reacting 0.5 nmole 1,1,3,3-tetraethoxy-propane with TBA reagent. Lipid peroxide in liver tissue was determined by the method described by Ohkawa *et al* (1979) involving photometric measurement of thiobarbituric acid complex extracted into butanol. Absorbance of the butanol extract was measured at 532 nm and compared with that of standard tetraethoxypropane, treated similarly.

Antioxidant molecules:

Total thiols in serum / liver were measured spectrophotometrically by using Ellman's reagent according to the method described by Sedlock and Lindsay (1968). Glutathione in liver was estimated by using Ellman's reagent according to Beutler *et al* (1963). Ascorbic acid in serum / liver was estimated spectrophotometrically by measuring the 2,4-dinitrophenyl-hydrazone derivative of dehydroascorbic acid according to Omaye *et al* (1973). α-Tocopherol in serum was determined by HPLC method described by Zaspel and Csallany (1983) using ODS column (C-18) and an UV-Visible detector (295nm) and a solvent system acetonitrile - methanol (1:1).

Statistical analysis:

Results are expressed as mean ± SEM and comparisons between groups were made by means of an unpaired Student's t-test (Snedecor & Cochran, 1976). Differences were considered significant when $p < 0.05$.

Results and Discussion

Curcumin loss from heat processing of turmeric by pressure cooking for 10 min was around 27 %, while capsaicin loss from red pepper was around 18% under similar conditions of cooking.

Influence of dietary turmeric and red pepper on serum lipid profile in normal as well as high cholesterol fed animals is presented in Table-1. High cholesterol feeding for 8 weeks resulted in a significant increase in serum total cholesterol concentration and this increase was observed predominantly in the LDL-associated fraction. The increase in serum total cholesterol was as much as 6.25-fold. The increase in serum LDL-cholesterol is about 9-fold. Dietary turmeric and red pepper, either raw or heat processed significantly countered the extent of hyper-cholesterolemia. Serum total cholesterol was 31 and 32% lower as a result of feeding raw and heat processed turmeric. The same was lower by 16 and 23% in animal groups fed raw red pepper and heat processed red pepper. The reduction in blood cholesterol brought about by these two dietary spices was predominantly in the LDL-cholesterol fraction. About 32% decrease in LDL-cholesterol was evidenced in turmeric fed animals, whereas dietary red pepper produced 17 and 24% decrease in the same. The HDL-cholesterol fraction essentially remained unchanged as a result of treatment with turmeric or red pepper.

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Table-1 Influence of dietary turmeric and red pepper on serum lipid profile

Animal group	Cholesterol			Triglycerides	Phospholipids
	Total	LDL-VLDL	HDL		
Basal-Control	70.2 ± 1.58	47.4 ± 1.64	22.8 ± 0.30	100.3 ± 4.92	93.7 ± 3.82
Basal-Turmeric (Raw)	71.9 ± 3.39	49.6 ± 2.30	22.3 ± 0.39	107.8 ± 7.17	95.7 ± 1.31
Basal-Red pepper (Raw)	77.3 ± 2.93	49.4 ± 1.84	27.9 ± 1.36*	99.6 ± 4.68	101.7 ± 3.27
HCD-Control	440.8 ± 14.4	429.5 ± 24.1	11.3 ± 1.14	117.1 ± 5.64	112.5 ± 6.50
HCD-Turmeric (Raw)	304.7 ± 28.6**	291.3 ± 18.2**	13.4 ± 0.91	116.1 ± 8.88	156.5 ± 9.92*
HCD-Turmeric (Cooked)	298.0 ± 38.7**	285.7 ± 20.8**	12.4 ± 0.53	110.4 ± 4.23	154.3 ± 9.39*
HCD-Red pepper (Raw)	369.4 ± 11.1**	358.2 ± 25.2**	11.2 ± 0.51	101.6 ± 5.60**	139.5 ± 6.46*
HCD-Red pepper (Cooked)	340.7 ± 29.3**	328.2 ± 28.0**	12.5 ± 0.94	98.5 ± 4.74**	150.7 ± 8.95*

Values expressed as mg/dl are mean ± SEM of 8 rats in each group.
LDL: Low density lipoprotein; HDL: High density lipoprotein; VLDL: Very low density lipoprotein; HCD: High cholesterol diet
* Significant increase compared to corresponding control. **Significant decrease compared to corresponding control.

Hypercholesterolemic rats registered a 17% increase in serum triglyceride concentration (Table-1). While dietary turmeric, either raw or heat processed did not affect the serum triglyceride content in these hypercholesterolemic rats, dietary red pepper, both raw and heat processed fully countered the increase in serum triglyceride content. Serum phospholipid concentration was 20% higher in hypercholesterolemic rats compared to basal control. Dietary turmeric, both raw and heat processed produced further increase in serum phospholipid concentration (38 - 39% increase) in hypercholesterolemic rats. Similarly both raw and heat processed red pepper feeding increased serum phospholipid concentration by 24 and 34% respectively.

Thus, there was practically no difference between raw and heat processed turmeric with regard to beneficial influence on serum cholesterol concentration in hypercholesterolemic rats. Even the influence of raw and heat processed turmeric on serum phospholipid content was also similar in hypercholesterolemic animals. The beneficial effect of red pepper on serum cholesterol concentration in hypercholesterolemic rats was even better in the case of heat processed spice when compared to the effect produced by raw red pepper. Similarly, the influence of heat processed red pepper on the serum phospholipid concentration in hypercholesterolemic rats was higher than that of corresponding raw spice. Dietary turmeric or red pepper generally did not have any influence on serum lipid profile in basal rats expect for a small increase in HDL-cholesterol by raw red pepper.

Liver lipid profile of normal and hypercholesterolemic rats as influenced by dietary turmeric and red pepper is presented in Table-2. Liver cholesterol was increased by 14.6-

Table-2 Influence of dietary turmeric and red pepper on hepatic lipid profile

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Animal group	Total lipids	Cholesterol	Triglycerides	Phospholipids
Basal-Control	51.1 ± 1.89	4.92 ± 0.11	25.4 ± 1.74	21.2 ± 1.66
Basal-Turmeric (Raw)	47.2 ± 0.96	5.07 ± 0.29	27.7 ± 1.19	23.8 ± 1.87
Basal-Red pepper (Raw)	44.1 ± 1.51**	4.24 ± 0.19**	18.5 ± 1.26**	20.6 ± 1.65
HCD-Control	138.3 ± 6.24	72.3 ± 2.78	52.8 ± 2.71	25.8 ± 1.63
HCD-Turmeric (Raw)	147.3 ± 5.71	71.8 ± 2.57	32.1 ± 1.37**	22.9 ± 1.88
HCD-Turmeric (Cooked)	153.4 ± 7.41	69.6 ± 3.41	27.9 ± 1.44**	23.6 ± 1.41
HCD-Red pepper (Raw)	135.9 ± 5.97	60.2 ± 2.98**	32.1 ± 1.41**	23.3 ± 0.89
HCD-Red pepper (Cooked)	147.9 ± 4.68	57.6 ± 3.20**	29.8 ± 2.99**	25.7 ± 1.61

Values expressed as mg/g liver are mean ± SEM of 8 rats in each group.
HCD: High cholesterol diet
**Significant decrease compared to corresponding control.

fold as a result of high cholesterol feeding for 8 weeks. This increase in hepatic cholesterol was moderately countered by dietary red pepper, either raw or heat processed by 17 and 20% respectively. Dietary raw red pepper also decreased hepatic cholesterol (by 14%) in basal rats. Dietary turmeric did not influence hepatic cholesterol level. Hepatic triglyceride concentration was elevated by 108% as a result of feeding the cholesterol enriched diet for 8 weeks. Both dietary turmeric as well as red pepper significantly countered the increase in hepatic triglyceride level in hypercholesterolemic rats. The decrease in hepatic triglyceride concentration evidenced was 39 and 47% by raw and heat processed turmeric respectively, while it was 39 and 44% in the case of raw and heat processed red pepper. Hepatic phospholipid concentration was not altered as a result of high cholesterol feeding, and the same was also not influenced by either of the spices. Liver total lipid was increased in high cholesterol fed rats by 171%. The same was not beneficially countered by either dietary turmeric or red pepper. Raw red pepper however decreased hepatic total lipid by about 14% in basal rats. The significant decrease in hepatic total lipid and triglyceride content caused by dietary 5% red pepper in normal rats observed in this investigation is in agreement with a similar observation reported earlier (Sambaiah & Satyanarayana, 1982).

Influence of dietary turmeric and red pepper on the concentration of various antioxidant molecules and lipid peroxides in serum is presented in Table-3. Total thiols in serum were slightly but significantly increased by raw turmeric and raw red pepper both in basal rats as well as in hypercholesterolemic rats. This effect was not seen in the case of heat processed spices. Among the antioxidant molecules, while serum ascorbic acid concentration was not influenced by either of the dietary spices, α-tocopherol was

Table-3 Influence of dietary turmeric and red pepper on serum antioxidant molecules and lipid peroxides

Animal group	Total thiols (mmole/dl)	Ascorbic acid (mg/dl)	α-Tocopherol (µg/dl)	Lipid peroxides (µmole/dl)
Basal-Control	17.3 ± 1.16	6.23 ± 0.20	0.783 ± 0.027	1.24 ± 0.024
Basal-Turmeric (Raw)	19.8 ± 0.73*	5.56 ± 0.27	0.852 ± 0.029	1.11 ± 0.039
Basal-Red pepper (Raw)	19.7 ± 1.19*	5.32 ± 0.09	0.800 ± 0.032	1.20 ± 0.033
HCD-Control	16.0 ± 1.30	5.20 ± 0.32	0.528 ± 0.037	1.01 ± 0.009
HCD-Turmeric (Raw)	20.0 ± 1.02*	5.22 ± 0.25	0.956 ± 0.050*	0.973 ± 0.008
HCD-Turmeric (Cooked)	14.6 ± 0.58	4.96 ± 0.22	1.127 ± 0.084*	0.978 ± 0.026
HCD-Red pepper (Raw)	20.8 ± 0.84*	5.89 ± 0.35	0.965 ± 0.025*	1.11 ± 0.019
HCD-Red pepper (Cooked)	19.7 ± 094	4.93 ± 0.08	1.001 ± 0.050*	0.928 ± 0.047

Values are expressed as mean ± SEM of 8 rats in each group.
HCD: High cholesterol diet
* Significant increase compared to corresponding control.

significantly enhanced by both dietary turmeric and red pepper in hypercholesterolemic animals. The extent of increase in serum α-tocopherol produced by dietary raw and heat processed turmeric was 81and 113 % respectively. Serum α-tocopherol was increased by 83 and 90 % in hypercholesterolemic rats by dietary raw and heat processed red pepper. Serum lipid peroxide concentration was not influenced by dietary turmeric or red pepper either in basal rats or in hypercholesterolemic rats. Influence of dietary turmeric and red pepper on the concentration of various antioxidant molecules and lipid peroxides in liver is presented in Table-4. Hepatic lipid peroxides were significantly lower as a result of dietary turmeric and red pepper both in normal and hypercholesterolemic situation (Table-4). The decrease in hepatic lipid peroxides produced was 28 and 13 % by dietary raw turmeric and red pepper respectively in normal rats. A decrease in the same by 15 and 11 % raw and heat processed turmeric was seen in the case of hypercholesterolemic rats, while raw and heat processed red pepper produced a decrease of 9 and 11 % respectively.

Ascorbic acid concentration in liver was favourably influenced by dietary turmeric and red pepper in normal rats (Table-4). The extent of increase in hepatic ascorbic acid was 12 and 64% in the turmeric and red pepper diet groups. These dietary spice principles did not show any beneficial effect on liver ascorbic acid in hypercholesterolemic rats. On the other hand, there were decreases in hepatic ascorbic acid of hypercholesterolemic rats in dietary turmeric and red pepper groups. The decreases in hepatic ascorbic acid were of the order of 12 to 29 % in these spice groups. Hepatic glutathione content remained unaffected by dietary turmeric and red pepper in both basal as well as in hypercholesterolemic rats. Hepatic total thiols were higher as a

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result of dietary turmeric and red pepper only in basal rats (Table-4). The increase in total thiols was 18 and 22 % in the respective diet groups as compared to control.

Table-4 Influence of dietary turmeric and red pepper on liver antioxidant molecules and lipid peroxides

Animal group	Total thiols (mmole/mg protein)	Glutathione (µg/mg protein)	Ascorbic acid (µg/mg protein)	Lipid peroxides (nmole/mg protein)
Basal-Control	0.491 ± 0.015	0.850 ± 0.040	0.444 ± 0.016	3.26 ± 0.18
Basal-Turmeric (Raw)	0.581 ± 0.034*	0.846 ± 0.050	0.496 ± 0.021*	2.35 ± 0.10**
Basal-Red pepper (Raw)	0.600 ± 0.042*	0.868 ± 0.052	0.728 ± 0.058*	2.85 ± 0.06**
HCD-Control	0.474 ± 0.021	0.994 ± 0.046	0.655 ± 0.027	2.52 ± 0.10
HCD-Turmeric (Raw)	0.396 ± 0.013	0.974 ± 0.058	0.523 ± 0.018**	2.13 ± 0.10**
HCD-Turmeric (Cooked)	0.423 ± 0.010	0.960 ± 0.048	0.578 ± 0.022**	2.23 ± 0.05**
HCD-Red pepper (Raw)	0.415 ± 0.013	0.896 ± 0.080	0.468 ± 0.021**	2.30 ± 0.06**
HCD-Red pepper (Cooked)	0.451 ± 0.011	1.020 ± 0.028	0.543 ± 0.019**	2.24 ± 0.08**

Values are expressed as mean ± SEM of 8 rats in each group.
HCD: High cholesterol diet
* Significant increase compared to corresponding control. **Significant decrease compared to corresponding control.

Table-5 Influence of dietary turmeric and red pepper on body weight gain and liver weight in hypercholesterolemic rats.

	Body weight (g)			Liver weight	
	Initial	Final	Gain	(g)	(g/100 g body)
Basal - Control	116.2 ± 1.05	195.2 ± 4.95	79.0 ± 2.00	4.98 ± 0.12	2.55 ± 0.06
Basal – Turmeric (Raw)	116.3 ± 0.98	186.2 ± 6.86	69.9 ± 2.57	5.27 ± 0.15	2.83 ± 0.08
Basal – Red pepper (Raw)	116.3 ± 0.87	172.7 ± 4.19	56.4 ± 1.36**	4.39 ± 0.10	2.54 ± 0.05
HCD - Control	124.5 ± 1.14	182.3 ± 2.85	57.8 ± 1.90	8.02 ± 0.23	4.39 ± 0.14
HCD – Turmeric (Raw)	124.6 ± 1.08	188.1 ± 4.45	63.5 ± 1.35	8.60 ± 0.25	4.61± 0.13
HCD – Turmeric (Cooked)	124.9 ± 0.97	182.3 ± 2.44	57.4 ± 0.76	8.44 ± 0.17	4.62 ± 0.09
HCD – Red pepper (Raw)	124.8 ± 1.03	182.3 ± 2.80	57.5 ± 0.88	7.24 ± 0.21	3.97 ± 0.15
HCD – Red pepper (Cooked)	124.9 ± 1.05	187.5 ± 3.71	62.6 ± 1.23	7.64 ± 0.26	4.07 ± 0.13

Values are ± SEM of 8 animals in each group.
**Significant decrease compared to corresponding control.

Conclusions

This is the first observation on the beneficial influence of dietary turmeric and red pepper on the antioxidant status of experimental animals with respect to antioxidant molecules and lipid peroxides in blood and liver, although such an effect has been documented for their active principles - curcumin and capsaicin. The beneficial influence of dietary turmeric and red pepper on the antioxidant status of experimental animals with respect to antioxidant molecules and lipid peroxides in blood and liver is practically the same irrespective of whether the spice is fed raw or after heat processing. Thus, the results of this animal study suggested that although heat processing of turmeric and red pepper by pressure cooking resulted in a considerable loss of the active principles – curcumin and capsaicin, the hypolipidemic potency or the antioxidant potency of the parent spices were not significantly compromised.

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CHAPTER-III

HYPOLIPIDEMIC AND ANTIOXIDANT EFFECTS OF DIETARY CURCUMIN AND CAPSAICIN

Section-A: **Hypolipidemic and antioxidant effects of curcumin and capsaicin in induced hypercholesterolemic rats**

Section-B: **Hypolipidemic and antioxidant effects of curcumin and capsaicin in high fat fed rats**

Hypolipidemic and antioxidant efficacy of dehydrated onion in experimental rats

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Summary

The hypolipidemic and antioxidant potency of dehydrated onion product developed in this Institute was evaluated in experimental rats maintained for 6 weeks at 5 and 10% dietary levels. Total serum cholesterol was significantly reduced (by 21-24%) in hypercholesterolemic rats maintained on dehydrated onion diet. This reduction was seen essentially in the LDL fraction of serum cholesterol. A beneficial increase of 11% in the concentration of HDL-cholesterol in blood was also evidenced in hypercholesterolemic animals as a result of dietary onion. Blood triglyceride concentration of hypercholesterolemic rats was 31% lower in onion treated groups compared to corresponding control. While hepatic triglyceride and phospholipid concentrations were unaffected by onion treatment in both normal and hypercholesterolemic rats, cholesterol was slightly higher in hypercholesterolemic rats as a result of onion treatment at 5% level which was also reflected in the total lipid content of liver tissue. Total thiols in the blood of hypercholesterolemic rats were profoundly higher in onion treatment at either level as compared to respective controls. Blood glutathione concentration was significantly increased by dietary 10% onion in hypercholesterolemic rats, the increase being around 18%. Blood α-tocopherol concentration was slightly higher in hypercholesterolemic rats as a result of onion treatment. Blood ascorbic acid concentration was significantly higher in both hypercholesterolemic animals as well as normal animals fed dehydrated onion. Blood lipid peroxides were lower by 15-16% in animals fed 10% dehydrated onion in

both hypercholesterolemic and normal rats. Liver ascorbic acid concentration was higher under onion treatment in both normal and hypercholesterolemic animals. Hepatic α-tocopherol concentration was significantly higher (53-77%) in normal rats maintained on dehydrated onion containing diets. Liver lipid peroxides were significantly lower in normal rats maintained on onion containing diets, the decrease being 24-26%.

Introduction

The importance of serum cholesterol levels and of lipoproteins in relation to atherosclerosis and coronary heart disease is well known. In view of this, there has been a continuous search for hypocholesterolemic agents. Since a dietary adjunct happens to be advantageous, some of the commonly used spices have been evaluated as possible hypocholesterolemics in a variety of experimental situations in animals and in humans. The spices - fenugreek, garlic, ginger, onion, red pepper and turmeric are evidenced to be effective as hypocholesterolemics under conditions of experimentally induced hypercholesterolemia and hyperlipidemia. All the studies in which spices have been shown to influence cholesterol and / or triglyceride levels in blood and liver, lipoprotein cholesterol levels, fecal excretion of sterols and bile acids and biliary secretion of cholesterol and bile acids have been recently reviewed (Srinivasan *et al*, 2004).

Onion (*Allium cepa*) is used both as a spice and as a vegetable. It has a strong flavour due to the sulfur compounds one of which has also been identified as the lachrymatory factor (Fenwick & Hanley, 1985). A large amount of work has been done on the hypocholesterolemic activity of onions in experimental animals (both rats and rabbits) as

well as in humans. Onion, onion juice, essential oil and aqueous extract of onion have been examined in these studies; decreased blood cholesterol concentration has been generally observed in majority of these studies under conditions of normal diet, high fat diet, high cholesterol diet (Srinivasan *et al*., 2004).

Among other health beneficial effects of onion, the decreased blood fibrinolytic activity observed in rabbits fed a high cholesterol diet was effectively countered in addition to hypocholesterolemic effect by dietary onion juice (lowering in blood fibrinogen concentration) (Sharma *et al*., 1975; Sharma *et al*., 1975a). The pronounced crenation and aggregation of erythrocytes in rabbits fed a high cholesterol diet was reversed by dietary onion extract (Vatsala & Singh, 1981; Vatsala & Singh, 1982). Enrichment of erythrocyte membranes with cholesterol was suppressed by dietary onion in hypercholesterolemic rats which was accompanied by changes in the erythrocyte membrane enzymes - alkaline and acid phosphatase, 5'-nucleotidase, total and Mg^{2+}-ATPase (Singh & Kanakaraj, 1984). Significant reduction in experimentally induced hypertriglyceride levels in blood, aorta and liver has been documented in rabbits as a result of administration of onion extract (Sebastian *et al*., 1979). The effects of onion have been ascribed to its sulfur containing principles, which oxidize thiol compounds either present free or combined in protein. Babu and Srinivasan (1997) have evidenced hypolipidemic effect of dietary onion in streptozotocin diabetic rats. They have also documented amelioration of diabetic renal lesions by dietary onion owing to this beneficial hypocholesterolemic influence (Babu & Srinivasan, 1999). Flavonoid

compounds present in plant foods have been increasingly understood to bestow antioxidant properties. Quercetin is the major flavonoid present in onions.

Dehydrated onion product was developed in this Institute which involved homogenization of the onion pulp along with corn starch and gum acacia, followed by drum drying. This product retained the major flavonoids present in onion - quercetin and kaempferol to the maximum extent (30 mg / 100g dehydrated onion). The objective of this animal study is to evaluate the hypolipidemic and antioxidant potency of the dehydrated onion product in experimental rats.

Materials and Methods

Materials:

Dehydrated onion powder product was developed from the locally purchased onions (*Allium cepa*; Bellary red variety) in this Institute's Department of Lipid Science and Traditional Foods, and the fresh product was used in this study. All fine chemicals used here were from M/s. Sigma-Aldrich Co., St.Louis, USA. All other chemical were of analytical grade obtained from M/s Qualigen Chemicals, Mumbai, India. The solvents were distilled before use.

Animal treatment:

Animal experiments were carried out taking appropriate measures to minimize pain or discomfort in accordance with the guidelines laid down by the NIH, USA, regarding the care and use of animals for experimental procedures and with due clearance from the Institutional Animal Ethics Committee. Male Wistar rats (10 per group) weighing 110-120 g and housed in individual stainless steel cages were maintained on various experimental diets *ad libitum* for 6 weeks. The basal diet consisted of (%): casein, 21; cane sugar, 10; corn starch, 54; NRC vitamin mixture, 1; Bernhardt-Tommarelli modified NRC salt mixture, 4, and refined peanut oil, 10. The hypercholesterolemic diet consisted of 0.5% cholesterol and 0.125% bile salts at the expense of an equivalent amount of corn starch in the basal diet wherein the peanut oil is also replaced with hydrogenated vegetable fat. The test material was incorporated into the basal diet / high cholesterol diet at 5 g and 10 g / 100g replacing an equivalent amount of corn starch. At the end of the experimental duration, overnight fasted animals were sacrificed under light ether anesthesia. Blood was collected by heart puncture and serum separated by centrifugation. Liver was quickly excised, weighed and stored frozen till lipid extraction.

Lipid profile:

Total lipids were extracted according to Folch *et al.* (1957) and estimated gravimetrically. Cholesterol (Searcy & Bergquist, 1960), triglycerides (Fletcher, 1968) and phospholipids (Charles & Stewart, 1980) were determned in the lipid extracts of serum and liver by using standard procedures. Serum cholesterol and triglyceride associated with HDL fraction were determined after precipitation of apolipoprotein-B containing lipoproteins with heparin-manganese reagent according to the method of

Warnick and Albers (1978). LDL-VLDL precipitate was extracted with chloroform : methanol (2:1 v/v) and used for cholesterol and triglyceride determination.

Lipid peroxides:

Plasma lipid peroxides were estimated by the fluorimetric measurement of thiobarbituric acid complex by the method of Yagi (1984). The fluorimetric measurement was carried out at an excitation wavelength of 515 nm and emission wavelength of 553 nm and compared with the standards prepared by reacting 0.5 nmole 1,1,3,3-tetraethoxy-propane with TBA reagent. Lipid peroxide in liver tissue was determined by the method described by Ohkawa *et al* (1979) involving photometric measurement of thiobarbituric acid complex extracted into butanol. Absorbance of the butanol extract was measured at 532 nm and compared with that of standard tetraethoxypropane, treated similarly.

Antioxidant molecules:

Total thiols in blood plasma / liver were measured spectrophotometrically by using Ellman's reagent according to the method described by Sedlock and Lindsay (1968). Glutathione in blood plasma / liver was estimated by using Ellman's reagent according to Beutler et al (1963). Ascorbic acid was estimated spectrophotometrically by measuring the 2,4-dinitrophenyl-hydrazone derivative of dehydroascorbic acid according to Omaye *et al* (1973). α-Tocopherol in liver and blood plasma was determined by HPLC method described by Zaspel and Csallany (1983) using ODS column (C-18) and an UV-Visible detector (295nm) and a solvent system acetonitrile - methanol (1:1).

Statistical analysis:

Results are expressed as mean ± SEM and comparisons between groups were made by means of an unpaired Student's t-test (Snedecor & Cochran, 1976). Differences were considered significant when $p < 0.05$.

Results and Discussion

Blood lipid profile of normal and hypercholesterolemic rats maintained on dehydrated onion containing diet is presented in Table-1. There was a 162% increase in blood total cholesterol concentration as a result of high cholesterol feeding. This increase in cholesterol in high cholesterol feeding was predominantly seen in the LDL-fraction. Total cholesterol in serum was significantly reduced in hypercholesterolemic rats maintained on either 10% or 5% onion diet. The extent of reduction was 20.7 and 23.6% in these respective diet groups. The reduction in blood cholesterol was seen essentially in the LDL fraction of serum cholesterol (33 and 37% decrease in the two onion groups respectively). Another favourable effect of dietary onion evidenced here in hypercholesterolemic animals was the increase in HDL-cholesterol in blood. This increase in HDL-cholesterol of hypercholesterolemic rats although was just around 11% was nevertheless significant. Moderate decreases in total cholesterol (12%) and LDL-cholesterol (23%) as a result of dietary onion at 10% level in the serum of normal rats was also evidenced.

Table-1 Influence of dietary dehydrated onion on blood lipid profile

Animal group	Cholesterol			Triglycerides			Phospholipids
	Total	LDL-VLDL	HDL	Total	LDL-VLDL	HDL	
Basal - Control	55.3 ± 0.40	35.8 ± 2.90	20.6 ± 0.19	113.9 ± 4.43	66.6 ± 2.99	47.3 ± 1.64	103.2 ± 2.12
Basal - Onion (10%)	48.5 ± 0.57**	27.4 ± 2.45**	20.2 ± 0.59	121.6 ± 5.89	76.9 ± 4.77	44.7 ± 1.46	112.0 ± 2.80*
Basal - Onion (5%)	52.9 ± 1.35	31.8 ± 3.09	21.2 ± 1.14	110.2 ± 3.70	78.1 ± 3.37	44.2 ± 1.23	122.1 ± 5.36*
HCD - Control	145.2 ± 4.62	117.8 ± 2.71	27.4 ± 0.57	148.6 ± 4.11	99.8 ± 3.71	48.8 ± 3.90	108.8 ± 3.74
HCD - Onion (10%)	115.2 ± 4.25**	84.8 ± 6.64**	30.4 ± 0.84*	117.7 ± 3.61**	73.5 ± 2.06**	44.2 ± 1.22	92.5 ± 2.06**
HCD - Onion (5%)	110.9 ± 4.15**	80.6 ± 3.98**	30.3 ± 1.15*	116.8 ± 1.81**	77.9 ± 2.78**	38.9 ± 4.30	87.0 ± 4.23**

Values expressed as mg/dl are mean ± SEM of 8 animals in each group

* Significant increase compared to corresponding control.
** Significant decrease compared to corresponding control.

Blood triglyceride concentration of hypercholesterolemic rats was significantly lower in both 10% and 5% onion treated groups compared to corresponding control (Table-1). The decreases in serum total triglyceride concentration were around 31% in onion treatment. This reduction was seen predominantly in the LDL-fraction (26 and 22% in the two onion groups respectively). Blood triglyceride concentration remained unchanged in normal rats as a result of onion treatment. Blood phospholipid concentration was higher in normal rats under onion treatment as compared to respective controls. On the other hand, blood phospholipid concentration was significantly lower in hypercholesterolemic rats under onion treatment as compared to respective control.

Liver lipid profile of normal and hypercholesterolemic rats maintained on dehydrated onion containing diet is presented in Table-2. Hypercholesterolemic rats featured higher concentrations of cholesterol, triglyceride and total lipid in the liver tissue when compared to their normal counterparts. Hepatic cholesterol was slightly higher in hypercholesterolemic rats as a result of onion treatment at 5% level. This increase over the control was also reflected in the total lipid content of liver tissue as a result of onion treatment at 5% level. Such an effect of onion on hepatic cholesterol or total lipid was however not evidenced when fed at 10% dietary concentration. Triglyceride and phospholipid concentrations were unaffected by onion treatment in both normal and hypercholesterolemic rats.

Table-2 Influence of dietary dehydrated onion on hepatic lipid profile

Animal group	Total lipids	Cholesterol	Triglycerides	Phospholipids
Basal - Control	45.4 ± 2.63	4.41 ± 0.22	12.3 ± 0.86	14.6 ± 0.83
Basal - Onion (10%)	50.5 ± 2.40	5.04 ± 0.38	14.0 ± 1.43	15.4 ± 0.50
Basal - Onion (5%)	51.2 ± 3.90	4.88 ± 0.11	13.7 ± 1.30	16.4 ± 0.89
HCD - Control	146.4 ± 5.56	55.7 ± 1.20	33.4 ± 1.48	14.5 ± 0.64
HCD - Onion (10%)	152.1 ± 6.21	58.6 ± 1.93	30.5 ± 1.75	14.8 ± 0.75
HCD - Onion (5%)	162.4 ± 4.09*	62.5 ± 1.79*	29.6 ± 2.14	13.1 ± 1.01

Values expressed as mg/g fresh tissue are mean ± SEM of 8 animals in each group

* Significant increase compared to corresponding control

Decreased cholesterol concentration both in blood as well as in liver has been reported in rats maintained on normal diet by treating with onion (Bakhsh & Chugtai, 1985) and aqueous extract of onion (Augusti & Mathew, 1973). Similarly, decreased cholesterol concentration both in blood as well as in liver has been reported in rats maintained on high sucrose diet and treated with essential oil of onion (Adamu *et al*, 1982) and in rats maintained on high fat diet and treated with allylpropyl disulfide (Wilcox *et al*, 1984). Decreased blood cholesterol has been evidenced in rabbits maintained on high cholesterol diet and treated with onion (Sharma *et al*, 1975; Sainani *et al*, 1979), onion juice (Jain, 1976) or essential oil of onion (Bordia *et al*, 1975). Decreased blood and liver triglyceride level has been reported as a result of onion treatment to rabbits maintained on high sucrose diet (Sebastian *et al*, 1979). Lowered blood cholesterol levels has been evidenced as a result of onion treatment in clinical trials involving normal subjects (Bhushan *et al*, 1977; Sharma & Sharma, 1979; Sainani *et al*, 1979) and in lipemic subjects (Jain & Andleigh, 1969; Jain, 1971; Bordia *et al*, 1974; Sharma *et al*, 1975; Jain & Vyas, 1977)

Sharma and co-workers (1975, 1975a) studied the effect of onion juice on rabbits fed a high cholesterol diet. Rabbits, when fed high cholesterol diet for 24 weeks, showed elevated levels of serum cholesterol and of plasma fibrinogen and decreased fibrinolytic activity. Addition of onion juice to the diet reversed these changes. The elevated levels of serum cholesterol and plasma fibrinogen were reduced, and blood fibrinolytic activity was increased. Onion juice at an equivalent of 25g of onion / kg body wt / day, when incorporated in the high cholesterol diet, prevented a rise in serum cholesterol.

Effects of an aqueous extract of onion on the sucrose fed rabbits have been investigated (Sebastian *et al*, 1979). Long term administration of sucrose significantly increased triglyceride levels in normal rabbits while administration of onion extract significantly reduced serum, liver and aorta triglycerides and serum and liver proteins. The effects of onion have been ascribed to its sulfur containing principles, which oxidize thiol compounds either present free or combined in protein. Babu and Srinivasan (1997) have evidenced hypolipidemic effect of dietary onion in streptozotocin diabetic rats. They have also documented amelioration of diabetic renal lesions by dietary onion owing to this beneficial hypocholesterolemic influence (Babu & Srinivasan, 1999).

Plant foods contain phytochemicals such as flavonoids, phenolic acids, etc., which show antioxidant activity. Onion is a major source of flavonoids, the major flavonoids being two quercetin glycosides, quercetin 4'-O-beta-glucoside and quercetin 3,4'-O-beta-diglucosides. There are only limited reports on the antioxidant potential of onions. The influence of dietary dehydrated onion on various antioxidant molecules and concentration of lipid peroxides in blood and liver was hence examined in this study. Influence of dietary dehydrated onion on blood lipid peroxides and various antioxidant molecules is presented in Table-3. Total thiols in the blood of hypercholesterolemic rats were profoundly higher in onion treatment at either level as compared to respective controls. This increase in total thiol concentration was as high as 106% and 260% in 10% and 5% onion groups respectively. Thus, onion at 5% dietary level produced a higher effect on blood total thiol concentration in hypercholesterolemic rats than when fed at 10% level.

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Table-3 Influence of dietary dehydrated onion on blood lipid peroxides and antioxidant molecules

Animal group	Lipid peroxides µmole/dl	Total thiols mmole/dl	Glutathione mg/dl	α-Tocopherol µg/dl	Ascorbic acid mg/dl
Basal - Control	36.25 ± 2.04	18.64 ± 3.20	0.366 ± 0.030	0.768 ± 0.032	0.391 ± 0.015
Basal - Onion (10%)	30.35 ± 1.90**	27.75 ± 2.70*	0.298 ± 0.034	0.943 ± 0.059*	0.480 ± 0.023*
Basal - Onion (5%)	34.55 ± 3.60	17.25 ± 2.70	0.310 ± 0.027	0.683 ± 0.044	0.475 ± 0.025*
HCD - Control	25.30 ± 1.70	7.60 ± 1.96	0.271 ± 0.013	0.953 ± 0.050	0.445 ± 0.030
HCD - Onion (10%)	21.50 ± 0.95**	15.61 ± 3.00*	0.320 ± 0.023*	1.160 ± 0.065*	0.585 ± 0.015*
HCD - Onion (5%)	27.30 ± 0.70	27.35 ± 0.65*	0.277 ± 0.016	1.120 ± 0.043*	0.602 ± 0.011*

Values are mean ± SEM of 8 animals in each group

 * Significant increase compared to corresponding control
** Significant decrease compared to corresponding control.

However, higher blood total thiol concentration (49%) was evidenced only in 10% onion treatment in the case of normal rats. Blood glutathione concentration was significantly increased by 10% onion in hypercholesterolemic rats, the increase being around 18%. α-Tocopherol concentration was slightly but significantly higher in hypercholesterolemic rats as a result of onion treatment (1.16 and 1.12 μg/dl respectively as compared to 0.953 μg/dl in the corresponding control). α-Tocopherol concentration was also significantly higher in normal rats under 10% onion treatments, the increase being around 23%. Ascorbic acid concentration was also significantly higher in hypercholesterolemic animals fed dehydrated onion (31 and 35% increase over corresponding control). Blood ascorbic acid concentration was similarly higher as a result of onion treatment even in normal rats (by 23 and 22% in the two onion groups). Blood lipid peroxides were lower by 15-16% in animals fed 10% dehydrated onion in both hypercholesterolemic and normal rats.

Influence of dietary dehydrated onion on liver lipid peroxides and various antioxidant molecules is presented in Table-4. Hepatic α-tocopherol concentration was significantly higher in normal rats maintained on dehydrated onion containing diets, but not in hypercholesterolemic animals. This increase in α-tocopherol concentration produced by 10% and 5% onion diet was as high as 53 and 77% in the respective experimental groups. Liver ascorbic acid concentration was higher under onion treatment in both normal (31 and 43% increase) and hyper-cholesterolemic (25 and 17% increase) animals. On the other hand, hepatic glutathione concentration was lower in experimental rats maintained

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Table-4 Influence of dietary dehydrated onion on hepatic lipid peroxides and antioxidant molecules

Animal group	Lipid peroxides nmole/mg protein	Total thiols mmole/mg protein	Glutathione µg/mg protein	α-Tocopherol µg/g liver	Ascorbic acid µg/mg protein
Basal - Control	4.70 ± 0.14	0.844 ± 0.042	0.667 ± 0.055	0.719 ± 0.090	0.397 ± 0.025
Basal - Onion (10%)	3.48 ± 0.24**	0.738 ± 0.048	0.496 ± 0.020**	1.097 ± 0.101*	0.521 ± 0.018*
Basal - Onion (5%)	3.58 ± 0.10**	0.928 ± 0.060	0.685 ± 0.034	1.274 ± 0.081*	0.568 ± 0.015*
HCD - Control	2.44 ± 0.12	0.761 ± 0.027	0.628 ± 0.027	1.920 ± 0.138	0.466 ± 0.018
HCD - Onion (10%)	2.64 ± 0.10	0.854 ± 0.038*	0.500 ± 0.017**	1.864 ± 0.078	0.583 ± 0.033*
HCD - Onion (5%)	2.90 ± 0.16	0.913 ± 0.050*	0.595 ± 0.032	1.822 ± 0.088	0.543 ± 0.036*

Values are mean ± SEM of 8 animals in each group

* Significant increase compared to corresponding control
** Significant decrease compared to corresponding control.

on dehydrated onion containing diet at 10% level. This decrease in hepatic glutathione concentration as a result of onion treatment was 26 and 20% respectively in the normal and hypercholesterolemic rats. Liver lipid peroxides were significantly lower in normal rats maintained on onion containing diets, the decrease being 24-26%.

Yamamoto *et al* (2005) have recently reported that Welsh onion reduces superoxide generation in rats fed with the high-fat high-sucrose diet when included at 5% level. Shon et al (2004) have demonstrated that the antimutagenicities and antioxidant properties of ethyl acetate extract of red, yellow and white onion against mutagens were related to their phenols and flavonoids, which are heat stable. Antoxidant effects of S-methyl cysteine sulfoxide isolated from onion were studied in alloxan diabetic rats after treating for two months (Kumari & Augusti, 2002). It lowered the levels of malondialdehyde, hydroperoxide and conjugated dienes in tissues exhibiting antioxidant effect on lipid peroxidation in experimental diabetes. This is achieved by their stimulating effects on glucose utilization and the antioxidant enzymes, viz. superoxide dismutase and catalase. The ability of flavonoids, especially quercetin to inhibit low-density lipoprotein oxidation *in vitro* has been demonstrated (O'Reilly *et al*, 2000).

In normal rats, feed intake in onion-10% and onion-5% animal groups were comparable to controls through out the experimental regimen. On the other hand, feed intake in onion-10% and onion-5% animal groups were lower than their control counterparts in hypercholesterolemic rats during 5th to 8th week (Table-5). Body weight gain was moderately lower as a result of 10% onion powder diet in hypercholesterolemic rats (Table-6). Liver weight was not affected as a result of onion treatment.

Table-5 Influence of dietary dehydrated onion on feed intake in experimental rats.

Animal group	Week 1-2	Week 3-4	Week 5-6	Week 7-8
Basal Control	11.8	16.5	15.3	14.9
Basal Onion 10%	11.5	13.0	14.3	13.6
Basal Onion 5%	12.4	15.2	14.7	12.7
HCD Control	13.5	14.5	16.6	17.3
HCD Onion 10%	12.9	13.0	12.9	13.8
HCD Onion 5%	13.3	15.3	12.9	13.0

Values expressed as average feed intake in g/ rat/ day

Table-6 Influence of dietary dehydrated onion on body weight gain and liver weight

Animal group	Body weight (g)			Liver weight	
	Initial	Final	Weight gain	(g)	(g/100 g body)
Basal Control	124.9 ± 3.0	278.6 ± 5.2	153.7 ± 2.9	8.07 ± 0.50	2.89 ± 0.18
Basal Onion 10%	124.5 ± 2.9	286.0 ± 6.4	161.5 ± 3.6	8.31 ± 0.38	2.89 ± 0.13
Basal Onion 5%	123.5 ± 2.2	287.2 ± 6.1	163.7 ± 4.0	8.42 ± 0.18	2.93 ± 0.07
HCD Control	123.5 ± 2.1	288.8 ± 5.8	165.3 ± 4.5	15.14 ± 0.64	5.29 ± 0.23
HCD Onion 10%	123.5 ± 2.1	269.0 ± 4.7**	145.5 ± 4.2**	13.41 ± 0.72	4.96 ± 0.21
HCD Onion 5%	123.0 ± 2.2	281.0 ± 6.9	158.0 ± 3.9	15.04 ± 0.51	5.35 ± 0.18

Values are mean ± SEM of 8 rats in each group.

** Significant decrease compared to corresponding control.

In conclusion, the present animal study has evidenced the health beneficial hypolipidemic potency of dehydrated onion product. The beneficial influence of the dehydrated onion product on the antioxidant molecules both in circulation as well as in liver is also evidenced here.

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CHAPTER – IV

PROTECTIVE INFLUENCES OF CURCUMIN AND CAPSAICIN ON EXPERIMENTALLY INDUCED HEPATOTOXICITY, INFLAMMATION AND OXIDATION OF LOW-DENSITY LIPOPROTEIN

Section-A: Protective effect of dietary curcumin and capsaicin on iron-induced hepatotoxicity and carrageenan-induced inflammation in experimental rats

Section-B: Protective effect of dietary curcumin and capsaicin on induced oxidation of low-density lipoprotein in normal and hypercholesterolemic rats

Hypolipidemic and antioxidant effects of curcumin and capsaicin in induced hypercholesterolemic rats

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Summary

The present animal study has evidenced that dietary curcumin, capsaicin or their combination uniformly countered (by around 20%) the extent of hypercholesterolemia brought about by high cholesterol feeding. Neither of the two dietary spice principles or their combination had any effect on blood cholesterol level in normal rats. Hepatic cholesterol concentration was significantly lowered (20 - 27%) as a result of dietary spice principles in normal rats, while the same was not influenced in hypercholesterolemic animals. Liver triglyceride was significantly lowered in both normal and hypercholesterolemic rats maintained on capsaicin diet. Blood lipid peroxide content in hypercholesterolemic rats was lowered by dietary curcumin and capsaicin, and this effect was additive with combination of curcumin and capsaicin. Hepatic thiols, glutathione and ascorbic acid were elevated in hypercholesterolemic rats with concurrent low titres of lipid peroxides. Both spice principles individually or in combination further depleted hepatic lipid peroxides. While hepatic ascorbic acid content was enhanced by dietary curcumin, capsaicin and their combination in normal rats, glutathione was enhanced by the combination of spice principles in hypercholesterolemic animals.

Activities of glutathione reductase, glutathione transferase and catalase in serum and hepatic glutathione reductase were enhanced by dietary curcumin, capsaicin and their combination in normal rats. Dietary curcumin, capsaicin and their combination increased the activity of serum glutathione peroxidase in hypercholesterolemic rats, while

glutathione reductase, glutathione transferase and catalase activities were higher only in curcumin feeding. Thus, the present study indicated that a high cholesterol diet compromises the endogenous antioxidant defense mechanisms as indicated by reduction in antioxidant molecules and antioxidant enzymes. Dietary curcumin and capsaicin were found to normalize these changes to a significant extent. Although this effect of spice principles was not generally additive when given in combination, the effect was certainly more pronounced than their individual effects in a few instances.

Introduction

Spices form an important class of food adjuncts consumed widely to enhance taste and flavour of foods. Besides, spices also possess several medicinal properties and hence find application in several indigenous systems of medicine. In recent years, spices are understood to impart several health beneficial physiological effects of which the hypolipidemic and antioxidant influence of a few spices are likely to have far-reaching implications [Srinivasan, 2005]. Curcumin, the yellow coloring principle of turmeric (*Curcuma longa*) and capsaicin the pungent principle of red pepper (*Capsicum annuum*), as well as the respective parent spices have been documented to have significant hypolipidemic influence in a variety of experimental animal systems [Srinivasan *et al*, 2004]. The importance of serum cholesterol levels and of lipoproteins in relation to atherosclerosis and coronary heart disease is well known. In view of this, there has been a continuous search for hypocholesterolemic agents. Since dietary adjuncts happen to be advantageous, the commonly used spices - turmeric and red pepper may possibly be exploited as hypocholesterolemics. The advantage, if any in terms of a possible synergy

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existing in this property between the active principles of these two spices remains to be understood.

Curcumin (diferuloyl methane) also exhibits antioxidant, anti-inflammatory and antitumour properties. Capsaicin (8-methyl-n-vanillyl-6-nonenamide) can induce body heat and possibly enhance blood flow as well as increase energy expenditure, and prevent oxidative stress. Our earlier study has shown that the structural integrity of red blood cells which is affected in hypercholesterolemic situation is effectively countered by dietary curcumin and capsaicin [Kempaiah & Srinivasan, 2002; 2005]. The antioxidant potential of spice principles – curcumin and capsaicin has been experimentally documented in *in vitro* systems and in a few *in vivo* studies [Srinivasan, 2005].

The present animal study examines the hypolipidemic influence of dietary curcumin and capsaicin individually and in combination in induced hypercholesterolemic rats. The study has also examined their influence on antioxidant molecules and a few antioxidant enzymes in blood and liver in hypercholesterolemic condition.

Methods and Materials

Animal treatment:

Animal experiments were carried out taking appropriate measures to minimize pain or discomfort in accordance with the guidelines laid down by the NIH (USA) regarding the care and use of animals for experimental procedures and with due clearance from the Institute's Animal Ethics Committee. Female Wistar rats (8 per group) weighing 110-120 g and housed in individual stainless steel cages were maintained on various experimental diets *ad libitum* for 8 weeks. The basal diet consisted of (%): casein, 21; cane sugar, 10; corn starch, 54; NRC vitamin mixture, 1; Bernhardt-Tommarelli modified NRC salt mixture, 4, and refined peanut oil, 10. The hypercholesterolemic diet consisted of 0.5% cholesterol and 0.125% bile salts at the expense of an equivalent amount of corn starch in the basal diet wherein the peanut oil is also replaced with hydrogenated vegetable fat. The spice principles were incorporated into the basal diet / hypercholesterolemic diet, replacing an equivalent amount of corn starch to give the various experimental diets containing: curcumin (0.2%), capsaicin (0.015%) and curcumin (0.2%) + capsaicin (0.015%). At the end of the experimental duration, overnight fasted animals were sacrificed under light ether anesthesia. Blood was collected by heart puncture and serum separated by centrifugation. Liver was quickly excised, weighed and stored frozen till lipid extraction.

Lipid profile:

Total lipids were extracted according to Folch *et al.* (1957) and estimated gravimetrically. Cholesterol [Searcy & Bergquist, 1960], triglycerides [Fletcher, 1968] and phospholipids [Charles & Stewart, 1980] were determined in the lipid extracts of serum and liver by using standard procedures. Serum cholesterol and triglyceride associated with HDL fraction were determined after precipitation of apolipoprotein-B containing lipoproteins with heparin-manganese reagent according to the method of Warnick and Albers (1978). LDL-VLDL precipitate was extracted with chloroform : methanol (2:1, v/v) and used for cholesterol and triglyceride determination.

Lipid peroxides:

Serum lipid peroxides were estimated by the fluorimetric measurement of thiobarbituric acid complex by the method of Yagi (1984). The fluorimetric measurement was carried out at an excitation wavelength of 515 nm and emission wavelength of 553 nm and compared with the standards prepared by reacting 0.5 nmole 1,1,3,3-tetraethoxy-propane with TBA reagent. Lipid peroxide in liver tissue was determined by the method described by Ohkawa *et al* (1979) involving photometric measurement of thiobarbituric acid complex extracted into butanol. Absorbance of the butanol extract was measured at 532 nm and compared with that of standard tetraethoxypropane, treated similarly.

Antioxidant molecules:

Total thiols in serum and liver were measured spectrophotometrically by using Ellman's reagent according to Sedlock and Lindsay (1968). Glutathione in serum and liver was estimated by using Ellman's reagent according to Beutler *et al* (1963).

Ascorbic acid was estimated spectrophotometrically by measuring the 2,4-dinitrophenyl-hydrazone derivative of dehydroascorbic acid according to Omaye *et al* (1973). α-Tocopherol in liver and serum was determined by HPLC method described by Zaspel and Csallany (1983) using ODS column (C-18) and an UV-Visible detector (295 nm) and a solvent system acetonitrile - methanol (1:1).

Antioxidant enzymes:

Glutathione reductase activity was assayed in serum and liver homogenate by measuring the oxidation of NADPH at 340 nm by oxidized glutathione as described by Carlberg and Mannervik (1985). Glutathione-S-transferase activity was assayed by measuring CDNB-GSH conjugate formed using 1-chloro-2,4-dinitrobenzene as the substrate as described by Warholm *et al* (1985). Glutathione peroxidase activity in serum and liver homogenate was determined by following NADPH oxidation in a coupled reduction system consisting of hydrogen peroxide and oxidized glutathione as described by Flohe and Gunzler (1984). Catalase activity in serum and liver homogenate was assayed according to the method of Aebi (1984) by following the decomposition of hydrogen peroxide at 240 nm.

Statistical analysis:

Results are expressed as mean $\pm$ SEM and comparisons between groups were made by means of an unpaired Student's t-test [Snedecor & Cochran, 1976]. Differences were considered significant when $p < 0.05$.

Results

Serum lipid profile

Influence of dietary curcumin, capsaicin and their combination on blood lipid profile in normal as well as high cholesterol fed animals is presented Table-1. High cholesterol feeding for 8 weeks resulted in a significant increase in blood total cholesterol concentration and this increase was observed predominantly in LDL-associated fraction. The increase in total blood cholesterol was as much as 6-fold. Dietary curcumin, capsaicin or their combination significantly countered the extent of hypercholesterolemia. Blood total cholesterol was 20, 22 and 21.5% lower in these respective animal groups compared to their corresponding control. The reduction in blood cholesterol brought about by dietary spice principles was predominantly in the LDL-cholesterol fraction. The HDL-cholesterol fraction essentially remained unchanged as a result of treatment with spice principles. While the effect of curcumin and capsaicin on blood cholesterol was quantitatively almost equal at their dietary levels used here, the combination of the two spice principles – curcumin and capsaicin did not have any additive effect on blood cholesterol level. Neither of the two dietary spice principles had any effect on blood cholesterol level in normal rats.

Serum total triglyceride concentration was significantly lower in high cholesterol treatment when compared to normal-Control (Table-1). This decrease was about 33%, and was due to a specific decrease in the LDL-associated fraction (38%). There was no change in the concentration of triglyceride associated with HDL as a result of high

Table-1 Influence of dietary curcumin and capsaicin on serum lipid profile

Animal group	Cholesterol			Triglycerides			Phospholipids
	Total	LDL-VLDL	HDL	Total	LDL-VLDL	HDL	
Basal Control	55.6 ± 2.00	34.9 ± 1.42	20.7 ± 1.74	112.3 ± 2.76	96.4 ± 3.41	16.0 ± 1.06	118.5 ± 4.50
Basal Curcumin	55.2 ± 3.55	33.1 ± 1.50	22.1 ± 1.48	101.3 ± 5.52	85.0 ± 5.67	16.2 ± 0.29	108.0 ± 3.81
Basal Capsaicin	57.8 ± 2.11	32.6 ± 2.39	25.2 ± 1.56	91.2 ± 8.01**	76.7 ± 4.94**	14.5 ± 0.47	108.8 ± 3.72
Basal Curcumin + Capsaicin	57.3 ± 3.33	36.7 ± 2.31	20.6 ± 0.99	98.0 ± 4.50**	82.7 ± 4.60**	15.3 ± 0.31	111.5 ± 1.77
HCD Control	398.0 ± 20.8	377.9 ± 22.0	20.1 ± 0.77	75.7 ± 3.46	59.5 ± 3.64	16.2 ± 0.85	159.6 ± 5.83
HCD Curcumin	317.8 ± 13.7**	298.2 ± 14.7**	19.6 ± 0.49	71.4 ± 4.50	55.7 ± 3.84	15.8 ± 1.49	228.8 ± 5.54*
HCD Capsaicin	309.2 ± 15.9**	289.0 ± 11.7**	20.2 ± 1.06	65.7 ± 5.23	52.1 ± 2.66	13.6 ± 0.96	185.4 ± 6.27*
HCD Curcumin + Capsaicin	312.6 ± 19.7**	291.7 ± 24.1**	20.9 ± 0.54	85.9 ± 2.70	69.0 ± 2.32	16.9 ± 0.63	201.6 ± 7.61*

Values expressed as mg/dl are mean ± SEM of 8 rats in each group.
LDL: Low density lipoprotein; HDL: High density lipoprotein; VLDL: Very low density lipoprotein; HCD: High cholesterol diet
* Significant increase compared to corresponding control. **Significant decrease compared to corresponding control.

cholesterol feeding. Dietary spice principles – curcumin, capsaicin or their combination did not alter the blood triglyceride concentration in these induced hypercholesterolemic animals. On the other hand, there was a statistically significant decrease in total triglycerides and LDL-associated triglycerides in normal rats as a result of dietary capsaicin and dietary curcumin + capsaicin. The decreases in blood total triglyceride concentration brought about in the normal rats belonging to the two respective diet groups were 19 and 13%, which was predominantly from the LDL-associated fraction.

While dietary spice principles did not affect blood phospholipid concentration in normal rats, considerable increase in the same was seen in hypercholesterolemic rats as result of dietary spice principles (Table-1). Hypercholesterolemic diet brought about 32% increase in blood phospholipid concentration compared to normal control. The increases in phospholipid concentration seen in hypercholesterolemic rats in the diet groups – curcumin, capsaicin, and curcumin + capsaicin were 43, 16 and 26% respectively, compared to HCD-control. No particular additive effect is seen when the two spice principles – curcumin and capsaicin were given in combination.

Liver lipid profile

Influence of dietary curcumin, capsaicin and their combination on hepatic lipid profile in normal as well as high cholesterol fed animals is presented in Table-2. Hepatic cholesterol concentration was significantly lowered as a result of dietary spice principles in normal rats. This decrease was 20, 27 and 23 % in curcumin fed, capsaicin fed and curcumin + capsaicin fed animals respectively. Liver cholesterol was not influenced by

Table-2 Influence of dietary curcumin and capsaicin on hepatic lipid profile

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Animal group	Total lipids	Cholesterol	Triglycerides	Phospholipids
Basal Control	65.6 ± 0.82	5.73 ± 0.19	27.8 ± 0.95	27.5 ± 0.30
Basal Curcumin	62.0 ± 0.63	4.58 ± 0.15**	24.4 ± 0.78	26.8 ± 0.42
Basal Capsaicin	48.4 ± 2.56**	4.18 ± 0.11**	13.7 ± 0.95**	24.6 ± 0.33
Basal Curcumin + Capsaicin	52.6 ± 3.55**	4.43 ± 0.19**	23.7 ± 2.53	26.6 ± 0.38
HCD Control	203.4 ± 5.96	59.3 ± 0.68	54.7 ± 2.97	22.8 ± 0.04
HCD Curcumin	199.5 ± 2.54	64.6 ± 1.60	47.3 ± 2.60	21.4 ± 0.04
HCD Capsaicin	172.6 ± 8.84**	53.4 ± 2.65	39.1 ± 2.57**	22.9 ± 0.77
HCD Curcumin + Capsaicin	206.3 ± 3.52	58.6 ± 0.79	46.6 ± 2.38	21.9 ± 0.07

Values expressed as mg/g liver are mean ± SEM of 8 rats in each group.
HCD: High cholesterol diet
**Significant decrease compared to corresponding control

either of the spices or their combination in hypercholesterolemic animals. Liver triglyceride was significantly lowered in both normal and hypercholesterolemic rats maintained on capsaicin diet. The decrease in hepatic triglyceride brought about by dietary capsaicin was 51 and 29% respectively in normal and hypercholesterolemic rats. Hepatic phospholipids concentration remained unaffected as a result of dietary spice principles in both normal and hypercholesterolemic rats. Total lipids in liver were lower by 15% in hypercholesterolemic rats and by 26% in normal rats as a result of dietary capsaicin treatment. Hepatic total lipid content was also lower in normal – curcumin + capsaicin group, which was about 20%.

Antioxidant molecules in blood

Influence of dietary curcumin, capsaicin and their combination on the concentration of various antioxidant molecules and lipid peroxides in serum is presented in Table-3. Among the various antioxidant molecules, while serum ascorbic acid concentration was not influenced by dietary spice principles in normal rats, the same was significantly enhanced in hypercholesterolemic animals. The extent of increase in serum ascorbic acid produced by dietary curcumin, capsaicin and their combination was 64, 34 and 44 % respectively. Thus, the effect of curcumin seen on serum ascorbic acid was more than that produced by capsaicin, and there was no additive effect when these two were fed in combination.

On the other hand, serum α-tocopherol concentration was significantly lowered in hypercholesterolemic animals by dietary curcumin, capsaicin, and their combination, the

Table-3 Influence of dietary curcumin and capsaicin on serum antioxidant molecules and lipid peroxides

Animal group	Total thiols (mmole/dl)	Glutathione (µg/dl)	Ascorbic acid (mg/dl)	α-Tocopherol (µg/dl)	Lipid peroxides (µmole/dl)
Basal Control	14.71 ± 0.52	0.221 ± 0.019	0.277 ± 0.010	0.570 ± 0.019	88.0 ± 5.13
Basal Curcumin	11.76 ± 0.46**	0.173 ± 0.013**	0.266 ± 0.014	0.527 ± 0.009	89.2 ± 3.15
Basal Capsaicin	11.91 ± 0.36**	0.208 ± 0.009	0.258 ± 0.017	0.499 ± 0.023	100.7 ± 6.08
Basal Curcumin + Capsaicin	12.08 ± 0.84**	0.170 ± 0.017**	0.300 ± 0.022	0.607 ± 0.013	102.1 ± 7.19
HCD Control	33.66 ± 1.14	0.391 ± 0.031	0.250 ± 0.015	1.698 ± 0.057	86.3 ± 3.22
HCD Curcumin	28.90 ± 1.53**	0.302 ± 0.025**	0.409 ± 0.040*	0.472 ± 0.081**	75.1 ± 3.02**
HCD Capsaicin	20.97 ± 0.43**	0.170 ± 0.016**	0.334 ± 0.023*	0.483 ± 0.049**	71.9 ± 3.53**
HCD Curcumin + Capsaicin	22.42 ± 0.55**	0.230 ± 0.026**	0.360 ± 0.012*	1.096 ± 0.080**	50.6 ± 5.15**

Values are expressed as mean ± SEM of 8 rats in each group.
HCD: High cholesterol diet
* Significant increase compared to corresponding control.
**Significant decrease compared to corresponding control.

effect being 72, 71.5 and 35% respectively (Table-3). Thus, the combination of the two spice principles produced a lesser decrease in the concentration of this antioxidant vitamin than the two individual spice principles. Serum α-tocopherol was not influenced by any of the spice principles in normal animals. Serum α-tocopherol was about 3-fold higher in the hypercholesterolemic animals (1.698 μg/dl vs. 0.57 μg/dl).

Total thiol content in serum was lower as a result of treatment with spice principles in both normal and hypercholesterolemic animals, the effect being more in the latter (Table-3). The decreases in serum total thiol concentration were 20, 19 and 18 % by dietary curcumin, capsaicin and curcumin + capsaicin respectively, in normal rats. The decreases in serum total thiol concentration produced in hypercholesterolemic animals by these respective diet groups were 14, 38 and 33 %. Significant decreases in serum glutathione concentration were also observed as a result of feeding these spice principles, more so in hypercholesterolemic animals. The decreases in serum glutathione concentration seen in hypercholesterolemic rats maintained on curcumin, capsaicin, and their combination were 23, 56 and 41% respectively.

Blood lipid peroxide content was lower in the hypercholesterolemic rats as a result of feeding either curcumin, capsaicin or their combination (Table-3). The extent of reduction in lipid peroxide was 13 %, 17 % and 41 % in the respective diet groups. Dietary spice principles did not have any influence on the blood lipid peroxide value in the case of normal rats.

Antioxidant molecules in liver

Influence of dietary curcumin, capsaicin and their combination on the concentration of various antioxidant molecules and lipid peroxides in liver is presented in Table-4. Ascorbic acid concentration in liver was favourably influenced by dietary curcumin, capsaicin and their combination in normal rats (Table-4). The extent of increase in hepatic ascorbic acid was 21, 14 and 11% in the respective diet groups. These dietary spice principles did not show any beneficial effect on liver ascorbic acid in hypercholesterolemic rats. On the other hand, there were marginal decreases in hepatic ascorbic acid of hypercholesterolemic rats in dietary curcumin, capsaicin and curcumin + capsaicin groups. The decreases were of the order of 23, 16 and 27 % in the respective diet groups.

Hepatic total thiols were higher as a result of dietary spice principles - curcumin, capsaicin or curcumin + capsaicin in normal rats (Table-4). The increase in total thiols was 11, 26 and 39 % in the respective diet groups as compared to control.   Thus, the combination of curcumin and capsaicin produced an effect higher than the two individual compounds. Total thiols were 87 % higher in high cholesterol treatment as compared to their normal counterpart. The spice principles did not have any influence on the same in hypercholesterolemic rats.

Hepatic glutathione content was higher in normal rats under the influence of dietary curcumin, capsaicin or the combination of these two (Table-4).   Dietary curcumin, capsaicin and curcumin + capsaicin produced an increase in hepatic glutathione of 29, 43

Table-4 Influence of dietary curcumin and capsaicin on liver antioxidant molecules and lipid peroxides

Animal group	Total thiols (mmole/mg protein)	Glutathione (µg/mg protein)	Ascorbic acid (µg/mg protein)	Lipid peroxides (nmole/mg protein)
Basal Control	0.512 ± 0.015	0.336 ± 0.030	0.269 ± 0.008	8.078 ± 0.329
Basal Curcumin	0.567 ± 0.016*	0.432 ± 0.034*	0.326 ± 0.007*	5.389 ± 0.519**
Basal Capsaicin	0.647 ± 0.033*	0.482 ± 0.022*	0.306 ± 0.009*	7.027 ± 0.231**
Basal Curcumin + Capsaicin	0.713 ± 0.019*	0.430 ± 0.016*	0.299 ± 0.005*	6.668 ± 0.439**
HCD Control	0.957 ± 0.024	0.528 ± 0.022	0.411 ± 0.007	3.959 ± 0.179
HCD Curcumin	0.870 ± 0.032	0.512 ± 0.011	0.318 ± 0.011**	3.157 ± 0.179**
HCD Capsaicin	0.892 ± 0.017	0.550 ± 0.029	0.345 ± 0.012**	2.896 ± 0.294**
HCD Curcumin + Capsaicin	0.925 ± 0.009	0.724 ± 0.031*	0.302 ± 0.008**	2.714 ± 0.248**

Values are expressed as mean ± SEM of 8 rats in each group.
HCD: High cholesterol diet
* Significant increase compared to corresponding control.
**Significant decrease compared to corresponding control

and 28 % respectively. Hypercholesterolemic rats displayed a higher hepatic glutathione content (0.528 µg/mg protein in HCD-control compared to 0.336 µg/mg protein in normal-control). Unlike in normal rats, only the combination of curcumin and capsaicin produced an enhancing effect on hepatic glutathione in hypercholesterolemic animals, the effect being 37 %.

Hepatic lipid peroxides were lower as a result of dietary spice principles both in normal and hypercholesterolemic situation (Table-4). The decrease in hepatic lipid peroxides produced was 33, 13 and 17% by dietary curcumin, capsaicin and their combination respectively in normal rats. A decrease in the same by 20, 27 and 31 % by the respective diet groups was seen in the case of hypercholesterolemic rats.

Antioxidant enzymes in serum

Influence of dietary curcumin, capsaicin and their combination on serum antioxidant enzymes is presented in Table-5. Activities of serum glutathione reductase and catalase were higher in hypercholesterolemic animals when compared to their normal counterparts. Activity of glutathione reductase was significantly enhanced by dietary curcumin, capsaicin and curcumin + capsaicin in normal rats. The increase in the enzyme activity produced by these diet groups was 41, 82 and 44 % respectively. However, in the hypercholesterolemic animals, there was no similar beneficial influence of these spice principles.

Dietary curcumin, capsaicin and their combination produced beneficial increases in the activity of glutathione peroxidase in hypercholesterolemic rats (Table-5). The

Table-5 Influence of dietary curcumin and capsaicin on serum antioxidant enzymes

Animal group	GSH Reductase (mmole/ min/ ml)	GSH peroxidase (mmole/ min/ ml)	GSH transferase (mmole/ min/ dl)	Catalase (mmole/ min/ dl)
Basal Control	25.4 ± 0.42	1.130 ± 0.015	1.970 ± 0.096	14.75 ± 1.03
Basal Curcumin	35.9 ± 1.74*	0.964 ± 0.015**	2.781 ± 0.078*	21.13 ± 1.80*
Basal Capsaicin	46.3 ± 1.98*	0.905 ± 0.038**	2.661 ± 0.212*	19.13 ± 1.54*
Basal Curcumin + Capsaicin	36.5 ± 1.74*	0.600 ± 0.070**	2.607 ± 0.168*	18.00 ± 0.48*
HCD Control	34.6 ± 3.06	1.246 ± 0.070	2.126 ± 0.126	22.25 ± 0.65
HCD Curcumin	33.3 ± 2.88	1.535 ± 0.060*	2.649 ± 0.216*	25.88 ± 1.04*
HCD Capsaicin	32.4 ± 3.24	1.538 ± 0.015*	2.084 ± 0.102	20.38 ± 1.21
HCD Curcumin + Capsaicin	33.6 ± 1.80	1.473 ± 0.063*	2.126 ± 0.150	18.75 ± 1.80

Values are expressed as mean ± SEM of 8 rats in each group.
HCD: High cholesterol diet
* Significant increase compared to corresponding control.
**Significant decrease compared to corresponding control.

enzyme activity was 23, 23 and 18 % higher in these diet groups. However, the same spice principles negatively influenced the activity of glutathione peroxidase in normal rats, the extent of decrease in the enzyme activity being 15, 20 and 47 % in the respective groups. This negative effect was much higher in the spice combination group compared to the individual spice principle groups.

Glutathione transferase activity was much higher as a result of dietary curcumin, capsaicin, and curcumin + capsaicin in normal rats (Table-5). The increase in the enzyme activity was 41, 35 and 32 % in the respective groups. The enzyme activity was similarly higher (by 25 %) as a result of curcumin feeding in hypercholesterolemic animals. Dietary capsaicin or the spice principles combination had no influence on the enzyme activity in hypercholesterolemic rats.

Serum catalase activity was similarly beneficially influenced by dietary curcumin, capsaicin and curcumin + capsaicin in normal rats (Table-5). The increase in catalase activity was 43, 30 and 22 % in the respective groups. Catalase activity was similarly higher (by 16 %) only in curcumin fed group of hypercholesterolemic animals. Dietary capsaicin or the spice principles combination had no influence on this enzyme activity in hypercholesterolemic rats.

Antioxidant enzymes in liver

Influence of dietary curcumin, capsaicin and their combination on activities of hepatic antioxidant enzymes is presented in Table-6. Activities of all the hepatic antioxidant enzymes examined here - glutathione reductase, glutathione peroxidase, glutathione

Table-6 Influence of dietary curcumin and capsaicin on liver antioxidant enzymes

Animal group	GSH Reductase (μmole/ min/ mg protein)	GSH peroxidase (mmole/ min/ mg protein)	GSH transferase (mmole/ min/ mg protein)	Catalase (mmole/ min/ mg protein)
Basal Control	29.6 ± 1.53	1.250 ± 0.075	1.025 ± 0.069	11.64 ± 0.54
Basal Curcumin	37.8 ± 1.10*	1.341 ± 0.052	0.988 ± 0.047	10.32 ± 0.66
Basal Capsaicin	36.6 ± 1.58*	1.152 ± 0.052	0.910 ± 0.049	10.08 ± 0.78
Basal Curcumin + Capsaicin	39.7 ± 2.84*	1.300 ± 0.051	1.038 ± 0.043	11.82 ± 0.48
HCD Control	22.5 ± 2.46	1.061 ± 0.088	0.693 ± 0.020	8.10 ± 0.42
HCD Curcumin	28.0 ± 2.28*	0.953 ± 0.050	0.809 ± 0.033*	5.52 ± 0.60**
HCD Capsaicin	22.8 ± 1.20	0.976 ± 0.019	0.746 ± 0.028	4.32 ± 0.48**
HCD Curcumin + Capsaicin	19.2 ± 1.55	0.937 ± 0.018	0.730 ± 0.043	4.08 ± 0.78**

Values are expressed as mean ± SEM of 8 rats in each group.
HCD: High cholesterol diet
* Significant increase compared to corresponding control.
**Significant decrease compared to corresponding control.

transferase and catalase were lower in hypercholesterolemic animals when compared to their normal counterparts. Dietary curcumin, capsaicin and their combination significantly enhanced the activity of glutathione reductase in liver of normal rats. The increases in this enzyme activity were 28, 24 and 34 % respectively as a result of feeding curcumin, capsaicin or their combination. Dietary curcumin also enhanced hepatic glutathione reductase activity in hypercholesterolemic animals (by 24 %). Hepatic glutathione peroxidase activity was unaffected by dietary spice principles in both normal and hypercholesterolemic animals.

Whereas, dietary spice principles had no effect on hepatic glutathione transferase activity in normal rats, only curcumin increased the activity of this enzyme in hypercholesterolemic animals (by 17 %). Activity of liver catalase was unaffected by any of the dietary spice principles in normal rats, which however was negatively influenced in all the three experimental groups of hypercholesterolemic rats (Table-6). The decreases in catalase activity observed in dietary curcumin, dietary capsaicin and dietary curcumin + capsaicin groups were 32, 47 and 50 % respectively.

Table-7 Influence of dietary curcumin and capsaicin on food intake in hypercholesterolemic rats.

Group/	0 - 14 days	15 - 30 days	31 - 44 days	45 - 60 days	Total per 60days
Basal Control	10.7 ± 0.35	11.8 ± 0.28	10.8 ± 0.23	10.5 ± 0.35	657
Basal Curcumin	10.9 ± 0.38	12.1 ± 0.35	10.8 ± 0.22	10.5 ± 0.46	665
Basal Capsaicin	8.27 ± 0.14	8.90 ± 0.31	8.56 ± 0.12	8.52 ± 0.35	515**
Basal Curcumin + Capsaicin	8.41 ± 0.10	8.59 ± 0.21	7.69 ± 0.08	7.94 ± 0.25	489**
HCD Control	10.6 ± 0.37	10.9 ± 0.27	11.8 ± 0.37	10.8 ± 0.37	662
HCD Curcumin	10.1 ± 0.34	11.1 ± 0.43	11.2 ± 0.30	11.0 ± 0.36	651
HCD Capsaicin	9.03 ± 0.13	10.2 ± 0.47	10.6 ± 0.44	9.88 ± 0.33	596**
HCD Curcumin + Capsaicin	8.93 ± 0.15	9.88 ± 0.27	9.47 ± 0.23	9.68 ± 0.50	570**

Values are indicated in terms of g / day / Rat.
**Significant decrease compared to respective control

Table-8 Influence of dietary curcumin and capsaicin on body weight gain and liver weight in hypercholesterolemic rats.

	Body weight (g)			Liver weight	
	Initial	Final	Gain	(g)	(g/100 g body)
Basal Control	107.0 ± 2.84	197.5 ± 6.57	90.5 ± 3.93	4.81 ± 0.10	2.49 ± 0.05
Basal Curcumin	107.9 ± 2.69	196.4 ± 6.55	89.2 ± 5.93	5.01 ± 0.19	2.61 ± 0.09
Basal Capsaicin	107.4 ± 2.94	185.4 ± 4.98	78.1 ± 2.40**	4.77 ± 0.07	2.64 ± 0.03
Basal Curcumin + Capsaicin	107.5 ± 2.94	178.1 ± 5.76	70.6 ± 2.08**	4.59 ± 0.14	2.66 ± 0.08
HCD Control	106.9 ± 2.34	206.4 ± 6.58	99.5 ± 4.44	8.46 ± 0.29	4.21 ± 0.14
HCD Curcumin	107.0 ± 2.40	196.1 ± 6.29	89.1 ± 4.19	8.41 ± 0.50	4.43 ± 0.26
HCD Capsaicin	106.9 ± 2.41	192.1 ± 5.67	85.4 ± 3.80**	7.30 ± 0.28	3.83 ± 0.15**
HCD Curcumin + Capsaicin	107.0 ± 2.42	190.5 ± 5.83	83.5 ± 3.60**	7.81 ± 0.22	4.26 ± 0.12

Values are ± SEM of 8 animals in each group.

**Significant decrease compared to corresponding control.

Discussion

In the present study, spice active principles have been fed to animals at levels corresponding to about 10 times the average dietary intake of the corresponding spices among Indian population [Thimmayamma *et al*, 1983]. The food intake was essentially similar in various spice principles fed groups and corresponding control group. Similarly the gain in body weights during the 8 weeks spice treatment was comparable to corresponding controls [Final weight (g) ranged from 178.1 ± 5.7 to 197.5 ± 6.6 in basal groups; and from 190.5 ± 5.8 to 206.4 ± 6.5 in hypercholesterolemic animals]. Thus, dietary spices have not negatively affected the feed intake and body weight gain.

The present animal study has evidenced that dietary curcumin, capsaicin or their combination significantly countered the extent of hypercholesterolemia brought about by high cholesterol feeding, and the effect was uniform (around 20%) in all the three experimental groups. The reduction in blood cholesterol brought about by dietary spice principles was predominantly in the LDL-cholesterol fraction. The combination of the two spice principles – curcumin and capsaicin did not have any additive effect on blood cholesterol level. Neither of the two dietary spice principles had any effect on blood cholesterol level in normal rats.

Subba Rao *et al* (1970) have reported that at 0.1 - 0.5% in the hypercholesterolemic diet, curcumin lowered serum and liver cholesterol levels of rats which were altered by cholesterol feeding. Further-more there was an increase in the fecal excretion of bile acids and cholesterol. Patil and Srinivasan (1971) evidenced the effect of curcumin at even 0.05% level in the diet and at as early as 4 weeks feeding. The anti-

hypercholesterolemic efficacy of dietary curcumin has been recently evidenced in rats fed an atherogenic high cholesterol diet, which also resulted in countering of the changes in membrane lipid profile in the erythrocytes [Kempaiah & Srinivasan, 2002]. Hypocholesterolemic and hypotriglyceridemic action of dietary curcumin (0.5%) has been evidenced in our laboratory in streptozotocin-induced diabetic rats [Babu & Srinivasan, 1997a].

Sambaiah *et al* (1978) have observed reduction in serum total cholesterol levels in rats on a normal (10% fat) diet incorporated with 1.5, 3.0 or 15 mg% capsaicin, the reductions being significant at both the higher levels. Sambaiah and Satyanarayana (1980) have reported that the serum cholesterol levels in rats on a 1% cholesterol + 15 mg% capsaicin did not influence serum cholesterol. On the other hand, liver cholesterol was lower in the capsaicin fed groups. Fecal excretion of free cholesterol and of bile acids was enhanced in animals fed capsaicin. In a subchronic toxicity study [Monsereenusorn, 1983] in rats administered 50 mg /kg body wt /day of capsaicin by stomach tube for 60 days, there was no significant change in plasma total cholesterol at 10 and 20 days, but at 30, 40, 50 and 60 days, the cholesterol levels were significantly reduced along with triglycerides and phospholipids. Negulesco *et al* (1983) found that intubation with 8 mg capsaicin / rabbit weighing 850g / day for 35 days did not have beneficial effect on plasma cholesterol and triglyceride when they were on a normal diet, while in rabbits on a 0.5% cholesterol diet, capsaicin had a beneficial lowering effect on plasma cholesterol and triglycerides. Young turkeys on a 2-3 mg capsaicin / kg feed for 9 days along with 0.5% cholesterol had lower total serum cholesterol than the controls [Ki *et al*, 1982]. Recently, the anti-hypercholesterolemic efficacy of dietary capsaicin has been evidenced in rats fed an

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atherogenic high cholesterol diet which resulted in countering of the changes in membrane lipid profile in the erythrocytes [Kempaiah & Srinivasan, 2002]. In streptozotocin-induced diabetic situation however, it was observed that dietary capsaicin did not show any beneficial hypolipidemic property [Babu & Srinivasan, 1997].

Hepatic cholesterol concentration was significantly lowered in the present investigation as a result of dietary spice principles in normal rats, while the same was not influenced by either of the spices or their combination in hypercholesterolemic animals. Liver triglyceride was significantly lowered in both normal and hypercholesterolemic rats maintained on capsaicin diet. Thus, no particular additive effect is seen when the two spice principles – curcumin and capsaicin were given in combination with regard to blood and liver lipid profiles.

Among the various antioxidant molecules, serum ascorbic acid and α-tocopherol concentrations were not influenced by dietary spice principles in normal rats, but were significantly affected in hypercholesterolemic animals. Serum ascorbic acid level was significantly enhanced by dietary spices in hypercholesterolemic animals while α-tocopherol concentration was significantly lowered. The effect of curcumin seen on serum ascorbic acid was more than that produced by capsaicin, and there was no additive effect when these two were fed in combination. The combination of the two spice principles produced a lesser decrease in the concentration of this antioxidant vitamin than the two individual spice principles. Serum α-tocopherol was about 3-fold higher in the hypercholesterolemic animals. This could probably be an adaptive mechanism by which

the oxidative stress is countered in the hypercholesterolemic situation, and as such the lipid peroxide level in blood was essentially the same as found in normal animals.

Blood lipid peroxide content was lower in the hypercholesterolemic rats as a result of feeding curcumin, capsaicin or their combination; the lowering of blood lipid peroxides was additive when curcumin and capsaicin were fed in combination. The increase in blood ascorbic acid concentration in these three diet groups may have contributed to the observed reduced lipid peroxide level. Hepatic antioxidants - thiols, glutathione and ascorbic acid were elevated in hypercholesterolemic rats with concurrent low titres of lipid peroxides. All the three dietary spice principle groups brought about further depletion in hepatic lipid peroxides. Ascorbic acid concentration in liver was favourably influenced by dietary curcumin, capsaicin and their combination in normal rats while the increase in ascorbic acid was countered by dietary spice principles in hypercholesterolemic rats. With respect to hepatic glutathione content, only the combination of curcumin and capsaicin produced an enhancing effect in hypercholesterolemic animals. Similarly, the combination of curcumin and capsaicin produced an effect higher than the two individual compounds in increasing the total thiol content in liver of normal rats.

There are several reports on the antioxidant effects of curcumin and capsaicin with regard to modulation of lipid peroxide level and antioxidant status. Curcumin has been reported to exert a protective effect against nicotine-induced lung toxicity by modulating the extent of lipid peroxidation and augmenting antioxidant defense system [Kalpana & Menon, 2004]. The enhanced circulatory lipid peroxides in nicotine-treated rats which

was accompanied by a significant decrease in the levels of ascorbic acid, vitamin E, reduced glutathione, glutathione peroxidase, superoxide dismutase, and catalase was significantly countered by the administration of curcumin ((80 mg/kg given simultaneously by intragastric intubation for 22 weeks) which significantly lowered the lipid peroxidation and enhanced the antioxidant status. Dietary supplementation of curcumin (2%) to male mice for 30 days has been reported to have significantly increased the activities of glutathione peroxidase, glutathione reductase and catalase in liver [Iqbal *et al*., 2003]. Curcumin has been reported to have a protective role in alcohol and δ-PUFA induced oxidative stress in male albino Wistar rats [Rukkumani *et al*., 2004]. The liver thiobarbituric acid reactive substances and antioxidants such as ascorbic acid, α-tocopherol, reduced glutathione, superoxide dismutase, catalase, and glutathione peroxidase were increased significantly in alcohol and δ-PUFA groups. Administration of curcumin abrogated this effect by effectively modulating the antioxidant status. *In vivo* antioxidative effects of curcumin have been reported in trichloroethylene-induced oxidative stress in mouse liver [Watanabe & Fukui, 2000]. Increases in the contents of peroxisome and thiobarbituric acid reactive substances and decreases in glutathione content of mouse liver by the trichloroethylene administration were suppressed by the pre-administration of curcumin. Iron induced liver lipid peroxidation was 29% lower in turmeric fed (1% in diet) male Wistar rats [Reddy & Lokesh, 1994]. The activities of superoxide dismutase, catalase and glutathione peroxidase were higher in liver homogenates of rats fed the turmeric-containing diet in comparison with the controls, suggesting that dietary turmeric lowers lipid peroxidation by enhancing the activities of antioxidant enzymes. Male Wistar rats administered *i.p*. with 3 mg/kg body weight

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capsaicin for 3 consecutive days showed a reduction of oxidative stress measured as malondialdehyde in the liver and other tissues suggesting that capsaicin can be a potent antioxidant [Lee *et al*., 2003].

In the present study, activities of glutathione reductase, glutathione transferase and catalase in serum were significantly enhanced by dietary curcumin, capsaicin and curcumin + capsaicin in normal rats. Dietary curcumin, capsaicin and their combination produced beneficial increases in the activity of glutathione peroxidase in hypercholesterolemic rats. Glutathione transferase and catalase activities were higher as a result of curcumin feeding in hypercholesterolemic animals. Dietary curcumin, capsaicin and their combination significantly enhanced the activity of glutathione reductase in liver of normal rats. Dietary curcumin also enhanced hepatic glutathione reductase and glutathione transferase activities in hypercholesterolemic animals.

SOD, catalase and glutathione peroxidase constitute the major defense against reactive oxygen species. Glutathione transferases are a group of enzymes capable of conjugating glutathione with diverse electrophilic compounds [Uysal *et al*, 1988]. Activities of hepatic antioxidant enzymes – glutathione transferase, glutathione reductase and catalase were significantly depleted under hypercholesterolemia. Cholesterol feeding has been reported to decrease the level of TBARS, SOD and catalase [Yi-Falu & Chia-Fung, 2001]. The decrease in hepatic glutathione transferase activity in rats fed high cholesterol diet suggests a deficiency in defense against electrophilic compounds.

Dietary spice principles were effective in reducing the oxidant stress, which was indicated by one or more of the following: (1) Enhancing one or more of antioxidant molecules in circulation, (2) Enhancing one or more antioxidant molecules in liver, (3) Increasing the activities of antioxidant enzymes in blood, or (4) Countering of the depleted hepatic antioxidant enzymes – glutathione reductase, glutathione-S-transferase and catalase. Induction of glutathione transferase activity by curcumin has been reported in mice and rats [Susan & Rao, 1992; Yokota *et al*., 1988]. Similar to the observation in the current study, cholesterol feeding has been reported to decrease liver glutathione peroxidase in rats but controversial results exist in studies involving lipid peroxidation [Mahfouz & Kummrow, 2000; Uysal *et al*., 1988].

Dietary curcumin and capsaicin or their combination in the present study have evidenced a significant stimulation of hepatic glutathione transferase activity in normal rats, while curcumin had a similar effect even in hypercholesterolemic animals. Earlier studies have shown that curcumin causes an increase in glutathione transferase activity in rodent liver which may contribute to its anti-cancer and anti-inflammatory activities [Piper *et al*, 1998]. When rats were fed curcumin at doses 25 - 50 mg kg^{-1} daily for 14 days, hepatic glutathione transferase activity towards 1-chloro-2,4-dinitrobenzene was maximally induced (about 1.5-fold). Glutathione transferase activity towards 4-hydroxynonenal was increased by curcumin in a dose dependent manner. Glutathione peroxidase activity towards cumene hydroperoxide in liver homogenate was also found to be increased in a dose-dependent manner by curcumin. Induction of enzymes (glutathione transferase and glutathione peroxidase) involved in the detoxification of the

electrophilic products of lipid peroxidation may contribute to the anti-inflammatory and anti-cancer activities of curcumin.

Thus, the present study which examined the effect of feeding two hypocholesterolemic spice principles individually and in combination along with a cholesterol enriched diet on lipid peroxide and antioxidant systems in blood and hepatic tissue has shown that a high cholesterol diet compromises the endogenous antioxidant defense mechanisms as indicated by reduction in specific antioxidant molecules and antioxidant enzymes. The spice principles were found to normalize these changes to a significant extent. These dietary spices were effective in reducing the oxidant stress, which was indicated by countering of the depleted antioxidant molecules and antioxidant enzymes in blood and liver. Although generally the beneficial effect of dietary curcumin and capsaicin was not additive when given in combination, in a few instances, the effect of the combination of curcumin and capsaicin was certainly more pronounced than their individual effects.

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Hypolipidemic and antioxidant effects of curcumin and capsaicin in high fat fed rats

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Summary

The present animal study has evidenced that dietary curcumin, capsaicin or their combination significantly countered the extent of hypertriglyceridemia brought about by high fat feeding, and the effect was 12 - 20%. The hypotriglyceridemic effect of dietary curcumin was more than that of capsaicin at the dietary levels examined. Dietary curcumin, capsaicin and their combination produced slight decrease in serum total cholesterol concentration in these high fat fed animals. While dietary curcumin and dietary capsaicin individually did not influence serum cholesterol concentration in normal rats, a combination of the two spice principles did produce a slight but significant reduction in the same. Serum α-tocopherol content was increased by dietary curcumin, capsaicin and their combination in high fat fed rats, the effect being greater by the combination of curcumin and capsaicin. Serum glutathione concentration was beneficially increased only by dietary capsaicin in the high fat fed animals. Serum total thiol content in high fat fed animals and serum ascorbic acid in normal animals was elevated by the combination of curcumin and capsaicin. Glutathione concentration in liver was increased by feeding curcumin, capsaicin or curcumin + capsaicin in normal animals. Hepatic glutathione and α-tocopherol concentration was significantly increased by dietary curcumin and dietary curcumin + capsaicin in high fat fed animals. As a result of beneficial enhancing influence on hepatic glutathione and α-tocopherol concentrations, dietary curcumin and dietary curcumin + capsaicin also reduced lipid

peroxide level in high fat fed animals. Hepatic lipid peroxide content was also lower in normal rats as a result of dietary spice principles. The activities of serum glutathione peroxidase and glutathione transferase in high fat fed rats were generally higher as a result of dietary curcumin, capsaicin and curcumin + capsaicin. Activities of hepatic glutathione reductase and glutathione peroxidase were significantly elevated by dietary spice principles in high fat fed animals. Additive effect of the two compounds was not evidenced in general when given together with respect to hypolipidemic or the antioxidant potential.

Introduction

Spices form an important class of food adjuncts consumed widely to enhance taste and flavour of foods. These spice ingredients impart characteristic flavour, aroma and colour to foods. Besides, spices also possess several medicinal properties and hence find application in several indigenous systems of medicine. In recent years, spices are understood to impart several beneficial physiological effects of which the hypolipidemic and antioxidant influence of a few spices are likely to have far-reaching health beneficial implication [Srinivasan, 2005]. Curcumin of turmeric (*Curcuma longa*), capsaicin of red pepper (*Capsicum frutescens*), fenugreek (*Trigonella foenum-graecum*), garlic (*Allium sativum*) and onion (*Allium cepa*) have been documented to have pronounced hypolipidemic influence in a variety of experimental animal systems and the later three spices are found to be efficacious in human studies too [Srinivasan *et al*, 2004]. The antioxidant potential of spice principles – curcumin, capsaicin and eugenol has been experimentally documented in *in vitro* systems and *in vivo* studies [Srinivasan, 2005].

Amelioration of diabetic nephropathy by dietary hypocholesterolemic and antioxidant spices – curcumin and onion has also been evidenced in experimental diabetes in our laboratory [Srinivasan, 2005a].

The importance of serum cholesterol levels and of lipoproteins in relation to atherosclerosis and coronary heart disease is well known. In view of this, there has been a continuous search for hypocholesterolemic agents. Some of the commonly used spices may be advantageous as possible hypocholesterolemic food adjuncts. Curcumin, the yellow pigment from *Curcuma longa*, is a major component of turmeric also exhibits anti-inflammatory and antitumour properties. Capsaicin (8-methyl-n-vanillyl-6-nonenamide), the pungent component of red pepper can induce body heat and possibly enhance blood flow as well as increase energy expenditure. Our recent studies have shown that the structural integrity of red blood cells and hence the osmotic fragility is affected in hypercholesterolemic and hypertriglyceridemic situation [Kempaiah & Srinivasan, 2002; 2005; 2006]. Dietary hypolipidemic spice principles – curcumin and capsaicin were evidenced to counter these changes in the osmotic sensitivity of erythrocytes to a significant extent [Kempaiah & Srinivasan, 2002; 2006].

The present animal study examines the beneficial hypolipidemic influence of dietary curcumin and capsaicin individually as well as in combination in high (30%) fat fed rats. In the current study, we have also examined if a high (30%) fat diet would have any detrimental influence on the antioxidant status of blood and liver, and evaluated the beneficial influence if any, of dietary hypolipidemic spice principles – curcumin, capsaicin and their combination on the antioxidant molecules and antioxidant enzymes of blood and liver tissue.

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Methods and Materials

Animal treatment:

Animal experiments were carried out taking appropriate measures to minimize pain or discomfort in accordance with the guidelines laid down by the NIH (USA) regarding the care and use of animals for experimental procedures and with due clearance from the Institutional Animal Ethics Committee. Male Wistar rats (8 per group) weighing 100 - 105 g housed in individual stainless steel cages were maintained on various experimental diets *ad libitum* for 8 weeks. The basal diet consisted of (%): casein, 21; cane sugar, 10; corn starch, 54; NRC vitamin mixture, 1; Bernhart-Tommarelli modified NRC salt mixture, 4 and refined peanut oil, 10. High fat diet consisted of (%): casein, 21; cane sugar, 10; corn starch, 34; NRC vitamin mixture, 1; Bernhart-Tommarelli modified NRC salt mixture, 4; hydrogenated vegetable fat, 25 and refined peanut oil, 5. The spice principles were incorporated into the Basal diet / High fat diet, replacing an equivalent amount of corn starch to give the various experimental diets containing: Curcumin (0.2%), capsaicin (0.015%) and curcumin (0.2%) + capsaicin (0.015%). At the end of the experimental duration, overnight fasted animals were sacrificed under light ether anesthesia. Blood was collected by heart puncture and serum separated by centrifugation. Liver was quickly excised, weighed and stored frozen till lipid extraction.

Lipid profile:

Total lipids were extracted according to Folch *et al*. (1957) and estimated gravimetrically. Cholesterol (Searcy & Bergquist, 1960), triglycerides (Fletcher, 1968) and phospholipids (Charles & Stewart, 1980) were determined in the lipid extracts of

serum and liver by using standard procedures. Serum cholesterol and triglyceride associated with HDL fraction were determined after precipitation of apolipoprotein-B containing lipoproteins with heparin-manganese reagent according to the method of Warnick and Albers (1978). LDL-VLDL precipitate was extracted with chloroform : methanol (2:1 v/v) and used for cholesterol and triglyceride determination.

Lipid peroxides:

Plasma lipid peroxides were estimated by the fluorimetric measurement of thiobarbituric acid complex by the method of Yagi (1984). The fluorimetric measurement was carried out at an excitation wavelength of 515 nm and emission wavelength of 553 nm and compared with the standards prepared by reacting 0.5 nmole 1,1,3,3-tetraethoxy-propane with TBA reagent. Lipid peroxide in liver tissue was determined by the method described by Ohkawa *et al* (1979) involving photometric measurement of thiobarbituric acid complex extracted into butanol. Absorbance of the butanol extract was measured at 532 nm and compared with that of standard tetraethoxypropane, treated similarly.

Antioxidant molecules:

Total thiols in blood plasma / liver were measured spectrophotometrically by using Ellman's reagent according to the method described by Sedlock and Lindsay (1968). Glutathione in blood plasma / liver was estimated by using Ellman's reagent according to Beutler *et al* (1963). Ascorbic acid was estimated spectrophotometrically by measuring the 2,4-dinitrophenyl-hydrazone derivative of dehydroascorbic acid according to Omaye *et al* (1973). α-Tocopherol in liver and blood plasma was determined by HPLC method

described by Zaspel and Csallany (1983) using ODS column (C-18) and an UV-Visible detector (295nm) and a solvent system acetonitrile - methanol (1:1).

Glutathione reductase activity was assayed according to Carlberg and Mannervik (1985). Glutathione-S-transferase activity was assayed by measuring CDNB-GSH conjugate formed using 1-chloro-2,4-dinitrobenzene as the substrate as described by Warholm *et al* (1985). Glutathione peroxidase activity was determined according to Flohe and Gunzler (1984). Catalase activity was assayed according to Aebi (1984) by following the decomposition of hydrogen peroxide. Superoxide dismutase activity was measured by quantitating the inhibition of cytochrome C reduction in xanthine-xanthine oxidase system as described by Flohe and Otting (1984).

Statistical analysis:

Results are expressed as mean ± SEM and comparisons between groups were made by means of an unpaired Student's t-test [Snedecor & Cochran, 1976]. Differences were considered significant when $p < 0.05$.

Results

Serum lipid profile

Table-1 presents the influence of dietary spice principles – curcumin, capsaicin and their combination on serum lipid profile. Serum total triglyceride concentration was 63 % higher in high fat feeding when compared to the normal control, and this increase was seen in both the LDL and HDL fractions. Dietary curcumin, capsaicin and their combination countered this hypertriglyceridemia produced by high fat diet. The decreases in serum total triglycerides brought about by curcumin, capsaicin and their combination were 20, 14 and 12 % respectively. The decrease in blood triglyceride concentration brought about by dietary spice principles was even more evident in the LDL-fraction, where the respective decreases were 33, 22 and 26%. Thus, the hypotriglyceridemic effect of dietary curcumin was more than that of capsaicin at the dietary levels examined. The serum triglyceride was not influenced by these spice principles in normal animals.

Serum total cholesterol concentration was slightly lower in high fat treatment, which was due to a predominant decrease in the LDL-associated cholesterol (Table-1). Dietary curcumin, capsaicin and their combination produced slight significant decrease in serum total cholesterol concentration in these high fat fed animals. The hypocholesterolemic effect produced by these three experimental diet groups was 12, 23 and 21 % respectively. The decrease in serum cholesterol concentration was due to the reductions produced in the LDL-cholesterol fraction. The decrease in LDL-cholesterol brought

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Table-1 Influence of dietary curcumin and capsaicin on serum lipid profile

Animal group	Cholesterol			Triglycerides			Phospholipids
	Total	LDL-VLDL	HDL	Total	LDL-VLDL	HDL	
Basal Control	88.3 ± 1.02	49.8 ± 1.88	38.5 ± 2.95	109.7 ± 4.50	79.4 ± 4.73	30.3 ± 4.32	76.9 ± 6.41
Basal Curcumin	81.7 ± 2.34	45.4 ± 0.33	36.3 ± 2.43	123.9 ± 6.87	94.2 ± 5.34	29.7 ± 1.37	83.6 ± 3.92
Basal Capsaicin	83.8 ± 2.58	45.6 ± 1.79	38.2 ± 2.50	124.1 ± 11.6	91.3 ± 2.67	32.8 ± 2.09	92.9 ± 4.33
Basal Curcumin + Capsaicin	76.4 ± 1.64**	37.1 ± 1.21**	39.3 ± 1.21	104.2 ± 6.38	76.8 ± 2.34	27.4 ± 2.08	87.5 ± 3.43
HFD Control	71.7 ± 1.44	38.0 ± 1.13	33.7 ± 1.15	179.2 ± 6.85	120.2 ± 8.22	59.0 ± 3.32	100.0 ± 1.31
HFD Curcumin	62.8 ± 1.54**	27.0 ± 2.44**	35.8 ± 0.95	143.3 ± 6.20**	81.1 ± 6.02**	62.2 ± 1.14	110.9 ± 6.34
HFD Capsaicin	55.0 ± 0.94**	21.4 ± 2.33**	33.6 ± 1.77	154.7 ± 3.58**	94.3 ± 7.39**	60.4 ± 1.65	106.4 ± 2.53
HFD Curcumin + Capsaicin	56.9 ± 1.52**	25.7 ± 1.38**	31.2 ± 0.77	157.8 ± 5.00**	88.7 ± 5.10**	69.0 ± 2.73	110.3 ± 4.35

Values expressed as mg/dl are mean ± SEM of 8 rats in each group.

**Significant decrease compared to corresponding control.

about by dietary curcumin, capsaicin and their combination was 29, 44 and 32 % respectively. Thus, the hypocholesterolemic effect was more in the case of dietary capsaicin compared to that of dietary curcumin at the dietary levels examined. HDL-cholesterol was not influenced by any of the dietary spices. While dietary curcumin and dietary capsaicin individually did not influence serum cholesterol concentration in normal rats, a combination of the two spice principles did produce a slight but significant reduction in the same. Reductions in total cholesterol and LDL-cholesterol concentrations observed in normal rats under dietary curcumin + capsaicin were 13 and 26 % respectively.

Serum phospholipids concentration was not influenced by dietary spice principles either individually or in combination both in normal rats and in high fat fed rats (Table-1). Serum phospholipids concentration was 30 % higher as a result of high fat feeding.

Liver lipid profile

Influence of dietary curcumin, capsaicin and their combination on hepatic lipid profile in normal and high fat fed rats is given in Table-2. Cholesterol concentration in liver was significantly lower as a result of high fat feeding (5.03 mg/g vs. 7.93 mg/g). Hepatic cholesterol content was significantly lowered in normal rats as a result of dietary spice principles. The reductions in liver cholesterol content brought about by dietary curcumin, capsaicin and curcumin + capsaicin were 31, 40 and 27 % respectively. Hepatic cholesterol was unaffected by dietary spice principles in high fat fed animals.

Table-2 Influence of dietary curcumin and capsaicin on hepatic lipid profile

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Animal group	Total lipids	Cholesterol	Triglycerides	Phospholipids
Basal Control	65.7 ± 1.99	7.93 ± 0.51	10.9 ± 0.48	26.7 ± 0.73
Basal Curcumin	51.6 ± 3.58**	5.47 ± 0.38**	5.58 ± 0.54**	26.1 ± 0.76
Basal Capsaicin	53.1 ± 2.55**	4.76 ± 0.49**	6.65 ± 0.38**	24.2 ± 0.76
Basal Curcumin + Capsaicin	54.2 ± 1.97**	5.75 ± 0.33**	5.84 ± 0.77**	27.9 ± 0.50
HFD Control	74.4 ± 3.81	5.03 ± 0.42	28.8 ± 1.61	28.3 ± 1.36
HFD Curcumin	80.8 ± 3.25	5.45 ± 0.45	17.9 ± 0.44**	31.4 ± 1.12
HFD Capsaicin	77.0 ± 1.70	4.67 ± 0.30	23.0 ± 1.39**	27.7 ± 0.82
HFD Curcumin + Capsaicin	62.0 ± 1.00**	4.23 ± 0.20	12.1 ± 1.95**	27.6 ± 1.33

Values expressed as mg/g liver are mean ± SEM of 8 rats in each group.

**Significant decrease compared to corresponding control.

High fat feeding resulted in a much elevated liver triglyceride concentration; which was nearly 3-fold that found in normal rats (Table-2). Hepatic triglyceride concentration was significantly lowered in all the three experimental diet groups both in normal and in high fat fed animals (Table-2). The reductions in hepatic triglyceride brought about dietary curcumin, capsaicin and curcumin + capsaicin were 49, 39 and 46% respectively in normal rats. Similarly, reductions of 38, 20 and 58% were brought about by these respective spice principles in high fat fed animals. Hepatic phospholipids concentration was not affected in high fat feeding (Table-2). None of the dietary spice principles had any influence on hepatic phospholipids content either in normal or in high fat fed animals.

As a result of reductions brought about in triglyceride and cholesterol contents by dietary spice principles, hepatic total lipid content was significantly lower in these diet groups of normal animals (Table-2). Decreases in hepatic total lipid content observed in these groups were 21, 19 and 18% respectively. In the high fat fed animals, only the curcumin + capsaicin combination treatment produced a significant reduction in liver total lipid content, the reduction being 17% compared to the respective control.

Antioxidant molecules in serum

Influence of dietary curcumin, capsaicin and their combination on antioxidant molecules and lipid peroxides in serum is presented in Table-3. Serum lipid peroxides were not influenced by any of these experimental diets either in normal or in high fat fed animals. Serum α-tocopherol content was increased by dietary curcumin, capsaicin and their combination in high fat fed rats, the extent of increase being 16, 20 and 161% in the

respective groups. Thus, the combination of curcumin and capsaicin produced a greater effect on serum α-tocopherol than the two individual spice principles. α-Tocopherol content in serum was significantly lower in high fat fed animals as compared to their normal counterparts (130.5 μmole / dl in HFD-Control vs. 231.3 μmole / dl in Normal-Control). Dietary spice principles had a negative effect on serum α-tocopherol concentration in the normal animals. The extent of decrease was 23, 29 and 36 % in curcumin, capsaicin and curcumin + capsaicin groups.

Serum ascorbic acid content was significantly elevated by dietary curcumin + capsaicin in normal rats (by 15 %), whereas the individual spice principles did not have any effect (Table-3). The spice principles did not have any effect on serum ascorbic acid in high fat fed animals either. Ascorbic acid content in serum was higher by 34 % in high fat fed animals as compared to their normal counter parts. Serum glutathione concentration was beneficially increased only by dietary capsaicin in the high fat fed animals, the increase being 32.5 %. Serum content of total thiols was increased by dietary curcumin and capsaicin by 37 and 39 % in normal rats. Serum total thiol content was elevated by the combination of curcumin and capsaicin in high fat fed animals (by 18 %). Glutathione and total thiol concentrations in high fat fed animals were comparable to the same in basal diet fed animals.

Table-3 Influence of dietary curcumin and capsaicin on serum antioxidant molecules and lipid peroxides

Animal group	Total thiols (mmole/dl)	Glutathione (mg/dl)	Ascorbic acid (mg/dl)	α-Tocopherol (nmole/dl)	Lipid peroxides (μmole/dl)
Basal Control	14.22 ± 0.63	0.539 ± 0.010	0.365 ± 0.010	23.1 ± 1.4.5	36.4 ± 1.13
Basal Curcumin	19.52 ± 1.39*	0.535 ± 0.014	0.376 ± 0.011	17.7 ± 0.52**	39.1 ± 1.63
Basal Capsaicin	19.82 ± 1.32*	0.571 ± 0.018	0.361 ± 0.029	16.5 ± 0.67**	41.3 ± 2.52
Basal Curcumin + Capsaicin	13.49 ± 0.24	0.565 ± 0.016	0.421 ± 0.010*	14.9 ± 0.56**	40.6 ± 3.70
HFD Control	15.62 ± 0.48	0.574 ± 0.012	0.489 ± 0.017	13.1 ± 0.21	36.2 ± 1.52
HFD Curcumin	16.29 ± 0.27	0.558 ± 0.016	0.485 ± 0.020	15.1 ± 0.39*	41.3 ± 2.47
HFD Capsaicin	16.12 ± 0.47	0.761 ± 0.063*	0.483 ± 0.014	15.6.± 0.62*	39.6 ± 1.85
HFD Curcumin + Capsaicin	18.49 ± 0.90*	0.582 ± 0.027	0.475 ± 0.015	34.1 ± 0.13*	35.5 ± 1.20

Values are expressed as mean ± SEM of 8 rats in each group.

* Significant increase compared to corresponding control.   **Significant decrease compared to corresponding control

Antioxidant molecules in liver

Influence of dietary curcumin, capsaicin and their combination on hepatic antioxidant molecules and lipid peroxides is presented in Table-4. While total thiol content remained unaffected by dietary spice principles in normal rats, the same was considerably lower in high fat fed animals as a result of feeding capsaicin or curcumin + capsaicin. The reduction in hepatic total thiol was 52 and 50 % respectively in these two diet groups. Glutathione concentration in liver was increased by feeding curcumin, capsaicin or curcumin + capsaicin in normal animals, the extent of increase being 15, 24 and 12% respectively. Hepatic glutathione was similarly increased by dietary curcumin and dietary curcumin + capsaicin in high fat fed animals by 22 and 35 % respectively.

Dietary spice principles did not influence liver ascorbic acid concentration either in normal or in high fat fed animals (Table-4). Hepatic α-tocopherol concentration was significantly reduced by dietary curcumin, capsaicin or curcumin + capsaicin in normal rats, the reductions being 51, 61 and 60 % respectively. On the other hand, in high fat fed animals, the same was significantly increased by dietary curcumin and dietary curcumin + capsaicin. The increase in liver α-tocopherol concentration produced in high fat fed animals by dietary curcumin was as much as 88 %, while dietary curcumin + capsaicin produced an increase of 33 %. High fat feeding by itself increased hepatic α-tocopherol concentration enormously. This increase was around 4-fold, being 1.195 mg/g liver in High fat control versus 0.314 mg/g liver in Normal control.

Table-4 Influence of dietary curcumin and capsaicin on liver antioxidant molecules and lipid peroxides

Animal group	Total thiols (mmole/mg protein)	Glutathione (µg/mg protein)	Ascorbic acid (µg/mg protein)	α-Tocopherol (mg/g liver)	Lipid peroxides (nmole/mg protein)
Basal Control	0.573 ± 0.020	1.044 ± 0.061	0.377 ± 0.030	0.314 ± 0.005	3.156 ± 0.072
Basal Curcumin	0.618 ± 0.010	1.198 ± 0.084*	0.325 ± 0.024	0.154 ± 0.013**	2.328 ± 0.136**
Basal Capsaicin	0.547 ± 0.030	1.290 ± 0.059*	0.347 ± 0.024	0.123 ± 0.011**	2.704 ± 0.079**
Basal Curcumin + Capsaicin	0.597 ± 0.016	1.174 ± 0.020*	0.362 ± 0.011	0.125 ± 0.008**	2.763 ± 0.067**
HFD Control	1.095 ± 0.042	0.926 ± 0.017	0.425 ± 0.011	1.195 ± 0.041	2.360 ± 0.060
HFD Curcumin	1.072 ± 0.058	1.126 ± 0.038*	0.420 ± 0.009	2.249 ± 0.146*	1.823 ± 0.061**
HFD Capsaicin	0.528 ± 0.022**	0.933 ± 0.037	0.383 ± 0.013	1.086 ± 0.053	2.331 ± 0.070
HFD Curcumin + Capsaicin	0.550 ± 0.013**	1.254 ± 0.122*	0.419 ± 0.014	1.595 ± 0.122*	2.054 ± 0.055**

Values are expressed as mean ± SEM of 8 rats in each group.
* Significant increase compared to corresponding control.
**Significant decrease compared to corresponding control.

As a result of beneficial enhancing influence on hepatic glutathione and α-tocopherol concentrations, dietary curcumin and dietary curcumin + capsaicin also reduced lipid peroxide level in high fat fed animals. The decrease in the concentration of hepatic lipid peroxides in these two groups was 23 and 13 % respectively. Hepatic lipid peroxide content was also lower in normal rats as a result of dietary spice principles (Table-4). The reduction in hepatic lipid peroxides produced by dietary curcumin, capsaicin and their combination was 26, 14 and 12 % respectively.

Antioxidant enzymes in serum

Activities of antioxidant enzymes – glutathione reductase, glutathione peroxidase, glutathione transferase and catalase in serum of normal and high fat fed animals as influenced by dietary curcumin, capsaicin and their combination are presented in Table-5. Serum glutathione reductase activity was not affected by any of the spice principles treatment both in normal and in high fat fed rats. The activity of serum glutathione peroxidase in high fat fed rats was generally higher as a result of dietary curcumin, capsaicin and curcumin + capsaicin. The increase in this enzyme activity was 21, 25 and 14 % in the respective groups. The same spice principles did not have any similar effect on serum glutathione peroxidase activity in normal rats.

Table-5 Influence of dietary curcumin and capsaicin on serum antioxidant enzymes

Animal group	GSH Reductase (mmole/ min/ dl)	GSH peroxidase (mmole/ min/ dl)	GSH transferase (mmole/ min/ ml)	Catalase (mmole/ min/ dl)
Basal Control	3.37 ± 0.15	80.4 ± 1.75	2.78 ± 0.09	15.12 ± 0.42
Basal Curcumin	3.49 ± 0.06	76.1 ± 3.24	3.41 ± 0.15*	10.20 ± 0.75**
Basal Capsaicin	3.42 ± 0.12	75.2 ± 2.77	3.24 ± 0.11*	12.58 ± 0.48**
Basal Curcumin + Capsaicin	3.20 ± 0.10	83.6 ± 3.34	3.13 ± 0.10*	12.95 ± 0.39**
HFD Control	2.95 ± 0.14	82.1 ± 1.33	2.32 ± 0.17	8.87 ± 0.51
HFD Curcumin	3.10 ± 0.06	99.7 ± 1.97*	2.94 ± 0.20*	7.63 ± 0.49
HFD Capsaicin	2.74 ± 0.12	102.9 ± 4.65*	2.49 ± 0.21	9.95 ± 0.82
HFD Curcumin + Capsaicin	2.79 ± 0.18	93.2 ± 1.57*	3.36 ± 0.29*	13.6 ± 1.11*

Values are expressed as mean ± SEM of 8 rats in each group.

* Significant increase compared to corresponding control.
**Significant decrease compared to corresponding control.

Serum glutathione transferase activity was generally enhanced by dietary spice principles both in normal and high fat fed animals (Table-5). The increases in the enzyme activity produced in normal rats by dietary curcumin, capsaicin and curcumin + capsaicin were 23, 17 and 13 % respectively. The increases in the enzyme activity produced in high fat fed rats by dietary curcumin and curcumin + capsaicin were 27 and 45 % respectively.

Serum catalase activity was negatively influenced by dietary curcumin, capsaicin and their combination in normal animals, where the activity was 33, 17 and 14 % lesser respectively when compared to normal control group (Table-5). Serum catalase activity was higher in high fat fed animals under dietary curcumin + capsaicin, where the activity was 53 % higher compared to High fat diet control.

Antioxidant enzymes in liver

Activities of antioxidant enzymes - glutathione reductase, glutathione peroxidase, glutathione transferase, catalase and superoxide dismutase in the liver of normal and high fat fed animals as influenced by dietary curcumin, capsaicin and their combination are presented in Table-6. Activity of hepatic glutathione reductase was significantly elevated by dietary curcumin, capsaicin and their combination in high fat fed animals. The extent of increase in the enzyme activity was 67, 32 and 58 % in the respective diet groups. The same spice compounds did not have any effect on hepatic glutathione reductase activity in normal rats.

Table-6 Influence of dietary curcumin and capsaicin on liver antioxidant enzymes

Animal group	GSH Reductase (μmole/ min/ mg protein)	GSH peroxidase (mmole/ min/ mg protein)	GSH transferase (mmole/ min/ mg protein)	Catalase (mmole/ min/ mg protein)	Superoxide dismutase (Units/min/ mg protein)
Basal Control	36.8 ± 1.21	0.204 ± 0.006	0.446 ± 0.013	16.02 ± 1.08	46.4 ± 3.51
Basal Curcumin	38.7 ± 1.82	0.244 ± 0.006*	0.406 ± 0.018	17.46 ± 0.96	39.6 ± 2.88
Basal Capsaicin	36.7 ± 1.13	0.278 ± 0.013*	0.398 ± 0.025	14.10 ± 0.96	39.1 ± 1.46
Basal Curcumin + Capsaicin	37.8 ± 2.28	0.214 ± 0.007	0.425 ± 0.014	17.16 ± 1.14	40.9 ± 4.03
HFD Control	27.8 ± 1.62	0.189 ± 0.007	0.406 ± 0.013	14.40 ± 0.66	58.3 ± 2.46
HFD Curcumin	46.5 ± 2.00*	0.209 ± 0.005*	0.439 ± 0.014	13.20 ± 0.72	50.3 ± 1.38**
HFD Capsaicin	36.8 ± 1.85*	0.222 ± 0.008*	0.410 ± 0.014	13.32 ± 0.66	46.1 ± 1.11**
HFD Curcumin + Capsaicin	44.0 ± 2.12*	0.195 ± 0.007	0.399 ± 0.015	13.56 ± 0.96	37.8 ± 0.82**

Values are expressed as mean ± SEM of 8 rats in each group.

* Significant increase compared to corresponding control.
**Significant decrease compared to corresponding control.

Activity of hepatic glutathione peroxidase was higher in dietary curcumin and dietary capsaicin groups of both normal and high fat fed animals (Table-6). The increase in the enzyme activity was 20 and 36 % by these two spice groups in normal rats, while the increase was 11 and 17 % in the respective groups of high fat fed animals. Activities of glutathione transferase and catalase in liver were not influenced by dietary curcumin, capsaicin or their combination either in normal or in high fat fed animals. Hepatic superoxide dismutase activity was higher as a result of high fat treatment (58.3 units / mg protein in HFD control as against 46.4 units / mg protein in normal control). While these spice principles did not influence the activity of hepatic superoxide dismutase in normal rats, dietary curcumin, capsaicin and dietary curcumin + capsaicin lowered the hepatic superoxide dismutase activity by 14, 21 and 35 % respectively.

Table-7 Influence of dietary curcumin and capsaicin on body weight gain and liver weight in hypertriglycerdemic rats.

Group	0 - 15 days	16 - 30 days	31 - 45 days	46 - 60 days	60 days
Basal Control	11.6± 0.34	14.1± 0.97	13.4 ± 0.51	13.8 ± 0.29	794
Basal Curcumin	11.1± 0.23	12.5 ± 0.30	12.6 ± 0.51	12.2 ± 0.28	726
Basal Capsaicin	11.4 ± 1.08	13.8 ± 0.19	12.6 ± 0.32	13.2 ± 1.05	765
Basal Curcumin + Capsaicin	10.4 ± 0.52	12.8 ± 0.18	12.4 ± 0.31	12.8 ± 0.29	726
HFD Control	8.72 ± 0.31	10.0 ± 0.13	11.4 ± 0.21	11.6 ± 0.31	626
HFD Curcumin	8.32 ± 0.44	10.5 ± 0.39	10.3 ± 0.18	10.6 ± 0.21	596
HFD Capsaicin	9.12 ± 0.37	10.3 ± 0.13	11.2 ± 0.25	11.6 ± 0.30	633
HFD Curcumin + Capsaicin	9.19 ± 0.32	10.1 ± 0.16	10.8 ± 0.37	11.2 ± 0.16	620

Values are indicated in terms of g / d / Rat

Table-8 Influence of dietary curcumin and capsaicin on body weight gain and liver weight in hypertriglycerdemic rats.

	Body weight (g)			Liver weight	
	Initial	Final	Gain	(g)	(g/100 g body)
Basal Control	104.9 ± 3.89	258.4 ± 5.57	153.5 ± 4.81	7.34 ± 0.66	2.84 ± 0.25
Basal Curcumin	102.9 ± 2.64	247.1 ± 5.44	144.2 ± 3.17	7.39 ± 0.25	2.99 ± 0.10
Basal Capsaicin	102.8 ± 2.37	240.9 ± 11.6	138.1 ± 6.20	6.90 ± 0.39	2.98 ± 0.16
Basal Curcumin + Capsaicin	102.4 ± 2.06	242.9 ± 11.5	140.5 ± 6.86	6.98 ± 0.30	2.87 ± 0.12
HFD Control	102.1 ± 1.59	272.6 ± 10.0	170.5 ± 7.96	8.79 ± 0.22	3.22 ± 0.08
HFD Curcumin	102.1 ± 1.45	271.0 ± 9.69	168.9 ± 12.2	8.54 ± 0.33	3.15 ± 0.12
HFD Capsaicin	101.9 ± 1.39	269.1 ± 10.3	167.2 ± 8.67	8.94 ± 0.45	3.32 ± 0.16
HFD Curcumin + Capsaicin	101.6 ± 1.30	268.3 ± 7.10	166.7 ± 5.74	9.30 ± 0.24	3.46 ± 0.09

Values are ± SEM of 8 animals in each group.

Discussion

In the present study, spice active principles have been fed to animals at levels corresponding to about 10 times the average dietary intake of the corresponding spices among Indian population [Thimmayamma *et al*, 1983]. The food intake was essentially similar in various spice principles fed groups and corresponding control group. Similarly the gain in body weights during the 8 weeks spice principle treatment was comparable to corresponding controls [Final weight (g) ranged from 242.9 ± 5.0 to 258.4 ± 5.6 in basal groups; and from 268.4 ± 5.1 to 272.6 ± 6.1 in high fat fed groups]. Thus, dietary spices have not negatively affected the feed intake.

The present animal study has evidenced that dietary curcumin, capsaicin or their combination significantly countered the extent of hypertriglyceridemia brought about by high fat feeding, and the effect was 12 - 20%. The hypotriglyceridemic effect of dietary curcumin was more than that of capsaicin at the dietary levels examined. The combination of the two spice principles – curcumin and capsaicin did not have any additive effect on blood triglyceride level. The serum triglyceride was not influenced by these spice principles in normal animals. Dietary curcumin, capsaicin and their combination produced slight decrease in serum total cholesterol concentration in these high fat fed animals. While dietary curcumin and dietary capsaicin individually did not influence serum cholesterol concentration in normal rats, a combination of the two spice principles did produce a slight but significant reduction in the same. High fat feeding resulted in a much elevated liver triglyceride concentration; which was nearly 3-fold that found in normal rats. Hepatic triglyceride concentration was significantly lowered in all

the three experimental diet groups both in normal and in high fat fed animals. The combination of curcumin and capsaicin produced an additive effect in high fat fed animals, in lowering the liver triglyceride concentration. Hepatic cholesterol content was significantly lowered in normal rats as a result of dietary spice principles while the same was unaffected in high fat fed animals. Dietary capsaicin produced higher effect on hepatic cholesterol content than dietary curcumin, while dietary curcumin produced a higher effect on hepatic triglyceride content than dietary capsaicin.

The antihypertriglyceridemic efficacy of dietary curcumin and capsaicin has been evidenced recently in rats fed an atherogenic high fat diet, and such an influence also resulted in countering of the changes in membrane lipid profile in the erythrocytes [Kempaiah & Srinivasan, 2006]. Hypocholesterolemic and hypotriglyceridemic action of dietary curcumin (0.5%) has been evidenced in our laboratory in streptozotocin-induced diabetic rats [Babu & Srinivasan, 1997]. As a result of this beneficial hypo-cholesterolemic influence, dietary curcumin was also found to ameliorate diabetic renal lesion [Babu & Srinivasan, 1998]. In streptozotocin-induced diabetic situation which was featured by hypertriglyceridemia and hypercholesterolemia, it was observed that dietary capsaicin did not show any beneficial hypolipidemic property [Babu & Srinivasan, 1997a].

Sambaiah et al (1978) have also observed that in rats on a high hydrogenated fat (40%) diet, 5% red pepper or equivalent level of capsaicin (15 mg%) showed a tendency to lower serum and liver cholesterol levels. In another investigation (Srinivasan et al, 1980), reduction in serum total cholesterol levels was reported in rats on a 10% peanut oil

diet incorporated with 15 mg% capsaicin.   In yet another study, Srinivasan and Satyanarayana (1987) observed that capsaicin at as low as 0.2 mg% in the diet led to a lowering of serum total cholesterol in both 10 and 30% fat fed rats. In another study (Monsereenusorn, 1983), administration of 50 mg /kg body wt /day of capsaicin by stomach tube to rats for 60 days produced no significant change in plasma total cholesterol at 10 and 20 days, but at 30, 40, 50 and 60 days, the cholesterol levels were significantly reduced along with triglycerides and phospholipids. Kawada *et al* (1986) did not observe any cholesterol-lowering effect of 14 mg% dietary capsaicin in a diet containing 30% lard for 10 days.

Serum α-tocopherol content was increased by dietary curcumin, capsaicin and their combination in high fat fed rats. The combination of curcumin and capsaicin produced a greater effect on serum α-tocopherol than the two individual spice principles. Serum glutathione concentration was beneficially increased only by dietary capsaicin in the high fat fed animals. Serum total thiol content was elevated by the combination of curcumin and capsaicin in high fat fed animals. Serum ascorbic acid content was significantly elevated by dietary curcumin + capsaicin in normal rats. Glutathione concentration in liver was increased by feeding curcumin, capsaicin or curcumin + capsaicin in normal animals, while the same was similarly increased by dietary curcumin and dietary curcumin + capsaicin in high fat fed animals. Hepatic α-tocopherol concentration was significantly increased by dietary curcumin and dietary curcumin + capsaicin in high fat fed animals.   High fat feeding by itself increased hepatic α-tocopherol concentration as much as 4-fold. As a result of beneficial enhancing influence on hepatic glutathione and

α-tocopherol concentrations, dietary curcumin and dietary curcumin + capsaicin also reduced lipid peroxide level in high fat fed animals. Hepatic lipid peroxide content was also lower in normal rats as a result of dietary spice principles. A similar increase in hepatic glutathione content and decrease in lipid peroxides, with no change in hepatic ascorbic acid concentration as evidenced here has been reported earlier in rats fed basal 10% fat diet containing 1% curcumin [Reddy & Lokesh, 1994a].

Levels of antioxidant enzymes, which constitute the major defense against reactive oxygen species, play a significant role in controlling lipid peroxidation [Allen & Venkataraj, 1992]. The activity of serum glutathione peroxidase in high fat fed rats was generally higher as a result of dietary curcumin, capsaicin and curcumin + capsaicin. Glutathione transferases are a group of enzymes capable of conjugating glutathione with diverse electrophilic compounds [Uysal *et al*, 1988]. Serum glutathione transferase activity was generally enhanced by dietary spice principles both in normal and high fat fed animals. Activity of hepatic glutathione reductase was significantly elevated by dietary curcumin, capsaicin and their combination in high fat fed animals. Activity of hepatic glutathione peroxidase was higher in dietary curcumin and dietary capsaicin groups of both normal and high fat fed animals. A similar increase in hepatic glutathione peroxidase has been reported in rats fed basal 10% fat diet containing 1% curcumin [Reddy & Lokesh, 1994a]. Curcumin which was fed at 0.2% level in the diet in the current study along with normal 10% fat diet did not elicit a similar effect on these hepatic antioxidant enzymes. Earlier studies have shown that curcumin causes an increase in glutathione transferase activity in rodent liver which may contribute to its anti-cancer and anti-inflammatory activities [Piper *et al*, 1998]. Dietary fat affects the susceptibility

of lipids to peroxidation and potential oxidative damage to cells. This suggests that the use of these spices at higher levels may offer beneficial influences on the antioxidant status. The induction of enzymes involved in the detoxification of the electrophilic products of lipid peroxidation may contribute to the anti-inflammatory activity of curcumin and capsaicin and to the anti-cancer activity of curcumin.

There are several reports on the antioxidant effects of curcumin and capsaicin with regard to modulation of lipid peroxide level and antioxidant status. Curcumin has been reported to exert a protective effect against nicotine-induced lung toxicity by modulating the extent of lipid peroxidation and augmenting antioxidant defense system [Kalpana & Menon, 2004]. The enhanced circulatory lipid peroxides in nicotine-treated rats which was accompanied by a significant decrease in the levels of ascorbic acid, vitamin E, reduced glutathione, glutathione peroxidase, superoxide dismutase, and catalase was significantly countered by the administration of curcumin ((80 mg/kg given simultaneously by intragastric intubation for 22 weeks) which significantly lowered the lipid peroxidation and enhanced the antioxidant status. Dietary supplementation of curcumin (2%) to male mice for 30 days has been reported to have significantly increased the activities of glutathione peroxidase, glutathione reductase and catalase in liver [Iqbal *et al.*, 2003]. Moderately increased activities of glutathione transferase, superoxide dismutase and catalase in the liver of 1% curcumin fed along with normal 10% fat diet has been reported {Reddy & Lokesh, 2004a]. Curcumin has been reported to have a protective role in alcohol and δ-PUFA induced oxidative stress in male albino Wistar rats [Rukkumani *et al.*, 2004]. The liver thiobarbituric acid reactive substances and antioxidants such as ascorbic acid, α-tocopherol, reduced glutathione, superoxide

dismutase, catalase, and glutathione peroxidase were increased significantly in alcohol and δ-PUFA groups. Administration of curcumin abrogated this effect by effectively modulating the antioxidant status. *In vivo* antioxidative effects of curcumin have been reported in trichloroethylene-induced oxidative stress in mouse liver [Watanabe & Fukui, 2000]. Increases in the contents of peroxisome and thiobarbituric acid reactive substances and decreases in glutathione content of mouse liver by the trichloroethylene administration were suppressed by the pre-administration of curcumin. Iron induced liver lipid peroxidation was 29% lower in turmeric fed (1% in diet) male Wistar rats [Reddy & Lokesh, 1994]. The activities of superoxide dismutase, catalase and glutathione peroxidase were higher in liver homogenates of rats fed the turmeric-containing diet in comparison with the controls, suggesting that dietary turmeric lowers lipid peroxidation by enhancing the activities of antioxidant enzymes. Male Wistar rats administered *i.p.* with 3 mg/kg body weight capsaicin for three consecutive days showed a reduction of oxidative stress measured as malondialdehyde in the liver and other tissues suggesting that capsaicin can be a potent antioxidant [Lee *et al*., 2003].

Thus, the present study which examined the effect of feeding hypolipidemic spice principles – curcumin, capsaicin or their combination along with a high fat diet on lipid peroxides, antioxidant molecules and antioxidant enzymes in blood and hepatic tissue has shown that a high fat diet compromises the endogenous antioxidant defense mechanisms. The dietary spice principles, which brought about significant hypotriglyceridemic influence, were also found to effectively reduce this oxidant stress in hyperlipidemic animals to a significant extent as indicated by countering of the depleted antioxidants and antioxidant enzymes and reducing the lipid peroxide content in liver. No particular

additive effect of the two compounds was evidenced in general when given together with respect to hypolipidemic or the antioxidant potential.

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Protective effect of dietary curcumin and capsaicin on iron-induced hepatotoxicity and carrageenan-induced inflammation in experimental rats

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Summary

An animal study was carried out to examine the beneficial influence of dietary curcumin, capsaicin and their combination on iron induced hepatotoxicity. Groups of rats injected with 30 mg/kg Fe^{2+}/kg body weight showed hepatic toxicity as measured by an increase in lipid peroxides which correlated with elevated serum enzymes, alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase. Dietary curcumin, capsaicin and their combination reduced the activities of these serum enzymes, as well lowered the liver and serum lipid peroxide level indicating that these spice principles reduce the severity of iron induced hepatotoxicity by lowering lipid peroxidation. In a separate study, a comparison of the extent of carrageenan-induced paw inflammation in spice principles fed animals showed that both dietary curcumin and capsaicin moderately lowered inflammation, while the spice principles in combination were more effective in countering the extent of paw inflammation. Dietary curcumin and capsaicin significantly decreased the activity of 5'-lipoxygenase activity in the PMNL cells in carrageenan-injected rats. The decrease in the enzyme activity was even higher in the case of combination of these two spice principles. Results suggest that dietary curcumin and capsaicin individually are protective to iron-induced hepatotoxicity and to carrageenan-induced inflammation. This beneficial effect was higher when the two compounds were fed in combination.

Introduction

Oxidative damage at the cellular and subcellular level is now considered to be an important event in disease processes like cardiovascular disease, inflammatory disease, carcinogenesis and aging. The antioxidant properties of several spice principles have been evidenced in rats both *in vivo* and *in vitro*. While curcumin (turmeric), capsaicin (red pepper) and eugenol (clove) were found to be more effective antioxidants, piperine (black pepper), zingerone (ginger), linalool (coriander) and cuminaldehyde (cumin) were only marginally inhibitory to lipid peroxidation (Reddy & Lokesh, 1992). These compounds inhibited lipid peroxidation by quenching oxygen free radicals (Reddy & Lokesh, 1994) and by enhancing the activity of endogenous antioxidant enzymes - superoxide dismutase, catalase, glutathione peroxidase and glutathione transferase (Reddy & Lokesh, 1994a). Dietary spice principles curcumin, capsaicin and garlic were found to be antioxidative by enhancing the antioxidant molecules and antioxidant enzymes in erythrocytes and liver of hyperlipidemic / hypercholesterolemic rats (Kempaiah & Srinivasan, 2004, 2004a).

The toxic effects of iron overloading leads to chronic liver disease, impaired cardiac function, endocrinopathies, skin pigmentation and orthropathy (Niederau *et al*, 1985; Stremmel *et al*, 1985). Hepatotoxicity is the most common finding in patients with iron overloading. The massive deposition of iron in hepatic parenchymal cells eventually produces fibrosis and ultimately results in cirrhosis (Weintraub *et al*, 1985). Spice principles – curcumin and capsaicin can effectively inhibit lipid peroxidation in rat liver by enhancing the antioxidant enzyme activities (As reported in previous chapter).

Curcumin has been shown to scavenge the reactive oxygen species and also prevent the oxidation of ferrous iron by hydrogen peroxide in the Fenton reactions (Reddy & Lokesh, 1994), which generates hydroxyl radicals involved in the initiation of lipid peroxidation (Girrotti & Thomas, 1984). Hence it would be relevant to examine if these two antioxidant spice principles could also have a protective role in iron induced hepatotoxicity.

Spice principles – curcumin (of turmeric), capsaicin (of red pepper) and eugenol (of cloves) have been understood to possess health beneficial anti-inflammatory property (Reddy & Lokesh, 1994; Joe & Lokesh, 1997). Curcumin and the volatile oil from turmeric are shown to reduce the edema in rats (Chandra & Gupta, 1972), and to moderately reduce the clinical symptoms in rheumatoid arthritis patients (Deodhar *et al*, 1980). Curcumin inhibits the formation of proinflammatory compounds like prostaglandins and leukotrienes (Huang *et al*, 1991). Dietary curcumin and capsaicin have been shown to lower the generation of proinflammatory mediators such as reactive oxygen species and nitric oxide released by macrophages (Joe & Lokesh, 1994).

The current study examines the beneficial antioxidant influence, if any, of dietary curcumin, capsaicin, and their combination in terms of protecting the damage caused to liver by iron overloading measured in terms of lipid peroxidation and elevation of plasma non-specific enzymes indicative of liver injury. The present study also investigates the anti-inflammatory property of curcumin and capsaicin when fed in combination on carrageenan-induced inflammatory responses in rats.

Materials and Methods

Curcumin, the yellow principle of turmeric (*Curcuma longa*) and capsaicin, the pungent principle of red pepper (*Capsicum frutescens*) was procured from M/s Fluka Chemie, Switzerland. Thiobarbituric acid was purchased from Sigma Chemical Company, St.Louis, USA. Ferrous sulfate ($FeSO_4.7H_2O$) was obtained from Qualigen Fine Chemicals Ltd., Mumbai, India. Other chemicals used were of analytical grade.

The animal experiments were carried out with due approval from the Institutional Animal Ethic Committee. Appropriate measures were taken to minimize pain or discomfort to the experimental animals and these experiments were carried out in accordance with the guidelines laid down by the NIH in the USA regarding the care and use of animals for experimental procedures.

(i) Protective effect of dietary curcumin, capsaicin and their combination on iron-induced hepatotoxicity

Male Wistar rats weighing 100 - 105 g (8 animals per group) housed in individual stainless steel cages were maintained on various experimental diets viz., 0.2% curcumin / 0.015% capsaicin / 0.2% curcumin + 0.015% capsaicin *ad libitum* for 8 weeks. The animals had free access to water. The basal diet consisted of (%): casein, 21; cane sugar, 10; corn starch, 54; NRC vitamin mixture, 1; Bernhart-Tommarelli modified NRC salt mixture, 4 and refined peanut oil, 10. The spice principles were incorporated into the Basal diet, replacing an equivalent amount of corn starch. At the end of the feeding period, the rats were starved for 16 h. Ferrous iron was injected i.p. (30 mg per kg body

weight as solution in saline) 1hr prior to sacrifice. The animals were sacrificed by cardiac puncture after anaesthetizing lightly with diethyl ether. The serum was separated by centrifuging blood and was used for analysis of lipid peroxides and activities of various plasma non-specific enzymes. The livers were perfused with saline and homogenized in 10 volumes of 0.15M KCl.

Lipid peroxides

Lipid peroxides in liver homogenates were measured as thiobarbituric acid reactive substances (TBARS) by the method described by Buege and Aust (1978). An aliquot of tissue homogenate in 1.54 mM potassium chloride solution was mixed with an equal volume of 8% sodium lauryl sulfate in a test tube. 1.5 ml of 20% acetic acid (pH 3.5) was added and mixed well. 2.0 ml of 8% aqueous thiobarbituric acid was also added, mixed well and boiled for 1 h, cooled. 5.0 ml *n*-butanol was added and mixed well; centrifuged at 3000 rpm for 10 min. Absorbance of butanol extract was measured at 532 nm. Values were compared with similarly treated 1,1,3,3-tetraethoxy-propane, used as standard. Serum lipid peroxides were determined fluorimetrically as described by Yagi (1984) using 1,1,3,3-tetraethoxy-propane as reference standard.

Serum enzymes

Plasma non-specific enzymes - Aspartate aminotransaminase (AsAT, EC.2.6.1.1) and alanine aminotransaminase (AlAT, EC.2.6.1.2) were determined by the colorimetric methods described by Bergmeyer and Bernt (1974; 1974a). Lactate dehydrogenase (LDH, EC.1.1.27) was assayed by the method of Kornberg (1974) following the rate of oxidation of NADH. Alkaline phosphatase and acid phosphatase activities in serum were

determined by the described by Walter and Schutt (1974) using *p*-nitrophenyl phosphate as the substrate.

Protein concentration of liver homogenate was measured according to Lowry's procedure using bovine serum albumin as reference standard (Lowry *et al*, 1951).

(ii) Protective effect of dietary curcumin, capsaicin and their combination on carrageenan induced inflammation.

To examine the post-local anti-inflammatory potential of the combination of spice principles - curcumin and capsaicin as compared to these individual compounds in rat models, groups of male Wistar rats (100 -110 g) were maintained *ad libitum* on semisynthetic diets containing 0.2% curcumin, 0.015% capsaicin and 0.2% curcumin + 0.015% capsaicin as described earlier for 10 weeks. At the end of the feeding period, inflammatory responses in the rats were followed by measuring the increase in the paw volume after injecting carrageenan (Singh & Mourya, 1972). Paw inflammation was induced by injecting χ-carrageenan (2.5 mg / kg body weight) as suspension in 200 µl sterile saline into the right hind paw under plantar aponeurosis. An equal volume of saline was similarly injected into the left hind paw of the same animals which served as parallel control. Extent of paw inflammation was measured by mercury displacement method (Otternest & Moore, 1988) at intervals of 1 hr up to 5 hr and at multiples of 5 hr thereafter. Simultaneously, the volume of the saline-injected left paw was also measured. After 20 hr of carrageenan injection, rats were sacrificed under light ether anesthesia. Blood was collected and centrifuged to obtain serum for further analysis.

5'-Lipoxygenase activity in PMNL cells.

Polymorphonuclear lymphocytes were isolated from rat blood collected in tubes containing 10% EDTA solution by centrifugation at 1800 rpm for 60 min using sterile Ficoll histoplaque gradient (1:1, v/v) as described by Boyum (1976). The middle opaque layer dense with PMNLs was taken in PBS for further purification and sonicated for 20-30 sec at 20 kHz to release the cytosolic 5'-lipoxygenase enzyme into solution. The suspension was centrifuged at 100,000 x g for 30 min at 4°C and the supernatant was used as source of lipoxygenase enzyme.

5'-Lipoxygenase was assayed according to the method of Aharony and Stein (1986). The reaction mixture for the assay contained 100 mM phosphate buffer, pH 7.4, 300 μM $CaCl_2$, 50 μM dithiotritol, 200 μM ATP, 150 μM arachidonic and the enzyme source. 5'-Lipoxygenase was measured as 5-HETE formed at 234 nm. The molar extinction coefficient of 28000 M^{-1} Cm^{-1} was used to calculate the activity of the enzyme. Lipoxygenase activity is expressed as μmol of HETE formed /min/ mg protein.

Histamine content of serum

Histamine content in serum was measured according to Siegel *et al* (1990) by reacting with *o*-phthalaldehyde. Proteins were precipitated by mixing serum with equal volume of 10% TCA followed by centrifugation. To 1 ml of the supernatant was added 300 mg of NaCl and 0.75 ml of butanol. The supernatant was made alkaline by the addition of 0.1 ml 10 M NaOH with simultaneous mixing. The mixture was vortexed for 1 min with intermittent vigorous shaking, and 0.5 ml of the butanol was recovered following

centrifugation at 1000 x g for 5 min. A Second 0.5 ml of butanol was added and the process repeated. Butanol extracts were pooled (1.0 ml) and placed in a tube containing 1.9 ml of heptane and 0.85 ml 0.12 HCl. This mixture was vortexed for 1 min and 0.75 ml of the aqueous phase containing histamine was recovered after centrifugation and stored at 4°C until derivatization. The histamine extract (0.5 ml) was placed in an ice bath, 0.09 ml of a 0.05 % o-phthalaldehyde in methanol and 0.3 ml of 0.75 M NaOH were added. After 40 min incubation, the reaction was stopped by the addition of 0.15 ml of 1M o-phosphoric acid. The reaction mixture was brought to room temperature in a water bath and the fluorescence was measured at excitation / emission filters of 360 / 450 nm, respectively.

Results are expressed as mean $\pm$ SEM and comparisons between groups were made by means of an unpaired Student's t-test (Snedecor & Cochran, 1976). Differences were considered significant when $p < 0.05$.

Results and Discussion

(i) Protective effect of dietary curcumin, capsaicin and their combination on iron-induced hepatotoxicity

One of the mechanisms by which iron induces the toxicity is by increasing oxidative stress and lipid peroxidation. Lipid peroxidation of membranes is the major damaging factor in iron toxicity (Jacob, 1980). The ability of iron to accelerate lipid peroxidation is well-established (Ryan & Aust, 1992). The primary mechanism for this acceleration is believed to be the iron-catalyzed decomposition of lipid peroxides. The role of iron in *in vivo* and *in vitro* lipid peroxidation has been well studied (Ryan and Aust, 1992). Iron

overload increased formation of urinary malondialdehyde, tissue thiobarbituric acid reactive substances and conjugated dienes (Dillard *et al*, 1984). In experimental animals, iron overload can be effected by *i.p.* injection of iron salts (Dillard *et al*, 1984).

Effects of dietary curcumin, capsaicin and their combination on iron-induced lipid peroxidation in rat serum and liver are presented in Table-1. The results of the present study demonstrated that excess iron introduced by *i.p.* injection induced oxidative stress by increasing lipid peroxide levels in liver as well as in serum. The *i.p.* injection of iron significantly elevated the hepatic lipid peroxides (418% increase in control group). The levels of TBARS in liver were lower in animals fed curcumin, capsaicin or their combination. These decreases were 28, 26 and 22% in the respective diet groups. Dietary curcumin, capsaicin and their combination significantly reduced the severity of iron induced lipid peroxidation in liver. The decreases brought about by dietary curcumin, capsaicin and their combination in liver TBARS in Fe^{2+}-injected rats were 26, 28 and 37% respectively. Intraperitoneal injection of Fe^{2+} to rats also resulted in higher lipid peroxides in serum (Table-1). The increase in serum TBARS value in control rats as a result of Fe^{2+} injection was 76%. Dietary curcumin, capsaicin and their combination lowered serum lipid peroxide levels by 24, 33 and 29% respectively in Fe^{2+}-treated rats. These dietary spice principles however did not influence the basal TBARS values in serum in saline-injected rats.

Table-1 Effect of dietary curcumin and capsaicin on iron-induced lipid peroxidation in rat serum and liver

Diet group	Serum (µmol TBARS / dl)		Liver (nmol TBARS / mg protein)	
	Saline-injected	Fe^{2+}-injected	Saline-injected	Fe^{2+}-injected
Control	77.0 ± 6.32	135.2 ± 10.2	4.52 ± 0.31	23.4 ± 1.50
Curcumin	63.8 ± 4.22	102.4 ± 9.24*	3.25 ± 0.10*	17.2 ± 2.12*
Capsaicin	60.1 ± 6.74	91.1 ± 5.80*	3.33 ± 0.07*	16.8 ± 1.93*
Curcumin + Capsaicin	68.4 ± 4.76	95.4 ± 7.40*	3.52 ± 0.32*	14.7 ± 2.45*

Rats were injected 1ml saline or $FeSO_4$ in 1 ml saline (30 mg per kg body weight) 1 hr before sacrifice.

Values are expressed as mean ± SEM of 6 rats in each group.

* Significantly different from control group

The serum enzymes are very important adjuncts to clinical diagnosis of diseases affecting specific organs and tissue damage. Liver damage by iron toxicity can be assessed by leakage of enzymes such as alanine aminotransferase (AlAT), aspartate aminotransferase (AsAT) and lactate dehydrogenase into blood (Shimuzu *et al*, 1989; Ozaki *et al*, 1995). Higher activities of all these three enzymes in blood have been found in response to iron-induced oxidative stress in the present study (Table-2). The i.p. injection of iron significantly elevated the serum AlAT, AsAT and LDH, and the increases were 150%, 172% and 215% respectively. Dietary curcumin, capsaicin and their combination reduced activities of serum enzymes – AlAT, AsAT and LDH, indicating that these spice principles reduce the severity of iron induced hepatotoxicity by lowering lipid peroxidation. Dietary curcumin, capsaicin and their combination lowered serum AlAT by 28, 37 and 34% respectively in Fe^{2+}-injected animals (Table-2). Dietary curcumin, capsaicin and their combination lowered serum AsAT activity by 18, 28 and 38% respectively in iron-injected rats. Similarly, the increase in serum LDH as a result of Fe^{2+} administration was countered by 21, 31 and 41% by dietary curcumin, capsaicin and their combination respectively. Thus, the combination of the two spice principles brought about greater protective effect against Fe^{2+}-induced hepatotoxicity when viewed in terms of the beneficial influence on serum AsAT and LDH. This is consistent with a greater countering influence of the spice combination on Fe^{2+}-induced liver lipid peroxides described above. There was no change in the activities of these enzymes as a result of curcumin, capsaicin or their combination in the saline injected animals (Table-2).

Table-2 Effect of dietary curcumin and capsaicin on serum enzymes in rats injected with iron salt

Treatment	Alanine aminotransferase[a]		Aspartate aminotransferase[a]		Lactate dehydrogenase[b]	
	Saline injected	Fe^{++} injected	Saline injected	Fe^{++} injected	Saline injected	Fe^{++} injected
Control	108.3 ± 5.28	270.8 ± 9.80	30.9 ± 2.18	84.2 ± 24.4	65.3 ± 5.78	205.7 ± 9.67
Curcumin	112.4 ± 6.32	$195.4 \pm 11.7^*$	28.6 ± 3.62	$69.3 \pm 26.0^*$	74.4 ± 6.38	$163.1 \pm 7.40^*$
Capsaicin	120.3 ± 7.41	$169.8 \pm 10.2^*$	35.6 ± 4.10	$60.6 \pm 18.5^*$	69.6 ± 7.24	$142.7 \pm 14.4^*$
Curcumin + Capsaicin	102.2 ± 4.80	$179.8 \pm 13.2^*$	26.4 ± 2.83	$51.9 \pm 14.1^*$	60.6 ± 4.30	$120.4 \pm 5.59^*$

Values are mean ± SEM of 6 animals in each group.

Rats were injected 1ml saline or $FeSO_4$ in 1 ml saline (30 mg per kg body weight) 1 hr before sacrifice.

Specific activity units: a: μmole pyruvate/ min/ dl; b: μmole NADH/ min/ dl

* Significantly different from control group

Among the activities of alkaline and acid phosphatases measured in the serum of Fe^{2+}-injected rats, only the latter was elevated by about 20% as a result of iron over-loading (Table-3). While individual dietary spice principles did not influence the activity of serum alkaline phosphatase and acid phosphatase in Fe^{2+}-injected rats, only the combination of spice principles significantly countered the elevated serum acid phosphatase in Fe^{2+}-injected animals (Table-3).

In general, iron induced liver injury resulted in a marked elevation in the activity of these enzymes. The extent of elevation in the activities of these enzymes which are indicators of hepatic injury was generally lower in various spice principles fed animal groups. Combination of the two spice principles was found to be more protective to liver in iron induced hepatotoxicity, when compared to the two individual spice principles.

(ii) Effect of dietary curcumin, capsaicin and their combination on carrageenan-induced paw inflammation in rats.

In control rats highest swelling was observed at 5th hour after carrageenan injection (Fig.1). A comparison of the extent of carrageenan-induced paw inflammation at 5th hour in various spice principles fed animals is shown in Fig.1. Dietary curcumin lowered inflammation to an extent of 12 %, while dietary capsaicin reduced the inflammation to an extent of 9 %. Spice principles in combination were more effective in countering the extent of paw inflammation compared to the two individual spice principles, where the paw inflammation at 5th hour was 84% of control. An earlier study has reported that supplementation of diets with 1% curcumin for 10 weeks did not affect the inflammatory

Table-3 Effect of dietary curcumin and capsaicin on serum enzymes in rats injected with iron salt (Contd.)

Treatment	Alkaline phosphatase[a]		Acid phosphatase[b]	
	Saline injected	Fe^{++} injected	Saline injected	Fe^{++} injected
Control	2.01 ± 0.091	2.22 ± 0.080	340.6 ± 24.4	410.5 ± 14.8
Curcumin	2.16 ± 0.127	2.46 ± 0.118	334.6 ± 20.9	437.7 ± 18.0
Capsaicin	1.92 ± 0.168	2.46 ± 0.120	324.8 ± 23.1	448.6 ± 22.9
Curcumin + Capsaicin	1.66 ± 0.151	2.19 ± 0.124	330.6 ± 12.6	319.9 ± 16.4*

Values are mean $\pm$ SEM of 6 animals in each group.

Rats were injected 1ml saline or $FeSO_4$ in 1 ml saline (30 mg per kg body weight) 1 hr before sacrifice.

Specific activity units: a: mmol p-nitrophenol / min / dl   b: μmol p-nitrophenol / min / dl

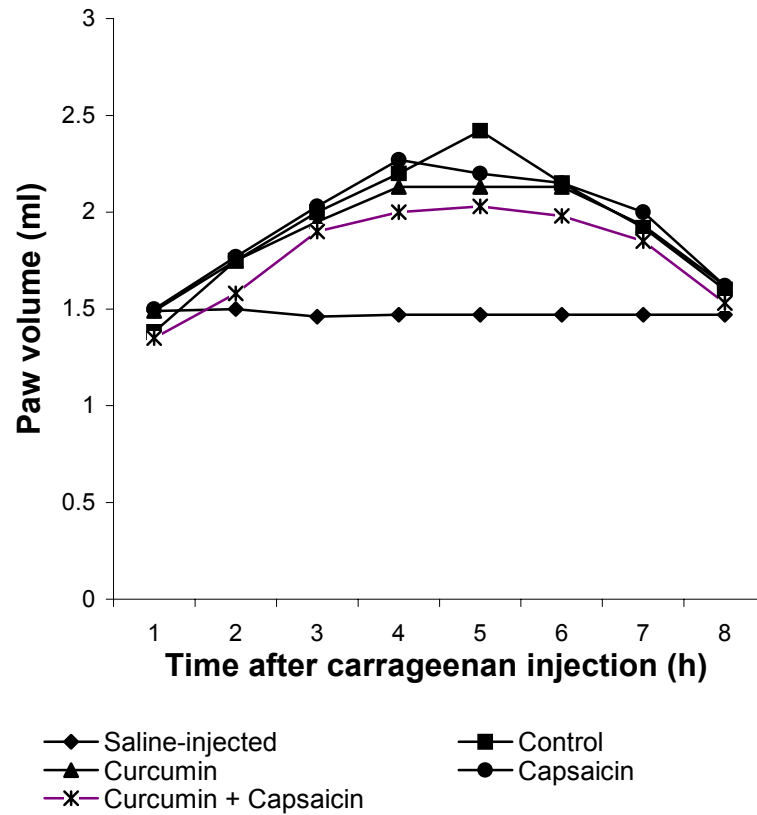* Significantly different from control group

Fig.1 Carrageenan-induced paw inflammation in rats fed spice principles

responses of animals to carrageenan injection (Reddy & Lokesh, 1994b). However, curcumin administered by gavage (15, 30 and 45 mg/kg body weight) 3 hr prior to carrageenan injection did show anti-inflammatory property (Reddy & Lokesh, 1994b). Similarly, capsaicin has been earlier shown to possess anti-inflammatory property against carrageenan-induced inflammation when given as a single oral dose (0.5 and 1.0 mg/kg body weight) 3 hr before carrageenan injection (Reddy & Lokesh, 1994b).

Influence of dietary curcumin, capsaicin, and their combination on 5'-lipoxygenase activity in the PMNL cells in carrageenan-injected rats is presented in Table-4. Dietary curcumin decreased the activity of 5'-lipoxygenase activity in the PMNL cells by 39 % in carrageenan-injected rats while dietary capsaicin produced 48% decrease in the enzyme activity. The decrease in the enzyme activity was even higher in the case of combination of these two spice principles (60%). Thus, the combination of spice principles curcumin and capsaicin had greater effect in countering the 5'-lipoxygenase activity in the PMNL cells as a result of carrageenan administration. Activity of 5'-lipoxygenase in the PMNL cells was also lower in saline-injected rats as a result of dietary spice principles, the decreases being 48, 26 and 49% respectively in curcumin, capsaicin and curcumin + capsaicin groups.

Table-4 Effect of dietary curcumin and capsaicin on 5'-lipoxygenase activity in polymorphonuclear lymphocytes of carraageenan injected rats

Animal group	Saline injected (nmole /min /mg protein)	Carrageenan injected (nmole /min /mg protein)
Control	2.988 ± 0.247	4.410 ± 0.205
Curcumin	1.550 ± 0.210*	2.700 ± 0.371*
Capsaicin	2.210 ± 0.165*	2.310 ± 0.187*
Curcumin + Capsaicin	1.520 ± 0.197*	1.770 ± 0.235*

Values are expressed as mean ± SEM of 6 rats in each group

* Significantly different from control group

Histamine concentration in serum was lower under the influence of dietary curcumin, capsaicin or their combination (Table-5). The decrease in serum histamine was 30, 37 and 21% lower in the respective groups among saline-injected rats. Serum histamine content was lower only in dietary capsaicin group among carrageenan-injected rats compared to respective controls (23% decrease). The low serum histamine titres in animals treated with dietary spice principles is consistent with their protective influence in response to carrageenan administration. There was no gross difference in the serum protein profile among rats of various diet groups injected with carrageenan as revealed by native PAGE (Fig.2).

Table-5 Effect of dietary curcumin and capsaicin on serum histamine content
     in carrageenan injected rats

Animal group	Saline injected (ng / dl serum)	Carrageenan injected (ng / dl serum)
Control	236.2 ± 15.4	286.1 ± 16.7
Curcumin	164.4 ± 11.8*	260.7 ± 17.8
Capsaicin	148.5 ± 14.1*	220.9 ± 20.0*
Curcumin + Capsaicin	187.0 ± 12.5*	299.2 ± 31.9

Values are expressed as mean ± SEM of 6 rats in each group

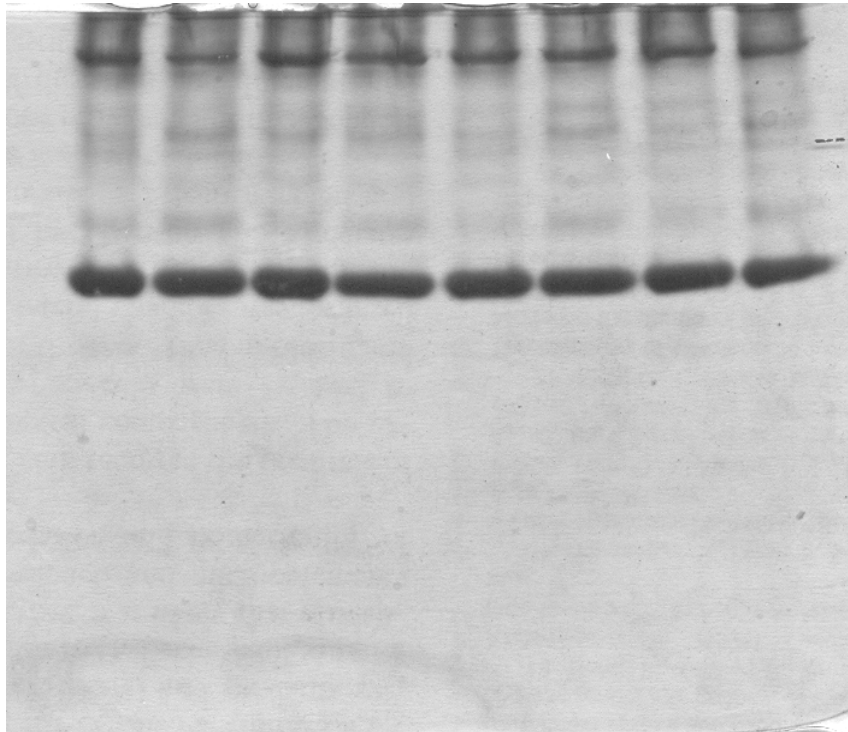* Significantly different from control group

Fig.2   Serum protein profile in carrageenan-injected rats of various diet groups as revealed by native PAGE

1. Control   (Saline-injected)   2. Control   (Carrageenan injected)
3. Curcumin (Saline-injected)   4. Curcumin (Carrageenan injected)
5. Capsaicin (Saline-injected)   6. Capsaicin (Carrageenan injected)
7. Curcumin + Capsaicin (Saline-injected)
8. Curcumin + Capsaicin (Carrageenan injected)

Conclusions

Results of this study suggest that dietary curcumin and capsaicin individually are protective to iron-induced hepatotoxicity and to carrageenan-induced inflammation. These beneficial effects generally appeared to be higher when the two compounds were fed in combination.

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Protective effect of dietary curcumin and capsaicin on induced oxidation of low-density lipoprotein in normal and hypercholesterolemic rats

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Summary

An animal study was carried out to examine the beneficial influence of dietary curcumin, capsaicin and their combination on the susceptibility of low-density lipoprotein to oxidation. Oxidation of low-density lipoprotein was induced either by ferrous ion *in vivo* or by copper ion *in vitro* after its isolation. Dietary curcumin and capsaicin individually produced significant inhibition in the in vivo iron-induced oxidation of LDL, as indicated by TBARS values. The extent of copper induced oxidation of LDL *in vitro* was also significantly lesser in the case of LDL isolated from curcumin or capsaicin fed rats. The protective effect of the combination of dietary curcumin and capsaicin on LDL oxidation both *in vivo* and *in vitro* was greater than that of the individual spice principles. The protective influence of dietary spice principles on LDL oxidation was also indicated by the relative anodic electrophoretic mobility of oxidized LDL on agarose gel. Results suggest that dietary curcumin and capsaicin individually are protective to LDL oxidation both *in vivo* and *in vitro*, this beneficial effect being even higher when the two compounds were fed in combination.

A separate animal study was carried out to examine the beneficial influence of capsaicin on the susceptibility of low-density lipoprotein to oxidation in hypercholesterolemic condition. In rats rendered hypercholesterolemic by maintaining them on a cholesterol-enriched diet for 8 weeks, inclusion of capsaicin (0.015 %) in the

diet produced significant hypocholesterolemic effect. Oxidation of low-density lipoprotein was induced either by copper ion *in vitro* after its isolation, or by ferrous ion *in vivo* in experimental rats under either normal or hypercholesterolemic situation and the beneficial effect of dietary capsaicin on the same was evaluated. LDL oxidation was measured by the thiobarbituric acid reactive substances (TBARS) formed and relative electrophoretic mobility of oxidized LDL. In the case of LDL isolated from hypercholesterolemic rats the extent of copper induced LDL oxidation was significantly lower than that of LDL isolated from normal rats. Dietary capsaicin which was protective to the LDL oxidation *in vitro* in the case of normal rats, did not make any difference to the extent of LDL oxidation *in vitro* in hypercholesterolemic rats. In high cholesterol feeding, Fe-induced *in vivo* oxidation of LDL was 73 % lower, while the same was still marginally lower in capsaicin fed hypercholesterolemic rats. While a significantly decreased level of lipid peroxidation was observed in hypercholesterolemic rats compared to normal rats, the same was not significantly altered by dietary capsaicin. Results suggest that dietary spice principle capsaicin is protective to LDL oxidation both *in vivo* and *in vitro* under normal situation, while in hypercholesterolemic situation where the extent of LDL oxidation is already lowered, capsaicin does not offer any further reduction.

Introduction

Oxidative damage at the cellular and subcellular level is now considered to be an important event in disease processes like cardiovascular disease, inflammatory disease, carcinogenesis and aging. In humans, plasma low-density lipoprotein is the major transport vehicle for cholesterol and its elevation is regarded as one of the principal risk factors for the development of atherosclerotic vascular disease (Goldstein & Brown, 1977; Grundy, 1986). A relatively large amount of cholesterol in the LDL fraction is atherogenic, whereas that in HDL fraction appears protective (Kannel *et al*, 1979). Oxidation of low-density lipoprotein has been suggested to play an important role in the development of atherosclerosis (Stamler *et al*, 1986). Inhibition of LDL oxidation can reduce the risk of atherosclerosis independent of lowering plasma cholesterol levels. The effectiveness of antioxidant vitamins C and E in the prevention of LDL oxidation has been well demonstrated (Sato *et al*, 1990). Phenolic compounds of red wine are shown to inhibit oxidation of LDL both *in vitro* and *in vivo* (Frankel *et al*, 1993; Miyagi *et al*, 1997).

The antioxidant properties of several spice principles have been evidenced in rats both *in vivo* and *in vitro*. While curcumin (turmeric), capsaicin (red pepper) and eugenol (clove) were found to be more effective antioxidants, piperine (black pepper), zingerone (ginger), linalool (coriander) and cuminaldehyde (cumin) were only marginally inhibitory to lipid peroxidation (Reddy & Lokesh, 1992). These compounds inhibited lipid peroxidation by quenching oxygen free radicals (Reddy & Lokesh, 1994) and by enhancing the activity of endogenous antioxidant enzymes - superoxide dismutase,

catalase, glutathione peroxidase and glutathione transferase (Reddy & Lokesh, 1994a). Spice active principles - curcumin (turmeric), capsaicin (red pepper), piperine (black pepper), eugenol (cloves) and allyl sulfide (garlic) have been shown to have protective effect on oxidation of human low-density lipoprotein *in vitro* (Naidu & Thippeswamy, 2002). Dietary spice principles curcumin, capsaicin and garlic were found to be antioxidative by enhancing the antioxidant molecules and antioxidant enzymes in erythrocytes and liver of hyperlipidemic / hypercholesterolemic rats (Kempaiah & Srinivasan, 2004, 2004a).

In the absence of any report on the *in vivo* effects of spice principles on LDL oxidation, the current study examines (1) the beneficial antioxidant influence, if any, of dietary curcumin, capsaicin, and their combination in terms of protecting the low-density lipoprotein from iron and copper induced oxidation in experimental rats. This investigation also makes (2) a study of the protective effect of dietary capsaicin on induced oxidation of low-density lipoprotein in hypercholesterolemic rats.

Materials and Methods

Curcumin, the yellow principle of turmeric (*Curcuma longa*) and capsaicin, the pungent principle of red pepper (*Capsicum annuum*) was procured from M/s Fluka Chemie, Switzerland. Thiobarbituric acid, agarose, Sudan black B and dialysis tubing were purchased from Sigma Chemical Company, St.Louis, USA. Ferrous sulfate (FeSO$_4$.7H$_2$O) was obtained from Qualigen Fine Chemicals Ltd., Mumbai, India. All other chemicals used were of analytical grade.

The animal experiments were carried out with due approval from the Institutional Animal Ethic Committee. Appropriate measures were taken to minimize pain or discomfort to the experimental animals and these experiments were carried out in accordance with the guidelines laid down by the NIH in the USA regarding the care and use of animals for experimental procedures.

Animal treatments

Experiment -1: Protective effect of dietary curcumin, capsaicin and their combination on iron induced LDL oxidation in vivo and copper induced LDL oxidation in vitro.

Male Wistar rats (8 per group), weighing 100 – 105 g, housed in individual stainless steel cages were maintained on various experimental diets viz., 0.2% curcumin / 0.015% capsaicin / 0.2% curcumin + 0.015% capsaicin *ad libitum* for 8 weeks. The animals had free access to water. The basal diet consisted of (%): casein, 21; cane sugar, 10; corn starch, 54; NRC vitamin mixture, 1; Bernhart-Tommarelli modified NRC salt mixture, 4 and refined peanut oil, 10. The spice principles were incorporated into the Basal diet, replacing an equivalent amount of corn starch. At the end of the feeding period, the rats were starved for 16 h and sacrificed under light ether anesthesia. Blood was drawn from the heart into tubes containing 0.1% EDTA.

Experiment-2: A study of the protective effect of dietary capsaicin on induced oxidation of low-density lipoprotein in hypercholesterolemic rats

Female Wistar rats (8 per group), weighing 100 – 105 g, housed in individual stainless steel cages were maintained on various experimental diets *ad libitum* for 8 weeks. The animals had free access to water. Food intake and growth of animals were monitored at regular intervals. The basal diet consisted of (%): casein, 21; cane sugar, 10; corn starch, 54; NRC vitamin mixture, 1; Bernhart-Tommarelli modified NRC salt mixture, 4 and refined peanut oil, 10. Hypercholesterolemia was induced by feeding a cholesterol enriched diet (0.5% cholesterol and 0.125% bile salts at the expense of an equivalent amount of corn starch in the basal diet). The other ingredients were similar to basal diet except for corn starch (34%). The spice principle - capsaicin was incorporated into the Basal diet, replacing an equivalent amount of corn starch to give the experimental diets containing: Capsaicin (0.015%). At the end of the feeding period, the rats were starved for 16 h and sacrificed under light ether anesthesia. Blood was drawn from the heart into tubes containing 0.1% EDTA.

In vivo induction of LDL oxidation

For *in vivo* LDL oxidation study, at the end of feeding period, rats were fasted overnight (16 h) and were injected i.p. with 30 mg iron as ferrous sulfate in 1 ml saline/kg body weight (Hu *et al*, 1990), 1h before animals were sacrificed.   Control animals were injected with the same volume of saline. Rats were sacrificed by cardiac puncture; blood was drawn from the heart into the tubes containing 0.1% EDTA and liver was excised quickly, perfused with saline and used for lipid peroxidation measurement.

LDL isolation

Plasma was separated by centrifugation at 600 x g for 15 min and adjusted to a density of 1.3 g/ml with potassium bromide. A discontinuous sodium chloride/ potassium bromide gradient was prepared by layering 1.5 ml of the adjusted plasma under 3.5 ml of normal saline (density = 1.006 / ml), in 5 ml Ultra clear quick seal tubes (Beckman Instruments Inc.). The tubes were centrifuged in Beckman L7 Ultracentrifuge at 4^0C using Beckman vertical rotor NVT65 at 1,25,000 × g for 2 h. Lipoprotein fractions were collected with the aid of a peristaltic pump and the LDL fractions with a density range of 1.020-1.080 g / ml were pooled and dialyzed extensively for 48 h against phosphate buffer saline (PBS) to remove potassium bromide and EDTA. LDL fraction (100μg/ml) suspended in 50 mM PBS buffer, pH 7.4 in a total volume of 4.0 ml. The purity of LDL fraction was tested by agarose gel electrophoresis.

Induction of LDL oxidation in vitro

LDL fraction (100 μg protein/ml) was suspended in 50 mM PBS buffer pH 7.4 in a total volume of 4 ml. The reaction was initiated with the addition of 10 μM $CuSO_4$ and 0.5 ml of aliquots were drawn at 3 and 12 h and the lipid peroxidation products were measured as thiobarbituric acid reactive substances (TBARS) according to the method described by Fairclough and Haschemyer (1978). To 0.5 ml of aliquots, 0.25 ml of 2.5% trichloroacetic acid and 0.25ml of 1.0% (w/v) 2-thiobarbituric acid were added, vortexed and kept in boiling water bath for 45 min. After cooling to room temperature, the fluorescent chromogen developed was extracted into 2 ml *n*-butanol and its fluorescence

intensity was measured spectrofluorimetrically at 515 nm excitation and 553 nm emission wave lengths.

LDL oxidation was measured in LDL isolated from Fe^{2+} injected rats by taking aliquots containing 400 μg protein in a total volume of 0.5 ml and fluorescence intensity was measured after developing fluorescent chromogen as above. TBARS concentration was calculated using 1,1,3,3-tetraethoxypropane as standard and expressed as nmole of MDA formed /mg protein of LDL.

Agarose gel electrophoresis

Electrophoretic mobility of LDL was examined by agarose gel electrophoresis according to the method of Noble (1968). Ten μl of LDL (200 μg of protein) was incubated in PBS pH 7.4 and oxidation was initiated by 10 μM of copper. After 12 h, the oxidized samples and LDL isolated from Fe^{2+} injected rats samples were electrophoresed in 1% agarose gel with Tris-barbital buffer pH 8.6 for 2 h at 50 volts. The gels were fixed for 30 min. in 5% acetic acid and 75% ethanol and stained with Sudan Black B.

Lipid peroxides in tissues

Lipid peroxides in tissues were determined by the method described by Ohkawa *et al* [1979]. An aliquot of tissue homogenate in 1.54 mM potassium chloride solution was mixed with an equal volume of 8% sodium lauryl sulfate in a test tube. 1.5 ml of 20% acetic acid (pH 3.5) was added and mixed well. 2.0 ml of 8% aqueous thiobarbituric acid was also added, mixed well and boiled for 1 h, cooled. 5.0 ml *n*-butanol was added and mixed well; centrifuged at 3000 rpm for 10 min. Absorbance of butanol extract was

measured at 532 nm. Values were compared with similarly treated 1,1,3,3-tetraethoxy-propane, used as standard.

Results are expressed as mean $\pm$ SEM and comparisons between groups were made by means of an unpaired Student's t-test (Snedecor & Cochran, 1976). Differences were considered significant when $p < 0.05$.

Results

Protective effect of dietary curcumin, capsaicin and their combination on iron induced LDL oxidation in vivo and copper induced LDL oxidation in vitro.

Oxidation of LDL observed in ferrous sulfate injected rats as measured by TBARS values is presented in Table-1. Dietary curcumin and dietary capsaicin significantly inhibited the oxidation of LDL, as indicated by TBARS values which were 71 and 62 % of control rats. Extent of iron-induced oxidation of LDL was considerably lower in curcumin + capsaicin fed groups when compared to curcumin fed animals (TBARS value 56 % of control). Extensive oxidation of LDL *in vitro* from control rats was noticed in presence of copper sulfate as measured by the time dependent increase in TBARS values over the period of 12 h (Table-1). The extent of copper induced oxidation of LDL *in vitro* was significantly lesser in the case of LDL isolated from curcumin fed rats. 17 and 18 % lesser TBARS formation at 3 h and 12 h of LDL oxidation respectively was seen in this case. The extent of copper induced oxidation of LDL *in vitro* was also significantly lesser in the case of LDL isolated from capsaicin fed animals. The decrease in TBARS formation was 29 and 21 % at 3 h and 12 h of LDL oxidation respectively in this case. A

Table-1 Effect of dietary curcumin and capsaicin on ferrous iron-induced (*in vivo*) and copper induced (*in vitro*) LDL oxidation in rats

Diet group	*In vivo* Fe^{2+}-induced	*In vitro* Cu^{2+}-induced	
		3 hr	12 hr
Control	1.036 ± 0.059	11.0 ± 0.45	19.6 ± 0.47
Curcumin	0.731 ± 0.022*	9.08 ± 0.40*	16.1 ± 0.52*
Capsaicin	0.640 ± 0.050*	7.84 ± 0.61*	15.5 ± 0.61*
Curcumin + Capsaicin	0.578 ± 0.055*	6.88 ± 0.16*	14.9 ± 0.47*

Rats were injected 1ml saline or $FeSO_4$ in 1 ml saline (30 mg per kg body weight) 1 h before sacrifice.

Values expressed as nmole TBARS / mg protein are mean ± SEM of 8 rats in each group.

* Significantly different from control group

decrease in TBARS formation of 37 and 24 % was seen at 3 h and 12 h of copper induced LDL oxidation in the case of LDL isolated from animals fed the combination of curcumin and capsaicin. Thus, the protective effect of the combination of dietary curcumin and capsaicin on LDL oxidation both *in vivo* and *in vitro* was greater than that of the individual spice principles.

Agarose gel electrophoresis revealed that LDL oxidation induced *in vivo* by ferrous ion caused an increase in the anodic mobility of LDL in the case of control rats (Fig.1). In the case of animals maintained on curcumin, capsaicin or curcumin + capsaicin, the anodic mobility of LDL oxidized *in vivo* by ferrous ion was slower compared to the control animals. The decreased anodic mobility of oxidized LDL in the case of spice principles fed animals is thus consistent with the observed protective influence on LDL oxidation by these compounds.

Protective effect of dietary capsaicin on iron and copper induced oxidation of low-density lipoprotein in hypercholesterolemic rats

The effect of dietary intake of hypolipidemic spice principle - capsaicin on plasma total cholesterol and LDL cholesterol in hypercholesterolemic rats is depicted in Fig.2. Plasma total cholesterol of hypercholesterolemic rats was effectively countered by dietary capsaicin from 240 mg% of HCD control value to 139 mg%. The increased blood cholesterol in hypercholesterolemic rats was predominantly in LDL fraction. LDL-cholesterol, which was about 7.8-times that of basal control value in dietary high cholesterol treatment, was also significantly countered in dietary capsaicin treatment.
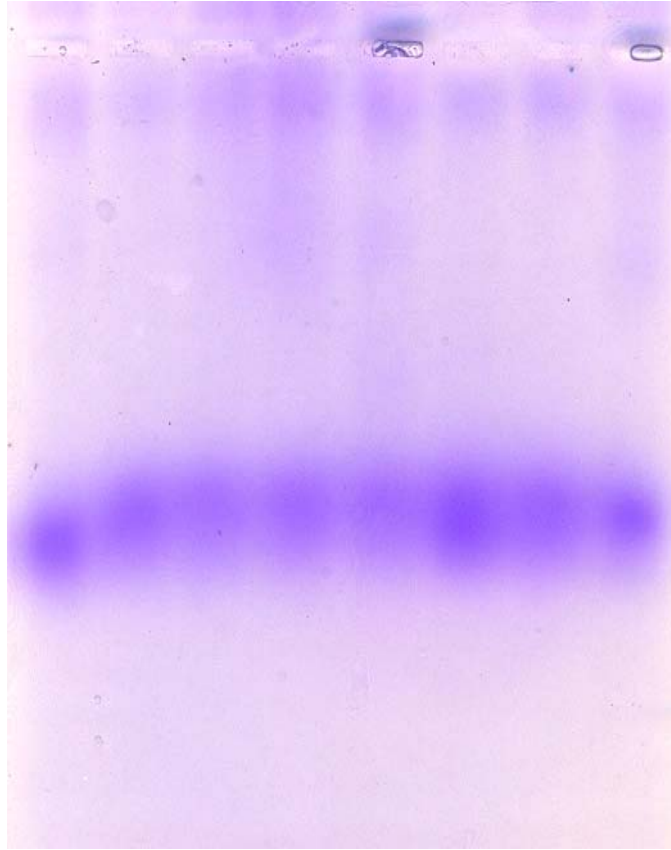
Fig.1   Agarose gel electrophoresis of LDL in different diet groups oxidized *in vivo* by ferrous ion.

(1) Control (Fe^{2+}-injected); (2) Control (Saline injected); (3) Curcumin (Saline injected); (4) Curcumin (Fe^{2+}-injected); (5) Capsaicin (Saline injected); (6) Capsaicin (Fe^{2+}-injected); (7) Curcumin + Capsaicin (Saline injected); (8) Curcumin + Capsaicin (Fe^{2+}-injected)
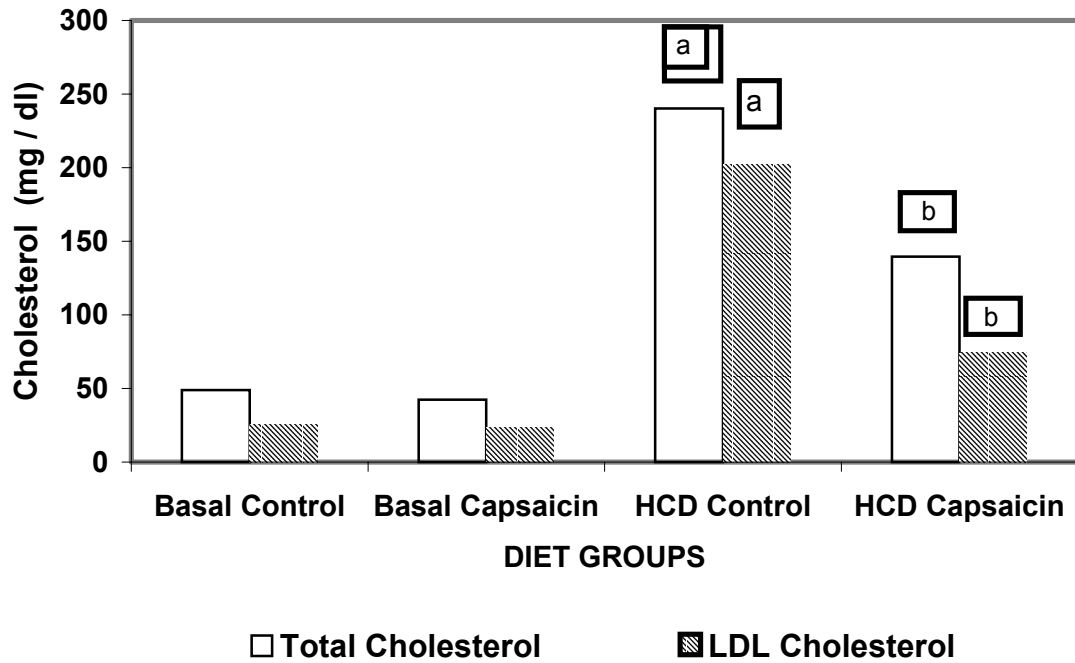
Fig.2 Effect of dietary capsaicin on blood cholesterol in hypercholesterolemic rats

    a: Significantly different from Basal Control;
    b: Significantly different from HCD Control

Extensive oxidation of LDL from basal control rats was noticed in presence of copper sulfate as measured by the time dependent increase in TBARS levels over the period of 12 h (Table-2). The extent of LDL oxidation induced by copper was significantly lesser in the case of LDL isolated from capsaicin treated rats. 40 % and 50 % lesser TBARS formation at 3 h and 12 h of LDL oxidation respectively was seen in this case. LDL oxidation as measured by TBARS values in hypercholesterolemic rats was significantly lower compared to Basal control, and the TBARS values at 3 and 12 h were 90 and 68 % lesser than control. Copper induced LDL oxidation / TBARS values were 89.5 % and 72 % lower than basal control at 3 h and 12 h in HCD-Capsaicin group and were thus comparable to HCD-Control group.

Oxidation of LDL observed in ferrous sulfate injected rats as measured by TBARS levels is presented in Table-2. Dietary capsaicin significantly inhibited the oxidation of LDL / TBARS values (71 % of basal control rats). In hypercholesterolemic rats also TBARS formation was significantly inhibited (73 % of basal control). In capsaicin fed hypercholesterolemic rats LDL oxidation was almost completely inhibited (TBARS value 84 % of basal control). Ferrous ion induced lipid peroxidation in liver of normal rats was also effectively inhibited by dietary capsaicin as shown in Table-2. Dietary capsaicin effectively reduced the TBARS value by 67 % in Basal control rats, while in hypercholesterolemic rats, which featured 77 % reduction in TBARS formation, there was 80 % reduction in the same by dietary capsaicin.

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Table 2. Effect of dietary capsaicin on Copper induced (in vitro) and Ferrous iron induced (*in vivo*) LDL oxidation in basal and hypercholesterolemic rats

Diet group	Cu^{++}-induced LDL oxidation *in vitro*		*In vivo* Fe^{++}-induced	
	3 h	12 h	LDL oxidation	Liver lipid peroxidation
Basal Control	2.68 ± 0.345	9.87 ± 0.616	0.528 ± 0.0564	2.50 ± 0.336
Basal Capsaicin	1.59 ± 0.058^a	4.90 ± 0.188^a	0.154 ± 0.0133^a	0.82 ± 0.077^a
HCD Control	0.255 ± 0.044^a	3.18 ± 0.393^a	0.142 ± 0.0158^a	0.57 ± 0.035^a
HCD Capsaicin	0.281 ± 0.038	2.78 ± 0.177	0.083 ± 0.022^b	0.50 ± 0.030^b

Values (TBARS) indicated are nmole / mg protein

'a' : Significantly different from Basal Control
'b' : Significantly different from HCD Control

Anodic mobility of LDL isolated from different rat groups is depicted in Fig.3 and Fig.4. Agarose gel electrophoresis indicated an increased anodic mobility of LDL on oxidation with copper sulfate in basal control rats. Dietary capsaicin significantly decreased the relative electrophoretic mobility (REM) indicating the antioxidant protection. In case of hypercholesterolemic rats, anodic mobility of LDL upon copper induced oxidation was unchanged moreover, in capsaicin fed hypercholesterolemic rats, anodic mobility was unaltered upon copper induced oxidation. Agarose gel electrophoresis revealed that LDL oxidation induced *in vivo* by ferrous ion caused an increase in the anodic mobility in the case of basal control rats. On the other hand, in hypercholesterolemic rats (both control and capsaicin fed groups) it remained unaltered. Dietary capsaicin significantly decreased the relative electrophoretic mobility of LDL in Basal control rats.
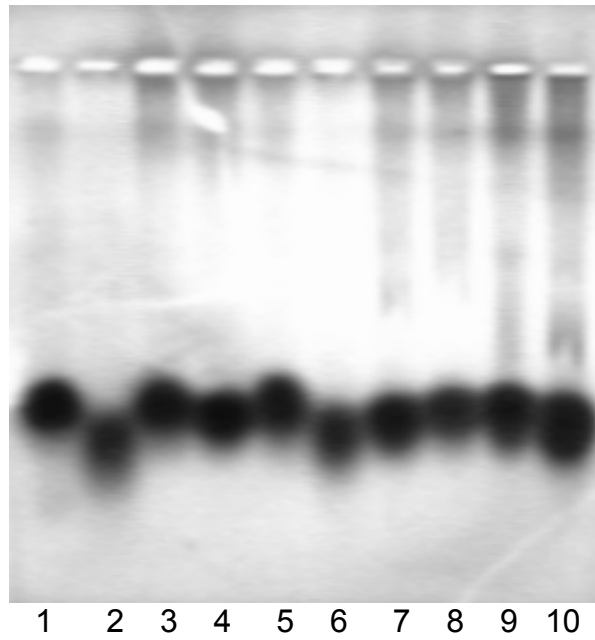
Fig. 3   Agarose gel electrophoresis of LDL isolated from different
           diet groups after oxidation by copper ion *in vitro*

1   Basal Control - Control   2. Basal Control - Oxidized
3. HCD Capsicin - Control   4. HCD Capsicin - Oxidised
5. Basal Control - Control   6. Basal Control - Oxidised
7. HCD Control - Oxidised   8. HCD Control - Control
9. Basal Capsicin - Control   10. Basal Capsicin - Oxidised
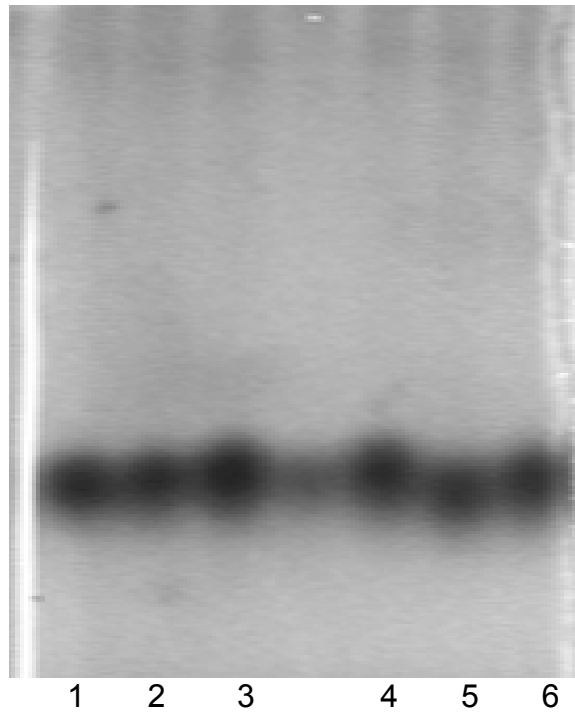
Fig.4 Agarose gel electrophoresis of LDL in different diet groups
oxidized by ferrous ion *in vivo*

1. Basal Capsaicin   2. Basal Capsaicin   3. HCD Capsaicin
4. HCD Control   5. Basal Control   6. Basal Capsaicin

Discussion

In humans, plasma LDL is a major transport vehicle for cholesterol and its elevation is regarded as one of the principle risk factor for the development of atherosclerotic vascular disease (Goldstein & Brown, 1977; Grundy, 1986). A relatively large amount of cholesterol in LDL fraction is atherogenic, whereas that in the HDL fraction appears protective (Kannel *et al*, 1979). Oxidation of low-density lipoprotein has been suggested to play an important role in the development of atherosclerosis (Stamler *et al*, 1986). It is also known that dietary factors influence plasma lipid levels and lipoprotein metabolism, altering the atherogenicity of lipoprotein profile (Grundy & Denke, 1990). The hypothesis states that the oxidative modification of LDL or other lipoproteins is central, if not obligatory to the atherogenic process. The important corollary is that inhibition of such oxidation should reduce the progression of atherosclerosis, independent of reduction of other factors, such as elevated LDL levels (Chisolm & Steinberg, 2000; Steinberg & Witztum, 1990).

It is universally accepted that hypercholesterolemia is an important independent risk factor for atherosclerosis (Lipid Research Clinics Program, 1984). Pathogenesis of atherosclerosis most likely involves free radical-mediated process. Oxidative modifications of LDL, which dysregulates the homeostasis between blood and vascular cells and alteration in endothelial function are considered among the early events in the pathogenesis of atherosclerosis (Banti *et al*, 2003). The alteration of oxidant/ antioxidant balance may affect the susceptibility of LDL to oxidation.

LDL oxidation can lead to its subsequent aggregation, which further increases cellular cholesterol accumulation (Aviram & Fuhrman, 1998). Factors that have been reported to affect the susceptibility of LDL to oxidation include antioxidant content, particle size and fatty acid composition. α-Tocopherol is the most abundant antioxidant in LDL (Esterbauer *et al*, 1991). And LDL isolated after individuals have been given α-tocopherol supplementation has been reported to exhibit increased resistance to oxidative modification (Jialal & Grundy, 1992; Jialal *et al*, 1995). Supplementing the corn oil and beef tallow enriched diets with moderate amounts of dietary cholesterol increased the susceptibility of LDL to oxidation but LDL α-tocopherol levels tended to be higher after consuming the diets with cholesterol supplementation (Schwab *et al*, 2000). However, although the LDL α-tocopherol content increased in the beef tallow diet supplemented with cholesterol, no significant relationship was observed between the α-tocopherol concentration of the LDL particles and the susceptibility of LDL to oxidation.

Active principles of spices such as curcumin (turmeric), quercetin (onion, garlic), capsaicin (red pepper), piperine (black pepper), eugenol (cloves) and allyl sulfide (garlic) have been shown to have protective effect on oxidation of human LDL *in vitro* [Naidu & Thippeswamy, 2002]. Spice principles curcumin, capsaicin and garlic were found to be antioxidative by enhancing the antioxidant molecules and enzymes in erythrocytes and liver of hyperlipidemic/ hypercholesterolemic rats [Kempaiah & Srinivasan, 2004, 2004a].

Hypercholesterolemia in rats induced by cholesterol enriched diet has been shown to feature resistance to lipid peroxidation in blood plasma, erythrocytes, and liver and also reduced activity of hepatic cytochrome-C reductase [Kempaiah & Srinivasan, 2004]. Inhibition of LDL oxidation can inhibit atherosclerosis independent of lowering plasma cholesterol levels. Many studies have demonstrated that inhibition of oxidation by pharmacologic and or genetic manipulations retards atherogenesis [Witztum & Steinberg, 2001]. In order to examine whether high cholesterol enhances the oxidation process in LDL or protective to oxidation of LDL we have examined the influence of the cholesterol-enriched diet as well as the hypolipidemic spice principle capsaicin on LDL oxidation in experimental rats.

LDL extracted from atherosclerotic tissue of both rabbits and humans has been shown to exhibit all of the physiological, immunological and biological properties of oxidized LDL *in vitro* [Yla-Herttuala *et al*. 1989]. Cardiovascular risk predominantly increases in individuals with increased levels of LDL-cholesterol [Rosengren *et al*. 1990]. Copper ion-induced *in vitro* oxidation of apo B-containing lipoproteins is used for detecting the risk for CVD. When lipoproteins are oxidized, hydroperoxides are released and are converted to reactive aldehydes (*e.g.*, MDA and 4-hydroxynonenal). Interaction of aldehydes with lysine residues in the apo B-100 moiety renders apo B-containing lipoproteins more negatively charged, which results in a decreased affinity for the LDL receptor and an increased affinity for scavenger receptors [Holvoet *et al*. 1998].

Catalysis of oxidation by copper ions as introduced by Esterbauer [Esterbauer & Ramos, 1996] is generally accepted and widely used for the determination of

oxidizability of LDL. In this test, the lag time of diene formation or production of thiobarbituric acid-reactive substances (TBARS) is measured, which is often used for characterization of the atherogenicity of LDL or of the therapeutic usefulness of antioxidants [Hense *et al*. 1993; Princen *et al*. 1992].

LDL particles contain many natural antioxidants, which are able to trap free radicals that can prevent or limit the extent of the chain reaction [Esterbauer & Ramos, 1996]. Among these natural antioxidants, vitamin E is the major chain-breaking lipophilic antioxidant in LDL and membrane [Esterbauer & Ramos, 1996; Daugherty & Roselaar, 1995] Supplementation with vitamin E and β-carotene were shown to reduce the susceptibility of LDL to oxidation in clinical trials [Esterbauer & Ramos, 1996; Hoffman *et al*. 1992]. But, some investigators found that vitamin E [Abbey *et al*. 1994] and β-carotene [Fuller & Jialal, 1994] were ineffective in reducing the extent of LDL oxidation. Increased α-tocopherol observed in plasma, erythrocytes and liver of hypercholesterolemic rats [Kempaiah & Srinivasan, 2004] may also be one of the factors responsible for the observed low TBARS values.

Total radical trapping activity increased after the feeding high cholesterol diet in rabbits, however, dropped significantly by 40 % during the time of regression with a trend to normalization after 40 weeks. The effect on TBARS formation during oxidation of isolated LDL was more pronounced showing, however, doubling of the time needed for half-maximal oxidation in high cholesterol fed rabbits [Schneider *et al*. 1999]. In our study, decreased TBARS formation has been found in LDL of hypercholesterolemic rats, and this may be due to cholesterol molecules getting oxidized by copper or iron ions.

Oxidized cholesterol accumulation in the LDL may lead to altered LDL which in turn leads to atherosclerosis. It is probable that cholesterol-enriched diet, which essentially consisted of hydrogenated fat, would have altered the unsaturation index of lipids in lipoproteins. We have earlier evidence that under similar atherogenic diet treatment, even the antioxidant status of animals is altered [Kempaiah & Srinivasan, 2004]. Lowered lipid peroxide concentrations in blood, erythrocytes and liver, reduced induction of lipid peroxidation in liver and higher contents of antioxidant molecules - ascorbic acid and vitamin E in liver were observed in induced hypercholesterolemic rats [Kempaiah & Srinivasan, 2004]. The observed lower oxidation of LDL, both *in vitro* and *in vivo,* in hypercholesterolemic rats may be attributable to the probability that cholesterol molecule itself acts as an antioxidant thus protecting LDL apoprotein from oxidation. Thus, higher circulatory cholesterol level in hypercholesterolemic animals resulted in lower LDL oxidation, compared to normal ones. Since the susceptibility of LDL for oxidation is already low in hypercholesterolemic rats, no further lowering effect is seen in capsaicin treatment, which is otherwise beneficial in normal condition.

Conclusions

Results of this study suggest that dietary spice principles - curcumin and capsaicin individually are protective to LDL oxidation both *in vivo* and *in vitro.* This beneficial effect generally appeared to be higher when the two compounds were fed in combination. Thus, the present study has evidenced the antioxidant effect of dietary capsaicin by virtue of having a protective effect on LDL oxidation. The current investigation also suggest that while dietary capsaicin is protective to LDL oxidation under normal situation, in

hypercholesterolemic situation where the extent of LDL oxidation is already lowered, capsaicin may not offer any further beneficial reduction.

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GENERAL SUMMARY

♦ Heat processing of spices – turmeric (*Curcuma longa*) and red pepper (*Capsicum frutescens*) by pressure-cooking generally resulted in a significant reduction in the concentration of the respective active principles – curcumin and capsaicin, which are well recognized to have several health beneficial physiological properties.

♦ Among the several health beneficial influences of turmeric and red pepper, hypolipidemic and antioxidant properties have far-reaching health implication. Since these beneficial physiological effects of spices are attributable to the active principles, studies were made to evaluate the extent to which the spices would retain their health beneficial effects after their heat processing as in domestic food preparation. Elaborate animal studies were made to quantitate the relative potency of cooked turmeric and red pepper with regard to their hypolipidemic and antioxidant properties.

♦ The hypocholesterolemic and antioxidant potency of both raw and pressure-cooked turmeric and red pepper were evaluated in experimental rats rendered hypercholesterolemic by feeding 0.5% cholesterol enriched diet and maintained for 8 weeks on 5 % spice diet.

♦ Dietary turmeric and red pepper, either raw or heat processed significantly countered the extent of hypercholesterolemia. Serum total cholesterol was 31 and 32% lower as a result of feeding raw and heat processed turmeric. The same was lower by 16 and 23% in animal groups fed raw and heat processed red pepper.

The reduction in blood cholesterol brought about by these two dietary spices was predominantly in the LDL-cholesterol fraction.

♦ Dietary red pepper, both raw and heat processed fully countered the increase in serum triglyceride content of hypercholesterolemic rats. Increase in hepatic cholesterol in hypercholesterolemic animals was moderately countered by dietary red pepper, either raw or heat processed.

♦ Both dietary turmeric as well as red pepper significantly countered the increase in hepatic triglyceride level in hypercholesterolemic rats.

♦ Total thiols in serum were slightly but significantly increased by raw turmeric and raw red pepper both in basal and in hypercholesterolemic rats, but not by heat processed spices. Serum α-tocopherol was significantly enhanced (81-113%) by both dietary turmeric and red pepper in hypercholesterolemic animals. Hepatic lipid peroxides were significantly lower (9-15%) as a result of dietary turmeric and red pepper in hypercholesterolemic situation.

♦ Thus, the results of this animal study suggested that although heat processing of turmeric and red pepper by pressure cooking resulted in a considerable loss of the active principles – curcumin and capsaicin, the hypolipidemic potency or the antioxidant potency of the parent spices were not significantly compromised.

♦ The hypolipidemic and antioxidant potency of dehydrated onion product developed in this Institute (which involved drum drying of this spice) was evaluated in experimental rats maintained for 6 weeks at 5 and 10% dietary levels.

♦ Total serum cholesterol was significantly reduced (by 21-24%) in hypercholesterolemic rats maintained on dehydrated onion diet. This reduction was seen essentially in the LDL fraction of serum cholesterol. A beneficial increase of 11% in the concentration of HDL-cholesterol in blood was also evidenced in hypercholesterolemic animals as a result of dietary onion. Blood triglyceride concentration of hypercholesterolemic rats was 31% lower in onion treated groups compared to corresponding control.

♦ While hepatic triglyceride and phospholipid concentrations were unaffected by onion treatment in both normal and hypercholesterolemic rats, cholesterol was slightly higher in hypercholesterolemic rats as a result of onion treatment at 5% level which was also reflected in the total lipid content of liver tissue.

♦ Total thiols in the blood of hypercholesterolemic rats were profoundly higher in onion treatment at either level as compared to respective controls. Blood glutathione concentration was significantly increased by dietary 10% onion in hypercholesterolemic rats, the increase being around 18%. Blood α-tocopherol concentration was slightly higher in hypercholesterolemic rats as a result of onion treatment. Blood ascorbic acid concentration was significantly higher in both hypercholesterolemic animals as well as normal animals fed dehydrated onion.

Blood lipid peroxides were lower by 15-16% in animals fed 10% dehydrated onion in both hypercholesterolemic and normal rats.

♦ Liver ascorbic acid concentration was higher under onion treatment in both normal and hypercholesterolemic animals. Hepatic α-tocopherol concentration was significantly higher (53-77%) in normal rats maintained on dehydrated onion containing diets. Liver lipid peroxides were significantly lower in normal rats maintained on dehydrated onion containing diets, the decrease being 24-26%.

♦ Since spices are often consumed in combination, and that the beneficial hypolipidemic and antioxidant potency of individual spice principles – curcumin and capsaicin have been understood, the advantage, if any in terms of a possible synergy existing in this property between the two spice active principles remains to be understood.

♦ An animal study has evidenced that dietary curcumin, capsaicin or their combination uniformly countered (by around 20%) the extent of hypercholesterolemia brought about by high cholesterol feeding. Neither of the two dietary spice principles or their combination had any effect on blood cholesterol level in normal rats.

♦ Hepatic cholesterol concentration was significantly lowered (20 - 27%) as a result of dietary spice principles in normal rats, while the same was not influenced in hypercholesterolemic animals. Liver triglyceride was significantly lowered in both normal and hypercholesterolemic rats maintained on capsaicin diet.

♦ Blood lipid peroxide content in hypercholesterolemic rats was lowered by dietary curcumin and capsaicin, and this effect was additive with combination of curcumin and capsaicin.

♦ Hepatic thiols, glutathione and ascorbic acid were elevated in hypercholesterolemic rats with concurrent low titres of lipid peroxides. Both spice principles individually or in combination further depleted hepatic lipid peroxides. While hepatic ascorbic acid content was enhanced by dietary curcumin, capsaicin and their combination in normal rats, glutathione was enhanced by the combination of spice principles in hypercholesterolemic animals.

♦ Activities of glutathione reductase, glutathione transferase and catalase in serum and hepatic glutathione reductase were enhanced by dietary curcumin, capsaicin and their combination in normal rats. Dietary curcumin, capsaicin and their combination increased the activity of serum glutathione peroxidase in hypercholesterolemic rats, while glutathione reductase, glutathione transferase and catalase activities were higher only in curcumin feeding.

♦ Thus, the present study indicated that a high cholesterol diet compromises the endogenous antioxidant defense mechanisms as indicated by reduction in antioxidant molecules and antioxidant enzymes. Dietary curcumin and capsaicin were found to normalize these changes to a significant extent. Although this effect of spice principles was not generally additive when given in combination,

the effect was certainly more pronounced than their individual effects in a few instances.

♦ Another animal study has evidenced that dietary curcumin, capsaicin or their combination significantly countered the extent of hypertriglyceridemia brought about by high fat feeding, and the effect was 12 - 20%. The hypotriglyceridemic effect of dietary curcumin was more than that of capsaicin at the dietary levels examined.

♦ Dietary curcumin, capsaicin and their combination produced slight decrease in serum total cholesterol concentration in these high fat fed animals. While dietary curcumin and dietary capsaicin individually did not influence serum cholesterol concentration in normal rats, a combination of the two spice principles did produce a slight but significant reduction in the same.

♦ Serum α-tocopherol content was increased by dietary curcumin, capsaicin and their combination in high fat fed rats, the effect being greater by the combination of curcumin and capsaicin. Serum glutathione concentration was beneficially increased only by dietary capsaicin in the high fat fed animals. Serum total thiol content in high fat fed animals and serum ascorbic acid in normal animals was elevated by the combination of curcumin and capsaicin.

♦ Glutathione concentration in liver was increased by feeding curcumin, capsaicin or curcumin + capsaicin in normal animals. Hepatic glutathione and α-tocopherol

concentration was significantly increased by dietary curcumin and dietary curcumin + capsaicin in high fat fed animals. As a result of beneficial enhancing influence on hepatic glutathione and α-tocopherol concentrations, dietary curcumin and dietary curcumin + capsaicin also reduced lipid peroxide level in high fat fed animals. Hepatic lipid peroxide content was also lower in normal rats as a result of dietary spice principles.

♦ The activities of serum glutathione peroxidase and glutathione transferase in high fat fed rats were generally higher as a result of dietary curcumin, capsaicin and curcumin + capsaicin. Activities of hepatic glutathione reductase and glutathione peroxidase were significantly elevated by dietary spice principles in high fat fed animals.

♦ Thus, no additive effect of the two compounds - curcumin and capsaicin was evidenced in general when given together with respect to hypolipidemic or the antioxidant potential.

♦ An animal study was carried out to examine the beneficial influence of dietary curcumin, capsaicin and their combination on iron induced hepatotoxicity. Groups of rats injected with 30 mg/kg Fe^{2+}/kg body weight showed hepatic toxicity as measured by an increase in lipid peroxides which correlated with elevated serum enzymes, alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase.

♦ Dietary curcumin, capsaicin and their combination reduced the activities of these serum enzymes, as well lowered the liver and serum lipid peroxide level indicating that these spice principles reduce the severity of iron induced hepatotoxicity by lowering lipid peroxidation.

♦ In a separate study, a comparison of the extent of carrageenan-induced paw inflammation in spice principles fed animals showed that both dietary curcumin and capsaicin moderately lowered inflammation, while the spice principles in combination were more effective in countering the extent of paw inflammation.

♦ Dietary curcumin and capsaicin significantly decreased the activity of 5'-lipoxygenase activity in the PMNL cells in carrageenan-injected rats. The decrease in the enzyme activity was even higher in the case of combination of these two spice principles.

♦ These results suggest that dietary curcumin and capsaicin individually are protective to iron-induced hepatotoxicity and to carrageenan-induced inflammation. This beneficial effect was higher when the two compounds were fed in combination.

♦ Another animal study was carried out to examine the beneficial influence of dietary curcumin, capsaicin and their combination on the susceptibility of low-density lipoprotein to oxidation. Oxidation of low-density lipoprotein was induced either by ferrous ion *in vivo* or by copper ion *in vitro* after its isolation.

♦ Dietary curcumin and capsaicin individually produced significant inhibition in the in vivo iron-induced oxidation of LDL, as indicated by TBARS values. The extent of copper induced oxidation of LDL *in vitro* was also significantly lesser in the case of LDL isolated from curcumin or capsaicin fed rats. The protective effect of the combination of dietary curcumin and capsaicin on LDL oxidation both *in vivo* and *in vitro* was greater than that of the individual spice principles.

♦ The protective influence of dietary spice principles on LDL oxidation was also indicated by the relative anodic electrophoretic mobility of oxidized LDL on agarose gel.

♦ Results suggest that dietary curcumin and capsaicin individually are protective to LDL oxidation both *in vivo* and *in vitro*, this beneficial effect being even higher when the two compounds were fed in combination.

♦ A separate animal study was carried out to examine the beneficial influence of capsaicin on the susceptibility of low-density lipoprotein to oxidation in hypercholesterolemic condition. In rats rendered hypercholesterolemic by maintaining them on a cholesterol-enriched diet for 8 weeks, inclusion of capsaicin (0.015 %) in the diet produced significant hypocholesterolemic effect.

♦ Oxidation of low-density lipoprotein was induced either by copper ion *in vitro* after its isolation, or by ferrous ion *in vivo* in experimental rats under either normal or hypercholesterolemic situation and the beneficial effect of dietary capsaicin on the same was evaluated. LDL oxidation was measured by the

thiobarbituric acid reactive substances (TBARS) formed and relative electrophoretic mobility of oxidized LDL.

♦ In the case of LDL isolated from hypercholesterolemic rats the extent of copper induced LDL oxidation was significantly lower than that of LDL isolated from normal rats. Dietary capsaicin which was protective to the LDL oxidation *in vitro* in the case of normal rats, did not make any difference to the extent of LDL oxidation *in vitro* in hypercholesterolemic rats.

♦ In high cholesterol feeding, Fe-induced *in vivo* oxidation of LDL was 73 % lower, while the same was still marginally lower in capsaicin fed hypercholesterolemic rats. While a significantly decreased level of lipid peroxidation was observed in hypercholesterolemic rats compared to normal rats, the same was not significantly altered by dietary capsaicin.

♦ These results suggest that dietary spice principle - capsaicin is protective to LDL oxidation both *in vivo* and *in vitro* under normal situation, while in hypercholesterolemic situation where the extent of LDL oxidation is already lowered, capsaicin does not offer any further reduction.