*Mallikarjun.B.C et al. / Journal of Pharmacy Research 2012,5(7),3613-3617*

**Research Article**
**ISSN: 0974-6943**

**Available online through**
**www.jpronline.info**

# Development of a sensitive and specific immunoassay to evaluate diabetic nephropathy and dietary modulation in experimental animals

**Mallikarjun.B.C[*], Smitha Bhatkar[†], Shylaja M Dharmesh[*], Rajan MGR[†], Salimath PV[*]**

[*] *Department of Biochemistry and Nutrition, Central Food Technological Research Institute,CSIR, Mysore -570 020, Karnataka, India*

[†] *Laboratory Nuclear Medicine Section Tata Memorial Hospital, Annexe Parel, Mumbai- 400 012, India*

**Received on:07-04-2012; Revised on: 12-05-2012; Accepted on:16-06-2012**

## ABSTRACT

Microalbuminuria, is commonly associated with the development of diabetic nephropathy (DN) in type 1 diabetes. An increase in albumin excretion rate leading to microalbuminuria is widely recognized as the earliest indicator of diabetic nephropathy and as a risk factor for the development of obvious renal and macrovascular complications. The current study was aimed at the development of a sensitive and specific immunoassay for Rat Serum Albumin (RSA) so that many nutraceutics can be screened and validated in experimental rats for their potential use as anti-DN nutraceutics in humans. PAb-RSA was generated, developed radioimmunoassay and ELISA that could detect as low as ~1 µg of RSA in the urine/serum and applicability was examined by evaluating the efficacy of dietary components - Curcumin and Quercetin, in offering protection against diabetic nephropathy. Curcumin and quercetin were found to be effective in amelioration of fasting blood glucose, urinary volume, urinary sugar and glomerular filtration rate. The results were compared with urinary albumin levels as estimated by RIA. A correlation coefficient (R2) ~ 0.95 - 0.97 was observed suggesting the possible use of these assays in monitoring of DN state as well as anti-DN nutraceutic's potency in experimental rat model.

**Key words:** Microalbuminuria, Curcumin, Quercetin, Radioimmunoassay, Diabetic nephropathy

## INTRODUCTION

Diabetic nephropathy (DN) is one of the major complications of long term diabetes characterized by progressive alterations in kidney structure and function (1). Approximately 25 to 40% of patients with Diabetes Mellitus ultimately develop DN (2). During diabetes, metabolism of food carbohydrates results in excessive accumulation of glucose in the blood and urine leading to hyperglycemia. Accumulated glucose enforces non-enzymatic glycation of proteins including albumin, a long lived and abundant protein in the blood leading to abnormal excretion through kidney; hence causing DN. The excretion of greater than 30 – 300 mg/day of albumin is characterized as microalbinuria and therefore is widely explored as a marker of DN (3). Filtration of glycated proteins through kidney during DN comprises of several manifestations, such as thickening of the glomerular basement membrane, diffuse or nodular glomerulosclerosis, arteriolar hylinosis, tubuointerstial fibrosis including increased excretion of albumin in the urine (or albuminuria). Of these, albuminuria is the most enigmatic form from a pathophysiologic standpoint (4).

Continuous excretion of increased albumin from kidney alters glomerular filtration rate, intraglomerular pressure, glomerular size, which occur due to cross – linking of glycated albumin and other affected membrane proteins (5). Indexing of elevated urinary albumin hence has been recommended as a routine and simple test to detect DN in diabetic subjects. Generally this can be detected by sensitive immunoassay technique, and the early diagnosis helps in controlling the progression to clinical nephropathy and end stage renal disease. Microalbuminuria not only predicts incipient DN but also reflects increased vascular permeability in hypertension and nondiabetic conditions (6, 7, 8)

***Corresponding author.**
***Dr. P.V. Salimath***
***Dept. of Biochemistry and Nutrition***
***Central Food Technological Research Institute***
***Mysore-570 020,India***

The concentration of albumin in urine is generally determined by immunoassays that are gaining importance due to their sensitivity and specificity. Since in our laboratory studies are directed towards the molecular understanding of basement membrane thickening in kidney, attempt has been made to raise polyclonal antibody to rat serum albumin (PAb-RSA) and develop sensitive immune assays that can be employed to follow diabetic state of experimental rats in addition to understanding the role of dietary modulation of DN. Anti-RSA antibodies have been employed to monitor the DN state and to understand the role of curcumin and quercetin in combating the DN state (9, 10).

### 2. Materials:

Rat serum albumin (RSA), Human serum albumin(HSA), Bovine serum albumin(BSA) and quercetin were obtained from M/s Sigma Pvt Ltd., USA, Freund's complete and incomplete adjuvants were obtained from M/s Genei Pvt. Ltd., Banglore, India, GOD/POD kit was from Span Diagnostics Limited (Surat, India). Curcumin (95% pure) was procured from Flavours and Essences, Mysore, India. Other chemicals were of analytical grade, obtained from SRL Pvt. Ltd., India.

### 2.1 Methods:

**Animals and diet**

Male Wistar rats (OUTB-Wistar IND CFTRI) weighing between 100 - 110 g were employed for the studies and was approved by Institutional Animal Ethical Committee. Animals were fed with AIN-76 diet (11) and six rats in control groups and 12 rats in diabetic groups. They were divided into a). starch fed control (SFC); b). starch fed diabetic (SFD) c). curcumin fed control (CFC); d). curcumin fed diabetic (CFD); e). quercetin fed control (QFC) and f). quercetin fed diabetic (QFD) groups. SFC and SFD groups received starch-based diet and CFC and CFD groups received diet supplemented with 0.5% curcumin and QFC and QFD groups received diet supplemented with 0.1% quercetin. The animals were fed with respective diet for a month after induction of diabetes.

**2.2 Induction and assessment of diabetic nephropathy**

Diabetes was induced by streptozotocin (STZ), prepared in 0.1 mol/ L citrate buffer, pH 4.5; injected intraperitonially in a single dose of 55 mg/ kg b.w. (10). Control rats received citrate buffer. Animals were kept in cages with free access to 5% glucose solution for 24 h subsequent to the injection and thereafter were replaced by tap water. Three days after STZ injection, blood glucose level was monitored using glucose oxidase/peroxidase (GOD/ POD) diagnostic kit method to assess the diabetic status and kidney function. Urine sugar (12), urine volume and glomerular filtration rates (GFR) (13) were measured.

**2.3 Production of Polyclonal Antibody to RSA**

Prior to immunization, sufficient preimmune blood was collected through the marginal ear vein of the rabbit. Serum was separated and stored at –20$^0$C. Briefly, 100 µg of RSA, dissolved in 1 mL of 0.01 M phosphate-buffered saline (PBS), pH 7.4, was mixed with an equal volume of Freund's complete adjuvant and injected subcutaneously at three sites. Two boosters at an interval of 15 days were given using Freund's incomplete adjuvant and postimmune serum was collected 6 days after the last booster dose, aliquoted, and stored at –20$^o$C. The post immune serum containing the antibody to RSA, designated as PAb-RSA (Polyclonal antibody to RSA) was used for subsequent experiments. The PAb-RSA was tested for its reactivity with HSA and BSA using Enzyme Linked Immunosorbent Assay - ELISA and Radio Immuno Assay (RIA). The amount of antibody present in the serum (antibody titre) was determined. PAb-RSA was examined for sensitivity, reliability and validation to detect micro / macro albuminuria condition in experimentally induced diabetic rats. Modulatory effect of curcumin and quercetin was also examined.

**2.4 Enzyme-linked Immunosorbent Assay (ELISA)**

The protocol was carried out as described by Dharmesh (14). Briefly, 96-well microtiter plates (Nunc; Roskilde, Denmark) were coated with HSA, RSA, and BSA using carbonate-bicarbonate coating buffer, pH 9.6, followed by incubation at 4$^0$C overnight. Next day wells were washed with PBS containing 0.05% Tween-20 (ELISA Wash buffer) and nonspecific binding sites were blocked with 5% of non-fat dry milk powder (Nakoda; Mumbai, India) in PBS (Blocking buffer) for 1 h at room temperature. Then, 100 µL of the postimmune serum and PAb-RSA at 1:100 to 1:50,000(v/ v) was added; unbound antibodies were removed by washing wells four times with ELISA wash buffer. Preimmune serum was tested at 1:1000 (v/ v). 100 µL of 1:5000 (v/v) alkaline phoshpatase-labeled goat anti-rabbit antibody (Genei, Banglore, India) was added and incubated at 37$^0$C for 1 h. Wells were then washed and immunoreactivity was measured by adding 100 µL of 1 mg/mL paranitrophenylphosphate as substrate in diaminoethyelene (DAE) buffer, pH 9.0 followed by incubation at 37$^0$C for 1 h. Reaction was stopped by 3N NaOH and color developed was measured at A405 nm in ELISA plate reader (Molecular devices, Spectra MAX 340). A concentration range of 1 to 100 µg/mL of native (RSA) and cross (HSA and BSA) antigens were examined.

**2.5 Radioimmunoassay for RSA**

Radioiodination of RSA (antigen) with $^{125}$I was done at the Radiation Medicine Center, BARC, Mumbai, India, according to Chloromine T method (15). The iodinated antigen was separated from free $^{125}$I using Sephadex G-50 column chromatography (column size 50 cm X 1 cm). Fractions were collected and emergence of iodinated RSA and free $^{125}$I was monitored by counting the fractions in scintillation counter. High molecular weight fraction representing $^{125}$I – RSA was isolated in first peak followed by peak containing free $^{125}$I peak. Labeling efficiency was calculated using the formula

$$\text{Labeling efficiency (\%)} = \frac{\text{Total counts in } ^{125}\text{I – protein + Reaction counts}}{\text{Total counts in crude fraction}}$$

Sensitivity of PAb-RSA by RIA was done by adding 200 µL of $^{125}$I labelled RSA to tubes containing 5 – 100 µg of unlabelled RSA as standards. To this 200 µL of PAb-RSA was added at 1:480 (v/v) and incubated for 2 h at 37ºC. The antigen-antibody complex was co-precipitated with 1 mL of 15% PEG containing 0.5% Bovine IgG (BoIg) and was centrifuged at 2000g, 10-15ºC for 30 min. The supernatant was removed and a count in the precipitate was measured in γ-counter.

**2.6 Applicability:**

Once PAb-RSA been generated and developed an assay system, applicability was examined by evaluating the efficacy of dietary components - Curcumin and Quercetin, in offering protection against DN. Various biochemical parameters such as fasting blood glucose, urinary volume, urinary sugar and glomerular filtration rate were determined between control, untreated diabetic and curcumin/quercetin treated animals. Results were compared with urinary albumin levels as estimated by RIA. Correlation coefficient (R2) was determined in order to understand the validity of the assay method developed in order to employ the same for routine monitoring of DN state in experimental animals.

**3. RESULTS:**

**3.1 Polyclonal antibody against Rat Serum Albumin (PAb-RSA)**

PAb-RSA was tested against the antigen RSA both by ELISA and RIA. ELISA showed that PAb-RSA has good antibody titer of 1:50,000 (v/v) and was sensitive to detect >5 µg/mL (Fig. 1). RIA was found to be effective at antibody dilutions < 1:1000 (v/v) (Fig. 2A) and sensitive, since it showed linearity with significant binding at concentration range ~ 5-20 µg/mL (Fig. 2B) at 1:480 (v/v) of PAb-RSA. Since detection of antigen by RIA is competitive unlike in direct ELISA, higher dilutions of the antibody did not show good binding particularly at lower concentration of antigen. However, data suggested that both ELISA and RIA can be operated at indicated concentration range of antigen with the antibody titre of 1:50,000 (v/v) and 1:480 (v/v) respectively.

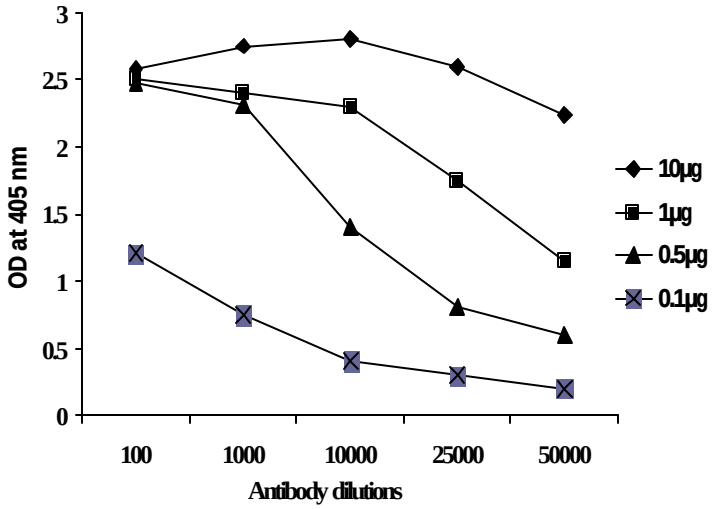


**Fig.1. Detection of antibody titer of PAb-RSA**

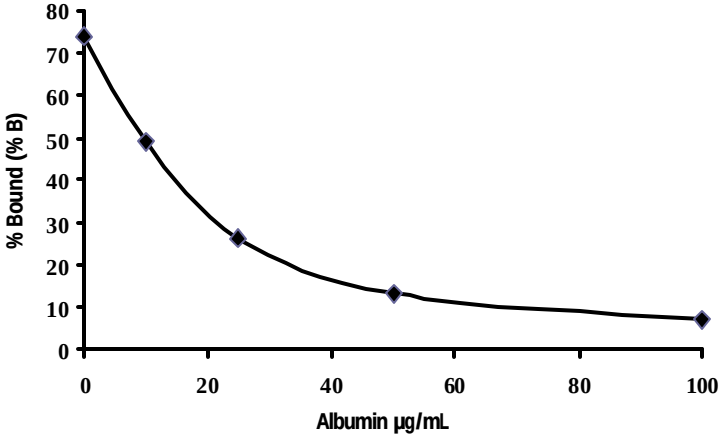


**Fig 2A. Standard graph for albumin by RIA**

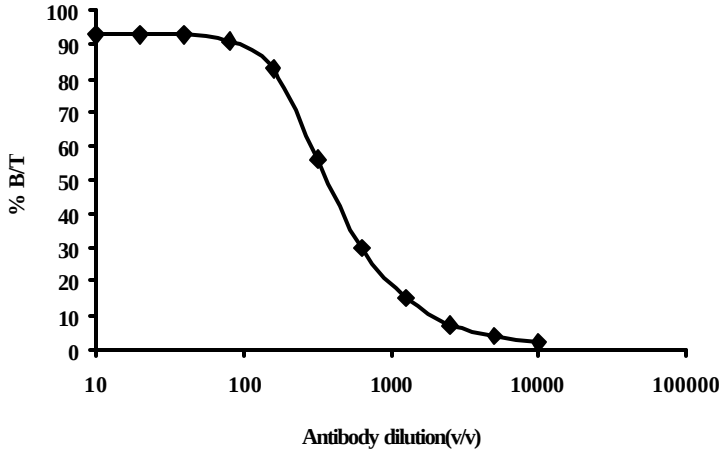


**Fig 2B. RSA antibody titre by RIA**

**3.2 Cross reactivity of polyclonal RSA (*PAb-RSA*) with HSA and BSA**

Cross reactivity of PAb-RSA to HSA and BSA was carried out by ELISA. Results in Fig. 3A clearly showed that PAb-RSA reacts best with the respective antigen RSA. At 1:50,000 (v/v), RSA showed better reactivity with increase in ~ 9-10 folds than HSA and BSA suggesting that PAb-RSA is specific to RSA. However at 1:10,000 (v/v) with 10 µg/mL of antigen concentration, ~ 40% of reactivity was observed with HSA and BSA (Fig. 3A) indicating that atleast at lower dilutions, this antibody can be also used for the analysis of human samples.

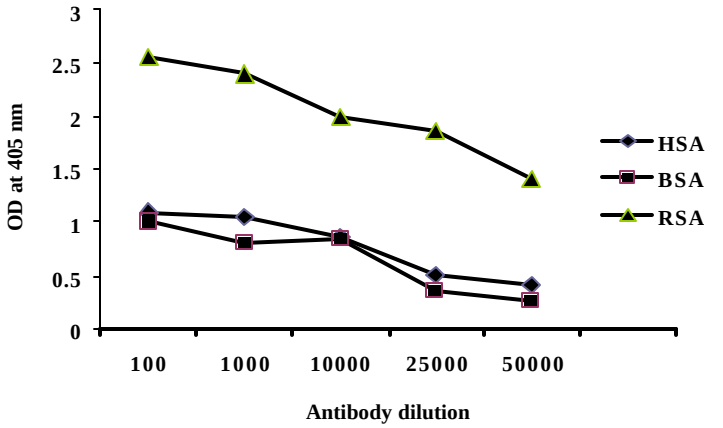


**Fig 3A. Cross reactive potential of PAb-RSA**

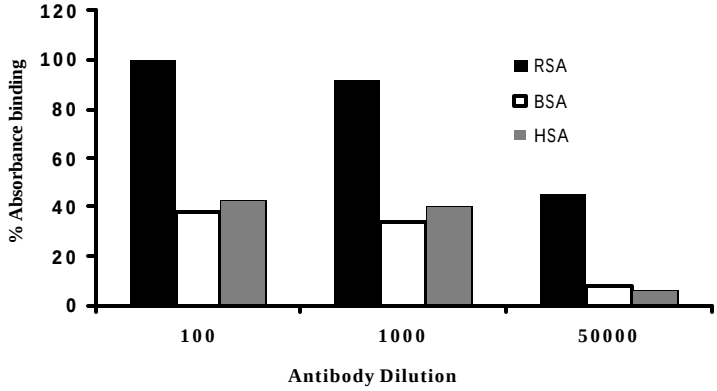


**Fig 3B. Cross reactive potential of PAb-RSA**

**3.3 Comparison between ELISA and RIA**

Fold increase in signal to noise ratio was calculated at different concentration of antigen by both ELISA and RIA at optimized PAb-RSA dilution. As indicated in Figs. 1 & 2, both ELISA and RIA are sensitive since similar range of albumin (1-100 µg) could be detected. It should be noted however that ~ > 20 fold differences in signal to noise ratio was obtained for RIA, when compared to ~ 10 folds in ELISA (Fig. 1 & 3). This clearly suggests that, for determining precise differences between the control and DN group of animals RIA can be preferred. Similarly for routine monitoring of DN state in diabetic animals, ELISA can be adopted, since it is easy-to-use and works out economically as it works at antibody dilution of 1:50,000 (v/v) as opposed to that of RIA, which requires 1:480 (v/v).

**3.4 Effect of curcumin and quercetin on DN**

RIA was employed to evaluate the effect of curcumin and quercetin on diabetic status in streptozotocin-induced diabetic rats. Validity of the results were established by comparing RIA data (Fig. 4) with conventional tests for diabetes such as measurement of fasting blood glucose level, urine sugar, urine volume and glomerular filtration rate (GFR) (Table 1). Results were compared between normal, diabetic and treated animal groups.

**Table-1. Effect of curcumin and quercetin on diabetic status**

| Groups | Fasting blood glucose(mL/dL) | Urine sugar (g/24 hr) | Urine volume (mL/24hr) | GFR mL/min | Albumin mg/day |
|---|---|---|---|---|---|
| SFC | 106.40 ± 02.23 | 0.06 ± 0.001 | 09.50 ± 1.09 | 0.41 ± 0.07 | 0.31 |
| SFD | 438.50 ± 19.59[a] | 8.30 ± 0.530[a] | 90.33 ± 7.26[a] | 02.1 ± 0.16[a] | 3.60 |
| CFC | 108.80 ± 02.60 | 0.04 ± 0.005 | 08.16 ± 1.05 | 0.48 ± 0.15 | 0.34 |
| CFD | 332.71 ± 26.33[b] | 5.75 ± 0.500[b] | 64.33 ± 6.33[b] | 01.2 ± 0.20[b] | 1.85 |
| QFC | 115.16 ± 04.71 | 0.04 ± 0.001 | 07.96 ± 1.08 | 0.44 ± 0.11 | 0.32 |
| QFD | 325.25 ± 26.20[b] | 5.17 ± 0.480[b] | 59.01 ± 5.09[b] | 01.7 ± 0.05[b] | 1.80 |

***All data were expressed as mean ± SD of control,diabetic and curcumin and quercetin treated rats. Statistical analysis of data was performed using one-way analysis of variance (ANOVA) with a Tukey's multiple comparison post-test and significance at***
***a P<0.05 indicates significant values when SFD was compared with SFC***
***b P<0.05 indicates significant values when SFD was compared with CFD and QFD groups***
***A correlation coefficient of R2***
***Albumin vs. Fasting blood glucose R=0.95***
***Albumin vs. Urine volume R=0.97***
***Albumin vs. Urine sugar R=0.97***
***Albumin vs. Glomerular filtration rate R=0.96***

The albumin excretion in urine in control, diabetic and curcumin and quercetin fed diabetic rats was measured by radioimmunoassay using the polyclonal antibody raised to rat serum albumin in rabbit. The results in Fig. 4 showed that in untreated or starch fed diabetic rats, the albumin excretion increased gradually from the 2nd week and at the 4th week it reached to a maximum of 3.5 mg/day compared to those of control rats (0.3 mg/day). It also showed ~ 4-10 folds higher glomerular filtration rate and urinary albumin excretion

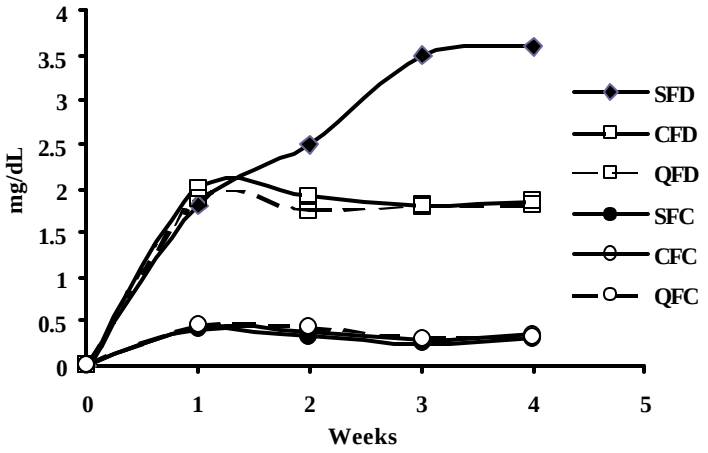


**Fig 4. Effect of curcumin and quercetin on albumin excretion in control and diabetic rats by radioimmunoassay**

when compared to control rats. Curcumin and quercetin fed control group showed values similar to that of starch fed control group. Curcumin and quercetin fed diabetic rats reduced the excretion of urinary albumin excretion by 40% at the 4th week, suggesting the efficacy of curcumin and quercetin in the control of DN. The developed assay being very sensitive and specific helps in monitoring the degree of kidney complications in DN state.

Correlation coefficient R2 was determined between urine albumin levels Vs. other characteristic diabetic parameters such as fasting blood glucose levels, urinary volume, urinary glucose and glomerular filtration rate. A correlation coefficient of R2 0.95 to 0.97 (Table – 1) was observed. Data thus suggest the validation of the developed assay method for the measurement of albumin levels with diabetic complication parameters.

## 4. DISCUSSION:

Screening of kidney function in individuals with type 2 diabetes not only provides a tool in identifying the progression or healing of disease process but enables the identification of patients who are at the risk of adverse renal complications due to diabetic nephropathy. Further, it also provides a scope for preventive interventions that can minimize the high risk of developing diabetic nephropathy in long term diabetic patients (16). In the present study attempt has been made to develop immunoassays for detection of DN and monitoring of the role of dietary components as modulators of DN, in experimental animal models, that are crucial for testing and validation of many potential antidiabetic and anti DN agents prior to employ in human use.

It is important to emphasize here that traditional method of detection of albumin involving microprecipitin method could detect the traces only in the advanced DN state in humans, where 30 – 300 mg of albumin per day is excreted. In case of rats as we have shown that DN state is achieved with the excretion of 3.5 mg/day which is difficult to detect by either colorimetic or dye binding or microprecipitin methods. Various investigators have developed analytical techniques such as HPLC and mass spectrophotometry (17, 18, 19). In these assays also the sensitivity ranges to > 5 µg/mL. However, such analyses pose problems in quantitating and comparing between the groups of animals. Currently developed PAb-RSA based immunoassay methods – both ELISA and RIA, however found to be useful as they were sensitive in detecting much lower levels of albumn that is ~1 µg/mL with a upper range of 100 µg/mL . Studies also substantiated the potential use of these assays in determining the modulation of DN state by dietary components such as curcumin and quercetin.

Curcumin, a major phenolic curcuminoid and quercetin, a bioflavonoid which is abundantly consumed, have been reported to have wide range of biological activities including hypoglycemic effect (20). In the current study we evaluate the effect of Curcumin/Quercetin upon feeding to diabetic rats as a validity testing for applicability. Results indicated significant lowering (2 mg/day) of urinary albumin concentration as measured by the developed RIA with the generated PAb-RSA. Data thus suggest the reliability of the assay to monitor the disease state during potential follow-up therapy and the ability of curcumin and quercetin in regulating the diabetic status in streptozotocin – induced diabetic rats. Similar efficacy of curcumin and quercetin at selected concentrations of 0.5% and 0.1% respectively as per previous standardizations (10) reveal that quercetin is a better modulator of DN than curcumin. Differences in the efficacy may be attributed to differences in the structure and increased bioavailability of quercetin (21).

Developed assays-RIA and ELISA helps also in monitoring of rate of accumulation of glycated albumin and its clearance from the serum in addition to the validation of nurtraceuticals against DN. Although ELISA assay kit is available, the limitations that it works at narrow range of signal to noise ratio poses difficulty in the precise indexing of anti DN potentials of various nurtraceuticals. Results of the current study validated the use of RIAs in distinguishing the degree of modulation of DN by nutraceutics.

It is to be noted that urinary albumin is very complex in nature and is biochemically modified by lysosomal enzymes into immunoreactive and immune - unreactive fragments due to extensive glycation (22, 23) before it is filtered by the kidney. The immunoreactive fragments therefore are the ones that are detected by antibodies. Immunoassays thus essentially measures albumin levels by virtue of immunoreactive domains and the reactivity being higher with increased immunoreactive albumin domains during advanced DN, correlation between the complexities of disease with RIA/ELISA reactivity may enable us also to make use of this RIA to determine the severity of the DN state and the extent of protection by varieties of antidabetic nutraceutics.

Early detection of DN has greater implications in terms of DN management (24, 25). It must be noted that the excretion of albumin into the urine is due to glycated albumin which mediates structural alterations in the kidney (26). Such serious changes encountered in the kidney enforced by glycated proteins are of complex type hence difficult to recover back by any kind of treatment. Early detection may put a break to constant alterations and hence prolong the life of the organ as well as the individual. In the light of the above, current investigation is significant, particularly in the early diagnosis and management of DN.

**ACKNOWLEDGEMENT:**

The authors thank Director, Central Food Technological Research Institute, Mysore, for his keen interest in the work and encouragement. Mr. Mallikarjun.B.C thanks Council of Scientific and Industrial Research (CSIR), India, for the award of fellowship.

## 5. REFERENCE

1) Zelmanovitz T, Gerchman F, Balthazar AP, Thomazelli FC, Matos JD, Canani LH. Diabetic nephropathy. Diabetol Metab Syndr, 1, 2009,10
2) National Kidney Foundation-Kidney Disease Outcomes Quality Initiative (NKF-KDOQI): Clinical practice guidelines and clinical practice recommendation for diabetes and chronic kidney disease. Am J Kidney Dis, 49, 2007, S1–S1.
3) American Diabetes Association: Standards of medical care in diabetes. Diabetes Care, 28, 2005, (Suppl.1):S4 –S36.

4) Viberti GC, Hill RD, Jarrett RJ, Argyropoulos A, Mahmud U, Keen H. Microalbuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. Lancet, 1, 1982, 1430–1432.
5) Dronavalli S, Duka I, Bakris GL. The pathogenesis of diabetic nephropathy. Nat Clin Pract Endocrinol Metab, 4, 2008, 444–452.
6) Endemann DH, Schiffrin EL. Endothelial dysfunction. J Am Soc Nephrol, 15, 2004, 1983–1992.
7) Matsushita K, van der Velde M, Astor BC, Woodward M, Levey AS, de Jong PE, Coresh J, Gansevoort RT. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. Lancet, 375, 2010, 2073–2081.
8) Wachtell K, Ibsen H, Olsen MH, Borch-Johnsen K, Lindholm LH, Mogensen CE, Dahlof B, Devereux RB, Beevers G, de Faire U, Fyhrquist F, Julius S, Kjeldsen SE, Kristianson K, Lederballe-Pedersen O, Nieminen MS, Okin PM, Omvik P, Oparil S, Wedel H, Snapinn SM, Aurup P. Albuminuria and cardiovascular risk in hypertensive patients with left ventricular hypertrophy: the LIFE study. Ann Intern Med, 139, 2003, 901–906.
9) Arun N, Nalini N. Efficacy of turmeric on blood sugar and polyol pathway in diabetic albino rats. Plant Foods Hum Nutr, 57, 2002, 41-51.
10) Shetty AK, Rashmi R, Rajan MGR, Sambaiah K, Salimath PV. Antidiabetic influence of quercetin in streptozotocin induced diabetic rats. Nutr Res, 24, 2004, 373-81.
11) Bieri JG, Stoewsand GS, Briggs GM, Phillips RW, Woolard JC, Knapka JJ. Report of the American Institute of Nutrition Ad Hoc Committee on standards for nutritional studies. J Nutr, 107, 1997, 1340-1348.
12) Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem, 31, 1959, 426-428.
13) Nandini CD, Sambaiah K, Salimath PV. Dietary fibers ameliorate decreased synthesis of HS in STZ induced diabetic rats. J Nutr Biochem, 14, 2003, 203-210.
14) Balasubramanian K, Vasudevamurthy R, Venkateshaiah SU, Thomas A, Vishweshwar A, Dharmesh SM. Galectin 3 in urine of cancer patients: stage and tissue specificity. J Cancer Res Clin Oncol, 135, 2009, 355-363.
15) Greenwood FC, Hunter WM, Glover JS, The preparation of 131I-labelled human growth hormone of high specific radioactivity. `Biochem. J, 89, 1963, 114–123.
16) Thomas MC. The assessment and management of albuminuria in primary care. Diabetes Res Clin Pract, 80, 2008, 83-88.
17) Babic N, Larson TS, Grebe SK, Turner ST, Kumar R, Singh RJ. Application of liquid chromatography-mass spectrometry technology for early detection of microalbuminuria in patients with kidney disease. Clin Chem, 52, 2006, 2155-2157.
18) Wayne D, Tanya MO, Malcom C, Richard JM, Macisaac, George J. Earlier detection of microalbuminuria in diabetic patients using a new urinary albumin assay. Kidney International, 65, 2004, 1850–1855.
19) McQueen MJ, Gerstein HC, Pogue J, Mann JF, Yusuf S. Re-evaluation by high-performance liquid chromatography: clinical significance of microalbuminuria in individuals at high risk of cardiovascular disease in the Heart Outcomes Prevention Evaluation (HOPE) Study. Am J Kidney Dis, 48, 2006, 889-896.
20) Suryanarayana P, Saraswat M, Mrudula T, Krishna TP, Krishnaswamy K, Reddy GB. Curcumin and turmeric delay streptozotocin-induced diabetic cataract in rats. Invest Ophthalmol Vis Sci, 46, 2005, 2092-2099
21) Manach C, Scalbert A, Morand C, Rémésy C, Jimenez L, Polyphenols:food sources and bioavailability. Am J Clin Nutr, 79, 2004, 727–747.
22) Osicka TM, Houlihan CA, Chan JG, Jerums G, Comper WD. Albuminuria in patients with type 1 diabetes is directly linked to changes in the lysosome-mediated degradation of albumin during renal passage. Diabetes, 49, 2000, 1579–1584.
23) Melissa JB, Sianna P, George J, Wayne DC. Alterations in renal degradation of albuminin early experimental diabetes in the rat: a new factor in the mechanism of albuminuria. Clinical Science, 95, 1998, 67–72.
24) Pavkov ME, Knowler WC, Hanson RL, Bennett PH, Nelson RG. Predictive power of sequential measures of albuminuria for progression to ESRD or death in Pima Indians with type 2 diabetes. Am J Kidney Dis, 51, 2008, 759-766.
25) Caramori ML, Fioretto P, Mauer M. Enhancing the predictive value of urinary albumin for diabetic nephropathy. J Am Soc Nephrol, 17, 2006, 339-352.
26) Cohen MP. Nonenzymatic glycation: a central mechanism in diabetic microvasculopathy? J Diabet Complications, 2, 1988, 214-217.

**Source of support: Nil, Conflict of interest: None Declared**