

**REGULAR ARTICLE****Elephant foot yam ameliorates activities of intestinal and renal disaccharidases in streptozotocin induced diabetic rats**

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ABSTRACT

Elephant foot yam (*Amorphophallus paeoniifolius* (Dennst.) Nicolson syn. *Amorphophallus campanulatus*) is a tuber vegetable used as an ingredient in ayurvedic preparations for various ailments. The present investigation deals with the effect of elephant foot yam extract on intestinal and renal disaccharidases in normal rats and streptozotocin induced diabetic rats. The specific activities of maltase, sucrase and lactase were measured in control and starch fed diabetic (SFD), elephant foot yam extract at 0.1% fed diabetic (YFD_{0.1}), elephant foot yam extract at 0.25% fed diabetic (YFD_{0.25}) and amino-guanidine fed diabetic (AFD) groups at the end of experimental period. Intestinal maltase, sucrase and lactase activities were high in SFD group compared to control, YFD_{0.1}, YFD_{0.25} and AFD groups. Amelioration of intestinal maltase activities by 18%, 26% and 48% was observed in YFD_{0.1}, YFD_{0.25} and AFD groups respectively when compared to SFD group. Intestinal sucrase activity was ameliorated in YFD_{0.1}, YFD_{0.25} and AFD groups to about 28%, 45% and 56% respectively. Lactase activity of intestine was improved by 36%, 52% and 64% in YFD_{0.1}, YFD_{0.25} and AFD groups respectively. On the contrary, specific activities of renal maltase, sucrase and lactase were decreased in SFD group compared to control groups. Supplementation of elephant foot yam extract in diet significantly ameliorated renal disachharidases activities in YFD_{0.1} and YFD_{0.25} groups. The results demonstrate the potential use of elephant foot yam for the management of diabetes.

1. Introduction

Diabetes mellitus is a chronic endocrine syndrome characterized by hyperglycemia and alterations in carbohydrate, protein and fat metabolism (Kumar et al., 2011). According to a press release by International Diabetes Federation (IDF) the number of people living with diabetes is expected to rise from 366 million in 2011 to 552 million by 2030 (International Diabetes Federation, 2011).

Effective management of diabetes without any undesirable effects is still a major problem to be addressed (Ayesha et al., 2008). Synthetic antidiabetic drugs are in use for the treatment of diabetes and exhibited significant therapeutic properties. However, usage of these drugs has been restricted due to adverse effects at higher doses and they are not suitable for use during pregnancy (Sunil et al., 2010). Hence, there is growing interest in the natural plant preparations which prevent side effects

(Suman et al., 2006).

Elephant foot yam (*Amorphophallus paeoniifolius* (Dennst.) Nicolson syn. *Amorphophallus campanulatus*) is a tuber crop commercially grown in India, Srilanka, China, Malaysia, Thailand, Indonesia and Philippines and in tropical regions of Africa. It is mainly used as vegetable and as an ingredient in ayurvedic preparations (Angayarkanni et al., 2007). The corm of elephant foot yam is restorative, carminative, stomachic and tonic (Chopra et al., 1958). It is used for piles, acute rheumatism, abdominal tumors, boils, asthma and enlargement of spleen (Yusuf et al., 1994). The tuber contains flavonoids, phenols, coumarins, terpenoids, sterols, tannins, steroids, alkaloids and sugars like glucose, galactose and rhamnose (Nataraj et al., 2009; Yadu and Ajoy 2010).

Elephant foot yam exhibited various functional properties like antibacterial, antifungal, cytotoxic (Angayarkanni et al., 2007; Khan et al., 2008b), anti-inflammatory (Dey et al., 2010), antioxidant (Angayarkanni et al., 2010) and hepatoprotective activity (Surendhra et al., 2011).

There is no scientific report available on the effect of elephant foot yam on intestinal and renal disaccharidases during diabetic condition. Hence, the present study was undertaken to evaluate the effect of phenolics rich acetone extract of elephant foot yam at 0.1% and 0.25% level in diet on intestinal and renal disaccharidases (maltase, sucrase and lactase) in streptozotocin induced diabetic rats.

2. Materials and Methods

2.1. Plant material

Fresh and healthy corms of elephant-foot yam (*A. paeoniifolius*) were procured from the local market of Mysore, Karnataka, India. The corm was identified and authenticated by Prof. Shivamurthy, Head, Department of Botany, University of Mysore, Mysore, India. The procured corms were washed, sliced into cubes and dried in a hot air oven at 40°C for 24 h and powdered to 60 mesh in an apex comminuting mill (Apex Constructions, London).

2.2. Chemicals

Streptozotocin, aminoguanidine, maltose, sucrose, lactose and p-dinitrosalicylic acid were obtained from Sigma chemicals, Co., (St. Louis, USA). All other chemicals used were of analytical grade.

2.3. Preparation of corm extract

The dried powder of elephant-foot yam was serially extracted with solvents (1:10 w/v) of increasing polarity, namely hexane, chloroform, ethyl acetate, acetone and methanol, on a shaker at 100 rpm for 48 h at room temperature. The extracts were filtered and concentrated by using rotary evaporator (Buchi Rota Vapor R-124). The concentrated extracts were lyophilized and stored in refrigerator. Acetone extract was used for the experiment because of its highest total phenol and flavonoid content when compared with other extracts.

2.4. Diet preparation

Acetone extract powder at 0.1% and 0.25% and aminoguanidine at 0.05% levels were incorporated in place of an equivalent amount of corn starch in AIN-76 basal diet containing 63.5% corn starch, 20% protein, 10% fat, 3.5% AIN-76 mineral mix, 1% AIN-76 vitamin mix and 0.2% choline chloride (Bieri et al., 1997). The prepared diet was stored at 4°C.

2.5. Animals

Male wistar rats (Breed-OUTB-Wistar, IND-CFT (2C)), weighing 140-160 g were obtained from Animal House Facility, Central Food Technological Research Institute (CFTRI), Council of Scientific and Industrial Research (CSIR), India, and housed in individual steel cages at animal house facility of the institute. The present study had the approval (Approval document number IAEC-92/06) of Institutional Animal Ethical Committee, CFTRI, Mysore, India.

2.6. Induction of diabetes

Animals were acclimatized to the laboratory conditions for at least 1 week before the experiment. After one week of acclimatization, the rats were subjected to 12 h fast and were rendered diabetic by a single intraperitoneal injection of streptozotocin (45 mg/kg body weight) in freshly prepared citrate buffer (pH 4.5, 0.1M). Animals with a fasting blood glucose level greater than 250 mg/dL were considered diabetic and included in the present study.

Rats were divided into eight groups, starch fed control and diabetic (SFC/SFD), acetone extract at 0.1% fed control and diabetic (YFC_{0.1}/ YFD_{0.1}), acetone extract at 0.25% fed control and diabetic (YFC_{0.25}/ YFD_{0.25}), and aminoguanidine (0.05%) fed control and diabetic (AFC/AFD). Control groups had 6 rats

and diabetic groups had 14 rats. The animals had free access to water and diet, which was in powder form. After twelve weeks of feeding, the rats were sacrificed under ether anesthesia.

2.7. Intestine and Kidney sample preparation

Small intestine and kidney were collected. The lumen of intestine was cut open longitudinally after washing with ice-cold saline. Intestinal mucosa was scraped with a glass slide and contents were collected into test tube. Final volume was made to 25ml using 0.9% saline and homogenized. The harvested kidney was homogenized by using 0.9% saline in the ratio of 1:10. The kidney homogenate was centrifuged at 3500 g for 10 min at 4°C. Supernatant thus obtained was used for the assay of disaccharidases.

2.8. Disaccharidases Assay

The specific activities of intestinal and renal sucrase, maltase and lactase were estimated and correlated to the amount of glucose released from sucrose, maltose and lactose, respectively. The assay was carried out at 37°C in maleate buffer (0.2 M, pH 6.0) at different time intervals as per the methodology described by Dahlqvist (Dahlqvist 1954). Protein content in the samples was determined by Lowry's method (Lowry et al., 1951).

2.9. Statistical Analysis

All the data are expressed as mean \pm SEM of 6 rats in control groups and 8 rats in all the diabetic groups. Data are analyzed by one-way analysis of variance (ANOVA) using Microsoft Excel XP^R (Microsoft Corporation, USA), and post-hoc mean separations were performed by Duncan's Multiple Range Test (DMRT) at $p<0.05$ (Harter, 1960).

3. Results

3.1. Specific activities of intestinal disaccharidases in control and diabetic groups

Fig. I, II and III show the effect of acetone extract of elephant foot yam on specific activities of maltase, sucrase and lactase in control and diabetic rats. The enzyme activities of SFC, YFC_{0.1}, YFC_{0.25} and AFC groups were not significantly ($P > 0.05$) different from one another. Maltase, sucrase and lactase activities were high in diabetic groups when compared to control groups. Specific activities of intestinal disaccharidases were high in SFD group when compared to YFD_{0.1}, YFD_{0.25} and AFD groups. Activity of maltase in SFD group was 692 nmoles/mg/

min, whereas, in YFD_{0.1}, YFD_{0.25} and AFD groups it was 569, 509.8 and 497.45 nmoles/mg/min, respectively. Sucrase activity in SFD group was 108.76 nmoles/mg/min and was ameliorated in YFD_{0.1}, YFD_{0.25} and AFD groups, which had sucrase activity of 78.86, 60.06 and 47.74 nmoles/mg/min, respectively. Similarly, intestinal lactase activity was reduced significantly ($P < 0.05$) in YFD_{0.1} (8.86 nmoles/mg/min), YFD_{0.25} (6.83 nmoles/mg/min) and AFD (5.13 nmoles/mg/min) groups when compared to SFD (14.38 nmoles/mg/min) group.

3.2. Specific activities of renal disaccharidases in control and diabetic groups

Unlike intestinal disaccharidases, the specific activities of renal sucrase, maltase and lactase decreased significantly ($P < 0.05$) in diabetic groups when compared to controls. There was no significant ($P > 0.05$) difference in the renal disaccharidases activity among control groups. The maltase activity of SFD group in kidney (Fig. IV) was 452.49 nmoles/mg/min, whereas, its activity was ameliorated to 316, 402 and 435 nmoles/mg/min in YFD_{0.1}, YFD_{0.25} and AFD group respectively. Sucrase activity in SFD group (Fig. V) was 0.81 nmoles/mg/min, whereas, in YFD_{0.1}, YFD_{0.25} and AFD groups it was 1.02, 1.33 and 1.46 nmoles/mg/min, respectively. Similarly, renal lactase activities (Fig. VI) was improved in YFD_{0.1} (3.24 nmoles/mg/min), YFD_{0.25} (4.08 nmoles/mg/min) and AFD (4.95 nmoles/mg/min) groups when compared to SFD (2.34 nmoles/mg/min) group.

4. Discussion

The mucosa of intestine is dynamic in nature that undergoes biochemical and morphological changes in its lifetime (Thomson and Wild, 1997a). The absorptive cells of the small intestine have the ability to adapt themselves with respect to changes in the intraluminal content of nutrients (Henning, 1981). Disaccharidases, such as maltase, sucrase, and lactase, localize in the brush border membrane of small intestinal mucosa. They hydrolyze disaccharides into monosaccharides and help in the terminal absorption of carbohydrates (Jamuna et al., 2010). The hydrolyzing activity of disaccharidases in the small intestine has been studied in humans and experimental animals (Tsuiji et al., 1986).

Intraperitoneal injection of streptozotocin, produced from *Streptomyces achromogenes*, damages β -cells of pancreas resulting in hyperglycemia (Kumar et al., 2011). Internal glucose levels are not

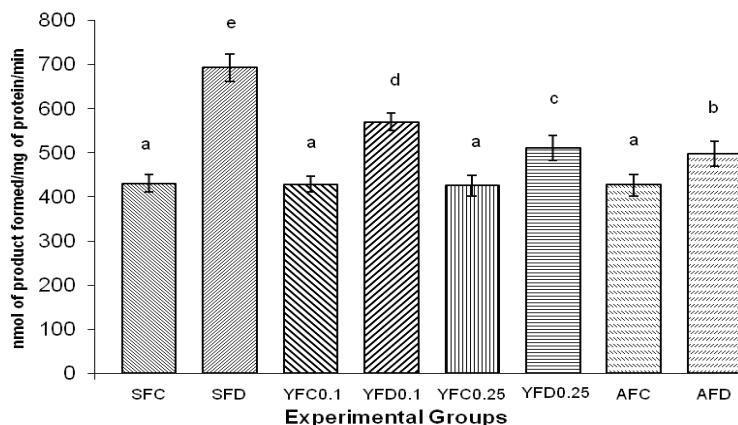


Figure 1: Effect of acetone extract of elephant foot yam on intestinal maltase activity (nmol of product formed/mg of protein/min) in control and diabetic rats. SFC: starch fed control, SFD: starch fed diabetic, YFC_{0.1}: elephant foot yam extract at 0.1 per cent fed control, YFD_{0.1}: elephant foot yam extract at 0.1 per cent fed diabetic, YFC_{0.25}: elephant foot yam extract at 0.25 per cent fed control, YFD_{0.25}: elephant foot yam extract at 0.25 per cent fed diabetic, AFC: aminoguanidine fed control, AFD: aminoguanidine fed diabetic. Values are expressed as mean ± Standard Deviation (SD) of control and diabetic groups (Value of 'n' is 6 for control groups and 8 for diabetic groups) at $P < 0.05$.

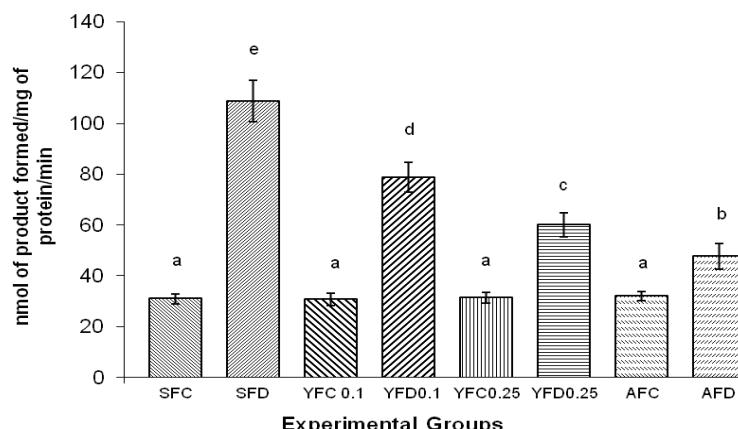


Figure 2: Effect of acetone extract of elephant foot yam on intestinal sucrase activity (nmol of product formed/mg of protein/min) in control and diabetic rats. SFC: starch fed control, SFD: starch fed diabetic, YFC_{0.1}: elephant foot yam extract at 0.1 per cent fed control, YFD_{0.1}: elephant foot yam extract at 0.1 per cent fed diabetic, YFC_{0.25}: elephant foot yam extract at 0.25 per cent fed control, YFD_{0.25}: elephant foot yam extract at 0.25 per cent fed diabetic, AFC: aminoguanidine fed control, AFD: aminoguanidine fed diabetic. Values are expressed as mean ± Standard Deviation (SD) of control and diabetic groups (Value of 'n' is 6 for control groups and 8 for diabetic groups) at $P < 0.05$.

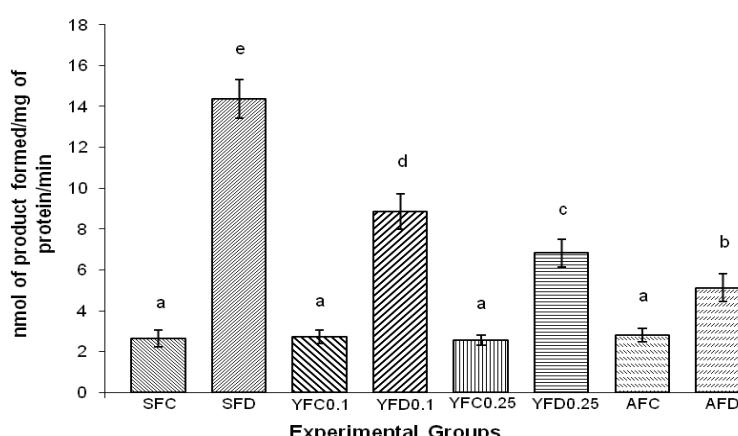


Figure 3: Effect of acetone extract of elephant foot yam on intestinal lactase activity (nmol of product formed/mg of protein/min) in control and diabetic rats. SFC: starch fed control, SFD: starch fed diabetic, YFC_{0.1}: elephant foot yam extract at 0.1 per cent fed control, YFD_{0.1}: elephant foot yam extract at 0.1 per cent fed diabetic, YFC_{0.25}: elephant foot yam extract at 0.25 per cent fed control, YFD_{0.25}: elephant foot yam extract at 0.25 per cent fed diabetic, AFC: aminoguanidine fed control, AFD: aminoguanidine fed diabetic. Values are expressed as mean ± Standard Deviation (SD) of control and diabetic groups (Value of 'n' is 6 for control groups and 8 for diabetic groups) at $P < 0.05$.

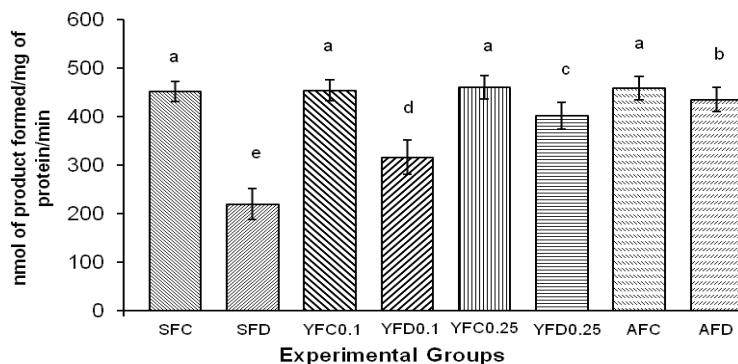


Figure 4: Effect of acetone extract of elephant foot yam on renal maltase activity (nmol of product formed/mg of protein/min) in control and diabetic rats. SFC: starch fed control, SFD: starch fed diabetic, YFC_{0.1}: elephant foot yam extract at 0.1 per cent fed control, YFD_{0.1}: elephant foot yam extract at 0.1 per cent fed diabetic, YFC_{0.25}: elephant foot yam extract at 0.25 per cent fed control, YFD_{0.25}: elephant foot yam extract at 0.25 per cent fed diabetic, AFC: aminoguanidine fed control, AFD: aminoguanidine fed diabetic. Values are expressed as mean \pm Standard Deviation (SD) of control and diabetic groups (Value of 'n' is 6 for control groups and 8 for diabetic groups) at $P < 0.05$.

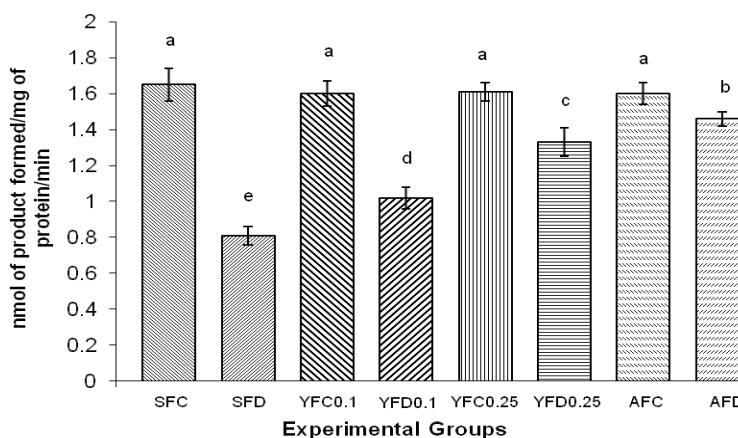


Figure 5: Effect of acetone extract of elephant foot yam on renal sucrase activity (nmol of product formed/mg of protein/min) in control and diabetic rats. SFC: starch fed control, SFD: starch fed diabetic, YFC_{0.1}: elephant foot yam extract at 0.1 per cent fed control, YFD_{0.1}: elephant foot yam extract at 0.1 per cent fed diabetic, YFC_{0.25}: elephant foot yam extract at 0.25 per cent fed control, YFD_{0.25}: elephant foot yam extract at 0.25 per cent fed diabetic, AFC: aminoguanidine fed control, AFD: aminoguanidine fed diabetic. Values are expressed as mean \pm Standard Deviation (SD) of control and diabetic groups (Value of 'n' is 6 for control groups and 8 for diabetic groups) at $P < 0.05$.

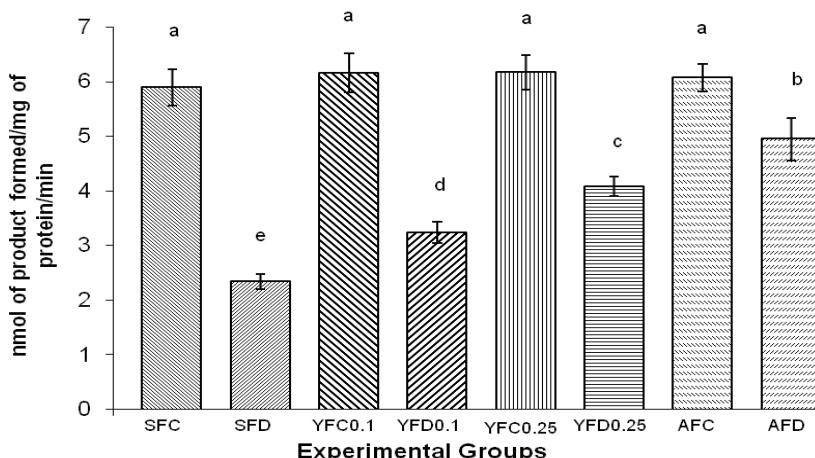


Figure 6: Effect of acetone extract of elephant foot yam on renal lactase activity (nmol of product formed/mg of protein/min) in control and diabetic rats. SFC: starch fed control, SFD: starch fed diabetic, YFC_{0.1}: elephant foot yam extract at 0.1 per cent fed control, YFD_{0.1}: elephant foot yam extract at 0.1 per cent fed diabetic, YFC_{0.25}: elephant foot yam extract at 0.25 per cent fed control, YFD_{0.25}: elephant foot yam extract at 0.25 per cent fed diabetic, AFC: aminoguanidine fed control, AFD: aminoguanidine fed diabetic. Values are expressed as mean \pm Standard Deviation (SD) of control and diabetic groups (Value of 'n' is 6 for control groups and 8 for diabetic groups) at $P < 0.05$.

constant in the cells damaged by hyperglycemia (Heilig et al., 1995). Intestinal absorption of glucose is high during diabetes due to increase in the length of villi and expression of the monosaccharide transporters (Dyer et al., 2002). Structural and functional changes like hyperplasia, increased disaccharidases activity and alterations in glucose uptake and transport were noticed in the intestine during diabetes (Fedorak et al., 1989; Burant et al., 1994). Alterations in the activities of disaccharidases were also observed to some extent in renal cortex (Murakami and Ikeda, 1998). This may be due to its involvement in the digestion or transport of sugars across the membranes (Taylor et al., 1972). Decrease in renal disaccharidases activity was observed during diabetes (Jamuna et al., 2010).

Alterations in intestinal and renal disaccharidases (maltase, sucrase and lactase) and increased intestinal absorption of glucose during diabetes result in the formation of advanced glycation end (AGEs) products by Maillard reactions (Vlassara et al., 2002). This further complicates the disease by generation of reactive oxygen free radicals and impairment of antioxidant defense mechanism (Jamuna et al., 2010). Hence, amelioration of disaccharidases activities is of prime importance in the management of diabetes.

Foods rich in phytochemicals and medicinal value were found to be beneficial in the dietary management of diabetes (Loizzo et al., 2011). Scientific investigations revealed that regular intake of phytochemical rich fruits and vegetables like apple, karonda, bitter gourd, garlic, pseudostem of banana and purple yam were proved to lower the risk of diabetes (Day et al., 1990; Platel and Srinivasan, 1997; Harunobu et al., 2009; Adyanthaya et al., 2010; Prakash et al., 2011; Jamuna et al., 2011; Maithili et al., 2011).

In the present investigation, feeding of acetone extract of elephant foot yam at 0.1% and 0.25% level in diet ameliorated intestinal maltase activities by 18% and 26% in YFD_{0.1} and YFD_{0.25} groups respectively, whereas, AFD group showed 48% amelioration when compared to SFD group. Intestinal sucrase activity was high in SFD group and was ameliorated in YFD_{0.1}, YFD_{0.25} and AFD groups to about 28%, 45% and 56% respectively. Intestinal lactase activity was high in SFD group and was ameliorated in YFD_{0.1}, YFD_{0.25} and AFD groups to about 36, 52 and 64%, respectively. In contrast specific activities of renal maltase, sucrase and lactase

were decreased in SFD group and were ameliorated in YFD_{0.1}, YFD_{0.25} and AFD groups. Similar results on activities of intestinal and renal disaccharidases during diabetes were reported (Ramachandra et al., 2005; Jamuna et al., 2010).

The beneficial role of elephant foot yam extract in the alleviation of intestinal and renal disaccharidases during diabetes may be attributed to its bioactive components. Phytochemical investigation of elephant foot yam revealed the presence of phenols and flavonoids (Khan et al., 2008b; Nataraj et al., 2009). Role of polyphenols in the management of diabetes has been well documented (Jalil et al., 2008; Adyanthaya et al., 2010). Gallic acid, an important polyphenol, was found to inhibit sucrase, lactase, maltase and trehalase activities in rodent intestine (Gupta et al., 2007). Phenolic compounds such as tannic acid and catechol inhibited sucrase activity in birds (Ahmed et al., 1991). Quercetin (bioflavonoid) present in fruits like apple and vegetables like onion alleviated the activities of intestinal and renal disaccharidases in streptozotocin-induced diabetic rats (Ramachandra et al., 2005).

5. Conclusion

The results clearly indicate that elephant foot yam extract play beneficial role by ameliorating the activities of intestinal and renal disaccharidases in streptozotocin induced diabetic rats. Elephant foot yam extract can be utilized in the development of functional foods for the management of diabetes and its related complications.

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