REMNANT B-CELL-STIMULATIVE AND ANTI-OXIDATIVE EFFECTS OF *PERSEA AMERICANA* FRUIT EXTRACT STUDIED IN RATS INTRODUCED INTO STREPTOZOTOCIN - INDUCED HYPERGLYCAEMIC STATE

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Abstract

Insulin-stimulative and anti-oxidative effects of *Persea americana* fruit extract were evaluated using streptozotocin (STZ). Ethanol extract of *P. americana* in the concentration of 300 mg/kg body weight/rat /day was orally administered to rats introduced into STZ-induced hyperglycaemic state for a period of 30 days. After the treatment with *avocado* fruit extract, the elevated levels of blood glucose, glycosylated haemoglobin, blood urea and serum creatinine seen in the hyperglycaemic rats, reverted back to near normal. Similarly, significantly decreased plasma insulin and haemoglobin levels went back to near normal after the treatment, suggesting the insulin-stimulative effect of *P. americana* fruit. Determination of thiobarbituric acid reactive substances (TBARS), hydroperoxides and both enzymatic and non-enzymatic antioxidants, confirmed the anti-oxidative potential of *avocado* fruit extract which, in turn, might be responsible for its hypoglycaemic potential. Changes in activities of enzymes such as serum aspartate transaminase (AST), serum alanine transaminase (ALT), and serum alkaline phosphatase (ALP) seen in the control and experimental rats, revealed the tissue-protective nature of *Persea americana* fruits, while all of the analysed biochemical parameters were comparable to those obtained with gliclazide as a standard reference drug.

Key words: Antioxidants. Insulin-stimulative. , Persea americana. *Avocado*. STZ-induced diabetes

Introduction

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action, or both **(**Bastaki, 2005). In turn, insulin deficiency leads to chronic hyperglycaemia accompanied by the disturbance in carbohydrate, fat, and protein metabolism; it is a global disease, prevailing throughout the world, although its prevalence differs across countries **(**Adeghate et al., 2006), India, China, and US being the top-three when it comes to the number of hyperglycaemic patients they host (Wild et al.,2004). Increasing ageing population, consumption of calorie-rich diet, obesity, and sedentary lifestyle have led to the tremendous increase in number of hyperglycaemic patients worldwide.

Current treatment includes insulin therapy which enables for a good glycaemia control, but can do very little when it comes to the prevention of secondary complications. In addition, these drugs are associated with side-effects or diminution in response after prolonged use (Chattopadhyay, 1999) Moreover, due to economic constraints, the provision of modern healthcare across the world is still a far-off goal. Thus, it is necessary for us to continue looking for a new and, if possible, more efficacious management, and the vast reserves of phytotherapy may just as well be an ideal area of interest in this regard.

For thousands of years, plants have played a significant role in maintaining human health and improving the quality of life. In particular, herbs have been used for centuries as food and medicinals. In herbal medicine, the term refers not only to seed–producing plants but also to bark, roots, leaves, seeds, flowers, and fruit. According to the World Health Organization, about three quarters of the world's population rely upon traditional medicine when it comes to their primary healthcare needs, and most of these treatments involve the use of plant extracts or their active components (Egan, 2002). However, the acting mechanism of most herbal medicines has not been fully understood yet, but the experience gained with their traditional use over the years should not be ignored (Elvin-Lewis, 2001). Therefore, it is prudent to look for herbal medicine-based options when it comes to diabetes treatment as well. East Africa is very rich in natural resources, while the knowledge on traditional medicine and the use of remedial plants represents an innate and very important component of the healthcare system.

The *avocado*, unflatteringly known in the past as alligator pear, midshipman's butter, vegetable butter, or sometimes as butter pear, and called by Spanish-speaking people *aguacate*, *cura*, *cupandra*, or *palta*; in Portuguese, *abacate*; in French, *avocatier*; is the only important edible fruit of the Laurel family, Lauraceae. From the botanical standpoint, this family is classified into three groups: A), *Persea americana* Mill. var. *americana* (P. *gratissima* Gaertn.), West Indian *Avocado*; B) *P. americana* Mill. var. *drymifolia* Blake (*P. drymifolia* Schlecht. & Cham.), the Mexican *Avocado*; C) *P. nubigena* var. *guatemalensis* L. Wms., the Guatemalan *Avocado*. It has traditionally been used due to its antibacterial, antifungal, hypotensive, anti-inflammatory, and immune-enhancing effect (Adeyemi et al., 2002). Furthermore, *avocado* juice made from ripe fruit was very popular due to its numerous health benefits. The *avocado* tree may be erect, usually up to 30 ft (9 m) but sometimes even up to 60 ft (18 m) or over 60 ft tall, with a trunk 12 to 24 in (30-60 cm) in diameter, (greater in very old trees), or short and spreading with branches beginning close to the ground. Almost evergreen, being shed briefly in dry seasons at blooming time, the leaves are alternate, dark-green and glossy on the upper surface, whitish on the

underside; variable in shape (lanceolate, elliptic, oval, ovate or obovate), and 3 to 16 in (7.5-40 cm) long. Because of the limited number of reports on the fruits of *avocado* available in the literature, it was deemed prudent and justified to systematically investigate the fruits of this plant. The present study was aimed at evaluating the insulin-stimulative and anti-oxidative potential of *avocado* fruit extract in experimental rat model introduced into STZ-induced hyperglycaemic state.

Avocado has a high lipid content ranging from 5 to 25%, depending on the cultivar. As regards saturated fatty acids, myristic acid share may amount to 1%, that of palmitic acid to 7.2, 14.1 or 22.1%, and that of stearic acid to 0.2, 0.6 or 1.7%. As for unsaturated fatty acids, palmitoleic acid share may range from 5.5 to 11.0%, while that of oleic acid may equal to 51.9, 70.7 or 80.97%, and that of linoleic acid to 9.3, 11.2 or 14.3%. Non-saponifiable fats are represented by the percent-share spanning from 1.6 to 2.4%. Iodine number is 94.4. Pulp amino-acids (N = 16 p. 100) were recorded to be represented as follows: arginine, 3.4; cystine, 0.1: histidine, 1.8; isoleucine, 3.4; leucine, 5.5; lysine, 4.3; methionine, 2.1; phenylalanine, 3.5; threonine, 2.9; tryptophan, 0.1; tyrosine, 2.3; valine, 4.6; aspartic acid, 22.6; glutamic acid, 12.3; alanine, 6.0; glycine, 4.0; proline, 3.9; serine, 4.1 (Kadam, and Salunkhe, 1995)

Materials and methods

Plant material

Fresh fruits of *P. americana* were collected from its natural habitat in the Agricultural Research Center, Tepi, Ethiopia, and authenticated in the Department of Biology, Mizan-Tapi University, Tepi. The seed was removed, while the edible part was chopped into small pieces, dried at 50-60^{0}C and grounded into powder. The known amount of dry powder was repeatedly extracted via maceration in an aspirator using 95%-ethanol as menstruum. The extract was concentrated under reduced pressure using rotary evaporator so as to obtain a thick syrup mass, and stored at 4^{0}C. The yield represented approximately 10% of the parental fresh fruit quantity. Before being used in the experiment, working extract concentrations were prepared in non-pyrogenic distilled water.

Experimental animals

Male Wistar albino rats weighing 160-180g were used in the present study. The rats were procured from Tepi Veterinary Centre, Tepi, Ethiopia. The rats were acclimatized and maintained over husk bedding in polypropylene cages. Throughout the experimental period, the rats were fed with a balanced commercial pellet diet (Hindustan Lever Ltd., Bangalore, India) composed of 5% of fat, 21% of protein, 55% of nitrogen-free extract, and 4% of fibbers (w/w) with both mineral and vitamin contents adequate to meet the needs of the animals used. Food and water were provided *ad libitum*. IAEC Adequate animal ethical care with taken via No. 01 / 013 / 08.

Toxicity and dosage fixation studies

Acute toxicity studies with *P. americana* fruit extract were performed in experimental rats. Graded doses of ethanol extract of *avocado* fruits (100, 250, 500, and 1000 mg/kg body weight) were administered orally, and the animals were subsequently observed for 2 weeks. Changes in body weight, food consumption, haematological, macroscopic, and clinical-biochemical findings, including the activities of enzymes, were noted. Dosage fixation studies were carried out by virtue of unequally long administration of graded doses of *P. americana* fruit extract (100, 200, 300, 400 and 500 mg/kg body weight), given to rats introduced into STZ-induced hyperglycaemia; it was found that the fruit extract shows its maximal hypoglycaemic effect at the concentration of 300 mg/kg body weight administered orally for 30 days. Hence, the dosage was fixed at 300 mg/kg body weight /rat/day and pursued for 30 days.

Experimental design

Rats were fasted overnight and experimental diabetes was induced by virtue of intra-peritoneal injection of streptozotocin (STZ) applied in a single dose of 50 mg/kg body weight. STZ was dissolved in a freshly-prepared 0.1M cold citrate buffer PH4.5 (Rakieten et al., 1963). The control rats were injected with citrate buffer in a similar manner. Since STZ is capable of inducing fatal hypoglycaemia arising as a result of massive pancreatic insulin release, 6 hrs post induction the STZ-treated rats were provided with 10% glucose solution for the next 24 hrs so as to prevent severe hypoglycaemia. Neither death nor any other adverse effect was observed. After 3 days needed for the development and aggravation of diabetes, rats with moderate diabetes (i.e. blood glucose concentration of 250 mg/dl) that exhibited glycosuria and hypoglycaemia were selected for experiment (Canepa et al., 1990). The animals were divided into four groups, each of them comprising six animals as follows: control rats (group I), rats introduced into STZ-induced hyperglycaemia (group II), hyperglycaemic rats treated with *P. americana* fruit extract (300 mg/kg body weight) aqueous solution for 30 days (group III), and hyperglycaemic rats given a single dose of gliclazide (5 mg/kg body weight) in an aqueous solution for 30 days (group IV) (Pulido et al .,1997). Changes in body weight seen across rat groups were recorded at regular intervals. After 30 days of treatment, rats were fasted overnight and sacrificed using cervical dislocation manoeuvre. Blood was collected and stored with and without an anticoagulant added.

Biochemical parameters

Wool blood was used for glucose (Sasaki et al., 1972) and urea (Natelson et l., 1951) estimation. Plasma was separated and used for insulin radioimmunoassay (RIA) kit for rats (Linco Research, Inc., USA). Levels of haemoglobin and glycosylated haemoglobin were estimated according to the methods of Drabkin and Austin (1932) and Nayak and

Pattabiraman (1981), respectively. Plasma was used for protein assay (Lowry et al., 1951), while serum served the purpose of determination of creatinine levels (Brod and Sirota, 1984). Activities of enzymes such as aspartate transaminase (AST), serum alanine transaminase (ALT), and serum alkaline phosphatase(ALP) were assayed using the method established by King (1965a,b).

Liver tissue was excised, washed in ice-cold saline, and then homogenized in Tris-HCl buffer (pH 7.4) using a Teflon homogenizer. Liver homogenate was then centrifuged at 5,000 x g to remove cellular debris; the supernatant was thence used for the determination of lipid peroxide and both enzymatic and non-enzymatic antioxidant levels. Lipid peroxidation was determined using thiobarbituric acid reactive substances and the method of Ohkawa et al., (1979), while hydro-peroxides were estimated using the method established by Jiang et al., (1992). The levels of *ascorbic acid*, *tocopherol*, and glutathione (GSH) were determined using the methods of Omaye et al., (1979), Desai (1984), and Sedlak and Lindsay (1968), respectively. Enzymatic antioxidants such as superoxide dismutase (Misra and Fridovich, 1972), catalase (Takahara et al., 1960), glutathione peroxidase (Rotruck et al., 1973) and glutathione-S-transferase (Habig et al., 1974) were assayed in the liver supernatant.

Statistical analysis

All-group data were statistically evaluated using SPSS 16.00 software. The hypothesis-testing methods included One-Way Analysis of Variance followed by the Least Significant Difference (LSD) test. The p-value of <0.05 was considered statistically significant. All of the results are expressed as means $\pm$ standard deviation (SD) referring to a six-rat group under consideration.

Results and Discussion

In the present study, all animals survived and showed no signs of toxicity or behavioural changes during the course of the acute toxicity studies; on the contrary, they all appeared to be healthy and gained weight. Clinical chemistry including AST, ALT, ALP, blood urea, and serum creatinine levels revealed no differences between the control and the fruit extract-administered rat groups. Similarly, no negative impact on haematological parameters had been noted. Thus, it is evident that oral administration of *avocado* fruit extract at the dose of 1,000 mg/kg body weight is not toxic to the system. Studies in Sprague-Dawley rats have showed that oral 50%-lethal dose (LD_{50}) of *avocado* fruits reaches above 15,000 mg/kg body weight.

Figure 1 shows changes in body weight of the control and experimental rat groups. Decreased body weight seen in hyperglycaemic, STZ-induced diabetic rats was improved following oral administration of both *avocado* fruit extract and gliclazide. Diabetes mellitus causes a drastic change in body weight (Al-Shamanoy et al., 1994), which may develop due to an excessive breakdown of tissue proteins and lipids caused by insulin insufficiency. The improvement in body weight seen in hyperglycaemic rats treated with *P. americana* extract might be underpinned by an improved metabolic activity, making the body system more capable of maintaining blood glucose homeostasis.

Table 1 shows the levels of blood glucose, haemoglobin, glycosylated haemoglobin, plasma insulin, total proteins, blood urea, and serum creatinine in the control and experimental rat groups. Blood glucose is the key marker utilised within diabetes mellitus diagnostics and prognostics. As a result of an excessive production of endogenous glucose by hepatic as well as by extra-hepatic tissues through gluconeogenic and glycogenolytic pathways and condensed utilization of glucose by various tissues, insulin deficiency causes radical elevations in blood glucose levels, i.e. the classical diabetes mellitus state **(**Soling and Kleineke, 1976). In the present study, oral treatment with *avocado* extract as well as that with gliclazide, appreciably decreased blood glucose levels and increased the insulin level in STZ-induced diabetic rats. Grover and Vats (2001) reported the anti-hyperglycaemic potential of medicinal plant extract to be normally reliant on the degree of β-cell damage. The anti-hyperglycaemic effect of *avocado* fruits may arise due to the stimulatory effect on remnant β-cells, making them capable of secreting more insulin, or due to the favourable effect of fruits in question on regenerated β-cells. This was evidently demonstrated by the increased levels of insulin seen in hyperglycaemic rat groups treated with *P. americana* fruit extract (Bartholomew, 2007).

Glycosylated haemoglobin is considered as a gold-standard marker utilised to the goal of an accurate and reliable measurement of fasting glucose; it is closely associated with the level of ambient glycaemia registered during a 3-month period and indicates the degree of protein glycation. Chronic hyperglycaemia results in glycosylation in which excess glucose non-enzymatically reacts with haemoglobin to form glycosylated haemoglobin (Koenig et al., 1976). This condition favours the reduction in haemoglobin levels and the concomitant increase in glycosylated haemoglobin levels, directly proportional to supra-physiological glucose (Alyassin and Ibrahim, 1981). The decrease in glycosylated haemoglobin levels observed in hyperglycaemic rats treated with *P. americana* fruit extract, may be witnessed due to the fruit's anti-hyperglycaemic activity, which in turn shows that *P. americana* fruit extract prevents the formation of glycosylated haemoglobin.

It is well known that absolute or relative shortage of insulin leads to a defective amino-acid/protein metabolism, which may pose as a factor more important than hyperglycaemia when it comes to the aetiology of certain hyperglycaemia-induced complications (Rosenlund, 1993). Diabetes experimentally induced in a rat model displays several alterations in amino-acid metabolism, which may be attributed to an increased muscle proteolysis, reduced protein synthesis, an energy-dependent liver progression, and stimulated hepatic gluconeogenesis utilizing gluconeogenic amino-acid (Fando et al., 1985). This readily accounts for the observed decrease in total protein content seen in STZ-induced diabetic rats. The administration of *P. americana* fruit extract significantly inhibited proteolysis caused by insulin deficiency in hyperglycaemic rats and raised total protein levels to near normal. This property of *P. americana* fruit extract is comparable to that of gliclazide.

Supra-physiological concentration of glucose in a hyperglycaemic state causes severe derangements in protein metabolism that result in the development of a negative nitrogen balance. This in turn elevates urea and creatinine levels

Table 1: Levels of blood glucose, haemoglobin, glycosylated haemoglobin, plasma insulin, total protein, blood urea, and serum creatinine in the control and experimental groups. Values are given as means ± SD for the each six-rat group. * Significant at $p< 0.05$ .Statistical significance was compared between the groups as follows: [a] Hyperglycaemic rats were compared to the control rats; [b] P. americana- treated arm was compared to the hyperglycaemic rats; [c] Gliclazide-treated hyperglycaemic arm was compared to the control rats

Groups	Blood Glucose (g/dl)	Haemoglobin (g/dl)	Glycosylated haemoglobin (%Hb)	Plasma Insulin (μUmL^{-1})	Total Protein (g/dl)	Blood Urea (mg/dl)	Serum Creatinine (mg/dl)
Control	98.23± 8.46	12.56± 0.78	6.18± 0.36	17.45± 0.79	7.89 ± 0.35	23.45± 1.33	0.56± 0.02
Hyperglycae-mic control	267.34± 18.4[a*]	6.09± 0.36[a*]	11.08± 0.42[a*]	4.56± 0.21[a*]	4.56 ± 0.19	41.66± 1.56[a*]	1.09± 0.012[a*]
Hyperglycae-mic+ P.americana	122.16± 5.3[b*]	9.13± 0.75[b*]	8.18± 0.13[b*]	11.52± 0.89[b*]	7.78± 0.55	34.49± 1.78[b*]	0.86± 0.032[b*]
Hyperglycaemic+ gliclazide	115.32± 4.78[c*]	10.48± 0.57[c*]	5.57± 0.17[c*]	12.36± 0.67[c*]	7.34 ± 0.35	25.78± 0.87[c*]	0.78± 0.05[c*]

Table 2 Activities of serum AST, ALT, and ALP in the control and experimental rat groups

Groups	**AST**	**ALT**	**ALP**
Control	84.34± 3.12	22.45 ± 1.37	76.23± 4.56
Hyperglycaemic control	126.45 ± 8.45[a*]	53.45± 2.09[a*]	152.34± 8.12[a*]
Hyperglycaemic+ *P.americana*	89.24± 3.56[b*]	26.47 ± 1.23[b*]	89.45 ± 5.78[b*]
Hyperglycaemic+ gliclazide	83.56 ± 2.45[c*]	28.23± 1.89[c*]	83.47 ± 4.88[c*]

Enzyme activities are expressed as: AST and ALT-µmoles of pyruvate/h/mg of protein; ALP-µmoles of phenol liberated/min/mg of protein. Values are given as means ± SD for the each six-rat group. * Statistically significant at $p<0.05$

Statistical significance was compared between the groups as follows: [a] Hyperglycaemic rats were compared to the control rats; [b]*P. americana*- treated hyperglycaemic rats were compared to the hyperglycaemic rats; [c]Gliclazide-treated hyperglycaemic rats were compared to the hyperglycaemic control

Table 3 s TBARS and hydroperoxide levels in the liver tissue of the control and experimental rat groups

Groups	TBARS (mM/100g of tissue)	Hydroperoxides (mM/100g of tissue)
Control	0.89 ± 0.02	74.34 ± 4.35
Hyperglycaemic control	1.89± 0.08[a*]	89.16± 6.49[a*]
Hyperglycaemic+ *P.americana*	1.27 ± 0.43[b*]	84.76 ± 6.04[b*]
Hyperglycaemic+ gliclazide	1.34 ± 0.05[c*]	78.12 ± 4.69[c*]

Values are given as means ± SD for each of the six-rat groups. * Statistically significant at $p<0.05$. Statistical significance was compared between the groups as follows: [a] Hyperglycaemic rats were compared to the control rats; [b] *P. americana*-treated hyperglycaemic rats were compared to the hyperglycaemic rats; [c]Gliclazide-treated hyperglycaemic rats were compared to the hyperglycaemic control rats

Table 4 Ascorbic acid, tocopherol and GSH in the liver of the control and experimental rat groups

Groups	*Ascorbic acid* (µg/mg protein)	*Tocopherol* (µg/mg protein)	GSH (mg/100g tissue)
Control	1.38 + 0.01	2.34 ± 0.12	49.17± 4.56
Hyperglycaemic control	0.38 ± 0.03[a*]	0.86± 0.14[a*]	22.56 ± 1.34[a*]
Hyperglycaemic+ *P.americana*	0.95± 0.03[b*]	1.87± 0.23[b*]	35.23± 2.78[b*]
Hyperglycaemic+ gliclazide	1.02± 0.01[c*]	1.89 ± 0.32[c*]	38.23± 3.23[c*]

Values are given as means ± SD for each of the six-rat groups. * Statistically significant at $p<0.05$. Statistical significance was compared between the groups as follows: [a] Hyperglycaemic rats were compared to the control rats; [b] P. americana-treated hyperglycaemic rats were compared to the hyperglycaemic rats; [c]Gliclazide-treated hyperglycaemic rats were compared to the hyperglycaemic control rats

Table 5 Superoxide dismutase, catalase, glutathione peroxidase, and glutathione-S- transferase activities seen in the liver of the control and experimental rat groups

Groups	SOD	CAT	GPx	GST
Control	13.45± 0.34	74.09± 3.78	13.73± 0.44	7.12± 0.22
Hyperglycaemic control	3.79± 0.08[a*]	32.12± 1.47[a*]	4.62± 0.08[a*]	3.28± 0.98[a*]
Hyperglycaemic+ *P.americana*	8.64± 0.67[b*]	62.33± 4.27[b*]	8.72± 0.27[b*]	6.97± 0.34[b*]
Hyperglycaemic+ gliclazide	8.98± 0.43[c*]	64.85 ± 2.37[c*]	8.15 ± 0.24[c*]	5.98± 0.14[c*]

Enzymatic activities expressed as 50%-inhibition of epinephrine auto-oxidation per min for SOD; µmoles of hydrogen peroxide decomposed per min per mg of protein for CAT; µmoles of glutathione oxidized per min per mg of protein for GPx; units per mg of protein for GST

Values are given as means ± SD for each of the six-rat groups. * Statistically significant at $p<0.05$. Statistical significance was compared between the groups as follows: [a] Hyperglycaemic rats were compared to the control rats; [b] P. americana-treated hyperglycaemic rats were compared to the hyperglycaemic rats; [c]Gliclazide-treated hyperglycaemic rats were compared to the hyperglycaemic control rats

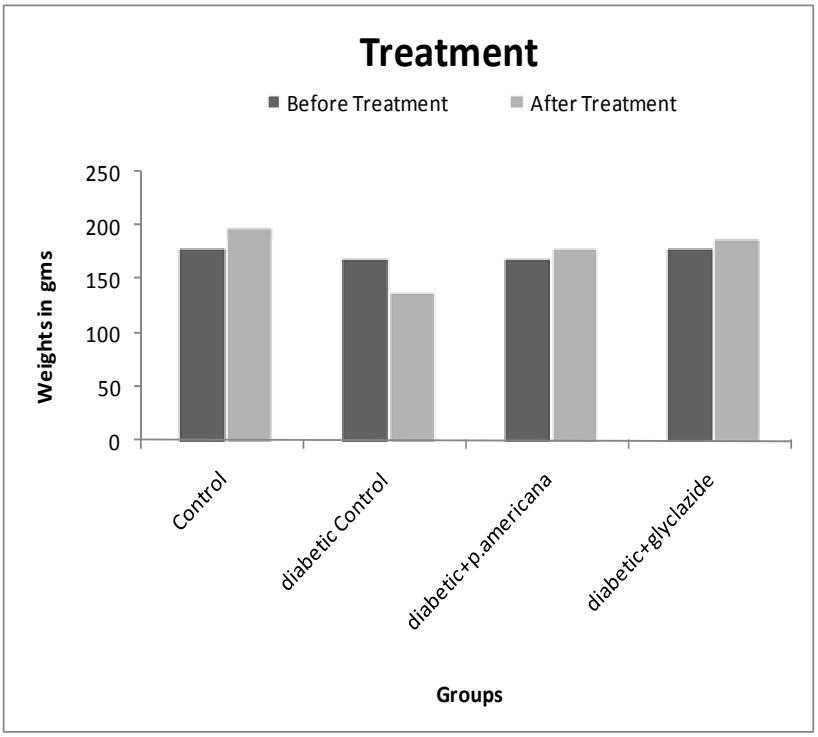

Figure 1: Body weight changes in the control and experimental rat groups. Values are given as means ± SD for each six-rat group. * Statistically significant at $p<0.05$. Statistical significance was compared between the groups as follows: [a] diabetic rats were compared to the control rats; [b] avocado-treated diabetic rats were compared to the diabetic rats; [c] gliclazide-treated diabetic rats were compared to the diabetic control rats.

(Asayama et al., 1994) which act as a biochemical diagnostic marker of renal impairment and drug-induced toxicity (Braunlich et al., 1997). Upon treatment wit *P. americana* fruit extract, the observed alterations in blood urea and serum creatinine levels in hyperglycaemic rats reverted to near normal, indicating the renal-protective nature of the extract as oppose to glucose toxicity.

Table 2 depicts the activity of enzymes such as AST, ALT, and ALP in the serum of the control and experimental rat groups. The activities of these enzymes were found to be increased in hyperglycaemic state, while oral treatment with *P. americana* fruit extract significantly reduced their activities to near normal. Aminotransferases are liver marker enzymes that leak into the circulation in cases of hepatocyte injury. Alkaline phosphatases act as markers of biliary function and cholestasis. It is assumed that increased ALT, AST, and ALP activities are predictors of diabetes mellitus. Furthermore, elevations in levels of these gluconeogenic enzymes, whose gene transcription is suppressed by insulin, could indicate an impairment in insulin signalling rather than a mere liver cell injury (O'Brien and Granner, 1991). Oxidative stress arising from reactive lipid peroxidation, peroxisomal β-oxidation, and recruited inflammatory cells, poses as another possible explanation for the elevation in aminotransferase levels seen with insulin resistance. An insulin-resistant state is also characterized by an increase in pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α), which may also contribute to hepatocellular injury (Grove et al., 1997). Thus, the observed increase in activities of ALT, AST, and ALP registered in the sera of hyperglycaemic rats may primarily arise due to the leakage of these enzymes from liver cytosol into the bloodstream consequential to STZ hepatotoxicity (El-Demerdash et al., 2005). Oral administration of *P. americana* fruit extract in hyperglycaemic rats significantly decreased the activity of these enzymes and put them down to their basal levels, suggesting the hepato-protective nature of the fruit extract in reference.

Decreased plasma insulin levels seen in hyperglycaemia, increase the activity of fatty acyl coenzyme A oxidase, which initiates β-oxidation of fatty acids resulting in lipid peroxidation. Increased lipid peroxidation impairs membrane activity by virtue of decreasing membrane fluidity and altering the activity of membrane-bound enzymes and receptors. Products of lipid peroxidation are injurious to most of the cells in the body, and are associated with a variety of diseases such as atherosclerosis and brain damage (Borek, 2001). In our study, significant increase in TBARS levels was observed in the liver of hyperglycaemic rats (Table 3). Oral administration of *P. americana* fruit extract in hyperglycaemic rats tends to bring liver peroxides down to near control levels, which could be the result of an improved antioxidant status.

Hydroperoxides are potentially toxic molecules capable of demolishing enzymes and cell membranes (Wang et al., 1996). The observed elevation in liver hydroperoxide level (Table 3) may be due to diminished activities of antioxidant enzymes which go in favour of an unrestrained production of free radicals and the subsequent production of lipid hydroperoxides (Matkovics et al., 1998). Oral administration of *P. americana* fruit extract significantly reduced hydroperoxide production in the liver of hyperglycaemic rats introduced into STZ-induced diabetic state. These observations lead one to contemplate on antioxidant and anti-lipid peroxidative potential of *P. americana* fruit extract.

In diabetes, oxidative stress coexists with the reduction in antioxidant capacity which can increase the deleterious effects of free radicals and consequently lead to the development of long-term diabetes mellitus-induced complications (Baynes, 1991). Non-enzymatic antioxidants such as *ascorbic acid* and E and reduced glutathione are known to be decreased in hyperglycaemic state because of their free radical- scavenging property (Garg and Bansal, 2000). The observed decline in *ascorbic acid* and E and reduced glutathione levels seen in the liver of STZ-induced diabetic rats (Table 4 and 5) arose on the grounds of the decreased capacity of non-enzymatic antioxidants to scavenge increasingly produced free radicals (Fang et al., 2002). However, the administration of *P. americana* fruit extract in hyperglycaemic rats resulted in a marked increase in levels of these non-enzymatic antioxidants, thereby diminishing the effects of free radicals in the liver. The phytochemicals present in *P. americana* fruit extract may contribute to the free radical-scavenging property of the extract.

Enzymatic antioxidants are also involved into the detoxification of free radicals and peroxides formed during the course of an oxidative stress, diabetes mellitus included. Enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) are crucial cellular components of the antioxidant defence system in the body, thus playing a crucial role in the maintenance of a balanced redox status (Evan and Littlewood, 1998). Diminished activities of enzymatic antioxidants in hyperglycaemic rats have been reported (Miyazaki et al., 2007). Similar results were observed in the present study. Oral treatment with *P. americana* fruit extract in STZ-induced diabetic rats resulted in increased activities of SOD, CAT, GPx, and GST enzymes. This may be attributed to free radical-scavenging and anti-hyperglycaemic activities of *P. americana* fruit extract.

Insofar, about 160 phytochemicals present in *P.americana* have been identified, the major micronutrients thereby being phenol compound, organic acids, and alkaloids. Thus, the observed hypoglycaemic and anti-oxidative effects of *P. americana* extract seen in STZ-induced hyperglycaemia in rats come as the result of synergistic effects of these biologically active extract ingredients, which, in turn, may arise due to the antioxidant nature of *P.americana* fruit extract. The present study also provides a rationale for the use of P.americana fruits in traditional medical treatment of diabetes mellitus.

Conclusion

The consumption of *P.americana* fruit is not toxic to the system and is hepato-protective. The presence of biologically active ingredients such as alkaloids, flavonoids, triterpenoids, minerals and vitamins, readily accounts for anti-hyperglycaemic and anti-oxidative properties of *P. americana* fruit

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