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Antidiabetic and Antihyperlipidemic Properties of *Phyllanthus emblica* Linn. (Euphorbiaceae) on Streptozotocin Induced Diabetic Rats

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Abstract: Diabetes is known to involve oxidative stress and changes in lipid metabolism. The present study was designed to investigate the therapeutic effects of an ethanolic extract of *Phyllanthus emblica* fruits on antidiabetic, antioxidant and lipid profile in plasma and tissues (liver and kidney) of experimental diabetes. Thirty rats were allocated randomly into 5 groups, each of 6 rats. Group I was acted as control group, group II rats were rendered diabetic by intraperitoneal injection of streptozotocin (40 mg/kg bw), group III rats received *Phyllanthus emblica* fruit ethanolic extract (PFEet) (200 mg/kg bw) by using an intragastric tube for 45 days, group IV rats received glibenclamide (600 µg/kg bw), group V rats given PFEet (200 mg/kg bw) alone. Ethanol extracts of *Phyllanthus emblica* fruits was administered orally at doses of 200 mg/kg body weight for 45 days resulted in a significant reduction in blood glucose and a significant increase in plasma insulin in diabetic rats. Diabetic rats had elevated levels of Total Cholesterol (TC), Very Low Density Lipoprotein-Cholesterol (VLDL-C), LDL-cholesterol, Free Fatty Acids (FFA), Phospholipids (PL), Triglycerides (TG) and decreased HDL-cholesterol. Diabetic rats fed PFEet showed a significant reduction in TC, VLDL-C, LDL-C, FFA, PL, TG and an elevation in HDL-C. In conclusion, the observations from this study show that *Phyllanthus emblica* has antidiabetic and its beneficial effects on lipid profile, thus it can be recommended for use as a natural supplementary herbal remedy in patients suffering from diabetes mellitus.

Key words: *Phyllanthus emblica*, streptozotocin-diabetes, plasma insulin, blood glucose, lipid profile

INTRODUCTION

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion or insulin action (Jayakar and Suresh, 2003). During hyperglycemia excessive production of free radicals is believed to be involved in many diabetic complications including diabetic neuropathy in diabetes mellitus (Sima and Sugimoto, 1999). Besides hyperglycemia, several other factors including dyslipidaemia or hyperlipidemia are involved in the development of micro and macro vascular complications of diabetes which are the major causes of morbidity and death (Bennet and Joslin's, 1998). Diabetes mellitus is also associated with hyperlipidaemia with profound alteration in the concentration and composition of lipid (Odetola *et al*., 2006). Changes in the concentration of lipid with diabetes contribute to the development of vascular disease. Excessive levels of blood cholesterol accelerate atherogenesis and lowering high blood cholesterol reduces the incidence of CHD (Grundy, 1986). One of the risk factors for coronary heart disease is elevated Total Cholesterol (TC), Low Density Lipoprotein-Cholesterol (LDL-C) and Lower High Density Lipoprotein-Cholesterol (HDL-C) (Moreno and Mitjavalia, 2003; Schaefer, 2002; Hu *et al*., 2001; Jain *et al*., 2007).

An ideal oral treatment for diabetes mellitus would be a drug that not only controls the glycaemic level, but also prevents the development of atherosclerosis and other complications associated with diabetes mellitus. A different type of oral hypoglycemic agents such as biguanides and sulphonylurea are available along with insulin for the treatment of diabetes mellitus (Holman and Turner, 1991), but has side effects associated with their uses (Kameshwara Rao *et al*., 1997; Valiathan, 1998). There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in and relatively low costs.

In spite of the presence of known antidiabetic medicines in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease possibly because they are considered to be less toxic and free from side effects compared to synthetic one (Momin, 1997). Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes (Pushparaj *et al*., 2000), but only a few have received scientific scrutiny. The World Health Organization has recommended the evaluation of the effectiveness of plants in conditions where safe orthodox drugs are scarce (World Health Organisation, 1980).

Phyllanthus emblica Linn. (Euphorbiaceae) commonly known as amla is an ancient herb used in folk medicine for the treatment of variety of human ailments,

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particularly diabetes. A decoction of the fruit with stems of *Tinospora cordifolia* is a well known remedy for various urinary diseases (Jayaweera, 1980). Amla is highly nutritious and could be an important dietary source of vitamin C, minerals and amino acids. The fruit pulp is being used in several indigenous medical preparations against a variety conditions such as headache (Jeena and Kuttan, 1995), liver injury (De *et al*., 1993), atherosclerosis (Thakur *et al*., 1998) and diabetes (Sabu and Kuttan, 2002). Mishra *et al*. (1981) reported that amla has hypocholesterolemic activity. Extract of *Phyllanthus emblica* and quercetin (a flavonoid isolated from *emblica*) for hepatoprotective action was assessed against paracetamol induced liver damage in albino rats and mice (Gulati *et al*., 1995). To the best of our knowledge, no detailed investigations had been carried out to shed light on the antihyperglycemic and antihyperlipidaemic effect of *P. emblica* in diabetic rats injected with STZ. The core aim of the present study was to assess the therapeutic effects of an ethanolic fruit extract of *P. emblica* on blood glucose, non-enzymic antioxidants and lipid profile in plasma, liver and kidney of STZ-induced diabetic rats. The effect of *P. emblica* ethanol extracts was compared to glibenclamide, which is often used as a standard drug.

MATERIALS AND METHODS

Animals: Male Albino Wistar rats weighing (170-220 g) were obtained from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University, Annamalai nagar. The animals were kept in clean and dry cages with a bedding of paddy husk, exposed to 12 h dark and night cycle, fed with rat pellets feed (Hindustan lever ltd, Bangalore, India) and water *ad libitum*. The study was conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Consens Statement, 1985). Animal studies in the work have been performed as per the Institutional Animal Ethical Committee of Rajah Muthiah Medical College (Reg no: 465/160/1999/CPCSEA), Annamalai University, Annamalai nagar.

Chemicals: Streptozotocin (STZ) was purchased from Sigma chemical company, St. Louis, Mo, USA. All other chemicals and solvents used were of analytical grade obtained from E-merck and Himedia, Mumbai, India.

Plant material: *Phyllanthus emblica* fruits were collected in and around areas of Chidambaram, Tamil Nadu. The Plant was taxonomically identified and authenticated by Dr. V. Venkatesalu, Reader, Department of Botany, Annamalai University. A voucher specimen was deposited at the Herbarium of Botany.

Preparation of methanolic fruit extract of *Phyllanthus emblica*: Amla fruits were cut into small pieces and ground into uniform powder using a blender. The methanolic extract of amla was prepared by soaking 100 g of dried powdered samples in 250 ml of methanol for 12 h. The extracts are filtered by using Whatman No. 1 filter paper. The filtrate was used for phytochemical screening.

Preliminary phytochemical analysis: Qualitative analysis carried out for *P. emblica* fruit crude extract showed the presence of phytochemical constituents such as flavonoids, tannins, terpenoids, alkaloids, carbohydrates and proteins with absence of steroids.

Chromatographic purification

Thin layer chromatography: The major compounds present in the *P. emblica* fruit extract were identified by using TLC/HPTLC.

Analytical method

Application of the sample in TLC plate: 2 µl of sample was applied on precoated silica gel GF254 aluminium plates (MERCK, 8cm * 7cm size). Develop the plate in Methanol: Chloroform (3:7) mobile phase. Run the mobile phase up to 9 cm of the plate. Remove the plate and dry in air. A spot in TLC was performed using a CAMAG is scanned in 2 Densitometer and linomatt IV applicator.

High Performance Thin Layer Chromatography (HPTLC): The methanol extract was determined in HPTLC/ (CAMAG TLC SCANNER II). HPTLC was performed using a CAMAG HPTLC spectrophotometer provided with a scanner II densitometry a Linomat applicator.

Preparation of *Phyllanthus emblica* alcoholic fruit extract for biochemical studies: The fruits of *Phyllanthus emblica* were dried and powdered. 100 g of powder was extracted with 250 ml ethanol for 72 h. After 72 h the suspension was filtered through a fine muslin cloth and concentrated at 40±5°C. A 13% solid material obtained was stored at 20°C prior to experimentation. Whenever needed, the residual extract was suspended in distilled water and used for treatment.

Experimental design: Diabetes was induced in a group of rats after an overnight fast by single intraperitoneal injection of STZ, which was freshly dissolved in 0.1M citrate buffer (P^H 4.5). The dose was 40 mg/kg body weight. STZ treated animals were allowed to drink 5% glucose solution over night to overcome drug induced hypoglycemia. After 48 h of STZ administration, the blood glucose range above 200-300 mg/dL were considered as diabetic rats and used for the experiment. In the experiment, a total of 30 rats were used. Animals were randomized and divided into five groups of six animals each.

Group A: Control rats received 3% gum acacia.

Group B: Rats were made diabetic by a single intraperitoneal injection of streptozotocin (40 mg/kg body weight) with citrate buffer (pH 4.5).

Group C: Diabetic rats treated with *Phyllanthus emblica* Fruit Ethanolic extract (PFEet) (200 mg/kg body weight) in aqueous solution daily using an intragastric tube for 45 days.

Group D: Diabetic rats received glibenclamide (600 µg/kg body weight) in aqueous solution daily using an intragastric tube for 45 days.

Group E: Control rats treated with PFEet (200 mg/kg body weight) in aqueous solution daily using an intragastric tube for 45 days.

At the end of the experimental period, all the rats were kept overnight fast and anesthetized using ketamine chloride (24 mg/kg body weight) by intramuscular injection and sacrificed by cervical decapitation between 8.00-10.00 am. Blood samples collected in potassium oxalate/sodium fluoride-containing tubes were used for the estimation of glucose. Plasma was separated for the estimation of insulin and other biochemical parameters. Liver and kidney tissues were dissected out and transferred to ice cold containers containing 0.9%NaCl and used for various biochemical estimations.

Biochemical investigations: Blood glucose was determined by the method of O-toluidine using the modified reagent of (Sasaki *et al*., 1972). Reduced glutathione in the plasma and tissues was estimated by the method of Ellman (1959). Ascorbic acid in the plasma and tissues was estimated by the method of Roe and Kuether (1943). α-Tocopherol in the plasma and tissues was estimated by the method of (Baker *et al*., 1980). Lipids were extracted from plasma and tissues by the method of (Folch *et al*., 1957). Total cholesterol were analyzed by the method of (Allain *et al*., 1974). Triacylglycerol in the plasma and tissues were estimated using the diagnostic kit based on the enzymic method described by (McGowan *et al*., 1983). Free fatty acids and phospholipids were estimated by the method of (Falholt *et al*., 1973) and Zilversmit and Davis (1950). HDL-cholesterol was estimated using the diagnostic kit based on the enzymic method described by (Izzo *et al*., 1981). Very Low Density Lipoprotein Cholesterol (VLDL-C) and Low Density Lipoprotein Cholesterol (LDL-C) fractions were calculated as VLDL-C = TG/5 and LDL-C = total cholesterol - (HDL-C + VLDL-C), respectively.

Data evaluation: Statistical analysis was performed using SPSS software package, version 11.5. The values were analysed by One Way Analysis Of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). All these results were expressed as mean±SD for six rats in each group: p-values <0.05 were considered as significant.

RESULTS

The qualitative analysis of methanolic crude extracts of *P. emblica* and the results were summarized in Table 1. It shows that flavonoids, tannins, carbohydrates, alkaloids, Proteins, terpenoids were present in extract. Steriods were absent in *P. emblica* extract.

The methanol extract of *P. emblica* fruit shows two major peaks. This indicates that there are two major compounds which may be present in the *P. emblica* fruit extract and it is shown in Table 2. Figure 1 shows HPTLC chromatogram of *P. emblica*.

Table 1: Qualitative analysis on phytochemical constituents in the methanol extract of *P. emblica* fruit

Flavonoids	+
Tannins	+
Carbohydrates	+
Alkaloids	+
Proteins	+
Steroids	-
Terpenoids	+

+ = Presence; - = Absence

Table 2: Analysis of methanol extract of *P. emblica* fruit by HPTLC

Peak	Retention Factor (R_f)	Area (%)
1	0.09	43.66
2	0.20	42.38

The methanol extract of *P.emblica* fruit shows 2 major peaks. This indicates that there are two major compounds may be present in the *P.emblica* fruit extract

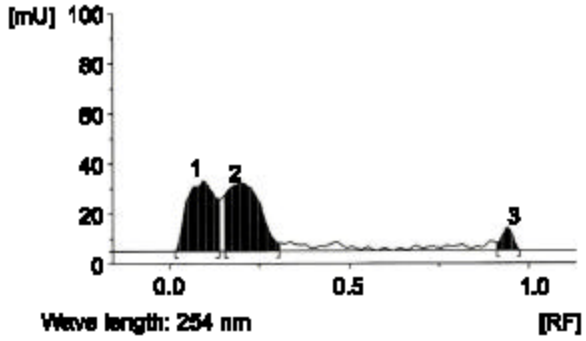

Fig. 1: HPTLC chromatogram of *P. emblica*

The body weight changes in control and experimental groups were illustrated in Table 3. The body weight of diabetic rats were significantly decreased ($p<0.05$) when compared with control group. Supplementation of PFEet and glibenclamide showed a significant improvement in the body weight of diabetic rats. There were no significant changes observed between control (group I) and control treated group (group V).

Table 3: Effect of PFEet on the changes of body weight of control and experimental rats

	Body weight (g)	
Groups	Initial (0 day)	Final (45 days)
Control (3% gum acacia)	175.12±6.50	202.76±8.08[a]
Diabetic control	184.07±4.54	147.06±6.46[b]
Diabetic + PFEet (200 mg/kg bw)	186.20±3.31	171.23±7.42[c]
Diabetic + glibenclamide (600 µg/kg bw)	189.06±5.43	175.43±9.08[c]
Control + PFEet (200 mg/kg bw)	180.21±6.54	203.54±5.70[a]

Values are given as mean±SD (n = 6 rats); Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)

Table 4: Effect of PFEet on the levels of blood glucose, plasma insulin in control and experimental rats

Groups	Blood glucose (mg/dL)	Plasma insulin (µU/mL)
Control (3% gum acacia)	80.52±7.00[a]	15.89±1.50[a]
Diabetic control	266.85±16.43[b]	6.87±0.54[b]
Diabetic + PFEet (200 mg/kg bw)	120.70±8.02[c]	13.92±0.61[c]
Diabetic + glibenclamide (600 µg/kg bw)	91.48±7.59[d]	14.90±0.79[a]
Control + PFEet (200 mg/kg bw)	82.08±4.81[a]	15.60±0.79[a]

Values are given as mean±SD (n = 6 rats); Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)

Table 5: Effect of PFEet on non-enzymatic antioxidants in plasma of control and experimental rats

	Plasma		
Groups	GSH (mg/dL)	Vitamin C (mg/dL)	Vitamin E (mg/dL)
Control (3% gum acacia)	24.94±1.90[a]	1.86±0.13[a]	1.74±0.12[a]
Diabetic control	14.82±2.62[b]	0.95±0.05[b]	0.85±0.06[b]
Diabetic + PFEet (200 mg/kg bw)	22.32±1.46[c,a]	1.12±0.04[c]	1.75±0.05[c]
Diabetic + glibenclamide (600 µg/kg bw)	20.71±1.90[d]	1.80±0.14[a]	1.60±0.03[a]
Control + PFEet (200 mg/kg bw)	22.07±1.28[a]	1.84±0.07[a]	1.65±0.14[a]

Values are given as mean±SD (n = 6 rats); Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)

Table 6: Effect of PFEet on non-enzymatic antioxidants in liver of control and experimental rats

	Liver		
Groups	GSH (mg/100 g wet tissue)	Vitamin C (mg/100 g wet tissue)	Vitamin E (mg/100 g wet tissue)
Control (3% gum acacia)	132.50±8.43[a]	0.67±0.05[a]	5.23±0.21[a]
Diabetic control	46.26±8.41[b]	0.30±0.02[b]	2.12±0.39[b]
Diabetic + PFEet (200 mg/kg bw)	116.86±8.00[c]	0.53±0.07[c]	4.20±0.31[c]
Diabetic + glibenclamide (600 µg/kg bw)	128.63±13.81[c,a]	0.63±0.05[a]	5.08±0.27[a]
Control + PFEet (200 mg/kg bw)	133.72±11.16[a]	0.69±0.04[a]	5.27±0.40[a]

Values are given as mean±SD (n = 6 rats); Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)

Table 7: Effect of PFEet on non-enzymatic antioxidants in kidney of control and experimental rats

	Kidney		
Groups	GSH (mg/100 g wet tissue)	Vitamin C (mg/100 g wet tissue)	Vitamin E (mg/100 g wet tissue)
Control (3% gum acacia)	118.42±8.10[a]	0.58±0.05[a]	3.28±0.10[a]
Diabetic control	47.32±5.14[b]	0.28±0.03[b]	1.12±0.15
Diabetic + PFEet (200 mg/kg bw)	95.53±10.68[c]	0.43±0.06[c]	2.10±0.34
Diabetic + glibenclamide (600 µg/kg bw)	110.21±10.68[a,d]	0.48±0.05[c]	3.01±0.20[a]
Control + PFEet (200 mg/kg bw)	125.86±13.85[a]	0.63±0.04[a]	3.10±0.21[a]

Values are given as mean±SD (n = 6 rats); Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)

To evaluate the effects of PFEet on blood glucose and plasma insulin levels of control and diabetic rats were summarized in Table 4. There was a significant (p<0.05) increase in the blood glucose and decrease in plasma insulin levels in diabetic rats as compared to control. After administration of PFEet and glibenclamide to diabetic rats were significantly reduced blood glucose and elevated plasma insulin levels. No variations were observed between control (group I) and control treated group (group V).

Table 5, 6 and 7 shows the levels of non-enzymatic antioxidants such as, vitamin C, vitamin E and GSH in the plasma and tissues of control and STZ-diabetic rats. The levels of non-enzymatic antioxidants were found to

Table 8: Effect of PFEet on the levels of total cholesterol in plasma and tissues of control and experimental rats

Groups	Plasma (mg/dL)				Total cholesterol	
	Total Cholesterol	HDL-C	LDL-C	VLDL-C	Liver (mg/100 g tissue)	Kidney (mg/100 g tissue)
Control (3% gum acacia)	76.32±5.24[a]	45.12±2.92[a]	17.42±1.12[a]	10.40±0.16[a]	3.76±0.13[a]	4.98±0.21[a]
Diabetic control	159.82±8.72[b]	24.53±1.98[b]	60.21±6.32[b]	21.62±1.41[b]	6.62±0.52[b]	7.85±0.56[b]
Diabetic + PFEet (200 mg/kg bw)	94.32±5.65[c]	36.15±2.11[c]	39.53±5.91[c]	18.12±1.14[c]	5.28±0.35[c]	5.63±0.68[c]
Diabetic + glibenclamide (600 µg/kg bw)	83.21±6.43[d,a]	43.10±3.20[a]	27.46±1.15[d]	12.31±0.09[d]	4.05±0.12[d,a]	5.02±0.43[a]
Control + PFEet (200 mg/kg bw)	72.98±5.55[a]	46.35±3.21[a]	12.63±1.04[a]	10.11±0.72[a]	3.42±0.31[a]	4.43±0.29[a]

Values are given as mean±SD (n = 6 rats); Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)

Table 9: Effect of PFEet on the levels of phospholipids in plasma and tissues of control and experimental rats

Groups	Phospholipids		
	Plasma (mg/dL)	Liver (mg/100 g tissue)	Kidney (mg/100 g tissue)
Control (3% gum acacia)	78.10±6.57[a]	21.46±1.77[a]	15.10±1.57[a]
Diabetic control	149.64±12.02[b]	56.74±3.89[b]	34.18±3.02[b]
Diabetic + PFEet (200 mg/kg bw)	109.54±0.78[c]	39.58±3.89[c]	20.25±1.39[c]
Diabetic + glibenclamide (600 µg/kg bw)	80.43±6.68[a]	28.56±2.05[d]	16.32±1.68[a]
Control + PFEet (200 mg/kg bw)	76.53±5.42[a]	24.52±1.97[a]	14.56±1.24[a]

Values are given as mean±SD (n = 6 rats); Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)

Table 10: Effect of PFEet on the levels of triglyceride in plasma and tissues of control and experimental rats

Groups	Triglyceride		
	Plasma (mg/dL)	Liver (mg/100 g tissue)	Kidney (mg/100 g tissue)
Control (3% gum acacia)	55.45±0.48[a]	4.21±0.38[a]	4.65±0.38[a]
Diabetic control	157.8±0.42[b]	7.2±0.22[b]	7.56±0.32
Diabetic + PFEet (200 mg/kg bw)	115.10±0.43[c]	5.65±0.43[c]	5.98±0.32[c]
Diabetic + glibenclamide (600 µg/kg bw)	60.45±0.43[d]	4.30±0.61[a]	4.03±0.28[d]
Control + PFEet (200 mg/kg bw)	57.34±4.54[a]	4.09±0.35[a]	4.86±0.76

Values are given as mean±SD (n = 6 rats); Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)

Table 11: Effect of PFEet on the levels of free fatty acid in plasma and tissues of control and experimental rats

Groups	Free fatty acid		
	Plasma (mg/dL)	Liver (mg/100 g tissue)	Kidney (mg/100 g tissue)
Control(3% gum acacia)	54.76±0.38[a]	7.54±0.48[a]	3.94±0.23[a]
Diabetic control	120.45±10.30[b]	17.15±1.33[b]	10.56±0.27[b]
Diabetic + PFEet (200 mg/kg bw)	69.54±3.89[c]	9.67±0.79[c]	7.87±0.61[c]
Diabetic + glibenclamide (600 µg/kg bw)	60.57±5.90[a]	7.96±0.69[a]	4.54±0.14[d]
Control + PFEet (200 mg/kg bw)	56.87±0.34[a]	7.25±0.41[a]	3.90±0.26[a]

Values are given as mean±SD (n = 6 rats); Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)

be significantly (p<0.05) decreased in the plasma and tissues of diabetic rats. Oral administration of PFEet and glibenclamide for 45 days resulted in the near normalization of the levels of vitamin C, vitamin E and GSH in the plasma, liver and kidney of diabetic rats. No significant changes were observed between control (group I) and control treated group (group V).

The results of the effects of PFEet on plasma and tissue total cholesterol of control and diabetic rats were depicted in Table 8. In diabetic rats, while the levels of TC, Low-Density Lipoprotein Cholesterol (LDL-C) and very Low-Density Lipoprotein-Cholesterol (VLDL-C) levels were found to be increased significantly (p<0.05), the level of High-Density Lipoprotein Cholesterol (HDL-C) was significantly (p<0.05) decreased.

Administration of PFEet and glibenclamide to diabetic rats decreased the TC, LDL-C and VLDL-C levels and significantly increased the levels of HDL-C. No significant changes were observed in the non-diabetic and non-diabetic rats treated with PFEet.

Influence of PFEet on PL, TG and FFA in plasma and tissues of control and experimental rats were presented in Table 9, 10 and 11. The levels of phospholipid, free fatty acid and triglyceride increased significantly (p<0.05)

in plasma and tissues of diabetic rats as compared to those of control rats. On the progression of treatment with PFEet and glibenclamide significantly decreased these parameters in diabetic rats. The control (group I) and control treated group (group V) did not show any significant changes.

DISCUSSION

Streptozotocin induced hyperglycemia in rodents is considered to be a good preliminary screening model. Streptozotocin is well known for its selective pancreatic ß-cell cytotoxicity and has been extensively used to induce Type-1 diabetes in experimental rat model. It interferes with cellular metabolic oxidative mechanisms (Papaccio *et al*., 2000). STZ-induced diabetes is characterized by severe loss in body weight of untreated rats. The characteristic loss of body weight in diabetic rats could be due to degradation and catabolism of fats and proteins (Hakim *et al*., 1997). Increased catabolic reactions leading to muscle wasting may be the cause for weight loss in diabetic rats (Rajkumar *et al*., 1991). In our study, the oral administration of PFEet and glibenclamide normalized the body weight in diabetic rats. This suggests that the protective effect of the extract in controlling muscle wasting is glycolysis.

Phyllanthus emblica is claimed to be useful in diabetes in folklore medicines, the results indicates that the plant extract was found to reduce the blood glucose level in STZ-induced diabetic rats. The observed significant increase in blood glucose was accompanied by a significant decrease in plasma insulin levels in diabetic rats when compared with control rats. Our observations are in complete agreement with the reports by several workers that STZ-induced diabetes mellitus and insulin deficiency lead to increased blood glucose (Chaude *et al*., 2001). This could be due to the result of impairment of peripheral tissues of liver to metabolize glucose. In the present study, diabetic rats treated with PFEet and glibenclamide showed a significant decrease in blood glucose with significant increase in plasma insulin level as compared to diabetic control rats. The possible mechanism by which PFEet bring about its antihyperglycemic action may be by increasing the pancreatic secretion of insulin from remnant ß-cells, which was clearly evidenced by the increased level of insulin in diabetic treated rats. A number of other plants have been reported to exert hypoglycemic activity through insulin release-stimulatory effects (Twaij and Al-Badr, 1988). The effect produced by the extract was compared with standard drug, glibenclamide.

Phytochemical analysis of the fruit revealed the presence of tannins, flavonoids, alkaloids, terpenoids, carbohydrates and proteins. These compounds are powerful antioxidants to scavenge the free radicals induced by hyperglycemia. Herbal extracts containing flavonoids and tannins were reported to demonstrate anti-diabetic activity (Suba *et al*., 2004). Inihibited α-glucosidase enzyme shows reduced blood glucose level due to the polyphenolic compounds such as tannins and saponins (Chakravarthy *et al*., 1982). On the basis of the above evidence, it is possible that the flavonoids and tannins present in this fruit may be responsible for the observed antidiabetic activity.

Free-radical induced oxidative stress has been implicated in the pathogenesis of a wide variety of clinical disorders, resulting usually from deficiency of natural antioxidant defenses. The non-enzymatic antioxidant defense systems are the second line of defense against free radical damage. Vitamin C, a potent water soluble non-enzymic antioxidant effectively intercept oxidants in the aqueous phase before they attack and cause detectable oxidative damage (Beter, 1994). Vitamin C plays an important role in the detoxification of reactive intermediates produced by $cytP_{450}$ which detoxifies xenobiotics. In our investigation, the decrease in vitamin C level may be due to increased utilization of vitamin C as an antioxidant defense against reactive oxygen species or to a decrease in GSH level, since GSH is required for recycling of vitamin C (Inofers and Sies, 1988). The observed decrease in the levels of vitamin C in the diabetic condition is consistent with previous reports (Prince and Menon, 1999). Vitamin E is an important radical scavenging antioxidant that interrupts the chain reaction of LPO by reacting with lipid peroxyl radical (Garry and Buethner, 1993). In our experiment, there is a decreased level of vitamin E in plasma and tissues of diabetic rats were noticed. Reduced vitamin E levels may be due to the decreased vitamin C level, because there is well established synergism between vitamin C and vitamin E.

Glutathione is one of the most abundant tripeptide, non-enzymatic antioxidant present in liver. It removes free radical species such as hydrogen peroxide, superoxide radicals and maintains membrane protein thiols. Moreover, it is substrate for glutathione peroxidase (Prakash *et al*., 2001). The levels of GSH in plasma and tissues decreases during diabetes represent the increased utilization due to oxidative stress. Treatment with PFEet and glibenclamide showed normalization of enzymic and non-enzymic antioxidant levels, which suggests the efficacy of extract to scavenge the reactive oxygen species overproduction during diabetes. The antioxidant potential of *P. emblica* which may be due to the presence of phenolic compounds and vitamin C. It is reported that phenolic compounds in plants possess strong antioxidant activity and may help to protect cells against the oxidative damage caused by free radicals (Kirakosyan *et al*., 2003).

Lipids play an important role in the pathogenesis of diabetes mellitus. The marked hyperlipidaemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of

lipolytic hormones on the fat depot (Casiglia and Palatini, 1998). In our study, we have noticed that the elevated levels of serum lipids such as Total Cholesterol (TC) and Triglyceride (TG) in plasma and tissues of diabetic rats when compared with that of control animals. This could be due to insulin deficiency, which results in failure to activate the lipoprotein lipase thereby causing hypertriglyceridaemia (Shirwakar *et al*., 2004). Under normal circumstances, insulin activates the enzyme lipoprotein lipase and hydrolyses Triglycerides (TG) (Suresh Kumar and Menon, 1992; Taskinen, 1987). The increased levels of TG and TC in diabetic rats are in agreement with other reports (Tunali and Yanardag, 2006). Upon administration of PFEet and glibenclamide reduced triglyceride in plasma and tissues of diabetic rats, this may be due to low activity of cholesterol biosynthesis enzymes or low level of lipolysis that are under the control of insulin (Sharma *et al*., 2003).

Fattyacids, an important component of cellular membranes are eicosanoid precursors and are therefore required for both the structure and function of every cell in the body (Rajasekaran *et al*., 2006). Apart from the regulation of carbohydrate metabolism, Insulin also plays an important role in the metabolism of lipids. Insulin is a potent inhibitor of lipolysis. Since it inhibits the activity of the Hormone Sensitive Lipase (HSL) in adipose tissues and suppresses the release of Free Fatty Acids (FFA) (Cohn and Roth, 1996). In our study it is clear that the plasma and tissues of the diabetic animal shows increased level of free fatty acids. Increased FFA in diabetic rats is mainly due to an increase in the mobilization of free fatty acid from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase (Goodman and Gilman, 1985). Our results are in accord with other reports (Krishnakumar *et al*., 2000). A significant reduction of FFA was observed in plasma and tissues of diabetic rats fed with PFEet and glibenclamide.

Phospholipids (PL) are vital part of biomembrane rich in Polyunsaturated Fatty Acid (PUFA), which are susceptible for free radicals such as O_2° and OH° radicals (Ahmed *et al*., 2001). Our present results indicate that the increased levels of phospholipids in diabetic rats may be due to the elevated levels of free fatty acid and total cholesterol. The phospholipids level tends to back towards normal after treatment with PFEet and glibenclamide.

Cholesterol is a powerful risk factor for many coronary heart diseases. The degree of hypercholesterolaemia is directly proportional to the severity of diabetes (Zavaroni *et al*., 1989). ß-Hydroxy-ß-Methylglutaryl Coenzyme A (HMGCoA) reductase catalyzes the rate-limiting step in cholesterol biosynthesis and its activity correlates closely with the rate of tissue cholesterol synthesis. According to our study, the total cholesterol concentration was remarkably increased in plasma and tissues of diabetic rats which is also associated with an increase in LDL-C, VLDL-C and decrease in HDL-C. In a diabetic state, plasma and tissue cholesterol levels were elevated due to lack of insulin, as the activity of HMG CoA as lowered in insulin deficiency. The increased concentration of cholesterol could be due to a decrease in HDL-C, Since HDL is known to be involved in the transport of cholesterol from tissues to the liver for its catabolism. The increased level of plasma and tissue cholesterol observed in our study is in agreement with the previous findings (Kweiterovich, 2000). Administration of PFEet and glibenclamide attenuated a significant reduction in total cholesterol, LDL-C, VLDL-C and significant increase in HDL-C in diabetic animals.

Conclusion: Our present investigation shows that *P. emblica* fruit extract possesses antidiabetic, antioxidant and antihyperlipidaemic effects in streptozotocin-induced diabetic rats. Therefore, *P.emblica* fruits show therapeutic promise as a protective agent against the development and progression of atherosclerosis and possible related cardiovascular complications in diabetes mellitus. It's worth emphasing that *P.emblica* has considerable potential for improving public health if used on a regular basis. Further pharmacological and biochemical investigations are underway to find out the active constituent responsible for the antidiabetic activity and to elucidate its mechanism of action.

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