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Full Length Research Paper

Standardized alcoholic extract of *Phyllanthus fraternus* exerts potential action against disturbed biochemical parameters in diabetic animals

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Alcoholic extract of *Phyllanthus fraternus* Webster whole plant (PFAE) prepared by successive solvent treatment was administered at a dose of 500 mg/kg body weight once in a day for 21 days to the alloxan induced diabetic albino rats. Certain biochemical parameters that is lipid profile (total cholesterol, high density lipoprotein, triacylglycerols), kidney functions (urea, creatinine) and liver functions (alkaline phosphate, alanine aminotransferase, aspartate aminotransferase) were evaluated and compared with normal and standard drug tolbutamide (200 mg/kg body weight) administered group. As a result, drug treatment has significantly improved the disturbed biochemical parameters at variable degrees when compared with standard drug. The phytochemical studies conducted for standardization of the extract showed the presence of tannins and flavonoids as major phytoconstituents. The total phenolics content was found to be 37.51 mg/g of drug extract. Quantitative estimation carried out on two major flavonoids by HPTLC confirmed a concentration of 1.706% w/w rutin and 5.614% w/w of quercetin present in the alcoholic extract. In conclusion, owing to the positive potential activity against disturbed biochemical parameters associated with diabetes, *P. fraternus* can be used effectively in the management of this deadly disease.

Key words: *Phyllanthus fraternus,* renal functions, liver functions tests, lipid profile, total phenolics.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder with vascular components that is characterized by disturbances in carbohydrates, lipids and protein metabolism (Pickup and Williams, 2003). Studies have shown that good metabolic control is beneficial in slowing the progression of these complications in diabetes (Floretto et al., 1998; Renu et al., 2004) . Several herbal drugs in different formulations have been experimented in search of an effective treatment for diabetes and certain claims of cure are on record (Jung et al., 2003). The plants of genus *Phyllanthus* (Euphorbiaceae) are widely distributed and long been used in traditional medicines and due to the presence of potential phytoconstituents, it has led to some promising findings in several disorders (Kirtikar and Basu, 1987; Calixto et al., 1998). A few species of this genus have also been reported to possess antidiabetic activity in addition to our earlier reported antidiabetic activity of the same plant (Higashino et al., 1992; Garg et al., 2008) . Based on the above, an attempt was made to screen the efficiency of PFAE on associated biochemical parameters in diabetic albino rats.

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MATERIALS AND METHODS

Plant material

The plant material of *Phyllanthus fraternus* Webster (whole plant) was collected from medicinal garden of University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, Authenticated and voucher specimen was stored in the department (Number ISF/Ph/VS-103). The material was then shade dried, powdered and stored at 25°C. Alcoholic extract of the whole plant were prepared by soxhlet extraction using petroleum ether as solvent by soxhlation. Extract was concentrated by rotaevaporator,

vacuum dried and stored. In animal studies, the extract was triturated with freshly prepared 0.3% w/v carboxyl methyl cellulose (CMC) solution to obtain a suspension of concentration 0.3 g/ml for oral administration to the animals.

Phytochemical analysis

Preliminary phytochemical screening of the alcoholic extract was carried out using standard method (Peach and Trecy, 1956). The total phenolics content were estimated by Folin-Ciocalteu reagent method. Total phenolic contents present in the plant extract were calculated as gallic acid equivalents (GAE) by applying the formula, C = c * V/m. Where, *C*-total content of phenolic compounds (mg/gm) plant extract in GAE, *c*-the concentration of gallic acid established from the calibration curve (mg/ml), *V*- volume of extract (ml), *m*- weight of pure plant extract (gm).

For fingerprinting studies, 10 mg/ml solutions of extracts in respective solvents were applied in triplicate on HPTLC plate (Silica gel 60 F 254 Aluminum sheets, 10 X 10 cm) and developed in solvent system benzene: ethyl acetate: formic acid (80:20:5) up to 90 mm in twin trough chamber. The developed chromatogram was scanned at 254 nm wavelength for detection of active compounds in the absorbance mode using CAMAG scanner III. Quantitative estimation of rutin and quercetin were carried out by HPTLC using solvent systems ethyl acetate: methanol: water: formic acid (100:13.5:10:2.5) and benzene: ethyl acetate: formic acid (40:10:2.5) respectively, by calibration curve method and compared with standard compounds rutin and quercetin.

Test animals

Albino rats of either sex (5 - 6 weeks) weighing 150 - 200 g were obtained from animal house, I. S. F. college of pharmacy, moga, punjab, kept in teflon cages and maintained under controlled conditions (22 - 28°C temp, 60 - 70% relative humidity) at 12 h dark/light cycle, fed with standard rat pellet diet (Hindustan Lever, India) and given water *ad libitum*. All drugs and chemicals were of analytical grade.

Diabetes was induced in animals by injecting freshly prepared alloxan monohydrate in sterile normal saline at a dose of 150 mg/kg body weight, intraperitoneally (Aruna et al., 1999). To prevent fatal hypoglycemia due to massive pancreatic insulin release, rats were treated with 20% glucose solution intraperitoneally after 6 h followed by supply of 5% glucose solution bottles in their cages for next 24 h (Barry et al., 1997). The animals shown blood glucose level >200 mg/dl after 72 h were considered as diabetic. Experiments performed were complied with committee for the purpose of control and supervision of experiments on animals (CPCSEA) New Delhi, India (registration no: 816/04/C/CPCSEA). The experimental protocol was duly approved by the institutional ethical committee.

Experimental design

In this experiment, a total of 24 rats (18 diabetic surviving rats, 6 normal rats) were used. The rats were divided into four groups (A-D) of six rats each. Group A and B served as normal and diabetic control while group C and D served as PFAE treated (500 mg/kg body weight) and standard drug tolbutamide (200 mg/kg body weight) treated groups, respectively. The dosage of the drug extract administered to the animals decided as 500 mg/kg body weight b.w. by oral administration on the basis of previously conducted studies (Khanna et al., 2002; Adeneye et al., 2006). The animals were administered drug for 21 days once daily by oral route using an intra gastric tube and weighed after every three days. On the last day before sacrificing the animals, the blood was withdrawn by cardiac puncture and placed into sterile container plastic tubes. Serum was separated by centrifugation (4000 rpm, 10 min) and transferred to Eppendorf tubes. All serum samples were stored at -80°C (deep freezer) until analysis. Total cholesterol, high density lipoprotein (HDL), serum triacylglycerols, urea, creatinine, serum alkaline phosphate (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) estimations were carried out as per methods described (Allain et al., 1974; Jacob and Denmark 1960; Talke and Schubert, 1965; Bartel et al., 1972; Varley, 1975). Data was statistically analyzed with one way ANOVA and Students't' test for paired observations. All data expressed as mean $\pm$ standard error.

RESULTS

In the phytochemical analysis, PFAE have shown presence of tannins and flavonoids as major phytoconstituents. Total phenolic contents were calculated as 37.51 mg/g present in the extract. HPTLC fingerprinting studies revealed six numbers of spots and showed the presence of 1.706% w/w of rutin and 5.614% w/w of quercetin contents, respectively. Changes in body weight in treated and untreated animals are shown in Figure 1. Significant reduction in body weight was observed in diabetic animals. Administration of PFAE improved the body weight and the values were quite comparable with the effect of standard drug. In biochemical studies, the results expressed in Table 1 reveal that PFAE administration has significantly reduced the elevated levels of serum cholesterol and triacylglycerols in alloxan-induced albino rats.

The HDL levels which were reduced in diabetic animals were significantly ($P < 0.001$) elevated by the drug treatment to the extent of normal animals and even better than standard drug. Serum urea and creatinine were observed (Table 2) as badly affected in diabetic rats and their values were quite elevated. However, PFAE administration for 21 days to the alloxan-induced diabetic animals has reduced the elevated levels significantly at variable degrees. The response in serum creatinine was better than the serum urea contents. The effect of PFAE administration on liver function tests are expressed in Table 3 which reveals that an elevation of alkaline Phosphate (ALP), Aspartate aminotransferase (AST) and (Alanine aminotranferase) ALT were observed in diabetic rats. The drug treatment has significantly controlled the elevated levels not to the extent of standard drug. However, a potential positive effect was observed on all the liver parameters as a whole.

DISCUSSION

The plants are considered as biosynthetic laboratory for a multitude of compounds that exert physiological effects. Secondary metabolites are the compounds which are responsible for imparting therapeutic effects. The plants of genus *Phyllanthus* have been reported to contain

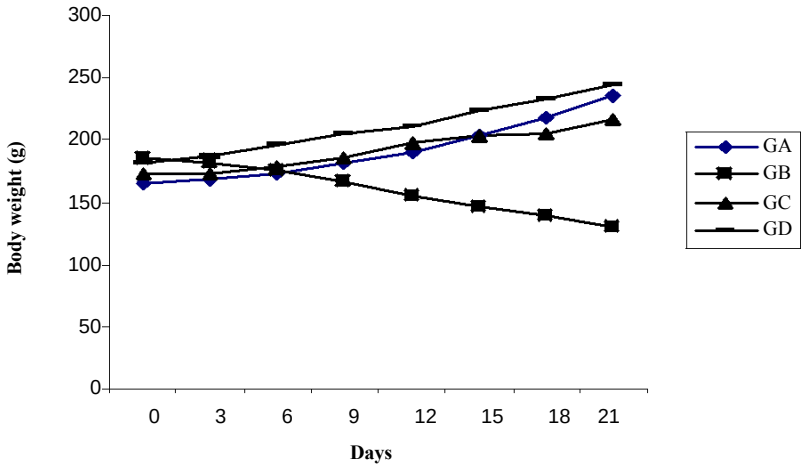

Figure 1. Effect of PFAE on body weight in diabetic albino rats after 21 days of treatment. GA = normal control, GB=diabetic control, GC = alcoholic extract treated and GD = standard drug tolbutamide treated group.

Table 1. Effect of PFAE on lipid profile in diabetic albino rats after 21 days of treatment (mg/dl).

Group	Serum cholesterol (mg/dl)	Serum triacylglycerols (mg/dl)	HDL (mg/dl)
Normal control	152.3 ± 3.76	61.3 ± 0.96	45.8 ± 0.70
Diabetic control	254.8 ± 9.62	102.0 ± 1.03	33.8 ±0.60
Alcoholic extract (500 mg/kg)	166.0 ± 8.01^{c}	70.6 ± 0.92^{c}	43.0 ± 0.68^{c}
Tolbutamide (200 mg/kg)	155.5 ± 7.29^{c}	66.8 ± 1.25^{c}	45.5 ± 0.76^{b}
F Value (ANOVA) (3,18)	26.59	147.21	51.22

Data are expressed as Mean ± SEM (n = 6). Statistical significance in comparison to control, ^{a} = P < 0.5, ^{b} = P< 0.01, ^{c} = P < 0.001, student's t-test.

Table 2. Effect of PFAE on renal profile in diabetic albino rats after 21 days of treatment.

Group	Serum urea (mg/dl)	Serum creatinine (mg/dl)
Normal control	25.5 ± 1.46	0.49 ± 0.036
Diabetic control	58.7 ± 3.11	1.2 ± 0.056
Alcoholic extract (500 mg/kg)	30.6 ± 2.20^{c}	0.59 ± 0.026^{c}
Tolbutamide (200 mg/kg)	28.7 ± 2.21^{c}	0.62 ± 0.064^{c}
F Value (ANOVA) (3,18)	24.05	21.81

Data are expressed as Mean ± SEM (n = 6). Statistical significance in comparison to control, ^{a} = P < 0.5; ^{b} = P < 0.01; ^{c} = P < 0.001, student's t-test.

Table 3. Effect of PFAE on liver functions tests in diabetic albino rats after 21 days of treatment.

Group	ALP (/L)	AST (/L)	ALT (/L)
Normal control	122.7 ± 4.25	72.8 ± 3.05	40.0 ± 2.12
Diabetic control	321.1 ±7.66	144.8 ± 1.99	96.5 ± 3.15
Alcoholic extract (500 mg/kg)	159.17±12.70^{c}	104.3 ± 2.75^{c}	76.7± 3.08^{b}
Tolbutamide (200 mg/kg)	131.50±4.98^{c}	81.7 ± 1.71 ^{c}	63.83 ± 3.1 ^{c}
F Value (ANOVA) (3,18)	98.22	133.79	45.83

Data are expressed as Mean ± SEM (n = 6). Statistical significance in comparison to control, ^{a} = P<0.5; ^{b} = P < 0.01; ^{c} = P<0.001, student's t-test. ALP = Serum alkaline Phosphate, AST = Aspartate aminotransferase, ALT = Alanine aminotranferase.

potential phytoconstituents like flavonoids, tannins, alkaloids and triterpenoids in earlier studies (Calixto et al., 1998). Our studies also confirm the claims and observed that potent compounds like flavonoids and tannins were present in the alcoholic extract. Significant amount of total phenolic contents and fingerprinting studies has further supported the earlier claims. Qualitative and quantitative studies carried out on two potent flavonoids that is quercetin and rutin confirmed that both compounds were present in substantial quantities in PFAE. Earlier reported studies have already confirmed that flavonoids and tannins are the class of compounds which are responsible for several therapeutic activities (Iwu, 1983).

Diabetic rats are observed with increased plasma lipids, which are responsible for several cardiovascular disorders (Nikkhila and Kekki, 1973; Chaterjee and Shinde, 1994). The higher lipid profile like cholesterol and triacylglycerols are also observed in tissues of liver, pancreas, kidney and intestine of diabetic rats, which are due to increase in mobilization of free fatty acids from peripheral depots and also due to the lipolysis caused by hormones (Murray et al., 2000). The results in the present study showed that administration of PFAE have reduced the hyperlipidaemia state significantly which may be due to the control of blood glucose level and thereby control on the lipolytic hormones. The main function of the kidneys is to excrete the waste products of metabolism and to regulate the body concentration of water and salt. Significant increase of total urea and creatinine levels indicated impaired renal function of diabetic rats leading to a negative nitrogen balance, enhanced proteolysis and lowered protein synthesis (Leonard et al., 2006; Alderson et al., 2004). Treatment of alloxanized-diabetic rats with PFAE induced a fall in the level of these metabolic parameters. Similar results are observed in the earlier studies using different plants (Kedar and Chakrabarti, 1983). The improvement of renal biochemical functions with PFAE in the present investigation could be due to its antidiabetic action, resulting in alleviation of altered metabolic status in animals and by the regenerative capability of the renal tubules (Kissane, 1985). In the current study, increased activities of ALP and ALT were observed in the diabetic animals. It has already been demonstrated that tissue antioxidant status is an important factor in the development of diabetic complications (Wohaieb and Godin, 1987). The increase in the level of these enzymes in diabetes may be as a result of leakage from the tissues and migration into the bloodstream (Chaudary et al., 1993).

Administration of PFAE brought about reduction in AST, ALT and ALP. Various mechanisms of action are involved in the antidiabetic effect of oral antidiabetic agents which includes, suppressing hepatic gluconeogenesis, stimulating glycolysis and inhibition of glucose absorption from the intestine, stimulation of insulin release, inhibition of dietary disaccharides to mono-saccharides and exerting transcription of fatty acids by activating a specific sub-class of proxisome-proliferator- activated receptor (Hardy and Nutty, 1997) . It is quite possible that a combination of these applies to the whole plant PFAE in which the presence of potent phytoconsti- tuents has been demonstrated. Although, exact biological active compound responsible for the observed thera-peutic activity is yet to be discovered however, observed activity points out the role of tannins, quercetin and rutin in the pancreatic and extra pancreatic mechanism of action. Due to this, enhancement of perpheral utilisation of glucose and increase insulin release may be associated in the resultant action of PFAE.

In conclusion, standardized PFAE containing estimated amounts of phenolic contents and two potent flavonoids that is, rutin and quercetin has demonstrated potential therapeutic activity against disturbed biochemical parameters in diabetic animals. It is therefore suggested that further work should be carried out to determine the exact mechanism of actions of the drug.

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