Effect of Phyllanthus Urinaria in Biochemical Profile of Experimental Hyperglycemic Albino Rats

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Abstract

Phyllanthus urinaria is a widely used plant in the folklore medicines in several disorders due to its excellent properties and potent phytocontituents. The aim of this study was to evaluate the effect of pet ether, alcoholic and aqueous extracts of this plant in renal, liver and cardiovascular complications in alloxan-induced diabetic rats. The altered levels of biochemical parameters in diabetic animals as compared to normal indicating impaired metabolic functions were significantly improved by oral administration of Phyllanthus urinaria extracts at the dose of 0.5 g/kg once daily for a period of 21 days. Present study shows that alcoholic extract of Phyllanthus urinaria was found to be most effective which could possibly be due to the presence of potent phytoconstituents like tannins and flavonoids.

Introduction

Diabetes mellitus is a chronic metabolic disorder with vascular components that is characterized by disturbances in carbohydrates, lipids and protein metabolism[1]. Studies have shown that good metabolic control is beneficial in slowing the progression of these complications in diabetes[2,3]. 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[4]. The plants of genus *Phyllanthus* (Euphorbiaceae) are widely distributed and long been used in traditional medicines[5]. Presence of potential phytoconstituents in the genus *Phyllanthus* has led to some promising findings in several disorders[6]. A few species of this genus are also reported to possess antidiabetic activity in addition to our previous findings related to the antidiabetic potential of the plant[7,8]. Keeping in view, the severity of disease and potential of *Phyllanthus* species, it was thought worthwhile to screen the efficiency of *Phyllanthus urinaria* plant extracts in the biochemical parameters associated with diabetes.

Material and Methods

Plant material: The plant material of *P. urinaria* was collected from medicinal garden of University Institute of Pharmaceutical Sciences, Punjab University, Chandigarh, authenticated and voucher specimen was stored in department (Number ISF/Ph/VS-104). Petroleum ether (yield 2.4 % w/w) and alcoholic extract (yield 3.8 % w/w) of the whole plant were obtained by soxhlet extraction procedure using successive solvent treatment. Aqueous extract (yield 5.3 % w/w) was obtained by maceration at room temperature for 24 hrs with regular stirring after every 2 hrs using the same marc left after

alcoholic extraction. The extracts were triturated with freshly prepared 0.3% w/v CMC solution to obtain a suspension of concentration 0.5 g/ml for oral administration to animals.

Preliminary phytochemical analysis and HPTLC fingerprints: All the extracts were subjected to preliminary phytochemical screening using standard methods[9] for presence/absence of different phytoconstituents. The 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.

Test animals: Albino rats of either sex (5-6 weeks) weighing 150-200g 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-hr 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.

Induction of diabetes: Animals were injected freshly prepared alloxan monohydrate in sterile normal saline at a dose of 150 mg/kg body weight, intraperitoneally. To prevent fatal hypoglycemia due to massive pancreatic insulin release, rats were treated with 20% glucose solution intraperitoneally after 6 hr followed by supply of 5% glucose solution bottles in their cages for next 24 hr. The animals shown blood glucose level >200 mg/dl after 72 hr were considered as diabetic.

Experiments performed were complied with the rulings of the European community guidelines and 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 the experiment total of 36 rats (30 diabetic surviving rats, 6 normal rats) were used. The rats were divided into six groups of six rats each: group A: normal control, group B: diabetic control, group C: diabetic rats + Pet ether extract of *P. urinaria*(0.5 g/kg), group D: diabetic rats + alcoholic extract of *P. urinaria*(0.5 g/kg), group E: diabetic rats + aqueous extract of *P. urinaria*(0.5 g/kg), group F: diabetic rats + tolbutamide (0.2 g/kg)

The dosage of the drug extracts administered to the animals decided as 0.5 g/kg body weight by oral administration on the basis of the thorough literature survey about the toxicity studies conducted earlier and use of *Phyllanthus* plant extracts in various studies[10,11]. The animals were administered drug for 21 days once daily by oral route using an intragastric tube. On last day, the rats were sacrificed by decapitation under light ether anesthesia. Blood samples were collected for biochemical parameters.

Biochemical estimation: The blood samples collected as above were used for analysis of various biochemical parameters: serum cholesterol level, high density lipoprotein (LDL) and low density lipoprotein (LDL)[12], serum triglycerides[13], urea and creatanine[14,15] were estimated asper methods described. Serum alkaline phosphate (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) estimation methods referred from Varley, 1975[16]. Readings of absorbance for standard and samples were taken by using Shimadzu UV-Vis double beam spectrophotometer.

Statistical analysis: Data were statistically analyzed with one way ANOVA and students 't' test for paired observations. All data expressed as mean $\pm$ standard error[17].

Results and Discussion

The preliminary phytochemical screening of extracts revealed the presence of flavonoids, triterpenoids and tannins as major phyoconstituent classes. HPTLC fingerprints of petroleum ether extract showed presence of 4 spots (R_f values 0.52, 0.65, 0.73, 0.83), alcoholic extract showed 5 spots (R_f values 0.01, 0.5, 0.73, 0.83, 0.89) and aqueous extract showed 3 spots (R_f values 0.08, 0.75, 0.89) at 254 nm wavelength. The alcoholic extract showed maximum number of spots.

Data expressed in table-1 reveal that *P. urinaria* has significantly reduced the elevated levels of lipids in alloxan-induced albino rats. The positive effect of drug was observed in serum cholesterol where alcoholic extract has best activity followed by aqueous extract. Pet ether extract could not show any significant difference in serum cholesterol. Serum triglycerides were significantly reduced by all the extracts. The HDL levels, which were reduced in diabetic animals, were slightly elevated by all the extracts and increased LDL levels were significantly reduced by alcoholic and aqueous extracts. In LDL levels pet ether extract could not show any significant changes.

The effect of *P. urinaria* expressed in table-II reveals that serum urea and creatanine were badly affected in diabetic rats and their values were quite elevated. However, the drug treatment for 21 days to the alloxan-induced diabetic animals has reduced the elevated levels significantly at variable degrees. The response in serum creatanine was better than the serum urea contents.

The effect of *P. urinaria* on liver function tests on alloxan-induced albino rats reveal that an elevation of ALP, AST and ALT were observed in diabetic rats. The treatment of drug has significantly controlled the elevated levels of ALP, AST and ALT with all the three extracts but not at the level of standard drug tolbutamide. Pet ether extract was least effective. A potential positive effect was observed on all the liver functions tests as a whole.

Diabetic rats are observed with increased plasma lipids, which are responsible for several cardiovascular disorders[18,19]. The higher lipid profile like cholesterol and triglycerides 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[20]. The results in the present study showed that administration of *P. urinaria* plant extracts has 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 creatanine levels indicated impaired renal function of diabetic rats[21,22]. Treatment of alloxanized-diabetic rats with *P. urinaria* induced a fall in the level of these metabolic parameters. However, the improvement was not sufficient to reach the levels observed in the normal rats and similar results are observed in the earlier studies using different plants[23,24]. The improvement of renal biochemical functions with *P. urinaria* treatment 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[25].

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[26]. 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[27]. Administration of *P. urinaria* brought about reduction in AST, ALT and ALP. As the drugs are reported to contain flavonoids and tannins as major phytoconstituents[6], the same are also indicated in the fingerprinting studies carried out in this study which may be responsible for the observed hepatoprotective activity due to their antioxidant actions.

Conclusion

Phyllanthus urinaria which is common drug in the Indian system of medicines used for various ailments demonstrated positive role in the management of associated biochemical parameters in diabetic experimental animals and thus holds the potential of a promising drug in the control of this deadly disorder.

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Table-1
Effect of phyllanthus urinaria plant extracts on lipid profile in diabetic albino rats after 21 days of treatment.

Treatment Group	Dosage (g/ kg)	Lipid Profile			
		Serum Cholesterol	Triglycerides	HDL	LDL
Normal Control	0.3% w/v CMC sol-5 ml	152.3 ± 3.76	61.3 ± 0.96	45.8 ± 0.7	95.8 ± 5.86
Diabetic Control	0.3% w/v CMC sol-5 ml	254.8 ± 9.62 [c]	102 ± 1.03 [c]	33.8±0.6 [c]	195.5 ±10.09 [c]
Pet ether extract	0.5 g/kg b.w.	250.1 ± 5.41	92.2 ± 0.83 [c]	36.7 ± 1.17	180.5 ± 5.84
Alcoholic extract	0.5 g/kg b.w.	177.2 ± 5.8[c]	80.0 ± 1.9 [c]	40.5 ± 2.46	142.3 ± 5.93[b]
Aqueous extract	0.5 g/kg b.w.	235 ± 4.98	83.2 ± 2.23 [c]	34.8 ± 2.21	167.2 ± 4.52[a]
Tolbutamide	0.2 g/kg b.w.	155.5 ± 7.29[c]	66.8 ± 1.25[c]	45.5 ± 0.76[c]	108.6 ± 4.92[c]
F Value (Degree of freedom)	--	*55.548 (5, 30)*	*108.686 (5, 30)*	*12.123 (5, 30)*	*38.276 (5, 30)*

Data are expressed as Mean ± SEM (n=6). Statistical significance in comparison to respective control i.e. diabetic control with normal control, rest of the groups with diabetic control, [a] = $P < 0.5$; [b] = $P < 0.01$; [c] = $P < 0.001$. Student's t-test.

Table-2
Effect of Phyllanthus urinaria plant extracts on liver functions tests and renal profile in diabetic albino rats after 21 days of treatment.

Treatment Group	Dosage (g/ kg)	Liver Functions Tests			Renal Profile	
		ALP	AST	ALT	Urea	Creatanine
Normal Control	0.3% w/v CMC sol-5 ml	122.2 ± 4.25	72.8 ± 3.05	40.0 ± 2.12	25.5 ± 1.46	0.49 ± 0.04
Diabetic Control	0.3% w/v CMC sol-5 ml	321.2 ± 7.66 [c]	144.8 ± 1.99 [c]	96.5 ± 3.15 [c]	58.7 ± 3.11 [c]	1.2 ± 0.06 [c]
Pet ether extract	0.5 g/kg b.w.	254.5 ± 6.12 [c]	103.3 ± 3.87 [c]	85.0 ± 4.6	47.8 ± 2.46[a]	0.93 ± 0.03 [c]
Alcoholic extract	0.5 g/kg b.w.	182.2 ± 3.83[c]	89.3 ± 3.62 [c]	75.8 ± 2.77[c]	38.8 ± 1.3	0.69 ± 0.03 [c]
Aqueous extract	0.5 g/kg b.w.	208.8 ± 7.03 [c]	123 ± 3.32 [c]	72.5 ± 3.35[c]	46.0 ± 2.18[b]	0.82 ± 0.04[c]
Tolbutamide	0.2 g/kg b.w.	131.5 ± 4.98[c]	81.7 ± 1.71 [c]	63.8 ± 3.1[c]	28.7 ± 2.21[c]	0.62 ± 0.06[c]
F Value (Degree of freedom)	--	*169.6 (5, 30)*	*80.012 (5, 30)*	*35.044 (5, 30)*	*32.7 (5, 30)*	*37.457 (5, 30)*

Data are expressed as Mean ± SEM (n=6). Statistical significance in comparison to respective control i.e. diabetic control with normal control, rest of the groups with diabetic control, [a] = $P < 0.5$; [b] = $P < 0.01$; [c] = $P < 0.001$. Students't-test.