Pharmacognosy Magazine
ISSN: 0973-1296

PHCOG MAG.
An official Publication of Phcog.Net

PHCOG MAG.: Research Article
Antidiabetic and antioxidant potential of *Phyllanthus fraternus* in alloxan induced diabetic animals

Munish Garg*[1], V. J. Dhar[1] and A. N. Kalia[2]

[1]*Indo-Soviet Friendship College of Pharmacy, Moga, Punjab, India.*

[2]*Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, M. D.University, Rohtak, Haryana, India.*

** Author's address for correspondence. Munish Garg,Assistant Director, All India Council for Technical Education, 4th Floor, NBCC Place, Lodhi Road, New Delhi*

***Author for Correspondence :** mgarg2006@gmail.com*

ABSTRACT

Implication of oxidative stress in diabetes due to oxygen free-radical generation and alteration in antioxidant enzymes is now well documented. *Phyllanthus fraternus* is a widely used folklore plant in several disorders due to its excellent properties and potent phytoconstituents. In this study, antidiabetic and antioxidant potential of petroleum ether, ethanolic and aqueous extracts of the whole plant of *Phyllanthus fraternus* was estimated in alloxan-induced diabetic albino rats. In a 21 days study, animals were divided into six groups (A-F) of six rats each. Group A served as normal control, group B as diabetic control, Groups C, D & E were administered with three different plant extracts (0.5g /kg), and group F was treated with standard drug tolbutamide (0.2g/kg). Administration of *Phyllanthus fraternus* extracts has remarkably improved the elevated levels of blood glucose. Ethanolic extract has reduced the blood sugar levels in a significant ($p<0.001$) and sustained manner throughout the study. Aqueous extract has also shown good activity during second week but could not sustain up to third week. Petroleum ether extract could not produce any significant results. Also the extracts tend to improve the altered levels of malondialdehyde, catalase and superoxide dismutase in liver and kidney. Ethanolic and aqueous extracts have shown maximum activity followed by pet ether extract against standard drug tolbutamide. In conclusion, *Phyllanthus fraternus* possess antidiabetic and antioxidant activity, which could exert a beneficial action against the deadly disease and its associated free radicals complications.

KEYWORDS : Phyllanthus fraternus, *Antidiabetic, Antioxidant activity.*

INTRODUCTION

Diabetes mellitus, a disease of metabolic disorders is associated with a number of chronic complications like nephropathy, neuropathy, retinopathy and cardiovascular diseases (1). Implication of oxidative stress in the pathogenesis of diabetes is suggested not only by oxygen free-radical generation but also due to non-enzymatic protein glycosylation and alteration in antioxidant enzymes (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). Since a few species of this genus are reported to possess antidiabetic potential (7, 8) therefore, in the light of above literature survey, it was felt to screen the antidiabetic potential along with antioxidant ability of *Phyllanthus fraternus* on liver and kidney of alloxan induced diabetic animals.

MATERIALS AND METHODS

Collection and identification of plant material

The plant material of *P. fraternus* was collected from medicinal garden of University Institute of Pharmaceutical Sciences, Punjab University, Chandigarh, identified by Dr M. P. Sharma, Taxonomist, Jamia Hamdard, New Delhi. Voucher specimen number ISF/Ph/VS-104 kept in department. Plant material was shade dried, powdered and passed through sieve number 40 to obtain uniform powder and packed in airtight sealed envelopes for further studies.

Test extracts

Petroleum ether (yield 1.4 % w/w) and ethanolic extract (yield 4.8 % w/w) 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 ethanolic extraction. The extracts were subjected to preliminary phytochemical screening (9) and mixed with freshly prepared 0.3% w/v CMC solution to obtain a suspension of concentration 0.5 g/ml for animal studies.

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^{o} C temp, 60-70% relative humidity) at 12-hr dark/light cycle (10), fed with standard rat pellet diet (Hindustan Lever, India) and given water *ad libitum*.

Drugs and Chemicals

All the drugs and chemicals used in this experiment were purchased from Sigma Company, St Louis, MO, USA. The 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 (11). 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 (12). The animals shown blood glucose level >200 mg/dl after 72 hr were considered as diabetic. Experimental procedure was followed strictly as per the guidelines of animal 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 *Phyllanthus fraternus* (0.5 g/kg)

Group D: Diabetic rats + Ethanolic extract of *Phyllanthus fraternus* (0.5 g/kg)

Group E: Diabetic rats + Aqueous extract of *Phyllanthus fraternus* (0.5 g/kg)

Group F: Diabetic rats + Tolbutamide (0.2 g/kg)

The rats were given drug for 21 days once daily by oral route using an intragastric tube. The glucose estimation was carried out at random on initial, seventh and fourteenth day by taking blood samples from cardiac puncture under light ether anesthesia. On last day, the rats were sacrificed by decapitation. Blood samples were collected for glucose estimation and each liver and kidney was dissected out, weighed and homogenized immediately with Teflon plunger in ice chilled 10% KCl solution (3ml/gm tissue). After centrifugation at 2000 rpm for 10 minutes, clear supernatant liquid was used for antioxidant studies.

Analytical Procedure

Blood glucose was estimated by oxidase peroxidase method using Shimadzu UV-Vis double beam spectrophotometer (13). Estimation of Catalase (CAT) and superoxide dismutase (SOD) levels was carried out as per procedure Beers and Seizer, 1952 (14) and Saggu *et al.*, 1989 (15) respectively. Estimation of malondialdehyde (MDA) level as thiobarbituric acid reactive substances (TBARS) was carried out as per method described by Chaturvedi *et al.*, 1999 (16).

Statistical analysis

Statistical comparison with respective control group was performed with students't' test for paired observations and data were expressed as mean $\pm$ standard error (17).

RESULTS

Results expressed in Table 1 reveal antidiabetic potential of the drug. Pet ether extract could not produce any significant results but ethanolic extract has shown significant and sustained results on second and third week estimation ($P<0.001$). Aqueous extract has also shown significant results on second week but could not sustain up to third week. Thus the drug tends to have good antidiabetic activity but not up to the level of standard drug tolbutamide.

There was a decrease in CAT and SOD level and increase in MDA level observed in liver and kidney of diabetic rats. Figure-I results reveal that ethanolic extract has elevated the SOD level and reduced MDA level significantly ($p<0.001$) in liver of diabetic rats and the observed results were quite comparable with tolbutamide but did not show any significant results in CAT levels. Aqueous extract only reduced the CAT levels in a significant manner. Petroleum ether extract could not produce any significant changes.

Results from figure-II reveal that administration of ethanolic and aqueous extracts has remarkably improved the levels of CAT, SOD and MDA in kidney of diabetic rats and results were comparable with tolbutamide. However, petroleum ether extract could only show positive results while reducing the MDA level. In the phytochemical studies, pet ether extract has shown presence of triterpenoids and steroids while ethanolic and aqueous extract has shown presence of tannins and flavonoids.

DISCUSSION

Phyllanthus fraternus is claimed to be useful in diabetes in folklore medicines. Results of the present study establish a scientific basis for the utility of *P. fraternus* in the treatment of diabetes. The drug proves to be an effective antidiabetic drug as many potential phytoconstituents like flavonoids, tannins and triterpenoids are present in the given extracts (6). Based on the evidences like, several plants and some of their active principles including flavonoids and tannin containing drugs reported as potent hypoglycemics (18-21), it is possible that the presence of these components are responsible for the observed activity. Studies are in progress to elucidate the detailed mechanism of action of this extract. However, the possible mechanism by which the drug brings about its hypoglycemic action may be either by potentiating the insulin effect or by increasing the pancreatic secretions of insulin from the cells of islets of Langerhan's.

During ß-oxidation of fatty acids by flavoprotein dehydrogenase, hydrogen peroxide is generated, which is accepted upon by CAT present in peroxisomes. CAT catalyses the rapid decomposition of hydrogen peroxide to water and protects the tissues from dangerous hydroxyl radicals (22). SOD scavenges the superoxides

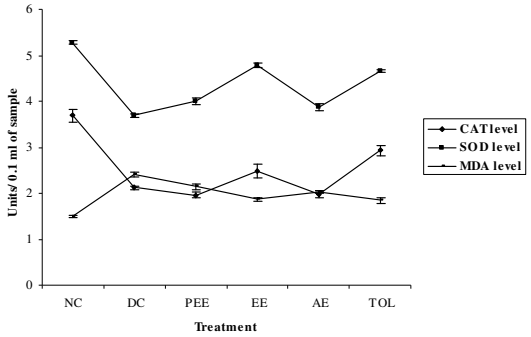
(O_2^-) and thus provides a first line defense against free radical damage. SOD catalyses the disputations of superoxide anions (O_2^-) to hydrogen peroxide and molecular oxygen (23). Treatment with *P. fraternus* extracts increased the CAT, SOD levels and thus help to counteract the damage by the free radicals generated during diabetes.

Table 1: Effect of Phyllanthus fraternus extracts on blood glucose levels in alloxan- induced diabetic rats.

Group	Blood Glucose levels (mg/dl)			
	1st Day	7th Day	14th Day	21st Day
Normal Control	109.6±1.99	111.0±1.96	104.8±2.39	106.8±2.86
Diabetic Control	243.0+1.96	241.0+1.77	242.6+3.39	234.5+3.99
Pet ether extract (0.5 g)	245.8±2.93	236.3±2.73	233.0±2.51	214.7±5.53
Ethanol extract (0.5 g)	244.8±3.66	235.8±3.56	218.2±1.74 *	172.5±2.43 *
Aqueous extract (0.5 g)	239.0±4.97	231.6±7.50	222.5±2.38 *	190.8±1.91
Tolbutamide (0.2g)	240.5±1.65	168.1±5.35 *	139.8±3.93 *	107.3±2.32 *

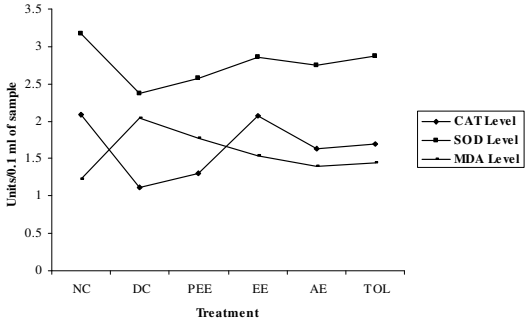
*Data expressed as mean ± SEM (n=6). Statistical significance in comparison to respective control, * = P<0.001. Students 't' test.*

Figure I: Effect of *Phyllanthus fraternus* plant extrcats on CAT, SOD and MDA levels in liver of diabetic rats.



*NC= Normal Control, DC= Diabetic Control, PEE= Petroleum ether extract treated group (0.5g/kg body weight), EE= Ethanolic extract treated group(0.5g/kg body weight), AE= Aqueous extract treated group (0.5g/kg body weight), TOL= Tolbutamide as Standard drug treated group (0.2g/kg body weight). Data expressed as mean ± SEM (n=6). Statistical significance in comparison to control, * = P<0.001. Students 't' test.*

Figure II: Effect of *Phyllanthus fraternus* plant extracts on CAT, SOD and MDA levels in kidney of diabetic rats.



*NC= Normal Control, DC= Diabetic Control, PEE= Petroleum ether extract treated group (0.5g/kg body weight), EE= Ethanolic extract treated group (0.5g/kg body weight), AE= Aqueous extract treated group (0.5g/kg body weight), TOL= Tolbutamide as Standard drug treated group (0.2g/kg body weight). Data expressed as mean ± SEM (n=6). Statistical significance in comparison to control, * = P<0.001. Students 't' test.*

Increased levels of lipid peroxidation in tissues of diabetic rats is one of the characteristic features of chronic diabetes (24). It has been observed that insulin secretion is closely associated with lipoxygenase derived peroxides (25, 26). The reduction of two electrons from alloxan gives dialuric acid, which undergoes oxidation and leads to generation of O_2^-, H_2O_2 and OH^-. Dialuric acid has been observed to stimulate lipid peroxidation *in vitro* (27). In this context, a marked increase in the concentration of MDA and hydroperoxides were observed in liver and kidney of diabetic rats. Increased lipid peroxide concentration in the liver and kidney of diabetic animals has already been reported (28). The administration of *P. fraternus* extracts has significantly reduced the MDA levels in diabetic rats.

The results of the present study indicate significantly increased lipid peroxidation of rats exposed to alloxan and its statistically significant attenuation by *P. fraternus* extracts treatment.

Since the phytochemical analysis in this study has shown the presence of potent phytochemicals like flavonoids and tannins which supports the earlier observations (6). This suggests the protective role of *P. fraternus* plant extracts due to antioxidative effect of flavonoids and tannins which act as strong superoxide radicals and singlet oxygen quenchers (29, 30).

In conclusion, the above observations show that *P. fraternus* extracts possess antidiabetic and antioxidant activity, which could exert a beneficial action against the deadly disease and its associated free radicals complications.

ACKNOWLEDGEMENTS

The authors are thankful to Mr. Parveen Garg, Secretary, ISF College of Pharmacy Managing committee for providing the facilities and moral support.

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Phcog Mag. Vol 4, Issue 14, Apr-Jun, 2008

Submitted on : 14th September, 2007
Revised on : 6th Dec, 2007
Accepted on: 10th January, 2008