

Preliminary Phytochemical Studies and Evaluation of Antidiabetic Activity of Roots of *Cayratia trifolia* (L.) Domin in Alloxan Induced Diabetic Albino Rats

Shikha Batra^{1*}, Nikhil Batra², and Badri Prakash Nagori³

Pharmacy Wing, Lachoo Memorial College of Science and Technology (Affiliated to Jai Narain Vyas University, Jodhpur) Sector 'A', Shastri Nagar Jodhpur-342 003, Rajasthan, India.

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ABSTRACT

The present study aims to investigate the antidiabetic activity of roots of *Cayratia trifolia* (L.) Domin in alloxan induced diabetic rats. Phytochemical studies showed the presence of steroids, flavonoids and alkaloids in petroleum ether, ethyl acetate and ethanol extracts, respectively. Antidiabetic activity was evaluated by observing blood glucose levels and analyzing the serum biochemical parameters after dosing the ethyl acetate extract. Blood samples were collected from overnight fasted diabetic rats on 5, 10 and 15 days of treatment to determine blood glucose levels. On day 16, blood samples were collected to estimate the biochemical parameters. In diabetic rats, both the doses (200 mg/kg and 400 mg/kg) of ethyl acetate extract were found to be significant ($P < 0.05$) when compared with control and favourable changes in biochemical parameters were also observed. It can be concluded from the study that the ethyl acetate extract of roots of *C. trifolia* possess potent antidiabetic activity.

INTRODUCTION

Diabetes mellitus is one of the most common chronic diseases in nearly all countries. There are an estimated 285 billion adults with diabetes in 2010; this number will continue to increase globally due to an aging population, growth of population size, urbanization, high prevalence of obesity and sedentary lifestyle (Zhang *et al.* 2010). World Health Organization (WHO) has reported 32 million people with diabetes residing in India, while the International Diabetes Federation (IDF) has reported an estimated 40.9 million people with diabetes for the same country (Ali *et al.* 2010).

Cayratia trifolia (L.) Domin is found in India from Jammu and Rajasthan to Assam, Tripura and West Bengal, extending into peninsular India, up to 600m (The Wealth of India, 1992).

In south-west region of Rajasthan, it is easily found in Sitamata wildlife sanctuary of Chittorgarh and Udaipur districts (Jain *et al.* 2005). It is commonly known as *Amal-bel* and *Ramchana* in Hindi and locally known as *Khhata Nimba* and

belongs to the family Vitaceae (Jain *et al.* 2005; Singh *et al.* 1998). The stem, leaves and roots of this plant contain hydrocyanic acid. Its leaves contain delphinidin, cyanidin and yellow waxy oil and sterols (Wealth of India, 1992). The bark extract has been reported to possess antiviral, antibacterial, antiprotozoal, anticancer and diuretic activities in animal models (Gupta *et al.* 2010). The extract of tuberous root of this plant along with the infusion of its seeds is given orally to diabetic patients to check the sugar level of blood (Swarnkar *et al.* 2008). In view of alleged antidiabetic potential of *C. trifolia*, we have investigated the antidiabetic activity of ethyl acetate extract of roots in alloxan induced diabetic Albino rats.

MATERIALS AND METHODS

Collection and authentication of plant material

The plant material including root was collected from the Udaipur district of Rajasthan and identified by Dr. P.J. Parmar, Joint Director, Botanical Survey of India, Arid Zone Regional Centre, Jodhpur and a herbarium specimen [LMC/PHARM/PC/CT/(08-09)/10819] has been deposited in the college for future reference.

* Corresponding Author

E-mail: shikhabatrapc@yahoo.co.in

Fax: 0291-2431820, Mob.: 094627-72264

Drying of plant material

The roots of *C. trifolia* were washed with water and dried under shade for three to four weeks. After complete drying, roots were subjected to size reduction to a coarse powder and stored in an airtight container in cool and dark place to prevent the deterioration by elevated temperature, light and moisture.

Extraction of plant material

The coarse powder of roots was successively extracted with petroleum ether (60-80°C), chloroform, ethyl acetate and ethanol using hot continuous extraction- Soxhletion. All the extracts were concentrated to dryness using rotary evaporator and preserved in refrigerator.

Preliminary phytochemical studies

Preliminary phytochemical studies were carried out to determine the presence of phytoconstituents of different categories - carbohydrates, proteins and amino acids, lipids, steroids, triterpenoids, alkaloids, flavonoids, glycosides, saponins and tannins in different plant extracts.

Animals used

Healthy adult Albino rats of Wistar strain weighing 180-200 gm were obtained from Animal House, Lachoo Memorial College of Science & Technology, Pharmacy Wing, Jodhpur. The animal house was well ventilated and animals had 12 ± 1 hour and day and night schedule with temperature between 15-20 ± 5°C. The animals were housed in standard polypropylene hygienic cages (three animals per cage). The animals were fed with standard rat pellet feed. The current work was carried out after approval by Institutional Animal Ethical Committee [Reg.No.541/02/C/CPCSEA].

Acute toxicity study of the extract

The acute toxicity study of the ethyl acetate extract was carried out according to the OECD guideline No. 420 (OECD, 2001).

Female Wistar rats (180-200 g) were used for this study. After sighting study, starting dose of 2000 mg/kg (*p.o.*) of the test extract was given to various groups containing five animals in each group.

The treated animals were monitored for 14 days, for mortality and general behavior. No death was observed till the end of the study (Jarald *et al.* 2008; Edwin *et al.* 2009).

Antidiabetic activity in alloxan-induced diabetic albino rats

Induction of diabetes mellitus

Diabetes was induced by the intraperitoneal (*i.p.*) injection of alloxan monohydrate (Shabeer *et al.* 2009) in normal saline to overnight fasted animals at a dose of 120 mg/kg body weight. The fasting blood glucose levels were determined after 72 hrs. of alloxan injection. Rats having blood glucose level above 250 mg/dl were used for the study.

Determination of antidiabetic activity

Diabetic rats were divided into four groups with six animals in each group. Group 1 served as control and received normal saline (2 ml/kg per day, *p.o.*). Group 2 served as standard and received glibenclamide (10 mg/kg per day, *p.o.*). Group 3 and 4 received ethyl acetate extract at the doses of 200 and 400 mg/kg per day, *p.o.* respectively. Fasting blood samples were collected on 0, 5, 10 and 15 days of the study by tail-vein method and blood glucose levels were estimated using an electronic glucometer and glucostrips (Abbott Diabetes Care Ltd., U.K.). On day 16, blood samples were collected under mild ether anesthesia from overnight fasted rats and separated serum was analyzed for various biochemical parameters (triglycerides, LDL, HDL, cholesterol, urea and creatinine) (Jain *et al.* 2010).

Statistical analysis

The obtained data were statistically analyzed by one way ANOVA and expressed as mean ± S.E.M. followed by Dunnett's *t*-test using computerized Graph Pad Prism 6.0, Graph pad software, U.S.A.

RESULTS

Preliminary phytochemical studies

The percentage yields of petroleum ether, chloroform, ethyl acetate and ethanol extracts were found to be 0.82%, 0.90%, 0.73% and 1.35%, respectively. Petroleum ether, ethyl acetate and ethanol extracts showed the presence of steroids, flavonoids and alkaloids, respectively.

Acute toxicity study

In this study, ethyl acetate extract was found to be safe up to the dose of 2000 mg/kg, and from the results, 400 mg/kg dose was chosen as the maximum dose for further experimentation.

Antidiabetic activity in alloxan-induced diabetic albino rats

The basal blood glucose levels of all the groups were not found significantly different from each other. Three days after alloxan administration, blood glucose levels were five-fold higher in all the groups and were not significantly different from each other. After 15 days, the blood glucose levels were decreased in all the treatment groups except the control group.

The administration of both the doses of ethyl acetate extract and glibenclamide restored the blood glucose levels significantly ($P < 0.05$) (Table 1).

The diabetic rats showed a significant increase in the levels of triglycerides, LDL, cholesterol, urea and creatinine and a significant decrease in HDL level. Treatment with ethyl acetate extract showed significant decrease in cholesterol and LDL levels ($P < 0.05$); at the same time, it showed an increase in HDL levels. Hypertriglyceridemia associated with hypercholesterolemia was also significantly prevented by treatment with ethyl acetate extract ($P < 0.05$).

Table 1: Hypoglycemic effect of ethyl acetate extract of roots of *C. trifolia* in alloxan induced diabetic albino rats.

Group	Dose	Fasting blood glucose level (mg/dl)			
		0 Day	5 th Day	10 th Day	15 th Day
Control	2 ml/kg	260.3 ± 2.9	266.16 ± 3.11	267.83 ± 2.9	269.66 ± 2.76
Glibenclamide	10 mg/kg	267.3 ± 3.68	119 ± 4.13*	100.8 ± 3.5*	85 ± 3.44*
Ethyl acetate extract	200 mg/kg	272 ± 4.04	230 ± 3.59*	199.7 ± 4.66*	167.16 ± 2.76*
Ethyl acetate extract	400 mg/kg	268.7 ± 3.84	226.7 ± 4.04*	172.8 ± 4.2*	124 ± 3.51*

Each value represents the mean ± SEM (n = 6), *P < 0.05 vs Control (ANOVA followed by Dunnet's *t*-test).

Table 2: Effect of ethyl acetate extract of roots of *C. trifolia* on biochemical parameters in alloxan induced diabetic rats after 15 days of treatment.

Group	Dose	Biochemical parameters (mg/dL)					
		TG	HDL	LDL	Cholesterol	Urea	Creatinine
Control	2 ml/kg	123.83 ± 1.44	10.5 ± 0.76	55.5 ± 1.41	81.5 ± 3.54	272.5 ± 3.66	1.89 ± 0.058
Glibenclamide	10 mg/kg	38 ± 0.73*	26 ± 1.12*	22.16 ± 0.70*	32.66 ± 0.71*	32.5 ± 0.76	0.61 ± 0.073
Ethyl acetate extract	200 mg/kg	76.45 ± 0.61*	16.06 ± 0.08*	37.43 ± 0.14*	54.38 ± 0.32*	74.5 ± 1.17	0.80 ± 0.154
Ethyl acetate extract	400 mg/kg	62.68 ± 0.22*	18.78 ± 0.18*	26.13 ± 0.07*	42.15 ± 0.25*	66.05 ± 0.45	0.63 ± 0.014

Each value represents the mean ± SEM (n = 6), *P < 0.05 vs Control (ANOVA followed by Dunnet's *t*-test).

Treatment with this extract significantly decreased urea and creatinine levels (P<0.05). The extract was found to be effective in alleviating the experimental diabetes and diabetes related complications (Table 2).

DISCUSSION

The present work was undertaken to evaluate the antidiabetic activity of roots of *C. trifolia*. As the phytochemical investigations showed the presence of flavonoids in the ethyl acetate extract and they are the known bioactive antidiabetic principles to regenerate the damaged β -cells in pancreas (Rahman *et al.* 1989), the ethyl acetate extract was further evaluated for antidiabetic activity. To the best of our knowledge, this is the first report on antidiabetic activity of this plant. Alloxan is a urea derivative which causes selective necrosis of the pancreatic β -cells. It is used to produce experimental diabetes in animals such as rabbits, rats, mice and dogs. (Etuk *et al.*, 2010). Our investigation indicated the efficacy of ethyl acetate extract in the maintenance of blood glucose levels in alloxan induced diabetic rats suggesting that the extract may act by regenerating the β -cells. The levels of serum lipids are usually elevated in diabetes mellitus and such an elevation represents a risk factor for coronary heart diseases. This abnormal high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots mainly due to the action of insulin. Under normal circumstances, insulin activates the enzyme lipoprotein lipase which hydrolyses triglycerides. However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridemia (Pushparaj *et al.* 2007) and insulin deficiency is also associated with hypercholesterolemia due to metabolic abnormalities that occur sequentially (Murali *et al.* 2002). In our study, the diabetic rats showed hypercholesterolemia and hypertriglyceridemia and the treatment with extract significantly (P<0.05) decreased the levels of cholesterol and triglycerides and increased the level of HDL. The diabetic hyperglycemia induced by alloxan produces elevation of plasma levels of urea and creatinine, which are considered as significant markers of renal dysfunction (Alarcon *et al.* 2005). Results showed that ethyl acetate extract also significantly (P<0.05) decreased the levels of

urea and creatinine. This further confirms the efficacy of this extract in reducing the complications of biochemical parameters seen in diabetics. We conclude that the plant has potential in decreasing the blood glucose level and other complications associated with experimental diabetes and the present work supports the folklore claim of this plant for possessing hypoglycemic activity. As the results indicated that *C. trifolia* possess antidiabetic potential; it could be used as a source of active principles for treating diabetes.

REFERENCES

- Alarcon A.F.J., Calzada B.F., Hernandez G.E., Ruiz A.C., Roman R.R. Acute and chronic hypoglycaemic effect of *Ibervillea sonorae* root extracts-II. *J Ethnopharmacol.* 2005; 97: 447-452.
- Ali M.K., Narayan K.M.V., Mohan V. Innovative research for equitable diabetes care in India. *Diabetes Res Clin Pract.* 2009; 86: 155-167.
- Edwin J., Joshi S.B., Jain D.C. Biochemical study on the hypoglycemic effects of extract and fraction of barks of *Cassia fistula* Linn. in alloxan-induced diabetic rats. *Indian Drugs.* 2009; 46 (7): 54-60.
- Etuk E.U. Animal models for studying diabetes mellitus. *Agr Biol J N Am.* 2010; 1 (2): 130-134.
- Gupta A.K., Sharma M. Review on Indian medical plants. New Delhi: Indian Council of Medical Research (2007) 879-882
- Jain A., Katewa S.S., Galav P.K., Sharma P. Medicinal plant diversity of Sitamata wildlife sanctuary, Rajasthan. *J Ethnopharmacol.* 2005; 102: 143-157.
- Jain S., Bhatia G., Barik R., Kumar P., Jain A., Dixit V.K. Antidiabetic activity of *Paspalum scrobiculatum* Linn. in alloxan induced diabetic rats. *J Ethnopharmacol.* 2010; 127: 325-328.
- Jarald E.E., Joshi S.B., Jain D.C. Antidiabetic activity of flower buds of *Michelia champaca* Linn. *Indian J Pharmacol.* 2008; 40 (6): 256-260.
- Murali B., Upadhyaya U.M., Goyal R.K. Effect of chronic treatment with *Enicostemma littorale* in non-insulin dependent diabetic (NIDDM) rats. *J Ethnopharmacol.* 2002; 81: 199-204.
- OECD (Organization for Economic co-operation and Development). OECD guideline for the testing of chemicals, 2001
- Pushparaj P.N., Low H.K., Manikandan J., Tan B.K.H., Tan C.H. Anti-diabetic effects of *Cichorium intybus* in streptozotocin-induced diabetic rats. *J Ethnopharmacol.* 2007; 111: 430-434.
- Rahman A.U., Zaman K. Medicinal plants with hypoglycemic activity. *J Ethnopharmacol.* 1989; 26: 1-55.
- Shabeer J., Srivatava R.S., Singh S.K. Antidiabetic and antioxidant effect of various fractions of *Phyllanthus simplex* in alloxan induced diabetic rats. *J Ethnopharmacol.* 2009; 124: 34-38.

Singh V and Pandey RP. Ethnobotany of Rajasthan, India. 1st ed. Scientific Publishers, Jodhpur, India (1998) 75-76

Swarnkar S., Katewa S.S. Ethnobotanical observation on tuberous plants from tribal area of Rajasthan (India). Ethnobotanical leaflets. 2008; 12: 647-666.

The Wealth of India. Vol. 3. Publications & Information Directorate, CSIR, New Delhi (1992) 39-40

Zhang P., Zhang X., Brown J., Vistisen D., Sicree R., Shaw J. *et al.* Global healthcare expenditure on diabetes for 2010 and 2030. Diabetes Res Clin Pract. 2010; 87: 293-301.

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