Original Article

Preliminary Phytochemical Studies and Evaluation of Antidiabetic Activity of Stem Bark of *Acacia senegal* (L.) Willd. in Alloxan Induced Diabetic Albino Rats

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ABSTRACT

The aim of present study was to investigate the potential of stem bark of *Acacia senegal* (L.) Willd for antidiabetic activity in alloxan induced diabetic albino rats. Phytochemical studies showed the presence of flavonoids in ethyl acetate extract. Antidiabetic activity of stem bark of this plant was evaluated by measuring blood glucose levels and serum biochemical parameters after dosing with ethyl acetate extract. Blood samples were collected from overnight fasted diabetic rats on 0, 5, 10 and 15 days of treatment to determine glucose levels. On day 16, blood samples were collected to estimate biochemical parameters. In diabetic rats, both the doses (200 mg/kg and 400 mg/kg) of ethyl acetate extract were found to be significantly ($P<0.05$) active in comparison to control and favourable changes in biochemical parameters were also observed. It can be concluded from this study that the ethyl acetate extract of stem bark of *A. senegal* possess significant antidiabetic activity.

Keywords: *Acacia senegal*, Hypoglycemic, Antidiabetic, Alloxan-induced diabetes.

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[1]. World Health Organization (WHO) had reported 32 million people with diabetes residing in India, while the International Diabetes Federation (IDF) had reported an estimated 40.9 million of diabetics for the same country[2].

Acacia senegal (L.) Willd. is a small deciduous tree having smooth bark, pale-greenish grey in colour that peels off in flakes in older twigs[3]. It is extremely hardy and resistant to drought and one of the main cash crops of the desert region[4]. It is native to Sudan, found growing throughout India particularly in Haryana, Gujarat and Rajasthan states. It is found on the hillsides and gravel throughout the Rajasthan[5,6,7]. It is commonly known as kumat, kumatio, kumta belongs to the family Mimosaceae[8,9]. This tree yields the true Gum Arabic which is used as a binding agent in the manufacture of cough pastilles in the pharmaceutical industry and for its demulcent and emollient action[10]. The Bhopas in Udaipur region prescribe the gum as a part of food for diabetic patients[9] and stem bark of the same plant had been proven to possess antioxidant activity. In view of these observations[11], we had investigated the antidiabetic activity of stem bark of this plant in alloxan induced diabetic albino rats.

MATERIALS AND METHODS

Collection and authentication of plant material

The plant material was collected from the outer skirts of Jodhpur district (Jodhpur - Jaisalmer National Highway). Taxonomic identification was carried out at Botanical Survey of India (BSI), Arid Zone Regional Centre, Jodhpur and a voucher specimen [LMC/PHARM/PC/AS (08-09)/10819] was deposited in the college herbarium for future reference.

Drying of plant material

Stem bark of *A. senegal* was dried under shade for three to four weeks. After complete drying, bark was pulverized into coarse powder. The comminuted bark was packed in airtight polythene bags and stored in cool & dark place to prevent deterioration by elevated temperature, light and moisture.

Extraction of plant material

The coarse powder of stem bark was successively extracted with petroleum ether (60-80^{o}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.

Phytochemical studies

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

Pharmacological studies

Animals used

Healthy adult albino rats of Wistar strain weighing 180-200 gm were obtained from Animal House, Lachoo Memorial College of Science and 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^{o}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 our after approval by Institutional Animal Ethical Committee [Reg.No.541/ 02/C/CPCSEA].

Acute toxicity study

Acute toxicity study of ethyl acetate extract of *A. senegal* was carried out according to the OECD guideline No. 420[12]. Female Wistar rats (180-200 gm) were used for this study. After the sighting study, starting dose of 2000 mg/kg BW (*p.o.*) of the test sample was given to various groups containing five animals in each group. The

treated animals were monitored for mortality and general behavior for 14 days. No death was observed till the end of the study[13,14].

Antidiabetic activity

Induction of diabetes mellitus

Diabetes was induced by the intraperitoneal injection of alloxan monohydrate[15] in normal saline to overnight fasted animals at a dose of 120 mg/kg BW. The fasting blood glucose levels were determined after 72 hours of alloxan injection. Rats having blood glucose level above 250 mg/dl were used for the study.

Diabetic rats were divided in four groups; each group comprised of six rats.

Group 1 (Control): Diabetic rats were given single oral dose of 0.5 ml/100 gm BW of normal saline.

Group 2 (Standard): Diabetic rats were given 10 mg/kg BW of standard drug - glibenclamide once a day.

Group 3: Diabetic rats were given 200 mg/kg BW of ethyl acetate extract once a day.

Group 4: Diabetic rats were given 400 mg/kg BW of ethyl acetate extract once a day.

Determination of hypoglycemic effect

Doses of ethyl acetate extract, standard drug and normal saline were calculated according to the body weight of each animal. Suspension of ethyl acetate extract, standard drug and normal saline were administered orally to each animal using stainless steel feeding needle fitted on a plastic syringe. Feeding needle was inserted through the oesophagus up to the stomach and the plunger was pressed slowly and steadily.

Treatment was continued for 15 consecutive days. Fasting blood samples (0.2 ml) were collected on day 0, 5, 10 and 15 of the study by tail-vein method and fasting blood glucose levels were estimated using Autoanalyzer.

Determination of biochemical parameters

On day 16, blood samples were collected from the retro-orbital plexus of overnight fasted rats under mild ether anesthesia and kept aside for ½ hr for clotting. Serum was separated and analyzed for biochemical parameters - triglycerides, HDL, LDL, cholesterol, urea and creatinine. These values were compared with the values obtained from the animals of control group.

Statistical analysis

The data obtained were statistically analyzed by one way ANOVA and expressed as mean ± S.E.M. followed by Dunnet's *t*-test using computerized Graph Pad *Prism version 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 0.59%, 0.71%, 2.65% and 1.93% respectively. Petroleum ether extract showed the presence of lipids, steroids and triterpenoids. Chloroform extract showed the presence of tannins. Ethyl acetate extract showed the presence of flavonoids and ethanolic extract did not show the presence of any phytoconstituent.

Acute toxicity study

Acute toxicity study revealed the non-toxic nature of ethyl acetate extract. There was no mortality and no toxic reactions found at any of the doses tested until the end of the study period. According to OECD guidelines, ethyl acetate extract came in category-5 having LD_{50} more than 2000 mg/kg BW. Therapeutic range was considered between 1/20 to 1/5 times of LD_{50}. Accordingly, 200 mg/kg and 400 mg/kg BW doses of ethyl acetate extract were selected for antidiabetic activity.

Antidiabetic activity in alloxan-induced diabetic albino rats

Experimental animals were made diabetic using alloxan which is a urea derivative that causes selective necrosis of pancreatic β-cells[16]. In diabetic rats, alloxan led to 1.5 fold elevation of fasting blood glucose level which was maintained over a period of two weeks. Basal blood glucose levels of all the groups were not found significantly different from each other. Daily treatment by ethyl acetate extract led to a dose dependent fall in blood glucose levels.

Administration of 200 mg/kg BW of ethyl acetate extract showed 29.43% decline in blood glucose levels of experimental animals on day 15. The same dose of this extract also decreased the serum cholesterol, serum triglycerides, serum LDL, serum urea and creatinine levels by 19.84%, 23.14%, 28.82%, 71.43% and 51.85% respectively, in animals of extract treated group in comparison to animals of control group on day 16 of the study. This dose increased the serum HDL levels by 31.71% in animals of extract treated group in comparison to control group on day 16 of the study.

Administration of 400 mg/kg BW of ethyl acetate extract showed 38.76% decline in blood glucose levels of experimental animals on day 15. The same dose of this extract also decreased the serum cholesterol, serum triglycerides, serum LDL, serum urea and creatinine levels by 25.97%, 31.36%, 45.96%, 73.69% and 58.73% respectively, in animals of extract treated group in comparison to animals of control group on day 16 of the study. This dose increased the serum HDL levels by 58.95% in animals of extract treated group in comparison to control group on day 16 of the study. Thus, the administration of both the doses of ethyl acetate extract and glibenclamide restored the blood glucose levels significantly ($P<0.05$) and also showed favourable changes in biochemical parameters (Table 1 and Table 2).

DISCUSSION

The present work was undertaken to evaluate the antidiabetic activity of stem bark of *A. senegal*. In phytochemical studies, ethyl acetate extract of stem bark of *A. senegal* showed the presence of flavonoids that are known bioactive antidiabetic principles and also known to regenerate the damaged β-cells in pancreas[17]. In our earlier investigations, the ethyl acetate extract of this plant was found to significantly ($P<0.05$) increase the uptake of glucose in isolated rat hemi-diaphragm *in vitro* model[18]. Therefore, in the present work only the ethyl acetate extract was further evaluated for antidiabetic activity in alloxan induced diabetic albino rats.

To the best of our knowledge, this is the first report on antidiabetic activity of this plant. 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 of pancreas. 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[19] and insulin deficiency is also associated with hypercholesterolemia due to metabolic abnormalities that occurs sequentially[20]. In our study, the diabetic rats showed hypercholesterolemia and hypertriglyceridemia and the treatment with ethyl acetate extract significantly ($P<0.05$) decreased the levels of cholesterol and

triglycerides and increased the level of HDL. Diabetic hyperglycemia induced by alloxan elevates the plasma levels of urea and creatinine which are considered as significant markers of renal dysfunction[21]. Results showed that the 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.

CONCLUSION

This study indicated that the ethyl acetate extract of stem bark of *A. senegal* has potential to decrease blood glucose level and to reduce the complications associated with experimental diabetes. This study also supports the folklore usefulness of this plant in the treatment of diabetic patients. It can be concluded that the stem bark of this plant could be further investigated for antidiabetic bioactive principles.

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Table 1. Hypoglycemic effect of ethyl acetate extract in alloxan induced diabetic rats after 15 days of treatment

Group	Dose	Fasting blood glucose level (mg/dl)			
		0 Day	5th Day	10th Day	15th Day
Control	2 ml/kg	260.30 ± 2.90	266.16 ± 3.11	267.83 ± 2.90	269.66 ± 2.76
Glibenclamide	10 mg/kg	267.30 ± 3.68	119.00 ± 4.13*	100.8 ± 3.5*	85 ± 3.44*
Ethyl acetate extract	200 mg/kg	267.00 ± 2.58	192.30 ± 2.40*	189.80 ± 1.27*	188.40 ± 1.30*
Ethyl acetate extract	400 mg/kg	276.30 ± 2.40	172.70 ± 2.38*	170.40 ± 1.23*	169.20± 0.80*

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 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	95.17 ± 0.703*	13.83 ±0.454*	39.50± 2.43*	65.33 ± 0.88*	77.83 ± 0.60*	0.91 ± 0.018*
Ethyl acetate extract	400 mg/kg	85± 0.574*	16.69 ± 0.47*	30 ± 0.57*	60.33± 0.76*	71.67 ± 1.05*	0.78 ± 0.025*

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