CODEN (USA): IJPB07 ISSN: 2320-9267

Indian Journal of Pharmaceutical and Biological Research (IJPBR)

Journal homepage: www.ijpbr.in

Original Research Article

Studies on antidiabetic activity of *Acacia ferruginea* DC. stem bark

Jhuma Deb[1*], Anoop Singh[1], Devendra Singh Rathore[1], Gouri Kumar Dash[2], Nilip Kanti Deb[1]

[1]Department of Pharmaceutical Sciences, NIMS University, Shobha Nagar, Jaipur -303121, India
[2]Faculty of Pharmacy and Health Sciences, Universiti Kuala Lumpur Royal College of Medicine Perak, 30450 Ipoh, Malaysia

ARTICLE INFO:

Article history:
Received: 30 October 2015
Received in revised form:
18 November 2015
Accepted: 16 December 2015
Available online: 30 December 2015

Keywords:
Acacia ferruginea ,
Alloxan,
Metformin,
Antidiabetic

ABSTRACT

The methanol extract of *Acacia ferruginea* (Family- Mimosaceae) was studied for possible antidiabetic activity on normoglycaemic, OGTT and alloxan induced diabetic rats at doses of 100, 200, and 400 mg/kg, p.o. The acute toxicity studies were carried out on Swiss albino mice to determine the LD_{50} values. The experiments were performed as per OECD guidelines. The results of the normoglycaemic, OGTT and hyperglycaemic studies revealed that the extract exhibited reduction in blood glucose concentration in a dose dependant manner as compared to the standard drug metformin (250 mg/kg, p.o.). The acute toxicity studies revealed no signs of mortality in animals treated with a single dose of 2000 mg/kg body weight. Preliminary phytochemical studies of the methanol extract revealed presence of alkaloids, steroids, triterpenoids, saponins, flavonoids, tannins and phenolic compounds, carbohydrates, gums and mucilages, proteins and amino acids. The present study justifies the use of the plant for treating diabetes as suggested in folklore remedies.

Introduction

Disease is undoubtedly the most fatal enemy of mankind. To prevent its approaches or to overcome its attacks is perhaps the most important concern of our lives and an acquisition that appears only attainable by the most natural and simple means [1]. It is the herbs that sustained our ancestors into this century and still provide the chief form of medicine for most of the world's people [2]. Diabetes mellitus (DM) is ranked seventh among the leading causes of death in the world [3]. The number of people afflicted with diabetes was 366 million in 2011; by 2030 this will have risen to 552 million worldwide [4, 5]. The number of people with diabetes is increasing due to population growth, urbanization, and increasing prevalence of obesity and physical inactivity. Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Though oral hypoglycemic agents are available along with insulin for the treatment of DM, there is an increased demand to use natural products [6]. The use of herbal medicine for the treatment of diabetes has gained importance throughout the world [7].

Acacia ferruginea DC. (Family- Mimosaceae) is a medium sized, drought-resistant, deciduous tree, 3.6 - 4.2m in height and grows up to 1.0 m in girth [8]. The plant has been used in different traditional systems of medicine for curing various diseases. In Ayurveda, it is used to treat '*Vata*' and '*Kapha*' disorders and the bark decoction is recommended for treating various skin infections. Traditionally, the decoction of stem and root bark is used externally as a cure from itching, leucoderma and ulcers. The paste of the stem bark along with water is used as a remedy for dysentery, piles and diabetes [9]. Thus, the present study was undertaken to evaluate the antidiabetic activity of methanolic extract of *Acacia ferruginea.* Reports on the antidiabetic studies of the stem bark are not found in the literature. Thus, in the present study, we report the antidiabetic activity of the stem bark in animal models.

Materials and Methods

Plant material

The fresh stem bark was collected from well grown and matured trees from Tirumala, Andhra Pradesh during January 2011 and authenticated by the Botanist Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. The collected plant material was washed, shade dried and pulverised to coarse powder.

Corresponding Author: Mrs. Jhuma Deb, Department of Pharmaceutical Sciences, NIMS University, Shobha Nagar, Jaipur, India.
E-Mail: jhumaniladi@gmail.com

Preparation of extract

The dried powdered plant material was defatted with petroleum ether (40 - 60^{0}C) and then extracted with methanol using soxhlet extractor [10, 11]. Following extraction, the methanol extract was concentrated under reduced pressure to yield dry solid residue (yield: 15.91%w/w).

Preliminary phytochemical studies

The methanol extract was subjected to preliminary phytochemical tests to find out different class of phytochemicals it contains [12, 13].

Experimental animals

Animals were selected as per the Organization for Economic Cooperation and Development (OECD) guidelines [14]. Healthy female young and nulliporous, non pregnant Swiss albino mice weighing from 20-25g were selected for acute toxicity studies [15]. Wistar albino rats of either sex weighing 150-200 g were used for antidiabetic activity. The animals were divided into different groups of six animals in each (three animals per step). The temperature, relative humidity, lighting in the experimental animal room was maintained as per the OECD guidelines (Table 1). For feeding, conventional laboratory diets were used with an unlimited supply of drinking water. To acclimatize with the laboratory conditions, randomly selected animals were marked to permit individual identification and kept in clean polypropylene cages for 5 days prior to commencement of the experiment.

Table- 1: Temperature, relative humidity, lighting in the experimental animal room

Sr.No.	Conditions	Requirements
1.	Room temperature	22^{0}C (+/-3^{0}C)
2.	Humidity	50-60%
3.	Light and dark period	12/12 hours
4.	Bedding	Clean sterilized husk
5.	Oral feed	Conventional Laboratory diets
6.	Distilled drinking water	Unlimited supply

Acute toxicity study

The OECD panel of experts has defined acute oral toxicity as "the adverse effect occurring within a short time of (oral) administration of a single dose of a substance or multiple doses given within a span of 24 hours". The purpose of acute toxicity studies is to determine the LD_{50} values which help in determining the safe dose range at which the drug can be used such that there is no harmful or lethal effect on the animal [16]. Test at one dose level of 2000 mg/kg body weight with methanol extract was carried out with three animals per step. The animals were fasted for 3-4 h prior to dosing but water was not withheld. Following the period of fasting, the animals were weighed and the test substance administered. The test samples were prepared by dissolving the extract in1% Tween-80 in normal saline just prior to dosing and were kept chilled and tightly capped. The test substance was administered in a single dose by gavage using specially designed mice oral needle. After the substance has been administered, food was withheld for an hour. The same procedure was again followed with the next three animals [17-20].

Evaluation of antidiabetic activity

The methanolic extract was administered to Wistar rats at doses of 100, 200, and 400 mg/kg. Metformin (250 mg/kg, p.o.) was used as reference standard for activity comparison of hypoglycemic activity. The test samples were administered through oral route.

Using normoglycaemic rats

The animals were fasted for 18 h, but were allowed free access to water before and throughout the duration of experiment. At the end of the fasting period, taken as zero time (0 h), blood was with-drawn from the tip of the tail of each rat and the blood glucose was estimated. The normal rats were then divided into five groups of six animals in each. Negative control was designated as group I and received vehicle, normal saline (2 ml/kg) through oral route. Group-II received metformin. Then the other groups received 100, 200 and 400 mg/kg of methanolic extract [22]. Blood glucose levels were monitored after 1, 2, 4 and 8 h of administration of single dose of test samples. The results are given in Table-2

Oral glucose tolerance test (OGTT) in rats

Fasted rats were divided into five groups of six rats in each. Group I served as a control and received only normal saline (2 ml/kg) through oral route. Group-II received metformin. The other groups received 100, 200 and 400 mg/kg of methanolic extract. After 30 min of treatment, rats of all groups were loaded orally with glucose (2 g/kg, p.o). Blood samples were collected before and at 30, 60, 150 and 180 min after glucose administration. The results are given in Table-3.

Using hyperglycaemic rats

The acclimatized animals were kept fasting for 24 h and injected intra peritoneally a dose of 120 mg/kg of alloxan monohydrate in normal saline. After one hour, the animals were provided feed *ad*

libitum. Animals were considered diabetic when the blood glucose level was raised beyond 200mg/100ml of blood. This condition was observed at the end of 48 h after alloxanisation. The animals were segregated into five groups of six rats in each. Group-I served as control and received vehicle (2ml/kg) through oral route. Group-II received metformin. Group-III-V received the plant extract at doses of 100, 200 and 400 mg/kg in a similar manner. Blood samples were collected and were estimated at 0 h, 1 h, 2 h, 4 h and 8 h. The results are depicted in Table-4.

Statistical analysis

All the results were statistically analysed using one way ANOVA followed by Dunnet's t-test. A 'P' value less than 0.05 was considered significant.

Results

Preliminary phytochemical studies

The methanol extract found to contain alkaloids, saponins, flavonoids and tannins.

Acute toxicity studies

After the extract was orally administered, animals were observed keenly for about 48 h. Wellness parameters of animals were observed continuously during the first 30 min after dosing and observed periodically (with special attention given during the first 4 h) for the next 24 h [21]. The acute toxicity studies revealed that the extract did not show any signs of mortality in animals treated with a single dose of 2000 mg/kg body weight. There were no clinical signs in the skin and fur, eyes and mucus membrane, respiratory rate, circulatory signs, salivation, perspiration, tremors and convulsion among mice. However the extracts induced mild purgation with the tested extract. Thus, the LD_{50} value may be higher than 2000 mg/kg body weight.

Using hyperglycaemic rats

In this study, the rise in the blood glucose level was observed after 24 h of alloxanization (Table 3). Single administration of the methanol extract at the tested dose level in diabetic rats showed significant reduction in blood glucose level. Metformin (250 mg/kg, p.o.) showed maximum reduction (58.75%) decrease in blood glucose level after 8 h and the methanol extract (400 mg/kg, p.o.) exhibited (57.19%) decrease in blood glucose level. The comparable effect of the extract with metformin may suggest similar mode of action, since alloxan permanently destroys the pancreatic ß-cells and the extract lowered blood sugar level in alloxanised rats, indicating that the extract possesses extrapancreatic effects.

Table-1: Effect in normoglycemic rats

Group	Treatment	Dose (mg/kg)	Fasting	Blood glucose concentration (mg / dl) (normoglycaemic study)			
				Time (h) after treatment			
				1	2	4	8
I	Control	2 ml/kg	95.33±2.65	96.16±2.32	98.66±2.35	97.16±2.21	97.5±2.99
II	Metformin	250 mg/kg	97.5±2.85	61.66±2.10** (36.82%)	52±2.42** (46.66%)	48.33±3.52** (50.437%)	44.66±3.75** (54.19%)
III	Methanol extract	100	98.33±3.54	95.16.±3.60 (3.22%)	91.16±3.75 (7.29%)	81.33±4.48 (17.28%)	80.5±2.41 (18.13%)
IV		200	97.83±3.32	92.66±2.56 (5.28 %)	83.16±2.46 (14.99%)	78.33±4.72 (19.93%)	67.16±4.976* (30.9%)
V		400	98.5±2.92	82.16±3.21 (16.58%)	70.16±4.76* (28.77%)	52.83±3.87** (46.36%)	46.33±3.47** (52.96%)

Results expressed as Mean ± SEM from six observations (n=6). *P<0.05, **P<0.01 as compared with control group (One way, ANOVA followed by Dunnet's t-test). Figures in parenthesis denote percentage reduction of blood glucose.

Table-2: Effect on oral glucose tolerance in normal rats

Group	Treatment	Dose (mg/kg)	Fasting	Blood glucose concentration (mg / dl) (oral glucose tolerance study)			
				Post treatment			
				30 min.	60 min.	150 min.	180 min.
I	Control	2 ml/kg	93.66±2.69	128.5±10.14	148.66±12.64	159.83±13.26	153.33±13.63
II	Metformin	250 mg/kg	95.33±2.34	127.83±5.42	104.66±7.54* (18.12%)	90.5±8.72** (29.20%)	76.16±33.02** (40.42%)
III	Methanol extract	100	91.33±8.65	125.16±10.76	122.66±9.22 (1.99%)	119.83±11.8 (4.25%)	111.66±10.67* (10.78%)
IV		200	95.83±4.78	131.66±9.91	128.16±10.67 (2.65%)	109.5±11.41* (16.83%)	104.16±10.76* (20.88%)
V		400	97.33±9.71	130.5±11.62	105.13±9.18* (19.44%)	97.33±8.69** (25.48%)	81.16±7.78** (37.80%)

Results expressed as Mean ± SEM from six observations (n=6). $^{*}P<0.05$, $^{**}P<0.01$ as compared with control group (One way, ANOVA followed by Dunnet's t-test). Figures in parenthesis denote percentage reduction of blood glucose.

Table-3: Effect of the bark on the blood glucose level in alloxan induced diabetic rats

Group	Treatment	Dose (mg/kg)	Fasting	Blood glucose concentration (mg / dl) (Hypoglycemic study)			
				Time (h) after treatment			
				1	2	4	8
I	Control	2 ml/kg	238.33±2.71	249.16±1.81	252.5±2.16	255.83±1.9	259.83±2.75
II	Metformin	250 mg/kg	239.16±10.2	201.16±14.03* (15.88%)	154.33±12.33** (35.46%)	114.66±9.63** (52.05%)	98.63±9.93** (58.75%)
III	Methanol extract	100	238.66±10.67	228±11.34 (4.46%)	224.16±10.56 (6.07%)	213.66±9.67 (10.47%)	196.5±12.43* (17.66%)
IV		200	239.16±9.31	199.5±9.33* (16.58%)	194±11.31* (18.88%)	190.16±10.86* (20.48%)	163.5±8.32** (31.63%)
V		400	236.33±12.11	195.16±10.43* (17.42%)	189.83±11.62* (19.67%)	157.33±9.28** (44.85%)	101.16±10.46** (57.19%)

Results expressed as Mean ± SEM from six observations (n=6). $^{*}P<0.05$, $^{**}P<0.01$ as compared with control group (One way, ANOVA followed by Dunnet's t-test). Figures in parenthesis denote percentage reduction of blood glucose.

Discussion

Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agent for the prevention of diseases and ailments. The results of the study indicated that the stem bark possess significant antidiabetic activity. *A. ferruginea* has been enlisted under the IUCN Red list of Threatened Species. Therefore there is an urgent need for conservation and research on this plant before it disappears from the earth. Following the WHO's recommendation for research on the beneficial uses of medicinal plants in treatment of diabetes mellitus, investigations on hypoglycemic agents derived from medicinal plants was undertaken. Currently available synthetic antidiabetic agents produce serious side effects like hypoglycemic coma and hepatorenal disturbances. Moreover they are not safe for use during pregnancy. Hence, the search for safer and more effective hypoglycemic agents has continued. The exact biological active constitutent(s) responsible for the said effect are neither reported nor was the exact mode of action of the hypoglycemic activity reported earlier, with the lone observation that it is used in folklore diabetic treatments. The results of the present study justify the use of the stem bark of the plant for treating diabetes as suggested in the folklore remedies.

Conclusion

Therefore the need of the hour is to call for research on this plant to isolate the constituents responsible for the antidiabetic activity and also to find out other possible pharmacological activities.

Acknowledgment: The authors are thankful to the Management of NIMS University, Jaipur for their constant help and support.

Conflict of interest: We declare that we have no conflict of interest.

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Cite this article as: Jhuma Deb, Anoop Singh, Gouri Kumar Dash, Nilip Kanti Deb. Studies on antidiabetic activity of *Acacia ferruginea* DC. stem bark. **Indian J. Pharm. Biol. Res.2015; 3(4):11-15.**