Recent Research in Science and Technology 2013, 5(1): 51-53
ISSN: 2076-5061
Available Online: http://recent-science.com/

Antidiabetic activities of *Cassia occidentalis*

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Abstract

The present investigation was carried out to evaluate the anti-diabetic activities of *Cassia occidentalis* (Seena, coffee weed), a well known medicinal plant commonly found in India and other tropical countries. Various medicinal properties have been attributed to this plant in the traditional system of Indian medicine. The aqueous and methanolic extracts of aerial parts, viz. leaves, stem and seeds of the plant, *Cassia occidentalis* possessed anti-hyperglycemic/ anti-diabetic activity against alloxan-induced animal model. All aqueous-treated rats showed no discernible behavioral changes up to 3000 mg/kg by oral route. No mortality was observed at this dose during 72 h observation period. Amongst all the extracts, potent anti-diabetic activity was observed in aqueous extracts of leaves of *C. occidentalis* followed by aqueous extracts of seeds and aqueous extracts of stem. In normal animals, significant (p<0.05) reduction in the blood glucose level was observed by the aqueous extracts as compared to the control and methanolic extracts. However, treatment of methanolic extracts of aerial parts of *C. occidentalis* could not bring back the sugar to normal levels. Acute and chronic treatment of the aqueous extract of aerial parts of *C. occidentalis* (3000 mg/kg) in alloxan-induced diabetic rats resulted in a significant (p<0.05) decrease in the elevated blood glucose levels as compared to the control, there was significant reduction in blood glucose level in the group treated with glibenclamide at 0.5 mg/kg. The results showed that blood glucose level gets decreased after varying the dose level. Thus the findings confirmed that level of blood glucose gets normal in dose-dependent manner.

Keywords: Anti-hyperglycemic/anti-diabetic activity, *Cassia occidentalis*, aqueous and methanolic solvent extracts, alloxan-induced diabetic rats

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder resulting from insulin deficiency, characterized by hyperglycaemia, altered metabolism of carbohydrates, protein and lipids, and an increased risk of vascular complication [1]. In conventional therapy, type I diabetes is managed with exogenous insulin and type 2 with oral hypoglycemic agents (sulphonylureas, biguanides etc). In traditional practice medicinal plants are used in many countries to control diabetes mellitus. Diabetes mellitus has recently been identified by Indian Council of Medical Research (ICMR) as one of the refractory diseases for which satisfactory treatment is not available in modern allopathic system of medicine and suitable herbal preparations are to be investigated. A large number of plant preparations have been reported to possess antidiabetic activity over last several decades. Researchers in India have documented the use of over 150 plants in various families with hypoglycemic activity [2]. *Cassia occidentalis* of family Caesalpiniaceae is a common weed scattered from foothills of Himalayas to West Bengal, South India, Burma, and Sri Lanka. The plant is a diffuse (usually annual) under shrub with loosely spreading branches 60-150 cm long, found throughout India, up to an altitude of 1500 m [3]. Different parts of this plant have been reported to possess anti-inflammatory, antihepatotoxic [4]. antibacterial, [5] and antiplasmodial activities [6]. They possess purgative, tonic, febrifugal, expectorant, and diuretic properties. The plant is also used to cure sore eyes, hematuria, rheumatism, typhoid, asthma, and disorder of hemoglobin and is also reported to cure leprosy. An infusion of the bark is given in diabetes [3]. A wide range of chemical constituents isolated from *C. occidentalis* including sennoside, anthraquinone glycoside [7], fatty oils, flavonoid glycosides, galactomannan, polysaccharides, and tannins [8]. In view of alleged antidiabetic potential of *C. occidentalis*, different extracts of the plant on fasting blood sugar levels were investigated. Histological examination was also carried out on pancreatic tissue of experimental animals.

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MATERIAL AND METHODS

The chemicals and reagents used were of Analytical grade and were procured from Ranchem and CDH, India. Alloxan monohydrate used for induced diabetes in rats was procured from Sigma Chemicals, USA.

Plant Materials

Plant parts were collected from Garhwal region of Uttarakhand, India. The collection was based on ethnophamocological and ethnobotanical literature, primarily Gaur, 1999 in consultation with Dr. G.S. Rajwar, Department of Botany, Govt. P.G. College, Rishikesh, Uttarakhand, India and further identified from Botanical Survey of India, Dehradun. Voucher specimens of plants were stored in the Institute herbarium for future reference. The collection took place in the flowering season of year 2010. The plant parts were dried in the shade and were pulverized to form the powder.

Preparation of Plant extracts

Plant parts, leaves, stem and seeds were separated, washed with distilled water, dried under shade and pulverized. Briefly 20 g portions of each of the powdered plant material was soaked separately in different solvents i.e. methanol and water on the basis of increasing polarity for 72 h. Each mixture was stirred every 24 h using a sterile glass rod. At the end of extraction, each solvent was passed through Whatmann filter paper No. 1 (Whatmann, England) The filtrates obtained were concentrated in vacuo using water bath at 30 ^{0}C .

Animals

Healthy male albino rats of body weight 150-180 g were used for the present studies. The animals were fed with commercial pellet diet and water *ad libitum.* The animals were acclimatized to laboratory hygiene conditions for 10 days before the start of the experiment. Induction of diabetes was performed by a single dose subcutaneous injection of freshly prepared alloxan monohydrate (120 mg/kg, Sigma chemicals, USA) dissolved in normal saline (0.85 % w/v NaCl in distilled water) to overnight fasted male albino rats (Dave and Katyare, 2002). Blood glucose level was measured by using glucometer and diabetes was confirmed after 72 h of alloxanisation. Rats showed fasting blood glucose levels more than 250 mg/dl was considered to be diabetic and were selected for experimentation.

Acute toxicity study

The acute toxicity of the extracts was determined according to the OECD guideline No. 421. After the sighting study, starting dose of 3000 mg/kg i.p. of the test samples was given to various groups containing six animals in each group. The treated animals were kept under observation for 14 days, for mortality and general behavior. If no death was observed till the end of the study then the test extract was found to be safe up to the dose of 3000 mg/kg.

Oral hypoglycemic activity

The rats were classified into five groups (n = 6). Group 1 was kept as control, and was given a single dose of 0.5 ml/100g of N-saline solution; Group 2 was treated with glibenclamide (0.5 mg/kg) hypoglycemic reference drug. Group 3 (a) and (b) was treated with leaves methanolic and aqueous extract separately at different doses (mg)/kg; Group 4 (a) and (b) was treated with seeds methanolic and aqueous extract at different doses (mg)/kg and Group 5 (a) and (b) was treated with stem methanolic and aqueous extract at different doses (mg)/kg. Blood samples were collected from the tail tip at 0 (after oral administration), 1, 2 and 3 h after administration. The blood sugar level was measured using glucometer.

Statistical analysis

All data was expressed as mean ± standard error of mean (SEM.) and analyzed by ANOVA and Student's 'T' test. Differences between groups will be considered significant at $p< 0.05$ levels.

RESULTS

Anti-diabetic activity

The present investigation elucidates that aqueous and methanolic extracts of aerial parts, viz. leaves, stem and seeds of the plant, *Cassia occidentalis* possessed anti-hyperglycemic/ anti-diabetic activity against alloxan-induced animal model. The results follow the same pattern as that of antimicrobial spectrum. All aqueous-treated rats showed no discernible behavioral changes up to 3000 mg/kg by oral route. No mortality was observed at this dose during 72 h observation period. Amongst all the extracts, potent anti-diabetic activity was observed in aqueous extracts of leaves of *C. occidentalis* followed by aqueous extracts of seeds and aqueous extracts of stem. The anti-diabetic effects of various extracts of *C. occidentalis* on the fasting blood sugar level of diabetic rats are shown in Table 1. In normal animals, significant (p<0.05) reduction in the blood glucose level was observed by the aqueous extracts as compared to the control and methanolic extracts (Table 1). However, treatment of methanolic extracts of aerial parts of *C. occidentalis* could not bring back the sugar to normal levels. Acute and chronic treatment of the aqueous extract of aerial parts of *C. occidentalis* (3000 mg/kg) in alloxan-induced diabetic rats resulted in a significant (p<0.05) decrease in the elevated blood glucose levels as compared to the control. Acute treatment of methanolic extract of aerial parts of *C. occidentalis* could not bring back the sugar to normal levels. There was no reduction in glucose content in control groups treated with solvent extracts. However there was significant reduction in blood glucose level in the group treated with glibenclamide at 0.5 mg/kg. The results showed that blood glucose level gets decreased after varying the dose level. Thus the findings confirmed that level of blood glucose gets normal in dose-dependent manner.

Table 1. The Effect of various extracts of *Cassia occidentalis* on blood glucose level in alloxan- induced albino rats

Group	Treatment/Dose	Blood Glucose Concentration (mg/dl) measured after dose treatment			
		0 hour	1st hour	2nd hour	3rd hour
I	Control (0.5 ml/100g of N- saline solution)	88.2±0.03	88.2±0.03	88.2±0.03	89.2±0.03
II	Glibenclamide (0.5 mg/kg)	85±0.04	78±0.04	75±0.04	72±0.04
III (a)	Leaves methanolic extract (3000 mg/Kg)	87±0.04	75±0.04	74±0.04	73±0.04
III (b)	Leaves aqueous extract (3000 mg/Kg)	85±0.04	78±0.04	75±0.04	70±0.04
IV (a)	Seeds methanolic extract (3000 mg/Kg)	89±0.04	73±0.04	77±0.04	75±0.04
IV (b)	Seeds aqueous extract (3000 mg/Kg)	89±0.04	76±0.04	75±0.04	73±0.04
V (a)	Stem methanolic extract (3000 mg/Kg)	88±0.04	72±0.04	75±0.04	78±0.04
V (b)	Stem aqueous extract (3000 mg/Kg)	85±0.04	79±0.04	74±0.04	76±0.04

*p<0.05 level of significance

DISCUSSION AND CONCLUSION

In the present investigation, antimicrobial and anti-diabetic/ anti-hyperglycemic activities of aerial parts viz. leaves, stem and seeds of *Cassia occidentalis* were screened. The results were found to be significant as, leaves and seeds showed prominent anti-diabetic activity. The results showed that aqueous extracts of leaves possessed potent anti-diabetic activity followed by aqueous extracts of seeds and stem. The methanolic extracts also follows the same pattern as that of aqueous extracts but were found to have less anti-hyperglycemic activity.

In light of the results, our study indicates that aqueous extract of *Cassia occidentalis* exhibited significant anti-hyperglycemic/anti-diabetic activity in normal and alloxan-induced rats. In normal rats, administration of aqueous extract showed 6.50%, 10.29%, and 7.21% decline in the blood glucose levels on 1, 2 and 3 h, respectively. Alloxan-induced diabetic rats administered with aqueous extract showed 4.15%, 6.52%, and 8.56% decline in the blood glucose level on 1, 2, and 3 h, respectively, whereas they showed 12.63%, 22.38%, 30.41%, and 38.19% decline in the blood glucose level on 4, 5, 6, and 7th day, respectively. The level of significance was found to be $p<0.05$. The study is a multi-directional pharmacological approach thus is a boon for the new researchers to investigate the molecule (s) responsible for antimicrobial and anti-hyperglycemic activities. Future elucidation of these molecules will thus may be utilized as important component in preparation of drug or can be utilized as such as medicine. In Conclusion *C. occidentalis* exhibited significant antihyperglycemic activities in normal and alloxan-induced diabetic rats. The aqueous and methanoic extract of *C. occidentalis* also showed a significant decline in blood glucose level and so might be of value in treatment of diabetes.

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