ORIGINAL ARTICLE

Antidiabetic effect of a black mangrove species *Aegiceras corniculatum* in alloxan-induced diabetic rats

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ABSTRACT

Earlier ethnopharmacological records divulged the traditional usages of mangrove *Aegiceras corniculatum* (Linn.) Blanco distributed in coastal and estuarine areas of Southeast India. Excluding scientific knowledge of *A. corniculatum* against diabetes an upgrowing endocrinal disorder, our present study evaluated the effect on alloxan-induced diabetic rats. Diabetes was induced in adult rats of the Wistar strain by intraperitoneal injection of alloxan monohydrate. The experimental rats were administered with leaf suspension of *A. corniculatum* post orally using an intragastric tube. On completion of the 60-day treatment, a range of biochemical parameters were tested including liver hexokinase, glucose-6phosphatase and fructose 1, 6 bisphosphatase in the liver of control and allaxon-diabetic rats. As a result, *A. corniculatum* leaf suspension showed moderate reduction in blood glucose (from 382 ± 34 to 105 ± 35), glycosylated hemoglobin, a decrease in the activities of glucose-6 phosphatase and fructose 1, 6-bisphosphatase, and an increase activity of liver hexokinase achieved through the oral administration of extract on 100 mg/kg. The present findings support promising results in terms of antidiabetic activities establishing its candidacy for further purification of individual compound in order to understand their mechanism of action.

Key words: Alloxan, antidiabetic, black mangrove, gluconeogenic enzymes, hexokinase

INTRODUCTION

Diabetes is increasing in a panic way all over the world and is relied on the use of traditional medicine which are largely derived from plants. About 200 million in the world are affected and 2.5 to 7% of the people are diagnosed by the most common endocrine disease diabetes.[1] The pathogenesis of insulin-dependent diabetes mellitus involves environmental causes that may activate autoimmune mechanisms on genetically susceptible individuals, leading to progressive loss of pancreatic islet β-cells resulting in insulin deficiency.[2] Non-insulin-dependent diabetes mellitus is associated with impaired insulin secretion, obesity, insulin resistance, and hereditary disposition in individuals over 40 years of age.[3] However, the increasing causes of commercially available anti-hyperglycemic drugs and insulin sensitizers, such as long-term adverse effects on blood vessels, nerves, and other organ systems, demand the discovery of new alternatives mainly insulin substitutes. The findings of insulin substitutes from the coastal medicinal plants with hypoglycemic effect have been a recent trend in marine pharmacological research.

Traditionally more than 100 numbers of mangroves and mangrove-associated plant used for the treatment of diabetes have been reported, but only a very few number of plants are evaluated and reported scientifically.[4] The antidiabetic effect of leaves of mangrove plants *Rhizophora mucronata* and *Ceriops decandra* had been documented and the gut perfusion studies on Long Evans rats reported the mode of action of *Rhizophora mucronata* leaves' hypoglycemic conditions.[5-7] Recently, the medicinal value of mangroves

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and associated plants persist to provide priceless therapeutic agents, both in modern medicines and in traditional systems.[8] Traditional applications of the black mangrove *Aegiceras corniculatum* (Linn.) Blanco has been selected for the present study, which belongs to the myrsinaceae family distributed in coastal and estuarine areas of India. Also, the ethnopharmacological consequence pointed out the study plant traditionally used for the treatment of rheumatism, painful arthritis, inflammation, asthma antioxidant, free radical scavenging, anti-inflammatory, antinociceptive, diabetes, and hepatoprotective actions.[9] However, there are no scientific reports regarding the effects of this plant on Diabetes mellitus. In consequence, we tried to establish mangrove floral therapy as antidiabetic drug instead of chemical drug. Hence, in the present study, we evaluated the effects of an ethanolic extract of *Aegiceras corniculatum* leaves (ACEt) in alloxan-induced diabetic rats for the potential use in the long-term treatment of diabetes.

MATERIALS AND METHODS

Chemicals and Drugs

Alloxan-monohydrate was obtained from Sigma-Aldrich Company (St. Louis, Missouri, U.S.A.). The other experimental chemicals used were analytical grade purchased from HiMedia (Mumbai, India).

Plant Material

Leaves of *A. corniculatum* were collected from Pichavaram mangrove forest (Tamil Nadu) Southeast coast of India. The specimen was botanically certified and a voucher specimen (AUCASMB02) deposited in the Herbarium of Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, India. Mature leaves were separated manually from the aerial part of the plant. Then, the leaves were dried and minced with a grinder into a powder in preparation for extraction.

Preparation of the Extract

The ACEt *was* prepared by cold maceration of 250 g of the shade-dried leaf powder in 500 ml of 80% ethanol for 2 days. This process was repeated three times. The extract was filtered, concentrated, dried in yield 1.7%, and the residue stored in a refrigerator at 3-7°C for use in subsequent experiments.

Experimental Animals

About 200 to 250 g weight of male albino rats purchased from the Central Animal House, Rajah Muthiah Medical College (RMMCH), Annamalai University were used in this study. The animals were fed on pellet diet (Hindustan Lever, India) water ad libitum and maintained at Central Animal House, RMMCH. All studies were conducted in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals and the study was approved by the Institutional Animal Ethical Committee of Rajah Muthiah Medical College and Hospital (Prop. No. 534, Reg.No. 160/1999/CPCSEA), Annamalai University, Tamil Nadu, India.

Acute Toxicity Studies

Enthusiastic adult albino male rats, be malnourished overnight, were divided into four groups (n = 6) and were orally fed with the ACEt in increasing dose levels of 50, 100, 250, 500, and 1 000 mg/kg body weight. The behavioral, neurological, and autonomic profiles of rats were observed continuously for 2 hours and after a period of 24 and 72 hours for any lethality or death.[10]

Experimental Design

The rats were intraperitoneally injected by alloxan monohydrate (150 mg/kg body weight) dissolved in freshly prepared citrate buffer (0.1M, pH 4.5) to induce diabetes after an overnight fast.[11] Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 30% glucose solution orally at different time intervals after six hours of alloxan induction, and 5% glucose solution was kept in bottles in their cages for the next 24 hour to prevent hypoglycemia. Hyperglycemia was confirmed by the elevated glucose levels (250 to 375 mg/dl) in plasma, determined at 72 hours and then on day 7 after injection. The rats found with permanent diabetes were used for the antidiabetic study. Animals were divided into six groups of six rats (namely a-f) each. The extract was administered for 60 days. Feed and water were provided ad libitum to the animals. The plant extract (ACEt) or glibenclamide were dissolved in water and administered post orally using an intragastric tube on the treatment of 60 days.

Group I: Normal control rats treated with saline daily for 60 days
Group II: Normal rats treated with (100 mg/kg) of ACEt
Group III: Diabetic control rats
Group IV: Diabetic rats treated with (25 mg/kg) of ACEt
Group V: Diabetic rats treated with (50 mg/kg) of ACEt
Group VI: Diabetic rats treated with (100 mg/kg) of ACEt
Group VII: Diabetic rats treated with glibenclamide (600 μg/kg)

Effect on Fasting Blood Glucose and Glycosylated Hemoglobin

Day 7 of induction was designated as day 1 for extract administration in diabetic rats. Fasting plasma glucose was estimated on days 1, 5, and 12 of extract administration. Blood was collected from rats by tail-puncture method. About 0.50 ml of blood was collected from each rat into a vial containing heparin (35 units/ml of blood) solution. The effects of ACEt on normal and diabetic rats were determined by measuring fasting blood glucose level that was estimated by Trinder using a reagent kit.[12] Glycosylated hemoglobin (HbA_{1c}) was estimated by Drabkin and Austin.[13]

Assay for Carbohydrate Metabolic Enzymes

After 60 days of treatment, the rats were anesthetized by intramuscular Ketamine (24 mg/kg/body) injection and sacrificed by cervical decapitation. The liver was dissected out and washed with ice-cold saline immediately to remove blood. The hepatic Hexokinase (EC2.7.1.1), Glucose-6-phosphatase (EC 3.1.3.9), and Fructose-1, 6-bisphosphatase (EC3.1.3.11) are the key enzymes in glucose homeostasis that were estimated to determine the effect of ACEt toward the cure and management of diabetes.[14-16] For these experiments, 1 g of fresh/frozen liver was chopped and homogenized in ice-cold sucrose (15 ml, 250 mM) with a Potter-Elvehjem homogenizer for 2minutes, centrifuged at 10 000 rev./min for 30 minutes, and the pellet was discarded and the supernatant was used as the source for the biochemical estimation of enzymes. Also, the protein content of the supernatant was determined by the method of Lowry *et al*.[17]

Statistical Analysis

Values were represented as mean ± SD. Data were analyzed using analysis of variance and group means were compared with Duncan's multiple range tests.

RESULTS

Acute Toxicity Studies

On treatment of enthusiastic adult albino male rats with *A. corniculatum* ethanolic extract, we could not find any lethality or toxic reactions while using different dose concentrations used in the present study, until the end of the study period.

Effect on Fasting Blood Glucose and Glycosylated Hemoglobin

Changes in blood glucose and body weight in diabetic control and on treatment of diabetic rats with *A. corniculatum*, glibenclamide are presented in Table 1. The results reveal a momentous increase in blood glucose and substantial reduction in body weight in diabetic rats when compared with control rats. Table 2 elucidated the effects of *A. corniculatum* and glibenclamide on hemoglobin, HbA_{1c}, and serum protein, in control and alloxan-diabetic rats. There is a considerable reduction in hemoglobin and serum protein while HbA_{1c} appreciably increased in diabetic rats when compared with control rats. Rats administrated orally with a leave extract of *A. corniculatum* (25, 50, and 100 mg/kg body weight) intensively attained the value to near normal.

Effect on Carbohydrate Metabolic Enzymes

Effects on the administration of *A. corniculatum* and glibenclamide on hepatic hexokinase, glucose-6-phosphatase, and fructose-1, 6-bisphosphatase of liver are presented in Table 3. The activity of hepatic hexokinase is appreciably decreased while glucose-6-phosphatase and fructose-1, 6-bisphosphatase are significantly elevated in alloxan-treated diabetic rats as compared with normal rats. There is an increased activity of hexokinase and decreased the activities of glucose 6-phosphatase and fructose- 1, 6- bisphosphatase in the administration of *A. corniculatum* (25, 50, and 100 mg of body weight) and glibenclamide compared with diabetic rats. There was no statistical significance in parameters estimated in normal animals. The antidiabetic activity was not found to be dose dependent as there was no significant difference between the 25, 50, and 100 mg/kg extract-treated groups.

DISCUSSION

Mangroves and associated plants provide a wide domain for therapeutic application in recent years, most yet to be explored. The leaves of *A. corniculatum* are reportedly rich in flavonoids with proven anti-inflammatory and antioxidant property.[18] Evaluation of physiological and toxic effects, solvent used for extraction, route of administration, and acute or chronic effect of *A. corniculatum* leave extract are quite diversified, which is encouraged by delineating the beneficial applications of the study plant and confines various assessments.

Alloxan is an oxygenated pyrimidine derivative which selectively destroys insulin-secreting beta-cells in the experimental animals, which results in alloxan diabetes.[19] In the present investigation, blood sugar level increased as expected in alloxan-injected animals, since alloxan causes a massive reduction in insulin release, by the destruction of the beta cells of the islets of Langerhans and inducing hyperglycemia.[20]

Oral administration of *A. corniculatum* (25, 50, and 100 mg/kg body weight) resulted in a significant reduction in the blood glucose and improvement in body weight. The decrease in body weight in diabetic rats clearly confirms a degradation of structural proteins due to diabetes. The structural proteins are known to contribute for the body weight.[21] The deficiency of the anabolic hormone insulin decreased protein synthesis in all tissues in alloxan-induced diabetic rats. Structural proteins are important constituents of the body and are required for the body's structure and proper function. The rate of structural protein synthesis affects organ protein mass, body size, and amino acid requirements. The ability of the *A. corniculatum* to protect from maximum body weight loss seems to be due to its ability to reduce hyperglycemia.

Within six to eight weeks, the blood sugar level of the patients had been determined by the amount of HbA_{1c} in the red blood cells. Normal human red cells contain 3.4 to 5.8% HbA_{1c}; during diabetes, the level has been increased within the red blood cells, which reflects the average level of glucose to which the cell has been exposed during the life cycle.[22] In the present study, diabetic rats have high blood glucose level due to the influences of level that was high in

Table 1: Changes in blood glucose and body weight in control and alloxan diabetic rats treated with *Aegiceras corniculatum* and glibenclamide

Groups	Blood glucose mg/dl		Body weight(g)		Changes body wt (g)
	Initial	Final	Initial	Final	
Control	80.2 ± 1.040	82.03 ± 2.311[a]	184.3 ± 0.90	213.3 ± 0.88	-29
Normal + ACEt (100 mg/kg)	81.8 ± 0.251	89.33 ± 1.150[a,c]	164.2 ± 1.12	180.16 ± 0.83	-15.96
Diabetic control	362.5 ± 0.503	386.6 ± 2.40[b]	118.90 ± 2.6	130.4 ± 0.60	-60
Diabetic + ACEt (25 mg/kg)	374.06 ± 0.901	120.6 ± 1.36[c,d]	182.8 ± 1.2	169.5 ± 0.60	-19
Diabetic + ACEt (50 mg/kg)	378.8 ± 1.250	108.33 ± 2.73[c,d]	184.7 ± 0.9	152.6 ± 2.13	+17.3
Diabetic + ACEt (100 mg/kg)	381.9 ± 1.70	102.9 ± 2.44[c,d]	185.8 ± 1.3	155.2 ± 2.6	+30.6
Diabetic + glibenclamide (600 μg/kg) (positive control)	273.9 ± 1.738	164.1 ± 1.42[a,d]	188.3 ± 3.32	154.4 ± 1.4	+33.9

Values are mean ± SD for six animals each. Values not sharing a common superscript differ significantly at $P<0.05$. DMRT: Duncan's multiple range tests

Table 2: Effect of *Aegiceras corniculatum* on hemoglobin, glycosylated hemoglobin and protein, in control, and alloxan diabetic rats

Groups	Hemoglobin (g%)	Glycosylated Hemoglobin (mg/g of Hb)	Protein (g/dl)
Control	13.43 ± 0.9	0.196 ± 0.02[a]	5.59 ± 0.05[a]
Normal + ACEt (100 mg/kg)	13.3 ± 1.5	0.196 ± 0.01[a]	5.73 ± 0.04[a]
Diabetic control	8.03 ± 1.09	0.603 ± 0.06[b]	4.17 ± 0.04[b]
Diabetic + ACEt (25 mg/kg)	14.26 ± 0.30	0.262 ± 0.02[c]	5.09 ± 0.08[c]
Diabetic + ACEt (50 mg/kg)	12.33 ± 0.68	0.220 ± 0.04[a]	5.37 ± 0.04[a]
Diabetic + ACEt (100 mg/kg)	12.53 ± 0.7	0.252 ± 0.00[a]	5.36 ± 0.04[a]
Diabetic + glibenclamide (600 μg/kg) (positive control)	11.16 ± 0.58	0.230 ± 0.02[a]	5.35 ± 0.01[a]

Values are mean ± SD for six animals each. values not sharing a common superscript differ significantly at $P<0.05$. DMRT: Duncan's multiple range tests

Table 3: Effect of *Aegiceras corniculatum* on the activities of hepatic enzymes in control and experimental animals

Groups	Hexokinase (Unit a/mg protein)	Glucose6phosphatase (Unit b/mg protein)	Fructose1,6-bis-phosphatase (Unit c/mg protein)
Control	0.252 ± 0.001[a]	0.186 ± 0.002[a]	0.421 ± 0.0025[a]
Normal + ACEt (100 mg/kg)	0.241 ± 0.023[a]	0.174 ± 0.002[a]	0.435 ± 0.004[a]
Diabetic control	0.081 ± 0.002[b]	0.403 ± 0.015[b]	0.688 ± 0.006[b]
Diabetic + ACEt (25 mg/kg)	0.236 ± 0.046[b]	0.220 ± 0.026[c]	0.484 ± 0.002[c]
Diabetic + ACEt (50 mg/kg)	0.221 ± 0.001[a]	0.231 ± 0.002[a,c]	0.474 ± 0.000[c]
Diabetic +ACEt (100 mg/kg)	0.233 ± 0.002[a]	0.224± 0.004[a,c]	0.459 ± 0.001[c]
Diabetic + glibenclamide (600 μg/kg) (Positive control)	0.233 ± 0.003[a]	0.223 ± 0.002[a,c]	0.464 ± 0.002[a,c]

Values are mean ± SD for six animals each. Values not sharing a common superscript differ significantly at $P<0.05$. DMRT: Duncan's multiple range tests. [a]p moles of glucose phosphorylated/h; [b]p moles of pi liberated/min; [c]u moles of pi liberated/min.

red blood cells. The sugar levels decreased immensely in *A. corniculatum*-administered diabetic rats.

The intracellular glucose has been utilized by insulin in several ways. The increased level of insulin influences the activity of gluconeogenic enzymes that results in the initiation of hepatic glycolysis. All types of cells contain hexokinase. Hexokinase D or glucokinase is more specific for glucose and differ with other forms of hexokinase in kinetic and regulatory properties, which has been found in hepatocytes.[23] Hexokinase plays a central role in the maintenance of glucose homeostasis, it catalyzes the conversion of glucose to glucose-6-phosphate. Also, hexokinase is an important regulator of glucose storage and disposal in the liver.[24] In the present study, the hexokinase activity was decreased in alloxan-diabetic rats which may be due to insulin deficiency (insulin stimulates and activates glucokinase). Treatment with *A. corniculatum* or glibenclamide elevated the activity of more effective therapeutic compounds in mangroves.

CONCLUSION

In conclusion, this is the first report on black mangrove *A. corniculatum* on antidiabetic activity in experimental model. Further bioactive compound isolation, clinical trials, hematological parameters, and drug development will be promising the development of antidiabetic products from mangroves with unidentified level of side effects.

REFERENCES

1. Seghrouchni JE, Drai J, Bannier P, Riviere I, Calmard J, Garacia Origiazzi A. Oxidative stress parameters in type I, type II and insulin-treated type 2 diabetes mellitus: Insulin treatment efficiency. Clin Chim Acta 2002;32:189-96.
2. Harrison LC, Honeyman MC. Cow's milk and type 1 diabetes: the real debate is about mucosal immune function. Diabetes 1999;48:1501-7.
3. Zimmet P, Dowse G, Finch C, Serjeantson S, King H. The epidemiology and natural history of NIDDM-lessons from the South Pacific. Diabetes Metab Rev 1990;6:91-124.
4. Bandaranayake WM. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetlands Ecol Manage 2002;10:421-52.
5. Ramanathan T, Hariharan B, Ganesan K. Antidiabetic activity of a coastal mangrove leaves of *Rhizophora mucronata*. Int J Plant Res Plant Arch 2008;8:931-3.
6. Nabeel MA, Kathiresan K, Manivannan S. Antidiabetic activity of the mangrove species *Ceriops decandra* in alloxan-induced diabetic rats. J Diabetes 2010;2:97-103.
7. Gaffar MU, Morshed MA, Azim Uddin, Saurov Roy, Hannan JMA. Study the efficacy of *Rhizophora mucornata* Poir. leaves for diabetes therapy in Long Evans rats. I J Biomol Biomed 2011;1:20-6
8. Kathiresan K, Ramanathan T. Medicinal Plants of Parangipettai Coast. Tamil Nadu, India: Annamalai University; 1997. p. 72.
9. Roome T, Dar A, Ali S, Naqvi S, Choudhary MI. A study on antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective actions of *Aegiceras corniculatum* (stem) extracts. J Ethnopharmacol 2008;118:514-21.
10. Ecobichon DJ. The Basis of toxicology Testing, New York: CRC Press; 1997. p. 43.
11. Katsumata K, Katsumata Y, Ozawa T, Katsumata K. Po-tentiating effects of combined usage of three sulfo-nylurea drugs on the occurrence of alloxan diabetic rats. Horm Metab Res 1999; 25:125.
12. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem 1969;6:24-7.
13. Darbkin DL, Austin JM. Spectrophotometric constants for common haemoglobin derivatives in human, dog and rabbit blood. J Biol Chem 1932;98:719-33.
14. Brandstrup N, Kirk JE, Bruni C. Determination of hexokinase in tissues. J Gerontol 1957;12:166-71.
15. Baginski ES, Foa PP, Zak B. In: Methods of Enzymatic Analysis. New York: Academic Press; 1974.2: 876-80.
16. McGilvery RW. Fructose-1,6-diphosphatase from liver. Methods Enzymol 1955;2:543-6.
17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
18. Banerjee D, Chakrabarti S, Hazra AK, Banerjee S, Ray J, Mukherjee B. Antioxidant activity and total phenolics of some mangroves in Sundarbans. Afr J Biotechnol 2008;7:805-10.
19. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetol 2008;51:216-26.
20. Bouwens L, Rooman I. Regulation of Pancreatic Beta-Cell Mass. Physiol Rev 2005;85:1255-70.
21. Rajkumar L, Govindarajulu P. Increased degradation of dermal collagen in diabetic rats. Indian J Exp Biol 1991;29:1081-3.
22. Paulsen EP. Experience in sulfonylurea therapy. J Metabolism 1973:22;381-5.
23. Lehninger AL, Nelson DL, Michael M. In: Principles of Biochemistry, 2nd Ed. New Delhi: CBS Publishers Distributors; 1993. p.406.
24. Ghosh MN. In: Fundamentals of Experimental Pharmacology. Calcutta: Scientific Book Agency; 1984. p. 153.

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