

Original Research Article

Antidiabetic Activity of *Bruguiera cylindrica* (Linn.) Leaf in Alloxan Induced Diabetic Rats

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Abstract	Keywords
<p>In this paper, different doses of ethanolic extract of leaves of <i>Bruguiera cylindrica</i> (BC) were evaluated for hypoglycemic and antihyperglycemic activity in normal and alloxan diabetic rats. The oral administration of ethanolic extract at a dosage of 0.15 g/kg body weight exhibited a significant antihyperglycemic activity in alloxan diabetic rats, whereas in normal rats no hypoglycemic activity was observed. The phytochemical screening of BC leaves revealed the presence of flavonoids, phenolic acids, sterols/triterpenoids, alkaloids, tannins and anthocyanins.</p>	<p>Alloxan diabetic rats Antidiabetic activity <i>Bruguiera cylindrica</i> Hypoglycemic activity</p>

Introduction

Diabetes mellitus is a global metabolic disorder characterized by hyperglycemia due to alterations in carbohydrate, fat and protein metabolism, Over 194 million people suffer from diabetes worldwide (Lau et al., 2009). The endocrine insulin plays a major role, total or virtual deficiency in insulin secretion or inability to promote its action leads to this disorder. Oral hypoglycemic agents are common treatment methodology along with insulin (Carver and Abrahamson, 2009); there is a growing interest in herbal remedies, due to the side effects associated with these therapeutic agents. Because of perceived effectiveness, minimal side effects in clinical experience and relatively low cost, herbal drugs

are widely prescribed even when their biologically active compounds are unknown (Samad et al., 2009).

The ecologically significant and biologically diverse mangrove forests, wedged between the land and sea, are known for the rich heritage of the flora. A number of plants with known and unknown medicinal values are available here, which have to be explored for the use in the effective treatment of diabetes mellitus (Anonymous, 2008). Mangrove plants like *Bruguiera cylindrica*, *Rhizophora apiculata*, *Rhizophora lamarkii* and *Rhizophora mucronata* are rich in polyphenols (Kulkarni et al., 2009). Some mangrove plants have been investigated for the production of tea. The

ashes of *Arthrocnemum indicum* have been prescribed to treat snakebites. Traditional healers in Africa use *Sesuvium portulacastrum* to treat kidney problems, fever and scurvy. India's traditional healers used *Avicennia officinalis* to treat smallpox infections in the past (Han et al., 2004). One among them is *B. cylindrica* Linn, belongs to the family Rhizophoraceae. It is an endangered mangrove plant commonly found in coastal areas of Pitchavaram, T.N., India and other parts of the world in both dry and rainy seasons (Chadha, 1976). The other physiochemical characteristic features of *Bruguiera* species are known to accumulate salt to levels higher than that of sea water (Kura-Hotta et al., 2001) *B. cylindrica* (BC) leaves have been in use for the treatment of diabetes by tribal people (information from local tribes). The scrapped skin of the fruit is used to stop bleeding. The leaves are used to control blood pressure in India (Mastaller, 1997). Maceration of leaves along with fruit powder is given orally for the treatment of diabetes by tribal population.

Materials and methods

Collection of plant material

The *B. cylindrica* (BC) plant were collected from Pitchavaram mangrooves near Chidambaram (11° 45' 0" North, 79° 45' 0" East), Cuddalore district, Tamilnadu, India, identified and authenticated by taxonomist Rev Fr Dr. John Britto, Director, Rapinart Herbarium St. Joseph's college, Trichy, India. The leaves were dried in shade, powdered and used for the extraction of antidiabetic principles.

Preparation of the extracts

The active principle/s of BC leaves was extracted into 95% ethanol. BC leaves powder was soaked in 95% ethanol in glass jar for 2 days at room temperature and soxhleted until the extract decolourize completely. The extract was distilled and concentrated under reduced pressure in the Buchi rotavapour and finally dried under reduced pressure (yield 4.5%). This extract was used for further studies. The phytochemical screening of the BC leaves was

carried out by the method of Brindha *et al.*, (1988) (Das and Bhattacharjee, 1970; Chhabra et al., 1984). Flavonoids were extracted according to the methods of Harborne (Harborne, 1973). Phenolic acids were extracted according to the methods of Bate-Smith (Bate-Smith, 1959). The Flavonoids and phenolic acids were quantitatively estimated by the method of Swain and Hillis (Swain and Hillis, 1959). The extracted principle/s was subjected to GC-MS analyzes and the data will be published elsewhere in future.

Induction of diabetes

Diabetes was induced in male wistar albino rats, body weight 110-140 g by intraperitoneal administration of ice-cold aqueous alloxan monohydrate (150 mg/kg body weight) by the method described earlier (Kameswara Rao et al., 1999). After a fortnight, rats with marked hyperglycemia (fasting blood glucose >250 mg/dl) were selected and used for the study. All the animals were allowed free access to water, pellet diet, and maintained at room temperature in polycarbonate cages.

Experimental design

The rats were divided into 11 groups and each group consisted of six rats.

- Group 1: normal untreated rats
- Group 2: Alloxan induced diabetic untreated rats
- Group 3: normal rats treated with 50 mg/kg b.w. of plant extract
- Group 4: normal rats treated with 100 mg/kg b.w. of plant extract
- Group 5: normal rats treated with 150 mg/kg b.w. of plant extract
- Group 6: normal rats treated with 200 mg/kg b.w. of plant extract
- Group 7: diabetic rats treated with 50 mg/kg b.w. of plant extract
- Group 8: diabetic rats treated with 100 mg/kg b.w. of plant extract
- Group 9: diabetic rats treated with 150 mg/kg b.w. of plant extract
- Group 10: diabetic rats treated with 200 mg/kg b.w. of plant extract
- Group 11: diabetic rats treated with 5 mg/kg b.w. of Glibenclamide.

After an overnight fast, the plant extract suspended in Tween-20 was fed to the experimental rats by gastric intubation, using a force-feeding needle. Group 1 and Group 2 rats were fed distilled water alone. Blood samples were collected for the measurement of blood glucose from the tail vein at 0, 1, 3, and 5 h after feeding the plant extract. Blood glucose was measured by using glucose strips with Ames glucometer and the results were compared with those of 11th Group of rats that were treated with 5 mg/kg b.w. of glibenclamide (oral hypoglycemic agent).

Statistical analysis

The results are expressed as mean \pm S.D. Significance of differences between normal and diabetic groups were determined using the Student's *t*-test.

Results and discussion

The preliminary phytochemical screening of BC leaves revealed the presence of flavonoids, phenolic acids, sterols, triterpenoids, and tannins. The BC leaves contained 4.19 mg of Flavonoids and 10.56 mg of phenolic acids per 100 g of dry weight of the leaf powder.

The effect of the different doses of ethanolic extract of BC fruit on the fasting blood glucose levels of both normal and diabetic rats are given in Table 1. The fasting blood glucose levels of diabetic untreated rats (Group 2) were significantly higher than normal untreated rats (Group 1). The ethanolic extracts of BC leaves at a dosage of 0.5 g/kg b.w. produced the maximum fall of 23.8% in the blood glucose levels of diabetic rats after 5 h of treatment. Nevertheless, none of the doses of ethanolic extract produced any hypoglycemic effect in normal treated rats. Treatment with glibenclamide at a dosage of 5 mg/kg b.w. in distilled water resulted in 31.1% fall in the blood glucose levels of diabetic rats after 5 h of treatment.

In the present study, ethanolic extract of leaves of BC at a dose of 0.5 g/kg b.w. could produce a significant fall in blood glucose levels by about 24% in diabetic rats, after 5 h of

treatment. However, none of these extracts could produce any hypoglycemic effect in normal rats. The aqueous and hexane extracts of leaves of BC have not shown significant antihyperglycemic activity (Shyam et al., unpublished data). Hence, the ethanolic extracts may be considered to have good antihyperglycemic active principles without causing any hypoglycemic effect unlike insulin and other synthetic drugs. The phytochemical screening of BC leaves revealed the presence of flavonoids, phenolic acids, sterols/triterpenoids, alkaloids, tannins and anthocyanins.

Flavonoids, sterols, triterpenoids and phenolics are well-documented bioactive antidiabetic phytochemicals (Oliver-Bever, 1986; Ivorra et al., 1989; Atta-Ur-Rhemann and Khurshid Zaman, 1989). Flavonoids were sought to be the principle factor to regenerate the damaged beta cells in the alloxanized diabetic rats (Chakravarthy et al., 1980). Phenolics are known to be effective antihyperglycemic agents (Manickam et al., 1997). In the present study, 4.19 mg of flavonoids and 10.56 mg of phenolic acids were found to be present in per 100mg of powdered BC leaves.

The antidiabetic effect of ethanolic extract of BC leaves may be due to the presence of more than one antihyperglycemic principle and their synergistic properties. In this study, the antihyperglycemic activity caused by glibenclamide in alloxanized diabetic rats is an indication of the presence of some beta cells, as glibenclamide is known to stimulate insulin secretion from beta cells. The ethanolic extract of BC leaves may exert stimulating effect on the leftover beta cells. However, further experiments are required to elucidate the exact mechanism of action and investigate the biochemical parameters. The decreased activity at the higher doses (>0.5 g/kg b.w.) of the plant extract could be due to reduced or no effect of the components present in the extract at higher doses. Albeit, the ethanolic extract of BC leaves did not produce any hypoglycemic effect in normal rats, the normal rats being in homeostasis, these plant extracts could cause less suppression of normal regulatory mechanisms involved in carbohydrate metabolism (Vats et al., 2002).

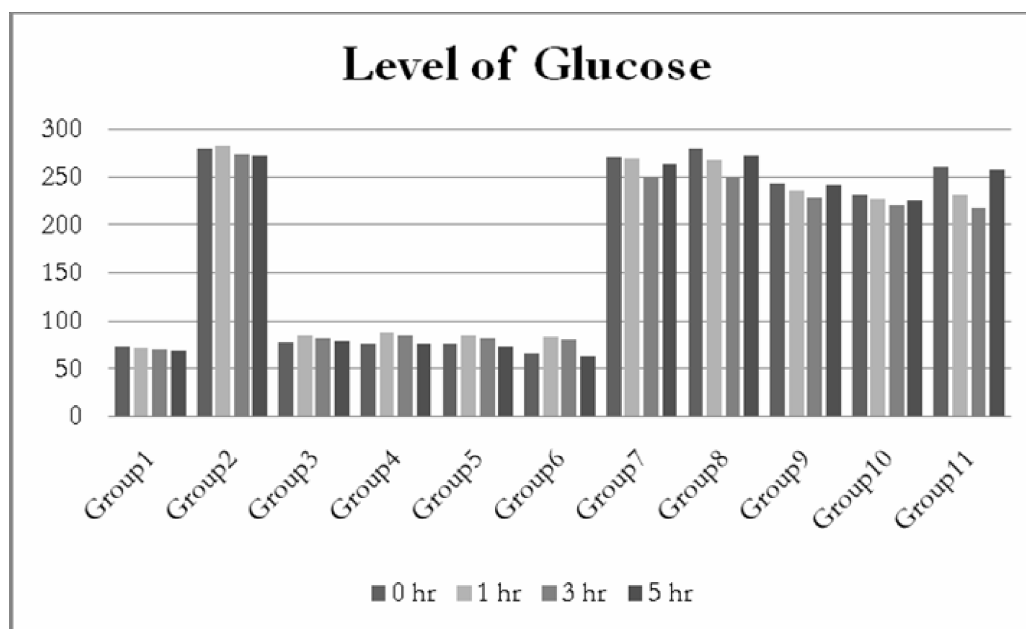
Further studies will be focused on determination of the mechanism(s) of action, as

well as on the isolation of bioactive principles and biochemical modulations.

Table 1. Effect of different dose of ethanol fraction of BC leaves on fasting glucose levels (mg/dl) of normal and diabetic rats (Mean ± SD).

Groups	Blood glucose at different hours after the treatment			
	0 h	1 h	3 h	5 h
1	72.7 ± 1.7	71.3 ± 1.1	69.9 ± 2.2	67.8 ± 0.7
2	279.4 ± 1.7	283.3 ± 1.0	274.4 ± 1	271.9 ± 1.6
3	76.9 ± 2.4	83.3 ± 1.6	80.8 ± 1.6	78.4 ± 1.0
4	75.9 ± 1.0	87.2 ± 1.8	84.3 ± 2.1	75.6 ± 1.2
5	75.2 ± 1.2	84.0 ± 1.8	81.7 ± 1.0	72.6 ± 1.7
6	64.6 ± 1.2	81.9 ± 1.1	79.6 ± 1.4	62.9 ± 1.3
7	270.9 ± 3.4	270.0 ± 1.5	251.1 ± 1.2	264.3 ± 1.4
8	280.4 ± 1.1	268.0 ± 1.0	249.0 ± 1.2	272.5 ± 1.2
9	243.0 ± 1.1	236.0 ± 1.7	229.6 ± 1.6	241.6 ± 1.0
10	231.1 ± 0.8	227.4 ± 3.6	220.8 ± 2.0	226.5 ± 2.3
11	261.4 ± 3.7	231.3 ± 2.3	218.0 ± 1.1	258.0 ± 8.8

Fig. 1: Effect of different dose of ethanol fraction of BC leaves on fasting glucose levels (mg/dl) of normal and diabetic rats (Mean ± SD).



Level of glucose at various experimental conditions (for details regarding grouping of animals; refer to section 2.4) clearly indicates the fact that the normal rats treated with plant extract at various dosage (group3 – group 6) doesn't show any significant decrease in the glucose level confirming that the BC extract doesn't produce hypoglycemic activity whereas the diabetic rats treated with plant extract at different dosage group 7 – group 10 is as effective as glibenclamide indicating the potency of anti diabetic effect.

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