Original Article

ANTIDIABETIC ACTIVITY OF AQUEOUS EXTRACT OF *PADINA BOERGESENII* IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Objective: The present study was to evaluate antidiabetic activity of *Padina boergesenii* extract in streptozotocin (STZ) induced diabetic rats.

Methods: Oral administration of the effective dose of *P. boergesenii* to the diabetic rats for 20 days showed abridged effects on fasting blood glucose, insulin and lipoprotein levels.

Results: Significant difference was observed in liver glycogen and total protein levels in diabetic rats after *P. boergesenii* extract treatment ($p<0.005$). The extract of *P.boergesenii* significantly increased the activities of the key glycolytic enzymes like hexokinase, aldolase and phosphoglucoisomerase and decreased the activities of gluconeogenic enzymes like flucose-6-phosphatase and fructose -1, 6-diphosphatase in liver and kidney of experimental rats.

Conclusions: *P. boergesenii* shows to have a potential value for the development of an effective phytomedicine for diabetes. Further comprehensive chemical and pharmacological investigations are needed to elucidate the exact mechanism of the hypoglycemic effect of *P. boergesenii* and compounds are accountable for its antidiabetic effect.

Keywords: *Padina boergesenii,* Antidiabetic activity, Streptozotocin, Glucose metabolism, Lipid metabolism.

INTRODUCTION

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces [1] the westernization of diet and other aspects of lifestyle in developing countries comprise uncovered major differences in genetic susceptibility across ethnic groups [2], which currently afflict 3 % of the world population. The issue of an increase in diabetes makes it even more important to diagnose and treat the disease earlier as the long-term microvascular complications are related to both the degree of hyperglycemia as well as the duration of the disease [3].

Medicinal therapy is the unique alternative and many kinds of antidiabetic medicines have been developed for diabetic patients, but almost all are chemical or biochemical agents; however it gives limited tolerability, and induces side effects [4, 5]. The medicinal plants have been used since ancient times to treat and manage diabetes mellitus in traditional medical systems of many cultures throughout the world [6-10]. During the last few decades, many studies have been made on biological activities of seaweeds [11].

 Traditionally, seaweeds have been used in the treatment of various infectious diseases and it could be the potential sources of natural antioxidants [12, 13] which offer rich sources of structurally diverse bioactive compounds with vast pharmaceutical and biomedical prospective. In particular, the brown algae have a variety of biological compounds, including pigments, fucoidans, phycocolloids and phlorotannins [14].

In our earlier studies, *Padina boergesenii* Allender & Kraft (Dictyotaceae), brown algae abundantly growing in Gulf of Mannar, southeast coast of Tamil nadu, India was found to have better antioxidant activity [15]. It has also been reported for hepatoprotective activity [16], chemopreventive effects [17] and herbivory effects [18].

To our data, there are no existing reports on the antidiabetic effect of this seaweed. Hence, the present study investigates the antidiabetic activity of aqueous extract of *Padina boergesenii* in streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Chemicals

Streptozotocin (STZ) was purchased from Sigma–Aldrich Co., USA. Solvents were purchased from SD-Fine Chemicals Ltd, India. Reagents and chemicals used in the present study were of analytical grade.

Seaweed material

Padina boergesenii (Allender & Kraft) was collected from Mandapam coastal region (78°8'E, 9°17'N), in Gulf of Mannar, Tamil nadu, South India on low tide during March 2012, the collected algae were immediately brought to the laboratory in polythene bags with seawater and washed several times with seawater to remove sand, mud and attached fauna. The algae were cleaned using brush for the removal of epiphytes with distilled water. After cleaning, algae were dried in shade at room temperature for one week. The dried algae materials were homogenized to fine powder and further subjected to extraction. The seaweed material was taxonomically identified and authenticated by the Botanical Survey of India and the voucher specimen (No.BSI/SRC/5/23/2012-13/TECH.486) was retained in our laboratory for future reference.

Extract preparation

About 50 g of powdered seaweed material was mixed with 250 ml of double distilled water in a 500 ml conical flask and was placed in shaker for 16 h. The solution was then extracted using a separating funnel and was concentrated by lyophilizer. A brownish-black powdered material was obtained (12 g) and stored in a dessicator and used for further experiments. The pH was adjusted to 7.5-8 and osmolarity was adjusted to 290-300 mOsm respectively.

Animals

Adult Wistar (albino) rats weighed between 150 to 200 g were obtained from animal house of Karpagam University, Coimbatore, India. The animal experiments were carried out according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The Institutional Animal

Ethical Committee approved added experimental design performed in this study for the use of Wistar albino rats as an animal model for antidiabetic activity. The animals were fed with standard pellet diet (Hindustan Lever Limited, Mumbai, India) and water freely available throughout the experimental period and replenished daily. The animals were housed in well ventilated large polypropylene cages under controlled conditions of light (12 hr light/12 hr dark), humidity (50-55 %) and ambient temperature (25 ± 2°C).

Induction of experimental diabetes

Experimental diabetes was induced by single intraperitoneal injection of 60 mg/ kg of streptozotocin (STZ), freshly dissolved in cold citrate buffer, pH 4.5 (Pandit *et al.,* 2010). Control animals received only citrate buffer. After two weeks rats with moderate diabetes having glycosuria, indicated by Benedict's qualitative test, were used for the study [19].

Acute toxicity study

To determine acute toxicity, single oral administration of *P. boergesenii* extract with different doses namely 50, 100, 250, 500, 1000 and 2000 mg/kg body weight were administrated orally to 6 groups of 5 animals each. Another group of 5 animals served as control which received 1 ml of physiological saline. The animals were observed continuously for 72 h for any signs of behavioral changes, toxicity and mortality.

Study design and does

Five groups of 6 rats each were used in this experiment. Group 1, normal control (the animals were given normal saline only). Group 2, diabetic group induced by streptozotocin (60 mg/kg body weight). Group 3, treatment group diabetic animals treated with *P. boergesenii* aqueous extract at 400 mg/kg body weight (effective dose of the extract). Group 4, Positive control (the diabetic rats treated with Glibenclamide at 2 mg/kg body weight). Group 5, control (animals were treated with *P. boergesenii* at 400 mg/kg body weight). The animals were weighed and dose was given through oral intragastric tube every day. The test sample and reference standard drugs were given orally and the experiment was terminated in overnight fasted rats at the end of 30 days. After the experimental regimen, the animals were sacrificed by cervical dislocation after giving mild anesthesia using chloroform. Blood was collected using EDTA as the anticoagulant and serum was separated by centrifugation at 2500 rpm. Liver and kidneys were immediately dissected out; washed and stored in 0.9 % ice cold saline and weight was recorded. A 10 % homogenate of the liver and kidney tissue were prepared with 0.1 M Tris - HCL buffer, pH 7.4. The homogenates were used to analyze the enzyme activities and biochemical parameters.

Analytical procedure

The body weight of control and experimental rats were checked up to 30 days. Serum blood glucose and insulin levels were estimated by the O-toluidine method [20, 21]. Liver glycogen was determined by the method of Rotruck *et al.* [22]. Protein level was estimated by Lowry *et al.* [23]. Estimation of hexokinase and aldalase were estimated according to the standard methods [24, 25]. Phosphogluco isomerase was assayed by the method of Horrocks, 1963 [26]. The glucose -6-phosphatase and fructose-1, 6-bisphosphatase were estimated by the method of Gancedo, 1971 [27]. The lipid profile of HDL by Warnick *et al.*1985 [28]. VLDL and LDL cholesterol were calculated by Friedewald's formula [29] as described below. LDL = TC-HDL-VLD; VLDL=TG /5

Statistical analysis

The results obtained were expressed as mean ± SD. The statistical comparison among the groups were performed with one way ANOVA and DMRT using statistical package (SPSS 10.0) at p<0.05.

RESULTS

Acute toxicity study revealed the non-toxic nature of *P. boergesenii* extract; there was no mortality, breathing, cutaneous effects, sensory nervous system responses and gastrointestinal effects or

signs of behavioral changes or toxicity observed after oral administration of up to the dose of 2000 mg/kg. This study showed the single dose of *P. boergesenii* extract even at higher dosage does not produce any toxic symptoms indicating high margin of safety of extract**.**

The body weight of control and experimental rats were checked up to 30 days and the results are represented in Figure 1. The body weight of the STZ treated rats were found to be significantly decreased when compared to normal control group.
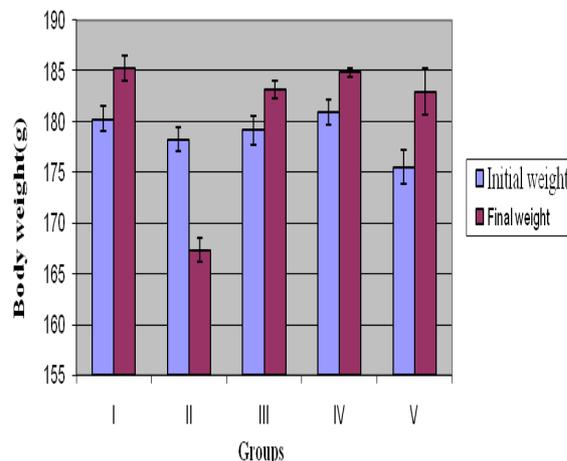
Fig. 1: Effect of aqueous extract *P. boergesenii* on body weight in streptozotocin induced diabetic rats. Values are given as mean ± SD from six rats in each group.

The body weight was slightly high in the normal control group and *P. boergesenii* extract alone group when compared to initial weight. Aqueous extract of *P. boergesenii* and the Glibenclamide treatment significantly prevented the weight loss.

Table 1 represents the levels of glucose and insulin in serum of control and experimental rats. The blood glucose level was significantly (P <0.05) higher in diabetic rats as compared to normal rats. Administration of *P. boergesenii* extract significantly lowered the serum glucose level as compared to diabetic rats. In the present study, STZ caused a significant decrease in serum insulin. Administration of *P. boergesenii* extract caused significant (P <0.05) increase in insulin levels at the end of the study, which was comparable to glibenclamide.

Liver glycogen levels in control and experimental animals are depicted in Table 2 Significant difference was observed in liver glycogen and total protein levels in diabetic rats after *P. boergesenii* extract treatment. Liver glycogen level was significantly increased with respect to diabetic control group which restored to the normal group glycogen level.

The activities of glycolytic enzymes like hexokinase, aldolase and phosphoglucoisomerase in liver and kidney of control and experimental rats are depicted in Table 3 the activities of glycolytic enzymes were significantly lowered in diabetes group. The enzyme activities were suppressed in STZ induced diabetic rats when compared to normal rats. Oral administration of *P. boergesenii* extract resulted in increased activity of these enzymes. Glibenclamide treatment to diabetes rats also brought the activities near normal as in Group I and Group IV animals.

The activities of gluconeogenic enzymes glucose-6-phosphatase and fructose -1,6-diphosphatase in liver and kidney of control and experimental animals are presented in Table 4. Their activities in liver and kidney increased significantly in diabetic rats. Treatment with *P. boergesenii* extracts significantly depressed the activity of these enzymes. Streptozotocin induced diabetic rats treated with glibenclamide also produced similar effect on Glucose-6-phosphate

and Fructose-6-phosphate activities in liver and kidney when compared to Group II. Table 5 represents the levels of HDL, LDL and VLDL in control and experimental rats. Serum LDL and VLDL were significantly increased whereas HDL cholesterol was significantly decreased in STZ induced groups, while they were significantly altered with extracts of *P. boergesenii* and standard drug.

Table 1: Effect of *P. boergesenii* on levels of serum glucose and insulin in serum of STZ induced diabetic rats

Groups	Glucose*	Insulin#
Group I	84.44 ± 2.10 [a]	17.72 ± 0.66 [c]
Group II	354.01 ± 18.48 [d]	11.20 ± 0.40 [a]
Group III	85.61 ± 2.35 [a]	17.90 ± 0.63 [c]
Group IV	116.77 ± 18.78 [b]	16.80 ± 0.56 [b]
Group V	121.40 ± 3.75 [c]	16.40 ± 0.48 [b]

Values are mean ± SD (n=6), values not sharing a common letter differ significantly at P<0.05 by DMRT, * mg/100 ml, # (µU/ ml)

Table 2: Changes in the levels of glycogen and protein in liver and kidney of control and experimental rats

Groups	Liver glycogen*	Liver protein*
Group I	12.21 ± 0.08 [c]	157.33 ± 6.20 [b]
Group II	8.68 ± 0.09 [a]	135.8 ± 4.009 [a]
Group III	11.83 ± 0.18 [b]	149.83 ± 5.16 [b]
Group IV	12.16 ± 0.22 [c]	157.52 ± 6.24 [b]
Group V	11.80 ± 0.17 [b]	149.60 ± 5.74 [b]

Values are mean ± SD (n=6), values not sharing a common letter differ significantly at P<0.05 by DMRT; * mg/g tissue.

Table 3: Changes in the activities of glycolytic enzymes in liver and kidney of control and experimental rats

Groups	Liver			Kidney		
	Hexokinase*	Aldolase**	Phosphogluco-isomerase#	Hexokinase*	Aldolase*	Phosphogluco-isomerase#
Group I	200.63 ± 4.40 [c]	17.44 ± 1.19 [c]	23.77 ± 0.95 [c]	119.96 ± 7.82 [c]	22.44 ± 1.34 [c]	18.78 ± 0.87 [c]
Group II	164.93 ± 7.98 [a]	12.59 ± 0.78 [a]	16.75 ± 0.62 [a]	68.31 ± 3.90 [a]	13.38 ± 0.45 [a]	12.89 ±0.59 [a]
Group III	184.72 ± 3.76 [b]	16.65 ± 0.07 [b]	20.49 ± 0.71 [b]	103.33 ± 1.64 [b]	19.34 ± 0.65 [b]	16.98 ± 0.73 [b]
Group IV	200.62 ± 13.21 [c]	17.36 ± 0.93 [c]	23.90 ± 1.91 [c]	119.17 ± 1.32 [c]	22.85 ± 1.61 [d]	18.85 ± 0.91 [c]
Group V	184.58 ± 5.80 [b]	15.66 ± 1.10 [b]	20.43 ± 0.17 [b]	106.78 ± 3.49 [b]	18.12 ± 0.82 [b]	17.05 ± 0.80 [b]

Values are mean ± SD (n=6), values not sharing a common letter differ significantly at<0.05 by DMRT, * nmoles of glucose of glucose -6- phosphate formed/min/ mg protein ** nmoles of glyceraldehydes formed/min/mg protein # nmoles of fructose formed/min/mg protein.

Table 4: Changes in the activities of gluconeogenic enzymes in liver and kidney tissue

Groups	Liver		Kidney	
	Glucose-6- phosphatase*	Fructose 1,6 diphosphatase*	Glucose -6- phosphatase*	Fructose 1,6 diphosphatase*
Group I	5.32 ± 0.22 [a]	13.07 ± 0.77 [b]	3.91 ± 0.17 [a]	10.49 ± 0.57 [b]
Group II	8.40 ± 0.42 [c]	27.54 ± 1.33 [c]	6.54 ± 0.33 [c]	17.70 ± 0.70 [c]
Group III	5.33 ± 0.33 [a]	13.04 ± 0.81 [a]	3.99 ± 0.11 [a]	10.75 ± 0.54 [a]
Group IV	6.27 ± 0.32 [b]	18.95 ± 0.92 [b]	4.40 ± 0.07 [b]	13.24 ± 0.70 [b]
Group V	6.22 ± 0.21 [b]	19.17 ± 0.85 [b]	4.50 ± 0.40 [b]	13.18 ± 0.68 [b]

Values are mean ± SD (n=6), values not sharing a common letter differ significantly at <0.05 by DMRT, * nmoles of pi liberated/min/mg protein

Table 5: Changes in the levels of serum HDL, LDL and VLDL in control and experimental rats

Groups	HDL*	LDL*	VLDL*
Group I	44.21 ± 2.88 [a]	27.01 ± 1.72 [a]	20.26 ± 0.08 [a]
Group II	28.84 ± 2.00 [b]	37.35 ± 2.81 [b]	33.63 ± 1.08 [b]
Group III	38.97 ± 3.73 [c]	38.45 ± 1.63 [c]	26.35 ± 0.37 [c]
Group IV	31.44 ± 1.50 [d]	34.08 ± 2.91 [d]	24.26 ± 0.14 [d]
Group V	45.88 ± 1.55 [a]	24.86 ± 2.10 [a]	21.82 ± 0.31 [a]

Values are expressed ± SD (n=6), values not sharing a common letter differ significantly at < 0.05 by DMRT,* mg/ dl/protein,*mg/ dl

No significance differences were observed in levels of lipid profiles in *P. boergesenii* treated groups alone when compared to control group. There was an increase in the levels of LDL and VLDL along with a decrease in the HDL level in case of Group II rats.

DISCUSSION

Antidiabetic effect of aqueous extract of *P. boergesenii* was evaluated in STZ induced diabetic rats at the dosage of 400 mg/kg for 30 days were compared with standard drug glibenclamide. The

STZ treatment rapidly produced the characteristic signs of diabetes such as increased intake of both water and food, failure to gain weight and increased blood glucose concentrations [30]. This may be due to increased muscle wasting and loss of tissue proteins. The enhancement of body weight was observed in treatment group this might be the effect of *P.boergesenii* on regulating the glucose metabolism.

In extract treated diabetic rats, reduction in blood glucose levels may be associated with degranulation in pancreatic β-cells,

peripheral utilization of glucose or decrease in glucose uptake in intestine, which is in line with earlier reports [31]. STZ is a potent DNA methylating agent and acts as a nitric oxide donor in pancreatic cells. The ß cells are particularly sensitive to damage by nitric oxide and free radicals because of their low levels of free radical scavenging enzymes [32]. The Streptozotocin causes the destruction of β-cells of the islets in diabetes, which lead to reduction in insulin releases [33] and an insufficient release of insulin leads to hyperglycemia. Normalization of increased blood glucose by *P. boergesenii* extract suggests that it may enhance glucose transport across the cell membranes and stimulate glycogen synthesis or enhance glycolysis. The extract might possess insulin like effect on peripheral tissues either by promoting glucose uptake and metabolism or inhibiting hepatic gluconeogenesis.

There was an improvement in *P. boergesenii* extract and glibenclamide treated rat's liver protein levels, which may be due to marked change in circulating amino acid level, hepatic amino acid uptake and muscle output of amino acid concentrations [34]. The increased hepatic glucose output in diabetes may be derived from glycogenolysis and/or gluconeogenesis [35]. In general, increased hepatic glucose production plus decreased hepatic glycogen synthesis and glycolysis are the major symptoms in type 2 diabetes that results in hyperglycemia [36]. Our results revealed an immense depletion in hepatic glycogen contents. These results are in accordance with those of Lavoie and Van de Werve, 1991 [37] and Ahmed *et al.* 2010 [38] who found that streptozotocin induced diabetes, reduced hepatic glycogen content and increased glucose - 6- phosphatase activity in diabetic rats.

In our study marked reduction in the levels of glycogen was observed in streptozotocin induced diabetic rats and was restored to normal by *P. boergesenii,* which is in line with previous reports [39].

Hexokinase is the prime enzyme catalyzing glucose phosphorylation. The first step in glycolysis is severely impaired during diabetes. Impairment of hexokinase activity suggests the impaired oxidation of glucose via glycolysis leading to its accumulation resulting in hyperglycemia [40]. In the present study, hexokinase activity was found to be decreased in diabetic rats which may be due to insulin deficiency. Treatment with *P. boergesenii* elevated the activity of hexokinase in liver and kidney. The *P.boergesenii* may stimulate insulin secretion, which may activate hexokinase, thereby increasing utilization of glucose leading to decreased blood sugar levels.

Aldolase, another key enzyme in the glycolytic pathway, increases in diabetes and this, may be due to cell impairment and necrosis [41]. In experimental diabetes, the cells are subjected to STZ induced damage and very often exhibit glycolysis after a period of increased oxygen uptake. Activities of phosphoglucoisomerase and ATP dependent phosphofructokinase enzymes are reported to be under regulated by citrate, [42] which is a TCA cycle intermediate. Decrease in activity of phosphoglucoisomerase might be expected to inhibit the proportion of glucose 6-phosphate metabolism via the glycolytic pathway [43]. The increased activities of glucose 6-phosphatase and fructose-1,6-diphosphatase in liver and kidney of the STZ induced diabetic rats may be due to insulin insufficiency. Insulin decreases gluconeogenesis by decreasing the activities of key enzymes, such as glucose-6-phosphatase, fructose-1,6-diphosphatase, phosphoenol pyruvate carboxykinase and pyruvate carboxylase [44]. In *P. boergesenii* treated rats, glucose-6-phosphatase and fructose-1, 6- diphosphatase were significantly reduced in liver and kidney. This may be due to improved insulin secretion, which is responsible for the repression of gluconeogenic key enzymes**.**

Hyperlipidemia is a recognized consequence of diabetes mellitus demonstrated by the elevated levels of tissue cholesterol, phospholipid and free acid [45, 46]. The Group III and Group IV animals received standard drug glibenclamide and *P. boergesenii* extract respectively; they were reverting back to normal lipid values. This implies the probable activation of the enzyme lipoprotein lipase by extract and glibenclamide. The normal functioning of lipoprotein lipase maintains the normal lipid profile in seaweed extract alone treated group.

In conclusion, the *P. boergesenii* extract showed a high-quality antidiabetic activity in STZ induced diabetic rats. The effective dose of *P. boergesenii* extract was found to be 400 mg/kg body weight. The action of *P. boergesenii* was comparable with antidiabetic drug glibenclamide. Results of this experimental study indicated that *P. boergesenii* has potent antidiabetic activity in STZ-induced experimental diabetes in rats. Further comprehensive chemical and pharmacological investigations are needed to elucidate the exact mechanism of the hypoglycemic effect of *P. boergesenii* and compounds are accountable for its antidiabetic effect.

ACKNOWLEDGEMENT

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