
Original Research Article***In vivo* antidiabetic efficacy of red algae *Portieria hornemannii* and *Spyridia fusiformis* methanol extract on alloxan stimulated hyperglycemic activity in Wistar albino rats**Subbiah Murugesan¹, Sundaresan Bhuvaneshwari¹, Vajiravelu Sivamurugan^{2*}¹Division of Algal Biotechnology and Bionano Technology, PG and Research Department of Botany, Pachaiyappa's College, Chennai – 600 030, India.²PG and Research Department of Chemistry, Pachaiyappa's College, Chennai –600 030, India.***Corresponding Author:**

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Abstract: To assess the effect of *Portieria hornemannii* and *Spyridia fusiformis* methanol extract for its anti-hyperglycemic activity against chemically induced diabetic rats by examining its influence on parameters such as blood sugar level, liver Glucokinase and Hexokinase activity, Glucose 6 phosphate activity, glycogen content. The methanolic residue of *P. hornemannii* and *S. fusiformis* at a dosage of 200 mg/kg of weight was given orally to Alloxan induced diabetic animals for about 28 days. The outcome of algae residue feeding in rats estimated by various biochemical as well as haematological parameters such as RBC, WBC, HB and platelets. Histopathological evaluation was made in the pancreas. The rats, pretreated with methanol residue of the above algal materials (200 mg/kg i.p) enhanced oral glucose tolerance against control. After feeding of methanol extract in diabetic rats for 28 days, the blood glucose has significantly decreased, while the increase in liver glycogen level was observed. In addition, the regeneration of the pancreas of the treated animals was noticed. The methanol residues of the experimental red algae *P. hornemannii* as well as *S. fusiformis* possess very effective hypoglycemic effect on the chemically induced diabetic rats compared to Glipizide.**Keywords:** *Portieria hornemannii*, *Spyridia fusiformis*, Alloxan monohydrate, Antidiabetic activity, Glucose tolerance, Oxidative stress

INTRODUCTION

Diabetes can be defined as a metabolic disease categorized by chronic hyperglycemia, with disorders in carbohydrate, fat, and protein metabolism, resulting from defects in insulin secretion, insulin action, or both [1]. The WHO has forecasted that the number of people with diabetes will increase by twice by 2025, to 300 million from the current number [2]. Managing diabetes in developing countries includes diet and insulin administration along with anti-diabetic agents. However, these procedures may be difficult for patients in developing countries because of socioeconomic conditions [3].

Along with the physical exercise and diet, nutritional factors which include antioxidants showed notable impact in managing diabetes and reducing problems to some extent [4]. The disparity among antioxidative defense and oxidative stress mechanisms in diabetics can cause tissue and cell damage and elevate diabetic complications.

Marine ecosystem is a mother of many biologically active metabolites which have led to the discovery of novel medicines for the treatment of hormonal based disorders such as cancer, diabetes mellitus etc., Seaweeds possess high nutritional contents that can be utilized as a supplement for the treatment of protein malnourishment conditions [5, 6]. It is a major source of wealth with a variety of diversity [7]. There are a few marine natural products are currently in the market or in clinical trials, marine organisms still remain unexploited source. But, the unusual diversity of chemical structures isolated from marine organisms, there is huge interest in screening marine natural products for their biomedical potential.

AIM AND OBJECTIVES

Based on the evidences, we attempted to find novel antidiabetic drugs from the marine red algae *P. hornemannii* and *S. fusiformis* and evaluate the anti-hyper glycaemic activity against diabetic rats.

MATERIALS AND METHODS

Collection of Seaweeds

In the present study *Portieria hornemannii* (Lyngbye) (Silva) and *Spyridia fusiformis* (Wulfen) was obtained from coastal regions of Kanyakumari, South India. The freshly collected seaweed samples were rinsed with sterilized sea water and shade dried to get constant weight. The methanolic residue of the above algae was obtained by soaking 1g of the dried algal powder in 100 mL of methanol overnight at 60 °C and the solvent extract was filtered followed by removal of solvents yielded 250 mg of extract residue.

Animal and Experimental design

The Wistar strain of male albino rats having 180 and 200 g body weight were used in the current study. After keeping the animals in the laboratory condition for a week for acclimatization, the experiment was initiated. The study was conducted after obtaining institutional animal ethical committee clearance and reference number is (IAEC/006/2011).

Chemical stimulation of diabetes

In Wistar rats diabetes was stimulated by freshly prepared solution of monohydrate of Alloxan was injected intraperitoneally at 150 mg. kg⁻¹ of body weight prepared in physiological saline after fasting overnight for 12 hrs [8]. The elevated glucose level in plasma was determined at 72 hrs and then on the 7th day, after injection, this data confirmed hyperglycemia. To diagnose the diabetes, the threshold value of fasting plasma glucose level was taken as >126 mg/dL.

Anti-diabetic study procedure

In the current study 30 rats (5 groups comprising 6 animals per group) were utilized and 3 days before commencement of the experiment the alloxan was injected to develop diabetes. The details of 5 groups as follows:

Experimental design

Group-I: (Normal untreated control) consist of normal rats treated with 10 mL/Kg with normal saline, orally.

Group-II: (Diabetic control) Diabetic control received 150 mg/Kg of Alloxan monohydrate through intraperitoneally (i.p).

Group-III: (Positive control) Diabetic rats given Glipizide (10 mg/Kg i.p) (Kavalaliet *al.*, 2003) for 28 days, orally.

Group-IV: (Treatment group) Diabetic rat received a methanol extract from *P. hornemannii* at a dose of (200 mg/Kg) daily using intra- gastric tube for 28 days.

Group-V: (Treatment group) Diabetic rat received a methanol extract from *S. fusiformis* at a dose of (200 mg/Kg) daily using intra-gastric tube for 28 days.

Sample collection

The treatment with algal residue was continued for 28 days. After that, the body weight and blood

glucose level were monitored. The blood was collected retro-orbitally under light ether anesthesia using capillary tubes. Blood sample was collected in new ampoules containing anticoagulant agents, EDTA and plasma was separated from the blood in a T8 electric centrifuge at a rotation of 2000 rpm for about 2 mins. The liver and pancreas were immediately removed by dissection from the animals after sacrifice and organs were washed in ice-cold saline and pancreas was subjected to histopathological studies and the enzyme activities are estimated from the liver.

Biochemical analysis

Estimation of blood glucose

Glucose assay kit (One Touch Ultra) provides direct measurement of glucose in various biological samples [9].

Hepatic glucokinase and hexokinase activity

The small portion of liver was washed in ice cold 150 mM solution of potassium chloride containing 1 mM of EDTA and homogenized using ice cold buffer (10 mM M of cysteine and 1 mM of EDTA in 0.1 mL of Tris-HCl at pH 7.4) and centrifuged for 20 mins at 4 °C. Glucose phosphorylation was assayed by means of Glucose 6 phosphate dependent spectrophotometric method [10].

In vivo Glucose-6-phosphatase assay

The small portion of the liver was homogenized using 40 times of its weight of the ice cold buffer (0.1 Citrate/KOH, pH 6.5) and cheese cloth used for filtration. Glucose-6-phosphatase activity was determined by phosphate release method (Marjorie, 1964) and concentration of H₃PO₄ was determined in the assay by colorimetric method [11].

Glycogen content

The tissue sample was washed using hot concentrated KOH (30%) and followed by anthrone reagent. Glycogen content was measured using a colorimetric method [12].

Determination of haematological parameters

The blood samples were evaluated for HB, WBC, RBC and Platelets by using an auto analyzer (MISPA-EXCEL, Japan).

Histopathological examination

The small portion of pancreas from each animal was removed after sacrificing the animal and was collected in 10% formalin solution, and immediately processed by the paraffin technique. Haematoxylin-eosin was used to stain the paraffin section [13]. Each pancreas sample was seen at 400 X using microscope and evaluated based on the injuries [14].

Statistical analysis

All the experimental outcomes were expressed as the mean \pm S.E.M. The biochemical parameters and haematological were subjected to statistical analysis by one way Analysis of Variance to determine the significant difference between the groups was done with Graph pad Prism software. All Pairwise Multiple Comparison Procedures by Student-Newman-Keuls Method. If the statistical significant difference was $p < 0.05$ data accepted for assessment.

RESULTS

The antidiabetic activity of *P. hornemannii* and *S. fusiformis* analyzed *in vivo* was taken as objective of the present study. The methanol extracts of the experimental algae were administrated to the Alloxan monohydrate induced diabetic animals, at the concentration of 200 mg/kg body for the experimental period of 28 days. From the results it is observed that the methanol extracts were able to restore the body weight, sugar level as well as the glycogen content as that of the normal rats (Group I).

Effect of MEP and MES on body weight

The diabetic rats mean body weights (150.42 ± 2.42 g) was decreased when compared to normal animals (216.42 ± 4.10 g) (Table 1). The methanol extract of *P.hornemannii* and *S. fusiformis* at a dose of 200 mg/kg was significantly increased the body weight of treated the animals to 218.30 ± 3.58 g and 220.50 ± 3.42 g respectively when compared to untreated animals. Glipizide treated (Group III) rats showed improve in their body weight.

Effect of MEP and MES on blood glucose levels

Prior to Alloxan administration, the level of plasma glucose of the rats were not significantly higher in the rats selected for the study in all the groups. The values shown in Table 2 provide that the effect of methanol extract of *P. hornemannii* and *S. fusiformis* at a dose of 200 mg/kg on level of plasma sugar. The level of blood glucose was increased to 175.26 ± 5.22 mg% and 216.9 ± 6.52 mg% at 14th and 28th day for *P. hornemannii* and *S. fusiformis* extracts respectively in the animals when compared to control animals.

The extract of *P.hornemannii* and *S.fusiformis* treated groups (200 mg/kg doses), showed antihyperglycemic effect from the 2nd week. The reduction in the blood glucose was higher at the end of the 4th week in the algal extract treating group. Similarly, in positive control group III the rats receiving Glipizide at a dose of 10 mg/kg returned to the normal blood sugar level.

Effect of MEP and MES on the glycogen content induced mice

The glycogen content in the liver tissue was assessed on the 28th day in all the V groups as summarized (Table.2). In diabetic control group, the glycogen in liver reduced significantly by 79.58%. On the other hand, the dose of 200 mg/kg of Glipizide and the experimental algal extracts showed a significant activity (73.06%, 63.36% and 67.31%) and increase in glycogen compared to Group II (Table.2).

Effect of MEP and MES on hepatic enzymes

The level of plasma glucose was determined 72 hrs after administration of Alloxan to check the diabetics. Animals with body weight above 180 mg were subjected in this study. At the end of 28th day, hepatic enzymes such as glucokinase, hexokinase, and substrate Glucose-6-phosphate were estimated in all the groups.

The result has been compiled in Table.3. As compared to non-diabetic control values, mean level of hepatic enzymes values decreased in diabetic control. The decrease in percentage was 56.94, 80.69 and 68.11% observed for hexokinase, glucokinase and glucose-6-phosphate respectively in diabetic control. The methanol extracts of *P.hornemannii* and *S. fusiformis* at a dose (200 mg/kg) for 28 days showed increase in the level of enzyme percentage by 22.50, 55.93 and 46.58 and 34.50, 67.54 and 48.34% ($p < 0.01$) (Table.3). Also, treatment with Glipizide at a dose of 10 mg/kg for 28 days exhibited significantly increased in the level of enzyme percentage by 27.34, 65.29 and 64.38% respectively ($p < 0.01$) as compared to diabetic control.

Effect of MEP and MES on haematological parameters

The effects of MEP and MES on haematological parameters in the rats showed in Table.4. It was shown that no significant differences were seen in the mean WBC and RBC counts, HB and platelet values at the end of the study period, when compared to the non-diabetic animals.

Histopathological studies

The normal acini and normal cellular population in the Islets of Langerhans in pancreas of non-diabetic rats (Group-I) (Fig.1a). The extensive damage and reduced number of Islets of Langerhans in the pancreas of diabetic rats (Group-II) (Fig.1b). The restoration of the normal cellular population size of islets with hyperplasia by Glipizide (Group-III) (Fig.1c). The partial restoration of normal cellular population and enlarged the size of β -cells with hyperplasia in the algal treated groups (Group IV & Group V) (Fig. 1d-e).

Table-1: Effect of algal extracts on body weight of animals

Groups	Initial Body Weight (g)	Final Body Weight (g)
Group I	210.12 ± 4.12	216.42 ± 4.10
Group II	202.96 ± 4.06	150.42 ± 2.42 ^{*a}
Group III	212.16 ± 4.26	220.32 ± 3.62 ^{*b}
Group IV	214.60 ± 4.45	218.30 ± 3.58 ^{*b}
Group V	208.45 ± 4.18	220.50 ± 3.42 ^{*b}

Table-2: Effect of treatment with MEP and MES on levels of glucose (mg%) and glycogen content in alloxan diabetic rats

Groups	Serum glucose levels (mg%)			Liver Tissue Glycogen Content (mg/g tissue)
	0 Days	14 th Day	28 th Day	
Group I	73.80 ± 3.23	76.80 ± 3.30	70.42 ± 3.40	40.26 ± 2.35
Group II	156.70 ± 4.60	175.26 ± 5.22 ^{*a}	216.9 ± 6.52 ^{*a}	8.22 ± 0.52 ^{*a}
Group III	186.50 ± 5.22	145.50 ± 3.22 ^{*b}	132.68 ± 3.70 ^{*b}	30.52 ± 1.88 ^{*b}
Group IV	196.22 ± 5.68	156.35 ± 3.95 ^{*b}	146.25 ± 3.55 ^{*b}	22.42 ± 1.25 ^{*b}
Group V	192.35 ± 4.89	160.28 ± 4.10 ^{*b}	150.25 ± 3.92 ^{*b}	25.15 ± 1.56 ^{*b}

Where, G₁– Normal control group G₄ – Treatment control (MEP)
 G₂– Diabetic control group G₅ – Treatment control (MES)
 G₃– Standard control (Glipizide) group

Table-3: Levels of hepatic enzymes involved in carbohydrate metabolism in rats

Groups	Hexokinase (µg/mg)	Glucose-6-Phosphate (µg/mg)	Glucokinase (µg/mg)
Group I	0.216 ± 0.012	0.392 ± 0.012	25.38 ± 1.40
Group II	0.093 ± 0.005 ^{*a}	0.125 ± 0.008 ^{*a}	4.90 ± 0.30 ^{*a}
Group III	0.128 ± 0.008 ^{*b}	0.351 ± 0.016 ^{*b}	14.12 ± 0.90 ^{*b}
Group IV	0.120 ± 0.006 ^{*b}	0.234 ± 0.009 ^{*b}	11.12 ± 0.52 ^{*b}
Group V	0.142 ± 0.007 ^{*b}	0.242 ± 0.010 ^{*b}	15.10 ± 0.94 ^{*b}

Table-4: Levels of haematological components

Groups	WBC × 10 ³ /µL	RBC × 10 ⁶ /µL	HB % gm/dL	Platelet × 10 ³ /MI
Group I	8.45 ± 0.62	6.55 ± 0.36	12.36 ± 0.62	314.46 ± 34.60
Group II	8.16 ± 0.70	6.94 ± 0.16	13.16 ± 0.42	296.40 ± 13.30
Group III	6.46 ± 0.42	6.52 ± 0.46	14.26 ± 0.34	280.42 ± 30.90
Group IV	7.22 ± 0.36	7.76 ± 0.22	12.82 ± 0.60	310.82 ± 18.92
Group V	8.70 ± 0.86	6.74 ± 0.34	11.76 ± 0.62	312.22 ± 13.70

Values are presented in Table are Mean ± SEM.

Data obtained by one way ANOVA followed by Newman Keul's multiple range tests.

*^aData notably varied from normal control (Group I)

*^bData notably varied from diabetic control (Group II)

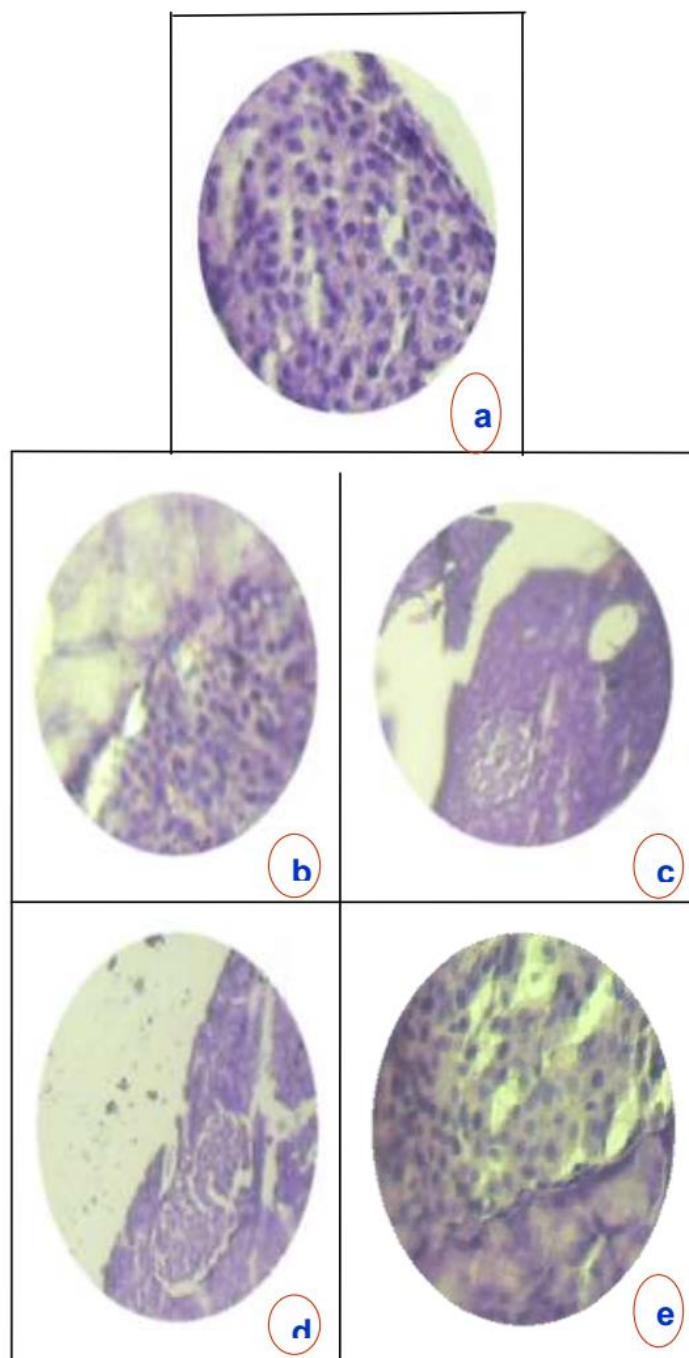


Figure 1 : Photomicrographs of a cross section of pancreas stained with haematoxylin and Eosin (magnification $\times 400x$).

- a) Normal untreated control (Saline) (Section shows Structure of pancreas with normal number of Islet cells)**
- b) Diabetic control (Alloxan monohydrate) (The number of islet cells was significantly decreased, islet cells were severely swelled)**
- c) Positive control (Alloxan monohydrate + Glipizide) (The number of islet cells was moderately decreased, islets cells were mildly swelled)**
- d) Treatment group (Alloxan monohydrate +MEP (The number of islet cells was moderately decreased, islet cells were moderately swelled)**
- e) Treatment group (Alloxan monohydrate+MES) (The numbers of islet cells were moderately decreased, islet cells were moderately swelled).**

DISCUSSION

The screening of the antidiabetic activity of natural products and synthetic compounds is performed

in experimental animal models after induction of diabetes by several methods such as biochemical, hormonal and morphological protocols. The drug

regimen currently being used have certain drawbacks on the management of diabetes [15]. Thus there is a need for more effective antidiabetic drugs without side effects. In the present study was to evaluate the anti diabetic activity of the methanol extract of the experimental algae using *in vivo* animals models.

The chemical agent, alloxan become the first chemical for inducing diabetes when Dunn *et al.*, [16, 17]. To induce diabetes in animals, Alloxan was used commonly at a dose of 150 mg/kg i.p. which produces moderate hyperglycemia with clinical symptoms.

In the present study, it was observed that a significant increase in level of plasma glucose in diabetic rats. The methanol extract of algal materials showed significant antihyperglycemic in mild hyperglycemia. In the case of mild diabetes, the highest percentage of reduction in glucose level was seen in groups receiving the algal extracts at a dose of 200 mg/kg per day. This may be owing to the insulin effect on plasma by enhancing their pancreatic secretion.

The body weight was found to be decreased in alloxan-induced diabetic rats [18]. The decrease in the body weight of diabetic rats in our study was due to the loss or degradation of structural proteins to provide amino acids for gluconeogenesis during insulin deficiency resulting in muscle wasting and weight loss [19, 20]. Due to insulin deficiency, protein content decreases in muscular tissue by proteolysis [21]. Murray *et al.*, (2003) have shown that protein synthesis is decreased in all tissues due to decreased production of ATP and absolute or relative deficiency of insulin [22]. The weight loss was reverted by administration of *P. hornemannii* and *S. fusiformis* methanol extract and Glipizide at a dose of 200 mg/kg and 10 mg/ per kg significantly increased body weights in diabetic rats compared to control rats. The ability of the algal extracts to protect massive body weight loss seems to be due to its ability to reduce hyperglycemia. The effect of 200 mg/kg of extract on the body weight of diabetic rats was more or less equal to that of Glipizide.

The present study confirms the reduction of intestinal glucose by inhibition of intestinal glucose absorption of the selected algae and their combinations and their mechanism was proved as hypoglycemic effect [23]. The hypoglycemic effect had been previously reported by Viquez-feiret *et al.*, [24]. Although the relationship between lipid and glucose metabolism suggested that the glycemia and triglyceride results ought to be related, a discrepancy, *in priori*, was found. The methanol extracts of *P. hornemannii* and *S. fusiformis* are effective in lowering and normalizing the blood glucose level in diabetic rats, and it was due to the presence of different secondary metabolites in the selected algae.

As reported earlier [25], in the present study also, the liver glycogen levels were lower in the diabetic animals when compared with the normal animals fed adequately, significantly the higher levels were found in the diabetic group when compared with the fasted condition. Treatment with *P. hornemannii* and *S. fusiformis* methanol extract prevented this alteration in glycogen content of liver tissue, but could not normalize the content of glycogen of the non-diabetic control. This prevention or depletion of glycogen in the liver is possibly due to either stimulation of insulin release from β -cells [26] or due to the insulin mimetic activity of some components of the plants resulting in direct peripheral glucose uptake.

The enhance in hepatic glycogen content in algal extracts administered animals may due to the activation of glycogen synthase with substrate glucose-6-phosphate could have been readily provided with an increased hexokinase activity [27 - 29]. It clearly indicates that the algal extracts could reduce gluconeogenesis both alone and in combination. Thus, *P. hornemannii* and *S. fusiformis*, have been shown to reduce levels of blood glucose and improved glycolysis and glycogenesis, reduce gluconeogenesis and to bring the glucose metabolism towards normal levels in diabetic rats. Moreover, the effect of *P. hornemannii* and *S. fusiformis* on the carbohydrate metabolism in diabetic rats is found to be similar to that of Glipizide. The results of the present study suggest that the slow disappearance of liver glycogen in fasted alloxan diabetic rats may be attributed to their hyperglycemia. On the problem of gluconeogenesis of the 48 hours-fasted diabetic rats, no evidence of enhanced gluconeogenesis compared to the normal rats was obtained.

In our investigation the diabetic animals showed lower GSH levels reflecting their increased utilization due to oxidative stress; while a substantial increase of GSH levels observed in *P. hornemannii* and *S. fusiformis* treated with diabetic animals showed decrease in peroxidation of lipids, it appears that the effect of the algal extract of GSH could be at two levels, either through increasing the biosynthesis of GSH or reducing oxidative stress [30-32].

In the present investigation, both SOD and CAT enzymes registered lower levels of activity in diabetic controls showing diabetes-induced stress [33 - 36]. The *P. hornemannii* and *S. fusiformis*, when administered to the diabetic animals improved both SOD and CAT activities significantly, showing the antioxidant potential of these algal extracts.

The oral administration of methanol extract of *P. hornemannii* and *S. fusiformis* showed significantly increased in hexokinase, glucokinase activities and Glucose-6-phosphate level in the liver, indicating an

increase in glucose influx and showed that the algal extracts have an overall effect of an increase in glucose utilization [37]. The present study also showed that algal extracts showed no unfavorable effect on WBC, RBC counts, Hb and platelets. Thus, the methanol extract of *P. hornemannii* and *S. fusiformis* can be safer to use without toxicological effects.

The presence of anthraquinones, steroid, cardiac glucoside, flavonoids, phenols and terpenoids of methanol extract of *P. hornemannii* and *S. fusiformis* may explain the normalization of the FBS (Fasting blood sugar) level in diabetic mice [38-43, 49]. The activity of these compounds could explain the effects of methanol extracts of *P. hornemannii* and *S. fusiformis* possessing secondary metabolites. Marine algae has shown to be a good source of unsaponifiable, non toxic sterols that have medicinal value [44 -46]. The steroid component of the fraction could support the FBS level lowering mechanism and assist indirectly the health of the diabetic mice since these metabolites have lipid lowering effect. Soodabeh Saeidnia *et al.*, reported that the red marine algae *Gracilariopsis persicaris* rich of sterols (fucosterol, stigmaterol and β -sitosterol) and are noteworthy for their effectiveness in decreasing the plasma cholesterol, glucose and inflammation [47, 48].

In animals treated with 200 mg/kg of methanol extract of *P. hornemannii* and *S. fusiformis* there were regenerative changes in tissue architecture of Islet cells of pancreas. The groups IV & V treated with *P. hornemannii* and *S. fusiformis* (200 mg/kg) and Glipizide (10 mg/kg) showed greater persistence of Islets of Langerhans and lesser degree of necrotic changes as compared to the untreated alloxan-induced diabetic rats. Microscopic examination shows abundant patches of β -cells in the pancreas of normal rats, which are absent in diabetic pancreas.

CONCLUSION

In conclusion, the methanol extract of *P. hornemannii* and *S. fusiformis* showed anti-hyperglycemic activity in diabetic rats. The extract also showed enhancement in body weight, liver glycogen content and carbohydrate metabolizing enzymes and renaissance of β -cells of the pancreas and has value in diabetes treatment. The histopathological results showed good signs of regeneration in the cells of the pancreas.

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