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Antidiabetic Activity of Methanolic Extract of *Halodule uninervis* in Streptozotocin-Induced Diabetic Mice

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Abstract

Diabetes mellitus (DM) is a global health problem and the incidence of DM is increasing at alarming rate all over the world. Many Indian medicinal plants have been reported to possess potential anti diabetic activity and could play important role in the management diabetes. The present study aimed to evaluate anti diabetic activities of methanolic extract of *Halodule uninervis* plant in streptozotocin-induced diabetic rats. Diabetes was induced in male Wistar rats by single intraperitoneal injection of streptozotocin (50 mg/kg b.wt.). The diabetic rats were administered orally with *Halodule uninervis* plant extract at two different doses (150 and 250 mg/kg b.wt./day) for 18 days. At dose levels of 150 and 250 mg/kg, glucose levels were decreased by 24.8% and 29.9% at the 6th hour, respectively. Anti diabetic effect of the extract was slightly decreased at the 8th hour, but remained statistically significant**.** An overall reduction of 26% was observed on the 18th day by 50mg/kg administration ($p<0.01$). 52.5% reduction of glucose level in the serum absorbed at 18th day administration at dose level of 150 mg/kg ($p<0.0001$). 250mg / kg extract administration is more effective from 6th day onwards with a reduction rate of 18.9% ($p<0.01$) and maximum reduction of serum glucose level by 61.9% on the 18th day. Rats treated with higher doses of the extract (150 and 250 mg/kg) showed significant improvements in hepatic and renal function. Additionally, these two dose levels recovered the weight loss and low white blood cell count observed in Streptozotocin-diabetic rats while decreasing liver glycogen. *Halodule uninervis.* extract (150 and 250 mg/kg) also showed a protective effect on liver oxidative status. The results of present study showed that *Halodule uninervis* plant extract possess significant antihyperglycemic activity and supports the traditional use of *Halodule uninervis* plant for the treatment of diabetes mellitus.

Key words: Anti diabetic, Blood glucose, *Halodule uninervis* plant, Glycogen, Kidney and liver oxidative status, Streptozotocin

INTRODUCTION

Diabetes mellitus is a group of syndromes characterized by hyperglycemia; altered metabolism of lipids, carbohydrate and proteins; and an increased risk of complications from vascular diseases. In the United States, about 90% of diabetic patients have Type-2 diabetes mellitus. There are more than 125 million persons with diabetes in the world today [1]. The treatment of diabetes mellitus-2 with oral hypoglycemic agents like sulfonyl urea and biguanides is always associated with numerous side effects. The major advantages of herbal medicine seem to be their good potential, low incidence of serious side effects and low cost [2]. The dependence of large rural population on medicinal plants for treatment of diabetes is because of its availability and affordability [3]. Additionally, after the approbation made by WHO on diabetes mellitus, exploration on hyperglycemic agents from medicinal plants has become more significant [4,5]. So, the present study was conducted to evaluate anti hyperglycemic activities of *Halodule uninervis* in streptozotocin induced diabetic rats.

Halodule uninervis is a sub littoral sea grass found from the mid-intertidal to a depth of 20 m. Its characteristic features include: shoots up to 30 cm long and erect, having 2-4 leaves in each branch, leaf linear, narrowed at base with sheath, margin entire, nerves 3, midrib conspicuous and lateral ribs inconspicuous, ending in well developed lateral teeth at leaf apex, teeth tridentate. Sea grasses are known to produce secondary metabolites as defence mechanism under stress conditions and these compounds are found to be anti-oxidative in nature. Biochemical analysis of *Halodule uninervis* estimated carbohydrate protein, lipid, tannin and phenol, Cardiac alkaloids, flavone glucosides, saponins, palmitic acid, linoleic acid, phenylethane derivative, (S)-methoxy-(3-,5-dimethoxy-4-hydroxyphenyl) ethanediol, 3,4,5-trihydroxy benzoic acid, (E)-3-(4-methoxyphenyl)-2-propenoic acid, syringin (7), 5-hydroxy-3-4 7-trimethoxyflavone, and 4'-hydroxy-3',5,7-trimethoxy flavones [6-9]. The plant contains many flavonoids and sterols/triterpenoids as its main constituents, which are known bioactive principles for anti diabetic potential [10,11]. Flavonoids are also known to regenerate the damaged β-cells in diabetic mice [12, 13].

MATERIALS AND METHODS

Collection of Plant Material

The plants were collected from around local costal area of Ramanathapuram, Tamilnadu during December and authenticated by the Botanist Prof.Chelladurai, Department of Botany, Govt. Siddha Medical College, and Tirunelveli. A voucher herbarium specimen number KMCP/HU/01/2015 was also preserved in the K.M.College of Pharmacy, Madurai. All the collected samples were washed immediately using native sea water to remove the adhering salts and sands, several times with distilled water. The whole plants were dried in shade for 3weeks. Then about 1 kg of the shade dried plant was made in to segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve. The powdered plant materials were stored in an airtight container, and used for further studies and investigation.

Preparation of Extracts

Previously soaked about 1000 gm of dried coarsely powdered plant material was extracted in soxhlet assembly with petroleum ether at 40◦ – 60◦ C by continuous hot percolation method. The extraction was continued for 72 hours. The petroleum ether extract was filtered and concentrated by using rotary vacuum evaporator to a dry mass (9.7 gm). A Dark green residue was obtained. The marc left, after petroleum ether extraction was taken for further 72 hours extraction with chloroform. The chloroform extract was concentrated to the dry mass. A greenish brown residue was obtained (12.3 gms).The marc left, after chloroform extraction was taken and further extracted with Ethyl acetate for 72 hour. The concentrated ethyl acetate extract was in Yellowish Brown residue (16.8 gms). The further extraction of marc from ethyl acetate was taken and extracted with Methanol for about 72hrs obtained Brownish green residue (65.3gms)

Phytochemical Screening

The extract was subjected to phytochemical analysis to test the presence of carbohydrates, glycosides, alkaloids, flavonoids, tannins, sterols, and triterpenoid in plant extracts [14].

Animals

Experimental animal Male Swiss albino mice (5-9 weeks old) mice, weighing about 20 to 30g, was kept under standard conditions, about 12 hours in both light and dark and fed with standard diet pellet, water, ad libitum. Animals were adapted to laboratory condition for a period of one day before conducting the experiments. All animal experiments were conducted according to the rules and regulations of institutional animal ethical committee clearance.

Acute Toxicity Studies

Acute toxicity study was carried out according to OECD guideline. Methanolic extract of *Halodule uninervis* a dose range of 50 mg to 450 mg / kg was administered intraperitoneally to different group of animals (six in each group). The animals kept under observation for 2h, to identify any symptoms of toxicity (behaviors like pattern, tremors, sleep, coma) and or death. The observation was continued for further 2 weeks of time [15,16].

Glucose tolerance

Overnight fasted animals were divided to group o three (n=6). 1 mL of Normal saline given orally to Group I. Concentrations of 50, 450 mg / kg *of Halodule uninervis* extract was administered to Groups II and III, respectively. Glucose (2 g/kg body weight.). Blood was administered and blood samples were taken at 0.5 hr, 1hr, 2hr and 4hr time from the vein in glucose administration. Blood Glucose was estimated and the Data was used as a hypothetical reference to determine the extract dose level. This data used in evaluation effects of *Halodule uninervis* extract on diabetic rats [17,18].

Treatment Protocol

Animals were divided in to four groups; six in each group received the treatment schedule as tabulated below.

Treatment schedule

Group	Study
I	Normal control (saline).
II	Streptozotocin treated control (150 mg/kg.ip)
III	Streptozotocin (150 mg/kg.ip) + *Halodule uninervis* extract (50mg/kg.ip)
IV	Streptozotocin (150 mg/kg.ip) + *Halodule uninervis* extract (150 mg/kg.ip)
V	Streptozotocin (250 mg/kg.ip) + *Halodule uninervis* extract (250 mg/kg.ip)

Anti diabetic effects

Treatment was given as per the protocol. Blood samples were collected at 2, 4, 6 and 8 hours intervals from the fasted rat's tail vein prior to administration of the extract. Glucose levels were measured from the separated Serum. According to the protocol the non-fasted animals daily treated with Methanolic extract of *Halodule uninervis* for 18 days. Blood samples were collected on 6^{th} day, 9^{th} day, 12^{th}, 15^{th} and 18^{th} day after Streptozotocin and glucose levels in blood serum were measured.

Liver and kidney functions

ALP (Alkaline Phosphatase), creatinine, GPT (glutamate pyruvate transaminase) and BUN (Blood urea nitrogen) were measured. Protein content was determined by the method of . Total WBC count was determined by using a heamocytometer.

Antioxidant status

GSH (reduced glutathione), GP (glutathione peroxidase), SOD (superoxide dismutase), catalase, and MDA (malondialdehyde) levels were determined in liver homogenates by spectrophotometrically using assay kits available commercially. Total nitrites present in aortic tissues were colorimetric ally estimated by using Griess reagent. Concentration of nitrite in the sample was calculated using sodium nitrite standard and normalized to the aorta protein content.

Statistical Analysis

Statistical evaluation for all the grouped data performed by ANOVA. Values were expressed as mean ± SEM (Standard Error of Mean) for six animals in each group. Unpaired student t-test is used for statistical comparison between the four different groups. Changes were considered to be statistically significant if the P-value was < 0.05. $+p < 0.05$, $++p < 0.01$, $+++ p<0.001$ and $*p<0.0001$ was considered statistically significant [19].

RESULTS

Effects of *Halodule uninervis* extract in the single dose study on blood glucose levels was estimated after Streptozotocin administration on the 3rd day. There is no reduction in glucose level due to *Halodule uninervis* extract (50 mg/kg). At dose levels of 150 and 250 mg/kg,

glucose levels were decreased by 24.8% and 29.9% at the 6th hour, respectively. Anti diabetic effect of the extract was slightly decreased at the 8th hour, but remained statistically significant (Table-1).

In parallel experiments, *Halodule uninervis* extract was administered to diabetic rats for 18 days as per the protocol. The glucose level reduction up to 26% on 9th day of *Halodule uninervis* (Forsk.) (50 mg/kg) treatment started to lower serum glucose on the 9th day. An overall reduction of 26% was observed on the 18th day by 50mg/kg administration ($p<0.01$). 52.5% reduction of glucose level in the serum absorbed at 18th day administration at dose level of 150 mg/kg ($p<0.0001$). 250mg / kg extract administration is more effective from 6th day onwards with a reduction rate of 18.9% ($p<0.01$) and maximum reduction of serum glucose level by 61.9% on the 18th day. Between the 12th and 18th days, anti diabetic effect of Halodule uninervis extract was in a concentration dependent manner(Table-2).

The effects of *Halodule uninervis* extract on hepatic and renal function in Streptozotocin-diabetic rats. As seen, treatment with 50 mg/kg did affect neither the significantly high levels of ALP, GPT, BUN and creatinine, nor the overall oxidative status. Conversely, rats treated with higher doses of the extract (150 and 250 mg/kg) showed significant improvements in hepatic and renal function (Table-3).

Table-1: Effects of *Halodule uninervis* on serum glucose levels in Streptozotocin -induced diabetic rats

Group	Serum glucose level (mg/dL)				
	0 hour	2 hour	4 hour	6 hour	8 hour
I	70.5 ± 5.6	67.2± 6.9	64.0± 7.9	67.4 ± 7.1	69.2 ± 6.9
II	$267.9 \pm 10.5_a$	$274.1 \pm 9.7_a$	$277.0 \pm 0.9_a$	$273.1 \pm 8.0_a$	$267.4 \pm 7.6_a$
III	261.4 ± 9.6	264.4± 8.6	258.2± 8.5	255.2 ± 9.9	252.5 ± 8.7
IV	258.2 ± 8.5	253.0 ± 8.1	237.4± 7.6	$204.9 \pm 9.2_b$	$214.2 \pm 9.0_c$
V	259.9 ± 10.9	241.9 ± 9.9	230.5± 7.9	$191.2 \pm 8.4_b$	$198.3 \pm 8.5_b$

After administration of Streptozotocin the serum glucose levels were obtained from fasted rats. Data are expressed as mean ± S.E; (n=6); ap<0.0001 (compared to normal group with the corresponding hours). bp<0.01 and cp<0.05 (compared to control group with the corresponding hours)

Table -2: Effects of *Halodule uninervis* (Daily treatment) on serum glucose levels in Streptozotocin -induced diabetic rats

Group	Serum glucose (mg/dL)					
	3 days	6 days	9 days	12 days	15 days	18 days
I	74.9 ± 7.3	72.8± 8.7	71.3 ± 10.6	74.1 ± 8.8	70.8 ± 6.8	75.0 ± 8.3
II	$272.4 \pm 13.0^{\#}$	$279.4 \pm 11.6^{\#}$	$271.5 \pm 11.6^{\#}$	$273.7 \pm 11.0^{\#}$	$268.6 \pm 11.0^{\#}$	$263.0\pm 9.5^{\#}$
III	269.9 ± 10.9	249.6±11.0	$230.5 \pm 9.4^{+}$	$218.5 \pm 9.1^{+}$	$208.7 \pm 9.9^{+}$	$199.7 \pm 9.1^{+}$
IV	275.3± 9.7	231.4± 10.5	$211.1 \pm 12.3^{++}$	$174.3 \pm 10.0^{+++}$,a	$151.0 \pm 10.7^{+++}$,b	$131.4 \pm 11.4_{*,c}$
V	271.5± 10.6	$220.7 \pm 9.3^{++}$	$166.4 \pm 11^{+++}$	$141.4 \pm 8.8_{*,d}$	$130.8 \pm 9.6_{*,e}$	$104.6 \pm 12.3_{*,f}$

Values of serum glucose levels were obtained from Streptozotocin induced diabetic rats in the absence and in the presence of 18 days of *Halodule uninervis* extract treatment (from the 3rd to the 18th day) and expressed as mean ± S.E; n=6; #p<0.0001 (compared to normal group with corresponding day); +p<0.05, ++p<0.01, +++p<0.001 and *p<0.0001 (compared to control group with corresponding day). ap<0.05, bp<0.01 and cp<0.001 (compared to Group III with corresponding day). d,e,f p<0.05 (compared to Group IV with corresponding day)

Table-3: Effects of *Halodule uninervis* extract on liver and kidney functions

Group	Liver		Kidney	
	ALP (KA/dL)	GPT (U/mg protein)	BUN (mg/dL)	Creatinine (mg/dL)
I	**34.9 ± 4.0**	**160.6 ± 11.3**	**10.4 ± 1.8**	**1.9 ± 0.2**
II	$51.5 \pm 7.9_a$	336.5 ± 23.1b	20. ± 3.4c	3.3 ± 0.6.c
III	43.8 ± 6.0	286.8 ± 21.0	17.6 ± 12.3	2.9 ± 0.4
IV	$36.5 \pm 3.9_e$	$245.4 \pm 13.5_e$	$14.8 \pm 1.9_f$	$2.7 \pm 0.8_e$
V	$32.3 \pm 13.3_f$	$207.3 \pm 10.3_f$	$12.7 \pm 1.8_g$	$2.4 \pm 0.6_g$

ALP: alkaline phosphatase; GPT: glutamate pyruvate transaminase; BUN: blood urea nitrogen. Liver and kidney markers were measured on the 18th day after Streptozotocin administration. Data are expressed as mean ± S.E; n=6; ap<0.01, bp<0.001 and cp< 0.0001; compared to normal group. ep<0.05, fp<0.01 and gp<0.001 (compared to control group)

Table-4: Effects of *Halodule uninervis* extract on body weight, total leucocyte count and liver glycogen

Group	Body Weight		Total leukocyte count (mm3)	Liver glycogen (µg/g tissue)
	Initial	Final		
I	240.5 ± 6.6	258.2 ± 7.9	13233.4 ± 455.5	75.7 ± 3.4
II	244.1 ± 8.4	$204.1 \pm 20.7_a$	$8566.0 \pm 388.0_c$	$56.9 \pm 3.6_d$
III	248.4 ± 8.9	$244.4 \pm 18.6_b$	9451.4 ± 510.0	66.6 ± 2.9
IV	242.2 ± 6.9	248.0 ± 11.1	$11102.4 \pm 656.9_e$	$73.3 \pm 3.9_e$
V	246 ± 10.9	261.9 ± 7.9	$13001.3 \pm 701.9_f$	$76.4 \pm 4.3_e$

Data were expressed as mean ± S.E; n=6; ap<0.001 (compared to initial body weight of the same group), bp<0.05 (compared to initial body weight of the same group), cp<0.0001 and dp<0.05 (compared to normal group), ep<0.05 and ^{f}p<0.001 (compared to control group)

Table-5: Effects of *Halodule uninervis* extract on oxidative status

Parameter	Group I	Group II	Group III	Group IV	Group V
GSH (nmol/mg protein)	9.6±1.6	$5.4 \pm 1.4_c$	5.7±1.5	$7.1 \pm 1.6^+$	$7.5 \pm 1.6^{+++}$
GSSG (nmol/mg protein)	1.5±0.2	2.1±0.3c	1.9±0.3	$1.8 \pm 0.6^+$	$1.5 \pm 0.4^{+++}$
GPx (U/mg protein)	1.4±.0.16	$1.3 \pm 0.5_a$	1.3±0.3	$1.2 \pm 0.1^+$	$1.1 \pm 0.1^+$
MDA (nmol/mg tissue)	355.4±11.4	$443.4 \pm 3.1_b$	421.5±14.3	$391.4 \pm 10.4^+$	$356.8 \pm 13.8^{++}$
SOD (U/mg protein)	7.5±0.6	$4.5 \pm 0.5_c$	4.8±0.5	$5.6 \pm 0.8^{++}$	$6.8 \pm 0.7^{+++}$
Catalase (U/mg protein)	154.6±7.9	$99.5 \pm 8.2_b$	120.4±9.5	$127.4 \pm 8.7^+$	$143.9 \pm 11.8^{++}$

GSH: reduced glutathione, GSSG: oxidized glutathione; GPx: glutathione peroxidase, MDA: malondialdehyde; SOD: superoxide dismutase. Data were expressed as mean ± S.E; n=6; ap<0.01, bp<0.001, cp< 0.0001 and $^{++}$p<0.01 (compared to normal group); $^+$p<0.05 and $^{+++}$p<0.001 (compared to control group)

Additionally, these two dose levels recovered the weight loss and low white blood cell count observed in Streptozotocin-diabetic rats while decreasing liver glycogen (Table-4). *Halodule uninervis* extract (150 and 250 mg/kg) also showed a protective effect on liver oxidative status (Table-5).

Antioxidants namely GSH, GPx, SOD and catalase were increased by *Halodule uninervis* extract administration. When compared to Streptozotocin-diabetic rats, MDA formation, as an indirect measure of lipid peroxidation, was found to be significantly low in high dose *Halodule uninervis* extract-treated rats.

DISCUSSION

In the present study, *Halodule uninervis* was selected for anti diabetic studies owing to its traditional uses. Therefore, the study was undertaken to justify its claimed uses. Wistar rats were selected as experimental animals for the anti diabetic activity. The extract was screened for Streptozotocin-induced anti diabetic activity. The methanolic extract of plant showed significant anti diabetic activity at both doses, that is, 150 and 250 mg/kg of body weight. This is further evidenced by percentage reduction in blood glucose levels after 18th day after administering the extract at both of the doses. The methanolic extract significantly increased the body weight of diabetic animal at higher doses.

During this prolonged study, various physical parameters were also observed such as body weight, food intake, water intake, and weight of internal organs. Generally, body weights are reduced in diabetic animals, but in this study, the decrease in body weights was diminished by the extract treatment; thus this effect may be useful for the diabetic animals. The phytochemical study showed the presence of saponin glycosides, steroids, and phenolic compounds in the extracts, which might be a reason for the good activity of extract.

However, this is a preliminary work, and more work is needed to determine the active ingredients in the extract which may help in improving management of the anti diabetic agents. The study reveals that the methanolic extract of *Halodule uninervis* could be added in list of herbal preparation, beneficial in diabetes mellitus. *Halodule uninervis* can be considered as an important addition to the therapeutic treatment of diabetes. The present investigation has also opened avenue for further research especially with reference to the development of potent formulation for diabetes mellitus from *Halodule uninervis*.

CONCLUSION

The experimental findings of the present study concluded that methanolic extract of *Halodule uninervis* is capable of exhibiting significant anti-hyperglycemic activity in STZ-induced diabetic mice. The extracts also showed improvement in body weight; biochemical parameters such as GSH, GPx, SOD and lipid profile and so might be valuable in diabetes treatment.

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