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ANTI-DIABETIC ACTIVITY OF METHANOLIC EXTRACT OF *GRACILARIA CORTICATA* J.AG. (RED SEAWEED) IN HARE ISLAND, THOOTHUKUDI, TAMIL NADU, INDIA.

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ABSTRACT

The present research was performed to investigate the anti-diabetic activities of *Gracilaria corticata* J.Ag., an important red seaweed collected from Hare Island, Thoothukudi, Tamil Nadu, India. The methanol extract of *Gracilaria corticata* J.Ag. was administered via intraperitoneal injection at a dose of 200 and 400mg/kg mice on alloxan induced hyperglycemic albino mice. The fasting blood glucose level, body weight and the glucose level after the treatment of diabetic mice were assessed. The animals treated with 200mg/kg methanol extract were shown the best result of decrease in blood glucose level at a regular interval when the time increased up to 7h compared with the dose of 400mg/kg methanol extract treated animals. The result of the present study showed that the anti-diabetic activity of the methanol extract was dose dependent.

Key Words:- *Seaweed, Anti-diabetic, Gracilaria corticata, Methanol extract, Wistar rats*.

INTRODUCTION

Diabetes mellitus is a systemic metabolic disease characterized by hyperglycemia, hyperlipedemia, hyperaminoacidemia and hypoinsulinaemia. It leads to decrease in both insulin secretion and insulin action. It is frequently associated with the development of micro and macro vascular diseases namely neuropathy, nephropathy, cardiovascular and cerebrovascular diseases (Altan, 2003). At present, there is growing interest in herbal remedies due to the side effects associated with the oral hypoglycemic agents for the treatment of diabetes mellitus. Hence the traditional herbal medicines are mainly used which are obtained from plants, it plays important role in the management of diabetes mellitus (Patel and Srinivasan, 1997).

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In past few decades, herbal medicines have started to gain importance as a source of hypoglycemic agents. Marles and Farnsworth estimated that more than 1000 plant species are being used as folk medicine for diabetes (Marles and Farnsworth, 1995). Biological actions of the plant products used as alternative medicines to treat diabetes are related to the chemical composition.

Herbal products or plant products are rich in phenolic compounds, flavonoids, terpenoids, coumarins and other constituents which show reduction in blood glucose levels (He *et al.*, 2005; Jung *et al.*, 2006; Ji *et al.*, 2009). Several species of herbal drugs have been described in the scientific and popular literature as having anti-diabetic activity (Valiathan, 1998). Due to the perceived effectiveness, fewer side effects in clinical experience and relatively low costs, herbal drugs are prescribed (Verspohl, 2002). Medicinal and herbal plant products are traditionally used from long ago in many countries for the treatment of diabetes mellitus. In the

present study, anti-diabetic activity of *Gracilaria corticata* J.Ag., an important red seaweed collected from Hare Island, Thoothukudi, Tamil Nadu, India was analyzed.

MATERIALS AND METHODS

Collection of Plant Sample

Gracilaria corticata J.Ag. (Figure-1) is red seaweed belonging to Rhodophyceae member showed much attention in the present study for anti-diabetic activity. *Gracilaria corticata* J.Ag. was collected from Hare Island, Thoothukudi, Tamil Nadu, India. The collected plant samples were rinsed with marine water to remove debris and epiphytes. The entire epiphytes were removed using soft brush. The plants were brought to the laboratory. In the laboratory, the plants were once again washed in freshwater and stored in refrigerator for further analysis (John Peter Paul and Shri Devi, 2014).

Figure 1: Natural Habit of *Gracilaria corticata* J.Ag.

Preparation of methanol extract

For the preparation of methanol extract of *Gracilaria corticata* J.Ag., the collected plant specimens were washed thoroughly and placed on blotting paper and spread out at room temperature in the shade condition for drying. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use. 3g powdered sample was packed in Soxhlet apparatus and extracted with methanol for 8h separately. The excess amount of methanol was evaporated and fine methanol crude powder was prepared and stored in the refrigerator for the analgesic activity (John Peter Paul and Yuvaraj, 2013).

Experimental Animals

Wistar albino rats (160-200g) and Swiss albino mice of either sex were procured from Venkateswara Enterprises, Bangalore, Karnataka, India. The selected animals were acclimatized for 7 days under standard husbandry conditions, i.e. room temperature 35±1°C, relative humidity 45-55% and light/dark cycle 12/12h. Animals were provided with standard rodent pellet diet and had free excess to water. The composition of diet is 10% protein, 4% *Arachis* oil, 1% fibers, 1% calcium, 1000 IU/gm vitamin A and 500 IU/gm vitamin D. All the animals were acclimatized to the laboratory conditions prior to experimentation. All the experiments were conduct between 10.00 and 17.00h and were in accordance with the ethical guidelines of the International Association for Study of Pain (Zimmerman, 1983). All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute toxicity test

Acute oral toxicity study was performed as per OECD-423 guidelines (Ecobichon, 1997). Albino mice (n=6) of either sex selected through random sampling technique was used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extract (50% methanolic extract) was administered orally at the dose level of 5 mg/Kg body weight by gastric intubation and observed for 14 days. If mortality is observed in 2 out of 3 animals, then the dose administered would be assigned as toxic dose. If mortality is observed in 1 animal, then the same dose would be repeated again to confirm the toxic dose. If mortality is not observed, the procedure would be repeated for further higher doses such as 50, 300 and 2000 mg/Kg body weight. According to the results of acute toxicity test, the doses were chosen for experiments.

Induction of diabetes and experimental design

Prior to the beginning of the experiment all the animals were not allowed for food for 18 hours but water was allowed without stoppage. Wistar albino mice received alloxan (150mg/kg), freshly prepared in 0.1M cold citrate buffer (pH 4.5). Normal control rats received citrate buffer only. 48h after alloxan administration, blood samples were collected from retro orbital plexus and plasma glucose was determined. The induction of diabetes mellitus was confirmed by determination of plasma glucose level (≥250mg/dl). Diabetic rats were kept untreated for four weeks. At the end of 4^{th} week, plasma glucose of diabetic mice ≥250mg/dl was selected for anti-diabetic studies.

Study design

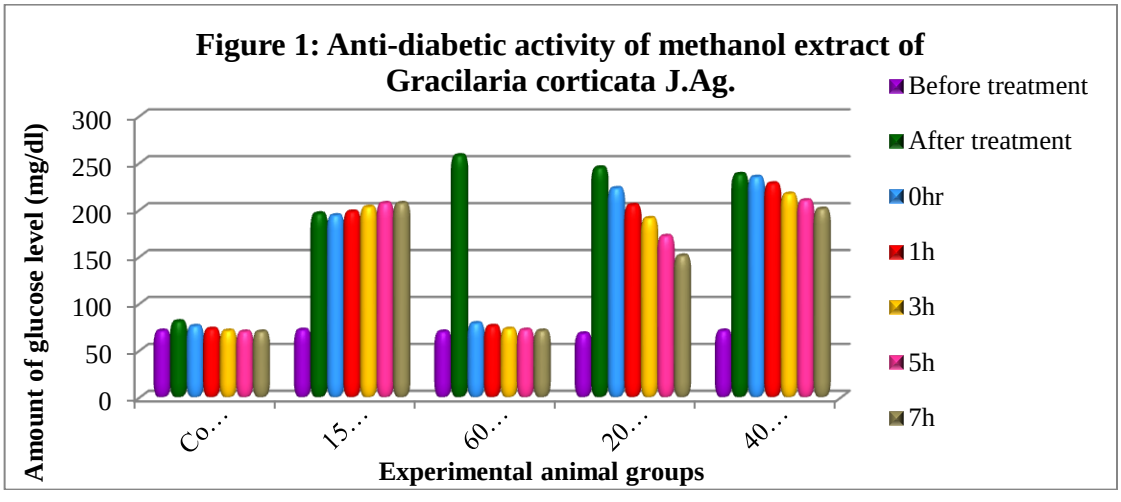
Wistar albino mice were randomly grouped into 5 groups (6 rats/group) and received the following treatment for 4 weeks. Group 1: Normal control which received normal saline (1ml/100g/day); Group II were alloxan induced diabetic rats, groups III and IV were alloxan induced diabetic rats administered with Glibenclamide (0.60mg/kg), methanol extracts 200mg/kg and 400mg/kg respectively. During the treatment, blood was collected from retro orbital plexus at every week interval and used for determination of blood glucose level. At the end of 4th week, before the sacrifice, blood was collected from retro orbital plexus for the measurement of glucose level.

RESULTS AND DISCUSSION

In the present study, alloxan induced anti-diabetic activity of methanol extract of *Gracilaria corticata* J.Ag. was studied using Wistar albino rats. The methanol extract at the dose level of 200 and 400mg/kg body weight were administered orally to the treated group and Glibenclamide at the dose level of 600µg/kg was administered orally to the standard group. The blood glucose levels were observed after 48h induction of alloxan. Table-1 and Figure-2 illustrated the effect of methanol extract of *Gracilaria corticata* J.Ag. on blood glucose levels of alloxan induced diabetes in rats. Administration of alloxan (150mg/kg) produced diabetes in the rats which was confirmed by the elevation of blood glucose levels. The diabetic animals were treated with methanolic extract of *Gracilaria corticata* J.Ag. and Glibenclamide by oral administration. After 48h, the mean blood glucose levels were measured during the test drug administration on 0h, 1h, 3h, 5h and 7h. It was observed that the glucose levels reached to moderate diabetes, thereafter distilled water had given to control group, Glibenclamide (600µg/kg) to the standard group and methanol extract (200 and 400mg/kg) to the test group. Blood glucose level was measured at 0h, 1h, 3h, 5h and 7h after administration of Glibenclamide to standard group which showed 79, 76, 73, 72 and 71mg/dl respectively.

Table 1. Alloxan induced anti-diabetic activity of methanol extract of *Gracilaria corticata* J.Ag.

Drug & Treatment	Blood Glucose level		Blood Glucose Level After Drug Administration (in h) mg/dl				
	Before treatment	After 48 h of treatment	0	1	3	5	7
Control 500mg/kg Tween 80	71±1.11	81.0±1.24	76±0.60	73±1.26	71±0.70	70±1.41	70±1.08
150mg/kg Alloxan	72±0.70	196±2.80	194±3.8	198±2.90	203±1.3	207±2.4	207±2.88
600µg Glibenclamide + 150mg/kg Alloxan	70±1.08	258±3.59	79±1.4	76±1.87	73±1.87	72±1.08	71±1.11
200mg methanol extract + 150mg/kg Alloxan	72±0.82	247±8.22	223±7.8	205±8.13	191±11.5	172±8.37	151±4.43
400mg methanol extract + Alloxan 150mg/kg	73±0.82	253±12.68	235±6.6	228±20	217±24.1	210±35.2	201±34.5



In the groups treated with 200mg/kg methanol extract of *Gracilaria corticata* J.Ag., there was a significant decrease in blood sugar levels to 223mg/dl in 0 hr, 205mg/dl in 1h, 191mg/dl in 3h, 172mg/dl in 5h and 151mg/dl in 7h. The diabetic animals treated with 400mg/kg methanol extract showed the decreased blood glucose level of 235mg/dl, 228mg/dl, 217mg/dl, 210mg/dl and 201mg/dl within 0h, 1h, 3h, 5h and 7h respectively. From the present study, it was found that 200mg/kg methanol extract of *Gracilaria corticata* J.Ag. showed the highest degree of anti-diabetic effect compared to 400mg/kg methanol extract.

Glucose is the important key physiological regulator of insulin secretion. Indeed, short-term exposure of β-cells to increasing glucose concentrations induces proliferation in a concentration dependent manner. In addition to its effect on β-cell turnover, hyperglycemia also impairs β-cell secretary function. This glucotoxic effect is evident before apoptosis leads to a significant decrease in β-cell mass (Stalmans and Hers, 1975). Alloxan induced diabetic rats exhibited severe glucose intolerance and metabolic stress as well as hyperglycemia due to a progressive oxidative insult interrelated with a decrease in endogenous insulin secretion and release (Donath and Halban, 2004). Treatment with anti-diabetic drugs based on their pancreatic anti-oxidant activity might be a protective strategy for protecting β-cell due to disproportionate generation of free radicals (Szkudelski, 2001; Johansen *et al.*, 2005).

The present investigation indicated that the single dose of alloxan (150mg/kg) intraperitoneally to adult male albino rats (160-200g) was suitable to induce the diabetics. A gradual loss of β-cells due to apoptosis significantly hinders insulin production and inhibits cell viability. During apoptosis, cells shrink, chromatin condenses, DNA is cleaved into pieces at inter nucleosomal regions. A proactive way to increase β-cell viability is to decrease apoptosis level in order to retain the cell population and increase insulin production (Lebedev *et al.*, 2007). Hence the methanol extract of *Gracilaria corticata* J.Ag. recovered the effect of alloxan induced anti-diabetics by providing the various secondary metabolites. Though the 200 and 400mg/kg of the methanol extracts showed the anti-diabetic effects, 200mg/kg methanol extract showed the highest activity compared to 400mg/kg.

CONCLUSION

The methanolic extracts of *Gracilaria corticata* J.Ag., an important red seaweed (Rhodophyceae) showed significant anti-diabetic activity which is evident by the data obtained. Among the two concentration of methanol extracts tested, 200mg/kg methanol extract had the highest effect than 400mg/kg. However further studies required to elucidate the exact mechanism of action and the structure of the secondary metabolites which is responsible for anti-diabetic activity for the development as potent anti-diabetic drug. These herbal drugs will help to develop new drug molecule for anti-diabetic therapy.

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CONFLICT OF INTEREST:
The authors declare that they have no conflict of interest.

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