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

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**ABSTRACT**

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

**Keywords**: Seaweed, Anti-diabetic, *Gracilaria dura*, Methanolic extract, Wistar albino rats.

## INTRODUCTION

Diabetes mellitus is caused by impaired production of insulin and or by decreased tissue response to the insulin [1]. Diabetes mellitus is a most common endocrine disorder affecting more than 336 million people worldwide. It was expected to increase the number to 552 million people in 2030 [2]. For this, therapies developed along the principles of western medicine are often limited in efficacy, carry the risk of adverse effects and are too costly, especially for the developing world. Therefore, treating diabetes mellitus with plant derived compounds which are accessible and do not require laborious pharmaceutical synthesis seems highly attractive. In this background, an attempt has been made to analyze the anti-diabetic activity of *Gracilaria dura* (Ag.) J.Ag., an important red seaweed collected from Hare Island, Thoothukudi, Tamil Nadu, India may be useful to the health professionals, scientists and scholars working the field of pharmacology and therapeutics to develop evidence based alternative medicine to cure different kinds of diabetes in man and animals. Isolation and identification of active constituents from the plant, can play a significant role in improving the hypoglycemic action.

## MATERIALS AND METHODS

### Collection of Plant Sample

*Gracilaria dura* (Ag.)J.Ag. (Figure 1) is red seaweed belonging to Rhodophyceae member showed much attention in the present study for anti-diabetic activity. *Gracilaria dura* (Ag.)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 [3].

### Preparation of methanol extract

For the preparation of methanol extract of *Gracilaria dura* (Ag.)J.Ag., the collected plant specimens

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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. 30g 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 [4].

**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 [5]. 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 perOEC D-423 guidelines [6]. 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. 48 hrs 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 4$^{th}$ week, before the sacrifice, blood was collected from retro orbital plexus for the measurement of glucose level.

**RESULTS AND DISCUSSION**

The increasing incidence of diabetes represents an enormous socio-economic burden in the developing countries. The World Health Organization (WHO) estimates that over 300 million people worldwide will have Diabetes mellitus (DM) by the year 2025 with alarming proportions from developing countries [7]. In this regard, the present investigation was undertaken to screen the alloxan induced anti-diabetic activity of methanolic extract of *Gracilaria dura* (Ag.) J.Ag. using Wistar albino rats. The methanolic extract at the dose level of 200 and 400mg/kg body weight were injected to the treated group and Glibenclamideat the dose level of 600µg/kg was administered to the standard group. The blood glucose levels were observed after 48h induction of alloxan.

Table-1 and Figure-2 showed the effect of methanolic extract of *Gracilaria dura* (Ag.) J.Ag. on blood sugar 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 sugar levels. The diabetic animals were treated with methanolic extract of *Gracilaria dura* (Ag.) J.Ag. and Glibenclamide by oral administration. After 48 hr, the mean blood sugar 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 methanolic 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.

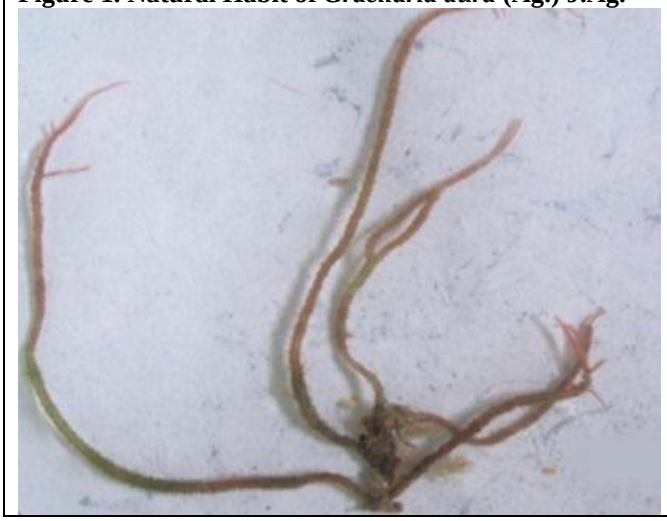
In the groups treated with 200mg/kg methanolic extract of *Gracilaria dura* (Ag.) J.Ag., there was a

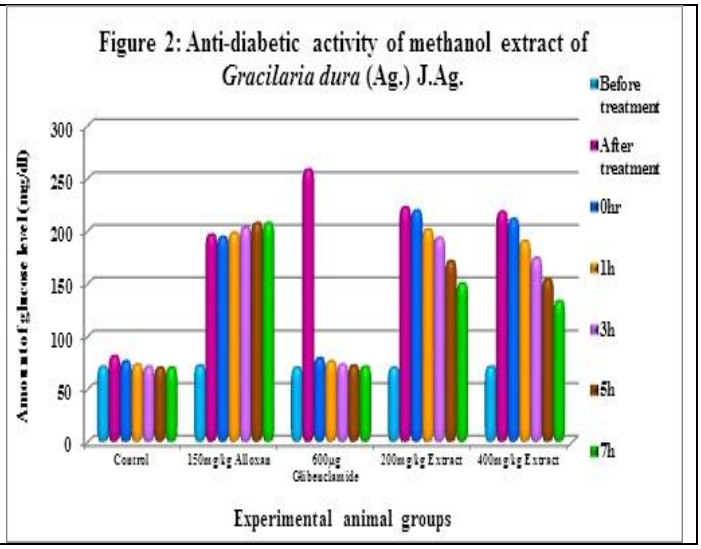
significant decrease in blood sugar levels to 211mg/dl in 0 hr, 190mg/dl in 1h, 174mg/dl in 3h, 154mg/dl in 5h and 133mg/dl in 7h. The diabetic animals treated with 400mg/kg methanolic extract showed the decreased blood glucose level of 219mg/dl, 201mg/dl, 193mg/dl, 171mg/dl and 150mg/dl within 0h, 1h, 3h, 5h and 7h respectively. From the present investigation, it was noted that 200mg/kg methanolic extract of *Gracilaria dura* (Ag.) J.Ag. showed the highest degree of anti-diabetic effect as compared to 400mg/kg methanolic extract.

**Table 1. Alloxan induced anti-diabetic activity of methanolic extract of *Gracilaria dura* (Ag.) 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 | 71±1.25 | 218±4.42 | 211±3.9 | 190±6.26 | 174±9.34 | 154±2.86 | 133±5.76 |
| 400mg methanol extract + Alloxan 150mg/kg | 70±0.72 | 222±4.67 | 219±4.53 | 201±3.65 | 193±5.73 | 171±9.03 | 150±3.76 |

**Figure 1. Natural Habit of *Gracilaria dura* (Ag.) J.Ag.**






## CONCLUSION

The methanolic extracts of *Gracilaria dura* (Ag.)J.Ag., an important red seaweed (Rhodophyceae) showed significant anti-diabetic efficacy which is evident by the results obtained. Among the two concentrations of methanolic extracts investigated, 200mg/kg methanolic 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 for the development of new drug molecule for anti-diabetic therapy.

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