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HEPATOPROTECTIVE ACTIVITY OF METHANOLIC EXTRACT OF GRACILARIA CORTICATA J.AG. IN HARE ISLAND, THOOTHUKUDI, TAMIL NADU, INDIA

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ABSTRACT

In the present study, screening of hepatoprotective activity of *Gracilaria corticata* J.Ag. collected from Hare Island, Thoothukudi in the south east coast of Tamil Nadu, India was analyzed. The methanolic extract of 200mg/kg and 400mg/kg of *Gracilaria corticata* J.Ag. showed significant hepatoprotective activity in both doses as evidenced by the data obtained. Among the two concentrations of methanolic extract studied, 200mg/kg methanolic extract was found to show more active as compared to 400mg/kg methanolic extract. The results showed for the first time that *Gracilaria corticata* J.Ag. showed hepatoprotective activity, a property that could lead to the application in useful health care.

KEYWORDS: Seaweed, Hepatoprotective, *Gracilaria corticata*, Methanolic extract, Hare Island.

INTRODUCTION

The liver is a huge, complex organ that is well intended for its central role in carbohydrate, protein and fat metabolism. It is the site where waste products of metabolism, such as ammonia are detoxified. In combination with the spleen, it is involved in the destruction of the remnants of the red blood cells and with the recycling of their constituents. It is

responsible for synthesizing and secreting bile and for synthesizing lipoproteins and plasma proteins, including clotting factors. It maintains a stable blood glucose level by taking up and storing glucose as glycogen during glycogenesis, breaking it down to glucose when needed through glycogenolysis and forming glucose from noncarbohydrate sources such as amino acids via gluconeogenesis.[1]

The liver also plays an significant role in drug elimination and detoxification and liver damage may be caused by many xenobiotics, such as alcohol and many medicines, malnutrition, infection and anaemia.[2,3] Liver damage is a prevalent disease which in most cases, involves oxidative stress and is characterized by a progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis and hepatocellular carcinoma.[4] The common scheme can be used to assess the hepatoprotective capability of a natural extract or isolated molecule. Hepatotoxicity is defined as an injury to the liver that is associated with an impaired liver function caused by exposure to a drug or another non-infectious agent.[5]

Hepatotoxic agents can react with the basic cellular components and consequently induce almost all types of liver lesions. Injury to the liver, whether acute or chronic, eventually results in an increase in serum concentrations of Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT) and Alkaline Phosphatase (ALP). The SGPT, SGOT and ALP are enzymes that catalyze the transfer of α-amino groups from aspartate and alanine to the α-keto group of ketoglutaric acid to generate oxaloacetic and pyruvic acids, respectively, which are important components of the citric acid cycle. In this background, an attempt was taken to study the hepatoprotective activity of the methanolic extract of the selected red seaweed *Gracilaria corticata* J.Ag. (Rhodophyceae) collected from Hare Island, Thoothukudi in the south east coast of Tamil Nadu, India.

MATERIALS AND METHODS

Collection of Plant Sample

Gracilaria corticata J.Ag. is red seaweed belonging to Rhodophyceae member showed much attention in the present study for hepatoprotective activity. *Gracilaria corticata* J.Ag. was collected from Hare Island, Thoothukudi in the south east coast of Tamil Nadu, India. The collected plant materials 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 with freshwater and stored in refrigerator for further analysis.[6]

Preparation of methanolic extract

For the preparation of methanolic 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. 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 hepatoprotective activity.[7]

Experimental Animals

Wistar albino rats (160-200g) 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 access 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.[8] 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.[9] Wister albino rats (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.

HEPATOPROTECTIVE ACTIVITY

Induction of paracetamol hepatotoxicity and experimental design

Thirty Wistar albino rat both male and female randomly selected and equally distributed into five groups. All the other groups of animals except control were treated with Paracetamol (2g/kg) for seven days. After seven days, it was confirmed that all the hepatic cells of animals were damaged. Group I served as normal control. Group II animals received Paracetamol (2g/kg) powder dissolved in distilled water given orally. Group III received Silymarin 100mg/kg orally as the standard reference for seven days. Group IV and V were given oral methanolic extract of the selected red seaweed at 200mg/kg and 400mg/kg respectively for seven days. Paracetamol was administered to the animals of group III, group IV and group V in a single dose of Paracetamol (2g/kg) suspension orally on the seventh day. All the experimental animals were euthanized on the eighth day under light ether anesthesia by cervical dislocation.

Biochemical assays

Blood samples were collected by intracardiac puncture in glass tubes and were used for the analysis of liver enzymes. Serum Glutamic Pyruvic Transaminase (SGPT) and Serum Glutamic Oxaloacetic Transaminase (SGOT) were estimated by Reitman and Frankel[10] method, Alkaline Phosphatase (ALP) activity by Kind and King[11] method and bilirubin was estimated by Malloy and Evelyn[12] method using the standard laboratory procedures.

RESULTS AND DISCUSSION

Screening of hepatoprotective activity of methanolic crude extract of *Gracilaria corticata* J.Ag. was studied by determining the effect on Wister albino rats. The methanolic extract of *Gracilaria corticata* J.Ag. showed the clear hepatoprotective activities which was dose dependent on albino rats. Acute toxicity studies showed that the methanolic extracts did not cause any mortality up to 2000 mg/Kg and were considered as safe.

In the hepatoprotective activity assay, there was a significant increase in Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT,) Alkaline Phosphatase (ALP) and bilirubin levels in toxicated group of Wistar rats than the normal control group and the methanolic extract of *Gracilaria corticata* J.Ag. showed reduction in the elevated levels. The control group showed 65.20 IU/L, 82.53 IU/L, 14.93 IU/L and 1.15mg/dL level of SGPT, SGOT, ALP and bilirubin respectively, whereas the Paracetamol (2g/kg) induced group of animals was found to have 125.14 IU/L, 136.93 IU/L,

86.97 IU/L and 2.66mg/dL level of SGPT, SGOT, ALP and bilirubin respectively. The standard drug Silymarin (100mg/kg) showed the reduction level of SGPT (72.15 IU/L), SGOT (80.74 IU/L), ALP (21.69 IU/L) and bilirubin (1.34mg/dL).

Table 1: Hepatoprotective activity of methanolic extract of *Gracilaria corticata* J.Ag.

Animal Group	SGPT (IU/L)	SGOT (IU/L)	Alkaline phosphatase (IU/L)	Bilirubin (mg/dL)
Control	65.20±1.61	82.53±1.95	14.93±0.89	1.15±0.09
Paracetamol (2g/kg)	125.14±2.92	136.93±3.55	86.97±1.46	2.66±0.15
Silymarin (100mg/kg)	72.15±1.55	80.74±2.59	21.69±0.68	1.34±0.05
200mg/kg Methanol extract	85.23±2.23	88.76±2.64	43.38±1.82	1.59±0.11
400mg/kg Methanol extract	97.54±1.38	93.12±2.51	56.37±1.42	1.75±0.16

The effect of methanolic extract of *Gracilaria corticata* J.Ag. on serum enzyme parameters such as SGPT, SGOT, ALP and bilirubin was shown in Table 1. The Paracetamol (2g/kg) treated Wistar rats were significant increased in serum enzymes SGPT, SGOT, ALP and bilirubin levels when compared to control group of the rats. Increases in serum SGPT, SGOT, ALP and bilirubin level by Paracetamol have been attributed to hepatic structural damage because these enzymes are normally localized to the cytoplasm and released into the circulation after cellular damage has occurred. The 200mg/kg methanolic extract of *Gracilaria corticata* J.Ag. treated rats were shown significant reduction the level of SGPT (85.23 IU/L), SGOT (88.76 IU/L), ALP (43.38) and bilirubin (1.59mg/dL) and The 400mg/kg methanolic extract of *Gracilaria corticata* J.Ag. treated rats were shown significant reduction in the level of SGPT (97.54 IU/L), SGOT (93.12 IU/L), ALP (56.37 IU/L) and bilirubin (1.75mg/dL) when compared to Paracetamol treated rats. Reduction in the levels of SGPT, SGOT, ALP and bilirubin towards the respective normal value was an signal of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by Paracetamol. Both the concentrations of the methanolic extract of *Gracilaria corticata* J.Ag. was found to possess hepatoprotective effect. Compared to the 400mg/kg methanolic extract of *Gracilaria corticata* J.Ag., 200mg/kg methanolic extract showed more hepatoprotective effect.

CONCLUSION

The methanolic extract of the selected red seaweed *Gracilaria corticata* J.Ag. showed a significant hepatoprotective activity on Wister albino rats. Among the two concentration (200

and 400mg/kg) tested, 200mg/kg methanolic extract was identified to be more effective as compared with 400mg/kg body weight of the albino Wister rats. From the present study, it was concluded that the methanolic extract of *Gracilaria corticata* J.Ag. could be a potential source as hepatoprotective agent.

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