Research Article **ISSN: 2349 – 7106**

Asian Journal of Research in Chemistry and Pharmaceutical Sciences

Journal home page: www.ajrcps.com

HEPATOPROTECTIVE POTENTIAL OF METHANOLIC EXTRACT OF *LOBOPHORA VARIEGATA* (LAMOUR.) WOMERSLEY EX OLIVIERA

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ABSTRACT

In the present study, screening of hepatoprotective potential of *Lobophora variegata* (Lamour.) Womersley ex Oliviera 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 *Lobophora variegata* (Lamour.) Womersley ex Oliviera 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 *Lobophora variegata* (Lamour.) Womersley ex Oliviera for hepatoprotective activity, a property that could lead to the application in useful health care.

KEYWORDS

Seaweed, Hepatoprotective, *Lobophora variegata*, Methanolic extract and Hare Island.

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INTRODUCTON

Liver diseases continue to be serious health problems and the management of liver disease is still a challenge to the modern medicine. Liver plays a vital role in regulation of physiological processes, involved in several fundamental functions such as storage, secretion and metabolism. It also detoxifies a variety of drugs and plays an important role in transforming, clearing the chemicals and is susceptible to the toxicity from these agents[1]. Drug-induced liver injury is a

potential complication of virtually every prescribed medication. Paracetamol (N-acetyl-p-aminophenol; APAP), a highly popular analgesic and antipyretic drug, is quickly absorbed from the gastric intestinal tract and reaches peak serum levels within a few hours. It is safe at therapeutic doses; however the overdose following accidental ingestion or suicidal attempt causes a toxic response leading to the necrosis in liver.

The use of plants for their therapeutic value is a part of the human history. Plant derived natural products have received considerable attention in recent years due to their diverse pharmacological properties[2]. Herbal drugs containing biochemical constituents are gaining importance in prevention and treatment of liver inked diseases[3]. However, there are currently few reports concerning their hepatoprotective activity on the scientific evidence. The scientific evaluation of these plants may provide modern medicine with effective pharmaceuticals for the treatment of liver diseases[4,5]. Therefore the current study was aimed to evaluate *in vitro* hepatoprotective potential in experimental rat model of paracetamol-induced liver toxicity.

MATERIAL AND METHODS

Collection of Plant Sample

Lobophora variegata (Lamour.) Womersley ex Oliviera is brown seaweed belonging to Phaeophyceae member showed much attention in the present study for hepatoprotective activity. *Lobophora variegata* (Lamour.) Womersley ex Oliviera 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 methanol extract

For the preparation of methanolic extract of *Lobophora variegata* (Lamour.) Womersley ex Oliviera, 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) and Swiss albino rats 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[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]. 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 rats 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 methanol extract of the selected brown 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. Immediately after the scarification, the liver was excised from the animals, washed in ice-cold saline. Thin cross sections were taken from a portion of the liver immediately and observed under the microscope.

RESULTS AND DISCUSSION

Screening of hepatoprotective potential of methanolic crude extract of *Lobophora variegata* (Lamour.) Womersley ex Oliviera was analyzed by determining the effect on albino rats. The methanolic extract of *Lobophora variegata* (Lamour.) Womersley ex Oliviera showed the highest 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 *Lobophora variegata* (Lamour.) Womersley ex Oliviera 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).

The effect of methanol extract of *Lobophora variegata* (Lamour.) Womersley ex Oliviera 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 *Lobophora variegata* (Lamour.) Womersley ex Oliviera treated rats were

shown significant reduction the level of SGPT (104.42 IU/L), SGOT (118.52 IU/L), ALP (61.79) and bilirubin (1.78mg/dL) and The 400mg/kg methanolic extract of *Lobophora variegata* (Lamour.) Womersley ex Oliviera treated rats were shown significant reduction in the level of SGPT (118.70 IU/L), SGOT (124.82 IU/L), ALP (66.47) and bilirubin (1.98mg/dL) when compared to Paracetamol treated rats. Reduction in the levels of SGPT, SGOT, ALP and bilirubin towards the respective normal value was an indication of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by Paracetamol. Both the concentrations of the methanol extract of *Lobophora variegata* (Lamour.) Womersley ex Oliviera was found to have hepatoprotective effect. Compared to the 400mg/kg methanol extract of *Lobophora variegata* (Lamour.) Womersley ex Oliviera, 200mg/kg methanol extract showed more hepatoprotective effect.

Histopathological findings in liver sections (100X) from the five experimental groups were shown in Figure No.1. Control group showed no significant lesions, liver sections treated with Paracetamol produced marked changes with white patchy acromolecular damages. Standard silymarin, 200mg/kg and 400mg/kg methanol extract of *Lobophora variegata* (Lamour.) Womersley ex Oliviera treated albino rats showed considerable reduction in the pathological changes when compared with Paracetamol induced animals.

Table No.1: Hepatoprotective potential of methanolic extract

S.No	Animal Group	SGPT (IU/L)	SGOT (IU/L)	Alkaline phosphatase (IU/L)	Bilirubin (mg/dL)
1	Control	65.20±1.61	82.53±1.95	14.93±0.89	1.15±0.09
2	Paracetamol (2g/kg)	125.14±2.92	136.93±3.55	86.97±1.46	2.66±0.15
3	Silymarin (100mg/kg)	72.15±1.55	80.74±2.59	21.69±0.68	1.34±0.05
4	200mg/kg Methanol extract	104.42±2.28	118.52±3.29	61.79±2.80	1.78±0.03
5	400mg/kg Methanol extract	118.70±2.19	124.82±2.18	66.47±4.15	1.98±0.03

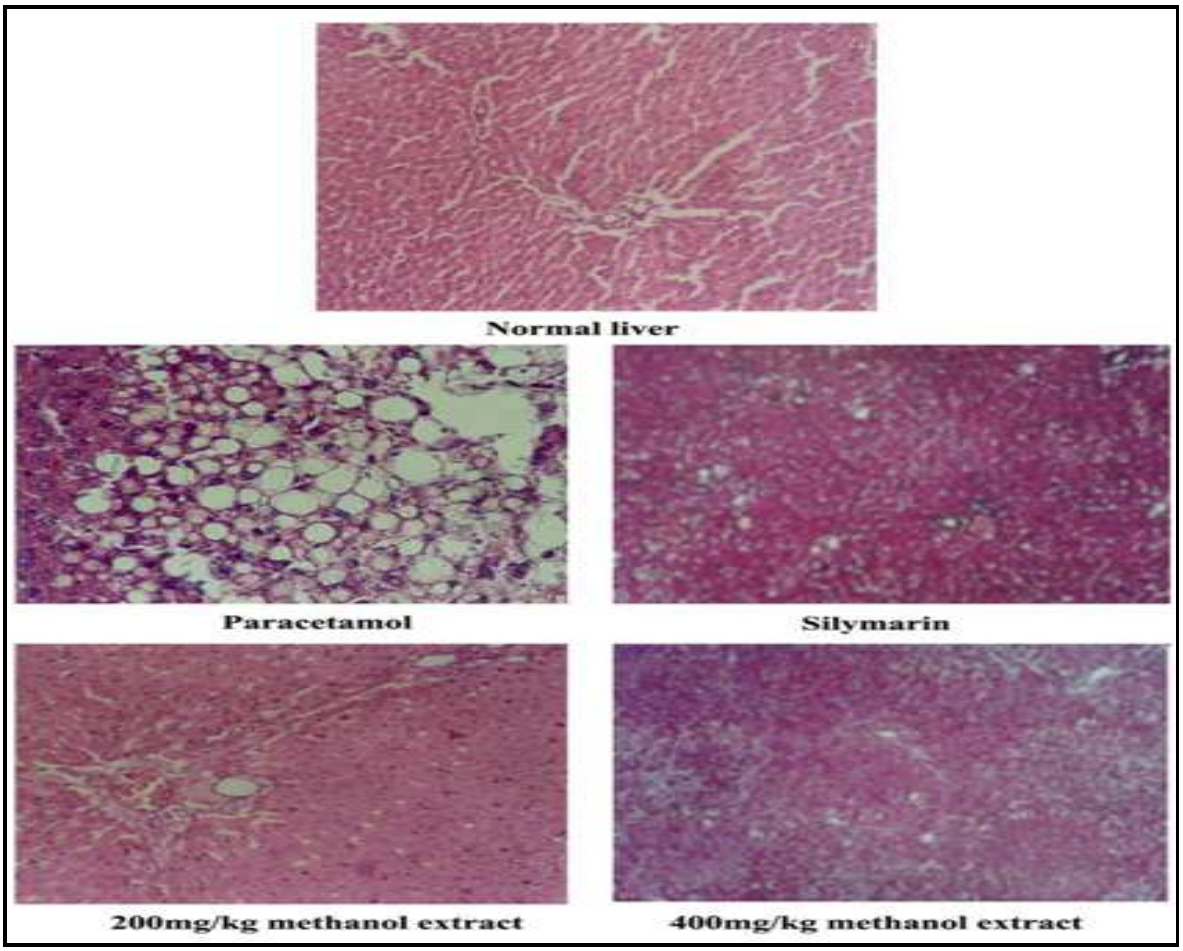

Figure No.1: Hepatoprotective activity of Methanol extract of *Lobophora variegata* t of *Lobophora variegata* (Lamour.) Womersley ex Oliviera

CONCLUSION

Lobophora variegata (Lamour.) Womersley ex Oliviera can be considering as a potential source of natural chemical compounds with hepatoprotective activity. From the present investigation, it can be concluded that 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. Further detailed investigations are required in order to identify and isolate the hepatoprotective components in the methanolic extract to justify its use in herbal formulations prescribed in the treatment of liver disorders. Moreover, the education of the public and medical profession is also desired to increase awareness of the potential toxic effects of paracetamol overdose.

ACKNOWLEDGEMENT

The authors are sincerely thanks to the Department of Botany and Director, Centre for Advanced Research in Plant Sciences (CARPS), St. Xavier's College (Autonomous), Palayamkottai – 627 002, Tamil Nadu, India for providing the facilities to complete this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: John Peter Paul J *et al.* Hepatoprotective potential of methanolic extract of *Lobophora variegata* (Lamour.) Womersley ex oliviera, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences,* 6(4), 2018, 188-193.