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AMELIORATIVE EFFECT OF RED ALGAE *ACANTHOPHORA SPICIFERA* AGAINST PUFFER FISH *AROTHRON HISPIDUS* INDUCED BIOCHEMICAL AND OXIDATIVE STRESS IN MICE.

K. MOHANA PRIYA AND SAMANTA S. KHORA*

Medical Biotechnology Division, School of Bio Sciences and Technology, VIT University, Vellore - 632014, Tamilnadu, India.

ABSTRACT

Acanthophora spicifera, red algae are known to possess antioxidant activities. The objective of this study is to evaluate the ameliorative effect of the ethyl acetate extract of *A. spicifera* against the puffer fish *Arothron hispidus* induced biochemical alterations and oxidative stress. 24 Mice were divided into four groups were used in this study. Group 1 received normal saline as control, Group 2 – 4 were administered with skin extract of puffer fish *A. hispidus*. Group 3, 4 were pretreated with the ethyl acetate extract of *A. spicifera*. Decreased level of AST, ALT, ALP, and Bilrubin were observed in the puffer fish extract administered group and it was significantly increased in drinking *A. spicifera* extract groups. A raise of TBARS and the decrease of antioxidant enzymes were observed in the skin extract treated group. TBARS and antioxidant enzymes SOD, Catalase levels were significantly restored in the group drinking the *A. spicifera* extract. Similarly it decreased the level of Cholestrol, Glucose, Protein, Creatinine, Urea The present study reveals the exposure of ethyl acetate extract of *A. spicifera* considerably reduced the effect of puffer fish *A. hispidus* induced toxicity in mice.

KEYWORDS: ***Acanthophora spicifera, Arothron hispidus,*** **Oxidative stress, Biochemical alterations.**

SAMANTA S. KHORA
Medical Biotechnology Division, School of Bio Sciences and Technology, VIT University, Vellore - 632014, Tamilnadu, India.

Corresponding author

INTRODUCTION

Puffer fish poisoning is caused by the consumption of the fish usually contaminated with the tetrodotoxin (TTX) [1]. Consumption of these fish usually causes food poisoning including fatal cases [2]. Poisoning by the puffer fish consumption is a major problem, because it leads to serious effects due to the presence of the tetrodotoxin (TTX) and numerous cases of intoxication were reported, particularly in Asia [3]. TTX accumulates in major parts of the fish liver, gonad, intestine, skin and muscle [4]. The consumption of the puffer fish *Arothron hispidus* causes biochemical and pathological disorders in rats [5]. Dietary factors plays a vital role in the antioxidant defense mechanisms against the reactive oxygen species [6]. Antioxidant compounds play an important role in various fields such as medical (to treat cancers, cardiovascular disorders, and chronic inflammations), cosmetics (anti- ageing process), food industries (food preservatives) and others. *Acanthophora spicifera*, a red algae which belongs to the family of Rhodophyceae widely distributed in tropical and subtropical areas [7]. Several studies have revealed the bioactivity of active compounds isolated from *Acanthophora spicifera* such as antibacterial [8], antioxidant [9], anti-viral [10], anti-inflammatory [11] and anti-fouling activities. *A. spicifera* extract was reported to be a potent free radical scavenger, hydroxyl and superoxide anion radicals [12]. Based on these results, this study was carried out to determine the ameliorative effects of *A. spicifera* extract against biochemical and oxidative stress damage induced in mice by intraperitoneal injection of *A. hispidus* extract (AH) (1ml/100g) for 10 consecutive days. Two groups of mice were pretreated with *A. spicifera* extract as a beverage to check the protective effect. In order to determine the effect, body weight was checked, liver function was determined, serum biochemical hepatic markers were assayed. Oxidative stress was estimated by the Lipid peroxidation (TBARS assay) and the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) in liver tissue were assayed.

MATERIALS AND METHODS

SAMPLE COLLECTION

White-spotted puffer fish *Arothron hispidus (*Linnaeus, 1758) and fresh Spiny seaweed *Acantophora spicifera* (Vahl) Borgesen, 1910; were collected from the Gulf of Mannar at Rameshwaram lying along the longitude from $78^0$08 'N to $79^0$30'E and along the latitude $8^0$35 to $9^0$25 during the low tide in the month of September 2012. The samples were washed thoroughly with sea water to remove the epiphytes and other dust particles present in it. Then, it was transported to the laboratory in the dry ice and washed with tap water followed by distilled water thrice. The puffer fish *A. hispidus* was stored in the deep freezer at -30^0C. The seaweed was shade dried in a blotting paper and powdered by using a Mechanical grinder. It was stored in the refrigerator for further use at 4^0C.

PREPARATION OF THE SEAWEED EXTRACT

10gms of powdered sample was soaked in a 100ml of organic solvent ethyl acetate. It was kept in a shaker at 120rpm for 48hrs.The extracts were concentrated under reduced pressure using a rotary evaporator and air dried in a laminar air flow to obtain a dried extracts. The dried extracts were then stored in the refrigerator for further use [13].

PRELIMINARY PHYTOCHEMICAL SCREENING

Phytochemicals present in the seaweed extract was analyzed by following the standard method of Harbone, 1979 [14].

TOTAL PHENOLIC CONTENT

Phenolic compounds present in the *A. spicifera* extract was estimated by the method described by Pal et al., 2013 [15.] 1 ml of extract was mixed with 1ml of Folin – ciocalteau reagent. After few mts, 1 ml of sodium carbonate solution was added to it and made upto 10ml with distilled water. Then, it was incubated at dark for 90min, after that absorbance was taken at 725nm. Gallic

acid was used as a standard. The results were expressed as mg/g Gallic acid equivalents.

DPPH RADICAL SCAVENGING ACTIVITY

The free radical scavenging activity of seaweed extract was measured by the DPPH method described by Blios, 1958[16, 17]. 0.5ml solution of DPPH (0.1mM in Methanol) was added to the extract (250-1000µg/ml) respectively. Ascorbic acid was used as a standard. The mixture was shaken gently and incubated at room temperature for 30min. 2ml of double distilled water was added and the absorbance was measured at 517nm.

AMELIORATIVE EFFECTS OF A. SPICIFERA

Experiment protocol

Swiss albino mice weighing 20-22g were used for the ameliorative studies. These animals were maintained at animal house, VIT University, Vellore – 632014, Tamilnadu, India under the standard laboratory conditions. Mice were fed with a commercial balanced food diet and drinking water. After acclimatizing to the laboratory conditions, 24 mice were divided into 2 groups, each group consists of 12 mice: one group served as a control drinking water and the experimental group drinking ethyl acetate extract of *A. spicifera* used as a beverage. Then each group was divided into two groups and group 1 received 1ml / 100g of saline solution. Group 2-4 received 1ml/100 g of skin extract of *A. hispidus.* At the 5^{th} and 10^{th} day mice were anesthetized by diethyl ether the blood was collected by cardiac puncture for biochemical serum tests. The liver tissue was dissected out and washed with phosphate buffer saline and were stored at -80 ^{0} C for further studies [19].

Serum parameters

The collected blood was centrifuged at 6000 rpm for 10min to obtain the serum. Biochemical parameters like Alanine transferase (ALT), Alkaline transferase (ALP), Alanine serum transferase (AST), Triglycerides (TG), Bilirublin (Bb), Glucose, Cholesterol, Protein, Creatinine, Urea were determined using the standard commercial kits (Saoudi *et al*., 2008) by following the standard procedure in it [20].

Oxidative stress analysis

The liver tissues was rinsed with phosphate buffer saline and homogenized in ice cold phosphate buffer saline, centrifuged at 12,000 rpm for 15min. Then, the supernatant was collected and were used for further studies. The total protein content present in the tissues was determined by the Lowry's method [21]. Lipid peroxidation (LPO) was determined by measuring the malonialdehyde content by TBARS assay [22], Superoxide dismutase (SOD) activity [23], Catalase (CAT) activity [24] were assayed.

STATISTICAL ANALYSIS

Experiments were performed in triplicates and the data were expressed as mean ± Standard deviation. Statistical analysis was performed by using the Graph pad software. Statistical significant values ($P < 0.001$) were used to compare the treated and control groups.

RESULT

Preliminary phytochemical screening of ethyl acetate extracts of Acantophora spicifera

The ethyl acetate extract of *A. spicifera* was examined for the phytochemical constituents reveals the presence of alkaloids, phenolics, flavonoids, sterols, tannins, terpenoids, saponins and sugars.

Table 1
Phytochemical constituents of A. spicifera.

Content	*A. spicifera* extract
Alkaloid	+
Phenolics	+
Flavonoids	+
Sterols	+
Tannins	+
Terpenoids	+
Saponins	+
Sugar	+

Total Phenolic content

The total phenolic content of the extract of *A. spicifera* using the Folin-ciocalteau reagent spectrophotometically. The results were found to be 55.1 ± 0.39 mg/g expressed as Gallic acid equivalent.

DPPH free radical scavenging activity

The scavenging activity of *A. spicifera* was measured by DPPH free radical scavenging method. The scavenging activity increases with the increasing of the sample. The higher scavenging activity exhibited by the extract was found to be (55.03 ± 0.5) % at the concentration of 1000µg/ml as compared with the standard Ascorbic acid as presented in the figure 1.

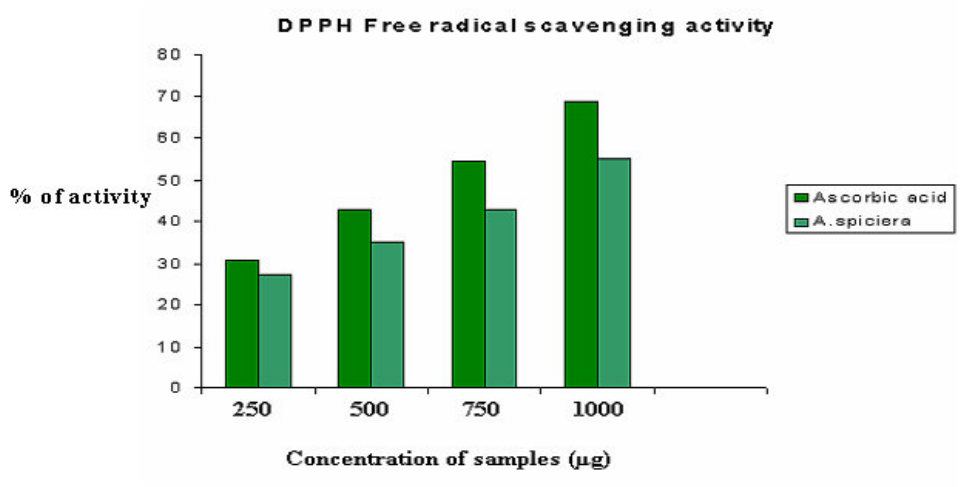


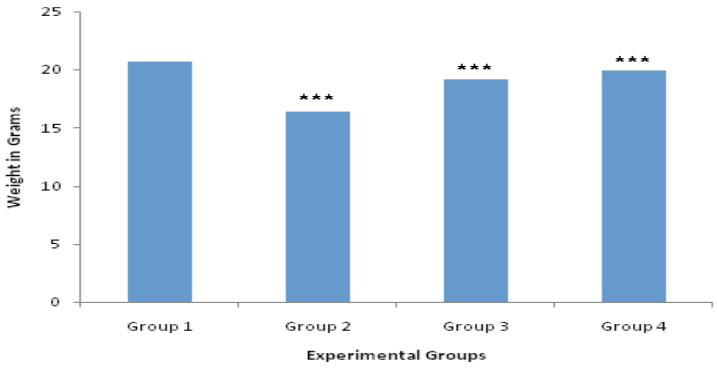
Figure 1
DPPH scavenging activity of A. spicifera extract compared with STD Ascorbic acid.

AMELIORATIVE EFFECT OF A. SPICIFERA EXTRACT

***Effect of body weight**:*

During the experiment a significant decrease in body weight was observed in the group treated with the skin extract of *A. hispidus* as compared to the control group. There was no significant reduction in body weight was observed in the group drinking the *A. spicifera* extract as shown in the figure 2.

Figure 2
Body weight of control and experimental groups



Values were expressed as ± SEM for 6 mice in each group.
Significant at *** P < 0.001 compared to control group.

Protective effect of ethyl acetate extract of A. spicifera on the hepatic marker enzymes.
Serum ALT, AST, ALP activities used as an indicator of hepatic damage. A significant decrease of the enzymes was observed in the skin extract treated group as compared with control. These changes were not obsered in the mice group drinking the *A. spicifera* extract as shown in the table 2. Serum bilrubin, urea, creatinine concentration significantly increased in the group treated with the skin extract compared to the control, indicates hepatic cell damage. *A. spicifera* extract prevented the increase of bilrubin, urea, creatinine level in the Group 3 & 4 as shown in the tables 3 & 4.

Table 2
Effect on Hepatic Marker enzymes

Groups	ALP (IU/ml)	AST (IU/ml)	ALT (IU/ml)	Bilirubin (mg/dl)
Group 1	461.58 ± 3.6	290.66 ± 0.43	85.53 ± 0.86	0.36 ± 0.12
Group 2	265.78 ± 5.4 ***	034.30 ±0.43***	32.9 ± 0.34***	1.93 ± 0.52***
Group 3	328.14 ± 2.7 ***	157.70 ±0.29***	54.78 ± 0.48***	0.62 ± 0.15***
Group 4	423.88 ± 3.4***	255.29 ± 1.31***	76.29 ± 0.63***	0.49 ± 0.18***

Values were expressed as ± SEM for 6 mice in each group.
Significant at *** P < 0.001 compared to control group.

Table 3
Effect on Protein, Bilirubin, Creatinine, Urea Parameters

Groups	Protein (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
Group 1	5.75 ± 0.31	35.14 ± 0.19	139.34 ± 1.2***
Group 2	2.38 ± 0.06***	105.91 ± 0.15***	38.68 ± 1.2***
Group 3	3.95 ± 0.11***	71.92 ± 0.10***	95.48 ± 1.3***
Group 4	5.18 ± 0.07	48.09 ± 0.12**	122.60 ± 1.0***

Values were expressed as ± SEM for 6 mice in each group.
Significant at *** P < 0.001 ** P<0.01 compared to control group

Table 4
Effect on Glucose, Creatinine, Urea Parameters

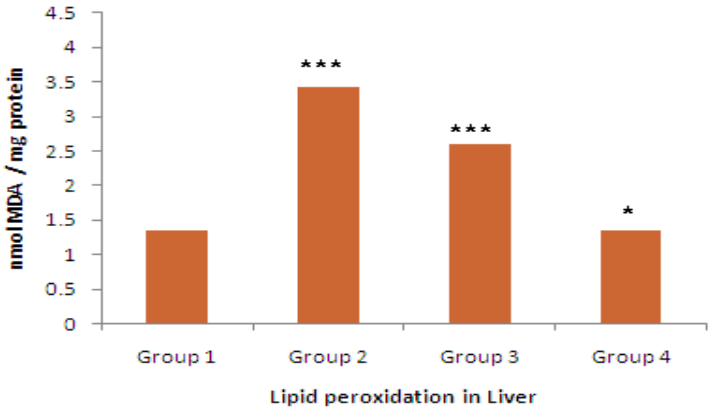
Groups	Glucose (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)
Group 1	77.9 ± 0.25	1.06 ± 0.01	2.94 ± 0.04
Group 2	147.0 ± 0.80***	4.10 ± 0.14***	5.44 ± 0.19***
Group 3	124.7 ± 1.04***	3.00 ± 0.13***	4.16 ± 0.02***
Group 4	88.9 ± 0.59***	1.70 ± 0.02 **	3.44 ± 0.02***

Values were expressed as ± SEM for 6 mice in each group.
Significant at *** P < 0.001, ** P <0.01 compared to control group.

Effect on Lipid peroxidation

To evaluate the oxidative stress induced by the skin extract and the ameliorative potential of *A. spicifera*, formation of Thiobarbutyric acid (TBA) a product of lipid peroxidation was assayed. Groups drinking the *A. spicifera* extract did not show the formation of TBA as compared to control. In contrast, exposure of the skin extract group alone increased the formation of TBA significantly as compared to control as shown in the figure 3.

Figure 3
Effect on Lipid peroxidation in treated groups

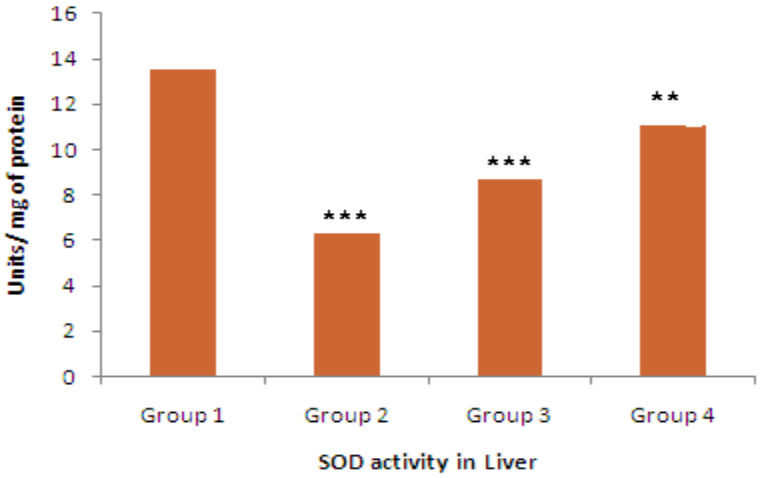


Values were expressed as ± SEM for 6 mice in each group.
Significant at *** P < 0.001, * P < 0 .5 compared to control group.

Effect on Antioxidant enzyme activities

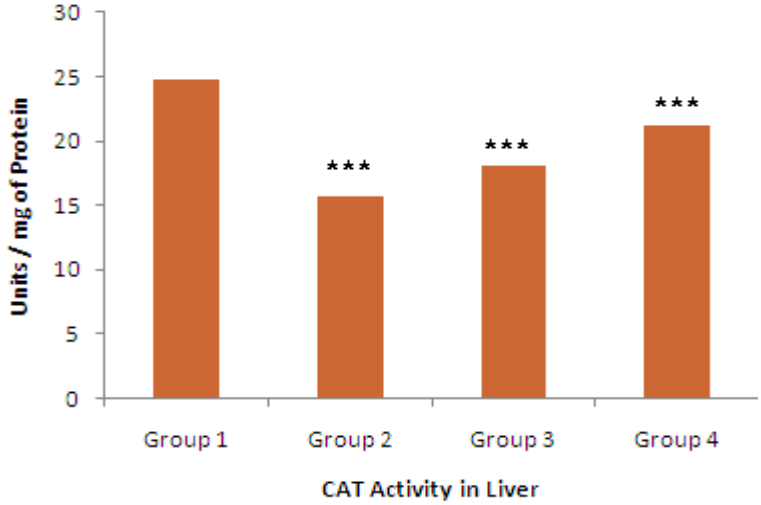
A significant decrease of Superoxide Dismutase (SOD), Catalase enzyme was found in the liver tissue of skin extract treated groups as compared to control. *A. spicifera* drinking group restored the enzyme level in the tissue as compared to control as shown in the figure 4 & 5.

Figure 4
Effect on Superoxide Dismutase (SOD) enzyme activity in treated groups



Values were expressed as ± SEM for 6 mice in each group.
Significant at *** P < 0.001, ** P < 0 .01 compared to control group.

Figure 5
Effect on Catalase (CAT) enzyme activity in treated groups



Values were expressed as ± SEM for 6 mice in each group.
Significant at *** P < 0.001, compared to control group.

DISCUSSION

The antioxidant activities of marine seaweeds have been drawn attention of the researchers all over the world because of the bioactive compounds present in them. Recently, seaweeds have been considered as a rich source of Reactive Oxygen Species (ROS) inhibitors. They also have the potential to inhibit oxidative damage [25]. Some evidences showed that the natural products may be useful in preventing the consequence of oxidative stress and protecting the biochemical functions [26]. The present studies showed the antioxidant, free radical scavenging activities and the hepatoprotective potential of *A. spicifera* against the puffer fish *A. hispidus* toxin induced biochemical and oxidative stress in mice. The

total phenolic content of the *A. spicifera* was found as 55.1 ± 0.39 mg/g as Gallic acid equivalents. The scavenging activity of the extract was found to be (55.03 ± 0.5) % by the DPPH method. Scientists have also been reported the phenolic content and the antioxidant activity of *A. spicifera* [16.] However, we have studied the phenolic content and free radical scavenging of the ethyl acetate extract of *A. spicifera*. Phenolic compounds present in the seaweeds are exhibiting high antioxidant activity and effective free radical scavenging activity, also some have been correlated the antioxidants with the phenolic contents [27]. Based on these studies, we evaluated the potential of *A. spicifera* to reduce the biochemical alterations and the oxidative stress induced by the puffer fish *A. hispidus*. Alanine transferase, Alkaline phosphatase, Aspartate transaminase serves as a hepatic markers, so these enzymes are significantly important in monitoring the liver cell damage which leads to the serious problems [28.] The skin extract of the puffer fish injected group showed a marked decrease of hepatic marker enzymes ALT, AST, ALP, Bilirubin indicate the liver damage. Similarly an increase in the cholesterol, urea, glucose, creatinine, protein in serum indicates the damage to tissue membrane and the liver damage. These studies are in agreement with the studies done in the puffer fish *Lagocephalus lagocephalus* by Saoudi *et al*., 2008. It indicates a down regulation of enzyme synthesis [20]. The hyperglycemia and hyperchloestromia were also found in the skin extract treated group is in accordance to the finding of Ramadan *et al.,* 1980 [29]. It may be occurred due to the severe stress on the body mechanisms and the liver glycogenolysis. A significant decrease in the protein and albumin level was found in the group treated with the skin extract of *A. hispidus* as compared to control group may be occurred due to the impaired synthesis of protein. These findings were agreed with the Mansour *et al.,* 1980 who injected the skin extract of the same fish in rabbits [30]. Serum urea and creatinine levels were significantly increased as compared to control group may be occured due to the increase in free amino acid turnover or protein catabolism, poor glomelular filtrate rate leads to renal disease.

However, *A. spicifera* extract drinking groups significantly restored the level of hepatic marker enzymes and the other biochemical functions. These findings revealsthat the extract has capability to prevent the alterations induced by the puffer fish skin extract. These findings are in accordance with the reports of Saoudi *et al.,* 2011 used *Artemesia campestris* for treating the puffer fish *Lagocephalus lagocephalus* induced biochemical alterations [19]. Hepatoprotective activity is having correlation with the antioxidant activity since it is free radical mediated damage [31]. The enhanced lipid peroxidation leading to tissue damage and the failure of antioxidant defense mechanisms leads to decrease in the important scavenging enzymes SOD, Catalase [32]. A significant increase in the Lipid peroxidation and a marked decrease in the enzymes Superoxide dismutase, Catalase was found in the puffer fish skin toxin treated groups. In contrast, *A. spicifera* extract drinking group in this study did not exhibit the change in the enzyme activity. This indicates skin extract of the puffer fish induces an oxidative stress that leads to tissue damage and *A. spicifera* has a potential to reduce the effect of the puffer toxin. Our study showed *A. spicifera* extract has high phenolic content and scavenging properties and also have a potential to prevent the oxidative stress induce liver damage by the toxin of the puffer fish *A. hispidus*. This has been evidenced by the restoration of the liver functional enzymes and the indicators of acute liver damage. This study concludes, Red algae *A. spicifera* may be an alternative source for neutralizing the toxicity effects produced by the puffer fish *A. hispidus*

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