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PROTECTIVE EFFECT OF *ASCIDIA SYDNEIENSIS* AGAINST CARBON TETRACHLORIDE INDUCED HEPATIC INJURY

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ABSTRACT

Liver toxicity is a major worldwide health problem. In recent years, in spite of tremendous scientific development in the field of hepatology, liver troubles are on rise. Jaundice and hepatitis are the most widespread and major hepatic disorders that account for high death rate. The protective effect of the ethanolic extract of Ascidia sydneiensis on carbon tetrachloride induced hepatic injury in male Wistar albino rats was assessed. Initial, final body weight, serum biochemical parameters (protein, albumin, globulin, A/G ratio, SGPT, SGOT, ALP), total, conjugated and unconjugated bilirubin, GGT and the level of antioxidant enzymes in plasma (LPO, GPX, GRD, SOD, CAT, GSH) were analysed following standard procedures. Histopathological evaluation of liver sections was also done. Effect of the extract at a dose of 50, 100 and 150 mg/kg bw was compared with control and standard drug Silymarin (100 mg/kg bw). Significant decrease in serum enzymes (SGPT, SGOT and ALP), total conjugated and unconjugated bilirubin, GGT and lipid peroxide was noted on treatment with the extract compared to group II. Body weight, Serum biochemical parameters (protein, albumin, globulin) and antioxidant enzymes (GPX, GRD, SOD, CAT, GSH) showed a significant increase. Treatment with Ascidia sydneiensis altered the above parameters to levels near to that of normal. The results revealed a dose dependent hepatoprotective effect with 150 mg/kg body weight possessing significant activity without any toxicity on liver. Histopathological studies also confirmed the protective nature of the extract on liver tissue. The present investigation demonstrates the hepatoprotective property of Ascidia sydneiensis and thus scientifically supports the usage of this marine species in various medicine preparations for treatment of liver disorders.

Keywords *– Ascidia sydneiensis; Ascidian; hepatoprotective activity; Silymarin*

1. INTRODUCTION

Liver, the key organ of metabolism and excretion is constantly endowed with the task of detoxification of xenobiotics, environmental pollutants and chemotherapeutic agents. Thus disorders associated with this organ are numerous and varied[1]. Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. This is one of the reasons for many people in the world over including those in developed

countries turning to complementary and alternative medicine (CAM)[2]. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed[3,4]. Though efforts have been made to search for valuable hepatoprotective agents, no such drugs are available in mainstream medicine till date, while in traditional system a number of herbal drugs are successfully being used in the treatment of liver disorders and are scientifically evaluated for their safety and efficacy[5]. The attention of pharmacologists throughout the world has been focused on finding out potent hepatoprotective drug. The natural products today symbolize safety in contrast to the synthetic drugs that are regarded as unsafe to humans and environment[6]. The present study was done to scientifically evaluate the hepatoprotective activity of *Ascidia sydneiensis* against CCl_4 induced toxicity in rats. *Ascidia sydneiensis* is a marine sedentary simple ascidian found in plenty in Tuticorin coast. Since the report of *Ascidia sydneiensis*, taxonomy[7], ecology, distribution, seasonal variation in the occurrence, breeding biology, recruitment and succession in the fouling community, role as bioindicators, food value[8], association with coral reef[9], chemical investigations[10-12], antibacterial, antimicrobial activity against human pathogens[13,14] and toxicity[15] have been studied. However so far there is no systematic study on hepatoprotective activity. Hence the present investigation focuses on evaluating the protective effect of ethanolic extract of *Ascidia sydneiensis.*

2. MATERIALS AND METHODS

2.1 Animal Material

Samples of *Ascidia sydneiensis* were collected from Tuticorin coast and identified using key to identification of Indian ascidians[16]. A voucher specimen AS 2252 has been deposited in the National Collections of Ascidians in the Museum of the Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin-628002.

2.2 Taxonomic Status

Ascidia sydneiensis is a simple ascidian belonging to the Phylum: Chordata, Subphylum: Urochordata, Class: Ascidiacea, Order: Enterogona, Suborder: Phlebobranchia, Family: Ascidiidae, Genus: *Ascidia*, Species: *sydneiensis*

2.3 Preparation of Extract

For hepatoprotective studies, 100 gram powder was extracted with ethanol using Soxhlet apparatus, cooled to room temperature, evaporated in a rotary evaporator under reduced pressure to obtain a brown residue.

2.4 Experimental Animals

Normal healthy adult male Wistar albino rats (180-200 g) were obtained from Central Animal House, Annamalai University, Chidambaram, Tamil Nadu, India. They were maintained under standard environmental conditions of temperature - 24±1°C, 12 h dark-light cycle, free access to drinking water and standard pellet diet. Rats were deprived of food except water 16-18 hour prior to the experiments. The rules and regulations of Animal Ethical Committee, Government of India were followed.

2.4.1. Experimental protocol - Induction of hepatotoxicity

2.5 ml/kg body weight of CCl_4 was dissolved in 7.5 ml of paraffin and administered intraperitoneally to all the treatment groups. CCl_4 induced hepatotoxicity model is widely used for the study of hepatoprotective effects of drugs and plant extracts[17].

2.4.2 Grouping of animals

Thirty rats were randomly selected and divided into six groups of five each. Group I and II acted as normal and CCl_4 intoxicated control which were given normal saline. Group III, IV, V and VI were administered with 50, 100, 150 and 100 mg/kg body weight of the ethanol extract of *Ascidia sydneiensis* and the standard drug silymarin respectively. A single dose of the extract was given orally using Intra Gastric Catheter daily. The experiment was carried out for 14 days. At the end of this period, animals were kept under overnight fasting and sacrificed. Blood samples were collected, serum separated and biochemical parameters estimated. For histopathological study, liver from each animal was dissected out and preserved in 10% formalin. Then representative blocks of liver tissues from each lobe

were taken and processed for paraffin embedding following standard microtechnique[18]. Sections (5µm) of liver were stained with hemotoxylin and eosin, observed microscopically.

2.4.3 Weight of body

The body weight of adult rat was monitored throughout the treatment period. 24 hours after the last treatment, the final body weight was recorded.

2.4.4 Estimation of Serum Biochemical Parameters

Protein, albumin, globulin, serum glutamate pyruvate transaminase (SGPT), glutamate oxaloacetate transaminase (SGOT) and alkaline phosphatase (ALP) were measured spectrophotometrically[19-22].

2.4.5 Estimation of total, conjugated, unconjugated bilirubin and GGT

Standard procedures were followed to determine total, conjugated bilirubin and GGT[23,24]. The difference between total and conjugated bilirubin gives the concentration of unconjugated bilirubin.

2.4.6 Estimation of Antioxidant Enzymes

Lipid peroxide (LPO), super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), reduced glutathione (GSH) and glutathione reductase (GR) were assessed by standard methods[25-30].

2.5 Statistical Analysis

Values are presented as mean ± S.E.M and statistically evaluated by one-way analysis of variance (ANOVA) followed by student's t - test to identify the differences between *hepatic control and extract treated group and ^{a}standard drug and extract treated.

3. RESULTS AND DISCUSSION

Reactive oxygen species (ROS) are causative factors of degenerative diseases including some hepatopathies. Liver plays a role in clearing and metabolizing chemicals through the process called detoxification which makes it a target organ for toxicity[31]. CCl_4 has been widely used for inducing experimental hepatic damage due to free radical formation during its metabolism by hepatic microsome, leading to lipid peroxidation and consequently, liver damage. The resulting hepatic injury is characterized by leakage of cellular enzymes into blood stream by necrosis and fibrosis[32].

In the present study, ethanolic extract of *Ascidia sydneiensis* was evaluated for its protective effect in CCl_4 intoxicated liver injury using rat model. CCl_4 is a well-known hepatotoxic agent and the preventive action of liver damage by CCl_4 has been widely used as an indicator of liver protective activity of drugs in general[33]. It is biotransformed by Cytochrome P-450 system to produce trichloromethyl free radicals. These free radicals may again react with oxygen to form trichloromethyl peroxyl radicals, which may attack lipids on the membrane of endoplasmic reticulum to elicit lipid peroxidation which finally results in cell necrosis and death[34].

The effect of the extract on the body weight before and after treatment in the normal, hepatic induced and drug treated rats are shown in Table 1. A significant increase in body weight was noted on treatment with the extract when compared to hepatic control indicating normal food intake. A reduction in the body weight of hepatic control can be attributed to reduced food consumption. Group treated with highest dose showed a higher mean weight gain and percentage difference.

Table 2 indicates the serum biochemical parameters and liver marker enzymes of albino rat treated with *Ascidia sydneiensis*. The level of protein, albumin and globulin decreased due to CCl_4 induced hepatotoxicity whereas treatment with the extract at 50, 100 and 150 mg/kg bw significantly increased and restored to normal values. These findings suggest that the extract has significantly neutralized the toxic effects of CCl_4 and helped in regeneration of hepatocytes[35]. The reduction noted in hepatotoxic conditions may be due to defective protein biosynthesis in liver[36]. CCl_4 intoxication causes disruption and disassociation of polyribosomes on endoplasmic reticulum and thereby reducing the biosynthesis of protein. In the present study treatment with the extract might have protected the

polyribosomes and assisted in normal protein synthesis. Marker enzymes such as SGPT, SGOT and ALP which are originally present in high concentration in the cytoplasm was measured to assess liver toxicity. During injury these enzymes are released into blood stream and their concentration is in proportion to the extent of hepatotoxicity. SGPT is a cytosolic enzyme primarily present in the liver tissue. Thus estimation of SGPT in serum is fairly specific for liver tissue is of great value. SGOT is a mitochondrial enzyme released from heart, liver, skeletal muscle and kidney which may rise in acute necrosis or ischemia of other organs such as the myocardium besides the liver cell injury[37,38]. Significant increase in the SGPT, SGOT and ALP levels in the CCl_4 treated group can be taken as an index of liver damage and return towards the normal value on administration of ethanolic extract of *Ascidia sydneiensis* as a proof of regeneration process. The decrease in serum transaminase concentration indicates the stabilization of plasma membrane and protection of hepatocytes against the damage caused by CCl_4. ALP is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules including nucleotides, proteins and alkaloids. It is the prototype of this enzyme that reflects the pathological alteration in biliary flow[39]. The cell membranes of hepatocytes become more permeable and a high level of ALP in the blood serum is related to the increased synthesis of it by cells lining bile canaliculi usually in response to cholestasis and increased biliary pressure[40]. A significant reduction of ALP in a dose dependent manner can be attributed to the protective role of *Ascidia sydneiensis*.

Table 3 indicates the total, conjugated, unconjugated bilirubin and GGT. Bilirubin is a yellow pigment produced when heme is catabolised. Hepatocytes render bilirubin water soluble and easily excretable by conjugating it with glucuronic acid prior to secreting it into bile by active transport. Serum bilirubin is considered as one of the true test of liver functions since it reflects the ability of the liver to take up and process bilirubin into bile. Increase in serum bilirubin levels may be found in hepatocellular damage, hemolytic jaundice or hepatitis. CCl_4 injury causes significant degeneration of hepatocytes and blockade of the bile ducts which results into significant increase in the serum total and direct bilirubin levels[41]. Extract of *Ascidia sydneiensis*, reduced the total, conjugated and unconjugated bilirubin level substantiating its defensive role over liver and improving its physiological efficiency suggesting the presence of a mechanism to overcome biliary dysfunction caused by CCl_4. A significant reduction of γ-glutamyl transferase (GGT) was noted. GGT is a microsomal enzyme which is widely distributed in tissue including liver. The activity of serum GGT is generally elevated as a result of liver disease and it is most useful in the diagnosis of hepatic function. The acute damage caused by CCl_4, increased the GGT level but the same attained normal after administration of *Ascidia sydneiensis* which may be due to its antioxidant activity.

The level of serum LPO, GPX, GRD, SOD, CAT and GSH are shown in Table 4. An increase in the level of LPO was found in the hepatic control. Treatment with the extract significantly decreased the LPO level near to that of normal. GC-MS study and the data on biological activities have revealed the presence of compounds like Tetradecanoic acid (acid), Bis-(2-methylpropyl)ester of 1,2-benzenedicarboxylic acid (ester), n-Hexadecanoic acid (acid), Diisooctyl ester of 1,2-benzenedicarboxylic acid (ester and acid), Cholest-5-en-3-ol(3)-carbonochloridate (steroid), Cholesterol (steroid), squalene (triterpene) and 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-methylester of cyclopropaneoctanoic acid (steroid) exhibiting antioxidant properties supporting the present findings. These compounds may have the role in hepatoprotective process. The GPX and GRD content in the liver reduced significantly in CCl_4 treated rats. GPX is a seleno enzyme. It protects the cells from damage due to free radicals like hydrogen and lipid peroxides[42]. GPX catalyzes the reaction of hydroperoxidases with reduced glutathione to form glutathione disulphide on reduction. Treatment with the extract of *Ascidia sydneiensis* was able to reverse such effects and bring back to normal indicating the role of bioactive principles in hepatoprotection. SOD, a metallo protein is the most sensitive enzyme index in liver injury and one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to from hydrogen peroxide and oxygen, hence diminishing the toxic effect caused by this radical[42]. In the present study, it was observed that the ethanol extract of *Ascidia sydneiensis* significantly increased the SOD activity in CCl_4 intoxicated rats thereby reducing oxidative damage and maintaining the normal functional status of the liver. CAT is an enzymatic antioxidant widely distributed in all tissues. The highest activity is found in red cells and liver. Catalase is a

heme protein, localized in the peroxisomes or the microperoxisomes. This enzyme catalyses the decomposition of H_2O_2 to water and oxygen, thus protecting the cell from oxidative damage. Therefore, a reduction in the activity of CAT may result in a number of deleterious effects due to accumulation of hydrogen peroxide[43]. Treatment with ethanol extract of *Ascidia sydneiensis* increased the level of CAT significantly in dose dependent manner. Liver damage is always associated with cellular necrosis, increase in lipid peroxidation and depletion in the tissue GSH levels. GSH extensively found in cells, protects cells against electrophilic attacks provided by xenobiotics such as free radicals and peroxides. GSH deficiency leads to cellular damage in kidney, muscle, lung, jejunum, colon, liver, lymphocytes and brain[44]. A dose dependent highly significant increase in GSH level was observed on treatment with the extract revealing its defending nature as an antioxidant in removing noxious radicals from tissues. Similar observations have been reported on studies with the ethanolic extract of *Microcosmus exasperatus*[45].

The histopathological changes recorded in the liver on treatment with ethanolic extract of *Ascidia sydneiensis* on CCl_4 induced hepatic injury in rats is shown in plate 1. In group I (normal control), the liver section shows normal arrangement of hepatic cells. Various pathological changes like steatosis, centrilobular necrosis and vacuolization with the destruction of hepatic architecture were noted in group II (hepatic control). Group III and V showed mild and full recovery of distorted and degenerated hepatocytes respectively revealing the protective effect of the extract. In group VI treated with standard drug, the architecture of the cells of the liver was brought back to normal.

Table 1: Effect of *Ascidia sydneiensis* on Body Weight

Parameter /Groups mg/kg	Initial Body weight (gm)	Final Body weight (gm)	Mean weight (gm) Gain ( ) / Loss ( )	% Difference
I-Normal control	212.15±4.85	232.56±5.88	20.41	8.77
II-Hepatic control	206.92±6.84	194.70±6.35	12.22	5.90
III-50 mg/kg	216.65±9.35	212.40±5.16	4.25	1.96
IV-100	202.40±6.95	224.95±7.35*	22.55	10.02
V-150	207.60±6.35	239.35±5.46*	31.75	13.26
VI- Silymarin 100	204.60±8.45	229.66±5.96	25.06	10.91

Data represented as mean ± SEM, (N=5). Significance between hepatic control and extract treated group $^{*}P>0.05$.

Table 2: Effect on Protein, Albumin, Globulin, SGPT, SGOT and ALP

Parameter /Groups mg/kg bw	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G Ratio	SGPT (u/L)	SGOT (u/L)	ALP (u/L)
I-Normal control	8.06±0.31	4.19±0.23	3.87±0.13	1.1:1	21.92±0.76	26.93±0.16	204.18±5.16
II-Hepatic control	6.24±0.11	3.68±0.16	2.56±0.35	1.4:1	59.12±1.84	61.36±1.98	258.16±8.24
III-50	6.18±0.15	3.45±0.21	2.73±0.11	1.3:1	41.65±3.18 ^{*a}	48.31±2.16 ^{*a}	236.65±4.86 ^{*a}
IV-100	7.98±0.67	4.38±0.14	3.60±0.53	1.2:1	29.65±1.13 ^{**aa}	37.39±2.09 ^{**aa}	204.16±4.13 ^{**aa}
V-150	8.09±0.18	4.26±0.11	3.83±0.21	1.1:1	21.18±0.86 ^{**aaa}	20.60±0.42 ^{***aaa}	186.34±1.16 ^{**aa}
VI-Silymarin 100	8.11±0.16	4.73±0.18	3.38±0.38	1.4:1	24.18±1.13	26.92±0.31	173.86±1.63

Data represented as mean ± SEM, (N=5). Significance between **Hepatic control and extract treated group.* $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, ^{a}*Standard drug and extract treated* $^{a}<0.05$, $^{aa}<0.01$, $^{aaa}<0.001$.

Table 3: Effect on Total, Conjugated, Unconjugated Bilirubin and GGT

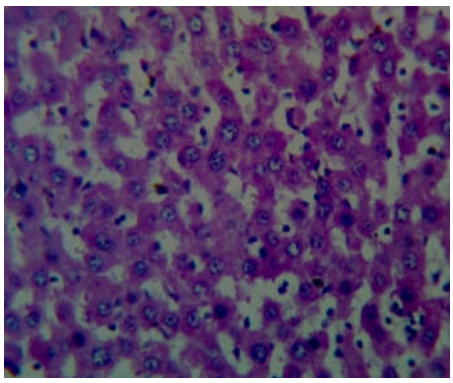
Parameter /Groups mg/kg bw	Total Bilirubin (mg/dl)	Conjugated Bilirubin (mg/dl)	Unconjugated Bilirubin (mg/dl)	GGT (mg/dl)
I-Normal control	0.73±0.06	0.21±0.06	0.52±0.02	7.56±0.13
II-Hepatic control	3.84±0.07	1.38±0.06	2.46±0.03	32.46±0.92
III-50	2.18±0.06^{*a}	0.46±0.05^{*a}	1.72±0.07^{*a}	16.84±0.81
IV-100	1.56±0.15^{**aa}	0.32±0.03^{**aa}	1.24±0.04^{**aa}	11.27±0.16^{*a}
V-150	1.14±0.08^{***aaa}	0.28±0.03^{**aa}	0.86±0.08^{***aaa}	7.24±0.11^{**aa}
VI-Silymarin 100	0.98±0.04	0.24±0.07	0.74±0.03	7.81±0.13

Data represented as mean ± SEM, (N=5). Significance between *Hepatic control and extract treated group. $^{*}p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.001, ^{a}Standard drug and extract treated ^{a}p <0.05, ^{aa}P <0.01, ^{aaa}p <0.001.

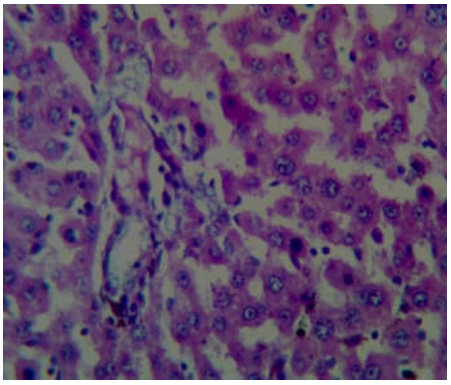
Table 4: Effect on LPO, GPX, GRD, SOD, CAT and GSH

Parameter /Groups mg/kg bw	LPO (n mole of MDA/mg protien)	GPX (u/mg Protien)	GRD (u/mg)	SOD (u/mg)	CAT (u/mg)	GSH (u/mg)
I-Normal control	1.94±0.036	816.16±11.46	91.84±1.93	491.78±7.84	36.55±1.31	21.65±0.98
II-Hepatic control	6.83±0.015	411.67±8.24	46.51±1.38	281.92±6.11	17.84±0.84	13.92±0.84
III-50	3.41±0.018 ^{*a}	592.16±2.93 ^{**aa}	82.16±1.04 ^{**aa}	413.36±4.18 ^{**aa}	26.88±1.64 ^{**aa}	21.81±0.36 ^{**aa}
IV-100	3.03±0.031 ^{**aa}	706.65±3.98 ^{*a}	89.24±1.31 ^{*a}	429.32±2.84 ^{*aa}	23.61±1.16 ^{*a}	18.16±0.3 ^{*a}
V-150	2.16±0.092 ^{**aa}	784.18±5.16 ^{*a}	82.65±1.93 ^{**aa}	436.14±4.07 ^{*a}	31.84±0.81 ^{***aaa}	26.81±0.94 ^{***aaa}
VI-Silymarin100	2.16±0.018	791.30±3.84	86.26±1.31	468.17±4.15	36.91±0 .91	25.96±0.42

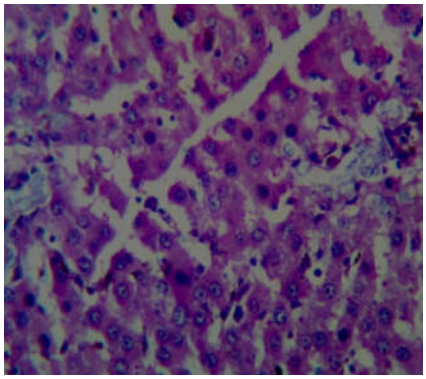
Data represented as mean ± SEM, (N=5). Significance between *Hepatic control and extract treated group. $^{*}p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.001, ^{a}Standard drug and extract treated ^{a}p<0.05, ^{aa}P<0.01, ^{aaa}p<0.001.



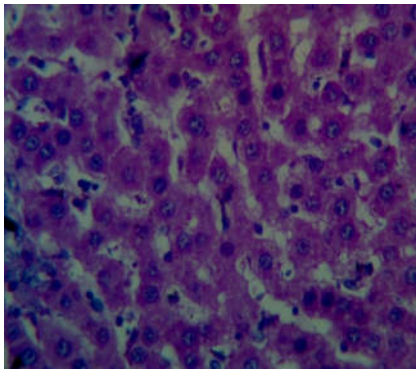
Group I – Normal control



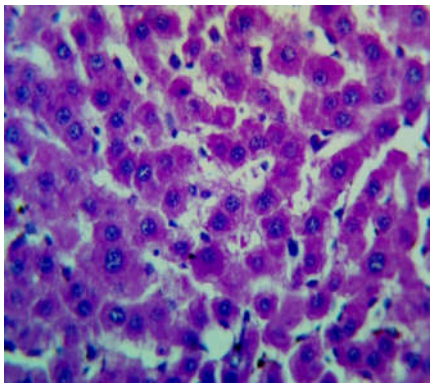
Group – II Hepatic control



Group III – Extract of *A. sydneiensis* (50 mg/kg bw)



Group V – Extract of *A. sydneiensis* (150 mg/kg bw)



Group VI– Silymarin (100 mg/kg bw)

Plate1. Photomicrograph showing histopathological changes in the Liver

4. CONCLUSION

The ethanolic extract of *Ascidia sydneiensis* has shown the ability to maintain the normal functional status of the liver. From the above results, we conclude that the extract in comparison with silymarin has a potent hepatoprotective action upon CCl_4 induced oxidative stress and hepatic toxicity in rat. The protective effect of the extract may be attributed due to the reduced lipid peroxidation and improved defence of the hepatocytes against the reactive oxygen species. The exact hepatoprotective mechanism of *Ascidia sydneiensis* is still unknown. However, it is necessary to determine other parameters such as oxidative stress markers and molecular biology assays to confirm our findings. Efforts are in progress to isolate and characterize the active principle, which is responsible for the hepatoprotective efficacy of this valuable marine species.

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