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Hepatoprotective activity of the ethanol extract of simple ascidian, *Microcosmus exasperatus* Heller, 1878

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ABSTRACT

The present study aims at analysing the hepatoprotective activity of the marine simple ascidian Microcosmus exasperatus. The animals were randomly divided into six groups of five individuals. Group I served as normal and Group II as hepatic toxicity induced control. Both were given normal saline. Group III, IV, V and VI were treated with Carbon tetrachloride to induce hepatic toxicity and were administered with 50, 100, 150 mg/kg of the ethanol extract of Microcosmus exasperatus and the standard drug silymarin at a dose of 100 mg/kg body weight respectively for 14 days. Initial, final body weight, protein, albumin, globulin, A/G ratio, Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Alkaline Phosphatase (ALP), total, conjugated, unconjugated bilirubin, GGT and Lipid Peroxide (LPO), Glutathione Peroxidase (GPX), Glutathione Reductase, Super Oxide Dismutase (SOD), Catalase (CAT), Reduced Glutathione (GSH) activities in serum were estimated. The results revealed a dose dependent hepatoprotective effect with 150 mg/kg body weight possessing significant activity without any toxic effect on liver and kidney. The extract treated groups were compared with that of hepatic control and standard drug.

Keywords: *Microcosmus exasperatus*, Ascidian, hepatoprotective activity, silymarin

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INTRODUCTION

Liver is a vital organ which plays a major role in metabolism and excretion of xenobiotics by regulating homeostasis and providing protection against foreign substances by detoxifying and eliminating them from the body. It is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy provision and reproduction [1]. The bile secreted by the liver plays an important role in digestion. Damage and malfunctioning of the liver is a major problem challenging medical professionals, pharmaceutical and drug regulatory bodies. *Microcosmus exasperatus* is a simple ascidian which belongs to the family Pyuridae. A review of literature shows that studies on the pharmacological activities like antimicrobial [2], antidiabetic [3], nutritive value [4], biochemical components [5], toxicity [6], GC- MS analysis [7,8] and HPTLC [9] are available. But a systematic screening for hepatoprotective activity of *Microcosmus exasperatus* has not been attempted at all.

MATERIALS AND METHODS

Animal material: *Microcosmus exasperatus* belongs to the Class: Ascidiacea, Order: Pleurogona, Suborder: Stolidobranchia and Family: Pyuridae. It has a hard leathery orange coloured tunic with two clearly visible siphons. The pharynx has 8-9 folds. There is one gonad in each side of the body, divided into three portions. It is a continuous breeder. Samples of *Microcosmus exasperatus* were collected from Thoothukudi coast, cleaned with sea

water, shade dried and powdered. A voucher specimen AS 2240 has been deposited in the museum, Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin - 628002.

Preparation of extract: 100 gm powder was extracted with ethanol using Soxhlet apparatus, cooled to room temperature and evaporated in a rotary evaporator to get a residue. This residue was used for further studies.

Experimental animal: Mature adult male Wistar albino rats weighing 180 - 200 gm were selected for the study. They were maintained in a well ventilated animal house with constant 12 hours of darkness and 12 hours light schedule, room temperature (24±2 ºC) and humidity (60-70%). Clean water and standard pellet diet "ad Libitum" (Hindustan Lever Ltd., India) were given to them. The animals were kept under fasting for 16 hours before the experiment.

Experimental protocol - Induction of hepatotoxicity: Carbon tetrachloride (CCl_4) 2.5 ml/kg body weight was dissolved in 7.5 ml of paraffin and administered intraperitoneally. CCl_4 induced hepatotoxicity model is widely used for the study of hepatoprotective effects of drugs and plant extracts [10,11].

Grouping of animals: The animals were randomly divided into six groups of five individuals. The experiment was carried out for 14 days. Group I served as normal and Group II as hepatic toxicity induced control. Both were given normal saline. Group III, IV, V and VI liver injured rats were administered 50, 100 and 150 mg/kg of the ethanol extract of *Microcosmus exasperatus* and the standard drug silymarin at a dose of 100 mg/kg body weight respectively. All the treatments were given orally by using IGC between 9.30 and 10.00 hour in the morning and were conducted in accordance with the guidelines established by the animal ethics committee, Government of India.
Weight of body: The body weight of adult mice was monitored throughout the treatment period. 24 hours after the last treatment, the final body weight was recorded.

Estimation of protein, albumin, globulin, SGPT, **SGOT, and ALP:** Protein, Serum albumin, globulin, SGPT, SGOT and ALP was estimated by standard procedure [12,13,14,15].

Estimation of total, conjugated, unconjugated bilirubin and GGT: Total, conjugated bilirubin and GGT were determined by standard methods [16,17]. The concentration of unconjugated bilirubin was calculated as the difference between total and conjugated bilirubin concentrations.

Estimation of LPO, GPx, GRD, SOD, CAT and GSH: Analysis of LPO, GPx, GRD, SOD, CAT and GSH were carried out with the serum [18,19,20,21,22,23].

Statistical analysis: Values are expressed as mean ± SEM. The statistical analysis was done by one-way analysis of variance (ANOVA) followed by Dunnett's test. P-values less than 0.05 were considered to be significant.

RESULTS AND DISCUSSION

Table 1 shows the effect of ethanol extract of *Microcosmus exasperatus* on the body weight of the rats before and after treatment in the normal, hepatic induced and drug treated rats. Ttreatments with the ethanol extract of *Microcosmus exasperatus* showed a marked increase in the body weight compared to the hepatic control which exhibited a decrease. In the present study, there was a gain in the body weight of rats treated with ethanol extract of *Microcosmus exasperatus*. This may be due to an increase in food intake. Similar observation of an increase in weight was noted on studies with the extract of marine algae *Gracilaria corticata* [24]. A reduction in the body weight of hepatic control can be attributed to reduced intake of food due to damage to liver cells leading to indigestion and anorexia. The mean weight gain and percentage difference was greater in the group treated with highest dose indicating a more effective protection bringing back the liver cells to normal.

Serum biochemical parameters and liver marker enzymes of albino rat treated with the extract of *Microcosmus exasperatus* is shown in Table 2. The level of protein, albumin and globulin were reduced due to the CCl_4 induced hepatotoxicity. The reduction can be assigned to the initial damage produced and localized in the endoplasmic reticulum which results in the loss of P450 leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides resulting in fatty liver [25, 26, 27, 28,]. Administration of the extract increased the

level of protein, albumin and globulin which may be due to the stabilization of the function of endoplasmic reticulum leading to normal protein synthesis.

Damage of liver cell is reflected by an increase in the levels of hepatospecific enzymes which are released into circulation after cellular damage [29]. 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 restoration towards the normal value on administration of ethanolic extract of *Microcosmus exasperatus* may be an indication of regeneration process. Similar observations have been reported with *Leucas ciliata* leaves [30].

ALP is the prototype of liver marker enzymes that reflects the pathological alteration in biliary flow [31]. Its activity on endothelial cell surface is responsible for the conversion of adenosine nucleotide to adenosine, a potent vasodilator and anti-inflammatory mediator that results from injury. Interlukin-6 is secreted by T cells and macrophages to stimulate immune response during tissue damage. Therefore, accumulation of interlukin-6 leads to the production of alkaline phosphatase from adenosine which may be the reason for the increment in ALP in hepatotoxic group [32]. A significant reduction of ALP in a dose dependent manner can be taken as a proof of the protective role of the extract. In CCl_4 induced toxic hepatitis the toxicity begins with the changes in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures [33]. A recovery against the toxic effects of CCl_4 was evident in the present study.

Total, conjugated, unconjugated bilirubin and GGT of normal, hepatic induced control, extract and standard drug silymarin treated rats is given in Table 3. Bilirubin is one of the most useful clinical clues to identify the severity of necrosis of hepatic cells and its accumulation is a measure of binding, conjugation and excretory capacity of hepatocyte. Quantity of total bilirubin, a byproduct of the breakdown of red blood cells in the liver, is a good indicator of liver function. High levels lead to jaundice and are indicative of damage to the liver and bile duct [34]. CCl_4 injury causes significant degeneration of hepatocytes and blockade of the bile ducts which may result in significant increase in serum total bilirubin [35]. Extract of *Microcosmus exasperatus*, reduced the total bilirubin level, indicating its protective effect over liver and improvement in its functional efficiency suggesting the possibility of a mechanism to stabilize bilary dysfunction caused by CCl_4.

A significant dose related decrease in the level of conjugated and unconjugated bilirubin was noted in the present study. A similar observation was reported on administration of the ethanol extract of *Clitoria ternatea* and *Cassia angustifolia* indicating that the conjugating function of the liver was improved [36]. It is also suggested that, the extracts may activate the constitutive androstane receptor which is a key regulator in bilirubin clearance in the liver as an evidence for the reduction in the level of bile. In the present experiment also treatment with the extract brought back the total bilirubin to normal indicating normalization of hepatocytes and removal of blocks in the bile ducts. A significant reduction of GGT was noted in the study. GGT is a hepatic microsomal enzyme which is useful in the diagnosis of liver diseases. An increase in the level observed in hepatic control indicates liver damage whereas on treatment with the extract a dose dependent decrease indicated the role of this enzyme as a marker of hepatic cell recovery due to its antioxidant nature.

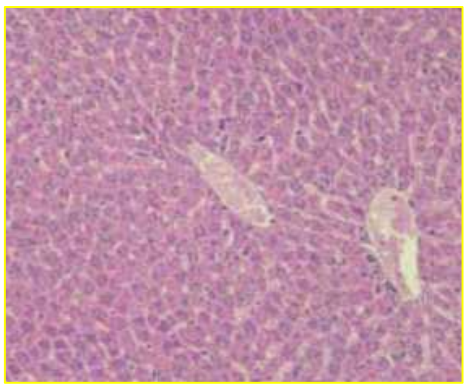
Table 4 indicates the serum LPO, GPX, GRD, SOD, CAT and GSH level of normal, hepatic, extract and standard drug treated groups. In the present investigation an increase in the level of LPO was noted in the hepatic toxicity induced rats. This can be taken as an indication of enhanced lipid peroxidation leading to damage to liver tissue by accumulation of excessive free radicals due to the failure of antioxidant defense mechanism. Administration of ethanol extract of *Microcosmus exasperatus* significantly decreased the LPO level near to that of normal showing that the extract may possess antioxidant properties. A study on the GC-MS analysis and the biological activities has revealed the presence of compounds like n-Hexadecanoic acid, Tetradecanoic acid, 26-Nor-5-cholesten-3á-ol-25-one, (Z,Z,Z)- phenylmethyl ester of 6,9,12-Octadecatrienoic acid, Cholestan-3-ol and N-[4-bromo-n-butyl]- 2-piperidinone exhibiting antioxidant properties supporting the present finding [7].

In CCl_4 treated rats the GPX and GRD content in the liver decreased significantly. These antioxidant enzymes play a key role in preventing oxidative damage to the liver [37]. Treatment with the extract was able to reverse the effects and bring back the level to normal indicating the role of bioactive principles in *Microcosmus exasperatus* extract in hepatoprotection.

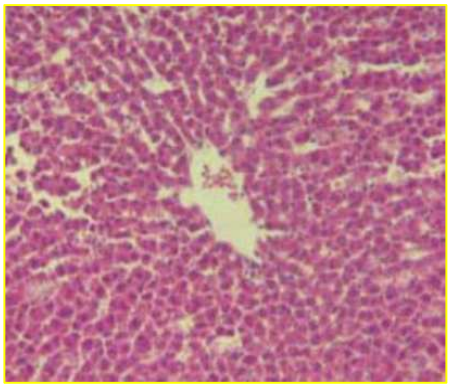
There was a decrease in the level of Superoxide Dismutase in the hepatic control which was gradually restored to normal on treatment with the ethanolic extract of *Microcosmus exasperatus*. SOD is a key defense enzyme which is responsible for dismutation of superoxide anions. An increase in the level of enzymes on administration of extract may be directly responsible for scavenging or neutralizing of radicals and this may be the reason for the normal functional status of the liver in the present study.

The reduction of H_2O_2 is catalysed by the haemoprotein catalase (CAT). This enzyme protects the tissues from highly reactive hydroxyl radicals by converting harmful hydrogen peroxide into water and oxygen [38]. The accumulation of highly toxic metabolites and H_2O_2 can induce oxidative stress leading to tissue damage. There was a reduction in the level of CAT in hepatic control whereas on treatment with the extract a gradual significant increase could be observed. The healing process of liver tissue observed here might be a result of detoxication of hydroxyl radicals or preventing the accumulation of free radicals by increasing the activities of catalase.

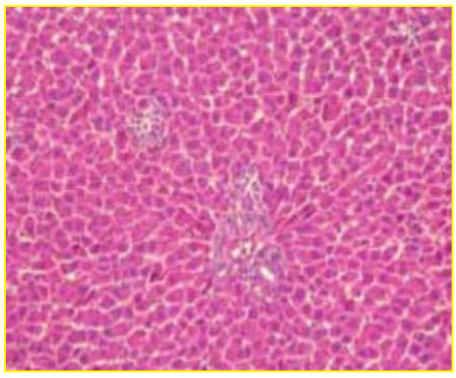
Liver damage is always associated with cellular necrosis, increase in lipid peroxidation and depletion in the tissue GSH levels. Deficiency of GSH leads to cellular damage of all vital organs [39]. The role of GSH is to protect the cells against attacks by toxic chemicals. A dose dependent highly significant increase in GSH level was noted on treatment with the extract indicating its protective nature as an antioxidant removing noxious radicals from tissues.
The hepatoprotective effect of the extract was further confirmed by histopathological examinations of the liver sections (Plate 1) which revealed that the normal liver shape was disturbed, distorted and degenerated by hepatotoxin intoxication in the CCl_4 treated rats. In the liver sections of the rats treated with ethanolic extract of *Microcosmus exasperatus* the normal cellular shape was retained with absence of distortion and degeneration of the hepatocytes. The bile duct epithelium was normal with absence of lymphocytic infiltration in the portal area as compared to silymarin, thereby confirming the protective effect.



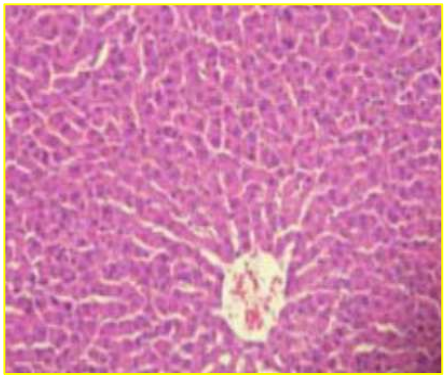
Group I - Normal control



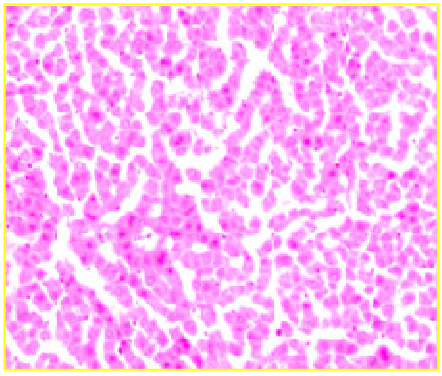
Group II - Hepatic control



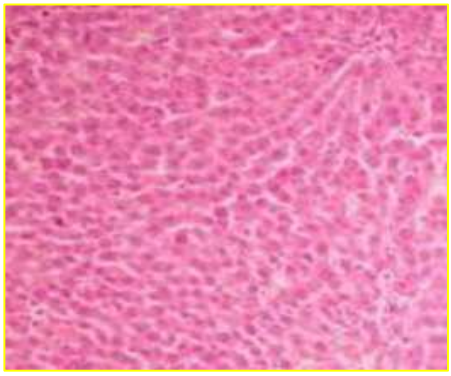
Group III - Extract of *M. exasperatus* (50 mg/kg bw)



Group IV - Extract of *M. exasperatus* (100 mg/kg bw)



Group V - Extract of *M. exasperatus* (150 mg/kg bw)



Group VI - Silymarin (100 mg/kg bw)

Plate1: Photomicrograph showing histopathological changes in the Liver

Group I - Central vein surrounded by hepatic cord of cells (Normal histology)
Group II - High distortion and degeneration of the hepatocytes, hyperplasia of bile duct epithelium, lymphocytic infiltration in the portal area
Group III - Mild recovery of hepatic cell distortion and regeneration of the hepatocytes
Group IV - Moderate recovery of hepatic cell distortion and regeneration of the hepatocytes
Group V - Good recovery of hepatic cell distortion and regeneration of the hepatocytes
Group VI - Normal histology

TABLE 1: EFFECT OF THE ETHANOLIC EXTRACT OF *MICROCOSMUS EXASPERATUS* ON BODY WEIGHT OF THE RATS BEFORE AND AFTER TREATMENT

Parameter/ Groups	Initial Body weight (gm)	Final Body weight (gm)	Mean weight (gm) Gain (↑) / Loss (↓)	% Difference
Group I Normal control	212.45±11.45	234.25±10.54	21.80↑	10.260
Group II Hepatic control	206.38±9.54	189.65±8.44	16.73↓	08.100
Group III 50 mg/kg bw	216.52±10.55	218.50±9.35	01.98↑	00.001
Group IV 100 mg/kg bw	202.14±8.36	226.74±10.15	24.60↑	12.160
Group V 150 mg/kg bw	208.35±12.26	238.65±11.45*	30.30↑	14.542*
Group VI Silymarin 100 mg/kg bw	204.18±10.84	229.25±9.35	25.07↑	12.278

*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	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G Ratio	SGPT (u/L)	SGOT (u/L)	ALP (u/L)
Group I Normal control	8.21±0.32	4.72±0.26	3.49±0.13	1.7:1	23.36±0.94	26.59±0.88	134.38±4.32
Group II Hepatic control	7.04±0.16	3.94±0.13	3.10±0.22	1.8:1	118.68±3.26	134.12±5.81	199.36±5.28
Group III 50 mg/kg bw	7.93±0.21	4.34±0.25	3.59±0.15	1.8:1	59.16±2.17*	62.38±4.83*	154.19±3.88*
Group IV 100 mg/kg bw	8.11±0.17	4.81±0.13	3.30±0.20	1.7:1	31.68±2.33**	34.51±3.94**	123.55±1.84**
Group V 150 mg/kg bw	8.39±0.11	4.93±0.17	3.46±0.11	1.7:1	29.74±1.84**	20.63±2.84***	118.14±2.13**
Group VI Silymarin 100 mg/kg bw	8.16±0.24	4.88±0.14	3.28±0.14	1.7:1	25.33±0.84**	29.15±1.36**	124.54±2.69**

*Data represented as mean ±SEM, (N=5). Significance between * Hepatic control and extract treated group.*
**p <0.05, **p <0.01, ***p <0.001.*

TABLE 3: EFFECT ON TOTAL, CONJUGATED, UNCONJUGATED BILIRUBIN AND GGT

Parameter/ Groups	Total Bilirubin (mg/dl)	Conjugated Bilirubin (mg/dl)	Unconjugated Bilirubin (mg/dl)	GGT (mg/dl)
Group I Normal control	0.73±0.07	0.23±0.03	0.50±0.16	9.31±0.81
Group II Hepatic control	4.56±0.96	2.06±0.64	2.50±0.11	43.69±1.56
Group III 50 mg/kg bw	2.13±0.46*[a]	0.94±0.04*[a]	1.19±0.34*[a]	25.14±1.31*[a]
Group IV 100 mg/kg bw	1.05±0.14**[aa]	0.31±0.07**[aa]	0.74±0.03**[a]	11.23±0.84**[aa]
Group V 150 mg/kg bw	0.93±0.11***[aa]	0.23±0.02***[aa]	0.70±0.08**[a]	9.14±0.78**[aa]
Group VI Silymarin 100 mg/kg bw	0.81±0.07***	0.20±0.04***	0.61±0.05***	7.84±0.35**

*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.*

TABLE 4: EFFECT ON LPO, GPX, GRD, SOD, CAT AND GSH

Groups	**LPO (n mole of MDA/mg protien)**	**GPX (u/mg Protien)**	**GRD (u/mg)**	**SOD (u/mg)**	**CAT (u/mg)**	**GSH (u/mg)**
Group I Normal control	2.219±0.054	3.814±0.107	0.492±0.24	0.324±0.175	3.988±0.081	32.66±0.24
Group II Hepatic control	5.688±0.017	1.036±0.057	0.206±0.35	0.101±0.094	1.114±0.035	10.69±0.15
Group III 50 mg/kg bw	3.103±0.084*	1.634±0.094*	0.294±0.17*[a]	0.146±0.021*	1.963±0.063*[a]	18.11±0.19*
Group IV 100 mg/kg bw	2.834±0.075*[a]	2.806±0.055**[aa]	0.358±0.28**[a]	0.228±0.054**[a]	2.871±0.074**[a]	26.08±0.61**[a]
Group V 150 mg/kg bw	2.011±0.026**[aa]	3.491±0.113***[aa]	0.448±0.17***[aa]	0.363±0.113***[aa]	3.618±0.024***[aa]	30.91±0.17***[aa]
Group VI Silymarin 100 mg/kg bw	2.431±0.390**	3.054±0.127***	0.487±0.34***	0.388±0.112***	3.504±0.019***	32.66±0.34***

*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.*

CONCLUSION

Liver is a key organ regulating homeostasis of the body. As a result it becomes a target for the accumulation of toxic byproducts and oxidative stress. Though various drugs are available in the market to treat liver damage, the efficacies of these are still questionable. The present investigation which is first of its kind using the extract of a simple ascidian has shown promising evidence of the presence of a principle with hepatoprotective activity. A chemical investigation of *Microcosmus exasperatus* already carried out showed the presence of flavonoids, tannins and saponins which may act as antioxidants. A further detailed study is needed to isolate and identify the exact compound and mechanism of action.

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