THE EFFECT OF THE ETHANOLIC EXTRACT OF *OCYPODE PLATYTARSI* ON PARACETAMOL INDUCED LIVER DAMAGE IN RATS

I.Rajalakshmy*1 and V. Venkata gangadhar 2

1Department of Pharmacology, Mother Theresa Post Graduate and Research Institute of Health Sciences, Puducherry – 605006.
2Pinnacle Biomedical Research Institute, Bhopal, 462003.

ABSTRACT

Background: Throughout world marine organisms were used to treat many diseases. Aims: To study the hepatoprotective potential of *Ocypode platytarsis* crab extract against paracetamol induced liver damage. Methods: Hepatotoxicity was induced by administration of paracetamol (3g/kg) orally on the 7th day. The extract of *Ocypode platytarsis* was administrated at a dose of 400 mg/kg and 200 mg/kg for 7 consecutive days. In order to evaluate the effect of hepatoprotective activity of *Ocypode platytarsis* extract the serum enzymes serum glutamate oxaloacetate transaminase, Serum glutamate pyruvic transaminase, Alkaline Phosphate, Bilirubin, and Total protein were determined. Results: The *Ocypode platytarsis* extract shows a significant and dose dependent decrease in elevated serum enzymes of serum glutamate oxaloacetate transaminase, Serum glutamate pyruvic transaminase, Alkaline Phosphate, Bilirubin, and increase the Total protein levels, which is compared with paracetamol alone treated rats. Histopathological studies also showed the significant improvement in *Ocypode platytarsis* extract. Conclusion: The results of the present study reveal the hepatoprotective activity of *Ocypode platytarsis* against paracetamol induced liver damage in rats.

Key words: Paracetamol, *Ocypode platytarsis*, hepatoprotective, silymarin, SGOT, SGPT, TOTAL PROTEIN, ALP, BILIRUBIN, rats.
INTRODUCTION
Now-a-days marine organisms play a major role in the management of various diseases. Sea possesses more than three lakes known species of plants and animals, which represents about 36 phyla. In case of biodiversity, marine species shows approximately half of total biodiversity marine product, only 10% of 25,000 plants are investigated [1]. About 7000 marine used in traditional system of medicine much before that. In most countries with ancient civilizations, such as India, a system of medicine, indigenous to the country concerned, exists [2]. In Ayurveda practices, marine organisms are grouped in three main categories, namely animals, plants and minerals. Corals, pearls, shells, conch, sea salt, sea coconut etc are the main sea animals used for the preparation of different kind of drugs in Ayurveda [3]. From these marine organisms near about 10,000 metabolites, many of them show pharmacological action on terrestrial biota to the discovery of number of potent pharmacological active products; many of them have been clinical application [1].

Among the marine organisms, *Ocypode platytarsis* is a crab belongs to Ocypodidae family and Genus: *Ocypode* which has been credited with therapeutic properties to treat several diseases. Its common name is Ghost crabs and it dominates sandy shores in tropical and subtropical areas, replacing the sandhoppers that predominate in cooler areas. The name "ghost crab" derives from the animals' nocturnality and their pale colouration. It is distributed throughout India. Traditionally it is used as hepatoprotective, but there is no scientific evidence so far. They are useful in vitiated conditions of vata and kapba, skin diseases, leprosy, scabies, syphilis, dyspepsia, constipation, jaundice, cough, bronchitis, cardiac debility. In our study we investigated the ethonlic extract of *Ocypose platytarsis* (OP) as a hepatoprotective agent against paracetamol-induced liver damage in rats.

MATERIALS AND METHODS
Collection of Animal material
The whole animals were collected from Chakicherla, Prakasam dist, Andhra pradesh during the month of December month 2011. Herbarium of Animal was prepared and submitted at Regional centre, Marine products export development authority for authenticated by Dr.R.Ramanjanayalu as *Ocypode Platytarsis* (OP).

Extraction method of the animal material:
The crab fresh were collected crabs (400g) and extracted with ethanol + water (80:20) in
soxhelt apparatus by simultaneous extraction for 48hrs. The dried extract was packed in air tight container.

**Phytochemical screening**
Detailed phytochemical testing was performed to ascertain the presence or absence of particular phyto- constituents.

**Test for alkaloids**
Small portion of the solvent ethyl alcohol extract of the *Ocypode Platytaurus* were added with few drops of dilute Hcl and filtered. Using this following tests for various chemical constituents were carried out, seperately. A drop of extract was spotted on a small piece of precoated TLC plate and the plate was sprayed with modified dragendorffs reagent. Orange coloration of the spot indicated presence of alkaloids. The extract was treated with a few ml of Hager’s reagent yellow precipitate indicated the presence of alkaloids [4].

**Test for saponins**
To 1ml of the extract was dissolved separately with distilled water to 20ml and shake with graduated cylinder for 15 minutes 1 cm indicated the presence of saponins [4].

**Test for glycosides**
The extract 0.1gm was dissolved in pyridine 2ml and sodium nitroprusside solution 2ml was added and made alkaline with sodium hydroxide solution pink to red colour solution indicated the presence of glycosides [4].

**Test for carbohydrates**
Small quantity 300mg of the extract was dissolved in 4ml distilled water and filtered .The filtrate was subjected to molisch test formation of reddish ring indicated the presence of carbohydrate [4].

**Test for phenolic compound and tannins**
To 2-3ml of the extract, 10%alcoholic ferric chloride solution was added. Dark blue or greenish gray coloration of the solution indicated the presence of tannins [4].

**Test for proteins and amino acids**
A small quantity of the extract was dissolved in a few ml of water and subjected to ninhydrin. Blue coloration indicated the presence of amino acids [4].
Test for flavonoids
To 2-3ml of the extract, a piece of magnesium ribbon and 1ml of concentrated hydrochloric acid were added. Pink red or red coloration of the solution indicated the presence of flavonoids [4].

Test for steroids/terpenoids
To 1ml of the extract of drug 1ml of chloroform, 2-3ml of acetic anhydride and 1 to 2 drops of concentrated sulphuric acid were added. Dark green coloration of the solution indicated the presence of steroids and dark pink or red coloration of the solution indicated the presence of terpenoids [4].

Test for fixed oils and fats
Small quantities of the extract were pressed separately between two filter papers. Oil stains on the paper indicated the presence of fixed oils [4].

Acute Toxicity Study
The acute oral toxicity study was carried out according to OECD 423 guideline (Organization for Economic Cooperation and Development). Healthy, young, female mice (23-25 gm) were used for this study. Animals were fasted prior to dosing. Group 1: Three animals were given 5 mg/kg of extract, orally. Group 2: Three animals were given 50 mg/kg of extract, orally. Group 3: Three animals were given 300 mg/kg of extract, orally. Group 4: Three animals were given 2000 mg/kg of extract, orally. Animals were observed individually 30 minutes after dosing, periodically during the first 24 hours and daily thereafter for total of 14 days [5].

Dose Selection
Dose was selected on the basis of maximum tolerable dose (NOAEL), as there was no lethality observed up to 2000 mg/kg. Thus dose was selected as 1/10th and 1/5th of 2000 mg/kg, i.e. 200 mg/kg and 400 mg/kg for further investigation.

Animals
Healthy Wistar rats (150 to 200g) of either sex were housed under uniform husbandry conditions and given pelleted diet and water ad libitum. All animals experiments were approved by Institutional Animal Ethical Committee (IAEC) of PBRI Regd. No. 1283/C/09/CPCSEA norms.
Experimental design
30 Rats were divided into five groups of six animals each, for hepato toxicity study, Biochemical studies, Anti-oxidant studies and Histopathological studies. Crab extract, paracetamol (PA), silymarin and saline were administered with the help of feeding cannula. Group I served as normal control, which received saline. Group II received paracetamol 3g/kg.body weight.p.o on the 7th day. Group III received silymarin (25mg/kg. body weight) for 7 days and paracetamol of (3g/kg.body weight) on the 7th day. Group IV animals received OP crab extract (200mg/kg) once daily for 7 consecutive days and paracetamol on the 7th day. Group V animals received OP crab extract (400mg/kg) once daily for 7 consecutive days and paracetamol of on the 7th day [6]. After 24 hrs of paracetamol administration, blood samples were collected from retro-orbital plexus under ether anesthesia. The blood samples were allowed to clot and the serum was separated by centrifugation at 15000 rpm at 37\(^{\circ}\)c and used for the assay of biochemical marker enzymes. Serum glutamate oxaloacetate transaminase (SGOT) [7], Serum glutamate pyruvic transaminase (SGPT) [7], Alkaline Phosphate (ALP) [8], Bilirubin [9], and Total protein (TP) [10] were determined by using commercially available kits.

Histopathological studies
The animals were sacrificed by carotid bleeding and the liver was dissected out. The liver were washed immediately with saline and then fixed in 10% buffered formalin. The livers stored in 10% buffered formalin were embedded in paraffin, sections cut at 5 mm and stained with hematoxylin and eosin. These sections were then examined under a light microscope for histoarchitectural changes.

Statistical analysis
The data of all the results were represented as Mean ± S.E.M. on statistically analysed by one-way ANOVA followed by Turkey Kramer post test was used for statistical analysis. P ≤ 0.001 was considered significant.

RESULTS
The result of percentage yield of crab extract
The dried granules of the Whole Animal of *Ocypode platytarsis* (OP) extracted with ethanol + water (80:20) in soxhelt apparatus by simultaneous extraction for 48hrs. The percentage yield of extract was 18.5 % of raw material (Table 1).
Phytochemical analysis OP extract

Phytochemical analysis of *Ocypode platytarsis* (OP) extract demonstrates the presence of carbohydrates, proteins, steroids, glycosides, flavanoids and alkaloids (Table 2). Saponins, phenolic compounds, tannins, fixed oil and fats were absent.

**Acute Toxicity**

None of the 3 rats died or showed any sign of toxicity at the limit dose of 2000mg/kg/oral in the first 48 hours and no evidence of toxicity was noted during the period of observation. The LD$_{50}$ in rats was therefore taken as above 2000mg/kg/oral.

**The result of OP on serum chemical parameters against paracetamol induced hepatotoxicity in rats**

The effects of ethanolic extract of *Ocypode platytarsis* in serum transaminases, alkaline phosphatase, bilirubin and protein level in paracetamol intoxicated rats are summarized. There was a significant ($p \leq 0.001$) increase in bilirubin level, SGOT, SGPT, and ALP, in paracetamol intoxicated group compared to the normal control group (Table 3). The total protein levels were significantly ($P \leq 0.001$) decreased in paracetamol-intoxicated rats. On the other hand group received both crab extracts and paracetamol showed highly significantly decreased the elevated serum marker enzymes when given orally and reversed the altered total protein to almost normal level (Table 3).

**Tables 1:** Results of the extractive value of the ethanolic extract of dried granules of the Whole Animal of *Ocypode platytarsis*.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Part of animal used</th>
<th>Solvent</th>
<th>colour</th>
<th>Extractive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Whole crab</td>
<td>Ethanol</td>
<td>Brown</td>
<td>18.5 %</td>
</tr>
</tbody>
</table>

**Table 2:** Results of the preliminary phytochemical constituents of the ethanolic extract of dried granules of the Whole Animal of the *Ocypode platytarsis*.

<table>
<thead>
<tr>
<th>Phytochemical constituents</th>
<th>Ethanolic extract of <em>Ocypode platytarsis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>+ve</td>
</tr>
<tr>
<td>Proteins</td>
<td>+ve</td>
</tr>
<tr>
<td>Steroids</td>
<td>+ve</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-ve</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+ve</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>+ve</td>
</tr>
<tr>
<td>Saponins</td>
<td>-ve</td>
</tr>
</tbody>
</table>
Table 3: Results of the effect of ethanolic extract of *Ocypode platytarsis* on serum biochemical parameters against paracetamol induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>BILIRUBIN (mg/dl)</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>TP (gm/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.8±0.145</td>
<td>245.5±11.54</td>
<td>258.5±16.07</td>
<td>528.5±3.82</td>
<td>6.4±8.48</td>
</tr>
<tr>
<td>PA alone</td>
<td>1.2±1.130*</td>
<td>675.5±33.71*</td>
<td>834.5±72.86*</td>
<td>690±4.63*</td>
<td>3.8±1.50*</td>
</tr>
<tr>
<td>Silymarin + PA</td>
<td>0.8±0.167***</td>
<td>483.5±12.88***</td>
<td>612±27.43***</td>
<td>368±4.75***</td>
<td>6.2±13.54**</td>
</tr>
<tr>
<td>OP 200mg/kg + PA</td>
<td>0.6±0.121***</td>
<td>274±13.54***</td>
<td>358±13.53***</td>
<td>385±5.71***</td>
<td>6±9.79***</td>
</tr>
<tr>
<td>OP 400mg/kg + PA</td>
<td>0.6±0.394***</td>
<td>207.3±6.81***</td>
<td>268.6±27.35***</td>
<td>360.3±2.42***</td>
<td>6.3±9.25*</td>
</tr>
</tbody>
</table>

Values were expressed as Mean ± S.E.M. number of rats = 6. *P*≤0.01 Compared with Control group, ***P*≤0.001 compared with paracetamol (PA) group.

*Ocypode platytarsis* (OP), Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvic transaminase (SGPT), Alkaline Phosphate (ALP), Bilirubin, and Total protein (TP).

Figure: 1 Histopathological changes occurred in the liver after paracetamol intoxication and prevention by the treatment with Ethanolic extract of *Mucuna capitata roxb* extract. a) control, b) Paracetamol alone, c) Silymarin, d) OP extract 200 mg/kg, e) OP extract 400 mg/kg.

**Fig 1a.** Histology of the liver sections of normal control animals showed normal morphology.

**Fig 1b.** The section of the liver tissue of rats treated with PA, severe hepatotoxicity was observed by Necrosis, degeneration and disarrangement in hepatic cells.

**Fig 1c.** In silymarin treated group normal architecture of all hepatic constitution was found.

**Fig 1d.** In group OP 200mg/kg inflammatory cells and damage in few hepatocytes was observed.
Fig 1e. In group OP 400mg/kg treated group showed less hepato damage.

Histopathological studies

The hepatoprotective potential of OP crab extract was confirmed by histological examination of normal and treated rats. The histological profile of normal rats showed normal cellular architecture (Fig 1a). In paracetamol intoxicated animals, there were drastic alterations in the histological architecture of the liver. Histological examination showed distended hepatocytes, fatty degeneration and area of necrosis (Fig 1b). The administration of OP (200mg/kg and 400mg/kg) extract and silymarin brought about significant recovery. There was less degeneration of hepatocytes as a result of marked regeneration activity (Fig 1 c - e).
DISCUSSION
Liver is the vital organ of metabolism and excretion. About 20,000 deaths found every year due to liver disorders [11]. Liver injury caused by toxic chemicals and certain drugs has been recognized as a toxicological problem. Hepatotoxicity is one of very common aliment resulting into serious deilities ranging from severe metabolic disorders to even mortality [12]. Exposure to drugs and chemicals often induce toxicity to living organisms. Factors determining the toxicity include the pharmacokinetics of the compound, the metabolic fate of the compound and the target organ ability to respond to the toxic insult. During the last decades considerable attention has been focused on the involvement of oxygen free radical (OFR) in various diseases. Active oxygen molecules such as superoxide and hydroxyl radicals have been demonstrated to play important role in the inflammation process produced by ethanol, carbon tetrachloride and paracetamol [13].

Paracetamol is widely used as antipyretic and analgesic drugs, causes hepatotoxicity if taken in the excess amount of dose. The mechanism of hepatotoxicity is firstly cause the necrosis of Centilobular hepatocytes and followed by lipid peroxidative degradation of glutathione and produces cell necrosis in liver due to the formation of intermediate oxidative product of paracetamol (N-acetyl-P-benzoquinoneimine) [14]. That means CYP2E1 converts Acetaminophen (APAP) into reactive intermediate, N–acetyl-p-benzoquinoneimine which is responsible for liver necrosis. NAPQI depletes glutathione and covalently binds to proteins loss of glutathione with an increased formation of reactive oxygen and nitrogen species in hepatocytes undergoing necrotic changes. Increased oxidative stress, linked with alterations in calcium homeostasis and beginning of signal transduction responses, causing mitochondrial permeability transition mitochondrial membrane potential, and loss of the ability ATP production, leads to the necrosis [15].

The results of present study demonstrated that Ocypode platytarsis (OP) crab extract had potent hepatoprotective activity against paracetamol induced liver damage in rats. Liver damage induced by PA is commonly used model for the screening of hepatoprotective drugs. In our study, we observed that raise in the serum levels of bilirubin, SGOT, SGPT and ALP showed the structural damage of liver in rats. Serum levels of these enzymes are very sensitive markers employed in the diagnosis of liver diseases. When the hepatocellular plasma membrane is damaged, the enzymes normally present in the cytosol are released into the blood stream. This can be quantified to assess the type and extent of liver injury. ALP is
excreted normally via bile by the liver. The liver injury due to toxins can result in defective excretion of bile by hepatocytes which are reflected as their increased levels in serum [16].

The co administration of silymarin with PA showed significant decrease in the marker enzymes like SGOT, SGPT, ALP and bilirubin and raised the levels of TP in serum. The prior administrations of OP extract prevented the increased serum marker enzymes SGOT, SGPT and ALP level and also bilirubin level in animals. OP extract of 200 mg/Kg and 400 mg/Kg were administered that showed the dose dependent prevention of the toxicity in liver. And we observed significantly increased the levels of TP in serum.

In PA treated animals histological examination reveal degeneration in centrilobular fatty section. Fatty changes were present moderately in centribular area. Necrosis, degeneration and disarrangement in hepatic cells were observed. Degeneration of cytoplasmic area was also found. Lobular inflammation, changes in lobular architect was also observed due to focal necrosis was present. Inflammation in sinusoids was also observed due to focal necrosis was present. Inflammation in sinusoids was also observed. There are extensive areas of patchy and confluent hepatocytes necrosis and lobular inflammation; sinusoidal spaces are flooded with inflammatory cells and RBC’s. Rat liver of PA treated group animals, revealed the dilated and congested blood cells and fatty acid changes. In silymarin treated animals normal architecture of all hepatic constitution was found. Necrosis, degeneration and damage was found to be non significant. Regeneration in hepatocytes was also observed. Central veins, fatty parenchyma, portal vein, hepatic artery, bile duct, sinusoids, all were found to be normal.

In samples treated group protection was significantly observed. Both the treated groups showed mild to moderate improvement in toxicity. In group OP 200mg/kg treated group showed inflammatory cells and damage in few hepatocytes was observed. Damage to all other structure was less. In group 400mg/kg of the Ocypode platytarsis (OP) crab extract treated group showed the highest protection with no significant necrosis although signs of inflammation were present central vein, portal vein were also found to be normal with normal sinusoidal space.

CONCLUSION

The Ethanolic extract of Ocypode platytarsis crab at a dose of 400mg/kg, p.o showed statistically highly significant hepatoprotective activity. The histopathology studies supported
these results by normalizing the paracetamol induced liver damage. The extract showed significant activity against paracetamol induced liver damage in rats when compared with that of standard drug silymarin. We conclude that the present study reveal the hepatoprotective activity of *Ocypode platytarsis* against paracetamol induced liver damage in rats.

**ACKNOWLEDGEMENTS**
The author is thankful to Dr. R. Ramanjanayalu, Regional centre, Marine products export development authority, Andhra Pradesh for identification and authentication of crab.

**REFERENCE**


