HEPATOPROTECTIVE ACTIVITY OF ETHANOL EXTRACTS OF 
PHALLUSIA NIGRA AGAINST CCl4 INDUCED HEPATOTOXICITY 
IN RATS

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ABSTRACT

Ascidians are marine sedentary organisms. Phallusia nigra is a simple ascidian belonging to the family Asciidiidae. The present study was designed to screen and evaluate the hepatoprotective activity of ethanol extracts of Phallusia nigra against CCl4 induced hepatotoxicity in rats. Liver functions were assessed by the activities of liver marker enzymes, SGOT, SGPT, ALP and bilirubins. Silymarin, a known hepatoprotective drug is used for comparison. The animal extracts were effective in protecting liver against injury induced by CCl4 in rats.

KEYWORDS: Phallusia nigra, hepatoprotective activity, Silymarin.

INTRODUCTION

Liver diseases have become one of the major causes of morbidity and mortality all over world. From among, drug induced liver injury (DILI) is one of the most common causative factor that poses a major clinical and regulatory challenge.[1] Hepatitis and cirrhosis are the two diseases that can badly damage the liver.[2] Hepatitis is an inflammation of liver caused by certain viruses. Cirrhosis changes the structure of the liver and hence it fails to function properly leading to retention of toxins in the blood. Therefore, damage to the liver inflicted by hepatotoxic agents is of great concern.[3] Drug induced liver toxicity is a common cause of liver injury.[4] Carbon tetrachloride (CCl4) induced hepatotoxicity model is widely used for the study of hepatoprotective effects of drugs and plant extracts.[5,6] Phallusia nigra is a simple ascidian belonging to the family Asciidiidae occurring as the major component of fouling community on the hull of ships, piers, pilings, harbour installations and materials
used for aquaculture operations in the Tuticorin Port Area. Previous studies show that the animal possesses antipyretic\(^7\), analgesic, anaesthetic\(^8\), anti-inflammatory\(^9\), wound healing\(^\text{10}\) and antimicrobial activities\(^11,12\). No reports are available on the hepatoprotective activity of the simple ascidian *Phallusia nigra*. Hence the present study aims to investigate the hepatoprotective activity of ethanol extracts *Phallusia nigra* of on carbon tetrachloride (CCl\(_4\)) induced liver toxicity in rats.

**MATERIALS AND METHODS**

**Collection and identification**

*Phallusia nigra* (Plate.1) was collected from Green Gate area (8°48’N and 78°11’E) of Tuticorin Port, Tamil Nadu by SCUBA diving and identified using Key to identification of Indian ascidians\(^13\). A voucher specimen (AS 2083) was deposited in the Museum of the Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin 628002, Tamilnadu, India.

![Plate. 1: Phallusia nigra Sav.](image)

**Extraction of the animal material**

Epibionts adhering to the test of *Phallusia nigra* were carefully removed, washed several times with sterile sea water, dried under shade and powdered. 100 g of *Phallusia nigra* was exhaustively extracted with ethanol in a soxhlet apparatus, concentrated in a rotary vacuum evaporator and 15 g of a brown sticky mass was obtained.

**Experimental animal**

Mature adult Wistar albino rats of either sex weighing about 180 - 200 g were maintained in a well ventilated animal house at 25°± 2°C and humidity 60± 5% with constant 12 h of darkness and 12 h of light schedule. Clean boiled water and standard pellet diet (Hindustan Lever Ltd., India) were given ‘ad libitum’. All the animals were acclimatised to laboratory conditions prior to experiments. 2ml of 1% Vanillin was used as a flavouring agent to enhance the acceptability of the extract.
Acute oral toxicity studies
To determine the minimum lethal dose, acute oral toxicity studies were performed as per OECD guidelines.[14] Adult albino rats of either sex weighing 180 - 200 g were used. The animals were divided into six groups of six each. Group I was given 2 ml of 1% saline and Group II received 2 ml of 1% vanillin both acted as control. The other four groups were administered 50, 100, 200 and 500 mg/kgbw of the ethanolic extract with 2 ml of 1% vanillin orally using Intra Gastric Catheter respectively. All the experimental rats were fasted overnight. They were observed continuously for any gross behavioural changes and toxic manifestations like hyperactivity, grooming, convulsions, sedation, hypothermia and mortality during the first three hours. Thereafter the animals were continuously monitored at regular intervals for 7 days. No adverse effect or mortality was detected in this study up to 500 mg/kg bw dose. Hence sub-lethal dose of 200 mg/kg bw doses of the extract were selected for the experiments.

Experimental protocol: Animals were divided into eight groups of six rats each and treated orally as below for 15 days.
Group-I: served as normal control received distilled water.
Group-II: CCl₄ induced control 2.5ml/kg bw
Group-III: 100 mg/kg bw of animal extract.
Group-IV: 150 mg/kg bw of animal extract.
Group-V: 200 mg/kg bw of animal extract.
Group-VI: Standard drug Silymarin 100 mg/kg bw.

Assessment of Hepatoprotective Activity
In the present study the hepatoprotective activity was evaluated biochemically and histopathologically. After 72 hours of drug treatment, the animals were dissected under ether anesthesia. Blood from each rat was withdrawn from carotid artery at the neck and collected in previously labeled centrifuging tubes and allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 3000 rpm for 15 minutes. The separated serum were used for the estimation of some biochemical parameters like Alanine aminotransferase (ALT/SGPT), Aspartate aminotransferase (AST/SGOT), cholesterol, bilirubin and glucose. For histopathological study, liver from each animal was removed after dissection and preserved in 10% formalin. Then representative blocks of liver tissues from each lobe were taken and possessed for paraffin embedding using the standard microtechnique.[15] Sections
(5μm) of livers stained with hemotoxylin and eosin, were observed microscopically for histopathological studies.

RESULTS AND DISCUSSION
The results of hepatoprotective activities of crude ethanol extracts of the animal were illustrated in the Table 1.

Table 1: Effects of ethanol extract of Phallusia nigra on various biochemical parameters in rats with carbon tetrachloride induced hepatotoxicity.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGOT Level (U/L)</th>
<th>SGPT Level (U/L)</th>
<th>ALP (U/L)</th>
<th>Bilirubin (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>42.56±2.51</td>
<td>43.26±2.11</td>
<td>164.56±10.21</td>
<td>0.83±0.11</td>
<td>108.66 ± 1.11</td>
<td>92.33 ± 3.48</td>
</tr>
<tr>
<td>Group II</td>
<td>136.53±2.6***</td>
<td>143.56±5.29***</td>
<td>269.36±8.4*</td>
<td>2.59±0.34*</td>
<td>229.67 ± 0.75</td>
<td>90.66 ± 2.45*</td>
</tr>
<tr>
<td>Group III</td>
<td>59.53±4.27a</td>
<td>52.26±1.98a</td>
<td>139.54±6.23a</td>
<td>1.14±0.21</td>
<td>181.21±0.74</td>
<td>78.24±4.05a</td>
</tr>
<tr>
<td>Group IV</td>
<td>36.53±4.29aa</td>
<td>41.53±1.33aa</td>
<td>129.36±2.6aa</td>
<td>0.79±0.03aa</td>
<td>156.26±8.21</td>
<td>70.98±7.82aa</td>
</tr>
<tr>
<td>Group V</td>
<td>30.83±3.67aa</td>
<td>36.27±2.98aa</td>
<td>117.62±2.3aa</td>
<td>0.62±0.98aa</td>
<td>123.45±3.68</td>
<td>65.63±3.21aa</td>
</tr>
<tr>
<td>Group VI</td>
<td>30.59±1.91aa</td>
<td>35.26±1.04aa</td>
<td>131.27±9.34a</td>
<td>0.87±0.11a</td>
<td>89.24±24.78</td>
<td>60.43±2.13aa</td>
</tr>
</tbody>
</table>

Each value is SEM ± 5 individual observations *p < 0.05; **p<0.01 Compared with normal control vs liver injured rats a: p < 0.05; aa p<0.01 Compared liver injured rats vs drug treated rats.

Carbon tetrachloride group significantly increased the serum level of SGPT, SGOT, Bilirubin and cholesterol shown in Table 1. Extract treated groups significantly reduced serum SGOT (36.27±2.98aa), SGPT (36.27±2.98aa), Bilirubin (0.62±0.98aa) and glucose (65.63±3.21aa) when compared to that of the standard. Results of histopathological studies provided supportive evidence for biochemical analysis.

Fig.1: Microscopical view of liver tissue of normal rat.
Liver damage induced by CCl₄ is commonly used model for the screening of hepatoprotective drugs. In the present study, there is significant hepatic damage in CCl₄ intoxicated rats as shown by the increase in the levels of SGOT, SGPT, Bilirubin etc. The rise in serum levels of AST, ALT and cholesterol has been attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation after cellular damages. Administration of rats with ethanol extracts of Phallusia nigra
significantly restored towards their normal value. The normalization of serum markers by ethanol extracts of *Phallusia nigra* suggests that they are able to condition the hepatocytes so as to protect the membrane integrity against CCl$_4$ induced leakage of marker enzymes into the circulation. The above changes can be considered as an expression of the functional improvement of hepatocytes.

Alkaline phosphatase, a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins and alkaloids. The alkaline phosphatase is the prototype of these enzymes that reflects the pathological alteration in biliary flow.$^{[18]}$ Total bilirubin, a by-product of the breakdown of red blood cells in the liver, bilirubin is a good indicator of liver function. High levels will cause jaundice and are indicative of damage to the liver and bile duct.$^{[19]}$ The ethanol extracts of *Phallusia nigra* induced suppression of the increased ALP activity with the concurrent depletion of raised bilirubins suggests the possibility of the extract to have ability to stabilize biliary dysfunction in rat liver during hepatic injury by CCl$_4$. The reduction of the bilirubins levels by the *Phallusia nigra* extracts also suggest that, the extracts may activate the constitutive androstane receptor (CAR) which is a key regulator in bilirubin clearance in liver.$^{[20]}$

Histopathology of liver section of normal control animal exhibited normal hepatic cells each with well defined cytoplasm, prominent nucleus and nucleolus and well brought out central vein (Figure 1), whereas that of CCl$_4$ intoxicated group showed total loss of hepatic architecture with very centrilobular hepatic necrosis, fatty changes, insignificant changes rather than produced toxicity vacuolization and congestion of sinusoids, kupffer apoptosis (Figure 2). The animal extract treated groups (Figure 3 &4) returned the injured liver to quite normal. Now, it could be decided that the hepatoprotective activity was dose and time dependent.

**CONCLUSION**
The results reveal that the ethanolic extract *Phallusia nigra* has shown most pronounced hepatoprotective effect. Further characterization and purification of the individual component in this animal is suggested in formulating the strategy of treatment.

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