Hepatoprotective Activity of Bark Extracts of Pajanelia longifolia (Willd.) K. Schuman against CCl₄ Induced Hepatic Damage in Mice

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The World Health Organization (WHO) estimates that 35 million individuals in the World die every year from chronic diseases of which a significant portion is due to chronic liver disease. Liver being involved in several vital functions such as metabolism and detoxification of toxic substances is the important organ of the body. Although, numerous medicinal plants and their crude extracts have been reported for hepatoprotective activity, very insignificant number of drugs have come out to the market from all those works till date. Aim of the present study was to validate the hepatoprotective effect of Hexane extract and Methanolic extract of bark of Pajanelia longifolia (Willd.) K. Schuman (Bignoniaceae) against Carbon tetrachloride induced hepatic damage in swiss albino mice using different biochemical parameters and histopathological studies. Crude extracts were obtained from plant materials by maceration process with n Hexane followed by methanol. CCl₄ was intoxicated intraperitoneally (0.5 ml/kg body weight) to the mice of each group except group I, to which normal saline and liquid paraffin (1 ml/kg body weight orally) were given. Group II, receive only CCl₄ (0.5 ml/kg b.w.) intraperitoneally which was denoted as a negative control. Where, group III, IV and V received silymarin (stand. drug) (50 mg/kg b.w.o.), Methanolic extract and hexane extract of Pajanelia longifolia (200 mg/kg b.w.o, p.o.) respectively before 30 minutes of CCl₄ administration. The entire study was carried out for 5 days and animals were sacrificed on the 6th day. Pajanelia longifolia (Willd.) K. Schuman bark extracts was found to show hepatoprotective activity against CCl₄ induced elevations of SGPT, SGOT, SALP and total bilirubin level. Administration of Pajanelia longifolia methanolic extract at an oral dose of 200 mg/kg b.w. shows better effect against CCl₄ intoxication. The histopathological observations also supported the hepatoprotective potential of bark extract of the plant. The overall data indicated that Pajanelia longifolia (Willd.) K. Schuman possesses a potent protective effect against CCl₄ induced hepatic damage in mice.

Key words: Biochemical markers, Hepatoprotective activity, Histopathology, Pajanelia longifolia.

INTRODUCTION

Liver being involved in several vital functions such as metabolism and detoxification of toxic substances is the important organ of the body. Naturally, damage of liver by toxic agents is associated with distortion of these metabolic functions [1]. Liver diseases are mainly caused by chemical substances and environmental pollutions, malnutrition, viral infections [2]. The World Health Organization (WHO) estimates that 35 million individuals in the World die every year from chronic diseases of which a significant portion is due to chronic liver disease. But the liver protective drugs used in the treatment of liver diseases are not adequate. Therefore, it is always necessary to search for alternative drugs for the treatment of liver diseases [4]. Searching of natural hepatoprotective drug leads from medicinal plants may be one of the ways to find an effective drug for the disease. Although, numerous medicinal plants and their crude extracts have been reported for hepatoprotective activity by several workers [5], very insignificant number of drugs have come out to market from all those works till date.

Present investigation aims to validate the hepatoprotective activity of different bark extracts of Pajanelia longifolia (Willd.) K. Schuman (Bignoniaceae), which is traditionally known to be very effective hepatoprotective agent in south Assam, India. Scientific literature survey however revealed that no scientific work has so far been carried out to establish the possible hepatoprotective activity of the plant. Present study is therefore, focused to evaluate the hepatoprotective potentials of the bark of the plant against CCl₄ induced liver injury in swiss albino mice.

MATERIALS AND METHODS

Plants material: Pajanelia longifolia (Willd) K. Schuman was collected from Cachar District of Southern Assam part of North East India. The plant belongs to the family Bignoniaceae. The plant was identified at the Assam University Herbarium, Silchar and a voucher specimen (H-17) was deposited for future reference.

Preparation of plant extract: The air-dried and powered bark (500 gm) was defatted at room temperature with n-hexane followed by extraction with methanol (1500 ml). The extract was then concentrated using a rotary evaporator in vacu (IKA, Fischer Scientific). Qualitative phytochemical screening of the crude extracts were performed following the method suggested by Siddiqui and Ali [6].

Animals: Ethical committee approval was sorted prior to the experiment. Swiss Albino mice (24-26 g) of either sex were used as animal model. The animals were maintained under controlled conditions with 12h light and dark cycle throughout the period of experiment. They were provided with standard laboratory diet and given tap water ad libitum.

Chemicals: All the chemicals were of analytical grade and purchased from Merck India Ltd., Mumbai, India.

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Biochemical Assay kit for Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), Serum alkaline phosphatase (ALP) and total bilirubin were obtained from Ozone Pvt. Ltd. India and Silymaran from Ranbaxy India Ltd.

Carbon tetrachloride induced hepatotoxicity: The hepatic injury was induced using carbon tetrachloride (CCl₄) as suggested by Ozbek et al.[8] with slight modifications. Animals were divided into five groups with six animals each as follows

**Group I**: normal control, received liquid paraffin (1ml/kg b.w.p.o.) for 5 days;

**Group II**: negative control, treated with CCl₄ (0.5ml/kg b.w. i.p.) for 5 days;

**Group III**: positive control treated with Silymarin (50mg/kg b.w. p.o.) and then after 30 minutes, 0.5 ml/kg b.w. of CCl₄ intoxicated intraperitoneally for 5 days;

**Group IV**: treated with methanol extract (200mg/kg b.w. p.o) and then after 30 minutes, 0.5 ml/kg b.w. of CCl₄ intoxicated intraperitoneally for 5 days.

**Group V**: treated with hexane extract (200mg/kg b.w.p.o) and then after 30 minutes, 0.5 ml/kg b.w. of CCl₄ intoxicated intraperitoneally for 5 days.

On the 6th day the animals were sacrificed to assess the liver function and histological changes.

** Behavioural effect and Toxicity:** Toxicity study was performed as per OECD guideline 423. Swiss Albino mice weighing 24-26g were used for toxicity studies. The animals were then treated with graded dose (200, 400, 600, 800, 1000, 1200, 1400, 1600 and 2000mg/kg b.w. p.o.) of the extracts. After dose administration the individual animals were under observation for any symptoms of toxicity and abnormality in the behavior up to 48 h, followed by daily observation for mortality up to 21 days. After 21 days, blood was collected and liver was excised out for histological observations.

**Histopathological observation:** Liver tissue were fixed in 10% formalin and were graded with ethanol. The paraffin embedded liver tissue were cut into thin sections, stained with Haematoxylin-Eosin dye and observed under microscope (BX41, OLYMPUS) to note the changes in the liver tissue.

**Biochemical assay:** Animals were anesthetized using chloroform and blood was collected after an overnight fast by cardiac puncture. Blood samples were allowed to coagulate at room temperature for 45 min. and centrifuged at 2500 rpm for 15 min for separation of the serum. The serum was used for biochemical analysis, such as the serum glutamic pyruvic transaminase (SGOT), serum glutamic oxaloacetic transaminase (SGPT), serum glutamic pyruvate transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), serum alkaline phosphatase (ALP) and total bilirubin. The SGOT and SGPT level were measured as per the method suggested by Reitman and Frankle[9]. The total bilirubin content was measured as per the method of Grof[10] and the SALP level was measured as per the method of Sood[11].

**Statistical analysis:** The data presented here were expressed as mean ± SE. Results were analyzed by student’s t-test. The level of significance was accepted at p<0.05.

### RESULTS AND DISCUSSION

The preliminary phytochemical screening of both the extracts revealed the presence of secondary metabolites like alkaloids, steroids, tannin, reducing sugar and flavonoids. The acute toxicity of hexane and methanol bark extracts did not show any visible behavioral changes at any of the treatment concentrations. No mortality in mice could be observed after 21 days of treatment. In accordance with the acute toxicity studies, the dose concentration of 200 mg/kg body weight per orally of hexane bark extract and methanol bark extract was selected. Mice treated with a single dose of CCl₄ developed significant hepatic damage as observed from elevated serum levels of different liver function parameters (Table 1). Level of SGOT, SGPT, alkaline phosphatase and bilirubin in serum were increased in CCl₄ intoxicated animals (Table 1). Treatment with methanol bark extract (200mg/kg body weight p.o.) of **Pajanelia longifolia** afforded a significant protection against CCl₄ induced increase in the serum enzyme levels and also in bilirubin level in comparison to bark extract with hexane. The liver histopathology of the control group showed normal hepatocytes (Fig. 1a) whereas the CCl₄ intoxicated treatment exhibited localized areas of necrosis (Fig. 1b) and the pre treatment with (50mg/kg body weight) of Silymarin showing normalization of liver architecture (Fig. 1 c) and 200mg/kg body weight of hexane extract and methanolic extract of **Pajanelia longifolia** exhibited healing of necrotic lesions (Fig. 1d, e respectively).

### Table 1. Effect of Pajanelia longifolia bark extract on CCl₄ induced hepatic damage in swiss Albino mice (n=3)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I (Normal control)</th>
<th>Group II (CCl₄ 0.5 ml/kg b.w.i.p.)</th>
<th>Group III (Silymarin 50 mg/kg b.w.i.p.)</th>
<th>Group IV (Methanol extract 200mg/kg b.w.i.p.)</th>
<th>Group V (Hexane extract 200mg/kg b.w.i.p.)</th>
</tr>
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<tbody>
<tr>
<td>SGOT</td>
<td>26.0±2.58</td>
<td>78.58±8.15</td>
<td>35.14±4.31</td>
<td>39.1±5.34</td>
<td>48.98±1.27</td>
</tr>
<tr>
<td>SGPT</td>
<td>18.85±1.81</td>
<td>57.88±2.62</td>
<td>23.08±3.04</td>
<td>34.27±3.13</td>
<td>51.85±2.33</td>
</tr>
<tr>
<td>SALP</td>
<td>41.67±2.51</td>
<td>127.24±12.2</td>
<td>62.12±4.50</td>
<td>73.58±3.41</td>
<td>90.13±4.34</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>0.31±0.02</td>
<td>3.75±0.25</td>
<td>0.74±0.14</td>
<td>0.86±0.15</td>
<td>1.63±0.30</td>
</tr>
</tbody>
</table>

Group II was compared with: Group I and all values were significantly different; Group III, Group IV and Group V were compared with Group II and p-values were calculated by student’s t-test and the level of significance was accepted at p<0.05.

### DISCUSSION

Since the changes associated with CCl₄ induced liver damage are similar to that of acute viral hepatitis[12], CCl₄ mediated hepatotoxicity was considered as experimental model for liver injury. It has been established that CCl₄ is accumulated in hepatic parenchymal cells and metabolically activated by cytochrome p450 dependent monoxygenase to form a trichloro methyl free radical CCl₃ which alkylates cellular proteins (including cytochrome p450) and other macromolecules[13] with a simultaneous attack on polyunsaturated fatty acids in the presence of oxygen to produce lipid peroxides[14] leading to liver damage[15]. A significant increase in the activities of SGOT, SGPT, Alkaline phosphatase and bilirubin was recorded after 5th day of CCl₄ intoxication, indicating considerable hepatocellular injury. Pretreatment with Silymarin and **Pajanelia longifolia** extracts attenuated these increased enzyme activities produced by CCl₄ and a subsequent recovery towards normalization of these enzymes strongly suggests the possibility of **Pajanelia longifolia** extracts being...
able to condition the hepatocytes so as to cause accelerated regeneration of parenchymal cells, thus protecting against membrane fragility decreasing the leakage of marker enzymes into the circulation. But the degree of protection was observed maximally with methanol extract (200mg/kg b.w. p.o.) compared to hexane extract (200mg/kg b.w. p.o.). However, the protection offered by Silymarin (50mg/kg b.w. p.o.) seemed relatively greater. The histopathological study also revealed that, the hepatic cells in Pajanelia longifolia methanolic extract (200mg/kg b.w. p.o.) treated group V in contrast with group II which received only CCl4.

CONCLUSION

The studies on various biochemical parameters like SGPT, SGOT, SALP and total bilirubin level validate that the methanolic extract of stem bark of Pajanelia longifolia at a dose of 200mg/kg body weight has hepatoprotective activity which is almost similar to that of standard drug silymarin at a dose of 50 mg/kg body weight. Thus, methanolic extract of bark of Pajanelia longifolia can be considered as an effective hepatoprotective agent, as it ameliorates almost to normalcy the damage caused by CCl4 to hepatic function. However, mechanism of action of the crude extract in keeping hepatic damage lower needs to be investigated further.

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