PUBLICATION
EFFECT OF METHANOL EXTRACT OF ACHYRANTHES ASPERA L. ON AFLOTOXICOSIS RATS

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Key words: Achyranthes aspera, Aflotoxin, Serum enzymes, Histopathology, Hepatoprotection.

Abstract—Methanolic extract of root parts of Achyranthes aspera L. (Amaranthaceae) as tested for its effect on aflotoxicosis rats at five dose levels. Aflotoxin intoxication in rats significantly (p < 0.001) elevated the levels of SGPT, SGOT, ALKP, and total bilirubin, which indicated acute hepatocellular damage and biliary obstruction. Methanol extract showed dose dependent decrease in the levels of SGPT, SGOT, ALKP and total bilirubin. Minimum effective dose of extract was found to be 100 mg/kg body weight. Results obtained from histopathological studies also supported hepatoprotective activity against aflotoxin induced hepatotoxicity. Thus the study demonstrates that A. aspera possess anti-hepatotoxic effect against aflotoxin.

INTRODUCTION

Aflatoxins are well recognized as a cause of liver cancer, but they have additional important toxic effects. In farm and laboratory animals, chronic exposure to aflatoxins compromises immunity and interferes with protein metabolism and multiple micronutrients that are critical to health. These effects have not been widely studied in humans, but the available information indicates that at least some of the effects observed in animals also occur in humans (Jonathan et al., 2004). Aflatoxin is a common contaminant of foods, particularly in the staple diets of many developing countries. The toxicity of aflatoxin also varies according to many nutritional factors (Pier et al., 1985) and recovery from protein malnutrition is delayed by exposure to aflatoxin (Rogers, 1993 and Adhikari et al., 1994).

Conventional or synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. So there is a worldwide trend to go back to traditional medicinal plants. Many natural products of herbal origin are in use for the treatment of liver ailments. Drug-induced hepatotoxicity is a potentially serious adverse effect of the currently used antitubercular chemotherapeutic regimens containing isoniazide, aflatoxin and pyrazinamide. It has been established in experimental animals that antitubercular drugs administered in toxic doses affect the liver, its membranes and organelles. This is supported by release of aspartate and alanine aminotransferases and alkaline phosphatase in serum (Parthasarathy et al., 1986 and Shakun and Tabachuk, 1982)

Achyranthes aspera Linn. (Family: Amaranthaceae) commonly known as Apamarga is herb, occurs throughout India and Tropical Asia (Nadkarni and Nadkarni, 1976). Various parts of the herb A. aspera have been studied for several pharmacological actions. Saponin isolated from A. aspera was tested for its effect on the phosphorylase activity of rat heart (Ram et al., 1971). Extracts of A. aspera were evaluated for their hypoglycemic effect in normal and alloxan diabetic rabbits (Akhtar and Iqbal, 1991).

Abortifacient principle was isolated from A. aspera (Pakrashi and Bhattacharya, 1977). Formulation containing equal parts of A. aspera, Chicorium intybus and Berberis aristata were put to therapeutic trial in paracetamol-induced hepatopathy in sheep (Bhaumik and Sharma, 1993). Therefore present study was aimed to evaluate anti-hepatotoxic effect of methanol extract of A. aspera against aflatoxin-induced hepatotoxicity.
MATERIALS AND METHODS

Plant material
Root parts of *A. aspera* were collected from outfield near Muthayammal College of Arts and Sciences, Rasipuram, Tamilnadu, India. The plant was authenticated by comparison with reference specimens preserved at the Rapmat Herbarium, St: Joseph's College, Tiruchirapalli. Voucher Herbarium specimens are kept in the Herbarium for future references. The root parts of the plant were properly cleaned and dried first in air and then artificially in oven at 60°C for approximately 4 hrs.

Extract preparation
Soxhlet extraction of powdered root parts afforded methanol extract in 13.0% (w/w) yield. Extract gave positive tests for alkaloids, flavonoids and saponins on phytochemical screening (Kokate, 1996).

Animals
Either sex of Wistar albino rats were purchased from our Animal Breeding Laboratory, Muthayammal College of Arts and Science, Rasipuram, Namakkal and were kept for one week on a commercial diet under environmentally controlled conditions (room temperature 22 ± 3°C relative humidity 60-80%. 12h light dark cycle with free access to food and water). Rats weighing 150-200g were used for further treatment.

Experimental design
Rats were divided into control, aflotoxin and test (aflotoxin + extracts) groups. Aflotoxin suspended in 5% acacia was administered 1 mg/kg, per oral (p.o.) (Kurma and Mishra, 1996). The control group of rats received 5% w/v acacia mucilage (1 ml/kg, p.o.) four times at 12 hr intervals. The aflotoxin group of rats received 5% w/v acacia mucilage (1 ml/kg, p.o.) four times at 12 hr intervals and a single dose of aflotoxin 1mg/kg, 30 minutes after the administration of first dose of acacia mucilage. The test groups received test suspensions (50-800 mg/kg, p.o.) four times at 12 hr intervals. Aflotoxin (1 mg/kg, p.o.) was administered 30 minutes after the administration of first dose of suspensions. Silymarin (100 mg/kg) was used as a standard hepatoprotective drug for comparison.

Forty-eight hours after aflotoxin administration blood was collected from retro orbital plexus. The blood samples were allowed to clot for 15 minutes at room temperature. Serum was separated by centrifugation at 2500 rpm for 15 minutes and analyzed for biochemical parameters such as serum glutamic oxaloacetate transaminase (SGOT) (Reitman and Frankel, 1957), serum glutamic pyruvic transaminase (SGPT) (Reitman, and Frankel, 1957), and alkaline phosphatase (ALKP) (Kind and King, 1954) and total bilirubin (T.Bil.) (Malloy and Evelyn, 1937). Liver from one animal of each group was isolated to study the histopathological changes.

Statistical analysis
Results of the biochemical estimations are reported as mean ± standard error of mean (S.E.M.). To assess variations, present in a set of data was estimated using one-way analysis of variance (ANOVA). Student's t-test was used for determining significance.

RESULTS
Rats treated with aflotoxin developed significant liver damage as observed from elevated serum levels of hepatospecific enzymes as well as several alterations in other biochemical parameters (Table 1). Activities of SGPT, SGOT and ALKP in serum were increased in aflotoxin-intoxicated rats. A marked elevation in the concentration of bilirubin, was observed in the hepatotoxic-treated rats. Treatment with methanol extract of *A. aspera* showed significant protection against aflotoxin-induced alterations in the serum enzyme levels and bilirubin (Table 1). Table 1 shows that methanol extract in dose dependent manner decreasing the increased levels of SGPT, SGOT, ALKP and total bilirubin due to aflotoxin. Minimum effective dose was found to be 100 mg/kg. The degree of protection was observed maximally with the highest dose of the *A. aspera* (i.e., 800 mg/kg body weight). Results obtained indicated the drug offers comparable protection to aflotoxin induced liver damage as compared to silymarin (Table 1). Histopathological examination revealed hepatic degeneration, necrosis and fatty infiltration in aflotoxin treated rats indicating liver damage (Fig. 1). Rats treated with *A. aspera* and Silymarin showed less necrosis and normal histoarchitecture compared to aflatoxin indicating hepatoprotective effect of the drug (Fig. 2).

DISCUSSION AND CONCLUSION
The levels of number of hepatic enzymes are used as diagnostic indicators of hepatic injury. SGOT, SGPT and serum bilirubin are the most sensitive tests employed in the diagnosis of hepatic diseases (Harper, 1961). Elevated levels of serum enzymes are
Table 1. Effect of methanol extract of *A. aspera* L. on aflatoxin induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGPT</th>
<th>SGOT</th>
<th>ALKP</th>
<th>T. Bil.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>19.90 ± 5.82</td>
<td>33.205 ± 5.14</td>
<td>36.41 ± 6.13</td>
<td>0.30 ± 0.06</td>
</tr>
<tr>
<td>Aflotoxin</td>
<td>137.71 ± 21.70***</td>
<td>169.15 ± 6.86***</td>
<td>193.21 ± 37.61***</td>
<td>2.02 ± 0.14***</td>
</tr>
<tr>
<td>Test ext. (50 mg/kg)</td>
<td>109.72 ± 7.80 N.S.</td>
<td>105.81 ± 9.46**</td>
<td>118.56 ± 9.18**</td>
<td>1.31 ± 0.17**</td>
</tr>
<tr>
<td>Test ext. (100 mg/kg)</td>
<td>56.24 ± 8.99***</td>
<td>92.32 ± 11.03***</td>
<td>83.57 ± 5.78***</td>
<td>0.96 ± 0.05***</td>
</tr>
<tr>
<td>Test ext. (200 mg/kg)</td>
<td>36.04 ± 5.93***</td>
<td>70.75 ± 10.18***</td>
<td>70.22 ± 11.91***</td>
<td>0.72 ± 0.07***</td>
</tr>
<tr>
<td>Test ext. (400 mg/kg)</td>
<td>37.56 ± 11.44***</td>
<td>59.54 ± 8.02***</td>
<td>71.12 ± 4.81***</td>
<td>0.75 ± 0.10***</td>
</tr>
<tr>
<td>Test ext. (800 mg/kg)</td>
<td>33.57 ± 5.20***</td>
<td>50.85 ± 4.83***</td>
<td>79.61 ± 8.01**</td>
<td>0.66 ± 0.13***</td>
</tr>
<tr>
<td>Silymarin</td>
<td>34.89 ± 3.22***</td>
<td>47.88 ± 4.57***</td>
<td>28.91 ± 2.72***</td>
<td>0.28 ± 0.05***</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. Aflotoxin group was compared with control group. Test extract groups were compared with aflotoxin group. **p<0.01; ***p<0.001; NS=Non Significant Values in parenthesis indicate the % reduction in relation to the aflotoxin group.

Fig. 1a. Photomicrograph showing normal liver architecture

Fig. 1b. Liver of rat receiving aflatoxin (Magnification 10X).

Effect of Methanol Extract of *Achyranthes Aspera* L. on Aflotoxicosis Rats

Oral administration of methanol extract of *A. aspera* at doses of 150-200 mg/kg body weight to rats caused a decrease in the activity of marker enzymes in serum in dose dependent manner, which may be a consequence of the stabilization of plasma membrane as well as repair of hepatic tissue damage caused by aflatoxin. The activity of serum alkaline phosphatase was also elevated during aflatoxin administration. Alkaline phosphatase is excreted normally via bile by the liver. In liver injury due to hepatotoxin, there is a defective excretion of bile by the liver which is reflected in their increased levels in serum (Roo, 1973).

Hyperbilirubinaemia is a very sensitive test to substantiate the functional integrity of the liver and severity of necrosis which increases the binding, conjugating and excretory capacity of hepatocytes that is proportional to the erythrocyte degeneration rate (Singh, 1998). Depletion of elevated bilirubin level together with the suppression of the activity of ALKP in the serum of rats treated with *A. aspera* suggests the possibility of drug being able to stabilize biliary dysfunction of rat liver during chronic injury with aflatoxin. Results obtained from histopathological study also shown signs of indicative of cellular leakage and loss of functional integrity of the cell membrane in liver (Drotman and Lawhorn, 1978). Administration of aflatoxin significantly raises the serum level of enzymes like SGPT and SGOT in rats as observed in our results.
Fig. 2. Photomicrographs showing effect of different doses of methanol extract of *A. aspera*.

- a - 50 mg/kg
- b - 100 mg/kg
- c - 200 mg/kg
- d - 400 mg/kg
- e - 800 mg/kg
- f - Silymarin, in aflatoxin-induced hepatotoxicity in rats. (Magnification 10X)
Effect of Methanol Extract of *Achyranthes Aspera* L. on Aflotoxicosis Rats

The hepatoprotective activity of drug against aflatoxin may be due to inhibitory effects on formation of the active metabolite which in turn reduces drug-metabolizing enzymes and actively and specifically binds to RNA polymerases and thereby inhibits the nucleic acid and protein synthesis (Bowman and Rand, 1982). Phytochemical screening of methanol extract revealed presence of saponins, alkaloids and flavonoids principally. However, the exact mechanism of action can only be explained when active principles are isolated and tested against hepatotoxins. It can be concluded from present study that methanol extract of *A. aspera* shows protective effect against hepatic injury caused by aflatoxin.

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