HEPATOPROTECTIVE POTENTIAL OF POLYHERBAL PREPARATION AGAINST CCl₄-
INDUCED LIVER TOXICITY IN RATS

Sarika Shrivastava* and Ritu Gilhotra

School of Pharmacy, Suresh Gyan Vihar University, Jaipur, Rajasthan, India.

ABSTRACT: Hepatoprotective activity of Polyherbal preparation against CCl₄-induced hepatic damage in rats was observed. The healthy control (normal), control, and standard drug Silymarin treated groups were also maintained for the comparison. The liver marker enzymes SGOT, SGPT, ALP and total Bilirubin were assessed in all the experimental groups. The changes in liver function parameters were significant in comparison to control group and the observed efficacy was comparable to standard drug. The efficacy of the polyherbal preparation was found to be dose dependent. From the present study it is evident that the Polyherbal preparation has no mortality in selected doses. Liver antioxidant markers were elevated significantly, while the serum and lipid parameters were maintained at normal levels compared to control groups. Histopathological examinations of the liver showed that extract and Silymarin have a protective role over the toxicity of paracetamol and carbon tetrachloride induced hepatotoxicity in rats. Hence Polyherbal preparation could be one of the best sources of natural hepatoprotective agents.

INTRODUCTION: Liver damage induced by CCl₄ is commonly used model for the screening of hepatoprotective drugs. The acute hepatotoxicity of free radicals which causes oxidative stress and membrane damage. These free radicals cause lipid peroxidation which results in hepatocellular damage and enhances formation of inflamed tissues. 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. Andrographis paniculata used widely to treat fever like chikungunya, swine –flu, typhoid, snake bite and common cold etc. It is an annual herb. The leaves are used traditionally in Asian traditional medicine and particularly in Ayurveda for treatment of various diseases and illness. Andrographis paniculata is an herbaceous plant in the family Acenthaceae, native to India and Sri Lanka. In North Eastern India the plant is known as Maha-tita literally “King of bitters”, known as various vermicular names. ¹⁻⁷

Annona muricata is a member of the Annonaceae family and is a fruit tree with a long history of traditional use. A. muricata, also known as sour sop, graviola and guanabana, is an evergreen plant that is mostly distributed in tropical and subtropical regions of the world. The fruits of A. muricata are extensively used to prepare syrups, candies, beverages, ice creams and shakes. A wide array of ethno medicinal activities is contributed to different parts of A. muricata, and indigenous communities in Africa and South America extensively use this plant in their folk. Nyctanthes arbor-tristis Linn. (Oleaceae) is popularly known as ‘Night Jasmine’
(English) or ‘Harsinghar’ (Hindi) due to the fact that its flowers emit a very strong and pleasant fragrance during the whole night. 8-14

MATERIALS AND METHODS:
Plant material: Plants materials were collected from the local market of Bhopal, Madhya Pradesh during the month of May –July, 2012. The specimens were identified and authenticated by Dr. Zia ul Hassan, Assistant professor, Department of Botany, Saifia College of Science & Education, Bhopal and their herbarium was deposited. The authentication number is safia/79.

Extraction: 15
Ethanolic Extraction: The plant materials so collected were cleaned properly and washed with distilled water to remove dust particles and dried in shade. The dried drugs were coarsely powdered and then exhaustively extracted with 50% ethanol in Soxhlet apparatus for 72 h. The ethanolic extracts so obtained were freed of solvent under vacuum. (Yield: 9.33 %)

Animals for experiment: Swiss albino rats were obtained from animal house VNS institute of Pharmacy with due permission from Institutional animal ethical committee (Registration Number. 778/03/c/cpcsa). Acute toxicity studies were conducted by using albino mice of either sex weighing between 20 and 25 gms and healthy adult male albino rats weighing between150 and 200 gms were selected for the study. The animals were acclimatized to standard laboratory conditions (temperature: 25±2 °C) and maintained on 12-h light: 12-dark cycle. They were provided with regular rat chow (Lipton India Ltd., Mumbai, India) and drinking water ad libitum.

Hepatoprotective activity: 16-22
Animals:
- Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%).
- Rats received standard rodent chow and water ad libitum. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h.

- Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.
- Individual animal was identified by marking on tail. Cage labels showing study number, sex, concentration, cage number etc.

Drugs and Chemicals:
- Carbon tetrachloride (Merck, Germany) was used in present study. Silymarin (Micro labs, India) was also used in present study as standard. All other chemicals and other biochemical used in the experiments were of analytical grade from different firms.

Acute toxicity studies:
- Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD, 2001). Animals were kept fasting providing only water, Andrographic paniculata, Night Jaismine, Annona squamosa and its Polyherbal preparation (50, 100, 150, 200, 300 mg/kg/day) was administered orally for 4 days of six groups of rats (n=6). The animals were kept under observation for mortality.

Carbon tetrachloride (CCL4) induced hepatotoxicity:
Treatment schedule: The Hepatoprotective activity was examined using the CCL4 induced hepatotoxicity. Animals were randomly divided into different groups comprising six rats each.

1. Group –I: Normal control (distilled water.)
2. Group –II: Carbon tetrachloride (CCL4, 1 ml/kg i.p.)
3. Group -III: Silymarin (50mg/kg, p.o.) + CCL4 (1 ml/kg i.p.)
4. Group -IV: Andrographic paniculata (100mg/kg, p.o.) + CCL4 (1 ml/kg i.p.)
5. Group -V: Andrographic paniculata (200mg/kg, p.o.) + CCL4 (1 ml/kg i.p.)
6. Group –VI: Night Jaismine (100mg/kg, p.o.) + CCL4 (1 ml/kg i.p.)
1. Group –I: Normal control (distilled water.)
2. Group –II: Carbon tetrachloride (CCl₄, 1 ml/kg i.p.)
3. Group -III: Silymarin (50 mg/kg, p.o.) + CCl₄ (1 ml/kg i.p.)
4. Group–IV: Polyherbal preparation (50 mg/kg, p.o.) + CCl₄ (1 ml/kg i.p.)
5. Group–V: Polyherbal preparation (100 mg/kg, p.o.) + CCl₄ (1 ml/kg i.p.)

**Polyherbal Preparation:**

- Group –VII: *Night Jaismine* (200 mg/kg, p.o.) + CCl₄ (1 ml/kg i.p.)
- Group –VIII: *Annona squamosa* (200 mg/kg, p.o.) + CCl₄ (1 ml/kg i.p.)
- Group –IX: *Annona squamosa* (200 mg/kg, p.o.) + CCl₄ (1 ml/kg i.p.)

**Administration of drug:** Animals were randomly divided into different groups comprising six rats each.

- Group I served as normal received distilled water.
- Group II was control, received vehicle for 5 days.
- Group III received standard silymarin (50 mg/kg p.o.) for 5 days.
- Groups IV -V were treated with *Andrographic paniculata* (100 & 200 mg/kg, p.o.) for 5 days.
- Groups VI-VII were treated with *Night Jaismine* (100 & 200 mg/kg, p.o.) for 5 days.
- Groups VIII-IX were treated with *Annona squamosa* (100 & 200 mg/kg, p.o.) for 5 days.
- Groups X -XI were treated with Polyherbal preparation (50 & 100 mg/kg, p.o.) for 5 days.

On third day, hepatotoxicity was induced in all groups by CCl₄ with olive oil 1 ml/kg, 1:1, i.p. not including normal group.

At the end of the experimental period the animals were sacrificed. The blood and liver tissue were used for the studies. Blood was collected after 48 h. Serum was separated by centrifugation at 2500 rpm at 30 °C for 15 minutes and utilized for the estimation of various biochemical parameters including Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Alkaline Phosphatase (ALP), Total bilirubin.

**Statistical analysis:** Each experimental value is expressed as the Mean ± SEM. Statistical calculations of the data were performed using ANOVA analysis. A probability of P < 0.05 was considered as significant.

**RESULTS:**

Carbon tetrachloride (CCl₄) induced hepatotoxicity:

**Effect of Polyherbal preparation on SGOT against carbon tetrachloride (CCl₄)** Induced Hepatotoxicity: Table 1 shows the effect of Polyherbal preparation (50 mg/kg and 100 mg/kg/ p.o.) on SGOT in CCl₄ induced hepatic damage. Hepatic injury causes elevated level of liver enzymes such as SGOT (serum glutamic oxaloacetic transaminase).

Treatment with Polyherbal preparation (50 mg/kg and 100 mg/kg) revealed comparable activity with reference standard silymarin (50 mg/kg). Polyherbal preparation, other plants extracts and Silymarin which was significantly (P<0.5) decreased the liver markers (Fig. 1 and 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (U/L)</th>
<th>Total Bilirubin (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group –I</td>
<td>92.08 ± 8.890</td>
<td>41.29 ± 5.607</td>
<td>116.38 ± 8.281</td>
<td>0.35 ± 0.063</td>
</tr>
<tr>
<td>Group –II</td>
<td>277.22 ± 14.516</td>
<td>155.90 ± 10.243</td>
<td>303.42 ± 18.403</td>
<td>1.92 ± 0.102</td>
</tr>
<tr>
<td>Group –III</td>
<td>119.97 ± 9.208***</td>
<td>58.47 ± 6.393***</td>
<td>154.57 ± 11.325***</td>
<td>0.54 ± 0.059***</td>
</tr>
<tr>
<td>Group –IV</td>
<td>162.12 ± 10.677**</td>
<td>89.86 ± 6.723**</td>
<td>200.72 ± 16.410**</td>
<td>0.83 ± 0.103**</td>
</tr>
<tr>
<td>Group –V</td>
<td>136.06 ± 8.687***</td>
<td>70.16 ± 7.726***</td>
<td>179.31 ± 12.008***</td>
<td>0.71 ± 0.097***</td>
</tr>
<tr>
<td>Group –VI</td>
<td>161.18 ± 11.356**</td>
<td>87.50 ± 5.366**</td>
<td>199.16 ± 11.380**</td>
<td>0.80 ± 0.060**</td>
</tr>
<tr>
<td>Group –VII</td>
<td>133.77 ± 10.822**</td>
<td>69.95 ± 5.878**</td>
<td>178.13 ± 11.460**</td>
<td>0.69 ± 0.078**</td>
</tr>
<tr>
<td>Group –VIII</td>
<td>161.55 ± 12.554**</td>
<td>88.90 ± 9.714**</td>
<td>197.17 ± 13.598**</td>
<td>0.81 ± 0.084**</td>
</tr>
<tr>
<td>Group –IX</td>
<td>136.12 ± 10.255***</td>
<td>71.67 ± 5.564***</td>
<td>180.75 ± 13.276***</td>
<td>0.72 ± 0.081***</td>
</tr>
<tr>
<td>Group X</td>
<td>130.09 ± 12.160***</td>
<td>69.29 ± 6.665***</td>
<td>168.44 ± 12.576***</td>
<td>0.65 ± 0.077***</td>
</tr>
<tr>
<td>Group –XI</td>
<td>125.45 ± 12.170***</td>
<td>63.50 ± 7.848***</td>
<td>159.45 ± 12.498***</td>
<td>0.59 ± 0.078***</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SEM at n=6, One way ANOVA followed by Dunnett's test. *P<0.05 compared to the CCl₄ control.
Effect of Polyherbal preparation on SGPT against against carbon tetrachloride (CCl₄) Induced Hepatotoxicity: Table 1 shows the effect of Polyherbal preparation (50 mg/kg and 100 mg/kg/ p.o.) on SGOT in CCl₄ induced hepatic damage. Hepatic injury causes elevated level of liver enzymes such as SGPT (serum glutamic pyruvic transaminase). Treatment with Polyherbal preparation (50 mg/kg and 100 mg/kg/p.o.) revealed comparable activity with reference standard silymarin (50mg/kg/p.o.). Polyherbal preparation, other plants extracts (100 and 200 mg/kg/p.o.) and Silymarin which was significantly (P<0.5) decreased the liver markers (Fig. 3 and 4).

Effect of Polyherbal preparation on ALP against carbon tetrachloride (CCl₄) Induced Hepatotoxicity: Table 1 shows the effect of Polyherbal preparation (50 mg/kg and 100 mg/kg/p.o.) on SGOT in CCl₄ induced hepatic damage. Hepatic injury causes elevated level of liver enzymes such as ALP (alkaline phosphatase). The present study revealed that, CCl₄ administration showed significant elevation in ALP which was significantly (P<0.5) reduced by treatment with Polyherbal preparation (50 mg/kg and 100 mg/kg/p.o.), other plants extracts (100 and 200 mg/kg/p.o.) and Silymarin (50 mg/kg /p.o.) treatments onwards till the end of the study (Fig. 5 and 6).
Effect of Polyherbal preparation on Bilirubin against against carbon tetrachloride (CCl₄) Induced Hepatotoxicity: Table 1 shows the effect of Polyherbal preparation (50 mg/kg and 100 mg/kg/ p.o.) on SGOT in CCl₄ induced hepatic damage. Hepatic injury causes elevated level of liver enzymes such as total bilirubin. The present study revealed that, CCl₄ administration showed significant elevation in percentage of bilirubin which was significantly (P<0.5) reduced by treatment with Polyherbal preparation (50 mg/kg and 100 mg/kg/p.o.), other plants extracts (100 and 200 mg/kg/p.o.) and Silymarin (50 mg/kg /p.o.) treatments onwards till the end of the study (Fig. 7 and 8).

DISCUSSION AND CONCLUSION: The effects of Polyherbal preparation on CCl₄-induced hepatotoxicity in rats were evaluated by recording changes in SGOT, SGPT, ALP and total bilirubin levels. The activities of SGOT, SGPT, ALP and total bilirubin in normal and all treated groups are shown in Table 3. The data in Table 3 demonstrate a trend of decreased levels of serum SGOT, SGPT, ALP and total bilirubin in the CCl₄-treated animals compared to the control. This effect was reversed in the animal groups that were given Polyherbal preparation only or and during treatment with CCl₄ (therapeutic group, protective group).
Carbon tetrachloride is a well-known hepatotoxic agent. It causes significant increases in serum levels of SGOT, SGPT, ALP and total bilirubin. However, serum levels fall to near normal levels as hepatic damage became more severe.

In the present study, the dose of CCl₄ was very high which may explain the contrary trend of decreased serum transaminases (SGOT and SGPT), ALP and total bilirubin which are probably due to decreased synthesis resulting from extensive damage of the hepatic cells. This damage appeared to inhibit or modulate by administration of Polyherbal preparation indicating restoration of hepatic cell function. The Polyherbal preparation significantly (P<0.05) reduced the liver enzymes levels in experimental animals shows that combined therapy has hepatoprotective action.

From the present study it is evident that the Polyherbal preparation has no mortality in selected doses. Liver antioxidant markers were elevated significantly, while the serum and lipid parameters were maintained at normal levels compared to control groups. Histopathological examinations of the liver showed that extract and Silymarin have a protective role over the toxicity of paracetamol and carbon tetrachloride induced hepatotoxicity in rats. Hence Polyherbal preparation could be one of the best sources of natural hepatoprotective agents.

In conclusion, Polyherbal preparation show pharmacological potential against CCl₄-induced hepatotoxicity due to synergism of plant extracts.

ACKNOWLEDGEMENT: I am thankful to staff of Suresh Gyan Vihar University for kind support.

CONFLICT OF INTEREST: The author declare no conflict of interest.

REFERENCES:


