Evaluation of Liver Protective Activity of Some Indigenous Plants Against Acute Paracetamol Toxicity in Rodents

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ABSTRACT

Aim: The aim of the present study was to evaluate the liver protective activity of some indigenous plants against acute paracetamol toxicity in rodents. Liver intoxication was induced by paracetamol drug at a dose level of 500 mg/kg b.w, p.o for 9 days. To conduct this study the hydroalcholic extract of Prunus persica, Calotropis procera and Canscora decussate were taken as test compounds. Methods: Rats (180-200 g) were used for all the study and they were divided into 9 groups containing 6 animals each. Rats in Group I served as normal control (distilled water) group, Group II served as toxic control (Paracetamol treated) group, Group III served as standard (Silymarin) group. The rats of groups IV, V, VI, VII, VIII and IX served as test control groups. Group IV, V received the hydroalcoholic extract of Prunus persica at the dose of 200 and 400 mg/kg b.w, p.o respectively for 9 days. Group VI, VII received the hydroalcoholic extract of Calotropis procera at the dose of 200 and 400 mg/kg b.w, p.o respectively for 9 days. Group VIII and IX received the hydroalcoholic extract of Canscora decussate at the dose of 200 and 400 mg/kg b.w, p.o respectively for 9 days. The degree of protection was measured by using biochemical parameters such as serum glutamate oxalate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), bilirubin and total protein and albumin. Results: Results of this study showed that the treatment of the toxic effect of the paracetamol were significantly controlled in the hydroalcoholic extract of plants treated groups. The hydroalcoholic extract of plants at 400 mg/kg b.w, showed significant reduction in elevated serum enzyme levels compared to paracetamol induced toxic group. The hydroalcoholic extract of Calotropis procera at a dose of 400 mg/kg, b.w showed the most significant hepatoprotective activity among all the test groups. Conclusion: From the results it was concluded that all the test plants extract possess significant Hepatoprotective activity which was manifested by restoration of serum biochemical parameters to nearer the normal values. On the basis of results obtained, it can also be concluded that the hydroalcoholic extract of Plants seems to have hepatoprotective activity which may be due to the presence of flavonoids.

Keywords: Prunus persica, Calotropis procera, Canscora decussate, Hepatoprotective activity, Serum enzymes, Silymarin.

INTRODUCTION

Liver is the major organ which plays a key roles in processing critical biochemical and physiological phenomena including metabolism and Detoxification of endogenous and xenobiotics compounds such as drugs and xenobiotics, homeostasis, growth, energy and nutrient supply. The toxicity of liver is a damage or injury to liver which is caused by various drugs, chemicals and other agents. Severity of liver damage or injury depends on degree of exposure, mild liver damage cause dysfunction but severe liver damage result in liver failure. However we do not have satisfactory liver protective drugs in allopathic medical practice for serious liver disorders. Nature has bestowed mankind with several plants which contains natural substances which cure diseases and promote health. Herbal drugs play a role in the management of various liver disorders most of which speed up the natural healing processes of the liver. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practice as well as traditional system of medicine in India. More than fifteen of these plants are evaluated for their hepatoprotective activities in light of modern medicine. Prunus persica, Calotropis procera and Canscora decussata were taken as test compounds to conduct this study.

Prunus Persica

Prunus persica L. (Peach) named as Amygdalus persica is a perennial and deciduous tree of the subfamily Prunoideae of the family Rosaceae. The leaves are insecticidal, sedative, diuretic, demulcent, expectorant, vermicidal and are used in leucoderma, and in piles. Leaf paste is used to kill worms in wounds, and fungal infections. The treatment of gastritis, whooping cough, and chronic bronchitis is carried out internally with leaves. Peach has an 86-89%
water content, a 7.5-8.5% sugar content (mainly sacarose, glucose and fructose), a 0.6-1.2% protein content, a 0.3% fat content, a 1.2-1.4% fibre content (mainly cellulose, pectins and hemicelluloses), a 0.63% acid content, a 0.8% mineral content (potassium being the main constituent), several vitamins (mainly from group B and C) and a 0.20-0.80% carotenoid content (mainly β-carotene). Table 1 shows the main components found in peas.

Total acids present in peaches are malic acid and, in a smaller proportion, citric and quinic acid. It also contains phenolic compounds, catequins and leucoanthocyanins. The presence of carotenoids and their derivatives is significant and more important than in other fruits. In particular, β-cryptoxantine, β-carotene and α-carotene (vitamin A precursors) have been detected, together with zeaxanthine, lycopene and xanthophyll, that gives peaches their characteristic colour.

**Calotropis procera**

Calotropis procera is a wild growing plant of family Asclepiadaceae. It is well known for its medicinal properties. A number of ethanomedicinal uses of the drug are reported. Whole plant was used either alone or with other herbs for the treatment of common diseases such as fever, rheumatism, indigestion, cold, eczema and diarrhoea. Paste of root bark was locally applied in the treatment of elephantiasis and Root bark powder was used to treat diarrhea and dysentery and it is an excellent substitute for ipecac. Traditionally it was used to treat cholera, extracting guinea worms and indigestion.

**Canscora decussata**

Canscora decussata is popularly known as “Shankhpushpi” and found throughout India, up to an altitude of 1300 m. It is also found to contain triterpines, alkaloids and

Table 1: Phytoconstituents present in hydroalcoholic extracts of *Prunus persica*, *Calotropis procera* and *Canscora decussata*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Chemical Constituents</th>
<th>Tests</th>
<th>Prunus persica</th>
<th>Calotropis procera</th>
<th>Canscora decussata</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>Mayer’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dragendorff’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wagner’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td></td>
<td>Hager’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Molisch’s test</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Carbohydrates</td>
<td>Benedict’s test</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fehling’s test</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Modified borntrager’s</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Legal test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Glycosides</td>
<td>Foam test</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Froth test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salkowski’s test</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LibermannBurchard test</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Saponins</td>
<td>Acetone-Water test</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Phytosterols</td>
<td>Filter Paper</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>Resins</td>
<td>Ferric Chloride test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Fixed oils</td>
<td>Gelatin test</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>Phenols</td>
<td>Alk. Reagent test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Tannins</td>
<td>Lead acetate</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zn-HCl acid reduction</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shinoda test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10.</td>
<td>Flavonoids</td>
<td>Xanthoproteic test</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>11.</td>
<td>Proteins</td>
<td>Ninyhydrin test</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biuret test</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Note: ‘+’ sign indicates presence and ‘−’ sign indicates absence
Table 2: Effect of hydroalcoholic leaves extract of some plants of Northern India against acute Paracetamol toxicity on Biochemical parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal Control</th>
<th>Toxic Control</th>
<th>Silymarin (100 mg/kg)</th>
<th>Prunus persica Extract (200 mg/kg)</th>
<th>Prunus persica Extract (400 mg/kg)</th>
<th>Calotropis procera Extract (200 mg/kg)</th>
<th>Calotropis procera Extract (400 mg/kg)</th>
<th>Canscora decussate Extract (200 mg/kg)</th>
<th>Canscora decussate Extract (400 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGOT (IU/L)</td>
<td>79.61±3.12</td>
<td>298.64±4.32 **</td>
<td>98.04±7.52 *</td>
<td>196.5±6.34 **</td>
<td>174.5±4.6 **</td>
<td>143.3±4.1 **</td>
<td>122.7±2.8 **</td>
<td>173.9±2.44</td>
<td>165.3±4.1</td>
</tr>
<tr>
<td>SGPT (IU/L)</td>
<td>71.23±6.56</td>
<td>201.63±4.44 **</td>
<td>82.52±3.22 *</td>
<td>184.7±4.4</td>
<td>125.4±3.44 **</td>
<td>132.8±6.34 **</td>
<td>97.81±4.5 **</td>
<td>167.6±5.50</td>
<td>100.5±4.55</td>
</tr>
<tr>
<td>SALP (IU/L)</td>
<td>129.44±6.34</td>
<td>318.21±6.34 **</td>
<td>138.11±5.34</td>
<td>197.4±6.44</td>
<td>173.6±2.2 **</td>
<td>153.3±4.53 **</td>
<td>146.42±7.52</td>
<td>178.23±2.2</td>
<td>158.43±7.52</td>
</tr>
<tr>
<td>Sr. (mg/dl)</td>
<td>0.34±0.21</td>
<td>5.22±0.21</td>
<td>0.73±0.1</td>
<td>1.82±0.0</td>
<td>1.21±0.1 **</td>
<td>1.10±0.11 **</td>
<td>0.92±0.06</td>
<td>1.43±0.12</td>
<td>1.19±0.2</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>21</td>
<td>1**</td>
<td>2 **</td>
<td>3 **</td>
<td>2 **</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>7.88±0.31</td>
<td>1.23±0.03</td>
<td>5.68±0.1</td>
<td>2.45±0.0</td>
<td>4.53±0.0</td>
<td>3.03±0.14</td>
<td>4.98±0.24</td>
<td>2.85±0.21</td>
<td>4.57±0.1</td>
</tr>
<tr>
<td>Sr. Total Protein (g/dl)</td>
<td>9.22±0.21</td>
<td>3.78±0.1</td>
<td>7.28±0.2</td>
<td>4.13±0.2</td>
<td>6.28±0.1</td>
<td>5.88±0.22</td>
<td>7.01±0.12</td>
<td>4.32±0.33</td>
<td>6.58±0.1</td>
</tr>
</tbody>
</table>

All values represent Mean ± S.E.M. of n=6/group; ## p<0.01 when compared with normal control and *p<0.05, **p<0.01 as compared with toxic control group.

xanthones. It is also a natural source of pentaoxygenated, hexaoxygenated and dimeric xanthones.

This plant contains bitter substances, oleoresin triterpenes, alkaloids and xanthones such as mangiferin. The leaves of Canscora decussata has been reported in the literature with presence of various phytoconstituents by adopting standard procedures. Animals

After prior approval from the Institutional Animal Ethical Committee (IAEC approval No. IAEC/Ph-16/TIPER/057), the rats were obtained from animal house facility of T.I.P.E.R. for evaluation of hepatoprotective activity of Calotropis procera, Canscora decussate and Prunus persica against paracetamol induced hepatic injury in rats. The healthy albino rats of Wistar strain of either sex weighing about 170-200 gm were kept at temperature 23±2 °C and humidity (50-55 %) with 12 hrs light and dark cycles. They were caged with a maximum of three animals in each polypropylene cage and were fed with standard pellet diet and water ad libitum.

Selection of Doses

As per Debjit, B et al., 2010, doses were selected on the basis of maximum tolerated safe doses found from toxicity studies. The doses were selected 1/10th and 1/5th of the maximum tolerated safe dose 2000 mg/kg, i.e. 200 mg/kg and 400mg/kg respectively and were administered orally once daily for twenty eight days.

Induction of Hepatotoxicity

The Paracetamol (PCM) induced hepatotoxicity model described by Parmer et al., (2010) was used with slight modifications. Rats were fasted overnight and hepatotoxicity was induced by administration of Paracetamol in pure form (API) in distilled water at the
dose of 500 mg/kg body weight by gastric gavage once daily for the entire duration of study. 

**Acute Model for Hepatoprotective Activity**

Animals were randomly divided into nine groups of six...

![Figure 4: Effect of hydroalcoholic extracts pretreatment on SGOT level in PCM induced Acute liver injury in rats.](image4)

![Figure 5: Effect of hydroalcoholic extracts pretreatment on SGPT level in PCM induced Acute liver injury in rats.](image5)

![Figure 6: Effect of hydroalcoholic extracts pretreatment on ALP level in PCM induced Acute liver injury in rats.](image6)
Figure 7: Effect of hydroalcoholic extracts pretreatment on Bilirubin level in PCM induced Acute liver injury in rats

Figure 8: Effect of hydroalcoholic extracts pretreatment on Serum albumin level in PCM induced Acute liver injury in rats

Figure 9: Effect of hydroalcoholic extracts pretreatment on Serum total protein level in PCM induced Acute liver injury in rats
animals each.
Group I- served as normal control and received distilled water (5 ml/kg) for 9 days.
Group II- served as toxic control and received Paracetamol in distilled water (500 mg/ kg body weight) for 9 days.
Group III- served as standard group and received silymarin (100 mg/kg) and Paracetamol in distilled water (500 mg/ kg body weight) for 9 days.
Group IV- treated with hydroalcoholic extract of Prunus persica leaves (200 mg/kg/day) and Paracetamol in distilled water (500 mg/ kg body weight) for 9 days.
Group V- administered with hydroalcoholic extract of Prunus persica leaves (400 mg/kg/day) and Paracetamol in distilled water (500 mg/ kg body weight) for 9 days.
Group VI- treated with hydroalcoholic extract of Calotropis procera leaves (200 mg/kg/day) and Paracetamol in distilled water (500 mg/ kg body weight) for 9 days.
Group VII- administered with hydroalcoholic extract of Calotropis procera leaves (400 mg/kg/day) and Paracetamol in distilled water (500 mg/ kg body weight) for 9 days.
Group VIII- treated with hydroalcoholic extract of Canscora decussate leaves (200 mg/kg/day) and Paracetamol in distilled water (500 mg/ kg body weight) for 9 days.
Group IX- administered with hydroalcoholic extract of Canscora decussate leaves (400 mg/kg/day) and Paracetamol in distilled water (500 mg/ kg body weight) for 9 days.

Analysis of liver biomarkers
After 24 hours of Paracetamol administration (on 10th day) blood samples were collected separately into sterilized dry centrifuge tubes by retro-orbital plexus puncture under mild ether anesthesia. The collected blood was allowed to clot at room temperature and serum was separated by centrifugation at 2500 rpm for 15 min. Then serum was used for the estimation of biomarkers such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total bilirubin (TB) and total protein (TP). The biochemical parameters were estimated as per the standard procedure prescribed by manufacturer’s instruction manual provided in the standard kit using autoanalyser.

Statistical analysis
All the data are expressed as mean ± SEM of six animals from each group. One-way analysis of variance (ANOVA) was used for statistical analysis of data followed by using Graph Pad Prism software. A probability of less than 5% (p<0.05) was considered statistically significant.

RESULTS AND DISCUSSION
The preliminary phytochemical screening confirmed the presence of alkaloids, carbohydrates, saponins, flavonoids, phenols and proteins in the hydroalcoholic leaves extract of Prunus persica. After phytochemical screening of the hydroalcoholic extract of calotropis procera, it was found that the extract contain alkaloids, glycosides, saponins, phytoesters, flavonoids and the hydroalcoholic extract of

Canscora decussate showed the presence of alkaloids, carbohydrates, glycosides, saponins, flavonoids, phenols and proteins.
The results of Hepatoprotective effects of hydroalcoholic extracts of some indigenous plants of Northern India against paracetamol toxicity in rats were shown in table 2. The levels of SGOT [(298.64±4.32) IU/L], SGPT [(201.63±4.44) IU/L], ALP [(318.21±6.34) IU/L] and bilirubin [(5.22±0.11) mg/dl] were significantly increased in toxic control group (Group II) when compared with normal control group but the levels of albumin [(1.23±0.16) g/dl] and total protein [(3.78±0.19) g/dl] were significantly decreased. Rats pre-treated with Prunus persica and Canscora decussata leaves extracts at dose 400 mg/kg showed significant reduction in the levels of SGOT, SGPT, ALP and bilirubin when compared with toxic control group. But the maximum reduction of SGOT and SGPT, ALP and bilirubin were observed in the rats of VI and VII groups, pretreated with hydroalcoholic extract of calotropis procera at the doses 200 and 400 mg/kg respectively.

DISCUSSION
The present study involves the evaluation of liver protective activity of some indigenous plants against acute paracetamol toxicity in rodents. Paracetamol or acetaminophen has been used as a tool to induce hepatic injury in the experimental rats. Acetaminophen (APAP) is one of the most frequently used drugs for its analgesic and antipyretic properties. It is safe and effective at recommended doses, whereas overdose may lead to hepatotoxicity and acute liver failure (ALF). Acetaminophen is bioactivated to a toxic reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI), by cytochrome 2E1 (Cyp2E1) and, to a much lesser extent, Cyp1A2 in the liver. NAPQI depletes glutathione (reduced glutathione [GSH]) and subsequently binds to liver proteins, leading to oxidative stress, mitochondrial dysfunction, and necrotic cell death. Cyp2E1 is critically involved in the bioactivation of APAP to form NAPQI and, thus, APAP hepatotoxicity.

This study showed that hydroalcoholic extracts of some indigenous plant leaves possess hepatoprotective activity, as evidenced by the
significant reduction in the elevated levels of serum biomarkers which was increased by PCM.
There was an increase in SGOT (↑275.12%), SGPT (↑183.07%), ALP (↑114.83%) and bilirubin (↑1435.2%) level of toxic control group treated with PCM when compared to that of normal control group whereas there was decrease in serum albumin (↓84.39%) and total protein (↓59 %) level. Rats pretreated with standard drug silymarin (100 mg/kg) exhibited decrease in SGOT (↓67.17%), SGPT (↓59.07%), ALP (↓56.5%), bilirubin (↓86.0%) level and increase in serum albumin (↑136.79%) and total protein (↑192.59%) level as compared to that of toxic control group. Further, rats pretreated with hydroalcoholic leaves extract of Prunus persica (200 mg/kg and 400 mg/kg) showed decrease in SGOT (↓34.20% and 41.57%), SGPT (↓37.9% and 45.4%), ALP (↓51.8% and 53.9%) and bilirubin (↓78.9% and 82.3%) level and increase in serum albumin (↑146.34% and 304.8%) and total protein (↑55.56% and 85.45%) level respectively when compared to that of toxic control group. Also, the rats pretreated with hydroalcoholic extract of Canescora decussata (200 and 400 mg/kg) showed decrease in SGOT (↓41.77% and 44.65%), SGPT (↓16.88% and 50.16%), ALP (↓43.99% and 50.21%), bilirubin (↓72.6% and 77.2%) level and increase in serum albumin (↑131.7% and 271.5%) and total protein (↑114.29% and 74.07%) level respectively as compared to that of toxic control group.
Thus hydroalcoholic extracts of Prunus persica, Calotropis procera and Canescora decussata leaves possess hepatoprotective properties in the dose dependant manner, against paracetamol intoxication in rats, after nine days pretreatment; at the dose level 200mg/kg and 400 mg/kg.

CONCLUSION
In present investigation it was found that the hydroalcoholic extracts of some indigenous plants of Northern India brought all the parameters affected by paracetamol toxicity near to normal. Thus, the hydroalcoholic extracts of Prunus persica, Calotropis procera, Canescora decussata has hepatoprotective effect which minimizes the hepatotoxicity induced by Paracetamol. Among all the plants Calotropis procera at the dose of 400 mg/kg showed maximum reduction in the level of biomarkers present in the liver.

REFERENCES


