Hepatoprotective effect of *Prunus armeniaca* L. (Apricot) leaf extracts on Paracetamol induced liver damage in Wistar rats

Varsha Raj*, Arun Kumar Mishra, Amrita Mishra, Najam Ali Khan

Department of Pharmacy, Pharmacology Research Lab, IFTM University, Moradabad, 244102, INDIA.

**ABSTRACT**

**Objective:** To evaluate the hepatoprotective effect of *Prunus armeniaca* L. (Apricot) leaf on paracetamol induced liver toxicity in rats. **Method:** Phytochemical investigation was performed to find active constituents of the plant extracts by the different phytochemical tests. After induction of liver toxicity, the biochemical parameters such as serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), serum alkaline phosphatase (sALP), serum bilirubin (SB), thiobarbituric acid reactive substances (TBARS), γ-glutamyl transferase (GGT), lactate dehydrogenase (LDH), total protein (TP), albumin. The physical parameters including liver weight, body weight and histopathological changes in the liver were studied with Ursodeoxycholic acid as standard hepatoprotective agents. **Results:** The phytochemical investigation of the extracts showed the presence of Alkaloids, volatile oil, saponin glycosides, condensed tanins, terpenoids, steroids and flavonoids. Methanol and aqueous extract before the paracetamol administration caused a significant reduction in the values of SGOT, SGPT, sALP, TBARS, GGT, LDH, TP, Albumin and SB (P<0.01) almost comparable to the Ursodeoxycholic acid. The hepatoprotective activity was confirmed by histopathological examination of the liver tissue of control and treated animals. **Conclusions:** The result concludes that *Prunus armeniaca* L. possesses the hepatoprotective effect against paracetamol induced liver toxicity in rats.

**Key words:** Apricot, Hepatotoxicity, Paracetamol, Liver toxicity.

**Correspondence:**
Ms. Varsha Raj, Department of Pharmacy, Pharmacology Research Lab, IFTM University, Moradabad, 244102, INDIA.
Email: varsharaj01@gmail.com
DOI: 10.5530/pj.2016.2.9

**INTRODUCTION**

*Prunus armeniaca* L. (Apricot or khubbani) belonging to family Rosaceae is an important edible, medicinal plant which is reported to contain polysaccharides, polyphenol, fatty acid, sterol derivatives carotenoids, carcinogenic glycosides, volatile component. The reported activities for this plant include antimicrobial, antimitagenic, cardio protective, anti-inflammatory, antinoceptive antioxidant activity. In very small amounts, the hydrogen cyanide present in apricot kernels has been traditionally prescribed in Chinese medicine for treating asthma, cough, and constipation.1

In present time, many newly developed drugs (e.g., Rimonabant, Propylthiouracil, or Corticosteroids) have been used for treatment of liver diseases; however, these drugs possess harmful side effects such as insomnia, vomiting, constipation, and depression. For that reason, further research on plants and herbs that could potentially substitute the chemical-based drugs are very crucial as many medicinal plants have been found to possess hepatoprotective properties.2

The liver is vital organ of paramount significance involved in the maintenance of metabolic functions and detoxification of the exogenous and endogenous challenges like xenobiotic drugs, viral infections and chronic alcoholism. It is involved in almost all the biochemical pathways related to growth, to fight against disease, nutrient supply, energy provision and reproduction.3 Diverse homeostatic mechanisms are affected if liver functions are impaired with serious consequences.4 Almost all types of liver injuries may lead to hepatic failure and ultimately death. Thus liver diseases are one of the most fatal diseases in the world today.5 It afflicts over 10% of the world population. This constitutes hepatitis, cirrhosis, fibrosis, hepatic steatosis (fatty liver) alcoholic liver disease and drug induced liver disease. Morbidity and mortality resulting from liver diseases is a major public health problem worldwide, especially in developing countries.4 Most of the hepatotoxic chemicals damage liver cells primarily by producing reactive species, which form covalent bond with the lipids of the tissue. However, inbuilt protective mechanisms combat the hazardous reactions associated with the free radicals. Due to excessive exposure to hazardous chemicals, sometimes the free radicals generated are so high that they overpower the natural defensive system leading to hepatic damage and cause jaundice, cirrhosis and fatty liver.7

Paracetamol (acetaminophen) is a widely used antipyretic and analgesic, which produces acute liver damage if overdoses are consumed. Paracetamol is mainly metabolized in the liver to excretable glucuronide and sulphate conjugates.9 At toxic doses of Paracetamol, the normal metabolic pathways become saturated, causing both more N-acetyl-p-benzoquinone imine (NAPQI) to be formed and hepatic glutathione to be rapidly depleted. Paracetamol toxicity is caused by the reaction metabolite, N-acetyl-p-benzoquinoneimine (NAPQI), which is partly metabolized by cytochrome P-450.10 This species causes severe oxidative damage and glutathione depletion leading to liver necrosis. Introduction of cytochrome or depletion of hepatic glutathione is a prerequisite for paracetamol-induced hepatotoxicity.11,12 The survey of literature reveals that the *Prunus armeniaca* L. is one of the most important Indian medicinal plants. Traditionally, it is used as an antimicrobial, antimitagenic, Antituberclousis, cardio protective, anti-inflammatory, antinoceptive and antioxidant. Presence of hydrogen cyanide in very small amount is also reported in earlier study in apricot kernels, which has traditionally prescribed in Chinese medicine for treating asthma, cough, and constipation. Furthermore, it is used in cosmetic industry as a astringents, for cooling effect on irritated skin, Its is also claimed to increase the fertility.13 The present study was aimed to evaluate the hepatoprotective potential of *Prunus armeniaca* L. (Leaves) against paracetamol-induced hepatotoxicity.

**MATERIAL AND METHODS**

**Drugs and chemicals**

Paracetamol was obtained from the Ranbaxy laboratory limited as a gift sample and Ursodeoxycholic acid was purchased from Embee diagnoses, Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT) (ERBA Diagnostics, Mannheim GmbH
Transasia bio-medical Ltd), Total bilirubin (Span diagnostics Ltd)-Surat. Other chemicals used in this experiment were also of analytical grade.

Plant material
The fresh leaves of *Prunus armeniaca* L. were collected in the month of July from Dist. Sirmour, Himachal Pradesh (India) and authenticated by NISCAIR New Delhi, India and the herbarium file of this plant (Voucher no -NISCAIR/RHMD/Consult/2011-12/1918/218) was submitted to the herbarium section of NISCAIR for future reference.

Preparation of extracts
The fresh leaves were cleaned, shade dried and then powdered. The dried coarse powder of the leaves (1000 g) was extracted by using the soxhlet apparatus to perform methanolic and aqueous extractions. Thereafter extracts were wiped in a vacuum according to the distillation method (Freeze Dryer). The extracts were preserved by placing to sample bottles wrapped with aluminum foil and kept at 4°C. The extracts were subjected to preliminary phytochemical investigation. Extracts were dissolved in water for injection (I.P), Paracetamol (3 g/kg) suspension prepared by using 40% Sucrose solution and subjected for hepatoprotective activity against paracetamol-induced liver damage.

Phytochemical Analysis
The methanolic and aqueous extracts were subjected to the phytochemical analysis using conventional protocol like alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, proteins, aminoacids, fixed oils, mucilage etc.

Experimental animals
A study was carried out by using Albino-Wistar rats either by sex, weighing 150 to 200 g. They were obtained from the animal house, IFTM University, Moradabad, India. The animals were grouped and housed in polyacrylic cages with not more than six animals per cage and maintained under standard laboratory condition (temperature 25 ± 2°C, with dark and light cycle (12/12 h). The animals were fed with standard pellet diet supplied by Hindustan Lever Ltd and fresh water ad libitum. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. All procedures described were reviewed and approved by the University Animal Ethics Committee.

Acute oral toxicity
The acute oral toxicity study was carried out as per the guidelines set by the OECD, revised draft guidelines 423, received from CPCSEA, Ministry of Social Justice and empowerment, Govt. of India.

Evaluation of hepatoprotective activity
The method used for evaluation of hepatoprotective activity was performed as per method described by Maity *et al.*, Mayuren *et al.*, Hegde *et al.* with some minor modifications. In the paracetamol-induced liver toxicity model, paracetamol (3 g/kg, p.o.) was administered on the 8th day to all animals except group 1. Paracetamol was suspended in 40% w/v sucrose solution. Ursodeoxycholic acid (250 mg/kg, p.o.) was used as a standard. The animals were segregated into five groups of six each. Group 1, which served as normal control and received 5 ml/kg b.w; p.o saline water daily for 7 days. Group 2 treated as group 1 for 7 days and served as negative control. Group 3 received Ursodeoxycholic acid (250 mg/kg, p.o.) for 7 days. Group 4 received a methanol extract (200 mg/kg, p.o.) for 7 days. Group 5 received an aqueous extract (200 mg/kg, p.o.) for 7 days. The animals were sacrificed 48 h after paracetamol administration under light anesthetic ether. Blood from each rat was withdrawn by retro orbital plexus under ether anesthesia for biochemical investigation of parameters such as i.e. sGOT, sGPT, sALP, LDH, TB, TBARS, GGT, Total Protein, Albumin. Serum was separated by the centrifugation of blood at 2500 rpm for 15 min at 40°C and supernatants were collected which were further used for the study of biochemical parameters. The liver of all the experimental animals was removed and processed immediately for histological investigation.

Histopathological studies
The livers were excised quickly and fixed in 10% formalin and paraffin embedded. Sections of about 4-6 µm were stained with haematoxylin and eosin (H & E) for the liver histopathological architecture. In brief, 4-6 µm thick sections of paracetamol embedded rat liver were dewaxed with distilled water for 2 min. Then the sections were stained with haematoxylin for 5 min at room temperature. After 15 min, the sections were counterstained with eosin for 2 min, dehydrated with alcohol, washed with xylene and blocked by eosin. Hematoxylin and eosin stained studies were observed under microscope. The staining procedure for histology was done by hematoxylin, a basic dye stains nuclei blue and eosin, an acidic dye, stains pink color to the cytoplasm.

Statistical analysis
All the results obtained were expressed as mean ± SD (Standard deviation) and analyzed by one-way ANOVA, followed by Dunnet’s t test between groups using GraphPad Instat version 3.06 (GraphPad Software, Inc. CA, USA). The results of all the extracts including the standard drug are compared with the result produced by normal group, and it is considered as significant as p<0.05. Data are presented as means + SEM (n=6), one way Annova followed by Dunnet’s t test.

* p<0.05, **p<0.01 vs.paracetamol treated group.

RESULTS
Extracts subjected for phytochemical study showed the presence of alkaloids, volatile oil, saponin glycosides, condensed tannins, terpenoids, steroids and flavonoids in both extracts.

Acute toxicity
Methanolic and aqueous extracts did not show any sign and symptoms of toxicity and mortality up to 2000 mg/kg dose.

Hepatoprotective activity
The hepatoprotective activity of methanolic and aqueous extracts of *Prunus armeniaca* L. on Paracetamol treated rats are shown in Table 1. Increase in the serum hepatic enzyme levels, such as sGOT, sGPT, sALP, LDH, TB and GGT by the Administration of paracetamol 3 g/kg, p.o) was compared to normal groups showed the marked increased value. While the other parameters such as albumin and total protein showed the marked reduction in the levels of liver enzymes as compared to the normal groups indicating liver damage. Body weight and liver weight also showed the significant results that indicate chronic liver damage.

Histopathological results
The hepatic architecture was present in normal group with features of polygonal nucleus with nucleus, abundant cytoplasm and bilobed nucleus and showed no visible changes, disarrangement in hepatic cells (Figure 1), these normal structures were absent in toxic control group showed the marked increased value. While the other parameters such as albumin and total protein showed the marked reduction in the levels of liver enzymes as compared to the normal groups indicating liver damage. Body weight and liver weight also showed the significant results that indicate chronic liver damage. (Figure 3).
**Table 1:** Biochemical assessment for the Paracetamol induced liver injury

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>TP (mg/dl)</th>
<th>AL (g/L)</th>
<th>LDH (U/L)</th>
<th>TB (mg/dl)</th>
<th>GGT (U/L)</th>
<th>B/W L/W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>46.17±1.755</td>
<td>60.49±0.635</td>
<td>8.18±0.356</td>
<td>6.07±0.75</td>
<td>8.89±7.37</td>
<td>3.08±0.66</td>
<td>0.79±0.11</td>
<td>0.20±0.25</td>
</tr>
<tr>
<td>Control</td>
<td>128.17±11.85**</td>
<td>120.99±5.78**</td>
<td>4.151±0.536</td>
<td>114.67±2.56</td>
<td>8.89±7.37</td>
<td>118.84±17.92**</td>
<td>118.84±17.92**</td>
<td>3.08±0.66</td>
</tr>
<tr>
<td>UDC</td>
<td>59.17±3.568</td>
<td>67.60±5.88</td>
<td>7.52±0.74</td>
<td>8.89±0.42</td>
<td>108.35±21.94</td>
<td>121.54±13.24</td>
<td>121.54±13.24</td>
<td>3.79±0.65</td>
</tr>
<tr>
<td>Methanolic extract</td>
<td>60.66±5.73**</td>
<td>75.37±1.370**</td>
<td>6.07±1.47**</td>
<td>108.35±21.94</td>
<td>311.28±35.11**</td>
<td>6.47±1.74**</td>
<td>6.47±1.74**</td>
<td>3.79±0.65</td>
</tr>
<tr>
<td>Aqueous extract (200 mg/kg)</td>
<td>88.90±12.79**</td>
<td>84.89±10.77**</td>
<td>5.58±0.415**</td>
<td>311.28±35.11**</td>
<td>347.72±22.48**</td>
<td>2.06±0.25**</td>
<td>2.06±0.25**</td>
<td>1.16±0.47**</td>
</tr>
<tr>
<td>Aqueous extract (200 mg/kg)</td>
<td>169.84±21.29**</td>
<td>169.84±21.29**</td>
<td>1.16±0.47**</td>
<td>169.84±21.29**</td>
<td>1.16±0.47**</td>
<td>1.16±0.47**</td>
<td>1.16±0.47**</td>
<td>1.16±0.47**</td>
</tr>
</tbody>
</table>

**Figure 1-5:** Histological monograph of extract and standard 1. Normal; 2. Paracetamol (3g/kg); 3. Paracetamol + Ursodeoxycholic acid (250 mg/kg); 4. Paracetamol + Methanolic extract (200 mg/kg); 5. Paracetamol + Aqueous extract (200 mg/kg)
DISCUSSION

Hepatotoxicity is the potential complication of paracetamol, which is widely used in general medicine and an assessment of its relative toxicity is important. The primary toxicity of paracetamol is the result of drug metabolism in liver. At therapeutic doses, paracetamol is metabolized via glucuronidation and sulfation reactions resulting in the water-soluble metabolites that are excreted via the kidney. The result of the metabolic conversion of paracetamol by the microsomal P-450 enzyme system is that, a highly reactive intermediate, namely, N-Acetyl-P-Benzoxquinone Imine (NAPQI) is produced. Glutathione (GSH) then reduces this metabolite. However, after Paracetamol overdose, capacity for glucuronidation and sulfation is exceeded with the formation of excess NAPQI via cytochrome P450 2E1.

This intern lead to depletion in glutathione, excess of NAPQI binds to hepatic cell protein and DNA resulting in mitochondrial dysfunction and the development of acute hepatic necrosis. More ever NAPQI can also make covalent bond with cellular proteins, can change the structure and functions of these proteins. This cellular damage causes a decrease in calcium ATPase activity and an increase in cytosolic calcium levels. As a result, cell permeability changes and lead to the loss of cellular integrity. In addition, it has been shown that NAPQI can inhibit NADH and succinate dehydrogenase function. It was considered that destruction of homeostasis was also responsible for high dose paracetamol induced hepatotoxicity. Because of destruction of homeostasis, intracellular Ca$^{2+}$ accumulates and an increase is shown in catalytic enzymes, which causes cell death. Nitric oxide, reactive oxygen species, lipid peroxidation and apoptosis are the major situations, which play roles in hepatotoxicity. In addition, peroxinitrite derivatives and protein nitrates also have roles in this condition. So in the present study, paracetamol was employed as toxic agent and the protective effect of Prunus armeniaca L. leaves against the paracetamol induced hepatoxicity was studied. Histopathological studies and biochemical enzyme markers like sGOT, sGPT, SALP, TB, LDH, GGT, Total protein and albumin levels estimated the extent of toxicity.

Reinforcing the above stated mechanisms, The histopathological study also supported the biochemical evidence for the hepatoprotection shown by Prunus armeniaca L. in the same group. The extent of hepatic damage is assessed by histological examination and the level of various biochemical parameters significant increase in the toxic control groups in the present study. The hepatic damage associated with Paracetamol is due to release of toxic metabolite N-acetyl-P-benzoquinone imine (NAPQI) and its free radical generation. The protective effect exhibited by both methanolic and aqueous extracts at a dose level of 200 mg/kg was comparable with the standard drug Ursodeoxycholic acid. The methanol extract showed more significant results as compared to aqueous extract Prunus armeniaca L. leaves.

After the phytochemical study, it is reported that, the plant extracts contain flavonoids, terpenoids, tannins and steroids. The presence of flavonoids in our extract may be responsible for its hepatoprotective activity. In conclusion, the results of this study demonstrate that Prunus armeniaca L. extract was effective for the prevention of Paracetamol-induced hepatic damage in rats. Our results show that the hepatoprotective effects of Prunus armeniaca L. extract may be due to marked changes in various parameters of liver enzyme, some physical parameters which proved that it’s a potent hepatoprotective in nature against the Paracetamol induced liver toxicity. The present study thus justifies the use of Prunus armeniaca L. in the treatment of liver diseases and points out that Prunus armeniaca L. warrants further detailed investigation as a promising hepatoprotective agent does. However, the exact mechanism(s) and the active compound(s) involved in these effects need to be clarified in future studies.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

ABBREVIATION USED


REFERENCES


PICTORIAL ABSTRACT

• The phytochemical study of the plant is reported that, the extracts contain flavonoids, terpenoids, tannins and steroids. The presence of flavanoids in our extract may be responsible for its hepatoprotective activity.

• The present study, paracetamol was employed as toxic agent and the protective effect of Prunus armeniaca L. leaves against the paracetamol induced hepatotoxicity was studied.

• Histopathological studies and biochemical enzyme markers like sGOT, sGPT, SALP, TB, LDH, GGT, Total protein and albumin levels estimated the extent of toxicity. The hepatic damage associated with Paracetamol is due to release of toxic metabolite N-acetyl-P-benzoquinone imine (NAPQ1) and its free radical generation.

• The protective effect exhibited by both methanolic and aqueous extracts at a dose level of 200 mg/kg was comparable with the standard drug Ursodeoxycholic acid. The methanol extract showed more significant results as compared to aqueous extract Prunus armeniaca L. leaves.

| ABOUT AUTHORS |
| Ms. Varsha Raj: Obtained her M. Pharm degree in Pharmacology in 2012 from Maharishi Markendeshwar University, Ambala Haryana. She is currently working on the post of Assistant Professor in Pharmacy Academy, IFTM University Moradabad. She works in the area of hepatoprotective activity of herbal plant in pharmacology. |
| Dr. Amrita Mishra: Phd from Birla institute of technology, Ranchi is working as a Assistant Professor at School of Pharmaceutical Sciences, IFTM University, Moradabad. She works in the area of Herbal drug standardization, Phytochemistry and Pharmacology. She have great knowledge of writing projects and research papers. |
| Dr. Arun K Mishra: Recipient of fellowship from All India Council of Technical Education (AICTE) Govt. of India for M.Pharm. He is also recipient of Shiri Devi Memorial award from IFTM administration for best performance in PG Course. Currently, he is working as Assistant Professor in School of Pharmaceutical Sciences. IFTM University, Moradabad, He has 39 research papers in national and international journals and 19 poster presentations in various National and International conferences. Presently he is supervising 6 Ph. D research scholars. |
| Dr. Najam Ali Khan: Phd from UPTU Lucknow. Working as a Associate Professor at School of Pharmaceutical Sciences, IFTM University, Mordabad. He works in the area of cardioprotective activity of herbal drugs in Pharmacology. He have great knowledge of writing projects and research papers. He has 19 research papers in national and international journals and 10 poster presentations in various National and International conferences. Presently he is supervising 8 Ph. D research scholars. |

SUMMARY

- The phytochemical study of the plant is reported that, the extracts contain flavonoids, terpenoids, tannins and steroids. The presence of flavanoids in our extract may be responsible for its hepatoprotective activity.
- The present study, paracetamol was employed as toxic agent and the protective effect of Prunus armeniaca L. leaves against the paracetamol induced hepatotoxicity was studied.
- Histopathological studies and biochemical enzyme markers like sGOT, sGPT, SALP, TB, LDH, GGT, Total protein and albumin levels estimated the extent of toxicity. The hepatic damage associated with Paracetamol is due to release of toxic metabolite N-acetyl-P-benzoquinone imine (NAPQ1) and its free radical generation.
- The protective effect exhibited by both methanolic and aqueous extracts at a dose level of 200 mg/kg was comparable with the standard drug Ursodeoxycholic acid. The methanol extract showed more significant results as compared to aqueous extract Prunus armeniaca L. leaves.