

## Hepatoprotective Potential of *Prosopis farcta* Beans Extracts against Acetaminophen-induced Hepatotoxicity in Wister Rats

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### ABSTRACT

**Background:** Hepatotoxicity by acetaminophen is the most frequent cause of acute liver failure in many countries. *Prosopis farcta* beans extract (PFE) has some antioxidant property and may alleviate hepatotoxicity. Therefore, the aim of this study was to evaluate effects of PFE against acetaminophen-induced hepatotoxicity.

**Methods:** Thirty-six male Wistar albino rats weighing  $220 \pm 30$  g were distributed into six groups. Two groups were pretreated with PFE (50 and 75 mg/kg) for 7 days before administration of acetaminophen (600 mg/kg). Two were given acetaminophen or PFE (50 and 75 mg/kg) alone, and the control received normal saline. One day after acetaminophen, administration blood samples were collected by cardiac puncture to determine liver function enzymes markers; aspartate aminotransferase and alanine aminotransferase (AST and ALT), cholesterol, triglyceride (TG), high, low, and very low density lipoproteins (LDL and VLDL).

**Results:** In acetaminophen-treated rat plasma AST ( $314 \pm 18.54$  vs.  $126.37 \pm 4.13$ ), ALT ( $304 \pm 49.24$  vs.  $187.33 \pm 3.71$ ), cholesterol, TG, LDL, and VLDL were increased by 149, 160, 37, 92, 60, and 94%, respectively. PFE at both doses significantly ( $P < 0.05$ ) attenuated the above biochemical indices to near normal.

**Conclusions:** *Prosopis farcta* beans extract (50 and 75 mg/kg) exhibited hepatoprotective activity against APAP.

**Keywords:** Acetaminophen, alanine aminotransferase, aspartate aminotransferase, lipid profile, *Prosopis farcta*

### INTRODUCTION

Liver plays a pivotal role in detoxification of xenobiotics, environmental pollutants, and chemotherapeutic agents. For this reason, this organ is subjected to diversity of diseases and disorders. Acetaminophen (APAP) is a widely used antipyretic and analgesic which is currently the most frequent cause of drug-induced hepatic failure in the United States with intentional and unintended overdose.<sup>[1]</sup> Metabolism of APAP to glucuronide and sulfate conjugate is mainly occurred in the

liver.<sup>[2]</sup> Cytochrome P450 enzymes convert a relatively minor portion of APAP to the highly reactive intermediate metabolite N-acetyl-p-benzoquinone-imine (NAPQI).<sup>[3,4]</sup> Under normal physiological conditions, NAPQI conjugates with glutathione (GSH) and detoxified.<sup>[5]</sup> APAP overdose increased NAPQI formation which exceeds the rate of detoxification by GSH.<sup>[5]</sup> In recent times, much attention was attracted to herbal medicines as alternative medicines. Quite a few plant remedies have been examined for the treatment of liver disorders for centuries<sup>[6,7]</sup> *Prosopis farcta* (PF) [Figure 1] from Leguminosae and sub-family *Mimosoideae* has some medicinal properties such as antiinflammatory effects, treating gastric ulcers, fetus abortion, dysentery, arthritis, larynx inflammation, heart pains, and asthma.<sup>[6]</sup> This plant is the indigenous to dry and semi-dry areas of America, Asia, and Africa<sup>[7]</sup> Some of the compounds existing in *Prosopis* plant are: Quercetin (flavonoids), tryptamine, apigenin 5-hydroxytryptamine (alkaloids), L-arabinose, and Lectin.<sup>[8,9]</sup> Moreover, a number of phenolic compounds with strong antioxidant activity has been identified in extracts of this plant such as vicenin-2, apigenin C-glycoside, iso-orientin, vitexin, luteolin 7-O-glucoside, isovitexin, quercetin 3-O-glucoside, rutin, kaempferol 3-O-rutinoside, caffeic acid derivative, and luteolin.<sup>[9]</sup> Antioxidants play an important role in inhibiting and scavenging free radicals and thus, providing protection against infections and degenerative diseases.<sup>[10]</sup> There is no *in vitro* or *in vivo* report to show an academic research to evaluate the protective effect of this plant so far.



**Figure 1:** *Prosopis farcta* beans

Since PFE has some antioxidant property and may alleviate hepatotoxicity, this study evaluated the possible preventive hepatoprotective effect of PFE on liver injury induced by APAP in rat.

## METHODS

Thirty-six male rats of Wister strain, weighing  $220 \pm 30$  g were randomly assigned to six groups of six. All animals were housed individually with a 12 h alternating light-dark cycle and had free access to food and water. Ambient temperature in the animal facility was kept at  $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$  with humidity between 30% and 50%. PF beans were collected from outskirts of Qom in Iran and were coded with the University of Birjand, Iran herbarium (herbarium code 1952). The fruit of PF was crushed to moderately coarse powder and for the extraction, 50 g of powder dissolved in 1000 ml ethanol 80% and placed on a shaker for 24 h. After 24 h, the solution is passed through a filter paper. To remove the solvent, the solution was filtered and placed in the oven for 1-2 days at  $40^{\circ}\text{C}$ .<sup>[11]</sup> After evaporation of the solvent, the samples maintained at  $-20^{\circ}\text{C}$ . A stock solution of 1 g/ml in 0.9% (w/v) normal saline was then prepared for the experiments. After 2 weeks of adaptation, animals were orally administered PFE (50 or 75 mg/kg) or saline once daily for 7 consecutive days. To induce hepatotoxicity, 1-h after the last pretreatment by PFE a single dose of APAP (600 mg/kg PO) was administered.<sup>[12]</sup> 5-6 ml of blood was obtained by cardiac puncture 24 h after induction of hepatotoxicity and plasma samples were separated by the heparinized syringe and analyzed for various biochemical parameters. Total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total protein, albumin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) concentrations were measured using standard techniques and commercial kits (Pars Azmoon Co., Iran) with an auto analyzer (Gcsan Chem 2000, Spain). Very low density lipoprotein (VLDL) was calculated by deduction of the sum of the cholesterol fractions from the TC plasma concentration as described by Salau *et al.*<sup>[13]</sup> All values were expressed as mean  $\pm$  standard error of the mean. Statistical significance between two groups of parametric data

was analyzed using one-way analysis of variance followed by Tukey's multiple comparisons test. All statistical analyses were performed with the SPSS version 16 (SPSS Inc., Chicago, IL, USA).  $P < 0.05$  was considered as statistically significant. The experiment was approved by the animal welfare committee of the Agriculture Faculty of Birjand University (research project number: 2131336).

## RESULTS

Tables 1 and 2 reveal that plasma AST and ALT levels were increased in rats administered with APAP when compared with controls indicating reliable induction of hepatotoxicity by APAP ( $P < 0.01$ ). While the groups received PFE at each dose alone showed no significant changes in any of the biochemical parameters. Treatment of rats with PFE before APAP administration, dose-dependently prevented the increase in AST and ALT. Furthermore,

administration of APAP significantly decreased plasma total protein and albumin levels ( $P < 0.05$ ) as well. As shown, pretreatments with different doses of the extract attenuated the reduction in plasma levels of total protein and albumin to some extent. Moreover, plasma cholesterol, TG, LDL, and VLDL levels markedly increased in rats administered with APAP when compared with controls ( $P < 0.05$ ). Pretreatment with PFE, especially at 75 mg/kg, provided marked protective effect and comparable improvement on these biochemical indices.

## DISCUSSION

The present study for the first time brings about the potential hepatoprotective activity of PFE against APAP-induced hepatotoxicity. The results of the study indicated that the PFE significantly reversed the enhancement of AST and ALT levels. Necrosis or membrane damage releases the intracellular enzymes into circulation and hence, it can be measured in the plasma.<sup>[14]</sup> Elevated levels of plasma enzymes are indicative of cellular leakage and loss of functional integrity of the cell membrane in liver prior to other indicators of liver function tests.<sup>[14]</sup> The most important enzymes are ALT and AST that are present in the liver parenchyma. ALT predominantly found in liver unlike AST which is also abundantly present in other organs namely, cardiac muscle and kidneys. For this reason, ALT is more specific indicator of liver inflammation than AST, though the parallel increases in ALT and AST often observe.<sup>[15]</sup> In the present study, plasma AST and ALT activity were significantly enhanced in rats receiving APAP. This enhancement may be due to disruption of hepatic cell as a result of necrosis or

**Table 1:** Effect of *Prosopis farcta* on liver enzymes in rats

Treatment	Dose (mg/kg)	AST (U/L)	ALT (U/L)
Control (normal saline)		126.37±4.13 <sup>b</sup>	187.33±3.71 <sup>b</sup>
APAP	600	314.87±18.54 <sup>a</sup>	490±56.75 <sup>a</sup>
PFE	50	111.53±5.04 <sup>b</sup>	187.67±10.80 <sup>b</sup>
PFE	75	110.13±9.53 <sup>b</sup>	176.67±3.76 <sup>b</sup>
APAP+PFE	50	163.97±17.90 <sup>b</sup>	304.67±49.24 <sup>b</sup>
APAP+PFE	75	137.37±19.70 <sup>b</sup>	248.00±27.62 <sup>b</sup>

Values are expressed as mean±SE. The values with different superscripts within a column represent significantly different means (<sup>a,b</sup> $P < 0.01$ ). ALT=Alanine aminotransferase, AST=Aspartate aminotransferase, SE=Standard error, APAP=Acetaminophen, PFE=*Prosopis farcta* beans extract

**Table 2:** Effect of *Prosopis farcta* on liver enzymes and total protein, albumin and lipid profile in rats

Treatment	Dose (mg/kg)	Total protein (mg/dl)	Albumin (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL (mg/dl)
Control (normal saline)		6.95±0.09 <sup>a</sup>	3.29±0.02 <sup>a</sup>	53.52±4.69 <sup>a</sup>	38.07±2.83 <sup>b</sup>	40.55±3.44 <sup>a</sup>	20±0.52 <sup>b</sup>	8.00±0.79 <sup>a</sup>
APAP	600	6.26±0.23 <sup>a</sup>	2.47±0.16 <sup>b</sup>	72.90±8.38 <sup>b</sup>	73.93±5.9 <sup>a</sup>	48.43±5.22 <sup>a</sup>	32.33±4.6 <sup>a</sup>	14.68±1.39 <sup>a</sup>
PFE	50	6.68±0.18 <sup>a</sup>	3.22±0.06 <sup>a</sup>	51±3.59 <sup>a</sup>	43.77±2.96 <sup>b</sup>	49.4±3.18 <sup>a</sup>	18±1.57 <sup>b</sup>	9.69±0.55 <sup>a</sup>
PFE	75	6.68±0.10 <sup>a</sup>	3.11±0.07 <sup>a</sup>	55.34±3.08 <sup>a</sup>	41.03±1.56 <sup>b</sup>	42.4±1.93 <sup>a</sup>	15±0.59 <sup>b</sup>	9.60±1.41 <sup>a</sup>
APAP+PFE	50	6.56±0.12 <sup>a</sup>	2.87±0.14 <sup>ab</sup>	54.96±3.81 <sup>a</sup>	51.03±3.24 <sup>b</sup>	43.93±1.29 <sup>a</sup>	22.33±2.18 <sup>b</sup>	9.76±0.44 <sup>a</sup>
APAP+PFE	75	6.48±0.20 <sup>a</sup>	2.92±0.10 <sup>ab</sup>	57.52±3.86 <sup>a</sup>	45.07±5.08 <sup>b</sup>	40.55±2.44 <sup>a</sup>	21±1.47 <sup>b</sup>	9.87±1.01 <sup>a</sup>

Values are expressed as mean±SE. The values with different superscripts within a column represent significantly different means (<sup>a,b</sup> $P < 0.05$ ). HDL-C=High density lipoprotein cholesterol, LDL-C=Low density lipoprotein cholesterol, VLDL=Very low density lipoprotein, SE=Standard error, APAP=Acetaminophen, PFE=*Prosopis farcta* beans extract



a consequence of altered membrane permeability. Pretreatment with oral doses of *P. farcta* extract attenuated the enzyme activities which are indications of the protective activities of the PFE against APAP hepatotoxicities. The most critical assessment if an intervention is used as a pretreatment is the evaluation of metabolic activation (GSH depletion and protein adduct formation) which has not been assessed in this study. Metabolism of paracetamol produces a highly toxic metabolite, NAPQI via the cytochrome P450 pathway<sup>[16]</sup> which induces the process of lipid peroxidation. This toxic metabolite normally conjugated with GSH and excreted in the urine. Although, accumulation of NAPQI occurs when the rate of formation exceeds the rate of detoxification by GSH or<sup>[17]</sup> the depletion of GSH stores. In addition, covalent bindings of NAPQI to hepatocyte macromolecules cause necrosis of liver cell and consequent release of the cytosolic liver enzymes particularly the aminotransferases that are regarded as signs of hepatocellular injury.<sup>[7]</sup>

Given the large number of compounds present in the extract, it is highly likely that several P450 inhibitors are present. Quercetin, a flavonoid antioxidant, is one of the compounds found in *Prosopis* with an excellent radical scavenger activity which attenuated oxidative stress induced by APAP, as well as other chemical.<sup>[18-20]</sup> It is possible that presence of polyphenols and flavonoids in PF might be responsible for its protective effect on APAP-induced liver damage in rats.<sup>[8,9]</sup>

Concerning plasma lipid profile, oral administration of APAP increased plasma cholesterol, TG and LDL with no significant alteration in plasma HDL level. Administration of PFE significantly decreased plasma cholesterol, triglyceride, and LDL levels. Lipid deposition in the liver may be as a result of excessive supply of lipids to the liver or interference with lipid deposition. Formation of phenoxyl radicals in the presence of peroxidases act as an LDL prooxidant is responsible of LDL elevation in APAP animals.<sup>[21]</sup> It is documented that lipid-lowering drugs have antioxidant properties which prevent LDL peroxidation.<sup>[22]</sup> PF with antioxidant property is capable of preventing LDL peroxidation and inhibiting elevation of LDL by APAP.

The liver is the major site of cholesterol synthesis. Cholesterol is made from acetate. Three molecules of acetyl CoA combined and produce

3-hydroxy-3-methyl-glutaryl-CoA, which is affected by different enzymes converted to malonic acid, and during different reactions, convoluted and converted to cholesterol.<sup>[23]</sup> HDL-C is responsible for reverse transport of cholesterol from peripheral cells to the liver cells. Cholesterol is transformed to bile acids, which are excreted into the intestine via the biliary tract.<sup>[24]</sup> The findings of Omidi *et al.*, while preliminary, suggests that the efficacy of PF beans powder as a beneficial agent to decrease LDL-C and increase HDL-C concentration.<sup>[25]</sup> The liver is also an important site for protein synthesis and degradation. It produces proteins for its own cellular needs, as well as secretory proteins that are released into the circulation. One of the most important of these secretory proteins is albumin.<sup>[15]</sup> In this study, total protein and albumin were used to assess the synthetic function of the liver. The decreased total protein and albumin levels by APAP were significantly improved in PFE-treated groups indicating of its hepatoprotective effect.

## CONCLUSIONS

Based on the results of the present study, it can be concluded that the aqueous extracts of PF beans exhibit hepatoprotective activity against APAP. However, the mechanism by which PFE exhibited protection is not clear by the present study. Further investigations on cellular and molecular mechanism of the plant may throw more light on the use of PF for hepatoprotective activity.

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