Effect of Hydro-alcoholic Extract from Prosopis Farcta Leaves on Liver Injury Caused by High-fat Diet in Rats
MR Hajinezhad¹, M Rasekh²

ABSTRACT

Objective: To determine the hepatoprotective and antioxidant effects of hydro-alcoholic extract of Prosopis farcta (P farcta) leaves on high fat diet-fed (HFDF) rats.

Methods: In this experimental study, 40 male Wistar rats were divided into four groups – group 1: normal control group; group 2: untreated control group, fed a high-fat diet; group 3: hyperlipidaemic + P farcta (500 mg/kg orally per day); and group 4: hyperlipidaemic + simvastatin (1.0 mg/kg). All groups were treated for 30 days. Liver enzymes, levels of total cholesterol, triglyceride, low-density lipoprotein (LDL), high-density lipoprotein, blood urea nitrogen and creatinine, antioxidant enzyme activity, lipid peroxidation and liver histopathology were assessed.

Results: Prosopis farcta extract reduced the elevated levels of total cholesterol, triglycerides, LDL and body weight. Catalase and superoxide dismutase activity were reduced in the HFDF animals, whose levels were increased statistically significantly by extract of P farcta leaves. The statistically significant increases in liver malondialdehyde in HFDF rats were reduced after treatment with P farcta. Histopathological findings also revealed positive effects of the extract.

Conclusion: These results indicate the lipid-lowering and antioxidative activity of extract of P farcta leaves.

Keywords: Catalase, hyperlipidaemia, lipid profile, Prosopis farcta, rat

Efecto del extracto hidro-alcohólico de las hojas de Prosopis Farcta en la lesión hepática causada en ratas por una dieta rica en grasas
MR Hajinezhad¹, M Rasekh²

RESUMEN

Objetivo: Determinar los efectos hepatoprotectores y antioxidantes del extracto hidro-alcohólico de las hojas de Prosopis farcta (P farcta) en ratas alimentadas con dieta rica en grasas (ADRG).

Métodos: En este estudio experimental, 40 ratas macho Wistar se dividieron en cuatro grupos – Grupo 1: Grupo de control normal; Grupo 2: Grupo de control no tratado, alimentado con una dieta alta en grasas; Grupo 3: hiperlipidémico + P farcta (500 mg/kg por vía oral por día); y Grupo 4: hiperlipidémico + simvastatina (1.0 mg/kg). Todos los grupos fueron tratados durante 30 días. Se evaluaron las enzimas hepáticas, los niveles de colesterol total, los triglicéridos, la lipoproteína de baja densidad (LBD), la lipoproteína de alta densidad (LAD),
el nitrógeno ureico y la creatinina en sangre, la actividad enzimática antioxidante, la peroxidación lipídica, y la histopatología hepática.

**Resultados:** El extracto de Prosopis farcta redujo los niveles elevados de colesterol total, los triglicéridos, la LBD, y el peso corporal. La actividad de la catalasa y el superóxido dismutasa se redujo en los animales ADRG, cuyos niveles se incrementaron estadísticamente en grado significativo mediante el extracto de hoja de P farcta. Los aumentos estadísticamente significativos en el malondialdehído hepático en ratas ADRG, disminuyeron después del tratamiento con P farcta. Los hallazgos histopatológicos también revelaron efectos positivos del extracto.

**Conclusión:** Estos resultados indican la actividad de reducción de lípidos y la actividad antioxidantes del extracto de las hojas de P farcta.

**Palabras clave:** Catalasa, hiperlipidemia, perfil lipídico, Prosopis farcta, rata

**INTRODUCTION**

Hyperlipidaemia and its attendant acute and long-term complications are among the most common health hazards in the world (1). Prevalence of diseases associated with hyperlipidaemia, including coronary artery disease, stroke and high blood pressure, is increasing in developing countries (2). An elevation in serum lipids (such as phospholipids, cholesterol and triglycerides) induces lipid peroxidation, which in turn might act as a possible mechanism of myocardial dysfunction. When the balance between the body’s antioxidant defence system and the production of free radicals is lost, the situation can be diverted to oxidative damage. Increased levels of oxygen-derived free radicals in the body leave the body vulnerable to cancer and heart attack (3). Low-density lipoprotein (LDL) carries most of the cholesterol from the liver to peripheral tissues. Low-density lipoprotein enters the cells by endocytosis. Circulating LDL cholesterol can accumulate beneath the endothelial layer of blood vessels. High-density lipoprotein (HDL) is involved in the transport of cholesterol mostly to the liver by both direct and indirect pathways (4). In recent decades, drugs such as gemfibrozil, atorvastatin and lovastatin are used to reduce blood lipid levels. These compounds have widespread side-effects such as digestive problems, headache and muscle weakness; in the long term, they cannot prevent complications of hyperlipidaemia (5). Medicinal and pharmaceutical products of plant origin can be replaced by synthetic drugs due to the low price, availability and fewer side-effects. Syrian mesquite plant with the scientific name Prosopis farcta belongs to the family of Leguminosae and subfamily of Mimosoideae. This plant is native to arid and semi-arid areas of West Asia and North Africa and can be found from large parts of western China to Iran and Iraq (6). Prosopis farcta has a wide range of medicinal properties. The bioactive compounds of this plant are typically concentrated in the root and leaves. The ethanol extract of the leaves of the plant contains alkaloids, tannins, Quercetin (flavonoids) glycoside and saponins (7). The fresh stem and leaves of this plant are used for the treatment of diabetes and a wide range of digestive, respiratory and skin diseases such as diabetic foot ulcers. There are other indications for this plant in the Middle East Arab countries such as stomach ulcers, colds, bronchitis and diarrhoea (8). Some applications of this plant have been scientifically studied in new experiments. However, most applications of the plant are based on the culture of the indigenous people and have not been studied academically. The leaves of this plant are used to treat ulcers caused by diabetes, but the exact mechanism of its effect is not well understood. In Jordan, the extract from the root of the plant is used in the treatment of diabetes (9). Recently, the neuronal protective effect of P farcta has been proven in sciatic nerve injury model (10). Moderate antibacterial activities were shown by leaf, pod and seed methanolic extracts of P farcta (11). Despite widespread medicinal use, there are few scientific reports about nausea and vomiting after accidental ingestion of seeds of P farcta (12). Herbs and medicinal compounds with herbal origin have a wide spectrum of antioxidants that can be used to treat diseases related to hyperlipidaemia. Fewer side-effects and reasonable prices of plant-derived drugs have resulted in an increased trend towards these types of products. In this study, the efficacy of the extract of P farcta leaves in reducing side-effects caused by hyperlipidaemia in male Wistar rats was studied.
SUBJECTS AND METHODS
In this experimental study, 40 adult male Wistar rats from the breeding colony of the University of Zabol, Zabol, Iran, were used. Rats were kept at 20–23ºC and 12-hour light/dark cycles. All animals were fed standard laboratory diet (Javaneh-Khorasan, Iran) and water ad lib. Rats were deprived of food for 12 hours with free access to water. The experimental procedures of this study were approved by the Animal Ethics Committee of the University of Zabol, Zabol, Iran. The air-dried leaves of *P. farcta* were purchased from an herbal medicine shop in Zabol, Iran, in May 2015. The leaves were identified at Mashhad University of Medical Sciences, Mashhad, Iran, by a plant taxonomist, Dr M Soozani. The voucher specimen was deposited at the Herbarium of the Faculty of Pharmacy of Mashhad University of Medical Sciences. The specimen number of the plant was 13070. The dried leaves were converted to powder by an electrical blender. The powder (400 g) was extracted with 800 ml of 50% ethanol and kept at room temperature for two days with constant shaking (30 rpm). The extract was concentrated in a vacuum oven at 40ºC for 24 hours and kept in a refrigerator until use. Preliminary phytochemical screening showed the presence of flavonoids, glycosides and phenols. Animals were randomly and equally divided into four groups. The control group was given standard laboratory rodent food. The second group was treated with a high-fat diet, which consisted of 20% sunflower oil, 2% cholesterol and 0.5% cholic acid added to ordinary rat chow (13). The third group was fed a high-fat diet plus extracts of *P. farcta* leaves (500 mg/kg/day) for 30 days by oral tube. The fourth group received a high-fat diet plus simvastatin (1.0 mg/kg body weight/day).

After 30 days, the rats were sacrificed, and blood samples were collected from the heart and centrifuged (3000 rpm for five minutes) for separating the serum. The serum was immediately frozen at −80ºC until use. Serum lipoproteins were measured using standard enzymatic methods. The body weight of the animals was recorded weekly throughout the period of the study. After euthanasia, abdominal fat pads were carefully removed and weighed.

Liver catalase activity was assayed by the method described by Aebi (14). The rate of H2O2 (the substrate of the enzyme) decomposition adrenochrome formation was determined spectrophotometrically at 240 nm (UNICO UV/VIS- 2100 Spectrophotometer). The hepatic superoxide dismutase activity was assayed based on the method described by Kakkar et al (15). Kits for total cholesterol, HDL-cholesterol and triglyceride (Pars Azmoon, Tehran, Iran) were used according to the instructions of the manufacturer. The LDL-cholesterol (LDL_C) was calculated by the standard equation:

\[
LDL_C = \frac{\text{total serum triglycerides} + \text{HDL}_C}{5}
\]

The hepatic level of malondialdehyde (MDA), a biomarker of lipid peroxidation, was assayed by the method described by Ohkawa *et al* (16). This method is based on the reaction between 2-thiobarbituric acid (TBA) and MDA. The absorbance of the MDA-TBA product was determined spectrophotometrically at 532 nm.

Histopathological examination
After euthanasia, liver specimens were sliced and preserved in 10% formalin and processed for histological staining. After paraffin embedding and block making, serial sections were stained with haematoxylin-eosin and examined under light microscope (Olympus, Tokyo, Japan) at 40 magnifications.

Statistical analysis
The collected results were analysed with SPSS software (version 20.0). All data were expressed as mean ± standard deviation. Multiple comparisons were performed using one-way analysis of variance followed by post-hoc Tukey’s test. Statistical significance was accepted at p < 0.05 or p < 0.001.

RESULTS
In this experimental study, the average body weight of the HFDF groups statistically significantly increased as compared to that of the normal control group (p < 0.001), suggesting the suitability of the administered diet. The increment in the mean body weight and mesenteric fat pad weight was reduced statistically significantly (p < 0.001) by the oral feeding of extract of *P. farcta* leaves (500 mg/kg) as well as simvastatin (1 mg/kg) in comparison with the untreated model control group. Compared to HFDF rats, administration of hydro-alcoholic extract from *P. farcta* (500 mg/kg) and simvastatin (1 mg/kg) statistically significantly reduced the elevated abdominal pad weight (p < 0.001 for both). Oral feeding of extract of *P. farcta* leaves markedly and statistically significantly decreased the hepatic level of MDA in rats submitted to hyperlipidaemia-inducing diet compared
to the untreated control group \((p < 0.05)\). According to Table 1, in rats submitted to HFDF, the serum levels of cholesterol LDL-C and triglyceride were statistically significantly increased when compared to normal controls \((p = 0.000\) for all).

Daily oral administration of extract of \(P\) farcta leaves statistically significantly decreased serum level of cholesterol, triglyceride \((p < 0.001)\) and LDL-C \((p < 0.05)\) compared to untreated control rats. No statistically significant change in serum HDL level was found between experimental and control groups. There were no statistically significant differences in the serum level of blood urea nitrogen between extract-treated and control groups. The serum levels of creatinine did not differ among the four studied groups (Table 1). The statistically significant reduction in catalase activity observed in untreated model control rats was restored by \(P\) farcta treatment \((p < 0.05)\) (Table 2). According to Table 2, oral administration of extract of \(P\) farcta leaves to HFDF rats statistically significantly decreased the reduced levels of superoxide dismutase activity when compared to untreated HFDF rats \((p < 0.001)\). Administration of extract of \(P\) farcta leaves statistically significantly decreased the activity of liver catalase and superoxide dismutase in HFDF rats (Table 2). Hydro-alcoholic extract from \(P\) farcta showed anti-lipid peroxidative properties by marked restoration of liver MDA content in HFDF rats, near to the level of the normal control group.

Liver sections of healthy control group rats showed normal lobular architecture with distinct hepatic cells and radiating hepatic cords (Fig. 1), while liver sections of the untreated control group (group 2) showed loss of cellular architecture with patterns of cell necrosis (Fig. 2). However, histological examination of liver sections of rats treated with \(500\) mg/kg of \(P\) farcta (group 3), and hyperlipidaemic rats treated with \(1.0\) mg/kg of

Table 1: The effects of extract of \(Prosopis fasrcta\) leaves on some biochemical parameters in control and experimental groups of rats (mean ± standard deviation; \(n = 10\))

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol ((\text{mg/dL}))</th>
<th>Triglyceride ((\text{mg/dL}))</th>
<th>Low-density lipoprotein-cholesterol ((\text{mg/dL}))</th>
<th>High-density lipoprotein ((\text{mg/dL}))</th>
<th>Blood urea nitrogen ((\text{mg/dL}))</th>
<th>Creatinine ((\text{mg/dL}))</th>
<th>Aspartate aminotransferase ((U/L))</th>
<th>Alanine aminotransferase ((U/L))</th>
<th>Alkaline phosphatase ((U/L))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal diet control group</td>
<td>74.4 ± 8.3</td>
<td>46.8 ± 11.6</td>
<td>43.10 ± 5.4</td>
<td>19.0 ± 4.2</td>
<td>7.2 ± 1.3</td>
<td>0.41 ± 0.1</td>
<td>42.5 ± 6.3</td>
<td>46.3 ± 6.8</td>
<td>105.3 ± 8.6</td>
</tr>
<tr>
<td>Negative control group</td>
<td>172.4 ± 8.7</td>
<td>150.8 ± 18.2</td>
<td>84.6 ± 9.2</td>
<td>15.6 ± 1.8</td>
<td>8.5 ± 0.9</td>
<td>0.49 ± 0.1</td>
<td>74.1 ± 9.3</td>
<td>70.5 ± 9.8</td>
<td>133.4 ± 11.5</td>
</tr>
<tr>
<td>Hyperlipidaemic + (Prosopis fasrcta)</td>
<td>95.4 ± 10.7</td>
<td>59.8 ± 7</td>
<td>62.5 ± 6.6</td>
<td>17.1 ± 2.4</td>
<td>7.4 ± 0.8</td>
<td>0.44 ± 0.1</td>
<td>53.1 ± 8.2</td>
<td>57.5 ± 9.2</td>
<td>121.7 ± 9.2</td>
</tr>
<tr>
<td>Hyperlipidaemic + simvastatin</td>
<td>85.3 ± 11.7</td>
<td>60.0 ± 6.73</td>
<td>53.1 ± 11.2</td>
<td>22.3 ± 3.3</td>
<td>7.3 ± 0.6</td>
<td>0.48 ± 0.05</td>
<td>57.4 ± 8.2</td>
<td>54.5 ± 7.2</td>
<td>94.5 ± 9.6</td>
</tr>
</tbody>
</table>

\(p < 0.05\) when compared to normal control
\(p < 0.05\) when compared to untreated model control
\(p < 0.001\) when compared to normal control
\(p < 0.001\) when compared to untreated model control

Table 2: Effect of extract of \(Prosopis fasrcta\) leaves on liver antioxidant enzymes, malondialdehyde content, body weight and fat pad weight in normal and hypercholesterolemic rats

<table>
<thead>
<tr>
<th></th>
<th>Normal diet control group</th>
<th>Negative control group</th>
<th>Hyperlipidaemic + (Prosopis fasrcta)</th>
<th>Hyperlipidaemic + simvastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase ((\mu\text{M}H_{2}O_{2} \text{decomposed/minute/g liver}))</td>
<td>82.1 ± 3.7</td>
<td>52.7 ± 5.9</td>
<td>60.1(^{ab}) ± 6.6</td>
<td>54.9 ± 5.9</td>
</tr>
<tr>
<td>Superoxide dismutase ((\text{Units/minute/g liver}))</td>
<td>260.8 ± 18.1</td>
<td>108.1(^{ab}) ± 8.3</td>
<td>202.8(^{ab}) ± 10.1</td>
<td>209.4(^{ab}) ± 12.9</td>
</tr>
<tr>
<td>Malondialdehyde ((\text{nmol/g liver}))</td>
<td>0.28 ± 0.8</td>
<td>0.55(^{a}) ± 0.13</td>
<td>0.43(^{ab}) ± 0.10</td>
<td>0.26(^{a}) ± 0.06</td>
</tr>
<tr>
<td>Body weight ((\text{mg/dL}))</td>
<td>235.4 ± 20.86</td>
<td>306.3(^{a}) ± 15.6</td>
<td>275.7(^{d}) ± 23.05</td>
<td>249.4(^{d}) ± 14.7</td>
</tr>
<tr>
<td>Mesenteric fat pad weight ((\text{mm}))</td>
<td>0.29 ± 0.07</td>
<td>0.55(^{a}) ± 0.06</td>
<td>0.32(^{b}) ± 0.07</td>
<td>0.30(^{a}) ± 0.05</td>
</tr>
</tbody>
</table>

\(p < 0.05\) when compared to normal control
\(p < 0.05\) when compared to untreated model control
\(p < 0.001\) when compared to normal control
\(p < 0.001\) when compared to untreated model control
simvastatin (group 4) revealed that most of the histological alterations were markedly alleviated (Figs. 3 and 4).

**DISCUSSION**

Herbs and medicinal compounds with herbal origin have an important role in reducing reactive oxygen species, therefore providing protection against atherosclerosis and degenerative diseases (17). In the current study, we investigated whether extract of *P farcta* leaves may attenuate liver injury caused by high-fat diet *in vivo* and whether there would be any potential for the development of a natural supplement to improve liver function against high-fat consumption. In the present study, 30 days’ oral administration of *P farcta* extract was able to protect the liver against high fat diet-induced oxidative damage in rats. The possible mechanisms by which the *P farcta* extract protects the liver against high dietary lipids include reducing MDA levels and enhancing the activity of antioxidant enzymes. It has been reported that this plant produces a variety of phenolic compounds with strong antioxidant activity such as iso-orientin, glycoside, isovitexin, rutin, caffeic acid derivative, tannins, 3-O-rutonoside, luteolin, and quercetin (18). Major chemical compounds found in leaf juice of *P farcta* include lectin, steroidal lactones, alkaloids, flavonoids, tannin, quercetin (flavonoids), and apigenin. The leaves are also a rich source of saponins which possess anti-hyperglycaemic properties (19). It is well accepted that saponin-containing compounds can reduce total cholesterol. As observed in this study, administration of extract of *P farcta* leaves ameliorated lipid profile and liver histology. The histological change of liver in HFDF rats treated with *P farcta* may relate to the alterations of lipid profiles. These findings are in accord with earlier studies and may reflect the ethnobotanical indication of *P farcta* extract on the reduction of chest pain (20). A previous report found that *P farcta* leaves possessed hepatoprotective and anti-lipid peroxidation activities in diabetic rats (21). The most unpredictable finding of the present study was a significant reduction in serum triglycerides, total cholesterol and LDL-C without a noticeable HDL-increasing effect. Many researchers have shown...
that natural antioxidants can reduce serum lipoproteins and diminish the risk of cardiovascular disease (22). Medicinal herbs and herbal antioxidants can reduce serum lipoproteins, stimulate the secretion of cholesterol through bile, and increase excretion of cholesterol through faeces (23). Given a large number of chemical compounds present in the extract of *P. farcta* leaves, it is suggested that several cytochrome-P450 inhibitors are present. Quercitin, the major flavonoid/antioxidant compound concentrated in the leaves of *P. farcta*, was proven to possess an excellent free radical scavenger activity which attenuated lindane-induced liver injury in rats (24). In the present study, activity of catalase and superoxide dismutase enzymes in liver tissue was significantly increased in HFDF rats which was restored after the administration of *P. farcta* extract. Increased plasma lipoproteins are associated with decreased activity of antioxidant enzymes, elevated levels of MDA and other markers of oxidative stress (25). Administration of extract of *P. farcta* leaves restored the activity of liver catalase and superoxide dismutase in HFDF rats. The findings of Asadollahi *et al* demonstrated the efficacy of extracts of *P. farcta* beans as a beneficial hepatoprotective agent, against acetaminophen-induced liver injury in rats (26). Preliminary experiments carried out by Omidi *et al* suggested the HDL-increasing efficacy of powder of *P. farcta* beans (27). *In vitro* and *in vivo* experiments carried out by Al-Jeboory and Alhusainy demonstrated the potential of alcoholic extract of leaves of *P. farcta* as positive inotropic and blood pressure-decreasing agents (28). In histopathological examination, cytoplasmic vacuolization was observed in the liver sections of hyperlipidemic rats. The increase in liver cell vacuolation and cell necrosis in hyperlipidemic group can be explained by increasing the amount of lipid accumulation in liver. Treatment with extract of *P. farcta* leaves slightly improved the histopathological changes induced by hyperlipidaemia. In the current study, all morphological findings were supported by serum biochemical parameters. It has been accepted that the hepatocyte damage in hyperlipidaemia depends on the formation of the free radicals in serum and liver tissue, which interact with oxygen to form more toxic compounds. These compounds are responsible for peroxidation of fatty acids of cell membrane phospholipids, leading to increased lipid peroxidation in serum and the liver tissue. Malondialdehyde content in liver homogenate reflects the degree of lipid peroxidation (29). In the findings of the current study, the MDA content in HFDF rats was restored to near-normal levels, and this is consistent with other studies indicating the efficacy of extract of *P. farcta* leaves in reducing serum peroxidation. It seems that flavonoid and alkaloids are responsible for the therapeutic effects of extract of *P. farcta* leaves. These antioxidant compounds can effectively neutralize free radicals by providing an extra electron, ending the electron-‘stealing’ reaction.

**CONCLUSION**

This study showed that *P. farcta* extract possessed hepatoprotective and antioxidative potential and will serve as a foundation for future experiments on the effects of *P. farcta* on the prevention and treatment of metabolic diseases.

**ACKNOWLEDGEMENTS**

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**REFERENCES**