Syrian Mesquite Extract Improves Serum Lipids and Liver Tissue in NFALD modelled Rabbits

Samira Keshavarzi (1)
Gholamreza Bahrami (1,2)
Bahareh Mohammadi (1)
Razieh Hatami (1)
Shahram Miraghaee (1)

(1) Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
(2) Pharmaceutical Sciences Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

Corresponding author:
S. Sh. Miraghaee
Medical Biology Research Center, P. O. Box 67155-1616, Kermanshah University of Medical Sciences, Parastar St., Daneshghah Blvd., Kermanshah, Iran
Tel: +98 833 427 4673
Mobile: +98 918 333 1385
Fax: +98 833 427 4671
Email: Shahram.miraghaee@yahoo.com

Received: December 25, 2017; Accepted: December 30, 2017; Published: March 1, 2018. Citation: Keshavarzi S. et al. Syrian Mesquite Extract Improves Serum Lipids and Liver Tissue in NFALD modeled Rabbits. World Family Medicine. 2018; 16(3):150-164. DOI: 10.5742/MEWFM.2018.93324

Abstract

Background: Prosopis farcta root has been proposed as an efficacious natural drug in traditional medicine for alleviation of chest pain.

Objectives: The present study evaluates the efficacy of aqueous extract of Prosopis farcta root on high cholesterol diet–induced NAFLD in rabbits as experimental model.

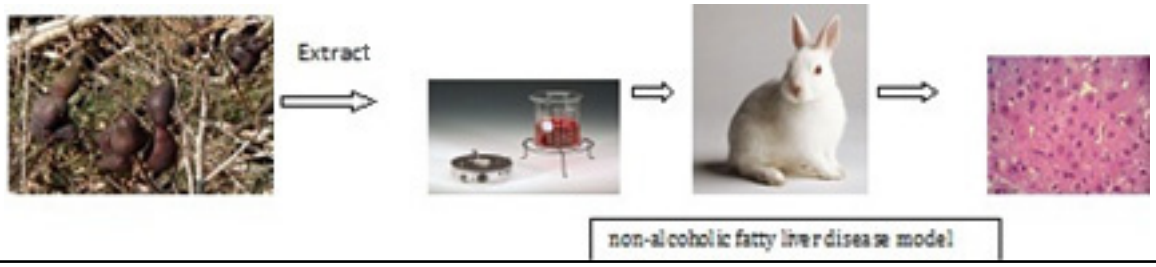
Methods: Male rabbits were randomly divided into 4 groups. The first group as control were fed by standard pellet and other groups were received 2% cholesterol amounts daily. Rabbits were fed with high cholesterol diet till the serum cholesterol level reached 1800 mg/dl, then, they were treated daily with distilled water, and 0.6 mg/kg Simvastatin, or 500 mg/kg/day Prosopis farcta root extracts orally by gavage for 30 days. Finally, the groups were compared considering serum lipid profile, serum enzymes and liver histopathological changes. Results: Serum lipid parameters and enzymes were significantly increased in the high cholesterol diet groups in comparison with the normal control group. Histopathological findings revealed that large lipid vacuoles were formed in hepatocytes. Treatment with Prosopis farcta root significantly improved rabbit lipid profile and decreased liver injury.

Conclusion: According to previously determined ingredients of Prosopis farcta root extract, it seems that phytosterols, Saponins and Flavonoids may play an important role in its hepatoprotective effect.

Key words: cholesterol; herbal medicine; hypercholesterolemia; non-alcoholic fatty liver disease; Prosopis farcta.

Summary: Prosopis farcta root has been proposed as an efficacious natural drug in traditional medicine. The present study shows the aqueous extract of Prosopis farcta root significantly improves rabbit lipid profile and decreases liver injury in rabbits fed a high cholesterol diet.

Abbreviations Used: NAFLD: Non-Alcoholic Fatty Liver Disease. NASH: Non-alcoholic Steato Hepatitis. CVD: Cardio Vascular Disease. TG: Triglyceride. LDL: Low-Density Lipoprotein. HDL: High-Density Lipoprotein. ALT: Alanine Amino Transferase. AST: Aspartate amino Transferase CPK: Creatinine Phospho Kinase. LDH: Lactate Dehydrogenase. CRP: C - reactive protein.



Introduction

Non-alcoholic fatty liver disease (NAFLD) is defined as storage of triglycerides in hepatocytes more than 5% of liver weight, less than 20 g/d of alcohol consumption and with exclusion of any other causes of chronic liver diseases(1, 2). In recent years, NAFLD has been suggested to be the most common cause of chronic liver disease throughout the world, with a suggested incidence of 10–24% in the general population in the USA and similar figures in Europe and Japan(2, 3). The prevalence of NAFLD and non-alcoholic steato hepatitis (NASH) in Iranians varies from 2.9% to 7.1% in the general population and 55.8% in patients with type 2 diabetes mellitus(4).

No effective pharmacological therapy is currently available for patients with NAFLD(5). There is a strong association between NAFLD and conditions related to the metabolic syndromes. Hence, drugs that have been used in hyperlipidaemia (such as simvastatin), and type II diabetes mellitus and some antioxidants now are receiving great attention in prevention of NAFLD. However, some of the drugs have been found to be potentially hepatotoxic in clinical trials(6). Moreover, some patients are resistant or intolerant to conventional pharmacotherapy. Therefore, alternative approaches are eagerly needed; plant-based therapies attract much interest as they are effective in reducing lipid levels (7, 8). In this line, the Syrian Mesquite (Prosopis farcta) root extract showed anti-hyperlipidemic effect that improved CVD risks (9). Prosopis is a genus of flowering plants in the Fabaceae family. Species of Prosopis are often spiny trees, 2 to 3 m or taller or small shrubs, well adapted to warm weather and drought. Prosopis farcta, commonly known as Syrian mesquite, is native to Asia and distributed from India to Iran(9). Species of this genus have several functions. They have been utilized for gum, paint, cordage, as dietary supplements for feeding ruminants, as well as medicinal purposes (10, 11). Beans and leaves of Prosopis farcta have been used for treatment of some diseases and disorders in traditional medicine including diabetes, inflammatory diseases, wounds and skin disorder, prostate disorders, measles, urinary diseases, diarrhea, and colds(12-14). Also, Prosopis farcta can be used to reduce cardiac or chest pain and for the management of cardiovascular disorders (15). However, to our knowledge, no report of its effect on liver health has been published. The objective of this study was to evaluate the hypolipidemic action of Syrian Mesquite root extract and its action on liver tissue and metabolic parameters of hypercholesterolemic rabbits.

Materials and Methods

1. Plant Material and Extract Preparation

Roots of Prosopis farcta were collected from Kermanshah province, west of Iran, and kept in standard conditions. The root parts of Prosopis farcta were powdered after drying. A total of 100 g of plant powder was added to 1000 ml boiling water and mixed for 15 minutes. The whole content of the mixture was first filtered through an ordinary filter paper, and the filtrate was then passed through a No.1Whatman filtering paper. The solution was transferred into a rotary evaporator for removing surplus water and about 80% of water was removed. The final solution was kept in a water bath at 30°C.

2. Animals and Experimental Design

Thirty-two young male New Zealand White (NZW) rabbits obtained from Pasteur Institute of Tehran and weighing about 180 - 200 g were utilized. After arrival in the laboratory, they were kept under standard conditions of temperature (23±1 °C), relative humidity (55±10%), 12-hour dark and12-hour light cycle, and were fed with ground laboratory Chow 5321 (Ralston Purina Co, St Louis, MO). Ethical rules of the investigation on animals were considered carefully, and the ethic committee's for animal study accepted the protocol of the present study. They were then randomly divided into 4 groups. The first group as control was fed by standard pellet (group I) and the other groups received 2% cholesterol amounts daily (9, 16). NZW rabbits were fed with high cholesterol diet till the serum cholesterol level reached 1800 mg/dl, then, they were treated daily by distilled water (group II), 0.6 mg/kg simvastatin (group III)(17), or 500 mg/kg/day Prosopis farcta root extracts (group IV) orally by gavage for 30 days.

3. Biochemical Analysis

Blood samples were taken from the marginal ear vein of un-anesthetized overnight fasted animals after the adaptation period (day 0) and at the end of treatment (day 30) with the simvastatin and plant extracts. Total cholesterol, triglyceride (TG), low-density lipoprotein(LDL), high-density lipoprotein (HDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST) andcreatinine phosphokinase (CPK), lactate dehydrogenase (LDH) and C-reactive protein (CRP) of serum were measured using a quantification kit (Roche Diagnostics, Mannheim, Germany) in automatic clinical chemistry analyzer. The formula VLDL=TGs/5 was utilized for VLDL measurement (18).

4. Histopathological Analysis of the Liver

In order to perform microscopic evaluation, rabbits were killed by chloroform (overdose) at the end of the investigation and the liver tissues were quickly removed and cut into small pieces. The fixed segments in 10% aqueous formalin were embedded in paraffin and stained with hematoxylin and eosin (9). The histopathological assessment was performed by one person who was blinded to the treated groups. All sections were evaluated microscopically for the extent of fat accumulation, steatosis, inflammation and necrosis.

5. Statistical Analysis

Statistical analysis was performed using the SPSS statistical package version 16.0. The analysis of the variance appropriate for the design was carried out to detect the significance of the differences ($P<0.05$) between the treatment and control groups. Duncan's multiple range test was also performed to compare the significant difference between groups. Difference from the control was considered significant. All the values presented in this article are expressed as the means ± standard deviation.

Results

1. Serum Lipid Parameters Profile

This study showed the changes on the serum level of total cholesterol, triglycerides, high density lipoprotein and low density lipoprotein; due to the effect of Prosopis farcta root extract. Simvastatin was used as a standard drug for improving the lipid profile (positive control). Table 1 shows the serum levels of triglycerides, cholesterol, HDL, LDL and VLDL in control and experimental groups of rabbits. The obtained results demonstrated that feeding the rabbits with high cholesterol diet for 30 days (group II) resulted in a remarkable increase in serum lipid parameters including triglycerides, total cholesterol, LDL, and VLDL when compared to normal controls, that is, rabbits receiving the normal feed (P< 0.01). This indicates the successful establishment of the NAFLD model in rabbits. Both the simvastatin and Prosopis farcta root extract treated groups (group III-IV) produced significant decrease in the serum triglycerides and VLDL levels as compared to the rabbits fed with high cholesterol diet group (P< 0.05). Interestingly, group IV rabbits had more significant decrease in the serum cholesterol and LDL levels than group III animals as compared to the high cholesterol diet group (Table 1). Although, the HDL level decreased in the group III and IV rabbits as compared to the high cholesterol diet group animals, but not statistically significant.

Table 1: Serum lipid profiles in normal and high cholesterol diet–fed rabbits treated with simvastatin and Prosopis farcta root extract for 30 days

Parameters	Group I	Group II	Group III	Group IV
Chol(mg/dl)				
Day 0	63±24	2050±671	2340±767	1920±628
Day 15	39±10	2282±601	1059±765	1149±1047
Day 30	32±10	2240±622	806±645*	530±365**
TG(mg/dl)				
Day 0	72±16	112±26	107±31	114±22
Day 15	55±10	161±21	108±27	107±36
Day 30	77±11	134±22	53±19*	57±17*
HDL(mg/dl)				
Day 0	28±12	443±60	463±12	434±71
Day 15	13±4	315±41	196±90	214±157
Day 30	22±10	106±20	144±98	123±37
LDL(mg/dl)				
Day 0	31±18	1790±484	1843±402	1767±538
Day 15	21±8	1938±524	1043±798	991±804
Day 30	18±3	2141±499	829±688*	512±239**
VLDL(mg/dl)				
Day 0	14±3	22±5	21±6	23±5
Day 15	11±2	32±4	22±5	36±7
Day 30	15±2	27±4	11±4*	11±3*

** P< 0.05 between group II and groups III or IV; **P< 0.01 between group II and groups III or IV*

2. Serum Enzyme Determination

The serum levels of CPK, LDH, ALT and AST were significantly increased in the rabbits fed with high cholesterol diet group as compared to control group receiving the normal feed (Table 2; P< 0.05). The serum CPK and LDH levels were decreased in group III and IV rabbits as compared to high cholesterol diet group, although they were not statistically significant. The ALT and AST enzyme activity in control, simvastatin and Prosopis farcta root extract treated groups revealed no statistical difference (values close to normal levels) whereas these groups showed statistically significant difference as compared to high cholesterol diet group animals (P< 0.05).

Table 2: Serum enzyme levels in normal and high cholesterol diet–fed rabbits treated with simvastatin and Prosopis farcta root extract for 30 days

Parameters	Group I	Group II	Group III	Group IV
CPK				
Day 0	582±175	861±329	676±250	943±338
Day 15	952±196	1980±520	1523±174	1663±962
Day 30	1087±306	2469±694	1577±153	1982±752
LDH				
Day 0	347±148	344±121	254±130	384±99
Day 15	484±173	1025±219	546±193	847±445
Day 30	872±93	1224±411	546±283	849±514
AST				
Day 0	44±19	55±15	43±26	45±9
Day 15	45±10	87±13	45±18	36±15*
Day 30	50±21	117±16	56±27*	54±21*
ALT				
Day 0	50±11	58±18	55±21	52±27
Day 15	45±11	93±23	44±4*	41±13*
Day 30	42±4	128±27	57±28*	42±20**
CRP				
Day 0	-	3	3	3
Day 15	-	3	2	2
Day 30	-	3	1	2

* P< 0.05 between group II and groups III or IV
**P< 0.01 between group II and groups III or IV

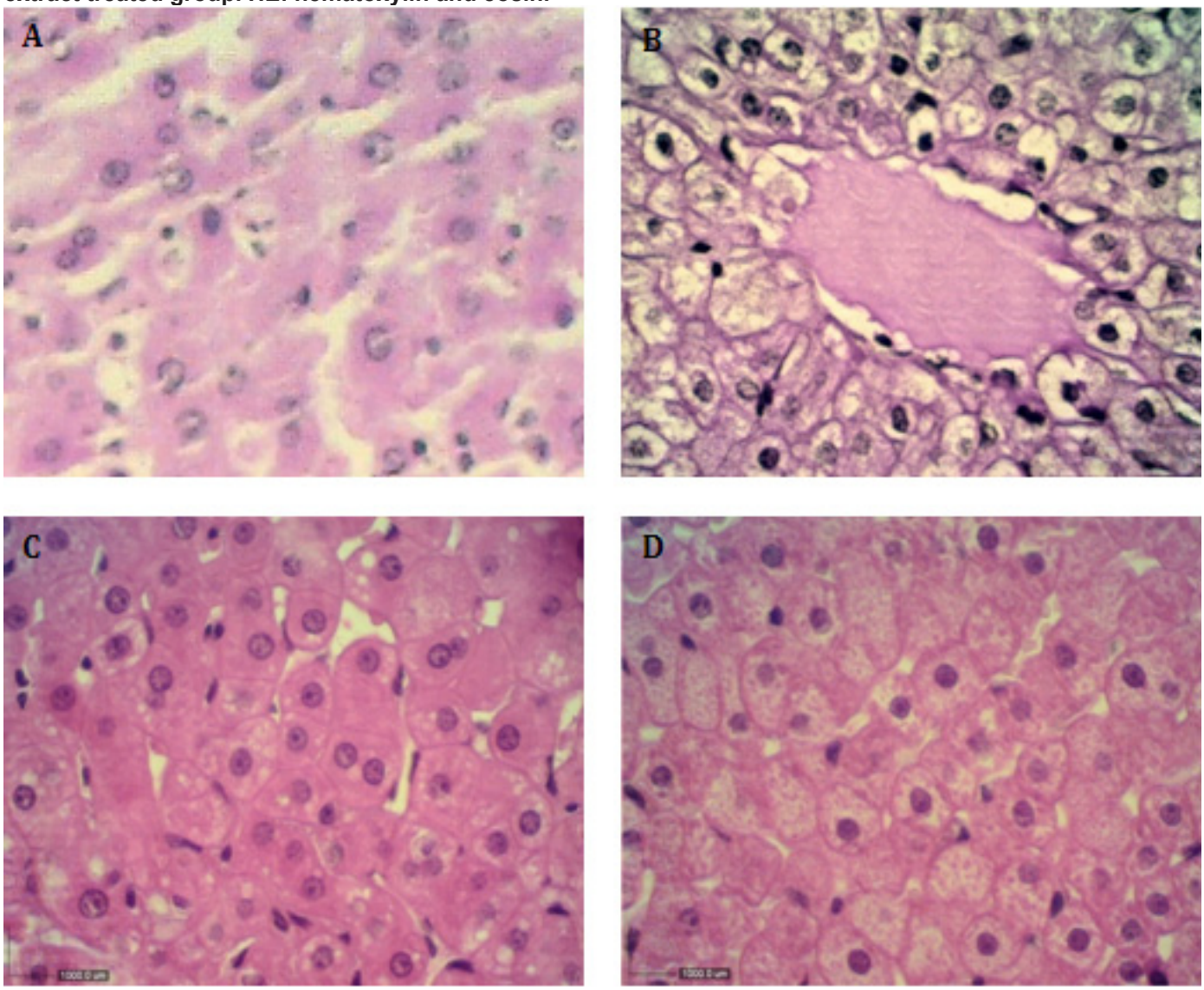
3. Histopathological observations of livers

The histopathological change of the hepatic tissue was examined by light microscope. As shown in Figure 1A, liver sections of normal rabbits showed distinct hepatic cells with abundant cytoplasm, prominent nucleus, and distinct nucleolus. However, the sections of hepatic tissues in the model group showed some degree of necrosis, inflammation and massive fat droplets in the cytoplasm of hepatocytes and centrilobular area, indicating fatty degeneration of the liver (Figure 1B). Although the small lipid globules were still observed in the simvastatin group, in both simvastatin and Prosopis farcta root extract treated groups, lipid degeneration and inflammatory response were significantly alleviated compared with the model group. The decrement of inflammation was confirmed by diminished serum CRP level (Table 2). Also, liver cell volume became smaller and the fat droplets were reduced (Figure 1C and D).

Discussion

NAFLD is a condition closely related to insulin resistance and metabolic syndrome, and associated with genetic susceptibility. NAFLD can start from non-alcoholic simple fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH), and then cause cirrhosis in the liver, and even hepatocellular carcinoma(19). The literature review revealed that high fat diet-induced obesity and abnormal lipid metabolism all collectively are associated with inflammation, congestion, and nonalcoholic fatty liver disease (NAFLD) leading to hepatic failure causing a boost in ALT, AST, and total bilirubin level in the serum (20). Currently the most appropriate and clinically validated methods to minimize and control the progression of this disease are acquisition of healthy life styles and proper food habits (21, 22). Nowadays, different types of therapeutic methods are being used in the treatment of NAFLD and its related disorders. Despite the current therapeutic developments more attention has been shifted towards plant origin therapy as a possible means of alleviating the NAFLD and its associated symptoms (7-9, 16, 21, 23, 24), which are easily available, cost effective, convenient and have limited side effects as compared to the synthetic drugs (22, 25). In Iran, Prosopis farcta has long been used as a therapeutic agent in order to reduce cardiac or chest pain and for managing cardiovascular disorder (9). Some reports showed dose-dependent and endothelium-dependent relaxation effects of Prosopis farcta on thoracic aorta of mice (15). Alcoholic extract of Prosopis farcta leaves reduced blood pressure in vivo and augmented contraction of heart in in vitro experiments(15). Prosopis farcta beans extract has demonstrated protective effects against acetaminophen-induced hepatotoxicity in an animal model (26). Also, according to the study of Mollashahi et al, Prosopis farcta's pod aqueous and ethanol extracts possess neuroprotective effect on rats (27). The findings of Narasimhacharya et al showed that Prosopis julifora leaves have anti hyperlipidemic effect and reverse the hypercholesterolemic conditions in hypercholesterolemic male Albino rats (28). So, this study explored the Prosopis farcta root extract ameliorating effect in minimizing the progression of NAFLD in animals fed with high cholesterol diet. Also, simvastatin, a standard drug for treatment of lipid disorder, was used as a positive control (9, 29). Simvastatin, a 3-hydroxy-3-methylglutaryl

Figure 1. HE staining of the hepatic tissue in all groups (×1000). A: the control group, was fed with normal diet; B: the model group, were induced by high cholesterol feed; C: simvastatin treated group; D: Prosopis farcta root extract treated group. HE: hematoxylin and eosin.



coenzyme A (HMG-CoA) reductase inhibitor, belongs to the class of drugs known as statins. Statins inhibit the synthesis of mevalonate, a rate-limiting enzyme in cholesterol biosynthesis (30). This results in the reduction of the plasma LDL levels with an increase in the hepatic uptake, thereby reducing the risk of CVDs (31).

To investigate the effect Prosopis farcta root extract on reducing blood fat, we reproduced a rabbit model with high cholesterol diet. In the model group, it is demonstrated that the liver index was significantly increased, serum total cholesterol, triglycerides level were increased; serum LDL level and ALT and AST activities were markedly increased. The pathological changes of fat degeneration and inflammatory cell infiltration in hepatic tissue suggested that high cholesterol diet could induce NAFLD pathological changes in the rabbits.

The current investigation demonstrated that treatment with Prosopis farcta root extract can significantly reduce serum levels of total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol ($P<0.05$; Table 1). Moreover, the results obtained in the present study established that high-fat diet causes hepatocellular damage, as clearly seen by the marked elevation of serum enzymes (ALT, AST, CRP) activities and histopathological studies of liver exaggerated with hepatic steatosis. The microscopic studies of liver tissue demonstrated that consumption of the plant root extract in hypercholestrolemic rabbits has significant effects on decreasing the size and abundance of lipid vacuoles. These results are in total agreement with the report of Asadollahi et al and Dashtban et al (26, 32). Asadollahi et al showed that Prosopis farcta bean extract decreased serum total cholesterol, triglycerides, LDL, ALT and AST levels in acetaminophen-induced hepatotoxicity in male Wistar albino rats (26), whereas Dashtban et al indicated that Prosopis farcta bean extract only reduced the serum lipid content (TG, TC and LDL) in streptozotocin-induced diabetic male Wistar albino rats and had no effect on ALT and AST(32).

The medicinal herbs exert their pharmacological effects through a multi-component and multi-target way (33). Preliminary phytochemical studies of the extracts of Prosopis farcta showed the presence of alkaloids, tannins, flavonoids, saponins, glycosides, phenols, resins and

sterols (34-36). Hence, according to the previous studies, Prosopis farcta extract components and our results, we review and summarize the probable mechanisms of Prosopis farcta root extract in the treatment/improving of serum lipid content and NAFLD as follows.

First, It is known that phytosterols with analogue structure to cholesterol may decrease cholesterol absorption displacing it from bile salt micelles and competing for intestinal absorption (37). Intestinal absorption plays a main role in the regulation of cholesterol homeostasis. It has been suggested that transporters can affect its intestinal absorption (38, 39). Silva et al observed a reduction of hepatic cholesterol after β-carotene supplementation to rats with a cholesterol-rich diet, an effect attributed to increased cholesterol fecal excretion(40). In the case of Prosopis farcta root extract, this could be the mechanism for serum cholesterol improvement. On the other hand, when phytosterols were given to hypercholesterolemic patients a significant reduction of LDL was produced (41). On the other hand, because after administration of plant extract the serum HDL level was decreased accompanying with LDL, it is likely that the biosynthesis or absorption/ transport of cholesterol has been impaired.

Secondly , the main ingredient of Prosopis farcta root extract is saponin, which plays the liver-protecting effects in a multi-target way which could well be related to increases in the expression of LDLR of liver, so as to decrease the serum level of LDL, TC and TG(29).

Thirdly, one of the Prosopis farcta extract main component is resins. Currently therapy-induced reduction in serum cholesterol and fatty liver are mainly ascribed to bile salt sequestrants such as the resin – cholestyramine. Resin bound bile salts do not enter the enterohepatic circulation, and this assists further biliary excretion of bile salts, the breakdown products of cholesterol (42). Also, the plant's anti-inflammatory effects may be an additional benefit when steatosis evolves into steatohepatitis (43).

Lastly, various extracts obtained from Prosopis farcta have demonstrated remarkable antioxidant properties which are attributed to flavonoid (especially quercetin) and phenolic components (9, 34, 35). The antioxidant activity may protect the liver from the high-cholesterol toxicity via diminishing of free-radicals production (44, 45). Pan et al have demonstrated that high fat diet can induce oxidative stress (due to increased lipid peroxidation and formation of free radicals (46)) with extensive liver steatosis in animals (47). On the other hand, the flavonoid and phenolic compounds of Prosopis farcta have an important role in improving serum lipid parameters in hypercholesterolemic rabbits through enzyme inhibition/activation. Flavonoids/quercetin inhibitacetyl-CoA carboxylase, diglyceride acyltransferase and HMG CoA reductase are the key enzyme involved in biosynthesis of fatty acids, triglycerides and cholesterol, respectively (48-50). Also, the lipid lowering effect of the flavonoids are due to activation of cytochrome p450 dependent 7a-hydroxylase which results in increased metabolism of cholesterol (51). Accumulating a body of evidence has confirmed the remarkable anti hyperlipidemic and hepato-protective potential of flavonoids; thus, it is suggested that these components are the principal agents responsible for the therapeutic effects of Prosopis farcta in hepatic disorders (29). However, the correct and definitive mechanisms of lipid-lowering and hepato-protective effects of Prosopis farcta root extract need to be further explored.

Conclusion

Overall, administration of Prosopis farcta root extract improved dyslipidaemia and lessened hepatic steatosis in high cholesterol diet-induced NAFLD rabbits. However, the mechanisms of these effects are unclear, and maybe the Prosopis farcta root extract not only regulates lipid intestinal absorption and transport between the peripheral adipose tissue and the liver, but also regulates the lipid metabolism and oxidative stress in the liver, and these were shown to be hepato-protective. This study can serve as a basis for future investigations on the other effects of this plant on human health. Also, phytochemical studies are suggested in order to identify active components of this plant responsible for its therapeutic effect in hepatic diseases. However, well-designed randomized clinical trials evaluating the efficacy and safety profile of these natural drug in patients are required.

References

1. Angulo P. GI epidemiology: nonalcoholic fatty liver disease. Aliment Pharmacol Ther. 2007;25(8):883-9.
2. Clark JM, Brancati FL, Diehl AM. Nonalcoholic fatty liver disease. Gastroenterology. 2002;122(6):1649-57.
3. Jimba S, Nakagami T, Takahashi M, Wakamatsu T, Hirota Y, Iwamoto Y, et al. Prevalence of non-alcoholic fatty liver disease and its association with impaired glucose metabolism in Japanese adults. Diabet Med. 2005;22(9):1141-5.
4. Bagheri Lankarani K, Ghaffarpasand F, Mahmoodi M, Lotfi M, Zamiri N, Heydari ST, et al. Non alcoholic fatty liver disease in southern Iran: a population based study. Hepat Mon. 2013;13(5):e9248.
5. Adams LA, Angulo P, Lindor KD. Nonalcoholic fatty liver disease. Can Med Assoc J. 2005;172(7):899-905.
6. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, et al. Nonalcoholic fatty liver disease a feature of the metabolic syndrome. Diabetes. 2001;50(8):1844-50.
7. Zhang X, Wu C, Wu H, Sheng L, Su Y, Zhang X, et al. Anti-hyperlipidemic effects and potential mechanisms of action of the caffeoylquinic acid-rich Pandanus tectorius fruit extract in hamsters fed a high fat-diet. PLoS One. 2013;8(4):e61922.
8. Mohajeri D, Monadi A. Effects of tomato pulp on hepatic steatosis in the rats fed with high fat diet Bratisl Med J. 2015 116(11):689-94.
9. Saidi MR, Farzaei MH, Miraghaee S, Babaei A, Mohammadi B, Bahrami MT, et al. Antihyperlipidemic Effect of Syrian Mesquite (Prosopis farcta) Root in High Cholesterol Diet–Fed Rabbits. J Evid Based Complementary Altern Med. 2016;21(4):62-6.

10. Andrade-Montemayor H, Cordova-Torres A, García-Gasca T, Kawas J. Alternative foods for small ruminants in semiarid zones, the case of Mesquite (Prosopis laevigata spp.) and Nopal (Opuntia spp.). Small Rumin Res. 2011;98(1):83-92.
11. Orozco-Villafuerte J, Cruz-Sosa F, Ponce-Alquicira E, Vernon-Carter E. Mesquite gum: fractionation and characterization of the gum exuded from Prosopis laevigata obtained from plant tissue culture and from wild trees. Carbohydr Polym. 2003;54(3):327-33.
12. Ali-Shtayeh MS, Jamous RM, Al-Shafie JH, Elgharabah WA, Kherfan FA, Qarariah KH, et al. Traditional knowledge of wild edible plants used in Palestine (Northern West Bank): a comparative study. J Ethnobiol Ethnomed. 2008;4(1):13.
13. Ezike A, Akah P, Okoli C, Udegbunam S, Okwume N, Okeke C, et al. Medicinal plants used in wound care: a study of Prosopis africana (Fabaceae) stem bark. Indian J Pharm Sci. 2010;72(3):334.
14. George C, Lochner A, Huisamen B. The efficacy of Prosopis glandulosa as antidiabetic treatment in rat models of diabetes and insulin resistance. J Ethnopharmacol. 2011;137(1):298-304.
15. Asadollahi K, Abassi N, Afshar N, Alipour M, Asadollahi P. Investigation of the effects of Prosopis farcta plant extract on rats aorta. J Med Plants Res. 2010;4(2):142-7.
16. Huseini HF, Anvari MS, Rabbani S, Sharifi F, Arzaghi SM, Fakhrzadeh H. Anti-hyperlipidemic and anti-atherosclerotic effects of Pinus eldarica Medw. nut in hypercholesterolemic rabbits. DARU. 2015;23(1):1.
17. Hu X, Shu X, Guo Y, Ma Y. Effect of an Ilex asprella root decoction on the related genes of lipid metabolism from chronic stress and hyperlipidemic fatty liver in rats. Chin Med J. 2012;125(19):3539-42.
18. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499-502.
19. Gentile CL, Frye MA, Pagliassotti MJ. Fatty acids and the endoplasmic reticulum in nonalcoholic fatty liver disease. BioFactors. 2011;37(1):8-16.
20. Čonková E, Laciaková A, Pástorová B, Seidel H, Kováč G. The effect of zearalenone on some enzymatic parameters in rabbits. Toxicol Lett. 2001;121(3):145-9.
21. Xiao J, Guo R, Fung ML, Liong EC, Tipoe GL. Therapeutic approaches to non-alcoholic fatty liver disease: past achievements and future challenges. Hepatobiliary Pancreat Dis Int. 2013;12(2):125-35.
22. Thoma C, Day CP, Trenell MI. Lifestyle interventions for the treatment of non-alcoholic fatty liver disease in adults: a systematic review. J Hepatol. 2012;56(1):255-66.
23. Tan Y, Lao W, Xiao L, Wang Z, Xiao W, Kamal MA, et al. Managing the combination of nonalcoholic fatty liver disease and metabolic syndrome with chinese herbal extracts in high-fat-diet fed rats. Evid Based Complement Alternat Med. 2013;2013.
24. Wani FA, Albahrawy AZ, Rahiman S. Hypolipidemic activity of olive oil (olea europaea) against high fat diet-induced nonalcoholic fatty liver disease (NAFLD) in mice. Open J Pathol. 2015;5(03):73.
25. Adams L, Angulo P. Treatment of non-alcoholic fatty liver disease. Postgrad Med J. 2006;82(967):315-22.
26. Asadollahi A, Sarir H, Omidi A, Montazar-Torbati MB. Hepatoprotective potential of Prosopis farcta beans extracts against acetaminophen induced hepatotoxicity in Wistar Rats. Int J Prev Med. 2014;5(10):1281–5.
27. Mollashahi M, Tehranipour M, Khayyatzade J, Moosavi BZJ. The neuroprotective effects of Prosopis farcta pod aqueous and ethanol extracts on spinal cord α-motoneurons neuronal density after sciatic nerve injury in rats. Life Sci J. 2013;10:293-7.
28. Narasimhacharya A, Nitesh K, Desai T. Prosopis juliflora as an antihypercholesterolemic agent. J Herb Med Toxicol. 2010;4(1):31-4.
29. Peng Q, Zhang Q, Xiao W, Shao M, Fan Q, Zhang H, et al. Protective effects of Sapindus mukorossi Gaertn against fatty liver disease induced by high fat diet in rats. Biochem Biophys Res Commun. 2014;450(1):685-91.
30. Pruefer D, Scalia R, Lefer AM. Simvastatin inhibits leukocyte–endothelial cell interactions and protects against inflammatory processes in normocholesterolemic rats. Atertio Thromb Vasc Biol. 1999;19(12):2894-900.
31. Bertolini S, Bon GB, Campbell LM, Farnier M, Langan J, Mahla G, et al. Efficacy and safety of atorvastatin compared to pravastatin in patients with hypercholesterolemia. Atherosclerosis. 1997;130(1):191-7.
32. Dashtban M, Sarir H, Omidi A. The effect of Prosopis farcta beans extract on blood biochemical parameters in streptozotocin-induced diabetic male rats. Adv Biomed Res. 2016;5:116.
33. Li X, Wu L, Liu W, Jin Y, Chen Q, Wang L, et al. A network pharmacology study of Chinese medicine QiShenYiQi to reveal its underlying multi-compound, multi-target, multi-pathway mode of action. PLoS One. 2014;9(5):e95004.
34. Sharifi-Rad J, Hoseini-Alfatemi SM, Sharifi-Rad M, Miri A, Sharifi-Rad M. Phytochemical screening and antibacterial activity of Prosopis farcta different parts extracts against methicillin-resistant Staphylococcus aureus (MRSA). Minerva Biotecnol. 2014;26(4):287-93.
35. Direkvand-Moghadam F, Ghasemi-Seyed V, Abdali-Mashhadi A-R, Lotfi A, Direkvand-Moghadam A, Delpisheh A. Extraction and measurement of the Quercetin flavonoid of Prosopis farcta in Khouzestan climatic condition. Adv Herb Med. 2015;1(1):29-35.
36. Jafarpour A, ElhamiRad A, Mirsaeedghazi H. Evaluation of physicochemical characteristic of Persian mesquite grain (Prosopis farcta) oil. Int J Biosci. 2014;5(1):308-14.
37. Zetina-Esquivel AM, Tovilla-Zárate CA, Guzmán-Garcia C, Rodríguez-Hernández A, Castell-Rodríguez AE, Ble-Castillo JL, et al. Effect of Carica papaya Leaf Extract on Serum Lipids and Liver Metabolic Parameters of Rats Fed a High Cholesterol Diet. Health (N Y). 2015;7(09):1196.
38. Anandhi R, Annadurai T, Anitha TS, Muralidharan AR, Najmunnisha K, Nachiappan V, et al. Antihypercholesterolemic and antioxidative effects of an extract of the oyster mushroom, Pleurotus ostreatus, and its major constituent, chrysin, in Triton WR-1339-induced hypercholesterolemic rats. J Physiol Biochem. 2013;69(2):313-23.
39. Pronin AV, Danilov LL, Narovlyansky AN, Sanin AV. Plant polyisoprenoids and control of cholesterol level. Arch Immunol Ther Exp (Warsz). 2014;62(1):31-9.

40. De Silva LS, de Miranda AM, de Brito Magalhães CL, dos Santos RC, Pedrosa ML, Silva ME. Diet supplementation with beta-carotene improves the serum lipid profile in rats fed a cholesterol-enriched diet. J Physiol Biochem. 2013;69(4):811-20.
41. Suanarunsawat T, Devakul Na Ayutthaya W, Songsak T, Thirawarapan S, Poungshompoo S. Lipid-lowering and antioxidative activities of aqueous extracts of Ocimum sanctum L. leaves in rats fed with a high-cholesterol diet. Oxid Med Cell Longev. 2011;2011.
42. Shetty S, Mengi S, Vaidya R, Vaidya A. A study of standardized extracts of Picrorhiza kurroa Royle ex Benth in experimental nonalcoholic fatty liver disease. J Ayurveda Integr Med. 2010;1(3):203.
43. Alsheikh-Ali AA, Lin J-L, Abourjaily P, Ahearn D, Kuvin JT, Karas RH. Extent to which accepted serum lipid goals are achieved in a contemporary general medical population with coronary heart disease risk equivalents. Am J Cardiol. 2006;98(9):1231-3.
44. Konan K, N'dah Kouamé Justin BL, Souleymane M, Francis YA. Hepatoprotective and in vivo antioxidant activity of Olax subscorpioidea Oliv.(Olacaceae) and Distemonathus benthamianus Baill.(Caesalpiniaceae). Pharmacogn Mag. 2015;11(41):111-6.
45. Senanayake GV, Fukuda N, Nshizono S, Wang Y-M, Nagao K, Yanagita T, et al. Mechanisms underlying decreased hepatic triacylglycerol and cholesterol by dietary bitter melon extract in the rat. Lipids. 2012;47(5):495-503.
46. Ozturk B, Ozer O, Durak ZE, Billur D, Kizil S, Durak I, et al. High cholesterol diet leads to oxidant load and peroxidation in the rabbit kidney tissue. Bratisl Med J. 2016;116(4):235-41.
47. Pan M, Song YL, Xu JM, Gan HZ. Melatonin ameliorates nonalcoholic fatty liver induced by high-fat diet in rats. J Pineal Res. 2006;41(1):79-84.
48. Singh DK, Banerjee S, Porter TD. Green and black tea extracts inhibit HMG-CoA reductase and activate AMP kinase to decrease cholesterol synthesis in hepatoma cells. J Nutr Biochem. 2009;20(10):816-22.
49. Ademosun AO, Oboh G, Passamonti S, Tramer F, Ziberna L, Boligon AA, et al. Phenolics from grapefruit peels inhibit HMG-CoA reductase and angiotensin-I converting enzyme and show antioxidative properties in endothelial EA. Hy 926 cells. Food Science and Human Wellness. 2015;4(2):80-5.
50. Gnoni G, Paglialonga G, Siculella L. Quercetin inhibits fatty acid and triacylglycerol synthesis in rat-liver cells. Eur J Clin Invest. 2009;39(9):761-8.
51. Bilal R, Zakaria M, Usman A, Aftab S, Zia A. Antihyperlipidaemic effects of Eugenia jambolana fruit in diet induced hyperlipidaemic rats. J Pakistan Med Assoc. 2011;61(5):433-7.