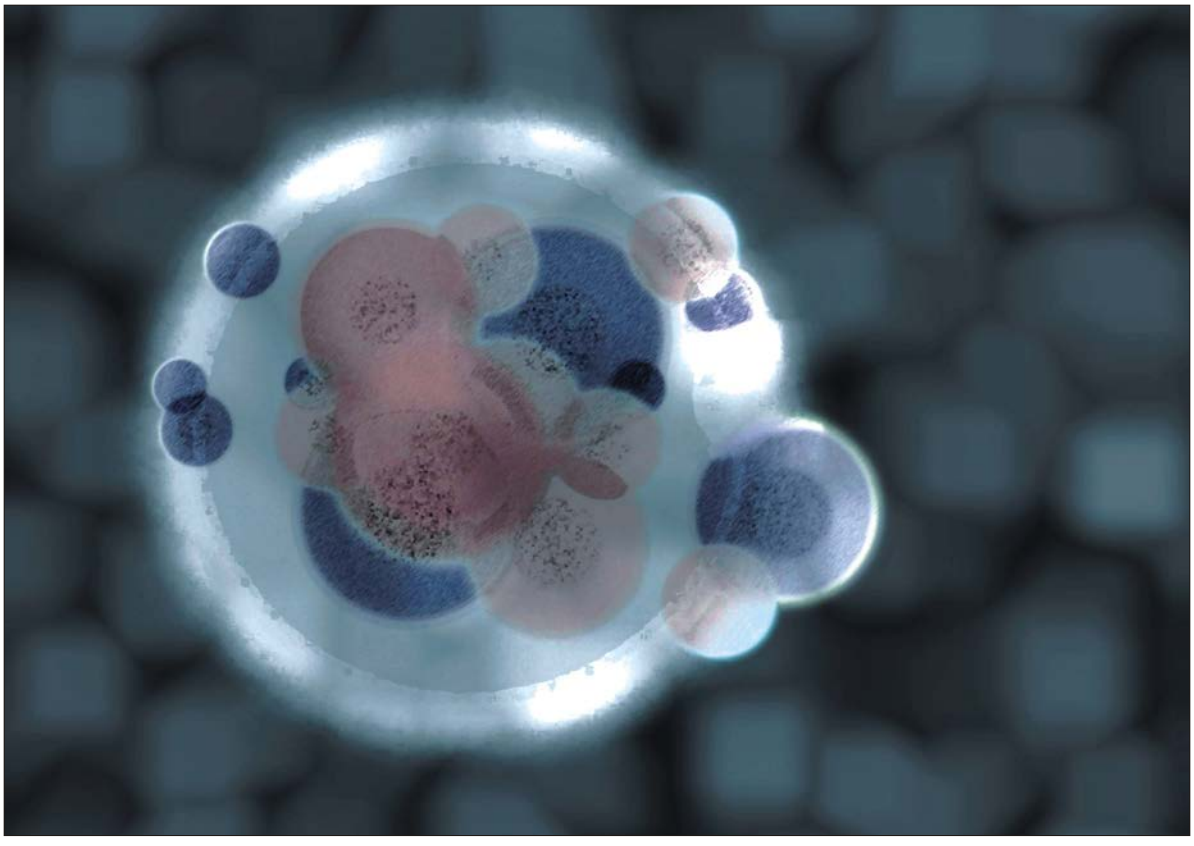

Journal of Biological Sciences

ISSN 1727-3048

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OPEN ACCESS Journal of Biological Sciences

ISSN 1727-3048
DOI: 10.3923/jbs.2017.35.41

Research Article
Hepatoprotective Effect of Methanolic Extracts of *Prosopis farcta* and *Lycium shawii* Against Carbon Tetrachloride-induced Hepatotoxicity in Rats

[1]Khaled B. Alharbi, [2]H.M. Mousa, [1]Z.H. Ibrahim and [1]I.M. El-Ashmawy

[1]Department of Veterinary Medicine,
[2]Department of Food Science and Human Nutrition, College of Agriculture and Veterinary Medicine, Qassim University, P.O. Box 6622, Buraidah, Al-Qassim, Saudi Arabia

Abstract

Background and Objective: Environmental pollutants and toxic chemicals are widely spread and can cause hepatotoxicity. In developing countries plant extracts are the main source for treatment of many ailments. The objective of this study is to investigate the protective effect of methanolic extracts of *Prosopis farcta* and *Lycium shawii* against carbon tetrachloride (CCl_4)-induced hepatotoxicity in rats. **Material and Methods:** The two plants were collected, identified and extracts were prepared. Phytochemical analysis for total phenolics, total tannins content, non-tannins phenolics and total flavonoids was performed. Liver damage was induced in rats by repeated administration of CCl_4 (30% in olive oil (3 mL kg^{-1} b.wt., i.p.). Liver protection was accomplished in 2 groups of rats by daily oral administration of extract of *Prosopis farcta* and *Lycium shawii* (250 mg kg^{-1} b.wt.,) for 15 days prior to CCl_4 administration. Liver marker enzymes, serum total bilirubin, malondialdehyde (MDA), reduced glutathione (GSH), glutathione-S-transferase (GST), lipid profile, total antioxidant capacity were determined together with histopathological damage evaluation. **Results:** The CCl_4 treatment increased significantly ($p \leq 0.05$) liver marker enzymes, total bilirubin, MDA, GST, lipid profile and decreased significantly total serum protein, total antioxidant capacity, high density lipoprotein cholesterol (HDL-C) and GSH. *Prosopis farcta* and *Lycium shawii* treatment significantly reversed the CCl_4-induced changes, towards normalcy. **Conclusion:** Liver protection capability of these two plants may be due to their high contents of various flavonoids that act as powerful antioxidants and free radical scavengers.

Key words: Heptotoxicity, *Prosopis farcta*, *Lycium shawii*, antioxidants, lipid profile

Received: November 02, 2016 Accepted: December 02, 2016 Published: December 15, 2016

Citation: Khaled B. Alharbi, H.M. Mousa, Z.H. Ibrahim and I.M. El-Ashmawy, 2017. Hepatoprotective effect of methanolic extracts of *Prosopis farcta* and *Lycium shawii* against carbon tetrachloride-induced hepatotoxicity in rats. J. Biol. Sci., 17: 35-41.

Corresponding Author: H.M. Mousa, Department of Food Science and Human Nutrition, College of Agriculture and Veterinary Medicine, Qassim University, P.O. Box 6622, Buraidah, Al-Qassim, Saudi Arabia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Environmental pollutants, toxic chemicals and some drugs can cause cellular injuries through various mechanisms[1]. Carbon tetrachloride (CCl_4) has been used to study liver damage in animal models because it generates lipid peroxides that can cause injuries to the liver and various other organs[2,3]. Metabolism of CCl_4 releases metabolites that can cause tissue injuries particularly in the liver. Exposure to CCl_4-induces an increase in free radicals concentrations that are highly reactive and cause injury[4].

Plant materials are used since ancient time for treatment of various diseases[5-7] . The plant *Prosopis farcta* (PF) from Leguminosae and sub-family Mimosoideae is indigenous to dry and semi-dry areas of Asia, America and Africa[8]. Previous studies on this plant have reported some medicinal properties[9]. Asadollahi *et al.*[10] reported that *Prosopis farcta* beans extracts have protective effects against induced hepatotoxicity in Wistar rats. However, the mechanism by which *Prosopis farcta* beans extracts exhibited protection is not clear. *Lycium shawii*, desert thorn or Arabian boxthorn is a species of thorny shrub adapted to desert environments. *Lycium* is a genus of flowering plants in the nightshade family, Solanaceae. They are xerophytic plants. Genus *Lycium* was reported to have some medicinal properties[11-14].

Studies on the hepatprotective effects of phytochemicals are scarce. In this study, the role of *Prosopis farcta* and *Lycium shawii* in protection against induced hepatotoxicity in Wistar rats was evaluated.

MATERIALS AND METHODS

Animals and diet: Twenty four adult male Wistar rats weighing between 150-180 g were purchased from the College of Pharmacy, King Saud University, Saudi Arabia. Rats were housed in polyacrylate cages. They were provided with standard commercial rat pellets (No. 648-General Organization for Grain Silos and Flour Mills, Riyadh and water *ad libitum*. All experiments were approved by the Institutional Animal Ethics Committee (IAEC) of Qassim University, Saudi Arabia.

Preparation of plant material: Two plants *Lycium shawii* and *Prosopis farcta* were collected at the flowering stage from Qassim region (KSA). The collected plant species were identified and confirmed by the Department of Plant Production and Plant Protection, College of Agriculture and Veterinary Medicine, Qassim University.

Shade dried and powdered plant materials were successively extracted by methanol. The extracts were concentrated until obtaining paste under vacuum. The extract was used for evaluation of phytochemical constituents and for the treatment of rats.

Experiment design: Rats were segregated randomly into 4 groups of 6 rats each:

Group 1: Animals of this group (Negative control) were fed standard pellet diet and received water *ad libitum*.

Group 2: The positive control, received 30% CCl_4 in olive oil (3 mL kg^{-1} b.wt., i. p.) after every 72 h for 3 days (3 doses).

Group 3: Animals in this group were given daily an oral aqueous extract dose of *Lycium shawii* (250 mg kg^{-1} b.wt.) for 2 weeks followed by CCl_4 in olive oil (3 mL kg^{-1} b.wt., i.p.) after every 72 h for 3 days (3 doses).

Group 4: Animals in this group were given daily an oral aqueous extracts dose of 250 mg of *Prosopis farcta* for 2 weeks followed by CCl_4 in olive oil (3 mL kg^{-1} b.wt., i.p.) after every 72 h for 3 days (3 doses).

Treatment with *Lycium shawii* and *Prosopis farcta* extracts continued for another 7 days for group 3 and 4, respectively. After that all animals were kept starved for 24 h. On the following day the animals were sacrificed by decapitation and blood was collected from the jugular vein. Serum was collected by centrifugation and stored at -20°C.

Following necropsy, liver specimens were harvested, preserved in 10% neutral buffered formalin and stored.

Phytochemical analysis

Determination of total phenolics and total tannins content: Total phenolics and total tannins contents of *Prosopis farcta* and *Lycium shawii* were determined as described by Singleton and Rossi Jr.[15].

Determination of total flavonoids: The flavonoid content was determined as described by Zhishen *et al.*[16] expressed as mg quercetin equivalents g^{-1} residue.

Biochemical estimations

Determination of malondialdehyde (MDA): Lipid peroxidation was determined in serum according to Ohkawa *et al.*[17].

Determination of glutathione-S-transferase (GST) activity: The GST activity was assayed as described by Habig *et al.*[18].

Evaluation of liver function: The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined spectrophotometrically according to the method of Schumann and Klauke[19]. While, the activities of alkaline phosphatase (ALP) was carried out according to the method of Tietz[20]. Total serum bilirubin concentration was estimated using commercially available kits (Boehringer, Mannheim, Germany).

Determination of reduced glutathione: Reduced glutathione was determined according to the method of Moron *et al.*[21].

Determination of serum total protein: Total serum proteins was determined by the method of Bradford[22].

Determination of serum lipid profile: Total Cholesterol (TC) was determined according to Allain *et al.*[23], triglycerides (TG) according to Stein and Myers[24], high density lipoprotein cholesterol (HDL-C) according to Sugiuchi *et al.*[25]. While low density lipoprotein (LDL-C) was calculated by Friedwald *et al.*[26] formula:

$$\text{LDL} = \frac{\text{Total cholesterol-HDL-(Triglycerides)}}{5}$$

Determination of total antioxidant capacity: Total antioxidant capacity was determined according to the method of Koracevic *et al.*[27].

Histopathology: Liver specimens were processed via paraffin embedding technique (dehydration in ethanol, clearing in xylene and impregnation in melted wax). Five micrometers thick sections were prepared and stained with hematoxylin and eosin (H and E) according to Suvarna *et al.*[28]. The sections were examined for pathological findings of hepatotoxicity.

Statistical analysis: Statistical analysis was performed using the statistical package (SPSS-16, Chicago, USA). The results were expressed as Mean±Standard Deviation (SD).

RESULTS

The phytochemical contents of *Lycium shawii* and *Prosopis farcta* are presented in Table 1. *Prosopis farcta* contain more total phenolics, tannins, non-tannins phenolics and total flavonoids (271.92, 81.24, 190.68 and 135.0 mg g^{-1}), respectively when compared with *Lycium shawii* (101.7, 47.66, 54.04 and 59.8 mg g^{-1}), respectively.

The levels of total protein, total cholesterol, triglycerides, HDL-C, LDL-C in sera of the experimental rats are shown in Table 2. Treatment with CCl_4 alone significantly ($p \leq 0.05$) decreased the level of total protein. Administration of *Prosopis farcta* and *Lycium shawii* increased the levels of serum protein.

Table 2 shows the effect of *Prosopis farcta* and *Lycium shawii* on the serum lipid profile in CCL_4 treated rats. There was a significant ($p \leq 0.05$) elevation of serum TC, TG and LDL-C with concomitant significant ($p \leq 0.05$) reduction in the level of HDL-C. Pre-treatment with *Prosopis farcta* decreased significantly ($p \leq 0.05$) the level of TC, TG and LDL-C and increased significantly ($p \leq 0.05$) the level of HDL-C.

While treatment with *Lycium shawii* resulted in a non-significant decrease in the level of serum TC, TG and LDL-C and a non-significant increase in the level of HDL-C.

The effect of *Prosopis farcta* and *Lycium shawii* on liver marker enzymes and bilirubin level in the serum of CCL_4 treated rats is shown in Table 3. The increased activities of ALP, AST and ALT and bilirubin level in the serum of

Table 1: Quantitative analysis of methanolic extracts of *Prosopis farcta* and *Lycium shawii* (mg g^{-1})

Plant name	Total phenolics	Tannins	Non-tannins phenolics	Total flavonoids
Lycium shawii	101.70	47.66	54.04	59.8
Prosopis farcta	271.92	81.24	190.68	135.0

Table 2: Effect of *Prosopis farcta* and *Lycium shawii* on serum total protein, total cholesterol, triglycerides, HDL-C, LDL-C in rats

Groups	Treatment	Total proteins (g dL^{-1})	Total cholesterol (mg dL^{-1})	Triglycerides (mg dL^{-1})	HDL-C (mg dL^{-1})	LDL-C (mg dL^{-1})
Negative control	Control	7.23±0.12^{a}	78.76±3.72^{c}	42.26±2.46^{c}	42.48±1.62^{a}	27.83±2.82^{c}
Positive control	CCl_4 (3 mL kg^{-1})	4.36±0.62^{c}	143.76±8.42^{a}	105.65±7.42^{a}	21.64±3.11^{c}	100.96±8.44^{a}
Lycium shawii	Extract 1+CCl_4	5.11±0.28^{c}	87.24±5.18^{b}	58.02±4.68^{b}	34.86±2.38^{b}	40.78±2.26^{b}
Prosopis farcta	Extract 2+CCl_4	6.66±0.42^{b}	82.68±6.14^{bc}	51.42±4.32^{b}	40.92±1.24^{a}	31.48±3.42^{C}

All values are expressed as Means±SD. Means on the same column with different letters are significantly different at $p \leq 0.05$, HDL-C: High density lipoprotein cholestrol, LDL-C: Low density density lipoprotein cholestrol

Table 3: Effect of *Prosopis farcta* and *Lycium shawii* on liver enzymes and serum bilirubin concentration in rats treated with CCl_4

Groups	Treatments	ALP (U L^{-1})	AST (U L^{-1})	ALT(U L^{-1})	Bilirubin (mg dL^{-1})
Negative control	Control	58.40±1.32^{d}	72.2±2.4^{d}	36.2±4.3^{c}	0.36±0.08^{c}
Positive control	CCL_4 (3 mL kg^{-1} b.wt.)	187.80±4.24^{a}	169.4±5.4^{a}	112.4±2.8^{a}	1.80±0.01^{a}
Lycium shawii	Extract 1+CCL_4	142.00±2.76^{b}	1 12.6±8.6^{b}	60.4±4.6^{b}	0.41±0.02^{b}
Prosopis farcta	Extract 2+CCL_4	96.46±3.44^{c}	88.2±6.6^{c}	52.6±4.82^{b}	0.38±0.04^{c}

All values are expressed as Means±SD. Means on the same column with different letters are significantly different at $p \leq 0.05$, ALP: Alkaline phosphatase, AST: Aspartate amino transferase, ALT: Alanine aminotransferase and CCl_4: Carbon tetrachlodie

Table 4: Effect of *Prosopis fracta* and *Lycium shawii* on serum oxidative stress parameters in rats treated with CCl_4

Groups	Treatments	MDA (µmol L^{-1})	GST (U L^{-1})	Total antioxidant capacity (mM L^{-1})	Reduced glutathione (GSH) (µmol L^{-1})
Negative control	Control	1.324±0.40^{c}	24.14±2.25^{d}	0.36±0.03^{a}	3.48±0.82^{a}
Positive control	CCL_4 (3 mL kg^{-1} b.wt.)	5.098±0.68^{a}	48.00±3.64^{a}	0.12±0.07^{c}	1.42±0.12^{d}
Lycium shawii	Extract 1+CCL_4	2.800±0.22^{b}	36.26±2.24^{b}	0.28±0.11^{b}	1.84±0.64^{c}
Prosopis farcta	Extract 2+CCL_4	1.490±0.23^{c}	29.14±1.44^{c}	0.32±0.08^{a}	2.96±0.72^{b}

All values are expressed as Means±SD. Means on the same column with different letters are significantly different at $p \leq 0.05$, MDA: Malondialdehyde, GST: Glutathione-S-transferase

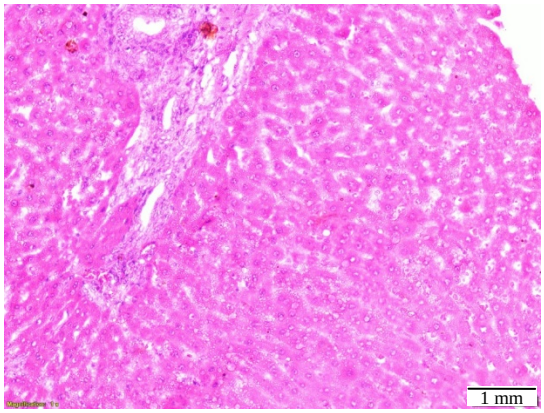

Fig. 1: Liver from rats given CCl_4 alone (group 2) showing mild fatty change, H and E X100

hepatotoxic rats were found to decrease upon treatment with *Prosopis farcta* and *Lycium shawii*. It can be observed that treatment with *Prosopis farcta* was more effective in reducing the hepatic marker enzyme activities and bilirubin compared to *Lycium shawii*.

The effect of *Prosopis fracta* and *Lycium shawii* on serum oxidative stress parameters in rats treated with CCl_4 is shown in Table 4. Administration of CCl_4 increased significantly ($p \leq 0.05$) the level of malondialdehyde (MDA) and the activity of GST and reduced significantly ($p \leq 0.05$) the level of the total antioxidant capacity and reduced glutathione (GSH). Treatment with *Prosopis farcta* and *Lycium shawii* resulted in improvement in the level of these parameters .In this study treatment with *Prosopis farcta* is more effective in improving the studied parameters when compared with *Lycium shawii*.

Histopathologic findings: The microscopic examination of livers of rats treated with (CCl_4) revealed multifocal mild

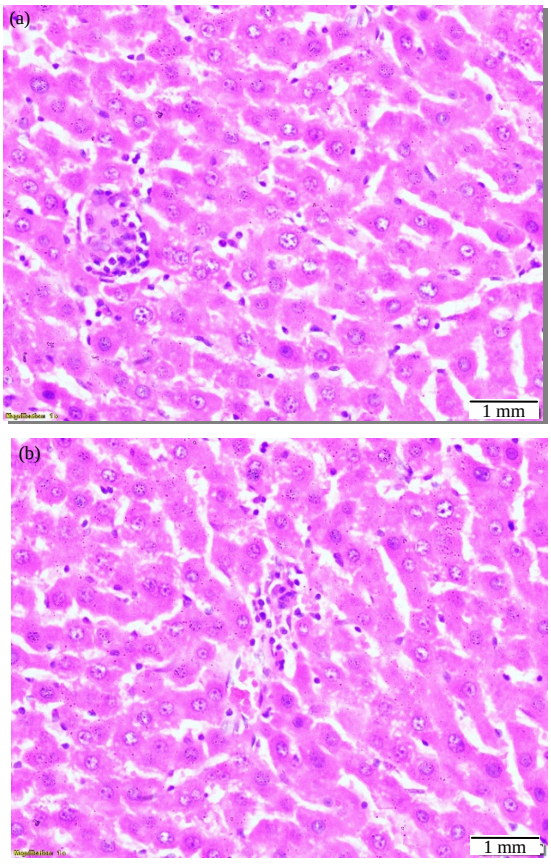

Fig. 2(a-b): Liver from rats given CCl_4 alone (group 2) showing small necrotic foci, H and E X160

fatty changes characterized by minute sharp cytoplasmic vacuolation of the hepatocytes (Fig. 1). Moreover, few minute foci of necrosis were evident (Fig. 2). Upon administration of *Prosopis farcta* extract (group 4), there was marked improvement of the histopathologic picture, severity and

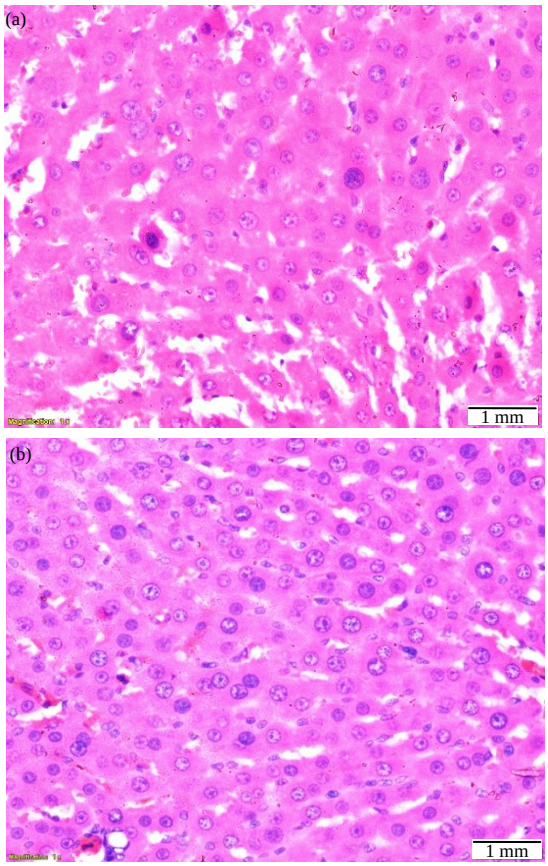

Fig. 3(a-b): Liver from rats given CCl_4 alone (group 2) showing nuclear hypertrophy and binucleation of some hepatocytes, H and E X160

distribution of the degenerative lesions were obviously reduced with disappearance of the necrotic lesions in addition to regenerative trials of the hepatic cells in the form of binucleation and nuclear hypertrophy (Fig. 3). Regarding rats that received *Lycium shawii* extract, both the dengenrative and necrotic changes were considerably diminished as seen in rats of group 3 but without hepatic cell regenerative capabilities.

DISCUSSION

The present study was carried out to investigate the protective effect of aqueous extract of *Prosopis farcta* and *Lycium shawii* against CCl_4-induced liver damage in rats.

Few plants were used for the treatment of hepatotoxicity[9]. Many plants are used as a folk remedy without academic confirmation of its effects. However, *Prosopis farcta* and *Lycium shawii* are used for the treatment of various ailments[10,29,30].

The present investigation showed that CCl_4 administration in rats significantly increased the activities of serum ALT, AST and ALP indicating hepatocyte injuries via altering membrane integrity by covalent binding of CCl_4 metabolites (i.e., CCl_3, CCl_3OO) to membranes. Similar findings, were reported by Weber *et al.*[31], Miyazaki *et al.*[32] and Preethi and Kuttan[3].

However, after treatment with *Prosopis farcta* and *Lycium shawii*, the observed elevation in ALT, AST and ALP were significantly restored indicating that *Prosopis farcta* and *Lycium shawii* have the ability to protect against hepatocytes injuries induced by CCl_4. The primary cause for hepatocytes injury is lipid peroxidation. Indications of lipid peroxidation in the present study included increases in the levels of MDA, GST and a decrease in the total antioxidant capacity and GSH in serum. Malondialdehyde (MDA) is an indicator of lipid peroxidation. An increase in free radicals causes over production of MDA. It is well known marker of oxidative stress[33]. Reduced glutathione (GSH) is a strong scavenger of free Radical Oxygen Species (ROS). The decreased concentration is an indication of GSH utilization in the neutralization of free radicals[34]. Administration of *Prosopis farcta* and *Lycium shawii* restored GSH and MDA levels to those in the control group.

Administration of CCl_4 increased serum TC, TG and LDL and decreased the level of serum HDL, GSH and total antioxidant capacity. Administration of *Prosopis farcta* and *Lycium shawii* significantly decreased serum TC, TG and LDL-C levels. This indicates that these two plant extracts have lipid-lowering effect and have antioxidant properties.

As presented in Table 1 these plants are very rich in phenolic compounds, mainly tannins and flavonoids that are major group of antioxidants acting efficiently as scavengers of free radicals[35]. Flavonoids are natural substances with variable phenolic structures. It was found that consumption of flavonoid is associated with a decreased risk of several types of diseases[36]. Hepatoprotective effects of various flavonoids were directly related to their antioxidant activities and reduction of lipid peroxidation[37,38]. It can be noticed in Table 2, that both *Prosopis farcta* and *Lycium shawii* contain phenolic compounds with strong antioxidant activity. However, the level of these phenolic compounds is higher in *Prosopis farcta* compared with *Lycium shawii*.

From pathologic point of view, it was clear that both extracts alleviated the toxic potentials of CCl_4 with additional hepatocytic regenerative capabilities for *Prosopis farcta* extract.

CONCLUSION

It can be concluded that *Prosopis farcta* and *Lycium shawii* have hepatoprotective and antihyperlipidaemic effect, which is evidenced by the decreased levels of TC, TG, LDL-C, MDA and elevated levels of HDL-C, total antioxidant capacity and GSH in the serum and in this respect, *Prosopis farcta* is more efficient .

SIGNIFICANT STATEMENT

The use of herbs to treat disease is widespread especially in poor societies. The study of plants chemical compounds is an effective way to discover future medicines:

- *Prosopis farcta* and *Lycium shawii* were found to contain high contents of various flavonoids well known as potent antioxidants and free radical scavengers that protected the liver from damage induced by CCl_4
- The study may provide baseline for use of these plants in food and in drugs manufacturing

ACKNOWLEDGMENT

The authors would like to thank King Abdulaziz city for Science and Technology (KACST) for the financial support of this study, which is a part of a research grant No. AT-32-1.

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