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AMELIORATIVE EFFECT OF FERMENTED GOAT'S MILK COMBINED WITH DESERT TRUFFLE AND BAOBAB AQUEOUS EXTRACT IN CCL4 INDUCED LIVER LESION IN RATS

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ABSTRACT

The aim of the present work was to investigate the synergistic hepatoprotective effect of fermented goat milk (FGM) combined with aqueous extracts of baobab (*Adansonia digitata* L.) (BE) and desert truffle (*Terfezia claveryi*) (DTE). Chemical analysis indicated that BE is rich in ascorbic acid (67.3 mg/100 g dry weight). Both extracts reduced the stable free radical DPPH (96.4 and for BE versus 70.2 % for BE and DTE, respectively). For examining the hepatoprotective effect of these treatments in vivo, thirty-six rats were randomly divided into six groups (n=6 per group). Group 1 served as the normal control (NC). Hepatotoxicity was induced in rats of the other five groups using CCl4. Group 2 served as the positive control (PC), while the remaining 4 groups received orally FGM, DTE, BE and their mixture (1:1:1) for 28 days. Hepatotoxicity was characterized by high liver weight as well as an elevation in serum levels of liver enzymes in the PC group. All treatments restored weight of liver to that of the NC group (P>0.05). Highest positive effect was recorded for DTE which lowered liver weight by 33.9% compared with PC group (P<0.05). Concentrations of liver enzymes were significantly reduced in all treatment groups compared to PC group. Moreover, the protective effect was further confirmed by histopathological as a considerable reduction in necrosis and fatty changes were noticed. In sum, FGM, Desert Truffle and Baobab could be potentially used as functional food to protect against liver lesion.

Keywords: Fermented Goat's Milk, Desert Truffle, Baobab, Antioxidant status, Hepatoprotective, Rats

INTRODUCTION

There is an increased interest in using healthy fermented milks and many naturally occurring botanicals and herbs as protective agents against chronic diseases. Nowadays, consumers are searching for healthier food products. Scientific evidence supported the health benefits of the active components of the food, consequently a rapid development in the production of healthy foods has been promoted. Due to its nutritional properties and health benefits, there is an increased interest in using goat milk as a healthy food in several developed countries **(Lad *et al.*, 2017)**.
Goat milk (GM) is a highly nutritive food compared to cow or human milk. GM has specific biological characteristics , such as high buffering capacity **(Park, 2009; Lad *et al.*, 2017)**, high digestibility and physiologically functional components. It showed several health benefits such as antioxidant properties **(Haenlein, 2004; Park *et al.*, 2007)**. In earlier work, GM protected from oxidative stress in rats **(Dı´az-Castro *et al.*, 2012)**. In addition, GM enhanced metabolism of iron **(Dı´az-Castro *et al.*, 2014)**. Some other investigations suggested hepatoprotective activity of GM, due to its anti-inflammatory and antioxidant properties **(Dı´az-Castro *et al.*, 2013; Miglani *et al.*, 2015)**. Fermented goat's milk (FGM) has higher nutritional value than the fresh goat's milk due to fermentation by lactic acid bacteria **(de Vrese *et al.*, 2011)**. FGM was showed high quality and sensory attributes that was comparable with fermented cow's milk **(Taufiq & Anindita, 2013)**. In addition to the health benefits of fresh goat's milk, FGM showed more health benefits, especially when the fermentation done by using probiotic bacteria **(Minervini *et al.*, 2009)**. Several trials were made to produce FGM by addition of putative probiotic strains to produce a healthy fermented dairy product **(Paz *et al.*, 2014; Moreno-Montoro *et al.*, 2018)**.
On the other hand, several reports showed health benefits of many naturally occurring botanicals and herbs and used as nutraceutical ingredients **(Farrukh and Mukhtar, 2002)**. Baobab (*Adansonia digitata L.*) is a member of Bombacaceae family that is found in the savannas of Africa and other locations around the equator **(Rahul *et al.*, 2015)**. Its fruit pulp can be eaten raw or as an ingredient in several food formulations. It is rich in micronutrients, vitamins (particularly vitamin C) and antioxidants **(Braca *et al.*, 2018; Althwab *et al.*, 2019)**. Compared with orange fruit pulp, Baobab fruit pulp showed more Antioxidant Capacity (10 times higher) **(Vertuani *et al.*, 2002)**.

Truffle is a desert fungus that grows in the northern part of Saudi Arabia and other desert parts of some Arab countries **(Hussain and Al-Ruqaie, 1999)**. Truffle is well known for its nutritional properties and health benefits. It is low in calories and fat and high in polyscharides (fibers), high-quality proteins, vitamins and minerals **(Murcia *et al.*, 2002)**. It showed protective effects due to its antioxidant and free radical scavenging properties **(Al-Laith, 2010)**. In addition, aqueous extract of desert truffle *Terfezia claveryi* showed a high hepatoprotective activity in rats treated with carbon tetrachloride **(Janakat and Nassar, 2010)**. CCl_4 when administered transforms into highly toxic trichloromethyl peroxyl radical; that disrupts polyunsaturated fatty acids in membrane lipids leading to rupture of membrane structures leading to lipid peroxidation in liver cells (Weber *et al.*,2003)
Therefore, the aim of the present work was to study the potential effects of FGM combined with an aqueous extracts of baobab (*Adansonia digitata L.*) and desert truffle (*Terfezia claveryi*) on liver of rats injured with carbon tetrachloride.

MATERIAL AND METHODS

Chemicals

Chemicals were purchased from Sigma-Aldrich (St. Louis, MO) Chemical Co. While Commercial kits were purchased from (bio-Merieux Laboratory Reagents and Products, France).

Experimental animals

Thirty-six male Wistar rats (140±10 g body weight) were obtained (College of Pharmacy, King Saud University, Riyadh, Saudi Arabia). Animals were housed in an experimental animal house (a control housing unit) at Dept. of Food Science & Human Nutrition, College of Agriculture and Veterinary Medicine, Qassim University, Saudi Arabia. Animals were kept under standard conditions of temperature and humidity (temperature at 25°C, 55% humidity and in a 12:12 h light: dark cycle). The animals were fed on the AIN-93-basal-diet according to **Reeves *et al.* (1993)**. They were provided with water *ad-libitum* during the experimental period. The animal study protocol was approved by the ethical committee, Qassim University.

Antioxidant status assay

Desert Truffle powder and Baobab fruit pulp powders were extracted by methanol:water (60:40 v/v) at solvent/powder ratio of 4:1 (v/w) as described by **Bloor (2001)**. To extract the bioactive compounds, each solutions was stirred and the contents were left to dissolve for ten minutes at room temperature then filtered with Whatman No. 4 paper to collect the filtrate. The quantification of Vitamin C (ascorbic acid), total phenolics, total flavonoid and DPPH radical scavenging activity were performed using an aliquot of these extracts. Vitamin C content was measured by the titration method against 2, 6-dichlorophenol indophenol **(AOAC, 2000)**. Total phenolic content was measured according to **Singleton *et al.* (1999)** using the Folin–Ciocalteu reagent. Gallic acid was applied as a standard, and calculations were expressed as as milligrams gallic acid equivalent (GAE).
The method of **Mohdaly *et al.* (2012)** was used to determined the total flavonoid content, and the calculations were expressed as milligrams quercetin equivalents (QE). The DPPH free radicals scavenging ability was determined according to the method described by **Blois (1958)**. Aliquots (100 µl) of each extract was mixed with 2.9 ml of 0.1 mM DPPH in methanol. The control samples contained all the reagents except the extract. The absorbance at 517 nm was measured after 30 min of incubation at room temperature. Radical scavenging capacity of each extracts was expressed as percent DPPH radical scavenging effect using the following equation:
Scavenging activity % = $[(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$

Preparation of FGM; Aqueous Extract of desert truffle and baobab

Goat milk samples were obtained from healthy lactating animals at the Agricultural Research Station, Qassim University. FGM was prepared by adding a starter culture of *Streptococcus thermophiles* and *Lactobacillus bulgaricus* (Chr. Hansen`s Laboratory, Copenhagen Denemark) to goat milk at 42 °C until coagulation.
Desert truffle *Terfezia claveryi* was purchased from local market, washed and dried in air oven at 65 °C. Then, 500 g of the dried truffle were mixed in water (3:1 w/v) at 4000 rpm in a mixer for 15 min according to **Janakat *et al.* (2004)**, then freeze-dried (freeze drieyer (CHRIST, Alpha 1-4 LD plus, German). The DTE was prepared by dissolving 50 mg of the freeze-dried powder in 100 ml of distilled water.
Edible portion of Baobab (pulp) was collected, hand pounded to pass through sieve 40 mesh size. The powdered pulp sample, 100 g was weighed, dispersed in 500 mL of deionized hot water. The mixture was then centrifuged and the supernatant filtered through rapid fluted filter paper. From the Baobab stock 10.0 % (V/V) solutions were prepared in distilled water and stored in refrigerator -20 °C till used. The mixture (MIX) was prepared from FGM: DTE:BE in1:1:1 ratio.

Biological experiment procedure

Rats were randomly divided into six groups (6 rats each) and fed on normal basal diet as the following:

1- **NC group**: normal control group.
2- **PC group**: Rats were intoxicated with CCl_4, and kept as a positive control group.
3- **FGM group**: Rats were intoxicated with CCl_4 + oral admiration of fermented goat's milk.
4- **DTE group**: Rats were intoxicated with CCl_4 + oral admiration of an aqueous extract of desert truffle (*Terfezia claveryi*).
5- **BE group**: Rats were intoxicated with CCl_4 + oral admiration of an aqueous extract of baobab (*Adansonia digitata* L.).
6- **MIX group**: Rats were intoxicated with CCl_4 + oral admiration of a mixture of goat's milk, desert truffle extract, and baobab extract (1:1:1).

Hepatotoxicity was induced in rats of 5 groups (PC, FGM, DTE, BE, and MIX) by using a (1:1) mixture of CCl_4: paraffin, administrated intraperitoneally at a single dose of 2 ml CCl_4 /kg body weight **(Janakat and Nassar, 2010)**. **Althnaian *et al.* (2013)** noticed no significant toxic changes on rats when received paraffin only. Therefore, there was no such rats' group in the present experiment. This is in consistence with other studies using the same animal model **(Ismail *et al.*, 2009; Cao *et al.*, 2005)**. All groups received oral treatments as described for 28 days. Changes in body weight were recorded weekly. At the end of the experimental period, blood samples were taken from the retro-orbital plexus of the eyes from all animals of each group in heparinized tubes. The animals were anesthetized with diethyl ether and rapidly decapitated. Livers were collected immediately after dissection and weighted. serump was obtained from blood samples by centrifugation at 1500 rpm/15 min at an ambient temperature for analysis. Animal procedures were performed in accordance with the ethics committee of Qassim University and according to the Guide for the Care and Use of Laboratory Animals of the National Institute of Health.

Evaluation of liver functions

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined colorimetrically according to the method of **Reitman and Frankel (1957)**. Alkaline phosphatase (ALP) was measured by using a commercially available ELISA kit (Human Co., Germany).

Tissue sampling

Tissue samples were taken from the liver of the sacrificed rats and fixed in 10% formalin saline solution for ten hours. Then washed in tap water for 12 hours. Serial alcohol (methyl, ethyl and absolute) were used for dehydration of the tissue samples. Tissue specimens were cleared in xylene and embedded in paraffin. The paraffin blocks were sectioned at 3-micron thickness by sledge microtome. The obtained tissue sections were collected on the glass slides and stained by hematoxylin and eosin stain **(Banchroft *et al*, 1996)** for histopathological examination by the light microscope.

Statistical analysis

Results are presented as mean ± SE. One-way analysis of variance (ANOVA) followed by Tukey multiple comparisons using a computer-based fitting program (SPSS statistical package ver. 22) were performed. Differences were considered to be statistically significant when $P<0.05$.

RESULTS AND DISCUSSION

A major global cause of morbidity and mortality is liver disease. Liver function can affect many other organs function **(Stärkel and Schnabl, 2016)**. Moreover, it was found that medications used for treatment of liver diseases have alot of side effects. As a result, it's crucial to search for prospective functional foods that can prevent liver damage while having little to no negative impacts on patients. Goats' milk is rich in nutrients compared to other milk types. It has smaller casein micelles and fat globules. It showed high content of medium- and short-chain fatty acids **(Zhang etal.,2017)**. Consumption of goats' milk can protect cells from lesion **(Dı´az-Castro *et al.*, 2013)**. This lead us to think that goats' milk alone or when mixed with rich sources of antioxidants may have hepatoprotective potentials that should be further studied.

Antioxidant status of BE and DTE

Vegetables and fruits are rich sources of antioxidants, such as vitamin C, flavonoids and phenolic compounds, that mitigate the risk of chronic diseases by preventing free radical damage. Therefore, consumption of foods high in dietary antioxidants is beneficial for prevention of liver lesion. Therefore, chemical components that supporting antioxidant activities were measured. Results of ascorbic acid, total phenolics, flavonoids and free radical scavenging expressed in dry mass basis were presented in Table (1). Ascorbic acid content (67.3 mg/100 g dry weight) in Baobab fruit pulp was higher than those for desert truffles (1.6 mg/100 g). These results are in agreement with the findings of **Braca *et al.*, (2018) and Althwab *et al.*, (2019)**. They reported that Baobab fruit pulp possesses higher vitamin C content and stronger antioxidant capacity than commonly consumed fruits.
From the data presented in Table (1), when compared to total flavonoid, the major antioxidant components found in the BE and DTE were the total phenolic compounds. On the other hand, the content of total flavonoids in the BE was found to be 42.7 mg QE/g and DTE contained 28.43 mg QE/g.

Table 1 Antioxidant status of Baobab fruit pulp and *Terfezia claveryi*

Components	Baobab Fruit Powder	Desert Truffle Powder
Vitamin C (mg/100g)	67.3 ± 0.11^a	1.6 ± 0.003^b
Total phenolic (mg of gallic acid equivalent/ g dry material)	48.10 ± 1.08^a	46.48 ± 0.029^b
Total flavonoid (mg of quercetin equivalent/ g dry material)	42.7 ± 0.43^a	28.43 ± 1.02^b
Scavenging activity (%)	96.4 ± 0.53^a	70.2 ± 0.023^b

Data are expressed as means ± standard error, (n = 3).
Means having different superscripts in the same row are significantly different ($P < 0.05$).

Antioxidants can inhibit lipid oxidation through the free radical-scavenging mechanisms. The absorption of free radical DPPH at 517 nm (purple color) decreases significantly on exposure to radical scavengers through providing hydrogen atoms or by electron donation. BE and DTE were subjected to DPPH radical-scavenging activity, presented in Table (1). These extracts were able to effectively reduce the stable free radical DPPH (96.4 % for BE versus 70.2 % for DTE). These results were in agreement with the findings of **Cheung *et al.* (2003)** who found that free radical-scavenging activity is greatly influenced by the

phenolic compounds of the sample**.** Therefore, Baobab fruit pulp and desert truffles powder can be used as functional ingredients due to their antioxidant properties.
In various studies it was found that CCl_4 induces lipid peroxidation of the liver cell membranes and this effect is believed to be an important factor causing hepatocyte lesion. We started the heptoprotective action of the different treatments by studying some growth parameters.

Some growth parameters

Growth parameters of rats in all experimental groups are shown in Table (2). All rat groups were having similar initial body weight (P>0.05). At the end of the feeding period, all groups showed similar final body weight (P>0.05). Indicating that treatment with CCl_4 did not affect the body weight of the experimental animals. However, treatment with CCl_4 increased significantly (P<0.05) liver weight (34.1%,) compared with negative control group. Oral treatment in all groups help maintaining normal weight of liver and to be similar with that of the NC group (P>0.05). The highest positive effect was recorded when rats were fed on DTE which maintened almost their normal liver weight when compared with the PC group (P<0.05). Similar trend was observed in the liver weight to body weight ratio (liver index). rats, fed with DTE,

Table 2 Effect of fermented goat milk (FGM), aqueous extract of baobab (BE), aqueous extract of dessert truffle (DTE) and (1:1:1) mixture (MIX) on some growth parameters of normal and CCl_4-treated rats.

Groups	Initial BW (g)	Final BW (g)	Liver weight (g)	Liver Index (g/100 g)
NC	218.6±12.9	278.1±21.0	8.8±0.26 [b]	3.19±0.18 [b]
IC	227.6±27.3	273.4±21.6	11.8±0.37 [a]	4.34±0.21 [a]
FGM	240.6±22.1	274.1±16.9	8.8±0.20 [b]	3.25±0.20 [b]
BE	244.8±16.1	284.8±19.1	8.4±0.51 [b]	2.90±0.17 [b]
DTE	243.8±16.8	292.1±12.2	7.8±0.20 [b]	2.62±0.08 [b]
MIX	236±16.4	282.4±17.9	8.2±0.58 [b]	2.93±0.19 [b]

The values were expressed as mean ± standard error. Means having different superscripts in the same row are significantly different (P < 0.05).
Liver index was calculated as (liver weight/body weight × 100).

Level of liver enzymes

Effect of feeding CCl_4-treated rats on FGM, BE, DTE and (1:1:1) MIX on activities of liver enzymes is illustrated in Fig. (1). Treatment with CCl_4- induced activities of ALT, AST and ALP in the serum of rats (PC group).
However, all experimental diets maintained normal levels of all liver enzymes (P<0.05). The lowering effect of these treatments on the liver enzymes levels is implying ability of goat milk, baobab and truffle to protect hepatocytes from liver lesion. Recently, fermented goat milk protect liver cells of mice exposed to acute liver lesion by CCl_4 **(Zhang *et al.*, 2018)**. In addition, **Janakat and Nassar (2010)** found that truffle have the ability to normalize the effect of CCl_4 on the liver enzymes levels. The protective effect of these treatments could resulted from either lowering degree of liver tissue damaging, or improved liver tissue repairing **(Hamid *et al.*, 2014)**.

Histopathological examination:

It is well established that CCl_4-induced lipid peroxidation causes hepatic lesion, and this model has been employed frequently in experiments to study the cellular mechanisms behind oxidative damage and to assess the therapeutic potential of dietary antioxidants **(Zamparelli *et al.*, 2016)**.
Histopathological analysis was also used to analyze and evaluate the liver lesion (Fig. 2). As it can be obsrved in NC group, the hepatocytes surrounding the portal region and central veins (CV) showed a normal histological structure (Fig. 2). Histopathological profiles of the liver from the CCl_4-treated rats (IC) showed intense necrosis with different degenerative changes in the hepatocytes with inflammatory cells. Inflammatory cells infiltration was detected surrounding the central vein (Fig. 2). It has been proposed that CCl_4 is converted into toxic substances through overexpression of Cytochrome P450 2E1 (CYP2E1) protein which located mainly surrounding the central vein of the hepatic lobule **(Zamparelli *et al.*, 2016)**.
The protective effect of FGM was confirmed by histopathological examination of the liver section of CCl_4 plus FGM treated group. There was a significant improvement as evident from considerable reduction in necrosis and fatty changes when compared with the PC group. Recently, fermented goat milk showed similar trend by lower expression of CYP2E1 protein **(Zhang *et al.*, 2018)** and lowering oxidative damage **(Moreno-Fernandez *et al.*, 2019)**. Furthermore, liver function can be affected by function of the gut and vice versa. **Stärkel and Schnabl (2016)** found that CCl_4-induced acute hepatic lesion in mice could be prevented by consumption of goats' milk. In addition, it improved the gut microbiota imbalance caused by CCl_4 in mice. A constant reduction in necrosis was also shown in liver sections of animals treated with CCl_4 plus BE. These specimens showed more regular liver architecture in which only dilatation (D) in wide area (Fig. 2). Recently, BE showed similar improvement in liver histology due to impairment of CCl_4-mediated lipid peroxidation and reduction free radicals and oxidative stress **(Althwab *et al.*, 2019)**.

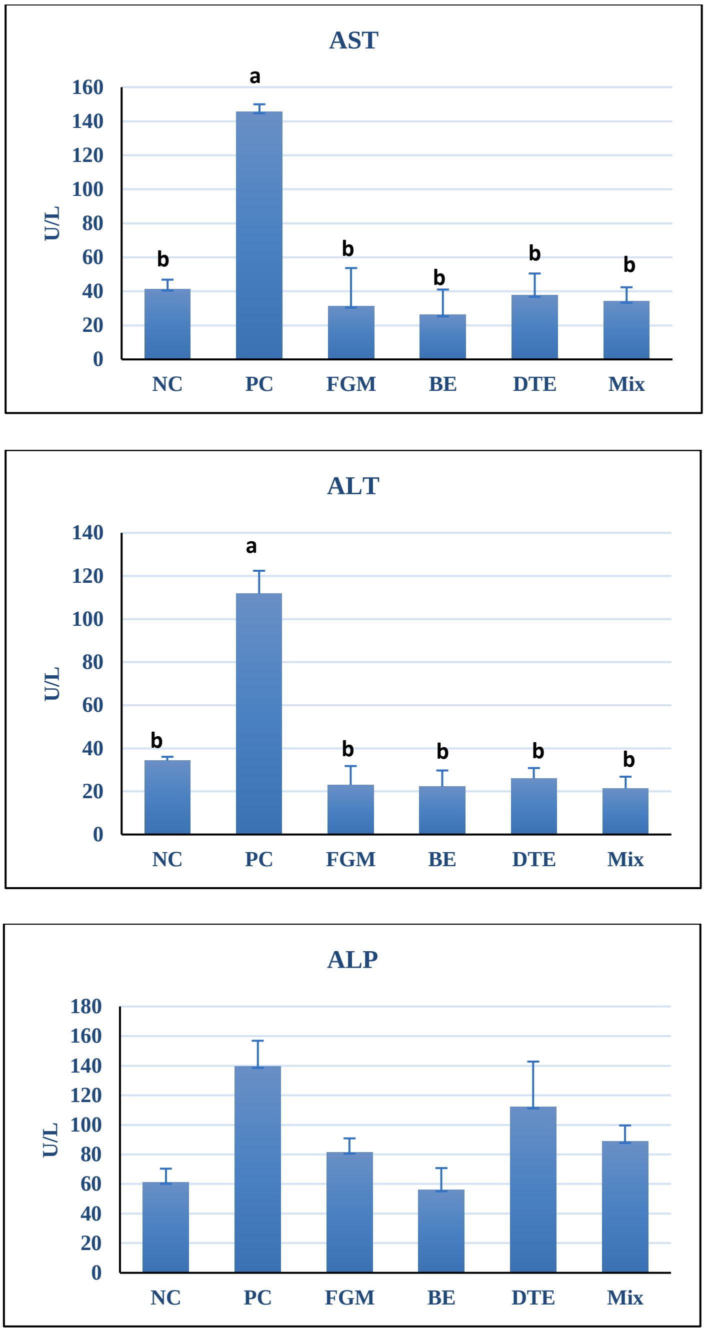

Figure 1 Effect of fermented goat milk (FGM), aqueous extract of baobab (BE), aqueous extract of dessert truffle (DTE) and (1:1:1) mixture (MIX) on the levels of liver enzymes in normal and CCl_4-treated rats.
Values are means for six rats per group, with their standard deviation represented by vertical bars. Means for groups having different letters are significantly different (P < 0.05).

The histopathological changes in the liver sections from animals treated with CCl_4 plus DTE were reduced. There was diffuse kupffer cells (K) proliferation in between the hepatocytes. These results suggest that DTE can diminish liver lesion induced by CCl_4 (IC). Indeed, there are antioxidant functions of DTE that inhibited the oxidative processes of lipids and lipoproteins in cell membranes. The liver sections obtained from animals treated with CCl_4 plus mixture showed mild inflammatory cells infiltration assisted with diffuse kupffer cells (K) proliferation in between the degenerated hepatocytes. These results suggest that the mixture showed better protection towards CCl_4 induced liver damage (Fig. 2 & Table 3).

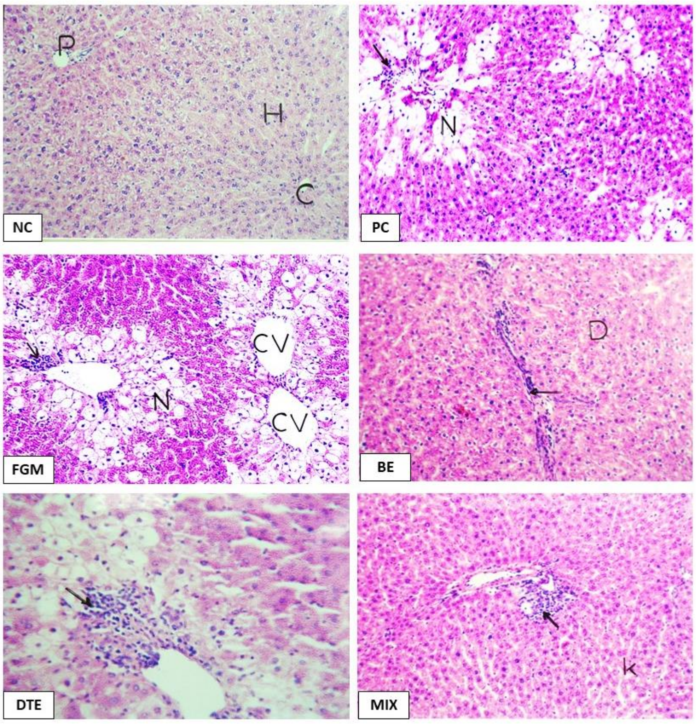

Figure 2 Effect of fermented goat milk (FGM), aqueous extract of baobab (BE), aqueous extract of dessert truffle (DTE) and (1:1:1) mixture (MIX) on the histopathology of liver from normal and CCl_4-treated rats.
(CV; Central veins, P; Portal vein, D; Dilatation, H; Hepatocytes, K; Kupffer cells).

Table 3 Histopathological alterations for liver of control and treated groups

Group	Histopathological alterations	Severity score
Normal Control	None	-
Positive Control	Degeneration and necrosis in the hepatocytes with inflammatory cell infiltration surrounding the central vein	++++
FGM	Diffuse fatty change in the hepatocytes with inflammatory cell infiltration surrounding the central vein	+++
BE	Focal fatty change in the hepatocytes at the periphery of hepatic lobules with dilatation in wide area	++
DTE	Fatty change in the hepatocytes in the portal area with diffuse kupffer cells proliferation in between the hepatocytes	++
MIX	Mild inflammatory cell infiltration in the portal area associated with diffuse kupffer cells proliferation in between the hepatocytes	+

- : Nil; +: Mild; ++: Moderate; +++: Severe; ++++ Very severe effect.

CONCLUSION

A mixture of FGM, DTE and BE improved the hepatic status of CCl_4 injured liver. This improvement was manifested by lowering of liver weight, liver index and activities of liver enzymes ($P<0.05$). Histopathological examination of the liver section showed better protection towards CCl_4 induced liver damage.

REFERENCES

Al-Laith AAA. (2010). Antioxidant components and antioxidant/antiradical activities of desert truffle (*Tirmania nivea*) from various Middle Eastern origins. J Food Compost Anal., 23(1):15–22. https://doi.org/10.1016/j.jfca.2009.07.005.
Althnaian, T., Albokhadaim, I., & El-Bahr, S. M. (2013). Biochemical and histopathological study in rats intoxicated with carbontetrachloride and treated with camel milk. SpringerPlus, 2(1), 57. https://doi.org/10.1186/2193-1801-2-57
Althwab, Sami A., ,Suleiman M. Alsattame , Tariq I. Al-mundarij , Essam M. Hamad and Hassan M. Mousa (2019) The Protective Effect of Baobab Fruit Pulp (*Adansonia digitata* L.) from Oxidative Stress Induced in Rats by High-Fat Diet. Life Science Journal 16(1) 63-71.
AOAC. (2000). Vitamins and other nutrients (Chapter 45). In Official methods of analysis (pp. 16–20, 17th Ed.). Washington, DC: AOAC International.
Banchroft J., Stevens A. and Turner, D. (1996). Theory and practice of histological techniques. Fourth Ed. Churchil Livingstone, NewYork, London, San Francisco, Tokyo. https://doi.org/10.1111/j.1365-2559.1990.tb00755.x.
Blois M.S. (1958). Antioxidant determinations by the use of a stable free radical. Nature, 181:1199-1200. https://doi.org/10.1038/1811199a0.
Bloor S. (2001). Overview of methods for analysis and identification of flavonoids, Methods in Enzymology, 335:3-14. https://doi.org/10.1016/s0076-6879(01)35227-8.
Braca, A., Sinisgalli, C., De Leo, M., Muscatello, B., Cioni, P.L., Milella, L., Ostuni, A., Giani, S. and Sanogo, R. (2018). Phytochemical profile, antioxidant and antidiabetic activities of Adansonia digitata L.(Baobab) from Mali, as a source of health-promoting compounds. Molecules, 23(12), 3104. https://doi.org/10.3390/molecules23123104.
Cao, A. H., Vo, L. T., & King, R. G. (2005). Honokiol protects against carbon tetrachloride induced liver damage in the rat. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 19(11), 932-937. https://doi.org/10.1002/ptr.1757
Cheung L.M., Cheung P.C.K. and Ooi, V.E.C. (2003). Antioxidant activity and total phenolics of edible mushroom extracts. Food Chem. 81:249–255. https://doi.org/10.1016/s0308-8146(02)00419-3.
de Vrese, M., Kristen, H., Rautenberg, P., Laue, C., Schrezenmeir, J., Probiotic lactobacilli and bifidobacteria in a fermented milk product with added fruit preparation reduce antibiotic associated diarrhea and Helicobacter pylori activity, J. Dairy Res. 78 (2011) 396-403. https://doi.org/10.1017/s002202991100063x
Dı´az-Castro J, Alf_erez MJ, L_opez-Aliaga I, Nestares T, Sanchez-Alcover A, Campos MS. (2013). Bile composition, plasma lipids and oxidative hepatic damage induced by calcium supplementation; effects of goat or cow milk consumption. J Dairy Res., 80:246-254. https://doi.org/10.1017/s0022029913000058.
Dı´az-Castro J, P_erez-S_anchez LJ, Ramı´rez L_opez-Frı´as M, L_opez-Aliaga I, Nestares T, Alf_erez MJ, Ojeda ML, Campos MS. (2012). Influence of cow or goat milk consumption on antioxidant defense and lipid peroxidation during chronic iron repletion. Br J Nutr;, 108:118. https://doi.org/10.1017/s0007114511005204.
Dı´az-Castro J, Pulido M, Alf_erez MJ, Ochoa JJ, Rivas E, Hijano S, L_opez-Aliaga I. (2014). Goat milk consumption modulates liver divalent metal transporter 1 (DMT1) expression and hepcidin during Fe repletion in Fe-deficiency anemia. J Dairy Sci., 97:147-154. https://doi.org/10.3168/jds.2013-7250.
Farrukh, A.; Mukhtar, H. (2002). "Photochemprevention by botanical antioxidants", Skin Pharmacol Appl Skin Physiol, , 15,297-306. https://doi.org/10.1159/000064533.
Haenlein GFW. (2004). Goat milk in human nutrition. Small Ruminant Res., 51:155-163. https://doi.org/10.1016/j.smallrumres.2003.08.010.
Hamid, M., Liu, D., Abdulrahim, Y., Khan, A., Qian, G., & Huang, K. (2017). Inactivation of kupffer cells by selenizing astragalus polysaccharides prevents CCl 4-induced hepatocellular necrosis in the male Wistar rat. Biological trace element research, 179(2), 226-236. https://doi.org/10.1007/s12011-017-0970-x.
Hussain G, Al-Ruqaie IM (1999). Occurrence, chemical composition, and nutritional value of truffles: an overview. Paki. J. Biol. Sci. 2(2):510-514. https://doi.org/10.3923/pjbs.1999.510.514.
Ismail, R. S., El-Megeid, A. A., & Abdel-Moemin, A. R. (2009). Carbon tetrachloride-induced liver disease in rats: the potential effect of supplement oils with vitamins E and C on the nutritional status. GMS German Medical Science, 7.
Janakat S, Nassar M. (2010). Hepatoprotective activity of desert truffle (*Terfezia claveryi*) in comparison with the effect of Nigella sativa in the rat. Pakistan Journal of Nutrition., 9(1):52 56. https://doi.org/10.3923/pjn.2010.52.56.
Janakat S., Al-Fakhiri S. and Sallal A.K. (2004) A promising peptide antibiotic from Terfezia claveryi aqueous extract against Staphylococcus aureus in vitro. Physiotherapy Res., 18:810–813. https://doi.org/10.1002/ptr.1563.
Lad, S. S., Aparnathi, K. D., Mehta, B., & Velpula, S. (2017). Goat milk in human nutrition and health–a review. Int J Curr Microbiol Appl Sci, 6(6), 1781-92. https://doi.org/10.20546/ijcmas.2017.605.194.
Miglani S, Patyar R R, Patyar S, Reshi M R. (2015). Effect of goat milk on hepatotoxicity induced by antitubercular drugs in rats. J of food and drug analysis, 24:716-721. https://doi.org/10.1016/j.jfda.2016.03.012.
Minervini, F., Bilancia, M. T., Siragusa, S., Gobbetti, M., & Caponio, F. (2009). Fermented goats' milk produced with selected multiple starters as a potentially functional food. Food Microbiology, 26(6), 559-564. https://doi.org/10.1016/j.fm.2009.03.008
Mohdaly A.A.A., Hassanien M.F.R., Mahmoud A., Sarhan M.A. & Smetanska I. (2012). Phenolics extracted from potato, sugar beet, and sesame processing by-products. International Journal of Food Properties, 16:1148–1168. https://doi.org/10.1080/10942912.2011.578318.
Moreno-Fernandez, J., Alférez, M. J., López-Aliaga, I., & Diaz-Castro, J. (2019). Protective effects of fermented goat milk on genomic stability, oxidative stress and inflammatory signalling in testis during anaemia recovery. Scientific reports, 9(1), 1-11. https://doi.org/10.1038/s41598-019-42851-1.
Moreno-Montoro, M., Navarro-Alarcón, M., Bergillos-Meca, T., Giménez-Martínez, R., Sánchez-Hernández, S., & Olalla-Herrera, M. (2018). Physicochemical, nutritional, and organoleptic characterization of a skimmed goat

milk fermented with the probiotic strain lactobacillus plantarum C4. Nutrients, 10(5), 633. https://doi.org/10.3390/nu10050633
Murcia, M.A., M.M. Tome, A.M. Jimenez, A.M. Vera, M. Honrubia and P. Parras, (2002). Antioxidant activity of edible fungi (truffle and mushrooms): Losses during industrial processing. J. Food Prot., 65:1614-1624. https://doi.org/10.4315/0362-028x-65.10.1614.
Park YW, Juarez M, Ramos M, Haenlein GFW. (2007). Physicochemical characteristics of goat and sheep milk. Small Rumin Res., 68:88-113. https://doi.org/10.1016/j.smallrumres.2006.09.015.
Park,Y.W. (2009) Bioactive components in goat milk Bioactive components in milk and dairy products, Wiley-Blackwell (2009), pp. 43-81. https://doi.org/10.1002/9780813821504.ch3.
Paz, N. F., Oliveira, E. G. D., Kairuz, M. S. N. D., & Ramón, A. N. (2014). Characterization of goat milk and potentially symbiotic non-fat yogurt. Food Science and Technology, 34(3), 629-635. https://doi.org/10.1590/1678-457x.6409
Rahul, J., Jain, M. K., Singh, S. P., Kamal, R. K., Naz, A., Gupta, A. K., & Mrityunjay, S. K. (2015). Adansonia digitata L.(baobab): a review of traditional information and taxonomic description. Asian Pacific Journal of Tropical Biomedicine, 5(1), 79-84. https://doi.org/10.1016/s2221-1691(15)30174-x.
Reeves, P.G., F.H. Nielsen and G.C. Fahey Jr. (1993). AIN-93 purified diets for laboratory rodents: Final report of the American institute of nutrition Ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr., 123: 1939-1951. https://doi.org/10.1093/jn/123.11.1939.
Reitman, S. and Frankel, S. (1957). A colourimetric method of the determination of plasma glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clins. Pathol., 28: 56-63.
Singleton V.L., Orthofer R., and Lamuela-Ravento´s R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology, 299:152–178. https://doi.org/10.1016/s0076-6879(99)99017-1.
Stärkel P, Schnabl B. (2016) Bidirectional communication between liver and gut during alcoholic liver disease. Semin Liver Dis 36:331–9. https://doi.org/10.1055/s-0036-1593882.
Taufiq, T. T., & Anindita, N. S. (2013). Fermented goat milk and cow milk produced by different starters of lactic acid bacteria: Quality studies. Journal of Agricultural Science and Technology. A, 3(11A), 904.
Vertuani, S., Braccioli, E., Buzzoni, V., & Manfredini, S. (2002). Antioxidant capacity of Adansonia digitata fruit pulp and leaves. Acta phytotherapeutica, 2(5), 2-7.
Weber LW, Boll M, Stampfl A. Hepatotoxicity and mechanism of action of halo alkanes: carbon tetrachloride as a toxicological model. Crit Rev Toxicol (2003) 33:105–36.
Zamparelli, S. M., Compare, D., Coccoli, P., Rocco, A., Nardone, O. M., Marrone, G… & Miele, L. (2016). The metabolic role of gut microbiota in the development of nonalcoholic fatty liver disease and cardiovascular disease. International journal of molecular sciences, 17(8), 1225. https://doi.org/10.3390/ijms17081225.
Zhang F, Wang Z, Lei F, Wang B, Jiang S, Peng Q, (2017). Bacterial diversity in goats' milk from the Guanzhong area of China. J Dairy Sci : 100:7812–24.
Zhang, J., Wang, Z., Huo, D., & Shao, Y. (2018). Consumption of goats' Milk Protects Mice From carbon Tetrachloride-induced acute hepatic injury and improves the associated gut Microbiota imbalance. Frontiers in immunology, 9, 1034. https://doi.org/10.3389/fimmu.2018.01034.