Research Article

Hepatoprotective effect of olive oil and camel milk on acetaminophen-induced liver toxicity in mice

Mahrous Abdel Basset Ibrahim[1,2], Farooq Ahmed Wani[3], Shaik Rahiman[4]

[1]Department of Forensic Medicine and Clinical Toxicology, College of Medicine, Aljouf University, Sakaka, Saudi Arabia.
[2]Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Suez Canal University (SCU), Ismailia, Egypt.
[3]Department of Pathology, College of Medicine, Aljouf University, Sakaka, Saudi Arabia.
[4]Department of Biochemistry, College of Medicine, Aljouf University, Sakaka, Saudi Arabia.
Correspondence to: Mahrous Abdel Basset Ibrahim, E-mail: mahrous_11@yahoo.com

Received September 07, 2016. Accepted September 12, 2016

Abstract

Background: Acetaminophen overdose is a major cause of drug-induced liver failure.

Objective: To investigate the hepatoprotective effects of Extra Virgin Olive Oil (EVOO) and camel Milk (CM) on histopathological changes and changes in oxidant &antioxidant systems in liver caused by acetaminophen(APAP)-induced liver toxicity in mice.

Materials and Methods: Mice were randomly divided into 6 groups, with 6 mice per group; control, EVOO,CM, APAP, EVOO + APAP, CM +APAP. In APAP group, mice were treated with single dose acetaminophen (500mg/kg).Prophylactic groups were given EVOO and CM for 28 days before APAP administration, in the same dose as that of the toxic group. Liver enzymes, lipid profile, malondialdehyde (MDA) and total antioxidant (TAC) activities were assessed.

Result: In the toxicity group, increased concentrations of MDA and decreased TAC activities were found compared to the control groups ($p<0.05$), with elevated liver enzymes. EVOO and CM treatment resulted in hepatoprotective effects as evident by a significant decrease in serum liver enzymes and liver malondialdehyde, with a synchronous increase in TAC compared to acetaminophen-treated group ($p<0.05$). Histopathological examination showed extensive centrilobular necrosis, hydropic degeneration of hepatocytes in APAP group. These changes were alleviated in the pretreatment groups with EVOO and CM.

Conclusion: The present study showed that olive oil and camel milk have hepatoprotective action against acetaminophen induced hepatotoxicity. Pretreatment with EVOO and CM markedly alleviates acetaminophen hepatotoxicity in mice possibly, in part, through anti-oxidative effects.

KEY WORDS: Acetaminophen, Extra Virgin Olive Oil, Camel Milk, Liver Damage, Oxidative Damage

Introduction

The liver is the largest body organ and plays a major role in the detoxification of deleterious materials. It regulates numerous metabolic functions and maintains body homeostasis.[1]

Access this article online	
Website: http://www.ijmsph.com	Quick Response Code:
DOI: 10.5455/ijmsph.2017.07092016614	

Acetaminophen is the commonly used drug as analgesic–antipyretic but excessive use causes liver necrosis, acute liver failure, and even death at higher doses.[2] Acetaminophen (paracetamol, N-acetyl-p-aminophenol; APAP) was discovered in 1889 and is an active metabolite of phenacetin.[3] Acetaminophen is believed to be safe at therapeutic doses, but hepatotoxicity can occur with misuse or overdose.[4]

Acetaminophen is extensively metabolized by the liver to sulphate and glucuronide conjugates that are excreted into bile.[5] However, the liver toxicity of acetaminophen has been referred to the formation of toxic metabolites when a small portion of the drug is metabolized via the hepatic cytochrome P-450 to a highly reactive arylating metabolite N-acetyl-p-benzoquinone imine (NAPQI).[6] NAPQI is initially inactivated by conjugation with glutathione (GSH).[7] However, when the

NAPQI formation rate exceeds the detoxification rate by GSH, it oxidizes tissue macromolecules such as lipid or SH group of proteins, and alters the calcium homeostasis after depleting GSH.[8]

Prevention of APAP toxicity is a curative defy and recognizing cheap natural products to achieve the goal is an additional and higher challenge.

Extra Virgin Olive Oil(EVOO) is the main origin of fat in the Mediterranean food, and its regular intake is thought to have many useful effects on human health.[9] EVOO is well known for its antioxidant properties, hypotensive, hypoglycemic, cardiovascular, and hepato-protective effects.[10] It is also known for its anti-microbial and anti-inflammatory properties.[11] Caruso, et al., showed that EVOO has a series of phenolic minor components that grant its particular aroma & taste, and possess important antioxidant activities.[12] Major phenols include hydroxytyrosol (HT), tyrosol (T), and their secoiridoid derivatives (oleuropein- and ligstroside related compounds).[9]

Camel milk (CM) is another agent that might represent such a potential candidate for hepatoprotective properties. CM is different from other ruminant milk; it has low levels of protein, cholesterol and sugar, but high levels of vitamins, minerals, and insulin.[13] It has no allergic features and can be used by lactose-intolerant persons as well.[14] It also has a relatively vast amount of polyunsaturated fatty acids and linoleic acids, which are fundamental for human nutrition.[15] Additionally, CM exhibits a wide range of biological activities; antioxidative, antimicrobial, antihypertensive, antithrombotic, and immuno-modulatory effect.[16, 17]

Not enough studies were done previously on the hepatoprotective activities of EVOO and CM against acetaminophen toxicity. This study aims to assess the postulated hepatoprotective properties of both EVOO and camel milk in acetaminophen induced liver toxicity in mice - utilizing standard biomarkers of hepatotoxicity and oxidative stress along with histopathological characterization.

Materials and methods

Chemicals

Acetaminophen (APAP) was purchased from (Sigma-Aldrich, St. Louis, MO, USA). Extra Virgin Olive Oil (EVOO) was purchased from the local market, Sakaka, Aljouf, KSA. Camel milk (CM) was obtained from local commercial sources or local camel milk venders and camel farms at Sakaka, KSA. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total protein, total cholesterol, high density lipoprotein (HDL-C), low density lipoprotein (LDL-C), triglycerides, total bilirubin, and malondialdehyde (MDA) kits were purchased from Biovision, USA. Total antioxidant capacity (TAC) kits were purchased from Sigma-Aldrich chemical co. Other than the commercially available assay kits, chemicals used of analytical grade from Sigma-Aldrich chemicals co. (St. Louis, MD, USA).

Experimental design

The experiment was performed on 36 male Swiss albino mice aged between 6 and 8 weeks and weighing approximately 25 g each. The mice were obtained from the College of Medicine, Aljouf University, Kingdom of Saudi Arabia (KSA). The animals were housed in animal house, College of Medicine, Aljouf University, KSA. The mice were kept in clean and dry plastic cages (6 mice per cage) in 12 h dark/light cycle under normal laboratory condition of temperature and humidity, fed with commercial rodent pellets purchased from Sakaka City, Aljouf, KSA and tap water *ad libitum*. The mice were acclimatized for one week before the experiments. Furthermore, the anesthetic procedures and handling of animals was carried out according to The European Communities Council Directive of 1986 (EC 86/609) and complied with the ethical guidelines of the Aljouf University Ethical Committee, Aljouf University, KSA.

Experimental protocol

The mice were divided into 6 groups (N=6) of 6 mice each (n = 6). APAP was dissolved in warm phosphate-buffered saline (PBS, 55°C) and cooled to 37°C before oral administration to mice.

Group 1 (control) (G1): Mice received 1ml saline once daily orally by orogastric tube for 28 days.

Group 2 (EVOO) (G2): Mice were treated with extra virgin olive oil (EVOO) (1.7 ml/kg) once daily by orogastric tube for 28 days.[18]

Group 3 (CM) (G3): Mice were treated with camel milk (CM) (33 ml/kg) once daily by orogastric tube for 28 days.[19]

Group 4 (APAP) (G4): Mice were treated with acetaminophen (APAP) (500 mg/kg) once by orogastric tube on 28th day.[20]

Group5 (EVOO + APAP) (G5): Mice were treated with extra virgin olive oil (EVOO) (1.7 ml/kg) once daily by orogastric tube for 28 days followed by APAP (500 mg/kg)at single dose orally on 28th day.

Group6 (CM+APAP) (G6): Mice were treated with Camel milk (CM) (33 ml/kg) once daily by orogastric tube for 28 days followed by APAP (500 mg/kg) at single dose orally on 28th day.

Blood and tissue collection

At the end of the experimental period, mice were sacrificed by cervical decapitation under diethyl ether anesthesia 24 h following APAP administration after the final dose of APAP for liver tissue collection and fixation period preceded by blood collection. Blood samples were allowed to clot, and the sera were separated by centrifugation at 1000g for 10 min and kept at -20°C till determination of liver function enzyme activities as well as triglycerides and cholesterol levels. Meanwhile, the abdominal cavity was dissected immediately; to dissect the liver that was wet weighed and divided into 2 portions: (a) the first portion was homogenized in 10 volumes of ice-cold double distilled water. An aliquot of this homogenate was mixed with 2.3% KCl and centrifuged at 600g for 15 min at 4°C. The resulting supernatant

was used for the determination of malondialdehyde (MDA) level, and (b) the second portion was fixed in phosphate buffered formalin for light histopathological processing and examination.

Estimation of biochemical parameters

The enzymatic activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP),lactate dehydrogenase (LDH),total protein levels,total bilirubin, triglycerides (TG), total cholesterol (TC), HDL-C, and LDL-C levels were measured in the serum by commercial kits as per manufacturer instructions using Chemwell Semi Biochemistry Analyzer (USA).

Estimation of lipid peroxidation (LPO) (Malondialdehyde, MDA)

The evaluation of LPO was analyzed according to the manufacture instructions. The reaction mixture consisted of 0.58 ml phosphate buffer (0.1 M, pH 7.4), 0.2 ml homogenate, 0.2 ml ascorbic acid (100 mM), and 0.02 ml ferric chloride (100 mM). The incubation of reaction mixture was maintained at 37°C in a shaking water bath for 1 h. The reaction was terminated by adding 1.0 ml of 10% TCA. After adding 1.0 ml of 0.67% thiobarbituric acid, the tubes were kept in boiling water bath for 20 min and then transferred to crushed ice bath before centrifuging at 2500g for 10 min. The malondialdehyde (MDA) produced in each of the samples was observed by analyzing optical density of the supernatant at 535 nm using spectrophotometer against a reagent blank. The results were expressed as nanomoles of MDA formed per minute per milligram tissue at 37°C using a molar extinction coefficient of $1.56 \times 105\ M^{-1}\ cm^{-1}$.

Estimation of total antioxidant capacity

Serum total antioxidant levels were detected by a total antioxidant capacity assay kit (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions and were expressed as milliequivalents per liter of Trolox per liter (mEqTrolox/l).

Determination of the liver histopathological changes

Liver specimens were fixed with 10% formaldehyde and processed routinely for embedding in paraffin. Sections of 5 μm were stained with hematoxylin and eosin (H&E) for routine histopathological examination[21] under the light microscope.

Statistical analysis

Numerical data obtained from each experiment were expressed as mean ± standard deviation (SD). Statistical differences between experimental groups were assessed using one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test and $p<0.05$ indicated statistically significant difference. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 22 software (SPSS® Inc, USA).

Result

No adverse effects and no mortality of the animals were observed during the period of study. The observations made on the different groups of experimental mice indicate that administration of APAP induced alterations in the biochemical parameters as well as the histopathology in liver that were analyzed at the end of the experimental period (28 days). Oral administration of extra virgin olive oil and camel milk reverted back all the alterations to near normal when compared with APAP induced toxicity in mice.

Effects of EVOO and camel milk on liver biomarkers

In the APAP treated groups, the ALT, AST, and ALP were significantly increased when compared to control ($p < 0.05$). Feeding the mice with EVOO in group 5 and camel milk in group 6 as protective agents before treatment with 500 mg/kg APAP significantly decreased the activity of ALT, AST, and ALP ($p < 0.05$) which indicating a hepatoprotective effect provoked by the APAP toxicity (Table 1).

We assess lactate dehydrogenase (LDH) activity as a tool to investigate the hepatic cells integrity (Table 1). It depicts the marked and significant elevation in serum LDH in APAP treated group as compared to control group($p < 0.05$). The groups pretreated with EVOO (G5) and camel milk (G6) before APAP dose recorded significant ameliorative effect of LDH value ($p < 0.05$).

Regarding, the serum total protein, it was found that the total protein content significantly decreased in APAP treated group($p < 0.05$) (Table 1). Meanwhile, pretreatment of EVOO and camel milk before APAP has ameliorated total protein content as compared with control (G1) and APAP (G4) groups ($p < 0.05$).

Data showed that mice treated with acetaminophen alone developed significant hepatocellular damage as evident from a significant increase in serum total bilirubin as compared to control group ($p < 0.05$) (Table 1). Pretreatment of the mice with EVOO and CM for 28 days prior to acetaminophen administration in groups 5 and 6 caused a significant reduction in the values of serum total bilirubin.

Effects of EVOO and camel milk on serum lipids

The mean serum lipoprotein and triglyceride concentrations of each group of mice are shown in Table 2. The mean total cholesterol concentration of mice treated with APAP was significantly higher than that of the other control groups ($p<0.05$). Meanwhile, pretreatment of EVOO and camel milk before APAP has ameliorated total cholesterol concentration as compared with APAP (G4) group ($p<0.05$). In the same context, the mean serum HDL cholesterol concentrations of mice in the APAP group were significantly lower than those in the EVOO and camel milk groups. The highest mean concentration of serum LDL cholesterol was observed in the APAP group. Mean serum triglyceride concentrations in mice from the APAP group were higher than in mice from the control group. Pretreatment of EVOO and camel milk before APAP

Table 1. Effect of extra virgin olive oil (EVOO) and camel milk (CM) on liver function parameters in serum of acetaminophen (APAP) – treated mice

Parameters	Control (G1)	EVOO (G2)	CM (G3)	APAP (G4)	EVOO+ APAP (G5)	CM + APAP (G6)
ALT U/L	59.67 ± 6.15	47.17 ± 5.67	58.33 ± 9.52	2430.33 ± 462.73^{abc}	149.67 ± 23.08^{d}	299.83 ± 38.47^{d}
AST U/L	94.67 ± 5.16	98.67 ± 7.65	103.17 ± 17.30	2223.67 ± 256.50^{abc}	135.33 ± 13.44^{d}	214.17 ± 45.85^{d}
ALP U/L	127.83 ± 12.04	125.50 ± 5.68	123.00 ± 9.84	146.67 ± 4.03^{abc}	129.83 ± 2.64^{d}	129.67 ± 6.25^{d}
LDH U/L	767.17 ± 21.05	1266.50 ± 93.15^{a}	1293.33 ± 45.26^{a}	3562.83 ± 222.77^{abc}	1736.33 ± 86.73^{abd}	1975.33 ± 91.91^{acd}
Total protein (g/dl)	7.47 ± 0.33	7.78 ± 0.15	7.70 ± 0.25	4.57 ± 0.31^{abc}	6.07 ± 0.47^{abd}	5.73 ± 0.25^{acd}
Total Bilirubin (mg/dl)	0.44 ± 0.04	0.47 ± 0.04	0.46 ± 0.08	1.15 ± 0.18^{abc}	0.45 ± 0.04^{d}	0.51 ± 0.06^{d}

Values represent means ± SD for 6 mice per experimental group. G1: control, G2: Extra virgin olive oil alone (EVOO) G3: Camel milk alone (CM), G4: Acetaminophen (APAP) (500 mg/kg b.w.), G5: EVOO + Acetaminophen (500 mg/kg b.w.), G6: Camel milk + Acetaminophen (500 mg/kg b.w.),
ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase; LDH: Lactate dehydrogenase.
a: compared to the control (G1) group- significant at $p \leq 0.05$. b: compared to EVOO (G2) group- significant at $p \leq 0.05$.
c: compared to the camel milk (G3) group- significant at $p \leq 0.05$ d: compared to the APAP alone (G4) group- significant at $p \leq 0.05$.

Table 2. Effect of extra virgin olive oil (EVOO) and camel milk (CM) on serum lipogram parameters of acetaminophen (APAP) – treated mice

Parameters	Control (G1)	EVOO (G2)	CM (G3)	APAP (G4)	EVOO + APAP (G5)	CM + APAP (G6)
Total Cholesterol (mg/dl)	73.67 ± 2.42	68.33 ± 2.07	68.67 ± 4.59	136.67 ± 5.05^{abc}	79.83 ± 2.48^{abd}	80.83 ± 5.12^{acd}
HDL-C (mg/dl)	24.00 ± 1.41	23.00 ± 1.41	24.00 ± 2.53	15.33 ± 1.51^{abc}	23.00 ± 1.79^{d}	20.33 ± 1.21^{acd}
LDL-C (mg/dl)	48.83 ± 3.06	45.50 ± 1.38	44.83 ± 1.33	117.67 ± 7.53^{abc}	53.50 ± 3.15^{bd}	58.50 ± 1.64^{acd}
Triglyceride (mg/dl)	128.17 ± 3.66	132.50 ± 5.09	134.00 ± 5.76	186.83 ± 4.67^{abc}	151.83 ± 6.31^{abd}	153.50 ± 2.59^{acd}

Values represent means ± SD for 6 mice per experimental group. G1: control, G2: Extra virgin olive oil alone (EVOO) G3: Camel milk alone (CM), G4: Acetaminophen (APAP) (500 mg/kg b.w.), G5: EVOO + Acetaminophen (500 mg/kg b.w.), G6: Camel milk + Acetaminophen (500 mg/kg b.w.), HDL, high-density lipoprotein; LDL, low-density lipoprotein ;
a: compared to the control (G1) group- significant at $p \leq 0.05$. b: compared to EVOO (G2) group- significant at $p \leq 0.05$.
c: compared to the camel milk (G3) group- significant at $p \leq 0.05$ d: compared to the APAP alone (G4) group- significant at $p \leq 0.05$.

has ameliorated HDL-C, LDL-C, and triglyceride concentrations as compared with APAP (G4) group ($p<0.05$).

Effects of EVOO and Camel milk on liver oxidant and antioxidant levels

Figure 1A and B shows the levels of total antioxidant capacity (TAC) in the serum of each group of mice and liver MDA levels. Compared to the control group (G1), TAC did not increased significantly at EVOO and CM groups (G2,3) ($p>0.05$), while TAC level was significantly decreased in the serum of acetaminophen treated mice alone (G4) ($p < 0.05$). The TAC regain their levels significantly in the protective groups (G5,6) when compared to G4 ($p < 0.05$). The activity of lipid peroxidation (MDA) levels was evaluated in all mice livers. The results were shown in Figure1A. MDA level significantly increased in acetaminophen administration

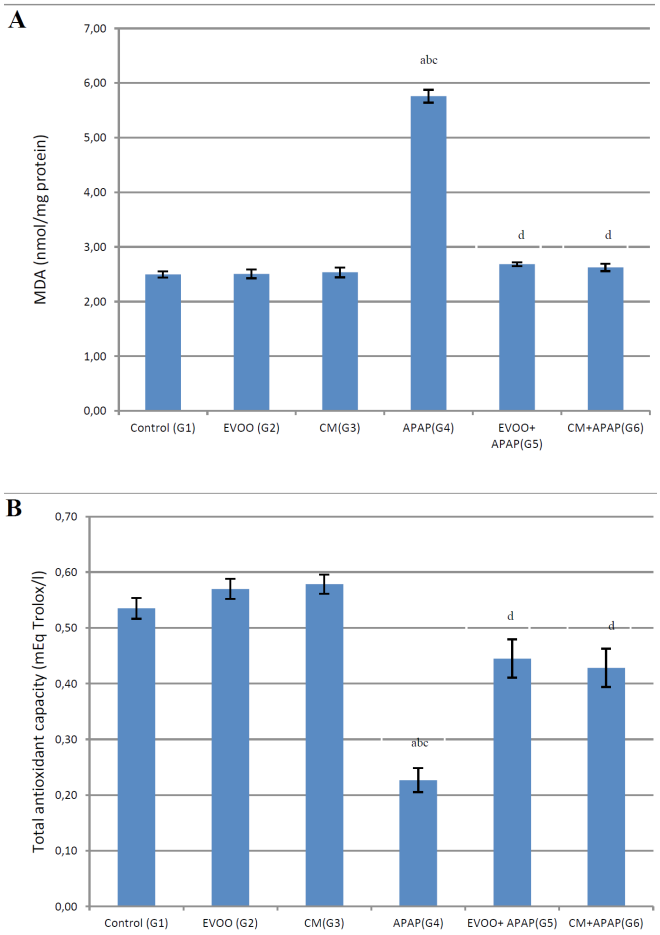

Figure 1: Effects of extra virgin olive Oil (EVOO) and camel milk (CM) on the levels of: A. Malondialdehyde (MDA); B. Total antioxidant activity (TAC) in the control and treated mice.
The values were reported as the mean ± SD of 6 mice per group.
a: compared to the control (G1) group b: compared to EVOO (G2) group c: compared to the camel milk (G3) group d: compared to the APAP alone (G4) group. a,b,c,d are significant at $p \leq 0.05$.

groups as compared to the control group ($p<0.05$). In contrast to the acetaminophen administration group, the MDA level was reduced by pretreatment of both EVOO and camel milk. Therefore, oral administration of the extra virgin olive oil and camel milk served as a factor that improved antioxidant defense and significantly reduce oxidative stress.

Effects of EVOO and camel milk on liver histopathology

Figure 2 showed photomicrograph of a section of liver histology (H&E; x100) which supported the results obtained from serum enzyme assays and showed hepatoprotective activity of EVOO and CM in acetaminophen induced liver injury. Figure 2a shows the normal control group with hepatocytes, presenting normal morphology and liver lobule structures were clear and regular. Figure 2d showed a mouse treated with acetaminophen with extensive centrilobular necrosis,

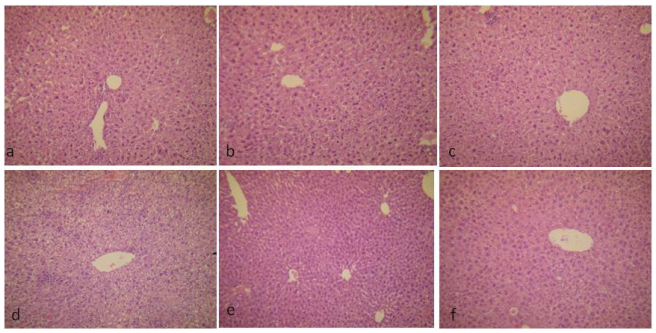

Figure 2: (a) Photomicrograph of a section of liver (H & E; x100) from a control group with normal lobular architecture and hepatocytes presenting normal morphology.(b) Photomicrograph of a section of liver (H & E; x100) from a mouse treated with olive oil shows normal morphology of liver lobules.(c) Photomicrograph of a section of liver (H & E; x100) from a mouse treated with camel milk shows normal morphology of liver lobules.(d) Photomicrograph of a section of liver (H & E; x100) from a mouse treated with acetaminophen shows extensive centrilobular necrosis, hydropic degeneration of hepatocytes, destruction of central vein endothelium, karyolysis, pyknosis, and karyorrhexis of nuclei.(e) Photomicrograph of a section of liver (H & E; x100) from a mouse treated with acetaminophen and prophylactic olive oil shows complete protection of hepatocytes and normal hepatic architecture. (f) Photomicrograph of a section of liver (H & E; x100) from a mouse treated with acetaminophen and prophylactic camel milk shows normal hepatic architecture.

hydropic degeneration of hepatocytes, destruction of central vein endothelium, karyolysis, pyknosis, and karyorrhexis of nuclei. Pretreated EVOO and CM groups (G5, G6) showed complete protection of hepatocytes and normal hepatic architecture (Figure2 e and f). There were no histopathologic changes in the EVOO and CM alone groups (Figure 2b and c).

Discussion

The liver plays a vital role in the metabolism of drugs and is susceptible to the toxicity from these agents. Acetaminophen is the drug most often involved in drug-induced liver injury. Davidson and Eastham were the first to adduce that APAP causes liver toxicity in overdose.[22] The molecular mechanism of APAP-induced liver toxicity is well attested. In normal circumstances, APAP is detoxified via glucuronidation and sulfation. Liver is the primary site of detoxification, and end-products are eliminated by the kidney. The toxic metabolite of APAP is NAPQI. In therapeutic dose, this metabolite is detoxified by conjugating with glutathione. In an overdose, a large amount of NAPQI is generated causing depletion of glutathione stores which overwhelms the detoxification process and leads to liver cell damage.[23]

This toxic metabolite prompts the generation of reactive oxygen species (ROS), which react with cellular proteins. The fatal process begins with activation of intracellular signaling pathways, lysosomal enzymes and caspases, and eventually results in lipid peroxidation, apoptosis, and cell death.[24] Muriel

reported that liver injury induced by transformation of agents such as acetaminophen in the liver caused increasing production of ROS and reactive nitrogen species (RNS) which play a potential role in cell death.[25]This central role of oxidative stress in APAP-induced hepatic toxicity presents the opportunity for natural antioxidants to overcome and scavenge free radicals to prevent the harmful effects of the toxicants.

The results of the present study showed that oral administration of olive oil and camel milk resulted in significant histopathological and biochemical antioxidant effects in mice. This was indicated by a marked decrease in MDA levels. In addition, ALT and AST levels were lower in the olive oil and camel milk-treated groups than in the APAP group.

The transaminase enzymes are the commonest liver enzymes in the identification of hepatocellular damage,[26] in particular ALT is the clinical chemistry gold standard of hepatotoxicity. The increase in liver enzymes after acetaminophen administration has earlier been reported.[27,28] In this study, the group treated with APAP showed significant increment of these enzymes in sera due to the hepatic damage induced by this drug, however, the simultaneous administration of acetaminophen, EVOO, and camel milk significantly lowered AST, ALT, ALP, and LDH concentrations when compared with those that received acetaminophen only, as evidence of protection against APAP-induced hepatotoxicity in animal receiving EVOO and camel milk.

The amelioration of rising serum enzymes in acetaminophen toxicity by olive oil and camel milk may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum transaminases levels return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes.[29, 30] Several studies[31-33] have provided an abundant support for evidencing the protective effects of camel milk on liver damage. This is in line with the work of Poudyal et al., and Kasdallah-Grissa et al. who stated the protective effect of olive oil against hepatotoxicity.[34,35] The mechanism by which EVOO and camel milk lowered liver enzymes may be referred to their ability to maintain liver cell integrity.[36] In the same vein, total protein decreased with acetaminophen administration while on the co-administration with olive oil and camel milk resulted in an increase in total protein. This is in line with the works of John Kennedy, and Adamma.[37]

Triglycerides and total cholesterol levels were significantly increased in the APAP intoxicated mice. The possible explanation of the observed hyperlipidemia might reflect the deterioration of liver cells to metabolize lipids or lipid peroxidation.[38] The increase in serum lipids may be attributed to the increased liver synthesis and/or diminished liver degradation; reduced lipoprotein lipase activity plays a role in the lipids increment.[39] Pre-treatment mice with EVOO or camel milk prevented the acetaminophen-induced rise in serum triglycerides and total cholesterol. These findings demonstrated their protective action on hepatic injury induced by APAP.

Camel milk can act in several ways to lower serum cholesterol and triglycerides. First, uptake of triglycerides and cholesterol in the gastrointestinal tract could be prohibited; second, LDL cholesterol could be eliminated from the blood via LDL receptor and finally, the potential increase of cholesterol degrading enzymes. Also,camel milk may have a direct effect on liver or an indirect one that influences thyroid hormones which affect lipid metabolism.[32] While some otherworkers found a decrease in serum cholesterol in the camel milk. The mechanism of lipid dropping effects of camel milk might be referred to an inhibitory activity on microsomal acyl coenzyme A: cholesterol acyltransferease *in vitro*. Acylation of cholesterol to cholesterol esters in liver occurs by this enzyme.[40]

Mice body has an effective mechanism to neutralize and prevent the free radical induced damage. This is carried out by a set of endogenous antioxidant enzymes. Biswas et al., suggested these compounds protect thiol groups of protein from oxidation by free radicals.[41] When ROS production overwhelming the antioxidant defense, oxidative stress results, which through a series of events deregulates the cellular functions leading to different pathological situations.[42] In the present study, the rise in the liver MDA level and the decline in the level of total antioxidant activities (TAC) in APAP-treated mice suggests enhanced lipid peroxidation during tissue damage and failure of antioxidant defense mechanism to prohibit formation of undue free radicals. This is in line with the works of Hinson et al., 2010 who stated that lipid peroxidation has been assumed to be the devastating process in liver injury due to acetaminophen administration. Nuttal et al., found that oral intake of acetaminophen over 14 days was associated with a drastic reduction of blood TAC in healthy subjects which is in support to our finding.[43] Kuratae al. reported that MDA is one of the end products in the lipid peroxidation process.[44] Pretreatment with olive oil or camel milk significantly reversed all of these changes induced by APAP. The possible mechanism by which olive oil and camel milk exhibited significant protection against APAP- induced hepatotoxicity may be due to the active ingredients like oleic acid and phenolic compounds in the olive oil as well as its free radical scavenging activity. In conformity with our works, Cheung et al., and Saito et al., suggested the antioxidant properties of triterpenoids, alkaloids, and flavonoids in liver injury.[45,46] Bonilla et al. proved that the resistance to lipid peroxidation and the antioxidant defense system is increased by EVOO more than refined olive oil.[47] These findings suggest that TAC of serum is part of a homeostatic mechanism and an efficient antioxidant defense system is important in the control of oxidative stress caused by oxygen- free radicals.

Strengthening the above mechanisms involved in the generation of toxicity by acetaminophen, there are significant increased levels of biochemical parameters in the toxic group of the current study, which is also obvious from the histopathological profile. Thus, it clearly indicates that toxicity is either due to depletion of glutathione or lipid peroxidation.

Virgin olive oil participates in the health-promoting properties of the Mediterranean food. Although the useful health effects of olive oil have been mainly referred to its high

content of oleic acid, recently greater awareness has focused on minor components like phenolic compounds, which have potent antioxidant activity.[48] The phenolic compounds have antioxidant features because of their ability to scavenge free radicals and active oxygen species such as free radicals, singlet oxygen, and hydroxyl radicals.[45,46] Olive oils are good source of several phenolic compounds, such as tyrosol, hydroxytyrosol ,caffeic acid, gallicacid, and oleuropein.[49, 50]

Besides the useful effect of its fatty acids, virgin olive oil provides a rich variety of natural antioxidants including tocopherols, carotenoids, and polyphenols. These minor components may act by various mechanisms to give an effective defense system against free radical attack. They appear to have a high antioxidant activity and provide greater stability to olive oil.[51] Antioxidant ability of olive oil can be attributed to the increase in tissue sensitivity to vitamin E which has lipid antioxidant effect.[52] Another possibility comes from some evidence that the polyphenols in olive oil and β-sitosterol inhibit the ROS formation[53] and reduce the susceptibility of LDL oxidation, erythrocyte membranes to lipid peroxidation.[49,54] Several studies have used isolated phenolic compounds. Antioxidant properties of hydroxytyrosol, have been reported to be responsible for the ability of olive oil to scavenge superoxide anion and hydrogen peroxide.[55, 56] Hydroxytyrosol has shown efficacy in preventing oxidative stress in cadmium-induced liver toxicity in rats.[57]

Camel milk prevents oxidative injury and cell damage by several mechanisms, including scavenging free radicals and inhibiting lipid peroxidation.[13, 58] The protective effect of camel milk against APAP-induced toxicity, oxidative stress, and tissue damage in this study could be referred to its composition of high levels of vitamins C, A, B2 and E and very rich in magnesium and other trace elements.[58] These vitamins are antioxidants that are found to be beneficial in preventing the tissues injury caused by toxic agent. Magnesium protects the cell against oxy-radical damage and assists in the absorption and metabolism of vitamins B, C and E,[59] which are antioxidants important in cell protection. Also, it has been documented that magnesium is very fundamental for biosynthesis of glutathione, because the enzyme Glutathione synthetase-needs glycine, γ-glutamyl cysteine, ATP, and magnesium ions to form glutathione.[60]

Also, camel milk is rich in zinc (Zn).[58] Zinc is a trace element fundamental for living organisms. Many enzymes require Zn for their activity. It also plays a vital role in the DNA replication, transcription, and protein synthesis, affecting cell division and differentiation.[61] It has been documented that Zn has a link with many of body enzymes and can prevent cell injury through activation of the antioxidant system.[62, 63]

Conclusion

In summary, the findings of the present study reveal that extra virgin olive oil and camel milk intake before APAP administration in mice reduced acetaminophen-induced toxicity in the liver by lowering the levels of lipid peroxides and improving antioxidant defense system, lipid profile, and histopathological changes caused by acetaminophen induced toxicity. The mechanism by which olive oil lowers oxidative stress in the liver could be due to its high content of oleic acid, tocopherol, and polyphenols, which exhibit antioxidant properties. This lowering mechanism in camel milk again could be referred to its contents of Vitamins C, A, B2 and E and magnesium. This finding suggests that including olive oil and camel milk in the diet may offer benefits in terms of reducing liver damage during APAP toxicity.

Acknowledgment

This work was supported by the grants of Deanship of Scientific Research of Aljouf University (Research Project No.35/311).The authors are greatly thankful to Deanship of Scientific Research of Aljouf University whose financial support made this study possible. The team is extremely thankful to the Pathology department for facilitating the working of the project.

References

1. Mayuren C, Reddy VV, Priya SV, Devi VA. Protective effect of Livactine against CCl4 and paracetamol induced hepatotoxicity in adult Wistar rats. N Am J Med Sci. 2010;2:491.
2. Zhu J-H, McClung JP, Zhang X, Aregullin M, Chen C, Gonzalez FJ, et al. Comparative impacts of knockouts of two antioxidant enzymes on acetaminophen-induced hepatotoxicity in mice. Exp Biol Med. 2009;234:1477-83.
3. Brown R. Hepatic and renal damage with paractamol overdosage. J Clin Pathol. 1968;21:793.
4. James LP, McCullough SS, Knight TR, Jaeschke H, Hinson JA. Acetaminophen toxicity in mice lacking NADPH oxidase activity: role of peroxynitrite formation and mitochondrial oxidant stress. Free Radic Res. 2003;37:1289-97.
5. Potter W, Thorgeirsson S, Jollow D, Mitchell J. Acetaminophen-induced hepatic necrosis V. Correlation of hepatic necrosis, covalent binding and glutathione depletion in hamsters. Pharmacology. 1974;12:129-43.
6. Vermeulen N, Bessems J, Van de Straat R. Molecular aspects of paracetamol-induced hepatotoxicity and its mechanism-based prevention. Drug Metab Rev. 1992;24:367-407.
7. Cover C, Mansouri A, Knight TR, Bajt ML, Lemasters JJ, Pessayre D, et al. Peroxynitrite-induced mitochondrial and endonuclease-mediated nuclear DNA damage in acetaminophen hepatotoxicity. J Pharmacol Exp Ther. 2005;315:879-87.
8. Sabina E, Samuel J, RajappaRamya S, Patel S, Mandal N, Pranatharthiiharan P, et al. Hepatoprotective and antioxidant potential of Spirulina fusiformis on acetaminophen-induced hepatotoxicity in mice. IJIB. 2009;6:1-5.
9. Waterman E, Lockwood B. Active components and clinical applications of olive oil. Altern Med Rev. 2007;12:331-43.
10. Benavente-Garcia O, Castillo J, Lorente J, Alcaraz M. Radioprotective effects in vivo of phenolics extracted from Olea europaea L. leaves against X-ray-induced chromosomal damage: comparative study versus several flavonoids and sulfur-containing compounds. J Med Food. 2002;5:125-35.

11. Bitler CM, Viale TM, Damaj B, Crea R. Hydrolyzed olive vegetation water in mice has anti-inflammatory activity. The Journal of nutrition. 2005;135:1475-9.
12. Caruso D, Visioli F, Patelli R, Galli C, Galli G. Urinary excretion of olive oil phenols and their metabolites in humans. Metabolism. 2001;50:1426-8.
13. Yousef MI. Aluminium-induced changes in hemato-biochemical parameters, lipid peroxidation and enzyme activities of male rabbits: protective role of ascorbic acid. Toxicology. 2004; 199:47-57.
14. Cardoso R, Santos R, Cardoso C, Carvalho M. Consumption of camel's milk by patients intolerant to lactose. A preliminary study. Review Allergy Mexican. 2010;57:26-32.
15. MS Gorban A, Izzeldin OM. Fatty acids and lipids of camel milk and colostrum. Int J Food Sci Nutr. 2001;52:283-7.
16. FitzGerald RJ, Meisel H. Milk protein-derived peptide inhibitors of angiotensin-I-converting enzyme. Br J Nutr. 2000;84: 33-7.
17. Saltanat H, Li H, Xu Y, Wang J, Liu F, Geng X. [The influences of camel milk on the immune response of chronic hepatitis B patients]. Xi bao yu fen zi mian yi xue za zhi= Chinese journal of cellular and molecular immunology. 2009;25:431-3.
18. 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 Journal of Pathology. 2015;5:73.
19. Bakhaat G, Abulfadle KA, Rahiman Shaik BA. Effect of Camel milk (Camelus dromedaries) on Milk induced Leukocytosis and Eosinophilia in the management of Asthma. Advances in Bioresearch. 2015;6.
20. Fouad AA, Al-Mulhim AS, Jresat I, Gomaa W. Therapeutic role of telmisartan against acetaminophen hepatotoxicity in mice. Eur J Pharmacol. 2012;693:64-71.
21. Bancroft J, Stevens A, Turner D. Theory and practice of histological techniques 4th Ed Churchill Living Stone, New York Edinburgh. Madrid, Sanfrancisco. 1996.
22. Davidson D, Eastham W. Acute liver necrosis following overdose of paracetamol. Br Med J. 1966;2:497.
23. Renton KW. Hepatic drug metabolism and immunostimulation. Toxicology. 2000;142:173-8.
24. Mansour MA, Nagi MN, El-Khatib AS, Al-Bekairi AM. Effects of thymoquinone on antioxidant enzyme activities, lipid peroxidation and DT-diaphorase in different tissues of mice: a possible mechanism of action. Cell Biochem Funct. 2002;20:143-51.
25. Muriel P. Role of free radicals in liver diseases. Hepatol Int. 2009;3:526-36.
26. Johnkennedy N, Adamma E, Austin A, Chukwunyere NE. Alterations In Biochemical Parameters Of Wister Rats Administered With Sulfadoxine And Pyrimethamine (Fansidar R). Al Ameen J Med Sci. 2010;3:317-21.
27. Ita S, Akpanyung E, Umoh B, Ben E, Ukafia S. Acetaminophen induced hepatic toxicity: protective role of Ageratum conyzoides. Pakistan Journal of Nutrition. 2009;8:928-32.
28. Ebong PE, Atangwho IJ, Eyong EU, Egbung GE. The antidiabetic efficacy of combined extracts from two continental plants: Azadirachta indica (A. Juss)(Neem) and Vernonia amygdalina (Del.)(African bitter leaf). American Journal of Biochemistry and Biotechnology. 2008;4:239-44.
29. Bessems JG, Vermeulen NP. Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues and protective approaches. Crit Rev Toxicol. 2001;31:55-138.
30. Darwish HA, Raboh NRA, Mahdy A. Camel's milk alleviates alcohol-induced liver injury in rats. Food Chem Toxicol. 2012;50:1377-83.
31. Al-Fartosi KG, Khuon OS, Al-Tae HI. Protective role of camel's milk against paracetamol induced hepatotoxicity in male rats. Int J Res Pharmaceut Biomed Sci. 2011;2:1795-9.
32. Al-Hashem F. Camel's milk protects against aluminum chloride-induced toxicity in the liver and kidney of white albino rats. Am J Biochem Biotechnol. 2009;5:98-109.
33. Dallak M. Camel's milk protects against cadmium chloride-induced hypochromic microcytic anemia and oxidative stress in red blood cells of white albino rats. Am J Pharmacol Toxicol. 2009;4:136-43.
34. Poudyal H, Campbell F, Brown L. Olive leaf extract attenuates cardiac, hepatic, and metabolic changes in high carbohydrate–, high fat–fed rats. The journal of nutrition. 2010;140:946-53.
35. Kasdallah-Grissa A, Nakbi A, Koubaa N, El-Fazaâ S, Gharbi N, Kamoun A, et al. Dietary virgin olive oil protects against lipid peroxidation and improves antioxidant status in the liver of rats chronically exposed to ethanol. Nutr Res. 2008;28:472-9.
36. Ahmed MB, Khater MR. Evaluation of the protective potential of Ambrosia maritima extract on acetaminophen-induced liver damage. J Ethnopharmacol. 2001;75:169-74.
37. John Kennedy N, Adamma E. The protective role of Gongronema latifolium in acetaminophen induced hepatic toxicity in Wistar rats. Asian Pacific journal of tropical biomedicine. 2011;1:S151-S4.
38. Saied NM, Hamza AA. Selenium ameliorates isotretinoin-induced liver injury and dyslipidemia via antioxidant effect in rats. Toxicol Mech Methods. 2014;24:433-7.
39. Mathur N, Chaudhary V, Mehta M, Gupta S. Sunset yellow induced changes in the lipid profile in male albino rat. Biochemical Cell Archive. 2005;5:197-200.
40. Matsuda K. ACAT inhibitors as antiatherosclerotic agents: compounds and mechanisms. Med Res Rev. 1994;14:271-305.
41. Biswas K, Kumar A, Babaria BA, Prabhu K, Setty RS. Hepatoprotective effect of leaves of Peltophorum pterocarpum against paracetamol Induced acute liver damage in rats. Journal of basic and clinical pharmacy. 2009;1:10.
42. Kuriakose GC, Kurup MG. Hepatoprotective effect of Spirulina lonar on paracetamol induced liver damage in rats. Asian J Exp Biol Sci. 2010;1:614-23.
43. Nuttall S, Khan J, Thorpe G, Langford N, Kendall M. The impact of therapeutic doses of paracetamol on serum total antioxidant capacity. J Clin Pharm Ther. 2003;28:289-94.
44. Kurata M, Suzuki M, Agar NS. Antioxidant systems and erythrocyte life-span in mammals. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry. 1993;106:477-87.
45. Cheung SCM, Szeto YT, Benzie IF. Antioxidant protection of edible oils. Plant Foods Hum Nutr. 2007;62:39-42.
46. Saito K, Kohno M, Yoshizaki F, Niwano Y. Extensive screening for edible herbal extracts with potent scavenging activity against superoxide anions. Plant Foods Hum Nutr. 2008;63:65-70.
47. Bonilla PA, Murillo RJ, González BJ, Sanz PB. [Variations in fatty acids, tocopherol and other quality parameters of virgin olive oil subjected to refining process]. Nutr Hosp. 1996;12:309-11.
48. Carrasco-Pancorbo A, Cerretani L, Bendini A, Segura-Carretero A, Del Carlo M, Gallina-Toschi T, et al. Evaluation of the antioxidant capacity of individual phenolic compounds in virgin olive oil. J Agric Food Chem. 2005;53:8918-25.
49. Visioli F, Galli C, Plasmati E, Viappiani S, Hernandez A, Colombo C, et al. Olive phenol hydroxytyrosol prevents

passive smoking–induced oxidative stress. Circulation. 2000; 102:2169-71.
50. Sohi KK, Mittal N, Hundal MK, Khanduja KL. Gallic acid, an antioxidant, exhibits antiapoptotic potential in normal human lymphocytes: a Bcl-2 independent mechanism. J Nutr Sci Vitaminol (Tokyo). 2003;49:221-7.
51. Morelló J-R, Motilva Ma-J, Tovar Ma-J, Romero Ma-P. Changes in commercial virgin olive oil (cv Arbequina) during storage, with special emphasis on the phenolic fraction. Food Chem. 2004;85:357-64.
52. Quiles JL, Ramírez-Tortosa MC, Ibéñez S, Gonzélez JA, Duthie GG, Fiuertas JR, et al. Vitamin E supplementation increases the stability and the in vivo antioxidant capacity of refined olive oil. Free Radic Res. 1999;31:129-35.
53. Moreno JJ, Carbonell T, Sanchez T, Miret S, Mitjavila MT. Olive oil decreases both oxidative stress and the production of arachidonic acid metabolites by the prostaglandin G/H synthase pathway in rat macrophages. The Journal of nutrition. 2001;131:2145-9.
54. Manna C, Della Ragione F, Cucciolla V, Borriello A, D'Angelo S, Galletti P, et al. Biological effects of hydroxytyrosol, a polyphenol from olive oil endowed with antioxidant activity. Advances in Nutrition and Cancer 2: Springer; 1999. p. 115-30.
55. Yvonne O, Driss F, Dang PM-C, Elbim C, Gougerot-Pocidalo M-A, Pasquier C, et al. Antioxidant effect of hydroxytyrosol, a polyphenol from olive oil: scavenging of hydrogen peroxide but not superoxide anion produced by human neutrophils. Biochem Pharmacol. 2004;68:2003-8.
56. D'Angelo S, Ingrosso D, Migliardi V, Sorrentino A, Donnarumma G, Baroni A, et al. Hydroxytyrosol, a natural antioxidant from olive oil, prevents protein damage induced by long-wave ultraviolet radiation in melanoma cells. Free Radic Biol Med. 2005; 38:908-19.
57. Casalino E, Calzaretti G, Sblano C, Landriscina V, Tecce MF, Landriscina C. Antioxidant effect of hydroxytyrosol (DPE) and Mn 2+ in liver of cadmium-intoxicated rats. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 2002;133:625-32.
58. Althnaian T. Protective Effect of Camel Milk Against Carbon Tetrachloride Hepatotoxicity in Rats. 2012.
59. Barbagallo M, Dominguez LJ, Tagliamonte MR, Resnick LM, Paolisso G. Effects of Vitamin E and Glutathione on Glucose Metabolism Role of Magnesium. Hypertension. 1999;34:1002-6.
60. Majerus PW, Brauner M, Smith M, Minnich V. Glutathione synthesis in human erythrocytes: II. Purification and properties of the enzymes of glutathione biosynthesis. J Clin Invest. 1971;50:1637.
61. Frederickson CJ. Neurobiology of zinc and zinc-containing neurons. Int Rev Neurobiol. 1989;31:145-238.
62. Ozturk A, Baltaci AK, Mogulkoc R, Oztekin E, Sivrikaya A, Kurtoglu E, et al. Effects of zinc deficiency and supplementation on malondialdehyde and glutathione levels in blood and tissues of rats performing swimming exercise. Biol Trace Elem Res. 2003;94:157-66.
63. Ozdemir G, Inanc F. Zinc may protect remote ocular injury caused by intestinal ischemia reperfusion in rats. The Tohoku journal of experimental medicine. 2005;206:247-51.

How to cite this article: Ibrahim MA, Wani FA, Rahiman S. Hepatoprotective effect of olive oil and camel milk on acetaminophen-induced liver toxicity in mice. Int J Med Sci Public Health 2017;6:186-194

Source of Support: This work was supported by the grants of Deanship of Scientific Research of Aljouf University, **Conflict of Interest:** There are no conflicts of interest to disclose for any of the authors.