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

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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

Abstract

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]

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.[8] 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. [6]

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. [10] 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. [11] It has no allergic features and can be used by lactose-intolerant persons as well. [10] It also has a relatively vast amount of polyunsaturated fatty acids and linoleic acids, which are fundamental for human nutrition. [10] Additionally, CM exhibits a wide range of biological activities; antioxidative, antimicrobial, antihypertensive, antithrombotic, and immuno-modulatory effect. [10, 11]

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 vendors 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. [10]
- **Group 3 (CM) (G3):** Mice were treated with camel milk (CM) (33 ml/kg) once daily by orogastric tube for 28 days. [10]
- **Group 4 (APAP) (G4):** Mice were treated with acetaminophen (APAP) (500 mg/kg) once by orogastric tube on 28th day. [10]
- **Group 5 (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.
- **Group 6 (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 manufacturer 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% thioarbituric 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 x 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

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

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 ≤ 0.05. b: compared to EVOO (G2) group- significant at p ≤ 0.05.
c: compared to the camel milk (G3) group- significant at p ≤ 0.05 d: compared to the APAP alone (G4) group- significant at p ≤ 0.05.

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

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

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 ≤ 0.05. b: compared to EVOO (G2) group- significant at p ≤ 0.05. c: compared to the camel milk (G3) group- significant at p ≤ 0.05 d: compared to the APAP alone (G4) group- significant at p ≤ 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.
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 (Figure 2e 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. 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.

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 caspasess, and eventually results in lipid peroxidation, apoptosis, and cell death.
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.[24] 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 other workers 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: acyltransferase 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 overwhelms 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] Kurata et al. reported that MDA is one of the end products in the lipid peroxidation process.[44] Pre-treatment 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.[46] 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.[15-46] 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.[54, 55] 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.[56, 57] 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.[15, 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] Zinc is an antioxidant 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-glutamylcysteine, ATP, and magnesium ions to form glutathione.[60]

Also, camel milk is rich in zinc (Zn).[59] 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.

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