Protective Effect of Camel Milk Against Aspirin Induced Oxidative Stress in Male Albino Rats

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Key words: Aspirin, Camil milk, Oxidative stress, Rats.

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Abstract:

The aim of this study is to evaluate the protective effect of camil milk on aspirin-induced oxidative stress in the liver of male albino rats. The current study included 24 male rats, which were divided into 4 groups, each group included 6 rats: group 1 as a control group, Group 2 was given aspirin at 100 mg/kg /day, orally via gavage, Group 3 was given camil milk orally via gavage at 1 mL/kg/day and group 4 were given aspirin at 100 mg/kg/day and (after 3 hours) camil milk at 1 mL/kg/day, orally via gavage for 30 days. The results show significantly increase in serum aspartate aminotransferase and alanine aminotransferase activity, which is associated with histopathological damage of the liver. Aspirin increased oxidative stress through the increase in malodialdehyde level and decrease in superoxide dismutase, catalase and glutathione peroxidase enzymes. This modulation of the biological parameter histological damage is significantly neutralized by the administration of the camil milk. From which we can conduct that administer of the camil milk reduce the toxicity and damage caused by the aspirin.

الاثر الحافظ لحليب الجمل على الجهد المؤكسد الناتج من الاسبرين في ذكور الجرذان

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مفتاح الكلمات: الاسبرين, حليب الجمل, الجهد المؤكسد, الجرذان

الملخص:

الهدف من الدراسة هو تقييم استخدام حليب الجمل لمعالجة جهد الأكسدة الناتج من استخدام الاسبرين.

في هذه التجربة تم استخدام 24 جرذًا. قسمت إلى أربع مجموعات كل مجموعة ضمت 6 جرذًا. المجموعة الأولى كانت المجموعة الضابطة, المجموعة الثانية كانت مجموعة الاسبرين في هذه المجموعة اعطيت للجرذان عقار الاسبرين عن طريق الفم بواسطة الانبوب المعوي بتركيز 100ملم/كم يوميا. المجموعة الثالثة كانت مجموعة حليب الجمل في هذه المجموعة اعطيت للجرذان حليب الجمل عن طريق الفم بواسطة الانبوب المعوي بتركيز 1ملم/كم يوميا. أما المجموعة الرابعة كانت مجموعة الاسبرين + حليب الجمل هذه المجموعة استقبلت الاسبرين بتركيز 100ملم/كم يوميا بالإضافة إلى ذلك وبعد ثلاث ساعات من تجريع الجرذان باستر الاسبرين جرعت بحليب الجمل بتركيز 1ملم/كم يوميا عن طريق الفم بواسطة الانبوب المعوي وجلات ثلاثون يومًا أظهرت النتائج زيادة معنوية في فعالية مصل الايثيل أمينو ترانسفيراز وباسبانت ترانسفيراز. وهذه زيادة مترامزة مع التلف في معملية خلايا الكبد. الاسبرين يزيد من الجهد المؤكسد في الجرذان من خلال زيادة مستوى المالدينيد وباثانث خلايا في فعالية انزيمات السوبراوكسيد، الكاتالاز والكولينثيون بروفيس. هذا الاختلاف في هذه التجربة يدل على استخدام حليب الجمل بصورة معنوية. من خلال النتائج التي تم التوصل إليها تبين أن تجنيح الجرذان المعرضة للاسبرين بحليب الجمل تكون مفيدة للتقليل من تسمم الكبد. ويمكن ان نقول بأن حليب الجمل له تأثيرات حافظة قوية.
Introduction:

Liver is the most important organ. It is a key organ regulating homeostasis within the body by various functions, such as metabolism, secretion, storage and detoxification. Liver injury caused by toxic chemicals and certain drugs has been recognized as a toxicological problem. Hepatotoxicity is one of very common ailments resulting into serious debilities ranging from severe metabolic disorders to even mortality. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages (1-4).

Aspirin is the most popular name of a salicylate ester of acetic acid. It has been used as one of the most famous, cheap, easily available and widely used non-steroidal anti inflammatory drug (5,6). Aspirin is used as anti-inflammatory, anti-platelets, analgesic and antipyretic (6). Long-term therapeutic administration of aspirin is associated with hepatotoxicity (7,8). Aspirin affects antioxidant system by shifting the equilibrium between oxidants and antioxidants in favor of oxidants causing hepatic damages (5).

Milk plays a important role in human’s nutrition for the wonderful cause that they are excellent source of various nutrients. Milk diet has been suggested in the management of many diseases (9). Camel milks reported to have antioxidant property, this is due to contain high concentration of vitamins A, B₂, C and E and is very rich in magnesium and other trace elements, these vitamins act as antioxidants and have been found to be useful in preventing toxicant – induced tissue injury and was found to be effective in various models of oxidative stress. It is prevents oxidative injury and cell damage by several mechanisms, including scavenging free radicals and inhibiting lipid peroxidation (10,11). In addition to that camel milks have antibacterial, antiviral activity (which may be due to higher concentration of lactoferrin in camel’s milk) (12,13).And anti-diabetic activity, (which may be due to insulin like activity content).The camel milk has been used as drug against tuberculosis, autoimmune diseases, asthma and antitoxic effect (14-20). Few studies have investigated the protective effect of camel milk against oxidative stress in rats. For this reason, the aim of this study is to investigate the protective effects of camel’s milk against aspirin -induced oxidative stress in rats by biochemical assaying and histopathology of liver tissues.

Materials and methods:

All chemicals used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA).Rats were treated with aspirin (100 mg/kg/day orally) via gavage for 30 days (21). Camel milk samples were collected daily early in the morning from a herd of camels, return to kerbala university, college of veterinary medicine. Milk was collected from camels by hand milking. The samples were collected in sterile screw bottles and kept in cool boxes until transported to the laboratory. Camil milk was given orally by intra gastric tube at a dose of (1mL/kg) as described (20).

The experimental protocols were conducted with the approval of the Animal Research Committee of Kerbala University, kerbala, Iraq. The study included 24 male albino rats, each weighing about 230 g. The animals were divided into 4 groups, each consisting of 6 members.
Group 1. Animals of this group were considered as controls (without any drugs).

Group 2. Animals of this group were given aspirin via gavage at a dose level of 100 mg/kg body weight, every day for 30 days.

Group 3. Animals of this group were given camil milk via gavage at a dose level of 1mL/kg body weight, every day for 30 days.

Group 4. Animals of this group were given aspirin at a dose level of 100mg/kg body weight plus (after 3 hours) camil milk at a dose level of 1mL/kg body weight via gavage every day for 30 days.

After 24 hours from the last treatment, blood were drawn from all rats by heart pincher method to determination of the biochemical tests, after that the rats were sacrificed the liver was removed immediately, washed with ice-cold 0.15 M phosphate buffer saline pH 7.4, a portion of ten percent of liver was homogenate with 1.15% KCl, the mixture centrifuged at 14000 rpm for 15 min and supernatant was used for measuring the parameters. liver is collected for assay the malondialdehyde (MDA) concentration and the activity of enzymatic antioxidant system in the tissue, and for histopathological study.

Biochemical analysis:

Serum alanine aminotrasferase (ALT) and aspartate aminotransferase (AST) activities were estimated according to Reitman and Frankel methods (22). Tissue protein levels were measured according to the method used by Lowry et al (23).Tissue MDA assays were performed according to the guidelines of Ohkawa et al (24).MDA is a product of lipid peroxidation that reacts with thiobarbituric acid (TBA) under acidic conditions at 95 °C, forming a pink complex that absorbs at 532 nm. 1,1,3,3-Tetraethoxypropane was used as the standard. The results are expressed as nmol/g tissue. Superoxide dismutase (SOD) activity was measured according to the method of Winterbourn (25).It is based on the ability of superoxide dismutase to inhibit the reduction of nitroblutetrazolum(NBT) by superoxide, absorbances were monitored at wave length 560 nm. Tissue catalase was assayed according to the method of Beers and Sizer (26).Catalase (CAT) catalyses the decomposition of hydrogen peroxide (H_{2}O_{2}) to water and oxygen, The enzyme activity was followed by the decreasing in absorbance at 240 nm at 15 second intervals. Tissue glutathione peroxidase was assayed according to the method of Leopold Flone etal (27). Oxidized glutathione formed during glutathione peroxidase reaction is instantly and continuously reduced by an excess of glutathione reductase activity for a constant level of glutathione. The concomitant oxidization of NADPH is monitored spectrophotometrically at 340 nm.

Histological procedure

Small portion of liver tissue were fixed in 10% formalin, dehydrate in graded alcohol and embedded in paraffin wax, sectioned at 5 μm thickness and stained with hematoxylin and eosin for light microscopic examination (28).
Statistical analysis:

The statistical analysis was performed using SigmaPlot version 11. Differences among groups were determined by one-way analysis of variance (ANOVA) followed by Fisher’s LSD test. All results are presented as means ± SD and significance is accepted at $P < 0.05$ (29).

Results:

As shown in table 1, there was a significant increase in the activity of ALT and AST in animal groups treated with aspirin as compared to control groups. However Co-administration of camil milk will decrease the rise in serum ALT and AST.

Table 1: The levels of serum AST and ALT in control, Aspirin induced hepatic damage , Camil milk treated, Aspirin induced hepatic damage and Camil milk treated hepatic damage groups rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (U/ml)</th>
<th>ALT (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>76.80 ± 7.93</td>
<td>24.41 ± 2.78</td>
</tr>
<tr>
<td>Aspirin</td>
<td>219.13 ± 22.64*</td>
<td>78.46 ± 7.99*</td>
</tr>
<tr>
<td>Camil milk</td>
<td>75.83 ± 8.22</td>
<td>23.21 ± 3.09</td>
</tr>
<tr>
<td>Aspirin+Camil milk</td>
<td>77.13 ± 9.93</td>
<td>25.28 ± 5.31</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six animals in each group.
*Level of significance $p < 0.05$.
AST = Aspartate transaminase, ALT = Alanine transaminase

Aspirin caused significant increases in the MDA level of liver when compared to the control group. This rise in MDA was decreased by camil milk. In the liver tissues the enzymatic antioxidant activity shows that in the aspirin treated rats there was a significant decrease in the activity of SOD, CAT and G-PX enzymes as compared to control group. However, oral administration of camil milk 1mL/kg/day for 30 days significantly decreased these anti-inflammatory drugs-induced adverse effects and maintained the rats at near normal status (Table 2).
Table 2: Show the MDA and antioxidant enzymes parameters in hepatic tissue.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA(nmol/g)</th>
<th>SOD(U/mg)</th>
<th>CAT(U/mg)</th>
<th>G-Px(μmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21.13±13.98</td>
<td>11.44±2.01</td>
<td>0.90±0.23</td>
<td>3.13±0.49</td>
</tr>
<tr>
<td>Aspirin</td>
<td>201.6±12.05*</td>
<td>3.2±0.48*</td>
<td>0.26±0.08*</td>
<td>0.61±0.17*</td>
</tr>
<tr>
<td>Camil milk</td>
<td>117.68±14.12</td>
<td>11.65±1.88</td>
<td>1.04±0.42</td>
<td>4.37±1.11</td>
</tr>
<tr>
<td>Aspirin+Camil milk</td>
<td>123.38±13.12</td>
<td>10.65±1.67</td>
<td>0.86±0.12</td>
<td>3.12±1.10</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six animals in each group.
*Level of significance p < 0.05.

Animals treated with aspirin (100 mg/kg orally) for 30 days showed necrosis, and fatty degeneration in liver (Figure 2). This effect was relatively decreased in animals co-administered with camil milk (Figure 4). However, histopathological changes were not observed on animals treated with camil milk when compared with the control group (Figure 3).

Figure (1): Control rat liver; normal architecture of liver.
Figure (2): Aspirin treated rat liver; severe cellular infiltrations, degenerative changes of hepatic cells with cell necrosis and disarrangement of normal hepatic cells.

Figure (3): Liver section from rat treated with camil milk showing normal morphology.
Figure (4): Aspirin + camil milk treated rat Liver; near normal appearance of hepatocytes (less cellular infiltrations and degenerative changes of hepatic cells with cell necrosis).

Discussion:
Liver is a versatile organ of the body that regulates internal chemical environment, and it is target for toxicity because of its role in clearing and metabolizing chemicals through the process called detoxification (2,30-32). Drug induced liver disorders occurred frequently can be life threatening and mimic all forms of liver diseases (2,33). Aspirin being a drug capable of causing liver disorders if overdoses are consumed.

In the present study the damage of liver due to aspirin was confirmed by elevated levels of biochemical parameters like AST, ALT, total cholesterol and serum triglycerides. This is due to the hepatic cells possess a variety of metabolic activities & contain a host of enzymes. Serum glutamatic pyruvate transaminase (SGPT), Serum glutamate:oxaloacetate transaminase (SGOT) found in higher concentration in cytoplasm & SGPT particularly in mitochondria. In liver injury the transport function of hepatocytes is disturbed, resulting in the leakage of plasma membrane (2,34), thereby causing leakage of such enzymes leading to the increased serum levels of them. And this result are in agreement with several studies (21,35-37). However, the elevated levels of enzymes are decreased to near normal levels after 30 days treatment of camil milk indicates that it offered protection by preserving the structural integrity of the hepatocellular membrane against aspirin.

The increase in serum biochemical parameters were well directly correlated with the hepatic histological damage. The main causative factor of tissue damage is lipid peroxidation which is motivated by the reactive oxygen species (ROS) formation, and this process is associated with the formation of malodialdehyde (MDA) because it is the by-product of lipid peroxidation (38-41).

Aspirin treatment causes significant increase in the level of MDA as compared to control group. This increase in MDA level may be due to the association with a lose
of balance between prooxidation and antioxidation, energy depletion and accelerated aging in the target organs such as heart, kidney and brain (20,42). And these results are agree with Shyamal (34) have showed that aspirin administration increases lipid peroxidation in the rat tissues. However, administration of camel milk along with aspirin caused significant decrease in MDA levels suggested the protective effects of camel milk. The protective effect of camel milk against aspirin-induced oxidative stress in the rat is due to its antioxidant properties, it was found to contain high concentrations of vitamins E,C,B2,C and A and is very rich in magnesium and other trace elements, these vitamins act as antioxidants and have been found to be useful in preventing toxicant-induced tissue injury (20,43,44). Magnesium protects the cell against damage from oxyradicals and helps in the absorption and metabolism of vitamins B,C and E, which play a large role in cell protection from free radicals by functioning as antioxidants (20,43-45).

The enzymatic antioxidant defense system, which includes SOD and CAT, helps protect cells from oxidative injuries (44,46). SOD catalyzes the rapid removal of superoxide radicals (44,47). Generating H2O2, which is eliminated by catalases (44,46) these enzymes are present in the peroxisomes of nearly all aerobic cells. CAT protects the cell from toxic effects of hydrogen peroxide by catalyzing its decomposition into molecular oxygen and water without the production of free radicals (44,48).

In the present study showed a significant decrease in SOD and CAT activity in liver tissues treated with aspirin. The inhibition of SOD and CAT activity during aspirin liver damage may be due to the increased generation of reactive oxygen radicals, which can create on oxidative stress in the cells. This results agree with others (36). On the other hand, there was a significant increase in SOD and CAT activities in liver tissues treated with camel milk. It has been reported that administration of camel milk significantly decreased lipid peroxidation and increased endogenous antioxidants, such as SOD and CAT (43,44). Our study shows that treatment with camel milk improved the activities of SOD and CAT in rat tissues. This improvement may have resulted from camel milk provided a significant recovery in the level of ROS in aspirin exposed animals in blood and tissues.

In the present study the above parameters analyzed, it was concluded that camel milk has significant hepatoprotective activity against aspirin induced oxidative stress in rat.

Reference:
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