Therapeutic value of camel milk as antiulcerogenic effect against ethanol-induced gastric ulcers in rats

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ABSTRACT

This study was performed to investigate the regular treatment of oral administration of raw camel milk 5 mg/kg b.wt. on ethanol- and naproxen-induced peptic ulcer in rats. The collected samples of raw milk were kept at room temperature 25°C and examined for physiochemical parameters as well as sensory evaluation. The result obtained showed that pH of the fresh camel milk was 7.34 ± 0.2, while the acidity was 0.187 ± 0.2. In sensory evaluation, camel milk was fairly acceptable. Oral administration of camel milk in rats with ethanol-induced peptic ulcers, significantly (p < 0.05) lowered the amount of long ulcers, average length ulcers, index and the volume of peptic juice. The total percentage of protein significantly increased (p < 0.05), while the pH of the gastric juice value differed significantly, the healing rate was 70.65% in camel milk ranitidine treated group compared to 4.5% in ranitidine-treated rats. Finally, the same positive effect of the oral administration of camel milk was observed in rats with naproxen-induced peptic ulcers: the value of healing rate was 60.03% compared to 34.03% in ranitidine-treated rats. These results suggested a possible benefit of milk supplementation in treating peptic ulcer.

Keywords: Camel milk, ethanol, naproxen, peptic ulcer, albino rats.

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INTRODUCTION

Over the past few years, improvement in clinical, increasing attention, which has been reported to the development bacteriological and radiological attributes were pronounced in the camel milk supplementation due to its higher content of protective proteins, because of their obvious therapeutic advantages of case of administration and better patient convenience.

There is a different formulation variable such as antiulcerogenic, so most of these agent caused several side effects such as impotence, arrhythmias as gynaecomastia and haematopoietic disorder. In addition recurrence rats are high (Ariyoshi et al., 1986).

Camel milk has medicinal properties. It is suggested that it contains protective proteins, which may have a possible role for enhancing the immune defense mechanism. Also antibacterial and antiviral activities of these camel milk proteins have been studied (El-Agamy et al., 1992). Otherwise camel milk destroys Mycobacterium tuberculosis (Donchenko et al., 1975). The inhibition of pathogenic bacteria by camel milk was also observed (Barhour et al., 1984).

In India, camel milk is used therapeutically for treating dropsy, jaundice, problems of the spleen, tuberculosis, anemia, asthma, piles and diabetes (Knoess, 1979). Beneficial role of camel milk in pulmonary tuberculosis has been showed (Mal et al., 2001). In USSR, camel milk was used in sanitaria for treating patients suffering from chronic hepatitis; they acquired improved liver function after drinking camel milk (Sharmanov and Fedotov, 1979).

Camel milk has high concentration of calcium and iron so the low pH of camel milk (from the ascorbic acid - vitamin C) allows enhanced absorption from the duodenum. However, camel milk is different from other ruminants’ milk, because it contains a larger concentration of insulin (Zogorski et al., 1998) and high
amounts of vitamins C, B2, A and E. Camel milk also contains enzymes which exert antibacterial and immunological properties (Kappeler et al., 1998). However, people with several food allergies improved with camel milk, because it can be consumed by lactase deficient patients and those with weak immune system. Other therapeutic effects of camel milk have been shown, such as improvement or normalization on characteristics of blood clinical and biochemical tests, same as of renal functions (Sharmanov et al., 1982), anti-diabetic effect (Redwan and Tabll, 2007; Saltanat et al., 2009). Also, it has a protective effect against hepatitis C virus (Redwan and Tabll, 2007). This study intends to explain and investigate the main action of camel milk on ethanol- and naproxen-induced gastric ulcers in rat.

MATERIALS AND METHODS

Ethanol induced gastric ulceration

Animals and treatment

In this study experiment, fifteen male white albino rats weighting (120 to 200 g b.w) were used. They were supplied by the animal house of central institute research of Khartoum, Sudan, and acclimated for at least 18 h prior to the experiment to ensure an empty stomach, and kept in aluminum cages to prevent coprophagy (Garg et al., 1993). The experimental animal were housed in air conditioned room at 21 to 23°C and 60 to 65 of relative humidity and kept on a 12 h night/dark cycle. To prevent dehydration during the fasting period, rats were supplied with sucrose (BDH) 8% (w/v) solution in NaCl (BDH) 0.2% (w/v) which was removed 1 h before experiment (Galvin and Mikhail, 1976).

Experimental group protocol

Rats were divided into three Groups (Table 1). In the starting first day, animals of group 1 (positive control) received 2 doses of distilled water (5 mg/kg) within 6 h intervals. Rats in groups 2 were administrated 2 doses of raw milk (5 mg/kg) with 6 h intervals. Rats in the third group received 2 doses of ranitidine (100 mg/kg orally), such as a same time period. The following day, control animals received 1 dose of distilled water (5 mg/kg), the second group received camel milk (5 mg/kg), while rats of the third group received one dose of ranitidine. After 90 min all rats were given a dose of 10 mg/kg of 80% alcohol. After one hour ethanol was given, all rats were euthanized by an overdose of chloroform. The stomachs were removed and opened along the greater curvature and carefully rinsed under running water. Then lesions were measured in the glandular part of the stomach under illuminated magnifying microscope (10x). Also, long lesions were counted and measured along the greater length. Petechial lesions were counted with the aid of a 1-mm squares grid (Ogle et al., 1985). Each five petechial lesions were taken as a 1 mm ulcer (Cho and Ogle, 1978). Finally, the total length sum of long ulcers and petechial lesions in each group of animals were divided by its number to calculate the ulcer index (mm). The treatment ratio was obtained by the following equation:

\[
\text{Treatment ratio} = \frac{\text{Control ulcer index} - \text{Test ulcer index}}{\text{Control ulcer index}} \times 100
\]

Naproxen induced gastric ulceration

Animals and treatment

Fifteen male Wister weighing (150 to 250 g b.wt.) were used. All animals were housed in standard conditions before starting the experiment. Rats were allocated into 3 equal groups (Table 1). The modified methodology (Goel et al., 1985) was used to imitate the gastric ulceration experiment. Animals of group 1 (positive control) received distilled water; after 3 h Naproxen (200 mg/kg) in solution of 1% carboxymethyl cellulose was administrated orally; then within 3 h distilled water was given again. Animals also received distilled water in groups 2 and 3 and orally camel milk (5 mg/kg) and ranitidine (100 mg/kg), respectively, in addition to naproxen. The treatments ended after three days. On the fourth day, to study the effect of naproxen-induced gastric ulceration, stomach was explored and the pylorus was closed and the animals were left to recover. Abdomen was closed and the animals left to recover with drinking water. After that, all animals were euthanized by an overdose of chloroform, the oesophagus was ligated and the stomach was removed. The gastric mucosa was cleaned with 3 ml of distilled water. The gastric juice and the washings were completely, homogenized and centrifuged at 5000 rpm for 5 min. Then, volume of gastric juice was measured and mention in ml/100 g body weight. The stomach was then cut along the great curvature and the glandular portion was examined under dissecting microscope. The number of ulcers was determined by counting and total length was measured to calculate the curative ratio as mention above.

Principals for study protein counted

To calculate the total protein (g/dl), gastric juice was determined by Biuret Reagents (Mehl, 1945) using commercial kits.

Statistical analysis

The study indicates the differences between treated animals and control group animals (Mean ± SD), which were examined for significance using a specific analysis of variance (ANOVA) followed by Duncan’s multiple range test. Difference were considered significant when P < 0.05.

RESULTS

Camel milk administered orally in animals with ethanol-induced gastric ulcers significantly lowered the number of long ulcers, the average length of ulcers and ulcer index and also the volume of gastric juice (p < 0.05). However, the pH of gastric juice was not significantly changed. The total of all protein was slightly increased. Ranitidine given orally in rats with alcohol-induced gastric ulcers slightly decreased the total protein. The curative ratio was 70.70% in camel milk treated animals in compared to 45.12% in ranitidine treated rats (Table 2).

Camel milk administered orally in animals with Naproxen induced gastric ulcers significantly (p < 0.05) reduced the number of long ulcers, ulcer index, volume of gastric juice and elevated the total protein. Moreover, the pH of gastric juice was not significantly modified.

Ranitidine given orally in pretreatment significantly
Table 1. Experimental Design.

<table>
<thead>
<tr>
<th>Interval</th>
<th>Groups</th>
<th>15 rats (Ethanol – induced ulcer)</th>
<th>15 rats (Naproxen – induced ulcer)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Day 1</td>
<td>D.w (2 doses)</td>
<td>C.M</td>
<td>Ran.</td>
</tr>
<tr>
<td>90 min</td>
<td>80% Alcohol (10 ml/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 h</td>
<td>Euthanasia</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Days 4</td>
<td>Pylorus ligation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D.W = Distilled water 5 ml/kg, C.M = Camel Milk 5ml/kg, Ran. = Ranitidine 100 mg/kg, Nap. = Naproxen 200 mg/kg suspended in 1% carboxymethyl cellulose.

Table 2. Antiulcerogenic effect of camel milk against ethanol-induced gastric damage in rats (Mean± SD, n = 5).

<table>
<thead>
<tr>
<th>No. of long ulcers</th>
<th>Length of ulcer (mm)</th>
<th>Ulcer index</th>
<th>Curative ratio (%)</th>
<th>Volume of gastric juice (ml/100 mg)</th>
<th>pH</th>
<th>Total protein (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.50 ± 1.12a</td>
<td>4.23±0.88b</td>
<td>1.02 ± 0.4b Standard</td>
<td>-</td>
<td>1.61 ± 0.26b</td>
<td>7.30 ± 0.55a</td>
</tr>
<tr>
<td>Camel milk (5 ml/kg)</td>
<td>1.8 ± 0.27a</td>
<td>1.26 ± 0.58a</td>
<td>0.33 ± 0.3a Standard</td>
<td>70.7</td>
<td>1.15 ± 0.27a</td>
<td>6.60 ± 0.55a</td>
</tr>
<tr>
<td>Ranitidine (100 mg/kg)</td>
<td>4.60 ± 1.3b</td>
<td>5.25 ± 0.27b</td>
<td>1.35 ± 0.4b Standard</td>
<td>45.12</td>
<td>1.89 ± 0.39b</td>
<td>7.0 ± 0.69a</td>
</tr>
</tbody>
</table>

Means with different letters in the same column are significant at p < 0.05. a, b refers to P < 0.05.

Table 3. Antiulcerogenic effect of camel milk on Naproxen-induced gastric ulcer in rats (Mean ± SD, n = 5).

<table>
<thead>
<tr>
<th>Number long ulcer</th>
<th>Ulcer index</th>
<th>Curative ratio (%)</th>
<th>Volume of gastric juice ml/100 g</th>
<th>pH</th>
<th>Total protein (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.3 ± 1.12b</td>
<td>3.41 ± 0.65c</td>
<td>-</td>
<td>2.22 ± 0.31b</td>
<td>5.66 ± 0.33a</td>
</tr>
<tr>
<td>Camel milk (5 ml/kg)</td>
<td>5 ± 0.55a</td>
<td>1.2 ± 0.51a</td>
<td>65.3</td>
<td>1.85 ± 0.25a</td>
<td>5.5 ± 1.35a</td>
</tr>
<tr>
<td>Ranitidine (100 mg/kg)</td>
<td>1.6 ± 0.44b</td>
<td>2.25 ± 0.50b</td>
<td>34.3</td>
<td>2.03 ± 0.11ab</td>
<td>6.8 ± 1.10a</td>
</tr>
</tbody>
</table>

Means with different letters in the same column are significant at p < 0.05. a, b refers to P < 0.05.

Reduced the number of long ulcers and ulcer index. The total protein was significantly (p < 0.05) elevated. Also, there was a slight modification in the volume of gastric juice. The curative ratio was 65.03% in camel milk–treated group compared to 34.03 in ranitidine-treated group (Table 3).

Macro-microscopic observation of gastric mucosa revealed large patches of severe hemorrhages observed in the stomach of alcohol–treated
animals (not previously existent). These lesions were markedly decreased in stomach of camel milk-treated animals. A moderate effect was observed in ranitidine treated rats.

DISCUSSION AND CONCLUSION

The present study indicates the protective effect of orally administration of camel milk against gastric destruction induced experimentally by ethanol or naproxen in rats. Two types of gastric ulcers were investigated, acute gastric damage was induced by ethanol and delayed onset of gastric ulceration was induced by the non-steroidal anti-inflammatory agent (NSAIA) such as naproxen.

Ethanol is the most widely used agent for evaluation of drugs in experimental models as anti-ulcerative effects in rats (Akhtar and Ahmed, 1995; Atta et al., 2005). The acute effect of ethanol has been justified to be due to protein precipitation of the cytoplasmatic components of the vasoactive mediators such as leukotrienes C4 (LTC4) and histamine (Jamal et al., 2006). The vasoactive mediators cause blood flow stasis in the microcirculation of the mucous membrane, an effect which may have contributed to the elevated lesions in this model (Konturek et al., 1988; Wallace, 2001). However, alcohol may also be used as source to induce solubilization in the mucous and stomach wall into lumen, increase the pepsin release and reduce the tissue levels injury (Robert et al., 1979). Moreover, ethanol has been indicated to releases the production of free radicals (Gazzieri, 2007). In the test, it was shown that the ironic and bizarre behavior of ranitidine in ethanol-treated animals in which the total protein was lowered, which indicated from the interaction between this drug and alcohol that curtails protein synthesis in the mucosal membrane.

Non-steroidal anti-inflammatory drugs (NSAID) being used as a model drug to induce experimental of gastric damage has been widely practiced (Atta et al., 2005). Inhibition of endogenous prostaglandins facilitated the stomach damage. These eicosanoids inhibit acid release by the parietal cells and permit secretion of cytoprotective mucous in the stomach (Robert, 1981). The mechanism of naproxen-induced ulcerogenic effect is by the inhibition of endogenous prostaglandins synthesis (White and Vane, 1983), which lowers the cytoprotective effect of prostaglandins.

Anti-inflammatory drugs, such as indomethacin, injury the gastric cyclooxygenase (COX) enzyme, obtaining in the elimination of prostaglandins production (Wallace et al., 2000).

Camel milk has been shown to have more antacid efficacy (Sharmanov et al., 1981). Camel milk has medicinal properties suggesting that it contains protective proteins high levels of vitamin C, A, B2 and E and is very rich in magnesium and zinc (Rahman et al., 2005). These vitamins are antioxidants that seem to be useful in reducing the oxidative stress caused by toxic agent (Sajitha et al., 2010; Traber and Stevens, 2011). Magnesium is very essential for biosynthesis of glutathione. Also, camel milk have a possible role for enhancing the immune defense mechanism. Recent studies also indicate that magnesium significantly enhances the antioxidant defense and was effective against alcohol-induced oxidative stress, disturbance of liver function and increased cholesterol, mainly when administrated with selenium (Markiewicz et al., 2011).

Another properties of the protective effect of camel milk studies in the present investigation showed that it is rich in zinc (Rahman et al., 2005). Zinc plays an important role as an essential element for the activity of many enzymes in the living organism. A protective effect of zinc has been showed against cadmium-induced cellular toxicity. This result is probably due to palliative studies effects on oxidative stress and apoptosis and reduced lipid peroxides (Goering and Klaassen, 1984; Jemai et al., 2007; Jihi et al., 2011). It also plays an important role in the DNA replication, transcription and protein synthesis, introduced in cell division and differentiation (Frederickson, 1989). Moreover, in various animals tissues, zinc deficiency increases lipid peroxidation, main trance supplementation corrected. It has been shown that zinc can protect cell damage through activation of the antioxidant system (Sato and Bremner, 1993; Powell, 2000; Ozturk, et al., 2003). The antiperoxide drugs have been investigated to possess a gastroprotective role against ethanol-induced gastric damage (Mizui et al., 1987). In another studies which indicated that camel milk can generate nitric oxide (Hashad et al., 2006) also similar to endogenous breast milk (Stevens et al., 2000). Nitric oxide plays an important role as a mediator in the gastric defense action in stimulated mucus release, inhibits the adherence of neutrophils to the endothelial cells, and mainly increases the blood flow to the gastric mucus membrane (Coruzzi et al., 2000; Olinda et al., 2008).

In conclusion, camel milk can protect gastric mucosa against ethanol or naproxen induced gastric damage. The current study indicates that the correct mechanism of gastroprotective needs further researches. Also, future investigation, analysis and evaluation of its content and sequences of amino acids and lipoproteins are necessary.

REFERENCES


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