

Effect of gout milk and bromelain on liver and hematological parameters in male rats

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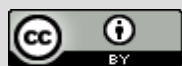
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ABSTRACT: Peptic ulcers occur owing to peptic acid damage to the gastrointestinal tract, resulting in the erosion of the mucosal layer, with lesions exceeding 3-5 mm in depth. Aim of the study: The main objectives of this study were to evaluate the effect of fortified goat milk on the blood, liver and hormone profile in rats by estimating them in blood plasma. To know the effect of goat milk fortified with bromelain on the stomach and intestines affected by ulcers by comparing it with the treatment of omeprazole. The research was conducted from September 2023 to January 2024. A sample of 30 sexually mature male animals was used, randomly distributed into six groups of similar weights, and each group included five rats obtained from the animal house of the College of Veterinary Medicine at Tikrit University. Division of rats occur as A1 healthy control group (untreated and provided only with water and food throughout the experiment period). A2 The infected control group (infected with a gastrointestinal ulcer after being given an oral dose of acetic acid). A3 given an oral dose of goat's milk. A4 given an oral dose of goat's milk fortified with bromelain enzyme. A5 given an oral dose of bromelain enzyme. A6 Group oral administration of omeprazole. The present study showed decrease RBC, Hb, and PCV in T2 group as compared with control(T1), while increased in T3, T4, T5, but no difference between T1 and T6. As for platelets increase in T1, while no differences between T1, T3, T4, T5, and T6. Both GPT and GOT increase in T2 as compared with T1, while no differences in T3, T4, T5, T6 as compared with T1. Furthermore, decrease T3, T4, T5, T6 as compared with T2. As for GPT decrease in T3, T4, T5, T6 as compared with T1 and T2. This study concluded the protective effect of goat milk on rats infected with peptic ulcer and synergical combination of both bromelain and gout milk were more effective than alone. This study also concluded increase PCV, Hb, RBC, and decrease GOT, and GPT in peptic ulcer.

Keywords: Male rats, peptic ulcer, gout milk, bromelain, omeprazole, hematological and liver function test



1. INTRODUCTION

Peptic ulcers are lesions that form in the mucosal lining of the stomach, lower esophagus, or small intestine. The primary factors are inflammation caused by *Helicobacter pylori*, consistent consumption of aspirin and ibuprofen, smoking, alcohol use, radiation therapy, and stomach cancer (1, 2). Goat milk is abundant in antioxidants and is effective in preventing and treating stomach ulcers (3, 4). Goat milk is exceptionally nutritious and possesses elevated concentrations of calcium, magnesium, and phosphorus compared to cow and human milk. Additionally, goat milk is more easily digestible than cow milk due to its smaller fat globules and somewhat lower lactose level(5).

It helps adults suffering from gastrointestinal disturbances and ulcers (6). Goat milk has been a staple in human diets for centuries, establishing its historical safety. Additionally, it's noteworthy that Whole Goat Milk is recognized as Generally Recognized as Safe (GRAS) by the FDA (7, 8). Bromelain is a proteolytic enzyme found in the pineapple plant. It is known for its anti-inflammatory properties in the gastrointestinal tract, making it a popular supplement for its many health benefits(9). The enzyme can break down proteins into smaller peptides Amino acids, which help speed up digestion and may reduce inflammation in the body, Bromelain has been traditionally used in South and Central America for centuries, where indigenous peoples consumed pineapple for its medicinal properties (10). The enzyme was first isolated in the late 19th century by researchers studying the enzyme composition of the fruit. Since then, bromelain has gained interest in the scientific and medical communities for its potential therapeutic applications. As a natural enzyme with diverse functions, bromelain remains an area of interest for researchers exploring its pharmaceutical and health-promoting benefits (11). The present study was conducted to ascertain the most effective methods for fortifying bromelain in goat milk for therapeutic purposes, including the treatment of intestinal problems such as ulcers, and to meet the daily requirements of the substance. Goat milk, which provides the consumer with a variety of nutrients including carbohydrates, proteins and fats, is one of the most widely consumed foods, and is inexpensive and readily available, in addition to being a staple or semi-daily diet for many individuals. In addition, the bromelain enzyme is considered a low-toxic substance.

2. MATERIALS AND METHODS

2.1 Study site

This study was conducted in the laboratories of the Department of Food Sciences at the College of Agriculture, Tikrit University, the Animal House at the College of Veterinary Medicine, Tikrit University, and the Central Laboratory at the Presidency of Tikrit University. The research was conducted from September 2023 to January 2024.

2.2 Experimental animals used

Albino white laboratory rats were obtained at the age of 9-11 weeks, and weighing between 190-200 g. In this study, before starting the experiment, the animals were observed and evaluated for 5 days in order to adapt and verify their ideal health status. Before using them in the experiment, the animals underwent a comprehensive examination by the specialized veterinarian at the center to ensure their safety and freedom from diseases and disabilities. A sample of 30 sexually mature male animals was used, randomly distributed into six groups of similar weights, and each group included five rats obtained from the animal house of the College of Veterinary Medicine at Tikrit University.

The animals were housed in plastic cages with a floor covered with sawdust, which was changed every four times a week. The animals were fed regularly with ready-made feed, as the light period was 12 hours, and the darkness period was also 12 hours, and the temperature was maintained at 24 ± 2 degrees Celsius. Each cage was assigned a number. The animals had continuous access to water and were fed the diet assigned to each treatment during the 42-day experimental period after confirmation of infection. They were continuously monitored under my supervision, along with the specialists at the center, until the end of the experiment. Each group received an oral dose of limited quantities of the materials that were purchased ready-made. 3-3-2 Induction of intestinal ulcers (12)

Four rats were isolated for the control treatment (without infection), and the remaining rats were dosed orally with Saudi-origin white vinegar, at a concentration of 1 ml/kg of body weight. White vinegar was given orally at a dose of 1 ml/kg (5%) for 14 days (Asrafiel et al., 2024). After confirming the presence of intestinal ulcers by examining blood images (CBC) and withdrawing a blood sample by cardiac puncture, the amount of blood withdrawn ranged from 0.5 to 5 ml, using a 5 ml injector, and was injected into test tubes containing EDTA to prevent clotting, the blood images included white blood cells (WBCs), red blood cells (RBCs), hemoglobin (HGB), blood cell volume (PCV), mean hemoglobin concentration (MCHC), and mean hemoglobin (MCH). The results also indicated a significant decrease in body weight and the pH value of gastric juice, in addition to a significant increase in the volume of gastric acid, total acidity, and ulcer index. The results of the complete blood analysis (CBC) also showed the presence of anemia caused by internal bleeding in the animal, and evaluation of C-reactive protein (CRP) and cytokine levels to evaluate systemic inflammation, stool analysis, and occult blood test in the stool to determine hidden intestinal bleeding. A decrease in the amount of food consumed by the animals and loss of appetite were also noted, along with Blood in the stool due to internal bleeding.

2.3 Experimental design:

After confirming that the animals were infected with intestinal ulcers through blood analysis and stool analysis, they were distributed into six plastic cages, and were distributed as follows:

- A1 The ideal healthy control group (untreated and provided only with water and food throughout the experiment period).
- A2 The infected control group (infected with a gastrointestinal ulcer after being given an oral dose of acetic acid while continuing to give them food and water throughout the experiment period).
- A3 The group of animals infected with a gastrointestinal ulcer and treated after being given an oral dose of goat's milk at a concentration of 5 ml in two doses, morning and evening.

- A4 The group of animals infected with a gastrointestinal ulcer and treated after being given an oral dose of goat's milk fortified with bromelain enzyme at a rate of 5 ml goat's milk with 2 ml bromelain enzyme in two doses, morning and evening.
- A5 The group of animals infected with a gastrointestinal ulcer and treated after being given an oral dose of bromelain enzyme at a rate of 2 ml in the morning and evening.
- A6 Group of animals with gastrointestinal ulcers treated after oral administration of omeprazole 1 ml.

2.4 Sources of materials

Goat milk Raw, untreated and unmodified goat milk was collected between October 2023 and February 2024 from the Awainat and Al-Alam areas in Tikrit district, Salah Al-Din Governorate. The samples were stored in tightly sealed, sterile containers at a temperature of 4-5°C for no more than 3-6 hours before starting the experiment.

2.5 Bromelain

A 500 mg (2400 IU) package of bromelain produced by Now Foods/USA was used, and the package was stored in the refrigerator until use at work.

2.6 White Vinegar

A 473 ml glass bottle of white vinegar produced by Al Faris Food Factory Limited/KSA was used, diluted with water to 5% acetic acid.

2.7 Omeprazole

Omeprazole capsules of 20 mg produced by Ajanta Pharma Limited, India were used, and stored at room temperature until use at work.

2.8 Statistical Analysis

The experiment was conducted according to a completely randomized design (CRD Complete Randomized Design) and the analysis of variance was conducted using the General linear Model within the ready statistical program. The (LSD) test was used to determine the significance of differences, if any, between the different averages at a probability level of 0.05.

3. RESULTS AND DISCUSSION

The present study showed decrease RBC, Hb, and PCV in T2 group as compared with control(T1), while increased in T3, T4, T5, but no difference between T1 and T6. As for platelets increase in T1, while no differences between T1, T3, T4, T5, and T6. As shown in Table (1).

Table (1): Level of Platelet, PCV, Hb, and RBC according to different groups

Parameters				Groups
Platelet	PCV	Hb	RBC	
7.44 ± 222.66 b	0.66 ± 34.50 b	0.32 ± 10.60 b	0.17 ± 4.93 b	T1
2.88 ± 305.00 a	0.11 ± 31.66 c	0.11 ± 9.50 c	0.05 ± 4.20 c	T2
21.37 ± 236.66 b	0.35 ± 37.33 a	0.17 ± 11.46 a	0.12 ± 5.90 a	T3
18.16 ± 207.66 b	0.69 ± 35.00 b	0.05 ± 10.90 ab	0.27 ± 5.87 a	T4
22.40 ± 242.33 b	0.57 ± 38.00 a	0.23 ± 11.36 a	0.12 ± 5.90 a	T5
22.66 ± 243.66 b	0.30 ± 34.60 b	0.12 ± 10.73 b	0.04 ± 5.07 b	T6

* Different lowercase letters within a single column indicate significant differences ($p \leq 0.05$) between treatments.

As shown in Table (1), both GPT and GOT increase in T2 as compared with T1, while no differences in T3, T4, T5, T6 as compared with T1. Furthermore, decrease T3, T4, T5, T6 as compared with T2. As for GPT decrease in T3, T4, T5, T6 as compared with T1 and T2.

Table (2): Level of Platelet, PCV, Hb, and RBC according to different groups

Parameters		Groups
GPT	GOT	
1.15 ± 43.00 b	2.18 ± 41.66 b	T1
0.33 ± 48.33 a	1.76 ± 52.66 a	T2
0.57 ± 39.00 bc	0.88 ± 39.66 b	T3
2.84 ± 37.66 bc	2.33 ± 41.66 b	T4
2.90 ± 34.00 c	2.08 ± 41.00 b	T5
1.73 ± 39.00 bc	2.60 ± 44.33 b	T6

* Different letters within a single column indicate significant differences ($p \leq 0.05$) between treatments.

Gastric ulcer arises from the erosion of the stomach's inner surface, primarily due to an imbalance between harmful substances (hydrochloric acid, *H. pylori*, and free radicals) and protective mechanisms. In gastric ulcers, it is crucial to rectify the cellular damage to the gastric mucosa and sustain the oxidant/antioxidant equilibrium. Goat milk provides antioxidant characteristics, nutritional benefits, enhanced digestion, and exhibits low allergenicity, along with dairy processing advantages (13). The current investigation demonstrated a decrease in PCV, Hb, and RBC, alongside an increase in platelets in peptic ulcer cases, likely attributable to bleeding and heightened blood loss during infection. Additionally, the use of goat milk enhances and treats infections. Moreover, the synergistic impact of bromelain. The current study concurs with (14), which demonstrates the effects of bromelain on hemoglobin (HB), red blood cells (RBC), and indices in Wistar rats. The results indicated a substantial increase ($p < 0.05$) in the overall values of Hb and RBC content in the experimental animals compared to the control group. The judicious administration of pineapple juice containing bromelain has demonstrated benefits, including a rise in hemoglobin levels (Hb), enhanced erythropoiesis, and immune system support, as seen by these results. A notable reduction in hemoglobin concentration was seen in group B relative to the control group. This contrasts with the findings published by (15). The antibacterial properties of goat milk are attributed to elevated amounts of Lactoferrin and lysozyme activity. Alf  rez MJ et al. (2001) (16) demonstrated that goat's milk alkalizes the digestive tract and raises the pH level in the bloodstream, hence avoiding stomach ulcers(16). Goat milk possesses a high biological value and nutritional qualities due to its improved digestion and dietary properties, characterized by smaller diameter fat globules. Besides its mineral and vitamin composition, it possesses a chemical structure that encompasses high-quality proteins and essential fatty acids (17, 18). Moreover, the advantageous medium-chain fatty acids present in raw goat's milk, including capric and caprylic acids, contribute to combating infections. Raw goat's milk is rich in selenium, a vital element known for its immune-boosting and antioxidant qualities. Adults with gastrointestinal issues and ulcers may find relief from soft curd derived from goat milk (17, 19). The robust buffering capacity of goat milk seems to be beneficial in the treatment of gastric ulcers (20). Nonetheless, increases in ALP, AST, and ALT are typically a consequence of tissue injury. This occurs because such damage causes the enzymes to seep from their intracellular reservoirs into the plasma. ALT is predominantly located in the liver, but AST is also present in the heart, skeletal muscle, and liver in almost equal proportions (21). Substantial elevations in transaminases are typically associated with liver conditions such as toxic hepatitis, acute liver necrosis, and hepatic cirrhosis. Oyesola et al. demonstrated a reduction in ALT and an elevation in AST at low concentrations of Bromelain(14).

4. CONCLUSION

This study concluded the protective effect of goat milk on rats infected with peptic ulcer and synergical combination of both bromelain and goat milk were more effective than alone. This study also concluded increase PCV, Hb, RBC, and decrease GOT, and GPT in peptic ulcer.

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