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ORIGINAL ARTICLE

Effect of Camel milk (*Camelus dromedaries*) on Milk induced Leukocytosis and Eosinophilia in the management of Asthma

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ABSTRACT

To study the effect of camel milk (Camelus dromedaries) in management of asthma. Camel milk at an oral dose of 33 ml/kg body weight was evaluated for asthma using milk induced leukocytosis and eosinophilia in mice.Oral administration of camel milk (33ml/kg body weight) showed a significant inhibition of milk induced leukocytosis and eosinophilia in mice when compared with the control group. It can be concluded that camel milk (Camelus dromedaries) may be used in the management of asthma.

Keywords: *Camel,Milk, Asthma, Leukocytosis, Eosinophilia, Dexamethasone.*

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INTRODUCTION

In most of Arab countries and in other areas of the world, camel milk (*Camelus dromedaries*) is used as a nourishing drink and is considered as an essential part of the everyday diet. Camel milk is widely consumed in Saudi Arabia both as fresh raw milk and as soured milk. Saudi Arabia is rated the second world's biggest camel milk producer after Somalia, with a yearly production of 89,000 tonnes [36].Camel milk is slightly saltier than cows' milk, three times as rich in Vitamin C and is known to be rich in iron, unsaturated fatty acids and B vitamins [3].Moreover, the nonprotein-bound amino acids in camel milk are easily digested by microorganisms; therefore, camel milk has a higher metabolic activity when used as a starter culture preparation[34].The interest and scope for research in the field of camel milk has significantly widened in recent years. Camel's milk is different from other ruminant milk and is an excellent source of well-balanced nutrients. It affects the metabolic responses and influences the nourishment of specific organs and resistance to several diseases. It is high in protein, minerals, vitamins, insulin and low in cholesterol [37]. It is also rich in essential polyunsaturated fatty acids (linoleic acid) required for human nutrition. Gorban and Izzeldin, [14]·Cardoso et al.[9] proved that, camel milk can be consumed by lactose-intolerant peoples due to its non-allergic properties. Moreover, camel milk exhibits numerous biological activities such as antihypertensive, antithrombotic, antioxidative, antimicrobial, and immune enhancing effects [11, 21, 28]. Asthma is a chronic inflammatory disorder of respiratory system, which is characterized by narrowing of airways hyper-responsiveness and changes in the levels of mast cells, cytokines, lymphocytes and other related inflammatory cell products [6].Currently, wide ranges of anti-asthmatic drugs are available for the treatment of asthma. However, these all are limited to short symptomatic relief and causes complex side effects. These adverse effects of synthetic drugs prompting a switch over to traditional complementary and alternative medicine [15].Now days, most of the scientists around the world look towards the camel milk and the researches upon it became more due to its

enormous therapeutic activities in management of different health conditions as it is used traditionally to treat jaundice, splenic problems, tuberculosis, diabetes and asthma [1, 21, 27]. Up to this moment, there is no scientific literature that shows the exact role of camel milk therapeutic effect to combat asthma. In the present study, the antiasthmatic activity of camel milk was studied using milk induced leukocytosis and eosinophilia in mice.

MATERIALS AND METHODS

Animals

A total of 30 sexually mature 12-week-old male Swiss albino mice, each of which weighed between 25and 30 g, were obtained from the Animal House of the College of Medicine at Aljouf University. The study protocol was approved by the Animal Ethics Committee of Aljouf University in accordance with the principles of Declaration of Helsinki. All of the animals were allowed to acclimate to the metal cages inside a well-ventilated room for 2 weeks prior to experimentation [12]. The animals were maintained under standard laboratory conditions (23°C, 60–70%relative humidity and a 12-hours light/dark cycle), fed a diet of standard commercial pellets and given water *ad libitum*.

Materials

Fresh camel milk was obtained commercially from a well known healthy camel farm. All other chemicals and reagents used in this experiment were research analytical grade from sigma USA.

Milkinduced leukocytosis and eosinophilia

Mice were divided into three groups with ten in each group. Blood samples were collected from retro-orbital plexus. Group I served as a control and received 1% Tween-80 solution, groups II received camel milk 33 ml /kg body weight orally[29]; group III received dexamethasone (50 mg/kg body weighti.p.). All the groups were injected 4 mL/kg body weights.c.of boiled cooled milk 30 min after treatments. Total leukocyte count was done in each group before administration of test compound and 24 hours after milk injection. Blood was sucked in WBC pipette up to mark and further diluted with WBC diluting fluid. Pipette was shaken for few seconds and kept aside for 5 min. Neubaur's chamber was charged with above fluid and total leukocyte count was done [35].In addition, eosinophil count was done in each group before administration of test compound and 24 hours after milk injection (boiled and cooled). Blood was suckedin WBC pipette up to mark and further diluted with eosin solution. The eosin solution facilitates destruction of all corpuscles except eosinophil. Pipette was shaken for few seconds and kept aside for 5 min. Neubaur's chamber was charged with above fluid and eosinophil count was done. Difference in total leukocytes and eosinophil count before and 24 hours after milk injection was calculated [10].

Statistical analysis

The data was expressed as mean ± standard error (SE) and they were analyzed by using (IBM SPSS Statistics Version 21 Software for Windows) for statistical significance using one-way ANOVA and Tukey HSD for Post Hoc Multiple Comparisons. $P<0.05$ was considered as statistically significant.

RESULTS

Effect of camel milk on milk induced leucocytosis in mice.

Table-1 and figure-1 show a maximum increase in the difference of leukocytes count (4040.2±86.37) in control group 24 hours after administration of milk (4 mL/kg, s.c). The group of mice pretreated with camel milk 33 ml/kg orally(2235.6 ± 55.11) show a statistically significant ($P< 0.001$)inhibition of milk induced leucocytosis 24 hours after treatment when compared with the control group. Also, the group of mice pretreated with i.p. dexamethasone 50 mg/kg (1655.2 ± 28.69) show a statistically significant($P<0.001$)inhibition of milk induced leucocytosis 24 hours after treatment when compared with the control group.

Effect of camel milk on milk induced eosinophilia in mice

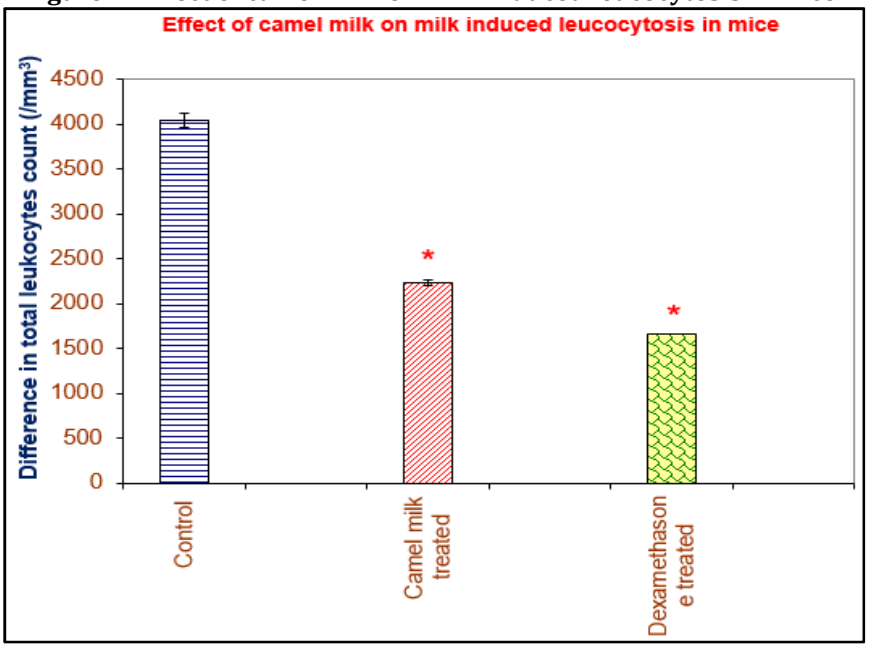
Table-2 and figure-2 show a maximum increase in the difference of eosinophil count (256.6 ± 9.64) in control group 24 hours after administration of milk (4 mL/kg, s.c). The group of mice pretreated with camel milk 33 ml/kg orally(118.8 ± 4.61) show a statistically significant ($P< 0.001$)inhibition of milk induced eosinophilia 24 hours after treatment when compared with the control group. Also, the group of mice pretreated with i.p. dexamethasone 50 mg/kg(86.6 ± 1.74) show a statistically significant($P<0.001$)inhibition of milk induced eosinophilia 24 hours after treatment when compared with the control group.

Table-1: Effect of camel milk on milk induced leucocytosis in mice.

Groups	Difference in Leukocyte count (/mm³)between that before and 24 hours after milk injection
Control group (1% Tween-80 solution and s.c. milk 4 mL/kg)	**4040.2 ± 86.37**
Camel milk treated group (oral camel milk 33 ml/kg and s.c. milk 4 mL/kg)	**2235.6 ± 55.11***
Dexamethasone treated group (i.p. dexamethasone 50 mg/kg and s.c. milk 4 mL/kg)	**1655.2 ± 28.69***

Data represent Mean ± SE * P< 0.001 compared with control group (1% tween-80 solution and s.c. milk 4 ml/kg)

Figure-1: Effect of camel milk on milk induced leucocytosis in mice.



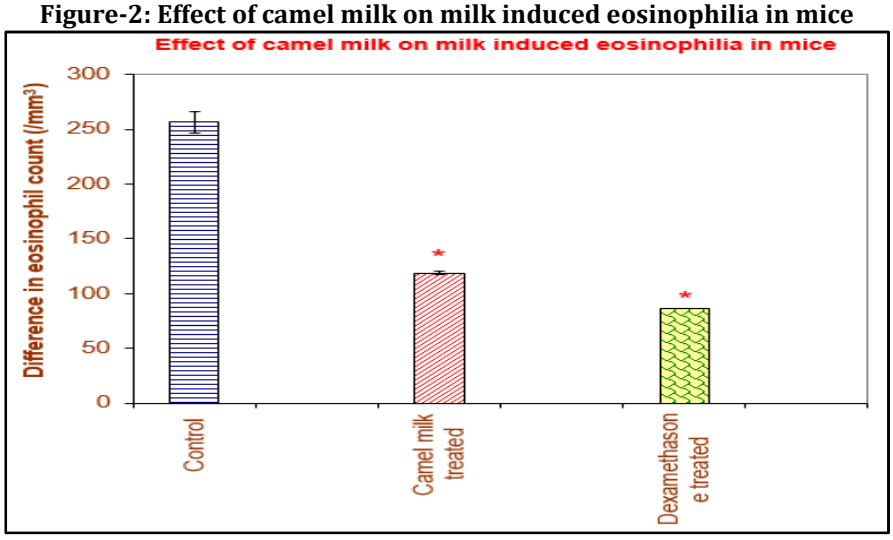
Data represent Mean ± SE. * P< 0.001 compared with control group (1% tween-80 solution and s.c. milk 4 ml/kg).

Table-2: Effect of camel milk on milk induced Eosinophilia in mice

Groups	Difference in eosinophil count (/mm³)between that before and 24 hours after milk injection
Control group (1% Tween-80 solution and s.c. milk 4 mL/kg)	**256.6 ± 9.64**
Camel milk treated group (oral camel milk 33 ml/kg and s.c. milk 4 mL/kg)	**118.8 ± 4.61***
Dexamethasone treated group (i.p. dexamethasone 50 mg/kg and s.c. milk 4 mL/kg)	**86.6 ± 1.74***

Data represent Mean ± SE. * P< 0.001 compared with control group (1% tween-80 solution and s.c. milk 4 ml/kg)

Figure-2: Effect of camel milk on milk induced eosinophilia in mice



Data represent Mean ± SE. * P< 0.001 compared with control group (1% tween-80 solution and s.c. milk 4 ml/kg)

DISCUSSION

In the present study, camel milk at a dose of (33 ml/kg, orally) was evaluated for management of asthma using milk induced leukocytosis and eosinophilia in mice. Physical and chemical stressors such as trauma, polluted air exposure, radiation etc. has been reported to concurrently produce immunodeficiency and oxidative stress. Suppression of immunity takes place due to exposure to polluted air and leads to respiratory diseases. Reactive nitrogen and oxygen species damages airways and play a role in pathophysiology of asthma. So, a drug having antistress activity induces a state of non-specific increased resistance against a variety of stress [18]. Asthma involves various types of mediator in pathology. It was demonstrated that parental administration of milk produces a marked increase in the leukocytes and eosinophils count after 24 hours of its administration [5, 32] and this stressful condition can be made normalized by administration of an antistress or adaptogenic drug [33]. Furthermore, leukocytes during asthmatic inflammation release the inflammatory mediators like cytokines, histamine, and major basic protein, which promote the ongoing of inflammation [7]. The infiltration of leukocytes potentiates the inflammatory process by the release of reactive oxygen species into the surrounding tissue, resulting in increased oxidative stress [25] and associated with many pathogenic features of asthma [24]. In the present study, we observed that leukocyte count was decreased in mice pretreated with camel milk at a dose of (33 ml/kg, orally) significantly as compared to control group. Result suggests that camel milk decreases milk induced leukocyte count by normalizing oxidative stress. The antioxidant activity of camel milk is mainly due to the presence of high concentrations of vitamins A, B_2, C and E and also it is very rich in magnesium and other trace elements, zinc and selenium [13, 17, 18, 22, 37, and 38]. Also, the magnesium which is present in high concentration in camel milk protects the cell against damage from oxyradicals and assists in the absorption and metabolism of vitamins B,C and E, which play a big role in cell protection from free radicals by functioning as antioxidants [4]. Moreover, the high mineral content in camel milk (sodium, potassium, iron, zinc, copper and magnesium) as well as the high vitamin C content act as antioxidants, thereby removing free radicals, which may provide a stress free situation to the mice [2]. Eosinophils are thought to mediate inflammatory and cytotoxic events associated with allergic disorders, including bronchial asthma, rhinitis and urticaria. The eosinophilic response has been identified as a key alteration in the pathogenesis of asthma and other allergic diseases [23]. Most of the asthmatic conditions including allergic and non allergic asthmatic have the feature of bronchial eosinophilia [31]. An abnormal increase in peripheral eosinophil to more than 4% of total leukocyte count is termed as eosinophilia. In asthmatic patient there is an increase in eosinophil count and mucus hypersecretion and airway hyperreactivity were stimulated [26]. The type-I hypersensitivity reaction leads to the development of edema, vascular dilation and eosinophilic infiltration [9]. Eosinophils infiltrating the airway also have an effect on mucus secretion by epithelial goblet cells [31].The mediators secreted by eosinophilssuch as tumor necrosis factor, eosinophil-derived neurotoxin, eosinophil cationic protein, and prostaglandin are responsible for shedding of epithelium, bronchoconstriction and promotion of inflammation in respiratory tract [8]. Also, eosinophils release toxic basic proteins and lipid mediators such as cysteinylleukotrienes that cause bronchial epithelial damage and airflow obstruction. Eosinophil-selective cytokines and chemokines including interleukin (IL)-5, eotaxin and RANTES may

represent targets for novel asthma therapies [30]. Subcutaneous administration of boiled and cooled milk causes eosinophil degranulation leading to the allergic inflammation such as cow's milk allergy conditions [16]. In our study, it was observed that mice pretreated with camel milk at a dose of (33 ml/kg, orally) show a significant decrease in milk induced eosinophil count as compared with the control. This probably indicates the camel milk may helps to reduce type I hypersensitivity in asthma. This is supported by Cardoso et al.[9] who proved that, camel milk can be consumed by lactose-intolerant people due to its non-allergic properties.

CONCLUSION

This is the first research study to screen the antiallergic activity of the camel milk against milk induced leucocytosis and eosinophilia in mice, whereas, the traditional medicine use of camel milk as a remedy for asthma in this region. Our studies results conclude that camel milk has antiasthmatic activity against milk induced leucocytosis and eosinophilia. Further working is going on various asthmatic models to evaluate its efficacy in the management of asthma.

COMPETING INTERESTS

The authors have declared that no competing interest exists.

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