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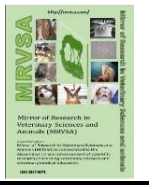

The effect of milk of the camel on some blood and liver parameters in formaldehyde induced arthritis in rats

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Abstract
This study was designed to evaluate the effects of camel's milk on some blood and liver parameters of formaldehyde induced arthritis rats. Forty-two adult male rats were used in two experiments. The preliminary study was done to induce arthritis and to evaluate the changing in blood and liver enzyme parameters. Twelve adult male rats were divide into two groups. All animals in group 1(G1/ control) and group (G2) were injected at the Plantar fascia of the left foot with 0.1 ml physiological saline and 0.1 ml formaldehyde twice (1st and 3rd day of the experiment) respectively. The experiment study was done to evaluate the anti-arthritic nature of camel's milk against the formaldehyde induced arthritis in rats and its effect on some blood and liver parameters. This experiment included 30 adult male rats and divided into five groups (G3, G4, G5, G6, and G7) each with 6 rats and received different treatment according to the design of the experiment that explained in the methods. The results of this study revealed significant elevation ($p< 0.05$) in the Leukocyte Count (WBC) especially in the neutrophil and monocyte and in the level of (AST, ALT, ALP). There were also significant decrease ($p< 0.05$) in the percentage of lymphocytes and monocyte in formaldehyde induced arthritis rats, when compare with the control group. The treated groups G6 in the second experiment revealed significant decrease ($p< 0.05$) in the in the WBC especially in the neutrophil and monocyte and in the level of (AST, ALT, ALP). Moreover, there was significant elevation ($P< 0.05$) in the percentage of lymphocyte. In conclusion, the results of this study revealed anti-arthritic activity of camel's milk against the formaldehyde induced arthritis in rats.

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Introduction

Although camels live in drought areas, it can produce an adequate amount of milk. The camel's milk has an importance for the young camel, and also for man, who drinks this milk. Data concerning the composition of milk vary greatly. The inherited capabilities of the animals are the important factors that attribute to the variation in the milk composition. In addition, the stage of lactation, age, and the number of calving also play a role. The feed and water quantity and quality play also special significance to the quality of the produced milk. Milk plays a vital role in man maturation, since it represents an essential source for different kinds of food, milk has been suggested as a nutrition system, in order to treat different kinds of diseases (*Kergoat et al., 1992*). Camel milk considered as rich source for vitamin and different kinds of metals. It characterizes by it low level of cholesterol and high concentration of insulin (Agrawal *et al.,* 2002). It has a medical activity against germs and viruses (El-ouardy *et al.,* 2011) and consists of high concentration of lactoferrin (Yagil *et al.,* 1994). In addition, it has the ability to inhibit the growth of *Salmonella, Brucella, Mycobacterium tuberculosis* and *Escherichia coli.* The milk of the camel has different distinctive features that make most people in the Arab world to use it for treat different kinds of diseases such as modulation of the immune system, babies suffering from malnutrition, liver disease especially the Jaundice, diabetes (Farah, 1993). The effectiveness of camel's milk against diseases like Brucellosis, Tuberculosis and Breast cancer and some kinds of immune diseases comes from that, this milk contains antimicrobials agents that destroys different kinds of germs. It is also used for treating some kind of spleen diseases, Asthma, Anemia and others (EL- Sayed *et al.*, 1992).

Inflammation is the response of the mammals living tissues against different kinds of injuries. In fact, it is the reaction of the defense part of the body in order to eliminate or stopping the causes of the disease. Arthritis is a group of diseases that affected the joints, tissues, synovial fluid and cartilage. Arthritis is also considered as kind of chronic disease, which spread all over the world. This disease consists of more than one hundred kind (Osteoarthritis, rheumatoid arthritis, septic Arthritis, gout, Juvenile Idiopathic Arthritis, Ankylosing Spondylitis and psoriatic arthritis). These kinds of disease have symptoms prevalent such as sclerosis around the joint, swelling in one or more than one of the joints, pains, redness, fever in the joint and not able to move naturally (Hafstrom *et al.,* 2001).

There is a very strong connection between blood and many rheumatoid diseases. Arthritis is one of important diseases that it has a very strong effect on some blood parameters. Leukocyte is considered as one of the main component of the immune system of the blood. Previous research reported that total leukocyte count is increased considerably in arthritis patients, the presence of (WBC) in great number is a sign of infection or inflammatory diseases (Jaijesh et *al.,* 2008; Norberg *et al.,* 2005). Review of literature revealed scarce information regarding the anti-arthritic effect of camel's milk. So, this study was designed to evaluate the activity of camel's milk on the total and differential leukocyte count and also the level of liver enzymes (ALT, AST, ALP) in the formaldehyde induced arthritis rats.

Materials & Methods

Animals

Forty-two, 10- 12 weeks old male rats (*Rattus norvegicus*), about (200-275) gm in weight were used in this study. Rats were kept under suitable environmental conditions and feed with standard laboratory animal food. This research study was approved by the research and animal ethical committees/ Biology Department / College of Science / University of Thi-Qar

Camel milk

The first Camel's milk samples were collected from a herd of camels in Al-Salman region about 160 km south of Samawa City/ Muthanna governorate. Hand milking was used to collect milk samples from the camels early morning. All samples were collected from healthy camels and neither suffer from mastitis nor received any kind of antibiotics. The samples were collected in sterile screw bottles and kept in cool box until transport to the laboratory.

Induction of arthritis

Arthritis in rats has been induced by using formaldehyde (HCHO), the induction has been done by injection 0.1 ml of 2% formaldehyde in the plantar of the left foot of the animals during the first and the third day of the experiment (Tirkey and Tiwari, 2012). This kind of induction of arthritis is a kind of chronic inflammation and its changes considerd as the same changes that take place in rheumatic arthritis that happens in human beings **(**Okoli *et al.,* 2008**;** Greenwald, 1991).

Experimental design

The current study consists of two experiments:

Preliminary study

Twenty-four rats were divided into two groups each with 12 animals and treated as follow:
1- (G1): Animals were injected twice with 0.1 ml of normal saline at the plantar fascia of the left foot at the first and third day of the experiment. This group was acted as affected control group.
2- (G2): Animals were injected twice with 0.1 ml of 2% formaldehyde solution at the plantar fascia of the left foot, at the first and third day of the experiment. This group was not treated and considered as non-treated control group. The preliminary study was done to investigate the blood and liver parameters. Blood samples with and without anticoagulant were collected from both groups after ten days and send to the laboratory for further investigation.

Experimental study

Thirty animals were used in this experiment. The animals were divided into 5 groups each with 6 animals and treated as follow:

1- (G3/ negative control group): Animals were injected twice with 0.1 ml f normal saline in the plantar fascia of the left foot, at the first and third day of the experiment and were left to the end of the experiment

2- (G4/ positive control group): Animals were drench orally 1 ml/ day milk of camel for fourteen days starting from the eleventh day until the twenty fourth day of the experiment.

3- (G5/ affected control group): Animals were injected twice with 0.1 ml of 2% formaldehyde solution in the plantar fascia of the left foot, at the first and third day of the experiment and left to the end of the experiment.

4- (G6):- Animals were injected twice with 0.1 ml of 2% formaldehyde solution in the plantar fascia of the left foot, at the first and third day of the experiment. Animals were drench orally 1 ml/ day milk of camel for fourteen days starting from the eleventh day until the twenty fourth day of the experiment.

5- (G7): Animals were injected twice with 0.1 ml of 2% formaldehyde solution in the plantar fascia of the left foot, at the first and third day of the experiment. Animals were drench orally 1 ml/ day milk of camel for seven days starting from the eleventh day by day until the twenty fourth day of the experiment.

Collection of blood samples

Blood samples were collected directly from the heart from all experimental animals (G3, G4, G5, G6, G7) after the twenty fourth day of the experiment. Samples were kept with anticoagulant and without anticoagulant tubes for the following test:

1- Total and differential leukocyte counts using Hematological analyzer according to (Brown, 1976).

3- Serum samples were separated from blood samples without anticoagulant by centrifugation. Serum samples were used to estimate ALT, AST and ALP enzyme using colorimetric method according (Reitmanand Frankel, 1957).

Statistical Analysis

SPSS version 14 was used to analyze the collecting data. The one-way analysis of variance (ANOVA) was used to determine significant differences between experimental groups to calculate the L.S.D. in the level of probability ($P<0.05$).

Results

The results of the preliminary study revealed significantly increase ($p<0.05$) in the total leukocyte (WBC) and the percentages of the neutrophils, monocytes in (G2) in comparison with (G1) (Table.1). The results also showed that there were significantly decreased ($p<0.05$) in the percentages of the lymphocytes in (G2) in comparison with to the control. The results of the experimental study revealed significant decreased ($p<0.05$) in the total leukocyte and the percentages of neutrophil and monocyte in (G6), however, non-significant decreased was observed in (G7) in compare to (G5) (Table.2). Moreover,

there was a significant increase ($p < 0.05$ (in the percentages of lymphocyte in G4, G6 and G7, in addition to the significant decrease in the percentages of neutrophil and monocyte in G4, G6, G7 in compare to G5 (Table.2). The results of this study revealed significant elevation ($P<0.05$) in the level of AST, ALT, ALP enzyme in G2 animals in compare to G1 animals (Table.3).

The results of estimation of the AST, ALT, ALP enzyme showed significant decrease ($P<0.05$) in G6 animals in compare with G5. However, non- significance difference was appeared in the level AST and ALP in the in G7 in compare with G5. Moreover, significantly difference were observed in AST, ALT, ALP in G6 in compare to G3 and G4. A decrease in the level of ALT in G7 as compare with G5 was also observed (Table.4).

Table (1) shows the effect of arthritis on the total number and the differential Count of white blood cells for the laboratory male rats

Groups	White blood cells / Mean ± standard error			
	WBCX10^3/uL	NEU %	MON%	LYM%
G1	7.33 ±3.74 b	10.5 ±0.8 b	2.19 ±0.4 b	86.11 ±5.41 b
G2	16.03 ±8.62 a	28.22 ±1.96 a	3.0 ± 0.2 a	67.38 ±9.74 a
LSD	8.55	1.7	0.398	10.14

*Different letters refer that there is incorporeal differences among groups ($P<0.05$).

Table. 2: shows the effect of camel milk on the total and differential leukocyte count in the experimental animals.

Groups	White blood cells / average ± standard error			
	WBCX10^3/uL	NEU %	MON %	LYM%
G3	7.42 ± 0.69 b	11.6 ± 0.97 b	1.34 ± 0.28 b	85.36 ± 5.46 a
G4	10.66 ±2.49 b	18.9 ± 0.58 b	2.64 ± 0.4 b	76.56 ± 6.56 a
G5	19.68 ±3.90 a	24.0 ± 0.55 a	4.3 ± 0.6 a	69.98 ± 2.01 b
G6	14.43 ±3.29 b	15.8 ± 0.3 b	2.9 ± 0.38b	79.71 ± 5.19a
G7	19.11 ±4.60a	12.1 ± 0.2b	1.4 ± 0.86b	85.0 ± 4.11a
LSD	4.011	0.68	1.05	5.847

*Different letters refer that there are significant differences between groups.
*Similar letters refer to similarity in significant differences.

Table. 3: Shows the effect of induced arthritis on the level of liver enzyme (AST, ALT, ALP) in the experimental animals.

Group	Liver enzymes / average ± standard error		
	AST (U/L)	ALT (U/L)	ALP (U/L)
G1	127.66 ±5.85 b	134.83 ±18.07 b	70.66 ±3.66 b
G2	171.16 ±13.40 a	168.5 ±8.31 a	332.16 ±176.81a
LSD	13.32	18.09	160.9

*Different letters refer that there is significant differences between groups (P<0.05).

Table. 4: Shows the effect of camel milk on liver enzyme (AST, ALT, ALP) in the arthritis induced experimental animals.

Groups	Liver enzymes / average ± standard error		
	AST (U/L)	ALT (U/L)	ALP (U/L)
G3	136.83± 6.94 b	132.33± 8.33 b	79.66± 5.0 b
G4	143.83± 39.09 b	130.83± 21.25b	88.33± 7.52 b
G5	189.33± 3.32 a	157.66± 3.23 a	579.0±53.58 a
G6	157.5± 26.39 b	131.16± 23.41 b	126.16±64.91 b
G7	186.33± 15.37 a	138.33± 3.98 b	572.0±119.3 a
LSD	26.7	17.61	77.8

*Different letters refer that there is incorporeal differences among groups.
*Similar letters refer to similarity in incorporeal differences.

Discussion

It is well known that the immunoglobulins (Igs) are large long and short-chained domains, having difficulties reaching and penetrating antigens. Researcher found that camel immunoglobulins have no short chains and small and they are active against antigens. The camel's immunoglobulins pass into the milk and so are available for fighting autoimmune diseases (Yagil, 2004).
The effect of drenching camel milk in reducing the effect of experimental induce arthritis on the total and differential leukocyte count and also on the levels of liver enzyme in rats were investigate in this study.
There were significant increase in total leukocytes count in G2 animals. Leukocytes are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders. The elevation was occurred due to the involvement of WBC in the arthritis that induced experimental in these animals. (Hassan and Jassim, 2011; VanderBorght *et al.*, 2001).
The results of this study revealed that there were increased in the percentages of neutrophil and decreased in the lymphocytes. This results is compatible with previous

studies that reported the changes in the percentages of differential leukocytes count with neutrophilia, lymphopenia in the in vivo induced arthritis. They reported that these changes occurred due to mobilised of the leukocytes fromthe blood to the inflammatory lesion, and exudate leucocytes show a markedly increased phagocytosis and metabolic activity (Kumar *et al.,* 2004; Siegal, 1980).

Formaldehyde was used in this study to induced arthritis. The results of this study approved the animal models of arthritis using formaldehyde, which can be used in the preclinical studies in the evaluation of anti-arthritic drugs such as milk of the camel. These animals' models were used in this study to investigate the anti-arthritic effects of camel milk. Animals injected with formaldehyde showed severe arthritis accompanied with lymphocytopenia and neutrophilia, which occurred due to increase in the cytokines and chemokines activities that attract the inflammatory cell to invade the affected areas (Karouzakis *et al.,* 2006; Kassab *et al.,* 1992).

The results of this study revealed a decrease in the total leukocyte count in all experimental animals that received camel milk. The decrease of the total leukocyte count might be occurred due to the effect of the milk camels components (camel's immunoglobulins) that pass into the milk and so are available for combating diseases (Agrawal *et al.,* 2005;. Carmen, 2002).

The lymphocytosis was observed in the experimental animals treated by camel milk. This result is compatible with studies that reported previously (Karakilcik et al., 2005; Fetrow and Avila, 2000). These studies approved that milk of the camel has high level of vitamins C& E. These vitamins work as antioxidant factors to prevent the harmful effects of the free radicals products such as peroxides that destroy the cell membrane. In addition, it work to protect the lymphatic cells genetic material (DNA) from the oxidization activities (Karakilcik *et al.,* 2005; Fetrow and Avila, 2000; Shlig, 2009; Coles, 1986).

There was also significant increase in leukocytes count in G7 animals, which might be occurred due to the reduction in concentration of camel's immunoglobulins, because these animals were treated day between days (each two days).

The effects of camel milk on the level of liver enzymes (ALP, ALT, AST) in the experimental animals revealed the clinical signs of induced arthritis and treated with camel milk. There were a significant increase in the levels of liver enzymes in the formaldehyde induced arthritis animals due to chemical irritation that led to decreases the level of cellular Gluathione (GSH). It is well known that glutathione is the body's own master antioxidant the preventing damage to important cellular components caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides and heavy metals (Al-Fartosi *et al.,* 2011; Stempel and Miller, 1977).

The elevation in the ALP enzyme in the untreated animal with arthritis was observed in this study. This result is agreement with previous studies that reported the relation between the performance of liver cells and arthritis. The elevation in ALP activity enzyme might be occurred in case of bones, liver, kidney diseases, in addition, there are 11 ALP analogues (Al-Fartosi *et al.,* 2011; Kaplan *et al.,* 2003; Gaw *et al.,* 1999).

The results of this study revealed a decrease in liver enzymes in experimental animals that suffered from in induced arthritis but treated with camel milk. This reduction might be resulted from the effect of camel milk that content a high concentration of minerals such as magnesium contained in the camel milk. Magnesium has also acted

like GSH enzyme. It protect the cells from the reactive oxygen species such as free radicals, peroxides, lipid peroxides and heavy metalsand its being necessary to produce Glauthione (Al-Fartosi *et al.,* 2011; Barbagallo *et al.,*1999).
In conclusion, this study revealed the anti-arthritic ability of camel milk to reduce the inflammatory reaction in experimentally animals that suffered from arthritis induced by formaldehyde.

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