



**ROLE OF CAMEL'S MILK WITH OR WITHOUT LEISHMANIA ANTIGEN AS IMMUNE IMPROVER AGAINST INFECTION OF *LEISHMANIA DONOVANI* IN MALE MICE**

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**ABSTRACT**

The present study aimed to evaluate the role of camel's milk with or without leishmania antigen As immune improver against infection of *Leishmania donovani* in male mice. 120 of mature male mice divided into five groups by 24 mice for each group. The first group was treated by 0.2 ml of normal saline for 15 days and non-injected parasite considered negative control, the second group was treated by 0.2 ml of normal saline for 15 days and injected with 0.2 ml of the parasite dose ( $1.2 \times 10^6$  parasite/ 0.2ml) considered a positive control, the third group treated with 0.2 ml of camel's milk, the fourth group injected by 0.2 ml of leishmania antigen only, the fifth group treated with 0.2 ml of camel's milk and injected with 0.2 ml of leishmania antigen. The third, fourth and fifth groups were injected with 0.2 ml dose of the parasite after treatment. Body weight was measured before and after the end of experiment. The each group dissected in two periods 45 days and 90days. The weights of liver and spleen was taken to a statement rates of infection in the organs. Blood samples collected to measure the level of malondialdehyde (MDA) and ceruloplasmin (Cp). The results in two turn 45 and 90 days showed a significant increases ( $P < 0.05$ ) in liver weight, length of spleen, ratio of spleen enlargement, MDA level and Cp level in second group compared with the first group. The third and fourth groups have shown a significant decrease ( $p < 0.05$ ) in liver weight, length of spleen, ratio of spleen enlargement, MDA level compared with the second group. The fifth group in two turn 45 days and 90 days, showed non a significant differences ( $p < 0.05$ ) in most of the above parameters compared with first group, and indicated a significant decrease ( $p < 0.05$ ) compared with the second, third and fourth groups.

**KEYWORDS:** Camel's milk, Leishmania antigen, *Leishmania donovani*, Male mice

**INTRODUCTION**

The leishmaniasis disease is a serious health problem invaded human societies since ancient times where it has become endemic in the Middle East and Central Asia, Africa South America and the European region, and epidemic increasingly as recorded every year about 1-1.5 million cases of cutaneous leishmaniasis and about 500,000 cases of visceral leishmaniasis. It has become endemic in 88 countries in the world (WHO, 2000). Leishmaniasis disease infected by single-celled haemoflagellate parasites of the leishmania, which belong to the family Trypanosomatidae and genus Kinetoplastida (Bsc, 2007; Van der Meide, 2008).

*Leishmania donovani* Causes Visceral leishmaniasis (black-fever) disease or so-called Kala-azar which is the most severe disease from other leishmaniasis symptoms appear after 2-8 months disease infected the internal organs such as the liver, spleen and bone marrow (Clem, 2010). The clinical symptoms of infection visceral leishmaniasis in humans is gradual or sudden rise in

temperature regularly or irregularly, weight loss, wasting and weakness continuously due to the imbalance in the intestine with the occurrence of diarrhea and vomiting in some cases (Sciaramella *et al.*, 1997).

The camel's milk is important nutrients for humans in many parts of the world, especially in the desert and semi-desert regions, and the remaining camels despite passing harsh conditions of high temperature and drought and the loss of water is capable of producing good quality of milk. The importance of camel's milk indicated by researchers, the study of Chemical composition and physical properties showed that camel's milk has a good quality of proteins effective preventive against bacteria and viruses such as Lactoferrin and Lysozyme Lactoperoxidase, which that will make it more distinction of cow's milk in terms of nutrients; (El-Gammal and Moussa, 2007; Hassan *et al.*, 2009; Mal and Pathak, 2010). There is a large medical uses for camel's milk, where it is used in the treatment of many diseases, high blood pressure and digestive disorders and other,

has been scientifically proven that camel's milk is used to treat many infectious diseases, whether bacterial such as Malta fever, tuberculosis or viral (Ani, 1997) as well as the great importance in the treatment of diabetes has proven effect where camel milk in reducing the level of sugar in the blood. (Kinani, 2010).

## MATERIALS AND METHODS

### Source of the parasite

It was obtained pure isolation and diagnosed of *Leishmania donovani* from the Department of Biology/ College of Science / University of Thi-Qar, Iraq .

### Source of camel's milk

Camel's milk samples were collected from different areas of Nassiriya city /Thi Qar province /Iraq by method of hand Milking and transfer immediately to the laboratory and keeping it in the refrigerator under the temperature of 2-5 m ° until use.

### Experimental design

120 of mature male mice (*Mus musculus*) of Balb /c strain aged (8-10 ). weeks and weighted (25- 35 )gm by divided into five groups (24 males for each group ) as following.

- 1- The first group was treated orally with of 0.2 ml of normal saline for 15 days (negative control).
- 2- The second group injected (I.P.) with 0.2 ml of the parasite dose and treated by 0.2 ml of normal saline for 15 days (positive control ) .
- 3- The third group treated orally with 0.2 ml of camel's milk for 15 days then injected (I.P.) with a 0.2 ml of the parasite.
- 4- The fourth group injected (I.P.) for ones with 0.2 ml of leishmania antigen, and after 21 days injected with 0.2 ml of the parasite.
- 5- The fifth group treated orally with 0.2ml camel's milk for 15 days and injected (I.P.) leishmania antigen and after 21 days its injected by parasite.

The animals in each group divided into two equal groups, the first group dissected after 45 days and second group 90 days to follow the development of infection.

### The present study included the following parameters

- 1-Changes in the liver weight (Stauber, 1966).
- 2-Changes in the length of the spleen (Stauber, 1966).
- 3- Enlarged spleen ratio = spleen weight (mg) \ body weight (g) ((Stauber, 1953).
4. Measurement of malondialdehyde (MDA) according to (Muslih *et al.*, 2002).
- 5- Measurement of ceruloplasmin (Cp) according to (Mendes *et al.*, 1977).

### Statistical analysis

Data was analyzed statistically using system Statistical Package for Social Science(SPSS) Version 17- ready using ANOVA analysis and extraction of the least significant difference LSD below the level of probability ( $p < 0.05$ ) .(Al- Baldawi, 2009).

## RESULTS

### Effect of camel's milk with or without leishmania antigen in liver weight of male mice infected with *Leishmania donovani*

The results of the present study in the infection period 45 days indicated a significant increase ( $p < 0.05$ ) in liver weight of male mice in the second group (positive control) compared with the first group (negative control) and other groups, Also the results indicated no significant differences ( $p < 0.05$ ) in liver weight between other groups when compared with each other(table 1).

The results of the present study in the infection period 90 days indicated a significant increase ( $p < 0.05$ ) in liver weight of male mice in the second group (positive control) compared with the first group (negative control), When the third and fourth groups indicated a significant increase ( $p < 0.05$ ) in the liver weight compared with the first group, but they indicated a significantly decreased ( $p < 0.05$ ) compared with the second group, the fifth group indicated no significant difference ( $p < 0.05$ ) compared with the first group, but indicated a significant decrease ( $p < 0.05$ ) compared with the second, third and fourth groups (table 1).

**Table (1): Effect of camel's milk with or without leishmania antigen in liver weight of male mice infected with *Leishmania donovani*.**

| Parameter         | Liver weight (gm)      |                        |
|-------------------|------------------------|------------------------|
|                   | 45 day                 | 90 day                 |
| Time of infection |                        |                        |
| Groups            |                        |                        |
| First group       | 1.46±0.26 <sup>b</sup> | 1.48±0.27 <sup>c</sup> |
| Second group      | 2.24±0.82 <sup>a</sup> | 2.38±0.69 <sup>a</sup> |
| Third group       | 1.75±0.5 <sup>b</sup>  | 1.91±0.60 <sup>b</sup> |
| Fourth group      | 1.79±0.33 <sup>b</sup> | 1.88±0.32 <sup>b</sup> |
| Fifth group       | 1.41±0.36 <sup>b</sup> | 1.42±0.14 <sup>c</sup> |
| LSD               | 0.34                   | 0.31                   |

- Values are means ±S.E.

-The different letters refer to a significant differences at ( $p \leq 0.05$ )

-The same letters refer to non significant differences at ( $p \leq 0.05$ ).

### Effect of camel's milk with or without leishmania antigen in the length of the spleen of male mice infected with *Leishmania donovani*

Table (2) showed in the period infection 45 days indicated a significant increase ( $p < 0.05$ ) in the length of the spleen in the second group (positive control) compared with the first group (negative control) and other groups, The third and fourth groups have a significant increase ( $p < 0.05$ ) in the length of the spleen compared with the first and the fifth group while indicated a significant decrease ( $p < 0.05$ ) compared with the second group but indicated no significant difference ( $p < 0.05$ ) compared with each other, the fifth group indicated no significant difference ( $p < 0.05$ ) compared with the first group, but showed a significant decrease ( $p < 0.05$ ) compared with the second, third and fourth groups(table 2).

The results of the present in the period infection 90 days indicated a significant increase ( $p < 0.05$ ) in the length of the spleen in the second group compared with the first group and other groups, The third and fourth groups indicated no a significantly difference ( $p < 0.05$ ) compared with the first group, but they have indicated a significant decrease ( $p < 0.05$ ) compared with the second group. The fifth group indicated no a significant difference ( $p < 0.05$ ) compared with the first group, but showed a significant decrease ( $p < 0.05$ ) compared with the second, third and fourth groups (table 2.).

**Table (2) Effect of camel's milk with or without leishmania antigen in the length of the spleen of male mice infected with *Leishmania donovani*.**

| Parameter                | The length of the spleen (cm) |                          |
|--------------------------|-------------------------------|--------------------------|
|                          | 45 day                        | 90 day                   |
| Time of infection Groups |                               |                          |
| First group              | 1.75±0.38 <sup>c</sup>        | 1.93±0.30 <sup>b,c</sup> |
| Second group             | 2.69±0.40 <sup>a</sup>        | 2.81±0.41 <sup>a</sup>   |
| Third group              | 2.17±0.42 <sup>b</sup>        | 2.45±0.30 <sup>b</sup>   |
| Fourth group             | 2.06±0.2 <sup>b</sup>         | 2.15±0.26 <sup>b</sup>   |
| Fifth group              | 1.84±0.41 <sup>c</sup>        | 1.97±0.42 <sup>c</sup>   |
| LSD                      | 0.26                          | 0.23                     |

-Values are means ±S.E. –

-The different letters refer to a significant differences at ( $p \leq 0.05$ )

-The same letters refer to non significant differences at ( $p \leq 0.05$ ).

**Effect of camel's milk with or without leishmania antigen in an ratio enlargement of spleen male mice infected with *Leishmania donovani***

The results of the present study in the period infection 45 days indicated a significant increase ( $p < 0.05$ ) in the ratio enlargement of spleen in the second group (positive control) compared with the first group (negative control), The third group indicated a significant increase ( $p < 0.05$ ) compared with the first group, but did not indicated no significant difference ( $p < 0.05$ ) compared with the second group, but fourth and fifth groups indicated a significant increase ( $p < 0.05$ ) when be compared with the first group, but they show a significant decrease ( $p < 0.05$ ) compared with the second and third groups, They also indicated no significant difference ( $p < 0.05$ ) compared with each other (table 3).

The result of the period infection 90 days indicated a significant increase ( $p < 0.05$ ) in an ratio enlargement of spleen in the second, third and fourth groups compared with the first group but these groups indicated no a significant difference ( $p < 0.05$ ) compared with each other . The fifth group indicated a significantly increase ( $p < 0.05$ ) compared with the first group, but indicated a significant decrease ( $p < 0.05$ ) compared with the second, third and fourth groups (table 3).

**Table (3): Effect of camel's milk with or without leishmania antigen in an enlarged spleen ratio of male mice infected with *Leishmania donovani***

| Parameter                | The ratio enlargement of the spleen (mg/gm) |                        |
|--------------------------|---|------------------------|
|                          | 45 day                                      | 90 day                 |
| Time of infection Groups |   |                        |
| First group              | 3.19±1.03 <sup>c</sup>                      | 3.85±1.08 <sup>c</sup> |
| Second group             | 6.22±0.86 <sup>a</sup>                      | 6.93±1.07 <sup>a</sup> |
| Third group              | 6.24±1.06 <sup>a</sup>                      | 6.15±0.88 <sup>a</sup> |
| Fourth group             | 4.88±0.86 <sup>b</sup>                      | 6.60±1.11 <sup>a</sup> |
| Fifth group              | 4.62±0.69 <sup>b</sup>                      | 5.06±0.96 <sup>b</sup> |
| LSD                      | 0.62  | 0.67                   |

-Values are means ±S.E.

-The different letters refer to a significant differences at ( $p \leq 0.05$ )

-The same letters refer to non significant differences at ( $p \leq 0.05$ ).

**Effect of camel's milk with or without antigen leishmaniasis in the level of malondialdehyde MDA of male mice infected with *Leishmania donovani*.**

The results of the present study in the period infection 45 days to malondialdehyde level indicated a significantly increase ( $p < 0.05$ ) in the second group compared with the first group, but it indicated no a significant differences ( $p < 0.05$ ) compared with the third and fourth groups, The third group indicated no significantly differs ( $p < 0.05$ ) with the first group when the fifth group indicated no significant difference ( $p < 0.05$ ) compared with the first group, The results of the period infection 90 days indicated a significant increase ( $p < 0.05$ ) in the second group compared with the first group, but indicated no significant differences ( $P < 0.05$ ) compared with the fourth group. The third and the fourth groups indicated no significantly difference ( $p < 0.05$ ) compared with the first group, while the fifth group indicated no significant difference ( $p < 0.05$ ) compared with the first group and the third and fourth groups but indicated significantly decrease ( $p < 0.05$ ) compared with the second group (table 4).

**Table (4): Effect of camel's milk with or without leishmaniasis antigen in the level of malondialdehyde MDA of male mice infected with *Leishmania donovani***

| Parameter                | The level of MDA (nmol/ml) |                          |
|--------------------------|----------------------------|--------------------------|
|                          | 45 day                     | 90 day                   |
| Time of infection Groups |                            |                          |
| First group              | 2.99±0.34 <sup>b</sup>     | 3.17±0.55 <sup>b,c</sup> |
| Second group             | 4.96±0.30 <sup>a</sup>     | 6.46±3.27 <sup>a</sup>   |
| Third group              | 3.97±0.66 <sup>ab</sup>    | 4.34±0.73 <sup>b</sup>   |
| Fourth group             | 4.82±2.10 <sup>a</sup>     | 5.79±2.00 <sup>ab</sup>  |
| Fifth group              | 3.05±0.29 <sup>b</sup>     | 3.34±0.50 <sup>b,c</sup> |
| LSD                      | 1.00                       | 1.75                     |

-Values are means ±S.E. –

-The different letters refer to a significant differences at ( $p \leq 0.05$ )

-The same letters refer to non significant differences at ( $p \leq 0.05$ ).

### Effect of camel's milk with or without leishmania antigen in ceruloplasmin level in male mice infected with *Leishmania donovani*

The results of the present study in the period infection 45 days indicated a significant increase ( $p < 0.05$ ) in the level of ceruloplasmin the second group compared with the first group, which indicated no significantly differ ( $p < 0.05$ ) compared with the third group and fourth group. The third and fourth groups indicated a significant increase ( $p < 0.05$ ) compared to with the first group, but they have a significantly decrease ( $p < 0.05$ ) compared with the second group, at the same time indicated no significantly difference ( $p < 0.05$ ) compared with each other, The fifth group indicated no significant differences ( $p < 0.05$ ) compared with the first group, but indicated significant decrease ( $p < 0.05$ ) compared with the second, third and fourth groups (table 5).

The results of the period infection 90 days indicated a significant increase ( $p < 0.05$ ) in the second group compared with the first group, but indicated no significant differences ( $p < 0.05$ ) compared with the third and fourth groups, The third group did not show a difference significantly ( $p < 0.05$ ) compared with the first group, while the fifth group indicated no significant difference ( $p < 0.05$ ) compared with the first group, but indicated a significant decrease ( $p < 0.05$ ) compared with the second group and fourth group (table 5).

**Table (5) Effect of camel's milk with or without leishmania antigen in ceruloplasmin level in male mice infected with *Leishmania***

| Parameter<br>Time of infection<br>Groups | The level of Cp (g/l)    |                         |
|--|--------------------------|-------------------------|
|  | 45 day                   | 90 day                  |
| First group                              | 1.74±0.48 <sup>b,c</sup> | 1.92±0.47 <sup>b</sup>  |
| Second group                             | 3.19±1.51 <sup>a</sup>   | 3.82±0.82 <sup>a</sup>  |
| Third group                              | 2.96±1.23 <sup>ab</sup>  | 2.72±1.56 <sup>ab</sup> |
| Fourth group                             | 2.13±0.84 <sup>b</sup>   | 3.68±1.75 <sup>a</sup>  |
| Fifth group                              | 1.83±0.56 <sup>b,c</sup> | 1.92±0.69 <sup>b</sup>  |
| LSD                                      | 0.99                     | 1.15                    |

#### *donovani*

-Values are means ±S.E. –

-The different letters refer to a significant differences at ( $p < 0.05$ )

-The same letters refer to non significant differences at ( $p < 0.05$ ).

#### DISCUSSION

The results indicated swelling in the liver and spleen in the second group in both periods (45day and 90day), and this swelling was less in the third and fourth groups. This result agree with Abreu-Silve *et al.* (2004) with swelling of liver and spleen of mice after 40 days of infection with *Leishmania amazonensis* as a results of increase of phagocytiz cells production. Also, the swelling of liver and spleen may be according to accumulation of parasites inside these organs (Stauber *et al.*, 1966; Rajesh and Latha, 2004). The fifth group

showed lower rate of this swelling which related with the role of camel's milk and leishmania antigen. The results agree with Al-Gizy (2014).

The results showed a significant increase ( $p < 0.05$ ) of MDA in the second group compared with first group, also there was a significant decrease ( $p < 0.05$ ) in the level of MDA in the third and fourth groups compared with the second group. The reduction of MDA level was attributed to the role of camel's milk against oxidation because its contains high level of vitamins, especially vitamin E and vitamin C which lead to inhibit of oxidation situation (Musamah *et al.*, 2002; Thephiniap *et al.*, 2007; Olayaki *et al.*, 2008).

The results showed a significant increase of ceruloplasmin in the second group as a result of infection especially in acute causes and presence of inflammatory antibodies (Osmand *et al.*, 1977; Rosales *et al.*, 2000). The decrease of ceruloplasmin in the groups treated with camel's milk related with the reduction of proteins activity associated with oxidation stress, also, the decline of Cp considered indicator for balance of free radicals with ions of iron and copper (Sirajwala *et al.*, 2007) this is consistent Al-Kinani(2010).

Leishmania antigen also play an important role in stimulating the phagocytic cells of macrophages in leishmaniasis disease ,the phagocytic cells responsible for killing the parasite cells and is also important as the location for the accommodation of these parasites within the body (Birnbaum and Craft, 2011), as well as leishmania antigen have role to raising the immune response against infection from during the process presentation of antigen, which play a very important role in the cellular response as each type of helper T cells patterns characteristic of units of different antigenic what distinguishes other styles (Cox, 1997), while dendritic cells stimulate the initial response of T cells by antigen presenting, therefore differ from function giant macrophages that are trying to eliminate the parasite (Flohe, 1997; Moll *et al.*, 2000).

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