

## CAMEL'S MILK AS AN IMMUNE IMPROVER AGAINST THE HYDATICYSTOSIS IN LABORATORY MICE

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### ABSTRACT

Hydatid cystosis is one of the most common diseases in the world, and had a zoonotic pattern, surgical intervention is one of the most important treatment attempts, despite the serious problems the patient experiences during surgery. The current study was aimed to use camel's milk as an immune improver against this disease in laboratory mice. Experimental study was conducted in the department of biology, College of Science, University of Thi-Qar, during the period from November 2015 till April 2016. So four groups of mice (*Mus musculus*) of Balb/c strain, age (6-8) weeks were used as follows. The first group was orally treated with 0.2 ml of physiological solution for one week (Negative control). The second group was orally treated with 0.2 ml of physiological solution for a week and then injected intraperitoneally with 0.2 ml of protoscolices of *Echinococcus granulosus* (3000 protoscolex / ml) (Positive control). The third group was orally treated with 0.2 ml of camel's milk for a week and then injected intraperitoneally with 0.2 ml of protoscolices (3000 protoscolex / ml). The fourth group was orally treated with 0.2 ml of camel's milk for two weeks and then injected intraperitoneally with 0.2 ml of protoscolices (3000 protoscolex / ml).

The results of the current study showed that the groups of animals treated with camel's milk for one week and two weeks had a decrease in the number and weights of secondary hydatid cysts, and the group treated with camel's milk for two weeks showed a significant decrease in the number and weights of cysts compared to the

positive control group. A significant reduction in weights and size ratio of liver and spleen was observed in the group treated with camel's milk for two weeks compared with control group .

It has been concluded that camel's milk has the ability to reduce the incidence of secondary hydatid cysts in infected mice and treated with camel's milk and the increased duration of treated with camel's milk significantly reduces the incidence of infection

## **INTRODUCTION**

Human cystic echinococcosis or hydatidosis is a zoonotic infection caused by the larval stage of cestode *Echinococcus granulosus* [1]. The disease is one of the most common diseases in various parts of the world and is endemic in the countries of the Middle East and parts of America, Australia, the Mediterranean, Central Asia and Central and Eastern Europe [2].

The final host of these parasites is Canine (Canidae) which is infected by the feeding of the hydatid cyst from the intermediate host as a domesticated animal [3], while the human is an accidental or incidental host and does not play a role in the continuation of the life cycle [4].

Children are more susceptible to infection with this disease especially those with close association with dogs [5]. In addition to the human health effects, this disease is still an endemic disease in Iraq and the economic impact which prompted many studies to investigate the means of treatment was surgical intervention is the most important method of treatment despite the serious problems experienced by the patient during surgery, the patient is not surgically qualified due to age, anesthesia or cyst in places where it is difficult for the surgeon to deal with it as in the brain cyst or in certain cases such as age or in the case of pregnancy or the patient has severe diseases such as heart disease, diabetes and hypertension [6]

The medicinal importance of camel's milk in the treatment of different diseases caused by fungi, parasitic worms and intestinal bacteria has been studied by many researchers and its chemical properties have been found that camel's milk contains a good quality of proteins that are effective against bacteria and viruses such as Lactoferrin, Lactoperoxidase, Lysozyme which makes it far superior to cow's milk in terms of

nutrients [7; 8; 9 ]. Several studies conducted in different areas in Iraq were used different materials such as medical plant to reduce the infectivity of infection and enhance the immune response , [ 10 ] studied the effect of volatile oil to *Cymbopogon citrates* with or without usage protoscolices antigen against infection in hydrated cysts of *Echinococcus granulosus*. [11] they studied the effect of the aqueous extract of the *Allium sativum* plant on the vitality of the protoscolices of *Echinococcus granulosus* .No one trialed to tested camel's milk therefore this study was aimed to use camel's milk as immune improver against the infection of hydatid disease in laboratory mice

## MATERIAL AND METHODS

### study design

Experimental study was conducted in department of biology , college of Science ,University of Thi-Qar, during the period from November 2015 till February 2016

### Source of hydatid cysts

The liver hydatid cysts of sheep were obtained from the Nasiriyah massacre and placed in a refrigerated case and transferred directly to the laboratory of the college of Science at Thi- Qar University within 10-30 minutes to conduct the isolation According to [12 ].

### Source of camel's milk

Milk was obtained from camel group in SiddhKhel area east of Thi-Qar up to 25 km. Milk samples were obtained by hand milking method. The milk was transferred directly to the laboratory and kept in the refrigerator under 2-5 ° C until use.

### Experimental design

Twenty eight of male mice (*Mus musculus*) of Balb /c strain age (6- 8) weeks and weighted (25-27)gm by divided into four groups (7 males for each group ) as following.:-

1-The first group was orally treated with 0.2 ml of physiological solution for one

week (negative control )

2-The second group was orally treated with 0.2 ml of physiological solution for a week and then injected intraperitoneally with 0.2 ml of protoscolices (3000 protoscolex / ml ) ( positive control ) (

3- The third group was orally treated with 0.2 ml of camel milk for a week and then injected intraperitoneally by 0.2 ml of protoscolices (3000 protoscolex / ml ) (

4- The fourth group was orally treated with 0.2 ml of camel milk for two week and then injected intraperitoneally by 0.2 ml of protoscolices (3000 protoscolex / ml).

On the day 90<sup>th</sup> of the treated with camel's milk and injections with protoscolices the animals were dissected. The growth or non-growth of the hydatid cyst was determined and their weights measured and determined changes in liver and spleen weights and determination of their enlarged ratio.

### **Statistical analysis**

SPSS program applied to find the Mean and SD with statistic significant by determine p value > 0.05 using 2-way ANOVA [13]

## **RESULT**

### **Examination of secondary hydatid cyst**

The results of the dissection of the groups of mice which treated with camel's milk and infected with the protoscolices of the *Echinococcus granulosus* and 90 days after the added It was noted that the second group ( Positive control group) and the presence of secondary hydatid cyst in the peritoneal cavity and distributed irregular and found hydatid cyst on the liver is not implanted in it has been the average number of 7 cyst of different sizes and a weight of 0.31 g the third group treated with camel milk for one week decreased the number of secondary hydatid cyst compared to the positive control group, the number of cysts in it to 4 and weight of 0.15 g. The fourth group treated with camel milk for two weeks the number of hydatid cyst decreased

significantly compared with the positive control group, where the number of cysts in it to 0.4 and a weight of 0.036 g . As shown in Table (1)

**Table (1) Changes in the numbers and weights of the hydatid cyst in study groups**

Group	Number of cyst (mean±SD) P (value)	Weight of cyst / gm(mean±SD) P (value)
Negative control	0±0	0±0
Positive control	7±1.58 0.000*	0.31±0.59 0.000*
Milk 1 week	1.4±0.54 0.096	0.15±0.051 0.002*
Milk 2 week	0.4±0.54 0.88	0.036±0.049 0.62

\* meaning statically significant comparing to the control P<0.05

**Changes in liver weight and its size ratio in treated mice and compared with positive and negative control**

According to result of Table (2) it is show that the fourth group treated with camel's milk for two weeks had the lowest weight in the liver and size ratio where the liver weight in this group was 1.64 gm and the size ratio 55.18 and the differences were significant between this group and the positive control group that recorded liver weight and size ratio 3.21 gm and 90.99 Respectively ,the fourth group did not record any significant differences from the negative control group, while the third group treated with camel milk for one week showed a decrease in weight and size ratio liver but less than in the fourth group.

**Table (2) Changes in liver weight and its size ratio in study groups**

Group	Liver weight /gm( mean±SD) P (value)	Liver size ratio( mean±SD) P (value)
Negative control	1.58±0.083	54.08±2.62
Positive control	3.21±0.318 0.003*	90.99±11.35 0.001*
Milk 1 week	1.94±0.114 0.04*	62.91±3.22 0.252
Milk 2 week	1.64±0.273 0.009*	55.18±7.83 0.99

\* meaning statically significant comparing to the control P<0.05

**Changes in spleen weight and its size ratio in treated mice and compared with positive and negative control**

The result of present study found that the groups treated with camel milk for one week and two weeks showed less weight and enlarged ratio in the spleen where the fourth group treated with camel milk for two weeks showed less weight of the spleen 0.11 g and the enlarged ratio 3.96 and the third group treated with camel milk for one week was the weight of the spleen has 0.17 g and the enlarged ratio 5.63 .The differences were significant between camel milk treatment groups and positive control group, which recorded weight of spleen 0.25 g and enlarged ratio of 7.3, while no significant differences noticed between fourth group and negative control group as shown in Table (3).

**Table (3) Changes in spleen weight and its size ratio in treated mice and compared with positive and negative control.**

Group	spleen weight/gm (mean±SD) P (value)	Spleen size ratio (mean±SD) P (value)
Negative control	0.105±0.011	3.58±0.402
Positive control	0.25±0.40 0.001*	7.3±1.589 0.002*
Milk 1 week	0.17±0.151 0.006*	5.63±0.577 0.39
Milk 2 week	0.11±0.36 0.898	3.96±1.31 0.94

\* meaning statically significant comparing to the control P<0.05

## DISCUSSION

The results of the current study showed that the groups of animals treated with camel milk for one week and two weeks have decreased the weights and number of hydatid cysts compared with positive control, this confirms the efficacy of camel's milk in reducing the numbers and weights of the hydatid cysts. The study agreed with the findings of [12], noting the low numbers and weights of hydatid cysts in animals infected with *E. granulosus* parasites and treated with milk and urine camel, compared to the positive control group, also this study agreed with other studies on the effect of camel's milk on other parasites. [14] concluded to prevent the infected of *Leishmania donovani* in animals treated with milk and urine camel, [15] concluded that camel's milk with or without leishmania antigen could be used as immune improver against infection of *Leishmania donovani* in male mice. This can be attributed to a high immune response that has an effect on controlling the growth and development of protoscolices to secondary hydatid cysts and eliminate them this effect may be attributed to the properties possessed by camel's milk as it has the inhibitory ability of different types of bacteria, viruses and parasites. This is because the immune molecules of camel's milk are smaller than in humans and other animals, so they can easily penetrate the protein layer around the parasite and weaken the vital effectiveness [16].

In this study, there was a significant decrease in liver weights and its size ratio in groups treated with camel's milk compared with positive control group, the decrease in liver weights and its size ratio is due to the immunological efficacy of camel's milk proteins of medical importance in the treatment of many diseases such as ascites, liver disease and liver function improvement [17]. The results of the reduction in liver weight and its enlarged ratio agree with the findings of [18] where she noted the apparent decrease in liver weights and its enlarged ratio in infected mice with Irradiated protoscolices compared to the positive control mice, which is due to reduced liver volume and hence the number of parasites. Thus reducing the granular tumor. This study agrees with the results of the study of [10], which showed a clear decrease in liver weights and its enlarged ratio of infected animals and treatment with volatile oil of *Cymbopogon citrates* plant by different concentrations of oil compared to positive control animals, which observed a large weight of the liver and high enlarged ratio. The increase in liver weight may be attributed to the intensity of the inflammatory reaction [12], as well as the increase in the number of lymphocytes as a result of their division and secretion of the cellular motor factors. The colony stimulating factor (csf) that secretes the macrophages stimulates their reproduction [19]

The current study shows a decrease in the rates of spleen weight and its size ratio among the groups treated with camel milk compared to the positive control group, which observed large enlarged of the spleen, changes in the weight of the spleen of infected mice are caused by the increase in the production of T lymphocytes and B lymphocytes [20] the study agrees with the findings of [21] that the weight of the spleen increases and amplifies its size in the group of mice infected with *E. multilocularis* due to the response of the spleen cells to the parasite and to the associated bacterial infections and lymphocytic infiltration as an immunological necessity. The decrease in spleen weights may be due to the chemical properties of camel milk for its contents of immune proteins such as lysozyme, lactoferrin and others [22].

It has been concluded that camel's milk has the ability to reduce the incidence of secondary hydatid cysts in infected mice and treatment with camel's milk and the increased duration of treatment with camel's milk significantly reduces the incidence of infection. Therefore, the current study recommends the completion of research and

research studies on the use of camel's milk as immune improver against other parasitic infections and study the effect of camel's milk in the treatment of various parasitic infections

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