ISSN-1994-697X

Effect of camel's milk on viability of protoscolices of Echinococcus granulosus in vivo

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Abstract

The present study was conducted to determine the effect of camel's milk in the vitality of protoscolices of Echinococcus granulosus in vivo, where noted in this study that camel's milk in the dosage animals infected with protoscolices and for various periods included one week and two weeks the results given different abilities in each group of aggregates in preventing the growth and development hyadtid cysts after 90 days .This study confirmed that camel's milk was useful of prevent of development of hyadtid cysts.

Key word:- Echinococcosis, camel's milk , Echinococcosis Treatment

Introduction:

The H.C.D (hydatid cystic disease) is one the of the common disease in different parts of the world, and is one of the endemic diseases in the Middle East and parts of America, Australia, the Mediterranean region and Central Asia and Central and Eastern European countries (Bitaretal., 2012), and is a zoonotic diseases among humans and animals (Dvorak et al., 2008).

The disease caused in the human and other intermediate host (sheep, cows, buffaloes, camels, horses and other animals) by larval stage (metacestode) of the parasites belonging to the genus echinococcosis Echinococcus, which includes

ISSN-1994-697X

many types most important medically the granular type E.granulosus which represents the predominant type responsible for bringing disease.

The disease is spread by high in rural areas where there is a closed contact between the final host, (the dog) various intermediate and domestic animals (Eckert et al., 2004)

The biggest spread of infections occur in these areas where there are large numbers of sheep and cattle, and ignorance is one of the factors leading to the occurrence of the infection, and a catalyst in the transition and the sustainability of this disease (Moro et al., 2005).

This disease is one of the endemic diseases and influential socially and economically as well as because of its effects on the health side of man, prompting many researchers to investigate the means of treatment and the surgical intervention of the most important methods of treatment in spite of the serious problems faced by the patient during surgery, Which are difficult to be made sometimes the fact that the patient is not eligible surgically because of age or anesthesia or the occurrence of the cyst in places difficult for the surgeon to deal with them as in the brain cysts or in certain cases, such as age or in the case of pregnancy or injury to the patient and other severe illnesses such as heart disease, diabetes and high blood pressure (Khuroo, 2002). the medical importance of camel milk in the treatment of many of causing disease fungi, and parasitic worms, and yeast-causing skin diseases, and bacteria gastrointestinal (Al-Elayane,2005) and the lack of studies on the impact of camel milk in this parasite, and from prophetic guidance in which he referred Prophet Muhammad (peace be upon him) in the therapeutic importance of camel milk, where such importance mentioned in the novel from Ibn Abbass

(may Allah be pleased with them) said: The Messenger of Allah (peace be upon him) said: (The in camel's milk and urine healing for sick stomachs) (Proved by Bukhari).

So study aimed to:

Impact statement camel milk in the development of protoscolices to secondary hydatid cysts in infected white mice and the study of changes in the liver weights and the coefficient of amplified and histological changes in the totals treatment mice after 90 days of infection.

Materials and methods of work

: Solutions used

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1- **physiological salt solution:** Phosphate Buffer Saline (PBS)

It has been prepared according to the method Collee et al., (1996) and consists of:

Chemical Symbol	Weight	Material
KH_2PO_4	0.2 gm	Potassium hydrogen phosphate bilateral
Na_2HPO_4	2.88 gm	Sodium hydrogen phosphate unilateral
NaCl	8 gm	Sodium chloride

Dissolved substances mentioned in the above in one liter of distilled water and the pH is set at 7.4

2- **Ringer SolutionKrep's:** Prepared according to the method Rotunno et al., (1974) consists of:

Material	Weight	Chemical Symbol
Sodium chloride	7.01gm	NaCl
Potassium chloride	0.36gm	KCl
Calcium chloride	0.03gm	$CaCl_2$
Sodium carbonate acid	0.56gm	$NaHCO_3$
Sodium hydrogen phosphate bilateral	0.23gm	NaH_2PO4
Na_2HPO4	0.98gm	Na_2HPO4
Water magnesium sulfate	0.19gm	$MgSO_4.2H_2O$
Streptomycin	0.18gm	Streptomycin(Injection)
Penicillin	0.10gm	Penicillin(Injection)

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Dissolve the above material in a liter of distilled water and the pH is set degree at pH 7.4, and sterilizes the solution in The incubator for 15 minutes under the pressure of 1.5 lb / Ang² and a temperature of 121 m º then leaves the solution to cool and add to it antibiotics and then kept in a refrigerator at a temperature of 16:00 º until use.

Stains used:

1- Aqueous Eosin Stain: It was dissolving 0.1 g of Eosin stain powder in 100 milliliters of distilled sterile water and then shake the solution well (Smyth and Barrett, 1980).

Collection of hydatid cysts:

livers of sheep was obtained out of Nasiriyah massacre were placed in plastic and refrigerated preservative transferred directly to the laboratory Faculty of Science, University of Dhi Qar, during the period of 30-10 minutes to hold the insulation process..

Isolation and separation of the protoscolices and cystic fluid collection: samples Were obtained from the massacre of Nasiriyah, the samples were a livers of infected sheep, where were filled with a large number of hydatid cysts with different weights and diameters ranged between 5-4sm, and the hydatid cysts filled with a large amount of hydatid sand and with a transparent membrane, hydatid cysts were isolated and placed in a Petri dish, was sterilizing the outer surface of it with cotton moistened with alcohol ethyl a concentration of 70%, then pulled the largest possible amount of hydatid sand by a medical syringe (10 mL) by making a hole in the top of the hydatid cyst, and then transfer the liquid withdrawn to clean and sterile and drag flasks the remaining liquid containing the largest possible amount of the protoscolices, and put in a clean and sterile beaker, and opening the hydatid cyst by scissors or scalpel and washed-generating layer of the cyst several times with a solution Krb- Rangers, and was nominated liquid product from washing delicate sieve allows the passage of the protoscolices , Having been sedimentation

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protoscolices were disposed from the filtrate, and transfer protoscolices with those isolated previously syringe and added to known means of the hydatid sand solution - amount of krb- Rangers 4: 1 ratio as a compromise maintained, then washing raw protoscolices three times with a solution of Phosphate Buffer Saline (PBS) by manual shake of the flask and leave for 10-5 minutes, until all protoscolices are deposited at a time, then were disposed from the filtrate and wash the sediment again, Then were disposed from the filtrate and wash the sediment again, and after the last wash process was suspended protoscolices given the size of the hydatid fluid solution krb- Rangers by 4-1, to maintain the vitality of theprotoscolices, and check vital by pulling 10 Maekerolatr from the From the solution after shake by a standard absorbent, and place on a glass slide and added to the same amount of dye water Aqueous eosin, for the purpose of knowing the live protoscolices, and examined mediated by light microscopy using a lens x10 and took the rate of 5 readings, then extracted the percentage of vitality for the living protoscolices in solution.

Camel milk source:

Was obtained camel milk from a camel in the east of the city of Nasiriyah area /thi- Qar up to 25 km, and the good southern city of Amarah / Maysan up to 30 km, as collected milk samples from the camels that the age of 6-4 years, as well as camels that old 9-7snh . Was obtained milk samples in a way manual milking hand Milking, it was transferred after milking mediated by directly to plastic refrigerated preservative to a laboratory, and then keeping it in the refrigerator under 2-5ºdegree heat until use.

Animals of the experience:

use of white mice of the Balb / C strain type Mosmossolos the age of 8-6 weeks, and the weight of 25-27 grams, put in crates and plastic furnished of sawdust and fitted with a lid metal and a place to put the water bottle with

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providing favorable conditions in terms of temperature, ventilation Animal House, and the provision of water and food and to maintain the cleanliness of the cages to change the sawdust and water at a rate of once every week.

Design experience in vivo:

It divided the mice into four groups each group of seven mice made up, and injected three groups in the peritoneal cavity with 0.2 ml of the protoscolices by 3000 head / ml and the fourth injected injected only with saline natural (PBS) to be a negative control a group , milk was given orally for the first and second groups with dose 0.2 ml in the next day, as follows:

The first group: injected orally with camel milk for a week.*

. The second group: injected orally with camel milk for two weeks*

Group: (positive control): injected orally with protoscolices only. Third *

*Fourth Group: injected orally orally with Physiological solution (PBS) only.

Standards used in the study:

In this study we examined the growth and lack of growth in the hydatid cysts in the injected mice orally after 90 days from the injected injection with protoscolices, since the animals were dissected after this period and the investigation of the presence of cysts, and their numbers, and weights. It was also set developments in the weights of the liver changes and the coefficient of amplified, and examination of liver histological changes in the animals after conducting transactions.

Anatomy of mice:

Were dissected rats after 90 days of injections and treatment camel milk, which was calculated animal weight after drugged, was anatomy and extraction hydatid cysts if present, measuring and weighing and extraction of the overall rate, were Hepatomegaly and the creation coefficient bulge by according to the following equation:

organ Weight

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User inflation coefficient =----------*1000

Body weight - the weight of the hydatid cyst

Kroes and Tanner, 1987), and saved grubber (liver) in formalin solution for the purpose of preparation of tissue sections later.

Histological sections preparation:

The preparation of histological sections of the liver of mice, according . .

.)Shao et al ., 1999(to

statistical analysis: Statistical analysis was performed using the results of the analysis of variance (F-test) the application of the computer system (Mini tab), were compared using the arithmetic mean (Duncan Test) polynomial level of probability 0.05P≤ (AL-Rawe, 2000).

Results:

check secondary hydatid cyst:

Control mice positive (infected), which have been autopsy showed after 90 days of infected having a secondary hydatid cysts in peritoneal cavity and distributed irregularly and found hydatid cysts on the liver which ingrained in it. Small hydatid cysts were having a transparent thin membrane and filled quantities of hydatid sand were grouped in blocks and reached to 4.00 cysts of different sizes, with an average weight of 0.2000 g as it shown in Figure (1) and Table (1)

Also noted the presence of cysts in the liver of mice treated one camel milk only for a week, and had fewer cysts, from those of which is found in the positive control group, While it was noted the absence of cysts in mice treated group camel milk only for two weeks, and this is what appears in Figure(3).

Table (1) changes in rates number and weights of hydatid cysts in treated mice and compared with positive and negative control.

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Animal Group	The average number of cysts)G) S.D±	The rate of the bags weight (g) S.D ±
Infected animals and the treatment of camel milk for a week	0.707 ± 1.000 c	0.015 ± 0.112 d*
Infected animals and the treatment of camel milk for two weeks	0.000 ± 0.000 c	0.000 ± 0.000 e
Infected animals only)Positive control(	2.345 ± 6.00 A	0.015 ± 0.2000 a
Normal animals)Negative control(	0.000 ± 0.000 C	0.000 ± 0.000 e

*Similar letters in each column means the absence of significant differences between them on the level of probability p≤0.05

Each value of the above values represent the rate of five replicates ± SD.**

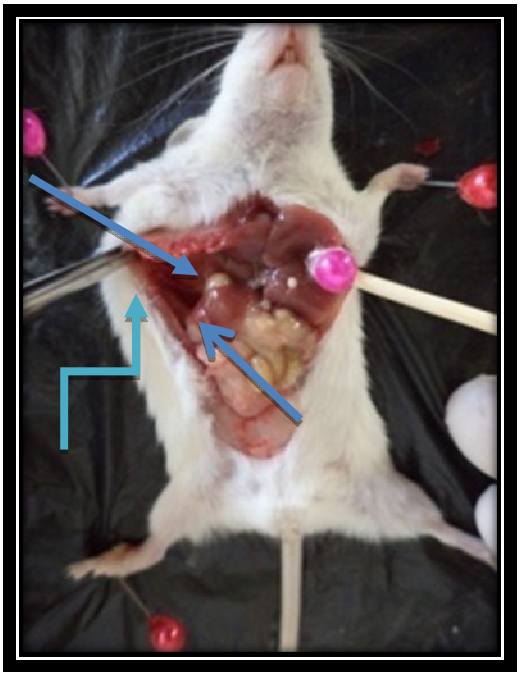

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Figure (1): illustrates the spread of hydatid cysts in the back cavity of the mouse in the positive control mice (infected) after 90 days of infection

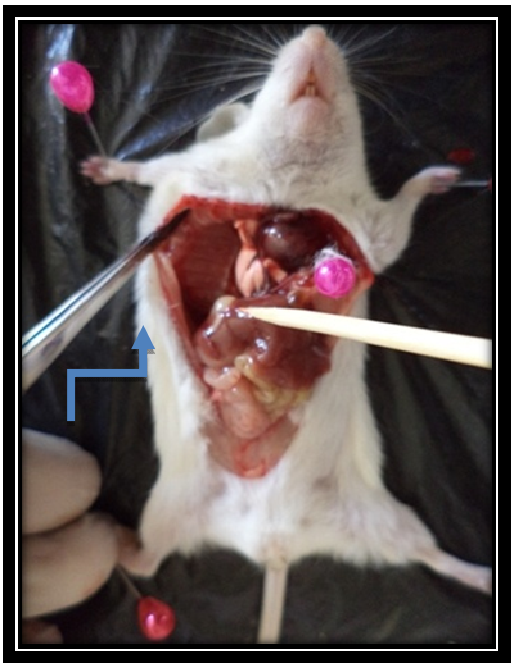
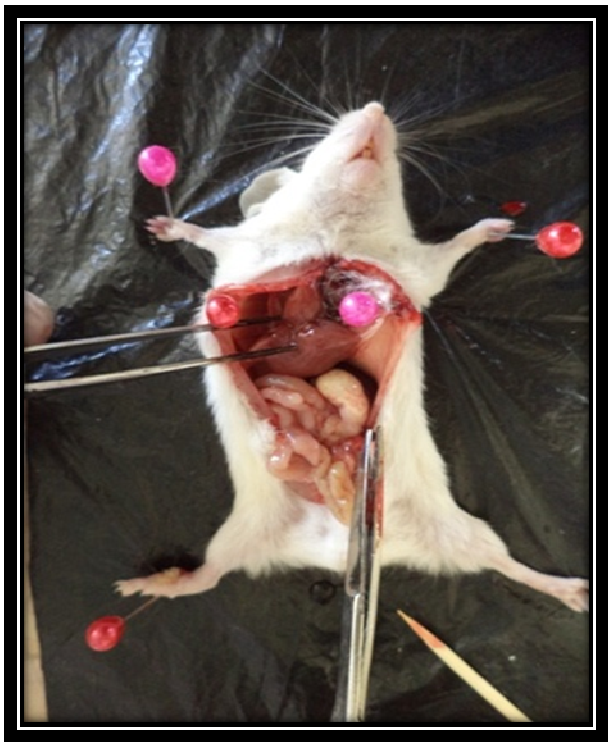


Figure (2): shows the presence of hydatid cyst in liver of mouse which treated with camel milk only for a week after 90 days of infection



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Figure(3): illustrates the lack of hydatid cysts in the cavity of the mouse treated with camel milk for two weeks after 90 days of infection

2- changes in the liver weights rates and the coefficient of amplified in mice compared to control treatment and the positive and negative groups: Notes from the table (2) that aggregates treatment with milk for two weeks, it showed less weight to the liver 1.530 g, And were significant differences between these groups and the positive control group and that gave weight to the liver 2.920 g it was not significant differences between this group and the negative control group and that the rate of liver weight was 1.55 g. It is noted that the totals treatment with milk for two weeks had given less coefficient of hepatomegaly 53.24, which differed significantly from the group positive control 114.07 while this group did not differ significantly from the range of negative control, in which the liver 58.74 inflation factor of, and notes that the liver weight and the coefficient of amplified least increase duration of the dosing.

Table (2) changes in the liver weights and the coefficient of amplified in the totals treatment mice after 90 days of infection and compared with totals for the positive and negative control..

Animal Group	The rate of liver weight (g(±S.D	Hepatomegaly coefficient S.D ± average
Infected animals and the treatment of camel milk for a week	0.186 ± 1.754 Cd	5.97 ± 67.47 Bcd
Infected animals	0.021 ±1.530	6.24 ± 53.24

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and the treatment of camel milk for two weeks	**d***	**E**
Infected animals only)Positive control(	**0.130 ± 2.920 A**	**14.53 ± 114.07 A**
Normal animals (Negative control)	**0.020 ± 1.554 D**	**2.36 ± 58.74 De**

*Similar letters in each column means the absence of significant differences between them on the level of probability p≤0.05

Each value of the above values represent the rate of five replicates ± SD.**

3- Histopathological changes in the liver:

Histological examination showed the liver of mice of negative control which treatment with a (Normal physiological salt solution) emergence of liver cells and nuclei at full size, and the distance between the bars of the cells sinusoid, and this is what is shown in Figure (1).

While histological sections of the liver of control mice positive occurrence of congestion of the central vein and vaculation and showed necrosis, and watery degeneration and lymphocytic infiltration of the cells in the liver lobule center, and this is what appears in Figure (2).

And it showed treatment with camel milk only for a week vaculation occurrence of moderation liver cells and this has been shown by Figure (3). While histological sections of rat liver treatment camel milk only for two weeks showed the emergence of liver cells within normal limits, and this is what is

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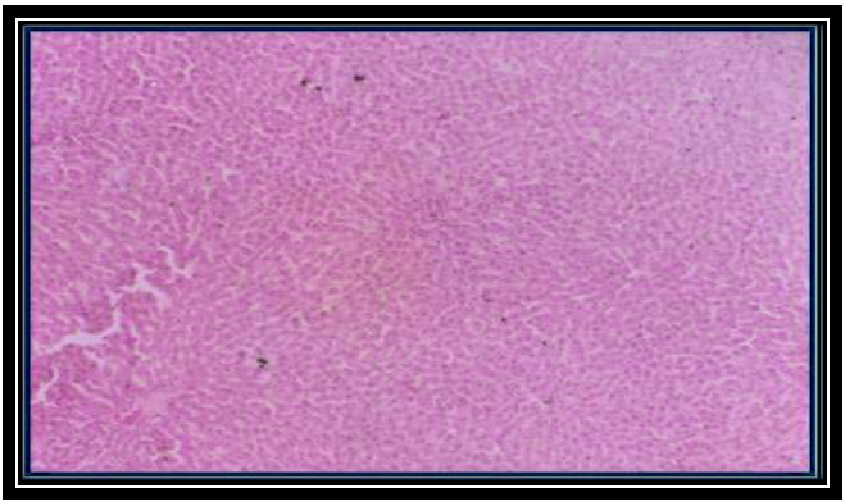

shown in Figure (4).

Figure (1): cross section of mouse liver showed tissue naturally represents a negative control shows the presence of hepatic cells within normal limits. (10x H & E).(

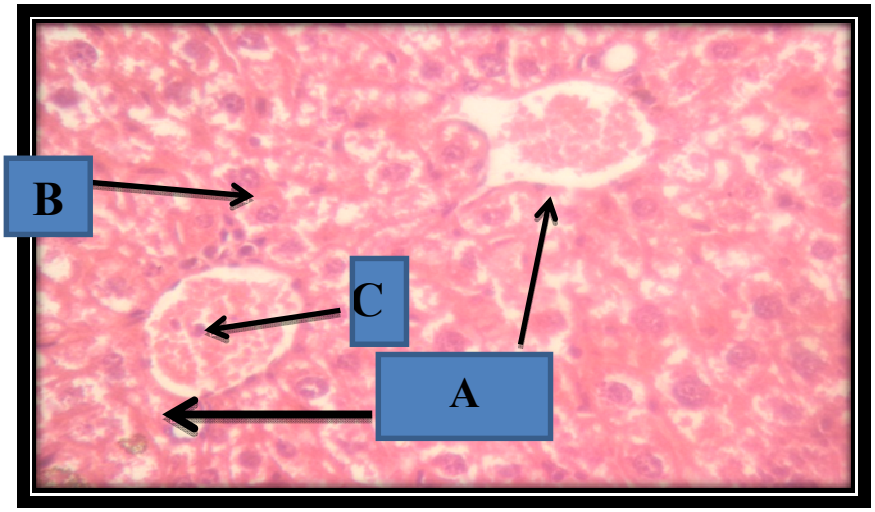

Figure (2) cross-sectional in infected mouse liver tissue (positive control), which explains A: congestion of central vein, B: degeneration water Hydropic degeneration C: lymphocytic infiltration of the cells in the liver lobule center area. 40 X H & E)

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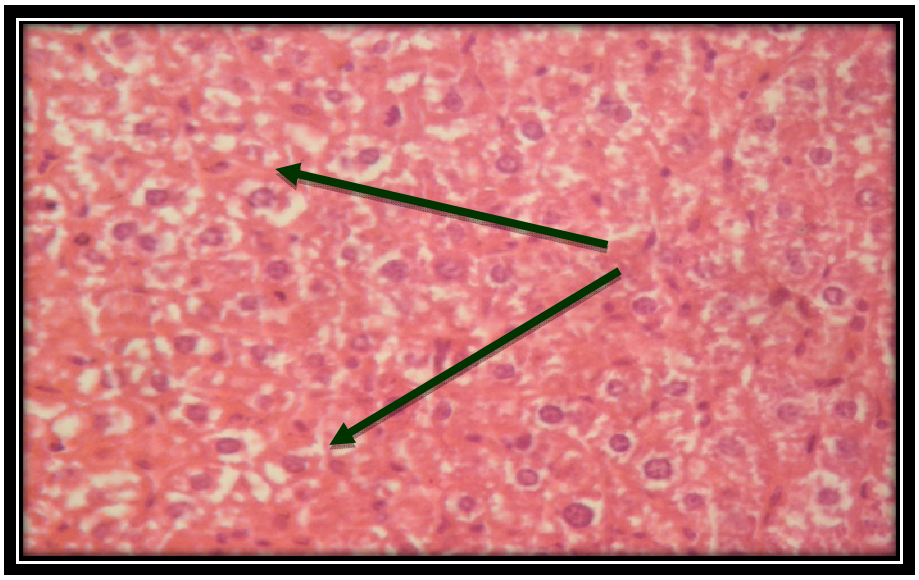

Figure 3 cross-sectional in infected mouse liver tissue treated with camel milk only for a week which shows moderation vaculation liver cells. (40X H & E)

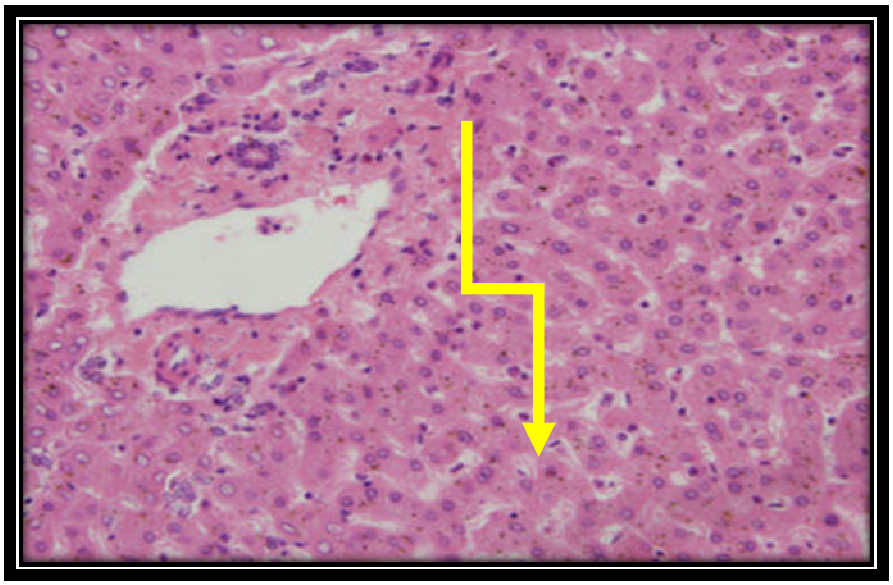

Figure 4) cross-sectional tissue in the liver of mouse infected and treated with camel milk only for two weeks in which cells shows within normal limits (40 x H & E)

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Discussion:

The present study showed the efficiency of milk in reducing the number and weight of hydatid cysts and prevent its appearance for two different period (one to two weeks), where showed the treated mice with camel milk only for two weeks, the lack of hydatid cysts in the peritoneal cavity , and can be attributed to a high immune response have an impact in to control the growth and development of protoscolices to secondary secondary hydatid cysts and eliminate them, , The impact of the properties owned by camel milk, since milk camels ability inhibitory different types of bacteria, viruses and parasites, and the reason for that may be due the fact that the immune molecules to milk camels smaller ones in humans and other animals so it can penetrate the protein layer around the parasite with ease and is working to weaken the effectiveness vital And it prevents the development of the protoscolices and the formation of the cysts as well as working to attack the generated layer (El-Agmay et al.,1992). As well as a clear decline observed in the rates of weights and hepatomegaly coefficient between the different groups compared to the positive control mice. The decline in liver weights rates and coefficient amplified due to the effectiveness of the immune proteins of medical importance of camel milk in the treatment of many diseases such as dropsy and liver disease, improve liver function (Rhoad and Elmore,2000). This is what was agreed with the findings of the Ahmadani (2002), as has been dosage 25 patients were suffering from cirrhosis of the liver, and some of them suffering from cirrhosis of the liver, doses of camel milk plus camel urine daily for two weeks, and after the end of the dosing observed all patients respond to treatment as stomachs dropped respondents and returned to normal and fully recovered from dropsy, and cirrhosis of the liver. Dropsy caused by a shortage of albumin or potassium, and milk and camel urine promise to sources rich in these two components as well as they include almagnseyoum Component (Oohaj.1998). The agreed reduced liver

ISSN-1994-697X

weight coefficient results amplified with the findings of Yaqub (2011) in for the dramatic reduction in liver weights and the coefficient of amplified in mice infected and protoscolices infrared (IR) compared with mice positive control, which is attributable to the reduction of size of the liver and then the small number of parasites and therefore lack of granuloma, may be due changes in the liver weight to increase the intensity of inflammatory reaction(Al-Sabawi,2001), In addition to increasing the numbers of macrophages and lymphocytes as a result of division and secreted factors cellular actuators, as the catalyst for the colonies colony stimulating factor works (csf), which is secreted from the macrophages to stimulate the composition (Roittetal, 1998). Showed histological examination of the liver of mice positive control group results, a significant expansion in the size of the central vein, and the appearance of congestion bloody, and the increase in the numbers and sizes of Coffer cells, and grew up in liver cells size, increase tissue severe and constriction in sinusoid so that it is almost non-existent and the infiltration of inflammatory cells and is groupings, and this is due to the fact that the hydatid cysts is a foreign body stimulates the immune reactions of the host tissue, leading to his portfolio fibrous infiltrated cells inflammatory, as well as exposing it to the vicinity of its cells to degeneration and changes formalism (Prasad and Prasad, 1980). This result for the approval referred to by Das et al (1995) who concluded that infection hydatid cysts lead to an increase in lymphocyte infiltration. These histological changes of the liver of mice infected took moderation when oral injection camel milk two different periods (one week, two weeks), where it was noted moderation vaculation liver cells, when the injection oral camel milk for a week, while it was noted the emergence of liver cells when the normal limits when increasing the treatment period for two weeks..

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