



## EVALUATION OF THE EFFECT OF CAMEL MILK WITH OR WITHOUT THE AQUEOUS EXTRACT OF THE *PEGANUM HARMALA* PLANT IN THE TREATMENT OF *ENTAMOEBIA HISTOLYTICA*

Enas Abdul Kareem Jabbar<sup>1</sup>, Bassad A. AL-Aboody<sup>2</sup>, Nihad H. Mutlag<sup>3</sup> and Ahmed S. Abdulhadi<sup>4</sup>

<sup>1,2</sup>College of Science-University of Thi Qar, Iraq, <sup>3</sup>College of Science-University of Kufa, Iraq,

<sup>4</sup>Agricultural Extension and Training, Iraq

E-Mail: nihad.alez@gmail.com

### Abstract

Camel milk contains antibacterial and viral properties. These properties may be attributed to certain substances such as proteins, fats and vitamins. The current study aimed An attempt to use camel milk with or without the aqueous extract of the plant *Peganum harmala* in the treatment of *Entamoeba histolytica*. Thirty *Mus musculus* mice of the Balb/c strain were divided into five groups each group consists of six mice. The first group was given of which 0.2 ml of the physiological solution was administered (negative control). The second groups were injected with *Entamoeba histolytica* with a concentration of  $5 \times 10^4$  parasite/ml. The next day treated with 0.2 ml of the physiological solution for one week and Considered positive control. The third group also injected with *Entamoeba histolytica* with a concentration of  $5 \times 10^4$  parasite / ml then treated with 0.2 ml of the aqueous extract of the plant and 0.1 mg/kg for one week. The fourth group treated with 0.2 ml of camel milk for one week after being injected with *Entamoeba histolytica* with a concentration of  $5 \times 10^4$  parasite/ml. The fifth group also injected with *Entamoeba histolytica* with a concentration of  $5 \times 10^4$  parasite/ml. and then treated with 0. 2 ml of camel milk with 0.2 of the aqueous extract of the plant. The results showed that the fifth group showed a significant decrease in *E. histolytica* infection. Infection rate was 11.1%. The third and fourth groups decreased the infection rate to 61.1% and 50% respectively when compared to the control group.

**Key words:** *Entamoeba histolytica*, *Peganum harmala*, camel milk, Biocontrol.

### Introduction

Amoebiasis caused by protozoan parasite called *Entamoeba histolytica* which infects predominately human and other primates (Lebbad and Svard, 2005). *E. histolytica* parasites are common in Iraq and the world as they are the third most common parasite that causes human death after malaria and bilharziasis, infecting nearly 50 million people annually in the world, of whom about 100,000 die (Tanyuksel and William 2003). Infection is widespread and endemic in poor and developing countries due to poor sanitary equipment, contamination of drinking water and food with sewage, and then contaminated with waste containing the cyst with four nuclus (Dimiceli, 2004). This parasite is very dangerous as it may damage the small intestine causing ulceration and may spread to the liver thus cause abscess if not treated (Regan, 2014).

Metronidazole is an effective antibiotic for amoeba and has become the chosen drug for the treatment of all forms of amoebiasis (Freeman *et al.*, 1997). However, the amoeba parasite may cause resistance to mitronidazole drugs. This has led to the search for new

drugs to treat these Common injury (Ordaz-Pichardo *et al.*, 2005). It is therefore necessary to obtain safe antiparasitic agents for human use from natural sources whether plant and animal sources, Camel's milk is one of the most important nutrients for humans in many parts of the world, especially desert and semi-desert areas, Camels, despite their cruel conditions of high temperatures, drought and loss of water, are able to produce good quality milk, Due to the importance of camel milk many researchers studied its composition and properties They found that camel milk contains a good quality of protective proteins against bacteria and viruses, such as Lysozyme, Lactoferrin, Lactoperoxidase, which make it more superior to cow's milk in terms of nutrients (El-gammal and Moussa, 2007; Hassan *et al.*, 2009; Mal and Pathak, 2010). *Peganum harmala* is a herbaceous plant belonging to the family Zygophyllaceae has multiple medical uses, used to treat many diseases such as Syphilis, hysteria, epilepsy, neuralgia, parkinsonism, rheumatism, colic, asthma. The fruits and seeds help digestion and are diuretic, sedative, emetic and stimulant (Al-obaidi, 2007). It was also found that the aqueous extract of the

seeds is effective against the virus (HSV.I) herpes simplex virus (Rashan *et al.*, 1989). The current study aimed at using camel milk with or without the aqueous extract of *Peganum harmala* plant in the treatment of *Entamoeba histolytica*.

## Materials and Methods

### Collection of samples

Stable stool samples were obtained from *Entamoeba histolytica* from the national medical laboratories in Thi-Qar province. The samples were collected in sterile glass containers and brought directly to the college laboratory, where they were later used to infect laboratory animals for research.

### Stool examination

The samples were examined by a direct wet smear (Tanyuksel *et al.*, 2005)

### Preparation of the parasite suspension used in the dosage of animals

The parasite used in the dosage of the animals was prepared by mixing 1 g of the stool sample with 3 ml of physiological saline solution by the centrifugal test tubes and then washed. The sample was concentrated twice using the centrifuge for 10 minutes and 500 cycles/minute to remove the waste residues. It is possible to use the sample directly in the dosage of animals after counting the number of cysts in this remaining sample concentrated using Haemocytometer (Stanley *et al.*, 1999).

### Source of camel milk

The camel milk was collected from different areas of Nasiriyah city / Thi Qar province / Iraq, which is 1ye about 360 km from the Baghdad. The hand milking method was used to collect the milk and transported directly by ice-cooled vials to the college laboratory and kept in the refrigerator under 2-5 Centrifuge until use.

### The preparation of *Peganum harmala* and prepare the aqueous extract

The seeds were obtained from local markets in Thi Qar province and the plant was washed then placed in a room with good ventilation for two days until dry, then grinded with an electric mill and stored until use.

The aqueous extract of *Peganum harmala* was prepared with 100 g of plant powder and add 500 ml of boiled distilled water for 1 hour. Then, sprinkle with four pieces of gauze and place the resulting leachate in the centrifuge tubes then centrifuges at 300 rpm for 15 min, and then put the supernatant liquid in glass dishes inside the oven drying degree of 40° and after the completion of drying Scrap the plant extract powder and

put in sterile glass containers in the room or refrigerator until use.

### Animals Infection

In this study, 30 male *Mus musculus* mice of the Balb/c strain were placed in plastic cages for animal breeding. Examination of the mice feces before starting the experiment to ensure that they were free of the infections, the animals were divided into five groups:

1. The first group was given of which 0.2 ml of the physiological solution was administered (negative control).
2. The second groups were injected with *Entamoeba histolytica* with a concentration of  $5 \times 10^4$  parasite / ml. The next day treated with 0.2 ml of the physiological solution for one week and Considered (positive control).
3. The third group also injected with *Entamoeba histolytica* with a concentration of  $5 \times 10^4$  parasite/ml then treated with 0.2 ml of 0.1 mg / kg the aqueous extract of *Peganum harmala* for one week.
4. The fourth group treated with 0.2 ml of camel milk for one week after being injected with *Entamoeba histolytica* with a concentration of  $5 \times 10^4$  parasite / ml.
5. The fifth group also injected with *Entamoeba histolytica* with a concentration of  $5 \times 10^4$  parasite / ml. and then treated with 0.2 ml of camel milk and 0.2 of the aqueous extract of *Peganum harmala* together for one week.

Infection mice were examined during and after the treatment to observe the reduced number of cysts and trophozoite stages .

## Results

The present results showed that the third group of infected mice with *Entamoeba histolytica* and then treated with the aqueous extract of *Peganum harmala* had a decrease in the rate of infection to 61.1%, as well as a microscopic examination of faeces also showed a reduction in the number of cysts and trophozoites to 11 parasite per sample compared to the second group ( positive control group), While the fourth group of infected mice with *Entamoeba histolytica* and then treated with camel milk showed a decrease in the infection rate to 50% where the microscopic examination showed reduce the number of parasites to 9 parasites in each sample, While the fifth group of infected mice with *Entamoeba histolytica* and then treated with camel milk and aqueous extract of *Peganum harmala* together, the proportion of infection decreased to 11.1% where the microscopic examination reduced the number of parasite to 2 parasites per sample, Table ( 1)

**Table 1** : Effect of camel milk and aqueous extract of the plant *Peganum harmala* in infection rate and stages of *E. histolytica* at the end of treatment period

GROUPS	infection ratio	number of parasites in faecal
The first group (Negative group)	0%	0
The second group (Positive group )	100%	18
The third group	61,1%	11
The fourth group	50%	9
The fifth group	11,1%	2

### Discussion

The present study results showed that camels' milk and aqueous extract of the plant *Peganum harmala* have a significant effect in reducing the infection ratio and numbers of *Entamoeba histolytica*, Where the number of parasites exiting with faeces decreased from 18 parasites in the case of infection to 11 parasites in the group treated with the aqueous extract of *Peganum harmala*, because it contains alkaloids, calcosides and flavons that have an antibiotic effect (AL-Wazan, 2009).

The inhibitory activity of the *Peganum harmala* is also due to the presence of alkaloids B-Carbolin including Harmalin and Harmine that have inhibition activity for DNA Topoisomerase which is necessary for DNA formation (Funayama *et al.*, 1996).

This study agreed with AL-Obaidi (2007) where the study showed that the aqueous extract of *Peganum harmala* has an anti amoeba activity with 1000, 1500, 2000 mg/ml concentration during two hours that obtained of mortality rate 40.3%, 62.1% and 90% respectively, Several studies were conducted using the aqueous extract of *Peganum harmala* in the elimination of other parasites. AL-Jubouri (2007) achieved the complete killing of the protozoal parasites of *Echinococcus granulosus* parasite when using the aqueous extract of *Peganum harmala* with concentrations 5 and 15 mg/ml.

While the group of infected mice with *Entamoeba histolytica* and then treated with camel milk showed a decrease in the infection rate to 50% where the microscopic examination showed reduce the number of parasites to 9 parasites in each sample this is because camel milk contains many proteins such as : Lysozyme, Lactoferrin, Casein, Lactoperoxidase, Lactoalbumins, Immunoglobulins, amylase which may have anti-parasite efficacy, Lactoferrin plays an important defense function alone or with the rest proteins against fungal, parasitic, bacterial and viral infection by acting with heparin sulfale on the cell surface thus preventing virus

adhesion or interacting with the virus itself (Longhi, *et al.*, 2006 and Jenssen, 2005).

This study is consistent with AL-Aboody *et al.* (2014) in their study on the effect of camel milk on the vitality of *E. histolytica* that growing in LEM media where they observed that the efficacy of camel's milk was directly proportional to the increase in concentration and incubation time in the media. The study also agreed with AL-Aboody *et al.* (2016), where they reached the role of protective camel milk against infection of *E. histolytica* in laboratory mice where camel milk had a role in reducing infection rate in groups of mice infected with *E. histolytica* and given camel milk compared with mice that infected with *E. histolytica* and not given camel milk.

This study agreed with other studies on other parasites where AL-Gizy (2014) found the inhibitory role of camel milk in the *Leishmania donovani* parasite *invitro* and *invivo*. And in studies on other organisms, Benkerroum *et al.* (2004) found that the camel milk has inhibitory effect against gram positive and negative bacteria such as *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella typhimurium*.

While the group of infected mice with *Entamoeba histolytica* and then treated with camel milk and aqueous extract of *Peganum harmala* together, the proportion of infection decreased to 11.1% where the microscopic examination reduced the number of parasite to 2 parasites per sample This confirms the essential and ingredients role of both milk proteins and aqueous extract of harmala in reducing the number of parasites.

The study, which is considered the first study of its kind in Iraq, shows that both camel milk and aqueous extract of *Peganum harmala* have an antimicrobial effect on the *E. histolytica* parasite through their ability to reduce the number of parasites in infected mice, but the doses used have not reached the limit Which leads to the complete killing of the parasite, which leads us to another study and a higher dosage of the current doses to reach the complete killing of the parasite and a faster period of time.

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