Tikrit Journal of Pure Science
ISSN: 1813 – 1662 (Print) --- E-ISSN: 2415 – 1726 (Online)
Journal Homepage: http://tjps.tu.edu.iq/index.php/j

Therapeutic effect of camel milk on albino mice *(Mus musculus)* colon experimentally infected with *Entameba histolytica*

Rana Hussein Al-Joboury[1], Fatima Shihab Al-Nasiri[1] , Ayad Hameed Ebraheem[2]
[1]*Department of Biology , College of Science , Tikrit University , Tikrit , Iraq*
[2]*College of Veterinary Medicine, Tikrit University , Tikrit , Iraq*
DOI: http://dx.doi.org/10.25130/tjps.26.2021.056

ARTICLE INFO.
Article history:
-Received: 4 / 7 / 2021
-Accepted: 8 / 8 / 2021
-Available online: / / 2021
Keywords: *E. histolytica*, colon tissue, camel milk, albino mice.
Corresponding Author:
Name: Rana Hussein Al-Joboury
E-mail:
ranahusseinmohammed1985@gmail.com
Tel:

ABSTRACT

The present study aimed to determine the therapeutic effect of camel milk on colonic tissues of albino mice experimentally infected with *E. histolytica*. Albino mice (*Mus musculus*) was infected experimentally with *E. histolytica* cyst stage at a dose $1x10^6$ cell/ml, which was isolated from faecal samples of infected children with *E. histolytica* who attended to Children's Hospital in the city of Kirkuk at period from May to July 2019. The infected group showed, sever lymphocytes infiltration, and trophozoit stage in the mucous layer with the presence of necrotic materials. In the group of mice which administered with 3 ml of camel milk, colon tissue was normal and there were no histopathological changes compared with mice infected with *E. histolytica*. While, in the infected group and later treated with camel milk, the histological sections confirm the improvement of colon tissue layers after treatment compared with infected group. Also, in this group, the stages of the parasite were absent. Therefore, from the current study it can be concluded that camel milk has a therapeutic role against the tissue lesions caused by *E. histolytica*.

Introduction

E. histolytica is a protozoan parasite, infecting about 45-50 million (persons)/ year, and causing 40,000 to 100,000 deaths annually [1]. Most cases are asymptomatic, but the parasite can invade the lining of the large intestine, leading to severe ulceration associated with amoebic dysentery and bloody diarrhea as well as other pathological symptoms [2, 3, 4]. Plant and animal products and derivatives are among the main sources of drugs for a long time. In addition, these products have a safe therapeutic effect due to the lack or decrease of their side effects [5]. Camel milk is one of the most important animal sources with therapeutic effects, preventing oxidative stress and cell damage through its role in scavenging free radicals and inhibition of lipid peroxidation [6]. In addition to the role of camel milk as antiparasitic, antibacterial and antiviral activity, camel milk contains a high concentration of lactoferrin [7]. Therefore, the present study aimed to induce an experimental infection in albino mice with *E. histolytica* by oral administrated of the infectious phase (cysts) and then using camel milk, after infection, to detect the role of camel milk in the treatment of damages colon tissues that caused by the parasite.

Materials and methods

Human faecal samples were collected from the Children's Hospital in Kirkuk during the period from May to July 2019. The sample was handled for the purpose of preserving the viability of the parasite according to the method described by WHO [8]. Fecal samples were examined within an hour after arrival to the laboratory of Biology Department, College of Science, Tikrit University. The isolation and purification of the cyst phase of *E. histolytica* was carried out according to the method that described by Snyder and Meleney [9] . Albino male mice (*Mus musculus*) used (age 5 weeks) was used to induce the infection experimentally with a dose 1X 10^6 cell/ml/ mice by using a gavage tube. On the 7^{th} day after administeration, the mice were dissected to study the histological structure of the colon. Another group of mice (positive control group) was administered with camel milk at a dose of 3 ml for 10

days, and dissected at 11th day to studying the histological structure of colon. In addition to the above two groups, the third group was experimentally infected (at a dose of 1X 10^6 cell/ml/ mice) with *E. histolytica*, then on 7th day, they were administered with camel milk at a dose of 3 ml for 10 days, and the mice were dissected at 11th day of the beginning of administration with camel milk. Also, negative control mice were administered with normal saline. In present study, two mice were used for per group. The histological sections of colon were prepared for all groups of experimental mice according to Luna [10] and Humason [11].

Results

Figure (1) shows the general histological structure of colon in negative control mice which comprised of tissue layers (mucosa, submucosa , muscularis and serosa) are a normal tissue structure. Figure (2) demonstrate the colonocytes and crypts of lieberkuhn that contain goblet cells located in the mucous layer. Lamina propria can also be distinguished within the histological structure of colon of negative control mice.

Figure (3) shows the general histological structure of the colon wall in experimentally infected group. The lymphocytes infiltration between the crypts of lieberkuhn, the trophozoit stage can be distinguished in the mucous layer with significant damage of lamina propria (Figure 3). Also, figure (4) shows the presence of the trophozoit stage in the mucosa layer between crypts of lieberkuhn, with the observation of sever lymphocytes infiltration between the interstitial tissue.

The histological sections of mice colon that adminstrated with camel milk (Figure 5) shows the general histological structure of the colon wall, without any tissue damages. Also, Figure (6) shows normal structure of colon mucosa layer, colonocytes or columnar absorptive cells with the presence of crypts of lieberkuhn that contain goblet cells. The normal interstitial cells which located between the colon glands (Figure 6) are also obvious.

The histological sections of the experimentally infected mice colon and treated with camel milk (Figure 7) shows the general histological structure of the colon wall (mucosa layer, submucosa layer, muscularis layer and serosa layer with lumen intestine). These histological sections confirm the improvement of colon tissue layers of this group after treatment with camel milk compared to the infected group with the parasite, where the tissue structures were close to the normal histological state. Also, figure (8) demonstrate improvement of colon tissue in treated mice, this improvement are clear in the colonocytes, crypts of lieberkuhn that contain goblet cells and lamina propria.

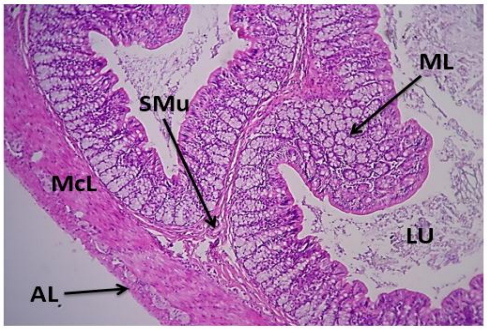

Fig. 1: Cross section of mice colon administrated with 3 ml normal saline for 10 days shows mucous layer (ML), submucosal layer (SMu), muscle layer (McL), Adventitia layer (AL) and colon lumen (LU); H&E, 100X.

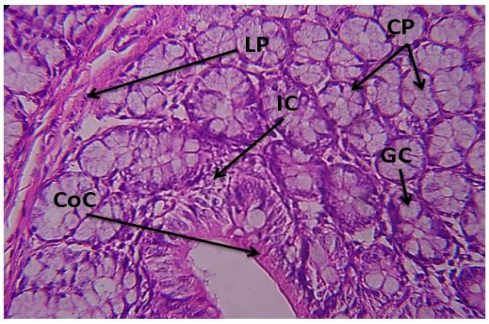

Fig. 2: Cross section of mice colon administrated with 3 ml normal saline for 10 days shows mucosal layer, which includes columnar cells (CoC), goblet cells (GC), lamina propria (LP) and interstitial cells (IC) H&E 400X.

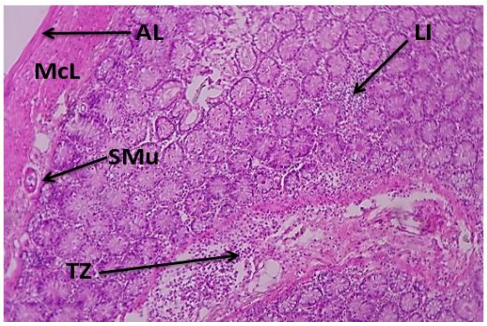

Fig. 3: Cross-section of mice colon infected with the parasite at 7th day shows the presence of trophozoit (TZ), lymphocyte infiltration (LI) in the mucous layer. The submucosal layer (SMu), muscle layer (McL) and adventitia layer was also observed (AL); H&E, 100X.

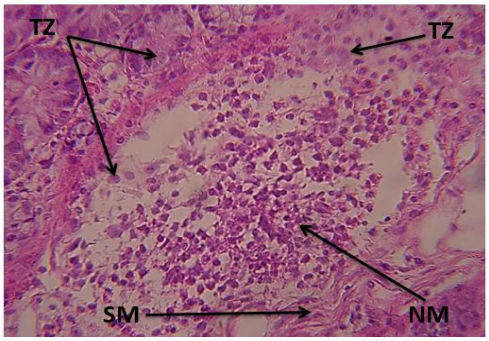

Fig. 4: Cross-section of mice colon infected with the parasite at 7th day shows the presence of trophozoit (TZ), reduced smooth cells (SM) in the lamina propria and presence of necrotic material (NM); H&E, 400X.

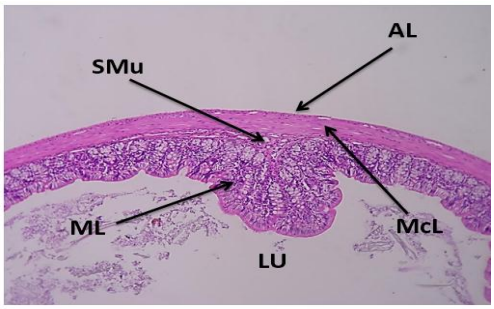

Fig. 5: Cross section of mice colon administrated with 3 ml camel milk for 10 days shows mucous layer (ML), submucosal layer (SMu), muscularis layer (McL), Adventitia layer (AL) and colon lumen (LU); H&E, 100X.

Fig. 6: Cross section of mice colon administrated with 3 ml camel milk for 10 days shows mucus layer, which includes columnar cells (CoC), goblet cells (GC) and interstitial cells (IC); H&E, 400X.

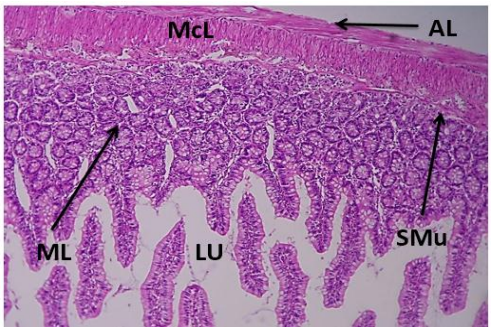

Fig. 7: Cross-section of mice colon infected with the parasite at 7th day and treated with 3 ml camel milk for 10 days showed mucous layer (ML), submucosal layer (SMu), muscle layer (McL), Adventitia layer (AL) and colon lumen (LU); H&E, 100X.

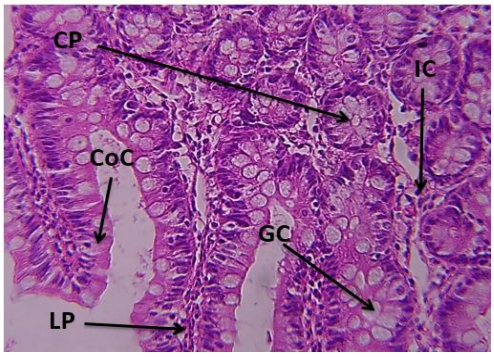

Fig. 8: Cross-section of mice colon infected with the parasite at 7th day and treated with 3 ml camel milk for 10 days shows mucus layer, which shows columnar cells (CoC), goblet cells (GC), lamina propria (LP) and interstitial cells (IC); H&E, 400X.

Discussion

The results of the current study showed sever lymphocyte infiltration in the colonic tissue, and damage of crypts in some colon regions of mice infected with *E. histolytica*. The results of this study are close to the study of Mahmood and Mohamed [12], who indicated that infection of mice with *E. histolytica* leds to various lesions in the colon, including shortening of the villi in some regions of the colon slides, degeneration and necrosis of mucous layer cells with lymphocyte infiltration. The results of the present study were in agreement with the study of Al-Kennany et al. [13], who observed the presence of histopathological changes of the large intestine (in the colon region) in mice injected with cysts of *E. histolytica*, represented by degeneration and necrosis of epithelial cells with hyperplasia of colon cells (columnar cell) that lining the glands and infiltration of inflammatory cells, as well as mucous degeneration of cells that lining the villi. In the current study, the sever infiltration of inflammatory cells in the colon can be explained by the secretion of pro-inflammatory cytokines and the infiltration of immune cells in the colon [14]. The intestinal inflammatory response sometimes contributes to the destruction of host tissue during the occurrence of infection with *E. histolytica* [15,16]. Ghosh et al. [17] indicated that infection with *E. histolytica* leads to colitis, and that persistent inflammation causes a classic flask-shaped ulcer even in the absence of severe infection of a large number of parasite individuals in the tissues. The results of the current study show that camel milk has an important role in treatment of the tissue lesions caused by *E. histolytica* in the colon tissues, as the results show a significant improvement in the colon tissue that appeared normal. Camel milk has the ability to inhibit various types of bacteria, viruses and parasites, and the ability to penetrate the protein layer that comprises the parasite membrane and thus works to weaken its vital activity as well as reducing the cystic process of the parasite [18]. Camel milk also contains many active substances, including lactoferrin, which acts as an antibiotic against many pathogens in the digestive system, and lactoferrin has anti-parasite activity against many species, such as *Pneumocystis carinii*, *Toxoplasma gondii*, *Trichomonas vaginalis* and *Entamoeba histolytica* [19]. The antiparasitic effect of lactoferrin is related to its ability to retain iron and destabilize the parasite's membrane and thus destroy it [18]. This may explain the ability of camel milk to treat the infection with *E. histolytica*. Camel milk contains high levels of vitamins (A, B2, C, and E) and minerals such as sodium, potassium, copper, magnesium and zinc [20]. Vitamin C has strong antioxidant activity [21]. Thus it eliminates free radicals [22], this support the results of the current study about the ability of camel milk to repair the tissue lesions that caused by *E. histolytica* in the colon tissue.

References

[1] Al-Oumashi, G. B.; Khairi, A. and Dawood, A. H. (2012). Evaluation of immunoglobulin m (IgM) and interleukin 2 (il-2) assay in true infection of *Entamoeba histolytica*. Iraqi J. Biotechnol.11(2):445-454.

[2] Blessmann, J.; Buss, H. and Nu, P. A. (2002). Real-time PCR for detection and differentiation of *Entamoeba histolytica* and *Entamoeba dispar* in fecal samples. J. Clin. Microbiol. 40: 4413-4417.

[3] Fotedar, R.; Stark, D.; Beebe, N.; Marriott, D.; Ellis, J. and Harkness, J. (2007). Laboratory diagnostic techniques for *Entamoeba* species. Clin. Microbiol. Rev. 20(3): 511–532.

[4] Al-Jnabee, N. A. B. and Mushtak, T.S. (2017). Molecular detection of *Shigella* spp. and *Entamoeba histolytica* causing bacillary and amoebic dysentery among children in Ramadi city. Al- Anbar Med. J. 14(1): 64-74.

[5] Yadav, A. K.; Kumar, R.; Priyadarshini, L. and Singh, J. (2015). Composition and medicinal properties of camel milk: a review. Asian. J. Dairy Food Res. 34: 83-91.

[6] Althnaian, G. (2012). Protective effect of camel milk against carbon tetrachloride hepatotoxicity in rats. J. Global Vet. 9(5): 564-570.

[7] El-Ouardy, K.; Mohamed, I.; Lorenzo, M.P.C.; Paula, F.B.; Nadia, S.S. and Jamal, A. (2011). Antimicrobial activities of the bacteriocin-like substances produced by lactic acid bacteria isolated from Moroccan dromedary milk. African J. Biotechnol. 10: 10447-10455.

[8] WHO: WHO/PAHO/UNESCO report (1987). A consultation with experts on amoebiasis. Mexico City, Mexico. Epidemiol. Bull., 18:13-14.

[9] Snyder, T. L. and Meleney, H. E. (1941). The excystation of *Endamoeba histolytica* in bacteriologically sterile media. American. J. Trop. Med. Hyg. 21, 63.

[10] Luna, L.G. (1968). Manual of histological staining methods of the armed forces institute of pathology. 3rd edn., McGraw Hill Book Co. New York: 258pp.

[11] Humason, G.L.(1979). Animal tissue techniques. 4th edn., freeman, W, San Francisco, 458 pp + xvi.

[12] Mahmood, S. H. and Mohamed, B. J. (2012). A histological study on the effect of aqueous extract of capsicum spp. On *Entamoeba histolytica* in albino mice. Iraqi J. Sci. 53(3): 54-59.

[13] Al-Kennany, E. R.; Rahawi, A. M. and Al-Jabori, S.A. (2012). Histopathological and histochemical study of intestinal cryptosporidiosis in mice. Kufa J. Vet. Med. Sci. 3(2): 36-42.

[14] Shirley, D. T.; Laura, A. F.; Laura, A. F.; Koji, W. and Shannon, M. (2016). A review of the global burden, new diagnostics, and current therapeutics for amebiasis. Infect. Dis. 5(7): 1-5.

[15] Moonah, S.N.; Jiang, N.M. and Petri Jr., W.A (2013). Host immune response to intestinal amebiasis. PLoS Pathog. 9(8): 1003489.

[16] Ghosh, S.; Leaton, L.A.; Farr, L.; Barfield, A., Moonah, S. (2018). Interaction between parasite-encoded JAB1/CSN5 and macrophage migration inhibitory factor proteins attenuates its pro-inflammatory function. Sci. Rep. 8:10241.

[17] Ghosh, P.K.; Mancilla, R. and Ortiz-Ortiz, L. (1994). Intestinal amebiasis: histo-pathologic features in experimentally infected mice. Arch. Med. Res. 25(3): 297–302.

[18] Elbarbary, H.A.; El-Nahas, E.M. and Effat, L.E.K.A. (2014). Anti-rotaviral activity of whey proteins derived from milk of different animal species. International J. Adv. Res. 2(4): 214-221.

[19] Alimi, D.; Abidi, A.; Sebai, E.; Rekik, M.; Maizels, R.M.; Dhibi, M. and Akkari, H. (2018). In vivo nematicidal potential of camel milk on *Heligmosomoides polygyrus* gastro-intestinal nematode of Rodents. Helminthologia. 55(2): 112–118.

[20] Nagy, P.; Thomas, S.; Markó, O. and Juhász, J. (2012). Milk production, raw milk quality and fertility of dromedary camels (*Camelus dromedarius*) under intensive management. Acta. Vet. Hung. 61 (1): 71–84.

[21] Abdel Gader, M.A. and Alhaider, A.A. (2016). The unique medicinal properties of camel products: a review of the scientific evidence. J. Taibah. Uni. Med. Sci. 11: 98-103.

[22] Kumar, D.; Chatli, M.K.; Raghvendar, S.; Mehta, N. and Kumar, P. (2016). Enzymatic hydrolysis of camel milk casein and its antioxidant properties. J. Dairy Sci. Technol. 96: 391–404.

التأثير العلاجي لحليب الابل على قولون الفئران البيض بعد اصابتها تجريبيا بأميبا الزحار

رنا حسين الجبوري[1]، فاطمة شهاب الناصري[1]، اياد حميد ابراهيم[2]

[1] قسم علوم الحياة، كلية العلوم، جامعة تكريت ، تكريت ، العراق

[2] كلية الطب البيطري ، جامعة تكريت ، تكريت ، العراق

الملخص

هدفت الدراسة الحالية الى تحديد التأثير العلاجي لحليب الإبل على انسجة قولون الفئران البيض بعد اصابتها تجريبا بأميبا الزحار. وقد استخدمت في الدراسة الحالية ذكور الفئران البيض السويسرية *Mus musculus*. حيث تم اصابتها تجريبيا بأميبا الزحار من خلال تجريعها بأكياس الطفيلي (بجرعة مقدارها 10^6X1 كيس/ مل) التي عزلت من عينات غائط الاشخاص المصابين بأميبا الزحار من المراجعين الى مستشفى الاطفال في مدينة كركوك خلال المدة من بداية شهر مايس ولغاية نهابة شهر تموز 2019. لوحظ في مجموعة الاصابة حدوث ارتشاح كثيف للخلايا اللمفية، كما وتم ملاحظة الطور المتغذي للطفيلي في الطبقة المخاطية، مع وجود مواد تتخريه. اما في المجموعة المجرعة 3مل حليب أبل، كانت انسجة القولون طبيعية ولم يكن هناك اي تغييرات نسجيه بالمقارنة مع الفئران المصابة بطفيلي اميبا الزحار. اما في المجموعة المصابة والمعالجة بحليب الابل، اظهرت المقاطع المفحوصة تحسن في الطبقة المخاطية mucous layer، الطبقة تحت المخاطية submucosal layer والطبقة العضلية muscle layer والطبقة الخارجية adventitia layer وتحسن في تركيب الخلايا القولون colonocytes والغدد المعوية النبيبية crypts of lieberkuhn الحاوية على الخلايا الكاسية goblet cells. كما انه في المجموعة الاخيرة لم يلاحظ وجود اي طور من اطوار الطفيلي. لذلك يستنتج من الدراسة الحالية بان حليب الإبل يمتلك دور علاجي ضد الآفات النسجية التي تسبب بها طفيلي اميبا الزحار.