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Histopathological Effects Resulting from the Experimental Infection of Laboratory Mice Affected with Amoebic Dysentery and its Treatment with Camel's Milk and Urine

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ABSTRACT

The study was carried out by isolating the *Entamoeba histolytica* cysts from the excrements and causing experimental infection in laboratory mice with amoebic dysentery by giving 1000 mature cysts as an oral dose. After two weeks of induction, the treatment was attempted using camel urine and milk separately and in doses 0.25, 0.5, 0.75 ml per treatment and comparing it with a group treated with Flagyl drug. The results of histological study showed occurrence of histopathological changes in rectum of laboratory mice, which were represented in the infectious groups by appearance of nodular lymphatic infiltration and necrosis and cellular abnormalities. As for the groups treated with camel milk and urine, they showed a marked improvement represented by the lack of histopathological effects such as necrosis, cellular leaching and cellular degeneration. The best results were with the therapeutic dose of the concentration 0.75 ml mixed with camel milk and urine.

KEYWORDS

Amoebic Dysentery, Histopathological Effects, Camel Milk, Camel Urine, Flagyl.

Introduction

The *Entamoeba histolytica* is eukaryotic monocellular cell parasites that causes Amobic dysentery, which is one of the oldest diseases known to mankind (Paniker, 2002). Tropical and sub-tropical regions are the most common areas of parasites due to availability of special conditions in terms of humidity and temperature (Markell and Krotoski, 2006). The parasite causes deep ulcers in large intestine of affected person, and the penetration of deep ulcers in intestine walls may sometimes lead to peritonitis which in turn leads to death (WHO, 1998). Allah Almighty has made camels one of its wonderful signs mentioned in Several verses in his glorious book (Quran), stating in an airtight text (do they not look at camels how they were created and to the sky how itwas raised and to the mountains how they were set up and to the earth how it was flattened) (17-20 Surah al-Ghashiyya). From this we infer the greatness of camels and how they are distinguished from other creatures. In addition to benefiting from camel meat and its hair and being used in carrying and riding, it has been mentioned in the noble hadith that the Prophet (peace and blessings be upon him) said ((Make use of camel's urine, they are healing for stomach problems (Sahih Muslim), and this is why researchers have gone to search for alternatives that work as antibiotics (Haque *et al*, 2003). The study aimed to know the histopathological effects of rectum in laboratory mice infected with *E. histolytica* and treated with different concentrations of camel's urine and milk, and comparing it with the control group treated with the Flagyl drug and untreated infected group.

Materials and Methods

A - Isolation of *E. histolytica* Cysts

The cysts were isolated from the excrements of patients at Tikrit Teaching Hospital / Salah Al-Deen Governorate.

B - Laboratory Animals

Laboratory mice were obtained from the Center for Drug Control and Monitoring at the Ministry of Health / Baghdad / Iraq and the excrements of the mice were examined to ensure that they were free of parasitic diseases. Their number was 36, and their weight ranged from 25-30 gm with ages ranging from 4-6 weeks.

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C - Experiment Design

Laboratory mice were divided into three main groups, with three mice for each group. Each group was administered with three concentrations of camel milk 0.25.,0.5 and 0.75ml and the same concentrations of camel urine. Another group was dosed with a mixture of camel milk and urine with same concentrations and an untreated infected control group and a group treated with Flagyl drug. The laboratory mice were dosed with 1000 cysts to cause experimental infection with *E. histolytica*. After two weeks, the laboratory groups of infected mice were given treatment doses with different concentrations of milk and urine. After three weeks, laboratory mice were dissected to study tissue changes occurring in large intestine (rectum) for infected and treated mice. The required parts, which are two containers, were preserved with formalin at a concentration of 10% until to be used in preparing the tissue sections.

D - Collecting Urine and Milk Samples

The samples were collected from a six-year-old white milking camel (Al-Attas, 2008) making sure that it did not drink water for a period of no less than three weeks and not have eaten wild plants (El-khalifa, 2004) from Samarra region. The samples were collected in sterile bottles and preserved in the laboratory refrigerator with a temperature of $4C^{o}$, then the milk and urine samples were filtered to get rid of the suspended substances and used purely 100% without dilution. The chemical analysis of camel urine was conducted to determine the physical properties such as hydrogen PH, specific density and chemical components such as (fats - proteins - lactose - and total solids). The measurement was carried out at the Food Industries department/ College of Agriculture / University of Baghdad using the EKomi Llkanalyser device and the scientific efficiency was measured according to a study by (Xiao *et al*., 1996).

E - Preparing the Tissue Sections

The tissue sections were prepared according to (Bancroft and Stevens, 1982), where the samples were fixed using 10% formalin solution for 42 hours and dried by passing it in increasing concentrations of ethyl alcohol and then to a mixture of pure alcohol and toluene, and finally covered with paraffin wax and appeared in paper forms as cubes. The wax cubes were cut to a thickness of 4 micrometers using Rotary Microtome. The loaded slides were transferred to the water bath for 3 minutes at a temperature of $37C^{o}$. Finally, they were transferred to a hot plate with a temperature of $37C^{o}$ for tissue spreading. Harris-Hematoxylin and eosin stains were used in staining tissue sections.

Discussion and Results

The results of the experimental study showed occurrence of infection in orally administered laboratory mice in cystic stages with a 100% infection rate for amoebic dysentery, where feeding and cystic phases were detected in the excrement of infected mice (Blessmann *et al*., 2003).

The histological sections of large intestine portion of infected mice with *E.* histolytica showed that infection occurred as a result of parasite adhesion to mucous tissue of intestine and start decomposition of the tissue and its penetration by feeding stages and the occurring of different histological effects of necrosis in mucous layer with its erosion and the arrival of the feeding stages to the submucosa layer. This is consistent with Stanley *et al*. (2001) study, where tissue changes appeared when compared with the tissue sections of the control group (figures 1,2,3). The tissue changes were represented by occurrence of disintegration and spoilage with focal necrosis of the upper surface of the mucosa and oral degeneration and lymph cell infiltration of the vertical lining of the elongated folds and transmission of infection to the sub-mucous layer with the grouping of the parasite in the vicinity of the striated tissue (figures 4,5).

There was a clear and good improvement in intestinal layers of the large intestine of infected mice and treated with camels' milk and urine with different doses compared to the treatment with Flagyl drug. The degree of improvement varied according to the amount of dose administered, as there was less improvement in the intestines of mice treated with 0.25 ml of camels' milk or urine (single dose) and better in the intestines of mice treated with the mixed dose. The improvement in the intestines of mice treated with a mixed dose (figures6,7,8,9). This is due to the fact that the given dose of milk in the groups treated with concentrations of 0.25ml and 0.05ml was insufficient in terms of proteins including lysosyme, lactoferrin, lactoperaxodase, immunoglobulins, casein and vitamins, fats and minerals. Each of milk components has an important role in building the cells of the body and compensating for lost proteins,

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fats and electrolytes during the occurrence of diarrhea and the protection of cells. As well as the lack of vitamins A, C and E in the treated groups with 0.05 ml, which play an important role as antioxidants that give vitality to the body. In addition, the more doses given from camel urine, the more it contains bacteria that possess the biological control feature (Al-Hashem, 2000).

The histological examination of the large intestine of treated mice with a concentration of 0.75 mL of single dose camel milk and urine showed a clear improvement. The mixed dose showed the most improvement and the best among all the doses of the above-mentioned treatment (figures10, 11). It was represented by restoring the intestinal layers to the normal shape as it appeared coated with simple vertical cells, and the muscular layer well-formed and arranged in its internal circular and longitudinal shape. Also, camel milk shows biological activity because it contains proteins and peptides and high levels of Linoleic acid which is regarded one of the unsaturated fatty acids which is important to prevent the occurrence of diseases (Badr El-Din *et al*., 2007).Camel urine has the important role in eliminating the pathogen because it contains a unique type of antibody called the Nanoparhcle, which is characterized by the presence of only the heavy chain, which makes it of a small molecular weight, which resulted in penetration of the most difficult tissues and cells.

The plants that camels feed on have proven their natural and curative importance and it is the wisdom of Allah Almighty that the concentrations of these active substances in plants are diluted and simple so that the bodies can interact with them gently. In addition, the presence of more than one active substance in one plant which cooperate together in treating diseases, and this gives urine an advantage by containing these combined effective resources.

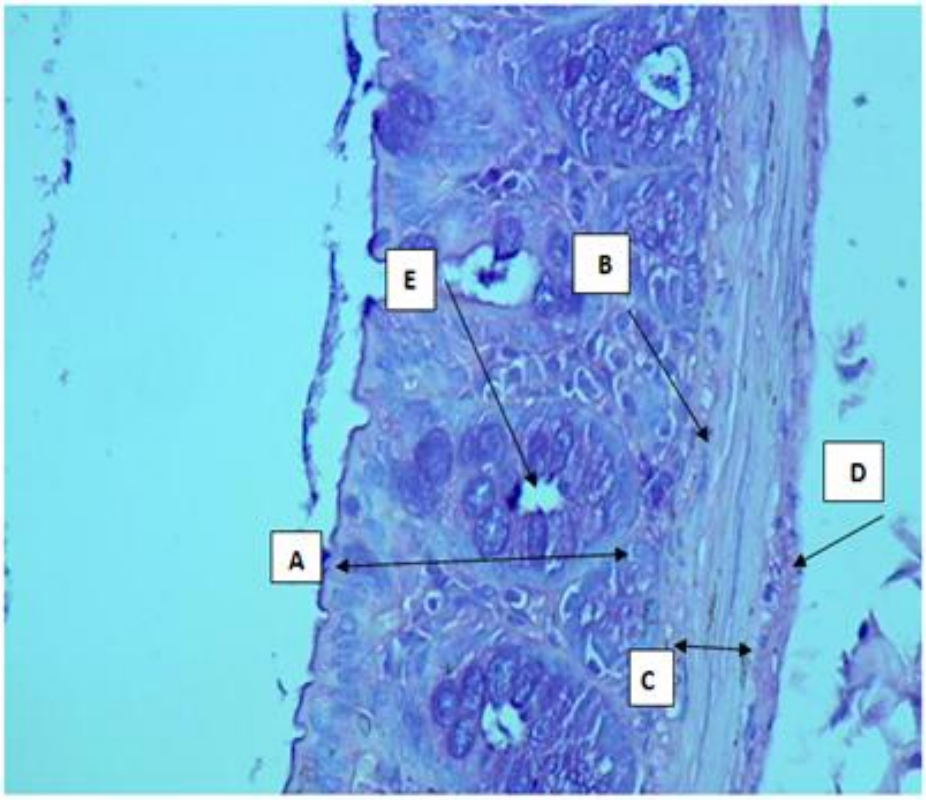

Figure 1. Rectum of mice (control): (A) mucose layer, (B) sub mucose layer, (C) muscle layer, (D) outer layer, (E) mucose gland, (H&E, 100X).

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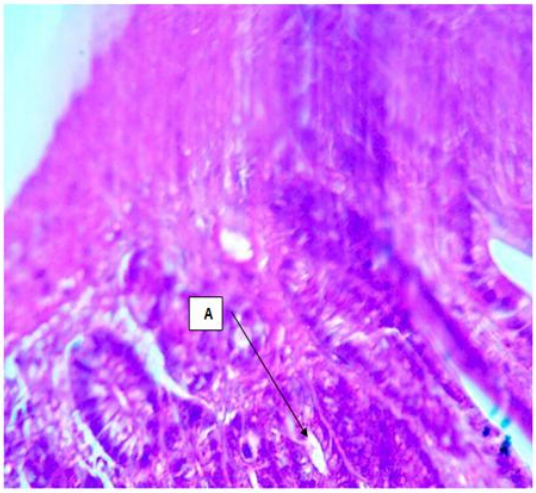

Figure 2. Rectum of mice (control) administer with camel urine (0.75 ml): (A)Goblet cells, (200 H&E200X).

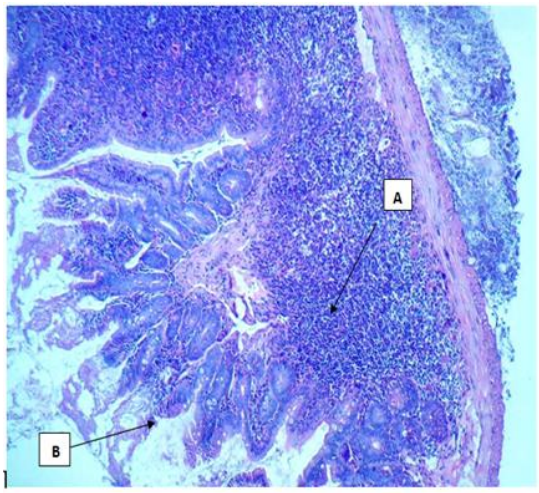

Figure 3. Rectum of mice (control) administer with camel urine and milk (0.75 ml): (A) hyperplasia of mucose layer, (B) degeneration of some epithelial cells of villi), (20 H&E 20X).

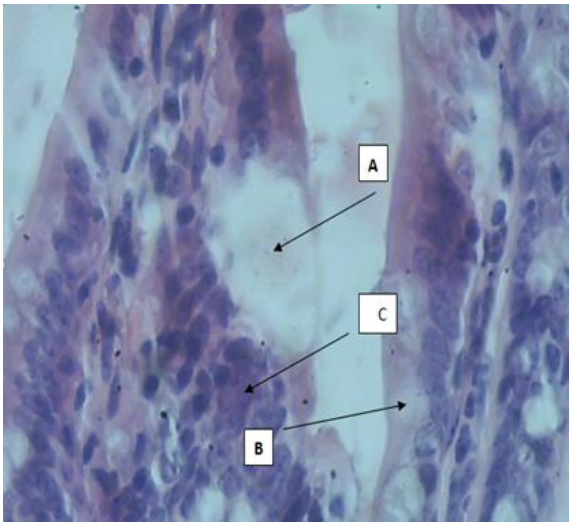

Figure 4. Rectum of infected mice: (A) cell alienation, (B) Goblet cells, (C) lymphocytes (H&E40X).

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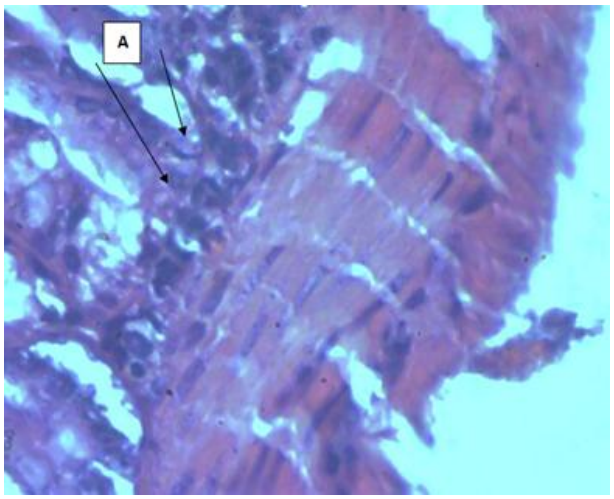

Figure 5. rectum of infected mice: (A) filteration of lymphocytes (H&E40X)

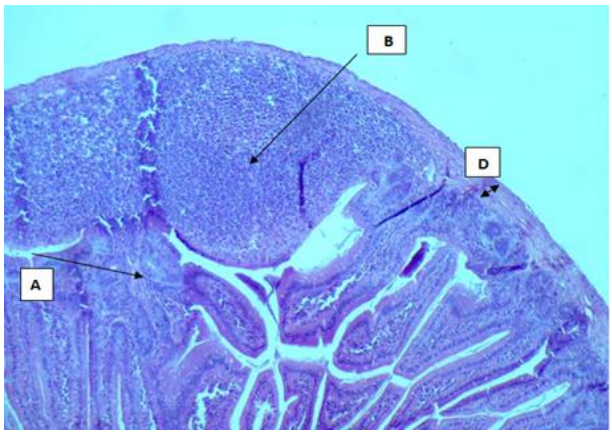

Figure 6. rectum of infected mice treated with antibiotic, (A) mucouse layer, (B) Hyper plasia of lymphnods, (C) less thickness of muscle layer, (H&E200X)

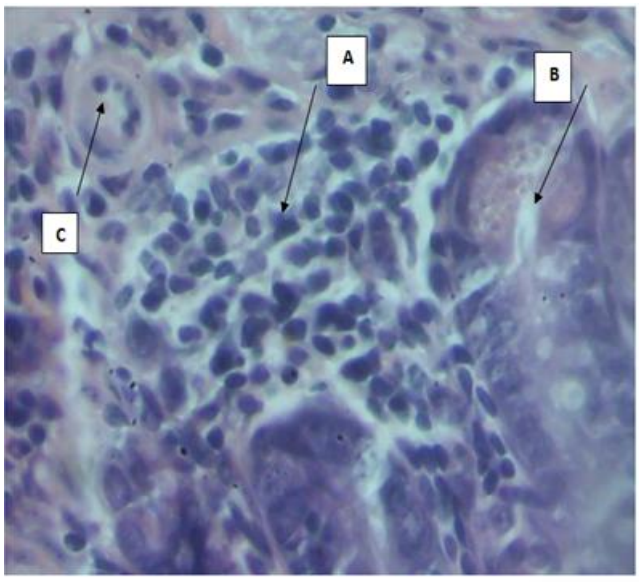

Figure 7. Rectum of infected mice treated with camel urine (0.5 ml): (A) filteration of lymphocytes, (B) mucosal gland, (C) blood vessles (H&E 40X)

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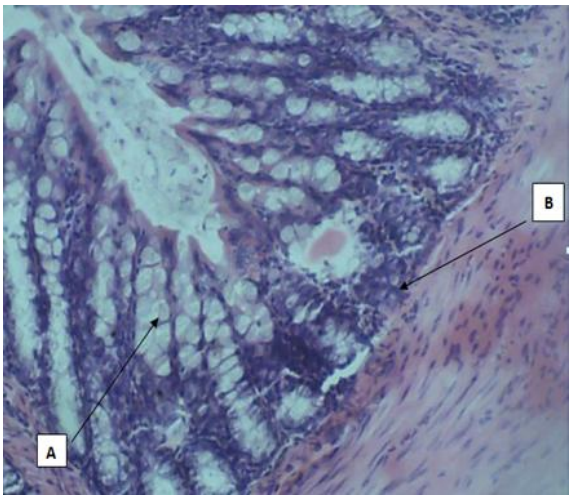

Figure 8. Rectum of infected mice treated with camel milk and urine (0.5ml): (A) diffusion of mucous gland, (B) lymphocytes, (H&E20X)

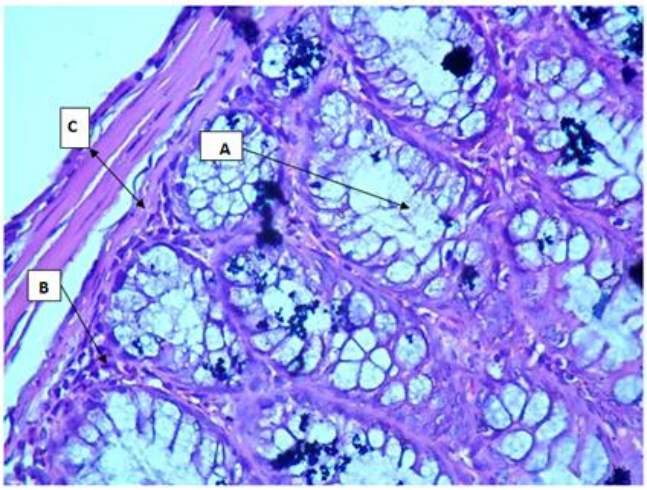

Figure 9. Rectum of infected mice treated with camel Milk and urine (0.5ml): (A) hyper plassia of mucose layer and glands and musine secration, (B) lymphocyte flteration, (C) dissociation muscle layer (H&E400X).

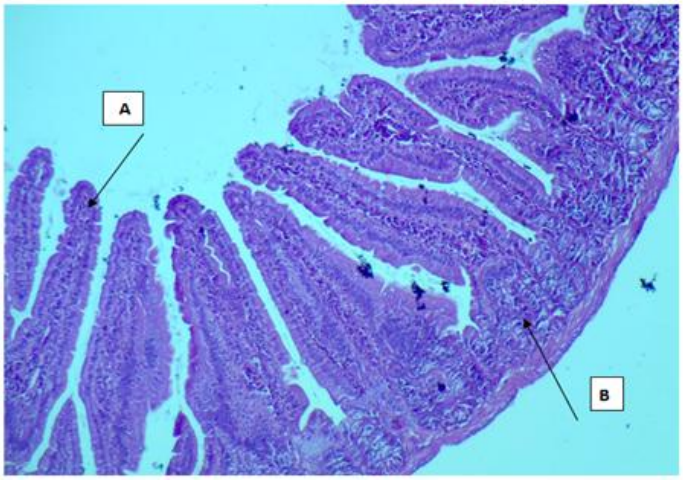

Figure 10. Rectum of infected mice treated with camel milk and urine (0.75ml): (A) hyper plassia of mucosal layer, (B) lymphocyte filteration, (H&E200X)

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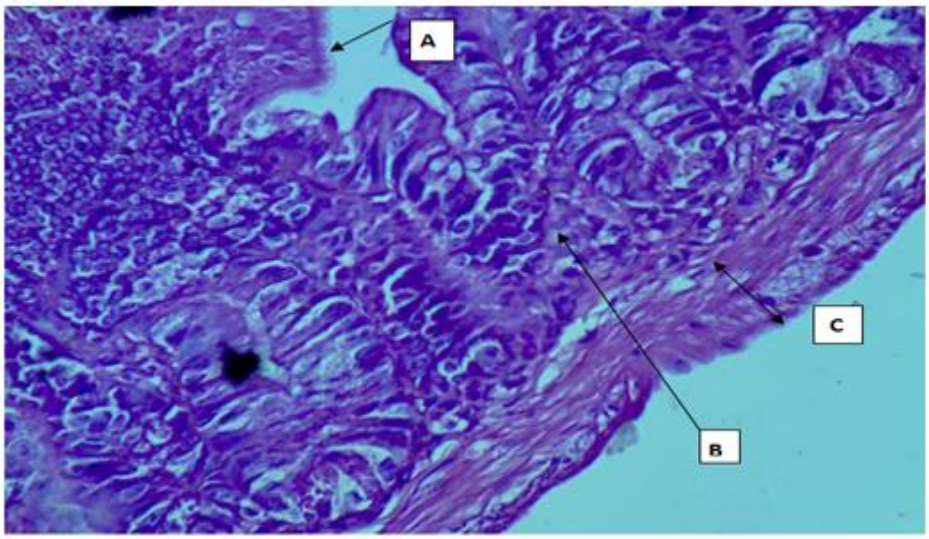

Figure 11. Rectum of infected mice treated with camel milk and urine (0.75ml): (A) epithelial cells, (B) hyper plassia of glands in of mucosal layer, (C) muscle layer (H&E200X)

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