ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

The Evaluation of IL-4, IL-6 and Histopathology Study in Mice Infected with *Entamoeba histolytica* and Treated with Proteins Extracted from Shrimp

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Abstract

Approximately 60% of the world's population is infected with intestinal parasites. *Entamoeba histolytica* constitutes one of the commonest protozoal infections in world, and it is estimated to infect 50 million cases of invasive amebiasis, and about 100,000 deaths and probably represent the third leading parasitic cause of death, behind only malaria and schistosomiasis. Oral inoculation of mice with ($1*10^3$ cell/0.1ml) cysts of *E. histolytica* isolated from the feces of 170 patients with amoebasis in Ibn Al-balady hospital, AL-Kindi Teaching hospital and Fatima Al-Zahraa hospital in Iraq resulted in an infection between (2-3) days after inoculation with the parasite .Among all the infected mice as shown by the presence of trophozoites in the large intestine and colon. Many histological abnormalities in the large intestine and the liver were detected in experimentally infected mice with *Entamoeba histolytica*. These abnormalities as necrosis, severe hemorrhage, increased in numbers of goblet cells, congested blood vessels. High level of IL-6 and IL-4 in the positive group compare with the negative group and the groups that treated with flagel and the group that treat with shrimp extracted proteins.

Keywords: *Entamoeba histolytica*, Shrimp extracted proteins, IL-4, IL-6, Histopathology study.

Introduction

Entamoeba histolytica is a most important protozoan parasite that cause the amoebasis disease [1].Which is the second leading cause of parasitic mortality worldwide. The major manifestation of disease is amebic colitis [2]. However, interestingly, only approximately 10 % of intestinally colonized individuals develop invasive disease. It usually causes destroying in the tissue (internal inflammation) of the large intestine and liver [3]. *E. histolytica* has two morphology, trophozoit the amoebic shape, active phase, (15-20) µm size, this phase has single nucleus with a small central karyosome, the chromatin is eventually distributed along in the peripheral of the nucleus [4].

Cyst are the dormant, can survive for prolong periods, spherical with retractile wall the cytoplasm of the cyst contain dark staining chromatoid bodies. The number of cyst, s nucleus [1, 4] each nuclei has central karyosome with peripheral chromatin [5]. Infection with *E.histolytica* done by the ingested with the cysts and other risk factors help the transmitted by fecal-oral route, contaminated water and food [6]. Intestinal amobasis is still an important health problem in the developing countries in world [7]. The chemical drugs some time have side effect over the years [8].

Shrimp considered from crustacean composed from protein which peptides binds to each other, these peptides most are small peptides less than 10 kd are positive and amphipathic and gives fast and immediate effect when invade microorganisms sometime used against the cancer disease [9]. In parasitic invasions, an increase is observed in the production of IgE antibodies, especially in helminth infections [10].

This defect results from disturbances in the regulation of antibody production by Th cells, which promotes local inflammatory reaction.

Via release of mediators from mast cells IgE participates in the reaction of antibody dependent cellular cytotoxicity (ADCC) [11]. Cytotoxic activity of eosinophil is increased under the influence of cytokines (TNF- α and IL-5) released by mast cells, lymphocytes, and macrophages. Th2 lymphocytes synthesize specific cytokines (IL-4, IL-5, IL-6, IL 10, IL-13, and IL-14), which play a major role in the pathogenesis of parasitic diseases [12].

Aim of this Study

This study was aimed to detect the rate of IL-6 and IL-4 in mice infected with *E.histolytica* and treated by the shrimp extracted proteins.

Materials and Method

Collection of the Sample

The present study included 170 stool samples were collected from patients actually infected with *Entamoeba histolytica* in Ibn Al-balady hospital, AL-Kindi Teaching hospital and Fatima zahraa hospital in Iraq from October 2017 to April 2018. The sample was collected in clean plastic cup. The sample was examined to ensure that have troph and cyst of *Entamoeba histolytica*.

Preparation of Extraction

This study include extraction of whole bodies of shrimp (fresh shrimp) in the first its washed with distilled water after clean it, and keep it in deep freezer (-1C) till it used .put the shrimp in mixer with 50%from acetic acid and mixed for 10 mint continuously, the mixture was left for 24h in (23-26)C^0.

The mixture was put in plan tube and centrifuged in centrifuge at 8000 cycle /mints for 15 mints .take the supernatants and put in dialysis tube in beaker with distilled water and exchange the water every 6 h for 48h. At the end of dialysis the solution and stored in (24-26) C^0.The concentration of the proteins of the shrimp extraction was masseur by nanodrop [13].

Preparation of Animal Laboratory

50 white swiss mice male and female were used, this mice was obtained from the national center for research and drug. The average of age to this mice was (6-13) weeks, the weight was (18-23) gm. all mice putted in a clean plastic cages that proved with sterile water and the special food of mice. All cages put in the animal house of the laboratory all feces of mice were examined to ensure that is free from any infection.

The Animal Experimental

50 mice divided to five group each group include ten mice, 30 mice was given ($1*10^3$ cell/0.1ml) to infection the mice by parasite and ensure the mice was infected .20 mice still without infection as negative control .Inoculated the mice orally by single dose every day through the experimental for 10 days.

- Group 1: infected group treated with 0.1ml from metredanozol.
- Group 2: infected group treated with 0.1ml from shrimp extraction 2000 mg/ml.
- Group 3: non infected group inoculate with 0.1ml from shrimp extraction 2000 mg/ml consider as positive control for the extraction.
- Group 4: infected group inoculate 0.1ml N.S this group consider as positive control.
- Group5:0.1ml N.S this group without infection as negative control.

At the end of treatment period, mice in each group were killed, and collected the blood from the ocular vain, the liver and large intestine the of each mice immediately fixed with 10% formalin for the histopathology examination

Result and Discussion

IL-6 and IL-4result

Table (1) showed the level of IL-6 in all treated groups after 10 days of treatment after the infection with the *E.histolytica* , the result was showed high level of IL-6 in the positive group compared with other groups (167.5±12.7)pg/m, the negative group concentration of IL-6 was the low rate among the groups(55.8 ± 4.0)pg/ml, the group that only inoculation with the shrimp extracted proteins show level that be near to the negative group (56.8 ± 6.7)pg/ml, the group that treatment with the shrimp extracted proteins was showed (162.3 ± 9.3)pg/ml and the group that treated with the flageal (metredanozol) was (114.8 ± 10.3)pg/ml.

Table 1: Compare between different groups of IL-4 rate

Group	Concentration of IL-4 pg/ml
	Mean ± S.D
Control positive	105.8 ± 8.
Control negative	29.7 ± 4.4
Flagyl	90.1 ± 6.8
Shrimp extracted protein	30.7 ±1.3
Control positive +shrimp extracted proteins	127.5 ± 4.7
LSD	7.38

The result of IL-4 concentration in the table (2) showed the result was high level of IL-4 in the positive group compared with other groups (105.8 ± 8.9)pg/ml, the negative group concentration of IL-4 was the low rate among the groups(29.7 ± 4.4)pg/ml, the group that only inoculation with the shrimp extracted proteins show level that be near to the negative group (30.7 ±1.3)pg/ml, the group that treatment with the shrimp extracted proteins was showed (127.5 ± 4.7)pg/ml and the group that treated with the flageal (metredanozol) was (90.1 ± 6.8)pg/ P<0.05,significant different

Table 1: Compare between different groups of IL-6 rate

Group	Concentration of IL-6 Pg/ml
	Mean ± S.D
Control positive	167.5±12.7
Control negative	56.8±6.7
Flagyl	114.8±10.3
Shrimp extracted protein	55.8±4.0
Control positive +shrimp extracted proteins	162.3±9.3
LSD	11.5

P<0.05, significant different

Histopathology Result

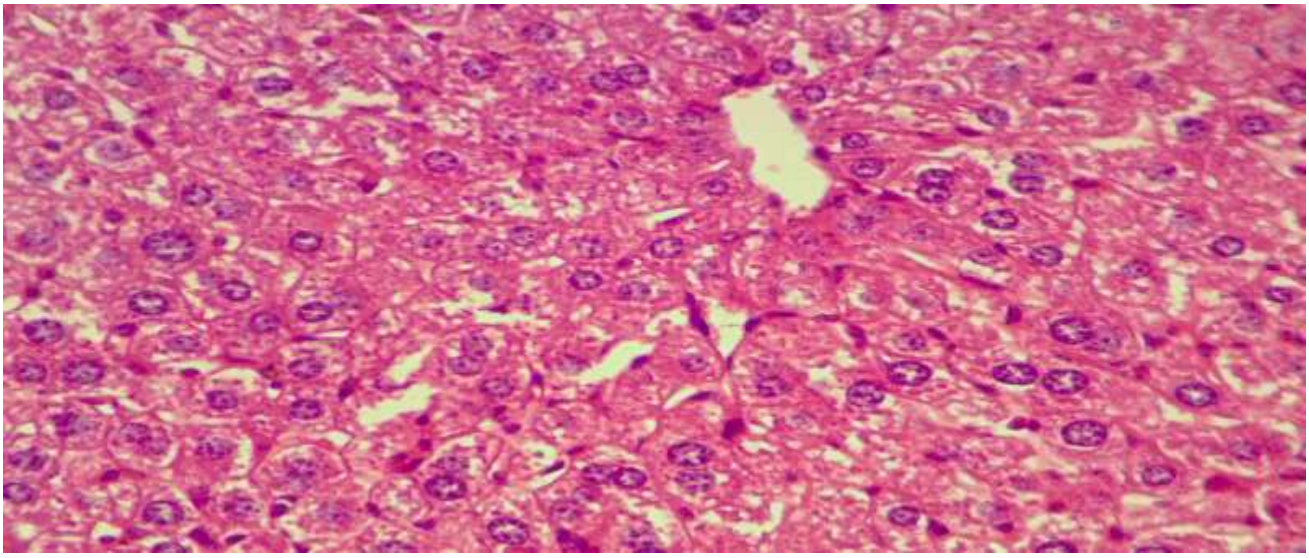

Fig. 1: A Section of liver in mice control negative was showing normal structure appearance of hepatic tissue with consisting central vain surrounded by thread of hepatocyte (400x)

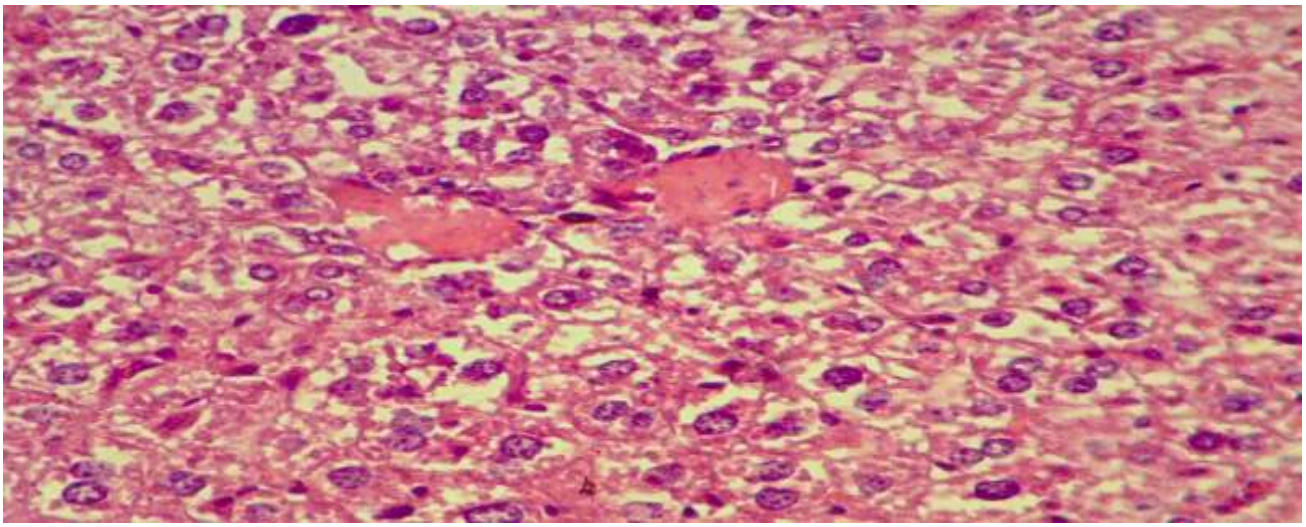

Fig. 2: A Section of liver in mice control positive was showing congestion of blood vessels with depletion of glycoprotein granules, some cells showing apoptosis (400x)

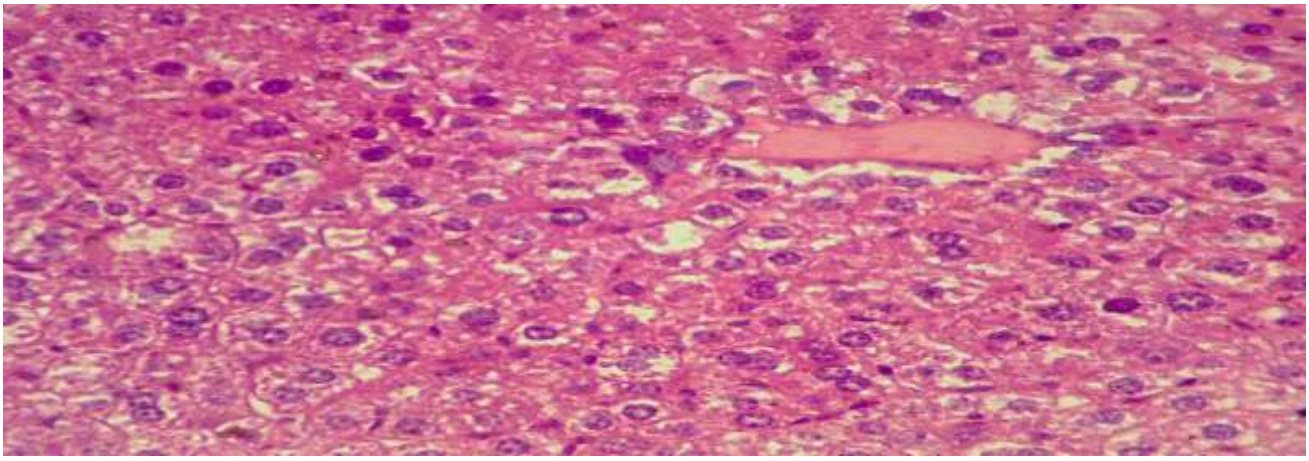

Fig. 3: A Section of liver in mice that inoculation by shrimp extracted protein was showing congestion of blood vessels with depletion of glycoprotein granules, and few apoptotic cells (400 x)

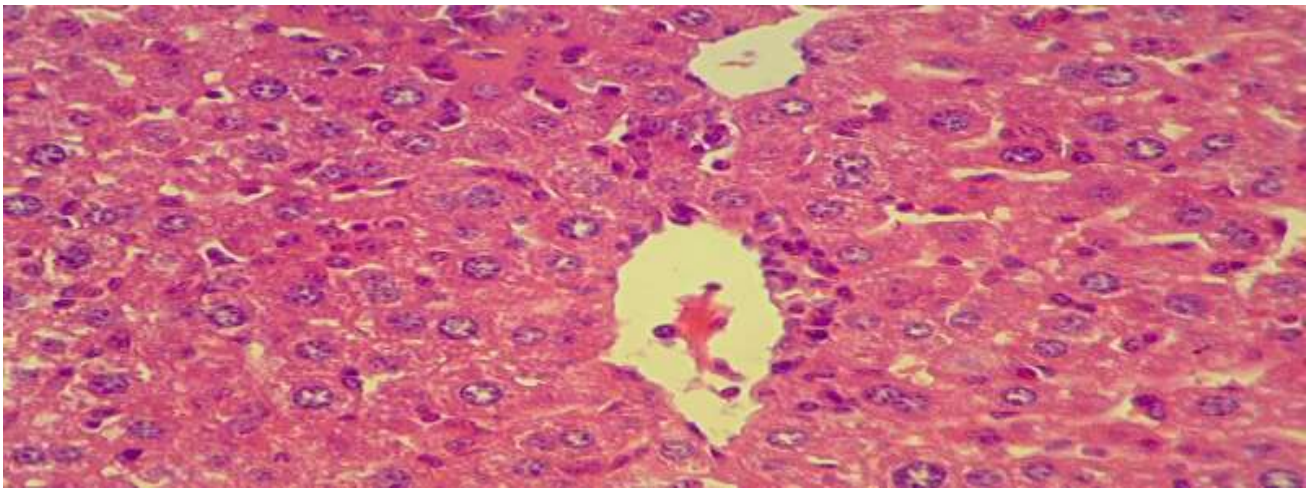

Fig. 4: A Section of liver in mice that infected with *E.histolytica* and treated with flagel was be near the normal appearance in which the mild of inflammation cells infiltration with increase the kupffer cells (macrophage cells) (400x)

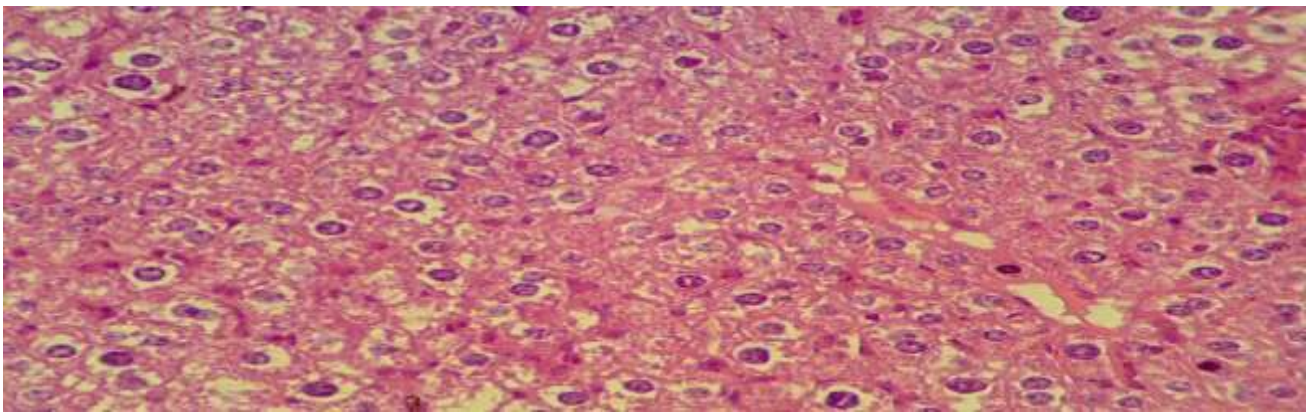

Fig. 5: A Section of liver in mice that infected with *E.histolytica* and treated with shrimp extracted protein was showing mild to modulate of glycoprotein granules depletion with still few apoptotic cells (400x)

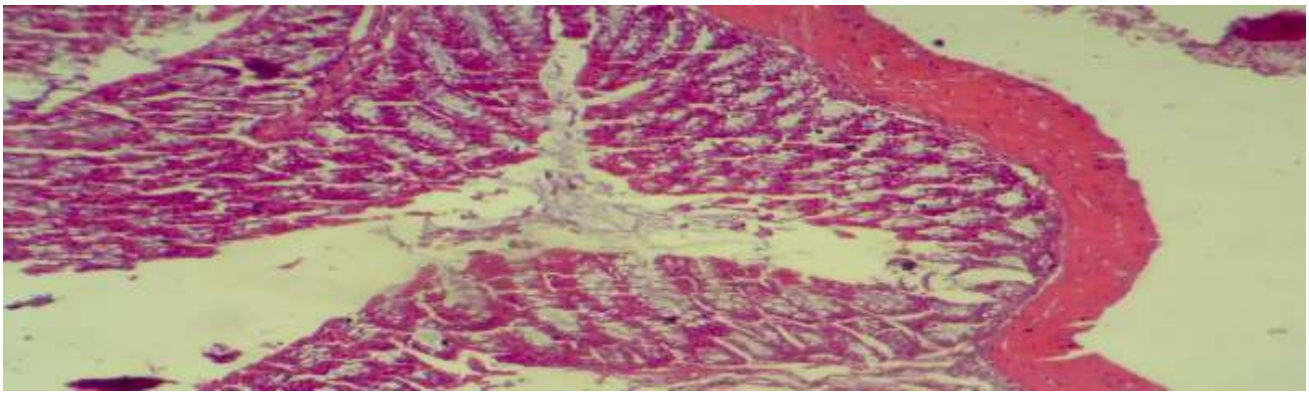

Fig. 6: A Section of large intestine mice control negative was showing normal structure appearance of colonic mucosa with it crypts (100x)

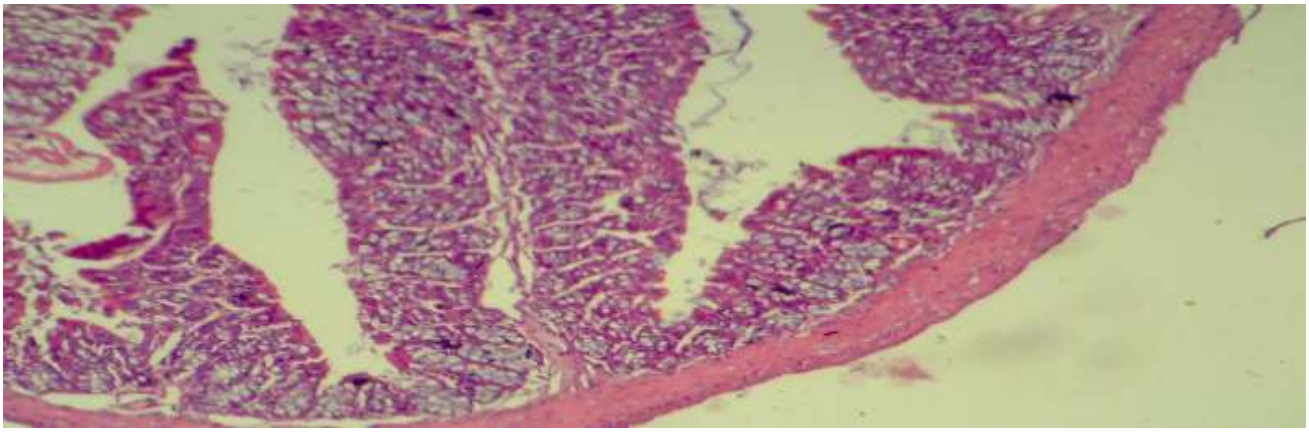

Fig. 7: A Section of large intestine mice control positive was showing slight (intermittent) shorting of the colonic mucosa (100x)

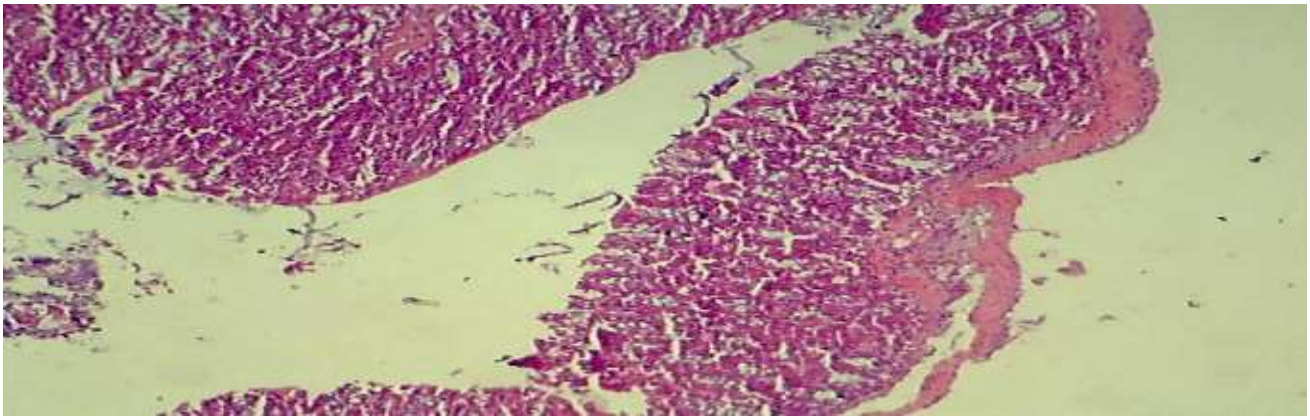

Fig. 8: A Section of large intestine (colon) mice control positive was showing slight shorting of the colonic mucosa, with presence of abundant of granules crypt's (100x)

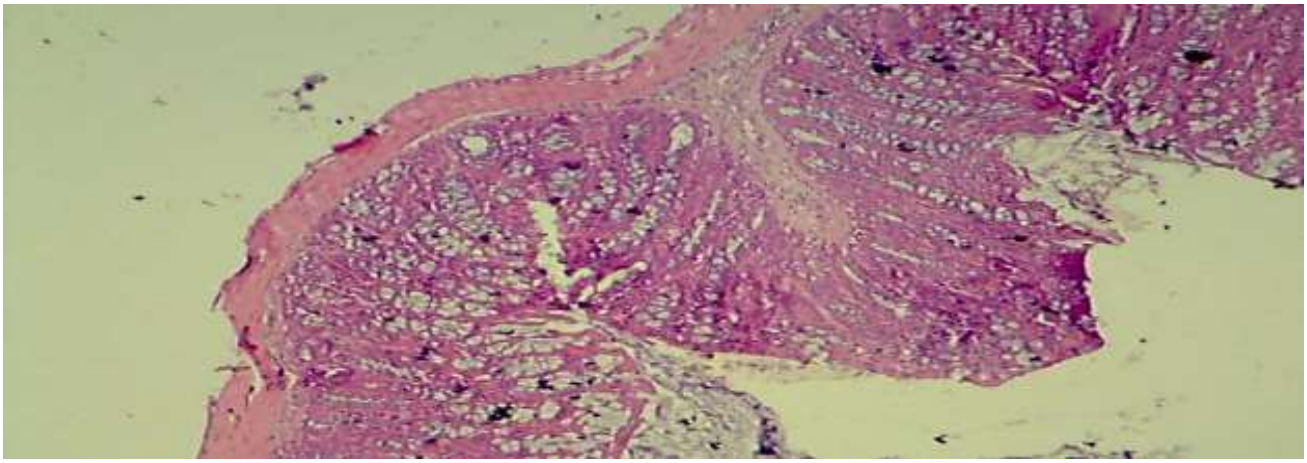

Fig. 9: A Section of colon in mice that infected with *E.histolytica* and treated with flagel lock like the normal appearance of colonic mucosa but with increase in the main secretion of mucus (100x).

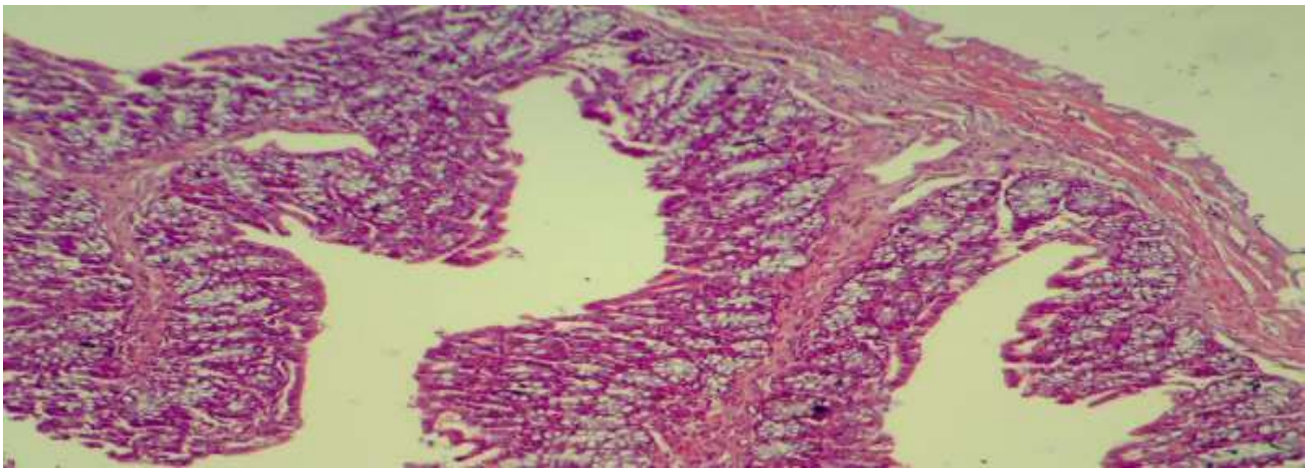

Fig. 10: A Section of large intestine in mice that infected with *E.histolytica* and treated with shrimp extracted protein showing slight shorting of the colonic mucosa (intermittent) (100x)

The Effect of the Shrimp Extracted Proteins on Liver Tissue in Mice

The histopathology study of the mice liver showed this result in different group of the study. In the group that do not infection whit any pathogen (control negative) the historical result was showing normal structure appearance of hepatic tissue with consisting central vain surrounded by thread of hepatocyte as in the Figure (3-1), in the group that infection with *E.histolytica* (control positive) the result was showing congestion of blood vessels with depletion of glycoprotein granules some cells showing apoptosis as in Figure (3-2), in the group that inoculation with the shrimp extraction proteins only the result was showing congestion of blood vessels with depletion of glycoprotein granules, and few apoptotic cell, in the group that infection with *E.histolytica* and treated with the flagel the liver section was be near the normal appearance in which the mild of inflammation cells infiltration with increase the kupffer cells (macrophage cells), while in the group that treated with shrimp extraction proteins the liver section was showing mild to modulate of glycoprotein granules depletion with still few apoptotic cells as in Figure (3-5).

The Effect of the Shrimp Extracted Proteins on the Large Intestine Tissue in Mice

The histopathology study of large intestine in mice with different group showed this result. in the group without any infection (control negative) showed that the Section of large intestine mice control negative was showing normal structure appearance of colonic mucosa with it crypts as in Figure (3-6), in the group that infection with *E.histolytica* (control positive) the result was showing slight (intermittent) shorting of the colonic mucosa in large intestine like Figure (3-7), in the group that inoculation with the shrimp extraction proteins only the result was showing that the Section of large intestine (colon) mice shorting of the colonic mucosa, with presence of abundant of granules crypt's like Figure (3-8), in the group that infection with the *E,histolytica* and treated with flagel the result was showing that the Section of colon in mice that infected with *E.histolytica* and treated with flagel lock like the normal appearance of colonic mucosa but with increase in the main secretion of mucus as in Figure (3-9) while while in the group that treated with shrimp extraction proteins the Section of large intestine in mice showing slight shorting of the colonic mucosa (intermittent) as in Figure(3-10).

Discussion

Amoebic colitis produced high levels of Th2 cytokines. Yet fundamental questions of whether humoral especially (systemic) or cellular (Th2) immunity or both induced during the course of intestinal amoebiasis The immune and inflammatory responses have a central role against E. histolytica infection there by the cytotoxic activity of the immune cells and their ability to produce a widerange of cytokines which play a crucial role in the deffance like interferon gamma IFN–γ , tumor necrosis factor alpha TNF–α and interleukins ILs which recruit the inflammatory process against parasite [14]. The serum concentration of one of Th2 cytokine or IL–4 in infection with intestinal amoebiasis was evaluated.

The results indicated that most of positive control group have a high concentration of IL-4 in their sera in comparison with the other control group in this study and these results were also indicated by [15, 16]. IL–4 represented as one of the cytokines which produced by Th2 cells and act as a cofactor in activation of humoral immunity by activation of B-cells and T-cells proliferation and differentiation [17, 18]. IL-6 is a proinflammatory cytokine, affects various processes including, the immune response reproduction, bone metabolism and aging. IL-6 is synthesized by mononuclear phagocytes, vascular endothelial cells, fibroblasts and other cells in response to trauma, burns, tissue damage and inflammation, IL-6 also has many regenerative or anti-inflammatory activities [19, 21].

The present study showed a higher rate of concentration of IL-6 in the positive group compared to the control group with significant differences ($p<0.05$) for the group of study .These results agree with studies of [22, 23]. The high level of IL-6 in positive group with amoebiasis suggests that local inflammatory processes may play a role in increasing level of IL-6 in amoebiasis patients, in early day of infestationIL-6 plays an important role in the immune response Its

manifestation of Th2 response assistant factor in the activation humoral immune cell by activating immune cell, IL-6 Cytokine production by inflammatory cells is also produced by primary intestinal epithelial cells in response T-cell and stimulating presence [24]. In most cases, trophozoites in the intestine live as commensals occasionally; however, trophozoites attack and invade the intestinal mucosa causing dysentery and or progress through the blood vessels to extra-intestinal locations like liver, brain and lungs, where they may form life threatening abscesses.

Most of trophozoites were found attached to interglandular epithelium, the trophozoites have been found associated with the micro ulcerations of the mucosa associated with thinning of the mucus layer, a shortening of the microvilli, bleeding, degradation of the extracellular matrix, cell vaculation, necrosis, hemorrhage with compression and distortion of individual cells resulting from the presence of large numbers of trophozoites [25]. Also, increased in numbers of goblet cells, cytoplasmic vacuoltions, congested blood vessels cysts undergo excystment and each cyst gives rise to eight trophozoites.

These migrate to and multiply in the colon. In most cases, trophozoites in the intestine live as commensals. Occasionally, however, trophozoites attack and invade the intestinal mucosa causing dysentery and/or progress through the blood vessels to extra-intestinal locations like liver, brain and lungs, where they may form life threatening abscesses. *E. histolytica* is cytotoxic to a variety of cell types, including neutrophils, T lymphocytes, macrophages and a variety of tissue culture lines.

Although cytotoxic activity is what the parasite was named for, the mechanism is an enigma. In a stepwise process, *E. histolytica* adheres to the target, induces its death, and then ingests the killed cell. Killing of the target cell appears to be primarily via activation of apoptosis, that is to say, the parasite 'tricks' the host cell into killing itself. *E. histolytica*-trophozoites reaching the liver create their unique abscesses, which are well circumscribed regions of cytolysed liver cells, liquefied cells, and cellular debris.

The lesions are surrounded by connective tissue enclosing few inflammatory cells and trophozoites. Parench ymal cells adjacent to the lesion are often unaffected. However, lysis of neutrophils by E. *histolytica* trophozoites might release mediators that lead to the death of liver cells, and extend damage to hepatocytes not in direct contact with the parasite. Studies have shown that in ALA in mice, most hepatocytes die from apoptosis, but necrosis is also pres ent.

In ALA from humans, the small numbers of amoebas relative to the size of the abscess suggests that E. *histolytica* can kill hepatocytes without direct contact [26]. The shrimp extracted proteins has shown beneficial effects because of the high protein material in the Shrimp composition from essential amino acids (methionine, tryptophan and lysine) this amino acid is antimicrobial activity (anti-fungal anti-bacterial) [27].

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