The effect of infection with *Entamoeba histolytica* on the level of some biological variables and histological changes in the liver

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ABSTRACT

Background: *Entamoeba histolytica* is an intestinal protozoan parasite that causes "dysentery," leading to Amebic colitis and a liver abscess. Infection begins with ingesting infective stages, represented by the cystic form found in contaminated food and drink; the parasite attacks the tissues by attaching to the epithelial lining of the intestine, and adhesion occurs through virulence factors. **Objective:** Detection of the *E. histolytica* parasite using the direct swab method, study the histological changes in the liver and some blood parameters of animals infected with *E. histolytica* and compare these with uninfected groups. **Material and method:** The current study was conducted between May 1, 2021, and October 30, 2022, to diagnose infection with *E. histolytica*. Samples were taken from clinically infected patients who suffered from diarrhea and examined microscopically using a direct wet swab. In the experimental study, *E. histolytica* was given to laboratory mice. Male laboratory mice were divided into four groups. The first group represented the negative control group, dosed with Normal saline only. **Results:** The negative control group showed normal liver histological sections. The hepatic lobule appeared to include hepatic cells, which were arranged radially, as they appeared as cords extending from the central vein, in addition to diagnosing normal liver cells. With central round nuclei and a homogeneous appearance of cytoplasm, hepatic lamellae, and hepatic sinusoids, the second group represented the positive control group. It was treated with the parasite *E. histolytica*, which recorded numerous histological changes in the livers of the group, which included irregular radiological appearance of the hepatic cells around the hepatic vein, infiltration of lymphocytes, and necrosis and swelling of some hepatic cells, there was an increase in liver enzymes, indicating infection with *E. histolytica*. **Conclusion:** Laboratory animals infected with *E. histolytica* had histological changes in the liver, represented by necrosis, nucleolytic, and amoebic liver abscesses. Although the parasite infects the intestines and settles there, it causes secondary infections through its transmission through the blood and lymph to the liver.

Keywords: Histological changes, liver, enzymes, E.histolytica.
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1-INTRODUCTION

The disease caused by the parasite *Entamoeba histolytica* is called intestinal amoebiasis (colitis). Ulceration begins in the intestine in the rectum and appendix area or at the top of the colon and may spread along the length of the colon. The increase in the number of parasites in the ulceration area increases the destruction of the mucous layer. The ulcer is at the basement membrane or musculoskeletal layer and then begins to erode gradually, causing a superficial area of coagulation to occur. This initial ulcer is accompanied by bacterial invasion, and the cellular response in the host is minimal (1).

In chronic infections, the amoeba invades the mucous layer, decomposes the submucosal layer, and penetrates the muscular and serous layers. Thus, the nutrient phase can be transmitted through the blood and lymph to other organs, hence forming secondary infections. A high percentage of deaths occur as a result of colon penetration with peritonitis. Sometimes, a granular mass called an amoeboma forms in the intestinal wall, which leads to intestinal obstruction. This is due to the host's cellular response to chronic ulcers, which usually contain active nutrient phases. Secondary infections can be found in all body organs, but the liver is the most common organ. It is most affected in about 5% of cases (2).

2-Material and Method

Parasite Diagnosis of macroscopic Examination:

This method included describing the shape and texture of stool samples as soft, light, watery, or semi-solid, indicating their type of parasitic organisms. The vegetative stages of intestinal primary animals are often found in a soft, light sample, while the cystic stages of these parasites appear in large, hard samples (3). It was also observed whether the stool sample was bloody or mucous, as the presence of blood and mucus indicates an infection, so samples containing blood and mucus are given special care. In such a case, the sample must be taken from the blood-stained and mucus-containing areas. It should also be noted whether the stool is greasy; the sample's color and smell, which may be moldy or spoiled, should be noted.

Laboratory animal groups

Examining the feces of the mice before starting the experiment to ensure that they were free from infection with parasitic intestinal infections by placing a small number of feces on a glass slide and mixing it with a bit of Logul's Iodine, the slide was covered and examined under a microscope, (10) and then mice were killed as they were considered as a negative control mice group. Then, ten mice were dosed with amoebic sludge after the dose was determined, as it was given orally using Gavage. The animal was dosed by holding it in a way that did not allow it to move. Gavage was introduced into the mouth, and after making sure that it was inserted into the esophagus, the dose was slowly pushed into the stomach of the animal. After the dose Mice infected with *Entamoeba histolytica* were placed in clean cages, and stool was collected and examined by microscope to determine the incidence of infection. The examination took place 48 hours after the dose, and after confirming the presence of infection, a group of mice was taken, and stool samples were collected from them and kept in a tube. They were considered a positive control group and were not treated with any treatment.

Testing Blood samples

Blood biochemical parameters were determined using a 1.5 ml blood sample collected by cardiac puncture, transferred to blood collection tubes, and centrifuged at 3,000 × g for 15 minutes. Serum was collected to determine aspartate aminotransferase (A.S.T) and alanine aminotransferase (A.L.T) by using RANDOX kits to estimate the effectiveness of the GOT and GPT enzymes in serum respectively as recommended by the manufacturing company according to the method of (4). The ALP (Alkaline phosphatase) enzyme level was estimated according to the method mentioned in the ALP determination kit, according to the method of (5).

3-Results

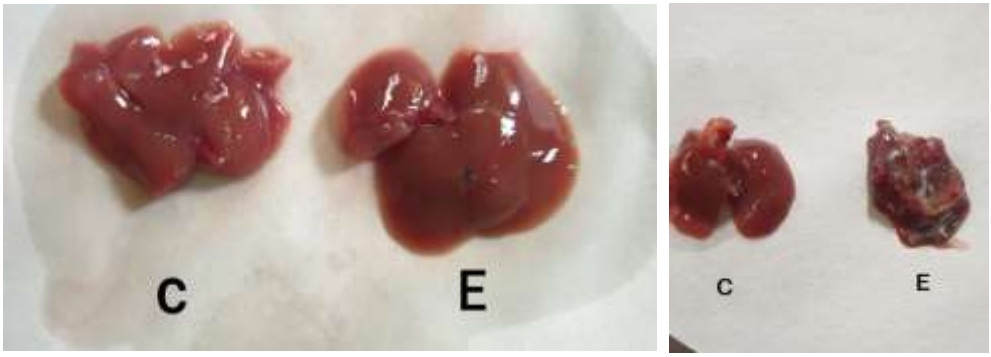
Table (1) shows the Variations in the levels of AST, ALT, and ALP in animals infected with *E. histolytica* infection:

			Enzymes / **Group**
ALP I.U/L	**(ALT) GPT I.U/L**	**(AST) GOT I.U/L**	
a 24.38 ± 2.47	31.2±1.61 a	32.75±4.79 a	Negative control
b 46.25 ± 4.95	47.67±4.23 b	57.44±2.87 b	Positive control

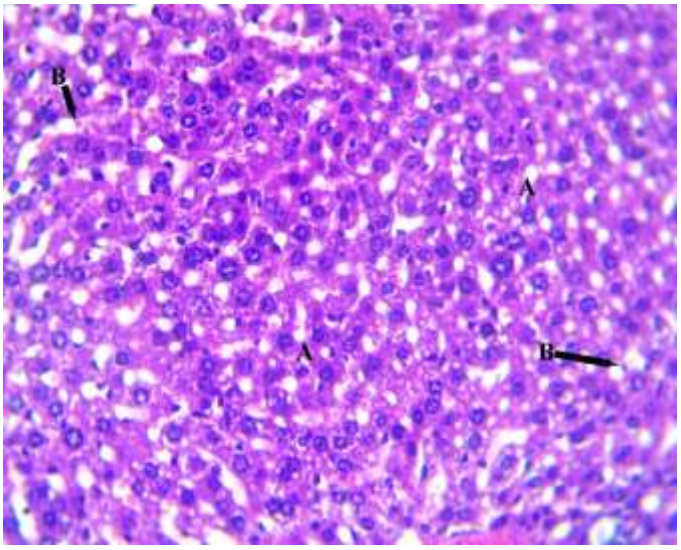
*Different letters indicate significant differences (probability ≤ 0.05) between the rates of the different groups.

E. histology Morphological changes of the liver

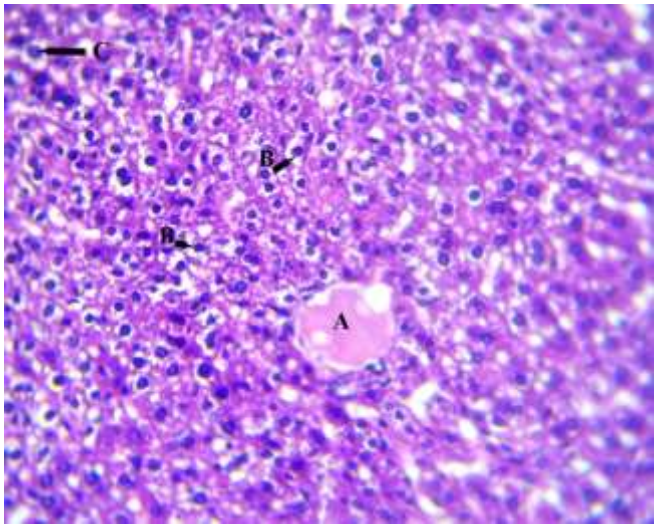
The results of the visual examination of mice infected with the parasite *E. histolytica* showed congestion and enlargement of the liver. Liver deformity reached a large degree in the infected groups compared to control groups in which the liver appeared normal and without deformities. Congestion was observed in the external appearance of the liver, with a change in color and the appearance of dark areas. The liver showed blood congestion and dark scarring.



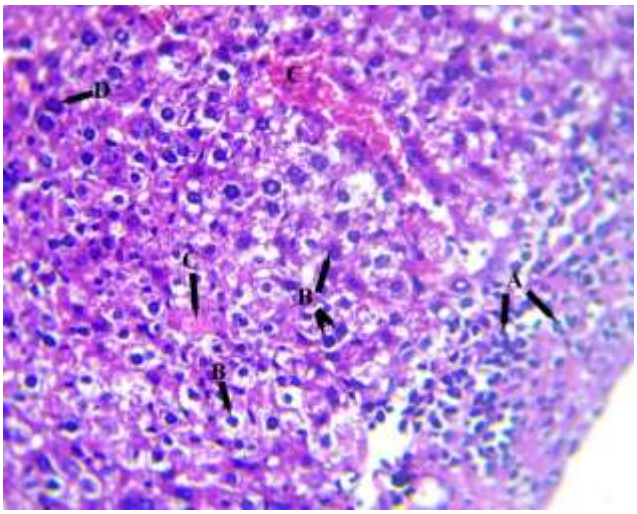
The letter C indicates the liver of the negative control group, while the letter E indicates the positive control group infected with *E. histolytica*.



Figure(1): A section of the liver tissue of the negative control group showing (A) the liver tissue containing rows of tangled liver cells, (B) the blood sinusoids containing red blood cells and Kupffer cells (H&E 400X).



Figure(2): Liver tissue of the positive control group infected with *E. histolytica*, showing (A) a central vein with decomposed blood, (B) hyperplasia of liver cells with bulging of the cytoplasm and thickening of their nuclei, (C) parasitic particles (H&E 400X).



Figure(3): Liver tissue of the positive control group infected with *E.histolytica*, showing (A) lymph node infiltration in the liver tissue, (B) hyperplasia of liver cells with cytoplasmic bulging and thickening of the nuclei, (C) blood congestion in the sinusoids, (D)) Parasitic particles (H&E 400X).

4-Discussion

The results of the current study recorded an increase in the concentration of aminotransferase enzymes (A.L.T, A.S.T) in groups infected with *E. histolytica* compared to the negative control group, as in Table (1). In general, the A.L.T enzyme is considered specialized for liver cell activity. In contrast, the A.S.T. enzyme is usually found in various tissues of the body, especially the heart, liver, kidney, and skeletal muscle. In general, any damage that occurs in liver cells due to the invasion of the parasite, which possesses some tissue-degrading enzymes, leads to the release of the enzyme into the blood, thus increasing its concentration. This explains the increased effectiveness of (A.L.T and A.S.T) for groups infected with *E. histolytica*; Liver alkaline phosphatase (ALP) levels and acute liver abscesses have increased AST and ALT levels, Aminotransferase levels are sensitive indicators of liver cell injury and help identify liver cell diseases like liver abscess; therefore, these enzyme levels serve as markers. More specifically, when the liver cell membrane is broken, both enzymes are released into the bloodstream at greater levels. Liver cell senescence also confirms the release of aminotransferases (6).

The ALP enzyme showed highly significant differences in the second group (Positive control) compared to the First group (Negative control) , However, the differences did not reach significance between all groups, and the initial group was also not noteworthy in contrast to the control group. These enzymes are typically found in muscle cells to a lesser degree and mostly in liver cells. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in the blood rise when the liver is injured or damaged because liver cells leak these enzymes into the bloodstream. ALP is a material in the bile ducts of the bones, intestines, and liver; it signifies liver disease. High levels of ALP can result from bile duct damage or obstruction, and these tests can reveal a variety of details regarding a range of pathological alterations (6). This is in line with the findings of (7), who noted that in patients with diarrhea, there was an increase in the level of AST and ALT enzymes in the serum, as 90% of cases had an increase in the ALP level, this outcome agreed with the findings of (8) because the serum levels of liver enzymes in patients with parasite infection are incredibly high, and (9) showed a rise in liver enzyme levels. However, this is at odds with the data that have been collected. (10) That patients' liver function tests showed elevated levels. The current study agreed with the study of (11), which indicated that the survey revealed that in addition to altering the biochemical parameters (ALP, AST, and ALT), the *E. histolytica* parasite also caused histological changes in the organs, particularly the liver, such as programmed cell death.

E. histology Morphological changes of the liver showed that the mucous layer of the intestine, with its hydrolytic enzymes, causes its perforation and is transmitted to other organs, such as the liver, causing abscesses. This is consistent with the results of (12) and (13), who indicated the occurrence of macroscopic changes and congestion in the internal organs of infected mice with *E. histolytica*.

An amoebic liver abscess (ALA) occurs most commonly after *E. histolytica* colonizes the large intestine, causing intestinal dysentery. In about 1% of cases, the parasite damages the intestinal mucosa and spreads to other organs, leading to various dysentery symptoms beyond the Intestines (14) and (15).

The destruction of host tissues and the survival of amoebae in the liver is associated with a robust adaptive response and regulation of proteins, including amoeboid virulence factors. (16).

The adhesion molecule Gal/Gal NAC lectin, cysteine proteins, and other virulence factors of *E. histolytica* have received the most research. (17), protein amoeba (18), and Liposomes phosphoglycans (19). Because of their significant contribution to ALA etiology in humans and animals.

Collagen, fibrinogen, elastin, and laminin are components of extracellular materials that the parasite must penetrate to cause invasive disease. *E. histolytic*'s cysteine proteins can break down these materials (15). These proteins also play a role in breaking down cellular monolayers (20). It has also been suggested that cysteine proteins (CP) contribute to creating the anaerobic environment that trophozoites require for growth in vivo during ALA development (21) and (22).

They cause eukaryotic cells and bacteria to lyse (18), have a proven cytolytic ability, and are implicated in inducing necrosis and apoptosis in vivo (23).

An amoebic liver abscess (ALA) formation following intraportal inoculation with *E. histolytica* consists of three successive stages: acute inflammation, abscess formation, and necrosis. Microabscess formation and hepatocyte damage have occurred, culminating in tissue necrosis a week after infection (24), demonstrating that distant hepatocytes die from necrosis and that amoebic particle diffusion takes place in the endothelium. These authors speculate that the secretion of amoebic particles, which have the ability to produce harmful effects at a distance, may be the cause of cytotoxicity, even in the absence of direct trophozoite-hepatocyte contact. This study found that the number of apoptotic cells increased with the length of incubation. It had fragmented nuclei, which are important characteristics of apoptosis. Indicating a gradual increase in apoptosis in infected slices during the increasing incubation period, the development of ALA causes severe liver tissue destruction, consistent with a study (15).

The appearance of abundant infiltration of inflammatory cells and the occurrence of bleeding and congestion are due to structural changes in the liver tissue that lead to the secretion of chemoattractive factors and then the infiltration of inflammatory cells, such as monocytes, to defend the body; Inflammation is part of a biological response. It is a complex process through which the body tries to get rid of pathogens and other factors to begin the healing process; when any foreign body or toxic materials enter the living body, the inflammatory process begins with the first step by identifying the foreign substance, and then blood vessels begin to expand, and fluids and inflammatory cells filter into nearby tissues, where they are attracted to the site of injury to treat inflammation (25).

The parasite's toxins affect the liver's metabolic activity, as metabolic activity increases when the liver is exposed to toxic substances resulting from the metabolism of pathogens and through the process of detoxification for the purpose of balancing Oxidative stress resulting from the action of toxins, and this increase is in order to release energy sources such as glucose, which is accompanied by cell death, decomposition, and necrosis, which occurs after severe degeneration or occurs directly (26).

5-Conclusion

Laboratory animals infected with *E. histolytica* had histological changes in the liver, represented by necrosis, nucleolytic, and amoebic liver abscesses. Although the parasite infects the intestines and settles there, it causes secondary infections through its transmission through the blood and lymph to the liver.

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تأثير الإصابة بـ *Entamoeba histolytica* على مستوى بعض المتغيرات البيولوجية والتغيرات النسجية في الكبد

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خلفية البحث: يعـد طفيلي *Entamoeba histolytica* من الطفيليات الابتدائية المعويه Intestinal protozoan parasites والتي تُسبب ما يُعرف بداء الزحار Amoebiasis . الذي يسبـب التهاب القولون الأميبي Colitis amoeba ، ويؤدي إلى حدوث خراج الكبد Liver abscess ، تبـدأ العدوى بابتلاع الأدوار المعدية infective stags والمتمثلة بالشكل المتكيس cystic form الموجود في الشراب والطعام الملوث ويهاجم الطفيلي الانسجة عن طريق التصاقه بالبطانة الظهارية للمعي ، ويحدث الالتصاق بواسطة عوامل ضراوة virulence factors. **هدف البحث** :الكشف عن طفيلي *E.histolytica* باستخدام طريقة المسحة المباشرة ودراسة التغيرات النسيجية للكبد وبعض المعايير الدموية للحيوانات المصابه بـ*E .histolytica* ومقارنتها مع المجاميع غير المصابه .**المواد وطريقة العمل** : أجريت الدراسة الحالية خلال الفترة ما بين 1ايار 2021 و 30 تشرين الاول 2022 بغرض تشخيص الإصابة بطفيلي *Entamoeba histolytica* وكان المرضى المصابون سريرياً يعانون من الإسهال عندما تم فحص العينات مجهريا باستخدام المسحة المبللة المباشرة ، وفي الدراسة التجريبية ، تم تقسيم ذكور فئران المختبر إلى مجموعتين. تمثل المجموعة الاولى مجموعة السيطرة السلبية المجرعة بمحلول بفر المنظم فقط والمجموعة الثانية تم تجريعها فمويا بطفيلي الحال للنسيج **.النتائج** : ظهرت أقسام نسيجية طبيعية للكبد. وأن الفصيص الكبدي يشمل خلايا كبدية مرتبة بشكل شعاعي ، حيث تظهر على شكل حبال ممتدة من الوريد المركزي ، بالإضافة إلى تشخيص خلايا الكبد الطبيعية. مع نوى مستديرة مركزية ومظهر متجانس للسيتوبلازم والصفائح الكبدية والجيوب الكبدية. ما المجموعة الثانية مثلت المجموعة الضابطة الإيجابية ، وتم معاملتها بالطفيلي *E. histolytica*، الذي سجل العديد من التغيرات النسيجية في كبد المجموعتين ، والتي شملت ظهور شعاعي غير منتظم للخلايا الكبدية حول الوريد الكبدي، وارتشاح الخلايا الليمفاوية ، و نخر وتورم بعض خلايا الكبد ، وقد حدثت زيادة في انزيمات الكبد دلاله على الإصابة بـ *E.histolytica* **الاستنتاجات** : إنّ الحيوانات المختبرية التي اصيبت بـ E.histolytica حدثت لها تغيرات نُسجية في الكبد التي تمثّلت بالتنخر وتحلل الانوية وخراج الكبد الاميبي وبالرغم من ان الطفيلي يصيب الامعاء ويستوطن فيها الا انه يحدث اصابات ثانوية بانتقاله عبر الدم واللمف الى الكبد.

الكلمات المفتاحية: تغيرات نسجية ، كبد ، انزيمات ، الاميبا الحالة للنسيج.