



Evaluation of the Therapeutic Potential of *Carica papaya* Leaf Extracts on Histopathological Changes in Experimentally Infected Rats with *Entamoeba histolytica*

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Abstract

The current research was designed to investigate the effect of the extract on the intestines and livers of 10-week-old male Balb/c white rats weighing 250-280 grams, obtained from 1-12-2025 to 1-2-2026. Laboratory rats were taken from the animal house of a recognized research center. They were placed in cages, with careful attention paid to cage cleanliness. The rats were divided into four groups: a negative control group; the positive group treated with an *amoeba* suspension, the third group was infected with the parasite and therapy with an aqueous plant extract of *papaya* at a concentration of 0.05 mg/kg and the fourth group was treated only with the extract at a concentration of 0.05 mg/kg. The results showed that sections prepared from the rat group infected with the suspension demonstrated changes in the intestinal and liver tissue cells in contrast to the uninfected control group. Sections of the intestinal and liver cells prepared from the infected and treated rat groups showed. The aqueous plant extract of *papaya* leaves was found intact and in long rows, indicating that the aqueous extract of *papaya* leaves has a strong effect on the intestines and liver of infected rats and may be a natural therapeutic alternative. No side effects were observed for the aqueous plant extract of *papaya* leaves at a concentration of 0.05 mg/kg on the intestines and liver of rats doused with the extract.

Keywords: *Entamoeba histolytica*, *Carica papaya*, change histological.

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Introduction

Parasitic diseases pose a widespread health challenge in developing countries, where multiple social, cultural, and economic factors hinder efforts to control them. In addition, the development of parasite resistance to drug treatments increases the difficulty of containing this problem (Eyayu et al., 2021). The parasite of *E. histolytica* is a protozoan that lives in the large intestine and infects humans and animals. It is one of the most common causes of bloody diarrhea, causing what is known as amoebiasis (Carrero et al., 2020). According to the World Health Organization, the *E. histolytica* parasite is one of the most prominent causes of parasitic diseases and is ranked as the third leading cause of death worldwide (Charles et al., 2022), with an estimated 350 million infections worldwide (Nasar, 2024). The parasite is transmitted through the ingestion of food or water contaminated with parasitic cysts, which are the infectious stage (Taherian et al., 2019). The parasite appears in feces and it causes disease symptoms. Symptoms include intestinal cramps, fatigue, bloody diarrhea, and fever (Matushkina et al., 2023). The parasite's damage to intestinal tissues leads to necrosis and atrophy of the villi, resulting in bloody diarrhea (Singh et al., 2023).

The optimal treatment for this parasite is metronidazole, which is highly effective in eliminating the parasite, but it has some adverse effects such as headache and a metallic taste in the mouth, and sometimes gastrointestinal disturbances and neurotoxicity (Hernández-Ceruelos et al., 201). Due to the limited number of currently available treatments, research has focused on new treatments without causing side effects (Zhang et al., 2023). The World Health Organization reports that plant-based bioactive compounds are used as traditional medicine by 90% of the world's population (Bhat & Sharma, 2022). *Papaya Carica* is a fast-growing tropical plant cultivated in diverse climatic zones (Heena & Sunil, 2019). The antiparasitic property of *papaya* is attributed to a variety of components, such as: steroids, terpenoids, tannins, alkaloids, flavonoids (Santana et al., 2019).

Materials and Methods

Laboratory Rats

Male white rats of the Balb/c breed, 10 weeks age and weighing 250-280 grams were employed. These rats were divided into 4 groups in clean, pathogen-free cages at the animal house of Tikrit University and provided with the necessary water and food During the 20-day trial period.

Isolation of the *Entamoeba histolytica* Parasite from Feces

Parasite cysts were isolated from human fecal samples obtained from individuals visiting laboratories in Tikrit/Salah al-Din City who were diagnosed with the parasite by microscopic examination. Using wooden sticks, four grams of human feces were collected, added the water and the mixture was filtered through six layers of filter paper. Then the residues were centrifuged at 4000 rpm for 7 minutes. The precipitate was collected from the tubes. 2 ml of zinc sulfate (ZnSO_4) were added to the precipitate, and the tubes were centrifuged at 4000 rpm for 3 minutes. The floating part was removed and disposed of and the precipitate was collected. 2 ml of zinc sulfate (ZnSO_4) were added to the precipitate, and it was conservation at -20°C (Clark & Diamond, 2002).

Determining the Dose of Parasite Injection in Rats

A pipette was used to collect approximately 0.3 ml of the filtrate containing the parasite cysts. This was loaded on a counting slide, and the number of parasite cysts was estimated to determine the dose of infection in rats according to the following equation: $200 \times (\text{number of cysts}) / (0.2)$ (Clark & Diamond, 2002).

Amoebic Suspension Treatment of Rats

The rats were placed in special cages and divided into four groups. They were administered amoebic suspension (4000 cysts) orally using a special needle. After the seventh day of induction, the rats were placed in clean cages, and fecal samples from the infected rats were examined microscopically for amoebic cysts. Following fecal diagnosis, the rats were therapy with aqueous extracts of *papaya*. At the end of treatment period, the rats were narcosis with chloroform and dissected. The organs were dissected, isolated, and then preserved in 10% formalin for a full day. They were then preserved in 70% ethyl alcohol for two months to study the histological changes in the organs.

Collection of *Carica papaya* Leaves and Preparation of the Aqueous Extract

The leaves were obtained from various growing regions, washed with water, and dried at a suitable temperature away from sunlight for 7-10 days. They were then sorted according to (Aqil, 2003) and ground using an electric grinder to obtain a fine powder. To prepare the aqueous extract, 50 grams of the leaf powder were supplemented to 500 ml of distilled water. The aqueous extract was left at room temperature for 24-48 hours with continuous stirring using a mechanical shaker. After that, the mixture



was filtered to obtain the crude extract. The extract was then stored in sterile bottles at a temperature of 4°C until its use in the experiment (Abdel-Halim et al., 2021).

Experimental Design of the Study

The rats were placed and divided into cages:

1. Negative Group (G1): Six rats were placed in a cage.
2. Positive Group (G2): Six rats were placed in a cage and administration with an amoebic suspension for one week.
3. Infected with the Parasite and administration with the Extract (G3): Six rats were placed in a cage and therapy with an amoebic suspension. After confirming infection, they were treated with an aqueous plant extract of papaya at a therapy of 0.05 mg/kg daily for 20 days.
4. Dosed with the Extract (G4): Six uninfected rats were placed in a cage and administered the plant extract of *papaya* at a dose of 0.05 mg/kg daily for 20 days, without infection with the parasite.

Histological Examination

The tissue slides were prepared according to method (Al-Hajj, 2010):

1. The large intestine and the liver of the experimental groups were fixed in 10% formaldehyde in glass vials for 24 hours to prevent autolysis.
2. The organ was washed to remove the formalin from the tissues (large intestine and liver).
3. The specimen was sequentially immersed in ascending ethanol one hour at each step.
4. The studied samples were placed in xylene for half an hour cause to its effectiveness in removing ethyl alcohol from tissues.
5. The tissues were placed in paraffin wax for one hour to infiltrate the tissue with wax.
6. The studied tissues were placed in special molds for embedding and left to dry at a suitable temperature, then stored in a refrigerator.
7. The organ was cut using a microtome into 5-micrometer-thick strips and the slides were placed to a water bath at 40°C for a few minutes to complete the preparation process. They were then placed on slides and left to dry on a heated plate at 37°C.

Staining of Samples with Eosine and Hematoxylin

Tissue staining was performed following the protocol described in (Bancroft & Stevens, 1982):

1. The tissue was immersed in xylene to remove paraffin wax from the tissues for 15 minutes.
2. They were rehydrated immersion in graded concentration of ethyle alcohol (100%, 90%, 70%) for 5 minutes, then stained with hematoxylin for 6 minutes and washed with water.
3. The sections were placed in an acidic alcohol solution, then washed with water until the nuclei stained blue.
4. The sections were then stained with eosin for 16 seconds.
5. The samples were placed in ethanol at increasing concentrations (70%, 80%.90%, 100%).
6. The samples were immersed in xylene solution for 6 minutes for clarification, followed by 6 minutes of deposition to remove ethyl alcohol from the organ tissues.
7. Drops of Distrene plastizer xylene were applied to the samples (large intestine and liver), the slide was covered, and the slide was allowed to dry. The results showed that the nuclei stained blue and the cytoplasm pink when examined under microscope.



The prepared slides were examined under the microscope to reveal tissue change in the organs.

Results and Discussion

The study revealed clear changes in the tissue cells of the infected rat group after the seventh day of infection, as confirmed by examination under microscope of rats' stool. It was found that the *Entamoeba histolytica* parasite had resulted pathological alterations in the rat tissues of both the liver and large intestine (colon). In the rats' positive group, results indicated necrosis of the lamina propria in the large intestine and degradation of the cells lining the intestinal glands. Smooth muscle fiber breakdown was observed in the intestinal wall, along with an increased number of leukocytes between the intestinal glands in the lamina propria. This contrasted with the negative (non-infected) control group, where the intestinal cell exhibited a normal. These observed pathological changes in the tissues were consistent with the findings of study (Khaleel et al., 2024) on rats, where similar alterations were recorded in the group infected with the *E. histolytica* parasite. Noticeable changes in intestinal cells, such as degeneration and breakdown of the mucous layer and the extension of inflammatory cells to the deep mucous membrane of the intestines, causing minor structural changes, are characteristics of infection with the parasite.

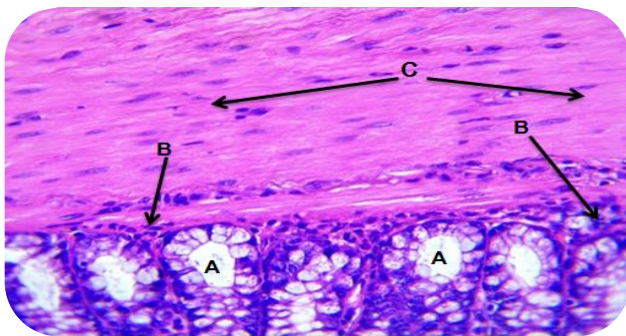


Figure 1. The Intestine of a Healthy Rat Shows the Intestinal Glands Appeared in The Deep Layer of The Intestine Lamina Propria (A). Leukocytes are Present Between the Intestinal Mucosa (B). The Muscle Layer of the Intestine (C) (400H&E×)

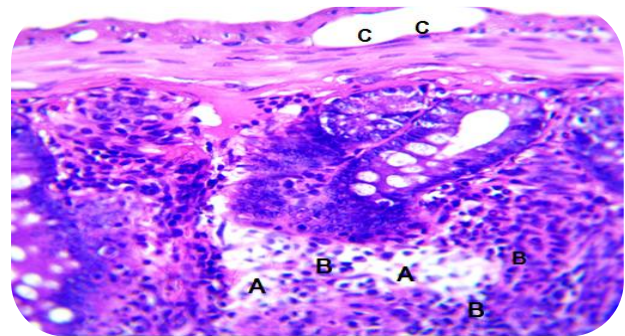


Figure 2. The Intestine of a Rat Oral Administration with the *E. Histolytica* Parasite Shows Necrosis of The Basic Lamina of the Intestinal Mucosa (A), Inflammatory Infiltration of Leukocytes Between the Intestinal Glands (B), and Breakdown of The Muscle Fibers of the Intestinal Mucosa Lining that Make Up the Muscular Layer of the Intestinal Wall (C) (400H&E×)

These pathological changes in the tissues are attributed to infection by the *E. histolytica* parasite. Upon reaching the intestine tract, it begins cell divided and adheres to the intestinal mucosa, secreting lytic enzymes that are among the most important virulence factors of the parasite, playing a role in invading and destroying tissues within the intestines (Tavares et al., 2005). In the negative (non-infected) control group, liver cells were observed in Figure 3, where white blood cells surrounded the central hepatic vein in the hepatic lobule in long rows, containing Copernicus cells and surrounded by blood sinusoids. In contrast, in the infected group, the liver cells, as shown in Figure 4, revealed the portal vein at the portal region, appearing longitudinally and containing some white blood cells and a mass of erythrocytes, with thickening of its walls was observed beside to the bile duct, where a pronounced leukocytic infiltration was detected in the peripheral area of the portal region. The portal vessels and bile ducts were embedded



within aggregates of hypertrophied hepatocytes, while hepatic sinusoids containing Kupffer cells were clearly evident. These observations are consistent with the results of (Khaleel et al., 2024), where they observed in their study on rats infected with the parasite notable tissue changes as a result of infection with the parasite, as liver cells are among the most pathogenic cells for infection with the parasite, since they are the first cells to which the absorbed substances reach via the digestive system through the portal circulation to the liver before entering the circulatory system via the inferior vena cava (Jahangeer et al., 2020).

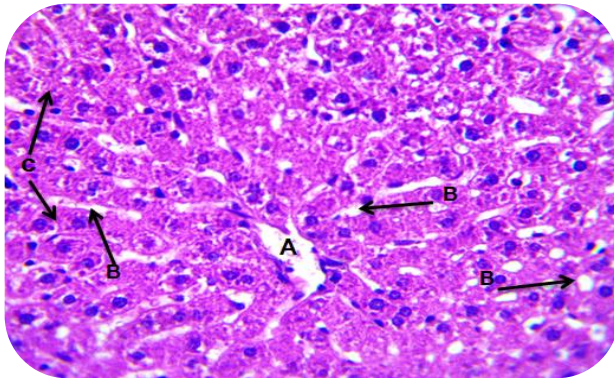


Figure 3. Illustrated Liver of Rats Showing the Organization of the Hepatobiliary Lobules and the Clarity of the Central Hepatocyte Vein (A) Showing Regular Rows of Liver Cells (B) Blood Sinusoids Containing Kupffer Phagocytic Cells (C)

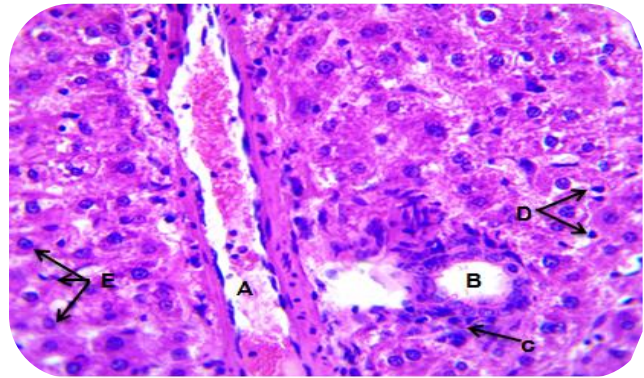


Figure 4. The Liver of a Rat Administration with the Parasite Shows the Portal Area of the Liver and the Portal Vein in Which a Blood Clot has Formed (A) The Bile Duct (B), Infiltration of Numbers of Leukocytes (C) Kupffer Inflammatory Cells (D), and Hepatocytes (E) (400H&E×)

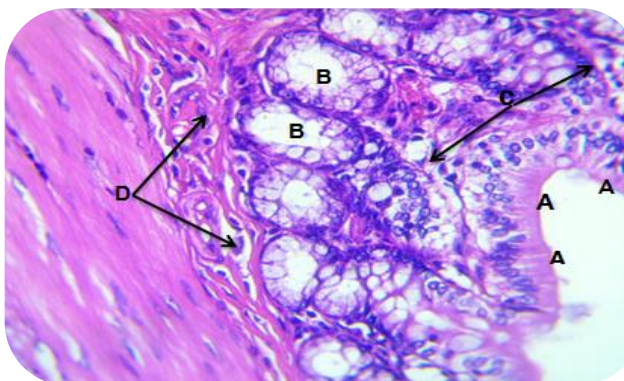


Figure 5. The Intestine of a Rat Oral Administration with Parasite and Therapy with an Aqueous Plant Extract at a Concentration of 0.05 Mg/Kg of Papaya Leaves Shows the Compaction of Intestinal Epithelial Cells Lining the Intestinal Wall (A), Intestinal Mucosal Glands (B), the Presence of White Blood Cells Between the Mucous Glands in The Lamina Propria (C) The Submucosa Layer Containing Loose (Soft) Lymphoid Tissue (D)

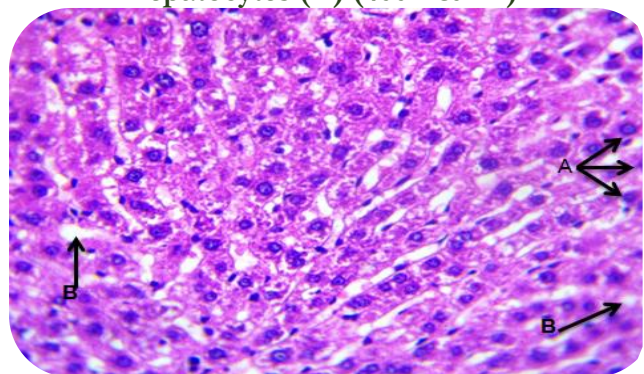


Figure 6. The Liver Oral Administration with the Parasite and Therapy Using an Aqueous Plant Extract at a Concentration Of 0.05 Mg/Kg of the Papaya Plant Shows the Hepatocytes in Rows (A) Blood Sinusoids (B) (400H&E×)



Histological sections of rats infected with the parasite and treated with a 0.05 mg/kg papaya extract, as illustrated in Figure 5, exhibited cellular improvement. The dorsal cells appeared tightly packed in the intestinal wall lining, and an infiltration of numerous leukocytes was observed beneath the epithelial layer and between the mucous glands. The sub mucosa contained loose (soft) lymphoid tissue with numerous white blood cells and small blood vessels, while the muscular layer consisted of regular bundles of smooth muscle fibers. These observations are consistent with those of study (Al-Dawoudi, 2019), which examined the effects of *Cyperus rotundus* (nutgrass) on the *E. histolyticus* parasite. In this study, the intestinal villi were observed to be intact, with lymphatic nodules present within the intestinal lamina. Furthermore, these results align with those of study (Sulaiman, 2024), which examined the intestines of rats infected with *E. histolyticus* and treated with *papaya* seeds, demonstrating its role in reducing the severity of intestinal infection caused by the parasite. Its direct effect on the parasite and its role in promoting the healing of damaged intestinal tissues.

In Figure 6, a rat liver infected with the parasite and treated with a 0.05 mg/kg aqueous extract of papaya was observed. The hepatocytes were arranged in rows arrangements. Every row contained homogeneous hepatocytes with a large, spherical nucleus and a polygonal appearance. The blood sinuses contained phagocytic Kupffer cells in the form of long, branching channels. This may be attributed to the remarkable therapeutic efficacy of papaya leaf extract, which plays a role in influencing the parasite and preventing its reproduction in the large intestine, thus halting the parasite's ability to cause infection. Furthermore, papaya leaves contain active compounds such as flavonoids, alkaloids, and enzymes that play a role in inhibiting the histolytic amoeba parasite (El-Sayed et al., 2025). This aligns with (Guzman et al., 2025) study on hamsters, where he observed that aqueous extracts of *papaya* possessed anti-hemolytic activity against parasite. His study showed that the extract reduced parasite viability and prevented the development of liver abscesses, along with an effect Similar to the drug metronidazole.

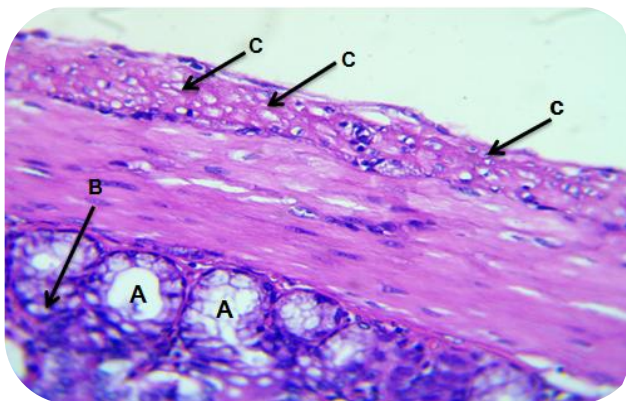


Figure 7. The Intestine of a Rat's Therapy with an Aqueous Plant Extract of Papaya at a Concentration of 0.05 Mg/Kg, Showing the Main Lamina of the Intestine Containing the Bases of the Mucosal Glands (A). Leukocyte Surrounded a Number of Intestinal Gland Bases (B). Muscular Fiber Cells are Ruptured in The Outer Part of the Smooth Muscular Layer (C)

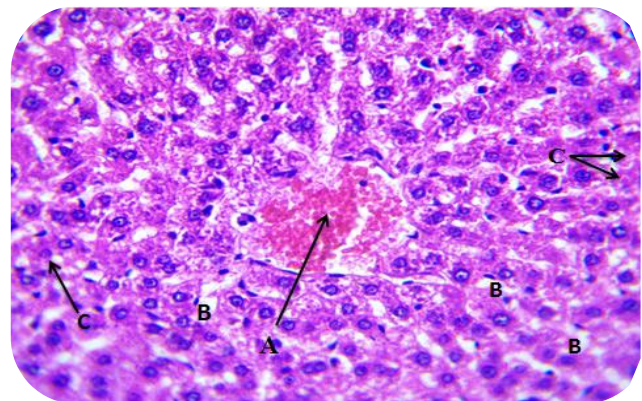


Figure 8. Shows the Liver of a Rat's Therapy Using Plant Extract Of Papaya at a Concentration of 0.05 Mg/Kg. Central Vein is Wide-Bore (A). Blood Sinuses are Visible (B). Rows of Hepatocytes with a Spherical Nucleus are Visible (C)

No pathological effects or other symptoms were observed in the studied results using the aqueous extract of papaya leaves. Figure 7 illustrated the intestine of a rat administered with the aqueous plant extract of



papaya leaves. The base of the lamina observed the bases of the glands in the intestine surrounded by a number of leukocytes in the interstitial tissue with the spread of leukocytes. The muscular layer is composed of bundles of smooth muscle fibers, circular in direction and outside in longitudinal direction, where some stenosis appeared. It is observed through Figure 8 of the liver treated with the aqueous extract of the papaya plant that the central vein is wide-bore with its connection to the blood sinuses at its ends and contains numbers of Kupffer cells and rows of hepatocytes that appear in long, regular rows, and each cell has a spherical nucleus.

Conclusion

A concentration of 0.05 mg/kg of aqueous papaya extract had a positive effect on the inhibition of cyst formation and the number of *E. histolytica* cysts excreted in the feces of rats in contrast to the infected group (dosed with the parasite and untreated). This signifies the efficacy of the aqueous *papaya* extract in improving histological changes. It also demonstrated the ability to prevent liver abscess formation and reduce necrosis, in addition to its role in rebuilding the mucous membrane of the affected intestine. This suggests it as an adjunct to conventional medicines.

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