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Article

The Efficacy of Allicin on *Entamoeba histolytica* in Experimentally Infected Rats

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ABSTRACT

Entamoeba histolytica, which came in second among parasitic infections that result in death after malaria, is the parasite that causes the common disease described as amoebiasis and is responsible for the major human gastrointestinal diseases. In the present study, the antiparasitic activity of allicin, one of the active principles of freshly crushed garlic (*Allium sativum*), on *Entamoeba histolytica* trophozoites was investigated in vitro. The type (*Rattus norvegicum*) was divided into five groups (A, B, C, D, E), with eight rates in each group weighing around 260-230 gm, with an average age of (14-16) weeks. (A) the control group Four groups (B, C, D, and E) were infected with suspension *E. histolytica*. Every rat gives cysts per milliliter through oral administration, and the group (C) was treated orally with metronidazole (20) mg/kg. Group (D) gives allicin after infection at a rate of 0.3 mg/kg. Group (E) gives allicin and metronidazole after infection. Results showed that allicin effectively inhibited the growth and development *of E. histolytica* and showed improvement in the growth of tissues treated with allicin and metronidazole compared to the rest of the groups.

Keywords: *Entamoeba histolytica*, Allicin, Efficacy, Protozoan.

INTRODUCTION

Amoebiasis is a common disease caused by the parasite Entamoeba histolytica, where the disease is second in the parasitic diseases that cause death after malaria [1]. It is a long-standing disease that was identified in 1875 by Russian physician Fedor Alexandrovich Lösch, who found the mutant in large numbers in a stool sample from a Russian peasant suffering from severe dysentery. Following that, the doctor attempted to infect dogs and infect one dog [2]. Amoebiasis can cause symptoms, including abdominal pain, diarrhea, and blood vomiting and diarrhea. Inflammation of the intestinal membrane might be one of the most severe implications. If the parasite enters the bloodstream, it can move throughout the body and eventually end up in the liver, causing amoebic liver abscesses. Without antecedent diarrhea, liver abscesses may develop. The most common method of

diagnosis is to examine the feces, which may suggest an increase in the number of leukocytes and red blood cells (Zulfiqar and Horrall,2018). When the Trophozoite stage crosses the mucous layer and penetrates the epithelial mucosal barrier, it damages intestinal cells, causing invasive illness. This damage causes inflammation and dysentery, followed by [3]. 90% of persons infected with amybate have no symptoms. Only 4 percent to 10% of those with asymptomatic amoebae who were checked for a year later developed colitis or an extraintestinal illness [4]. In the tropics and subtropics, amoebiasis remains a major health concern (Roshdy et al., 2017). Poor sanitation, hygiene, and cramped living circumstances are common causes of amoebiasis spread in impoverished nations. Individuals visiting from endemic nations in wealthier countries estimate that 500 million people globally are affected with amoebiasis. According to previous studies, 50 million people are affected with amoebiasis each year [5].

MATERIAL AND METHODS

Infection of animals:

The animals were housed in special cages at the Animal House Department-College of Education for Pure Science / The Qar University. On female white rats, the type (Rattus norvegicum) were divided into five Groups(A, B, C, D, E) with eight Rate in each group weighing around 260-230 gm, with ages (14-16) weeks. (A) control Group Four groups were infected with suspension E. histolytica that bring from stool patients attending the Bint AL-Huda teaching hospital and Al-Moussawi Hospital for Children in Thi-Qar province, south of Iraq. The animals were divided into five groups:

A-First group: control Group: consisting of Eight (8) rats that were treated with normal saline NaCl (0.5 ml).

The second group: infection with 10^4cysts per milliliter through oral Administration Entamoeba histolytica as a positive control group for 20 days.

The third group: metronidazole (20 mg) dissolved in distilled water after infection at a rate of (20) mg/kg per animal for 20 days.

The fourth group was dosed with allicin after infection at a rate of (0.3) mg/kg for each animal for 20 days.

The fifth group was dosed with Allicin(0.3) mg/kg and metronidazole (20 mg) for each animal for 20 days.

Clark and Diamond (2002) prepared a suspension solution from the parasite Entamoeba histolytica for animal injection. E. histolytica cysts and trophozoites were added to this stool in a final concentration of 1:1 with normal saline. One milliliter of suspension containing roughly 1,000 cysts was infected rats orally administration. The prevalence of infection was confirmed fifteen days later by inspecting animal excrement and finding the presence of cysts in stool rats. The animals were then anesthetized with chloroform, and the abdominal cavity was opened with medical scissors until the sternum was reached.

Tissue preparation and histology

Tissue preparation was made according to [17.] A small piece of the targeting organ (intestines and liver) of the rates was kept in 10% formalin till tissue preparation for histological study.

RESULTS

Rats dosed orally with parasite suspension of Entamoeba histolytica after fifteen days of oral dosing showed a high infection of Cyst and Trophozoite in the feces of infected animals**,** Figer 1(A) Trophozoite stage (B) Cyst stage

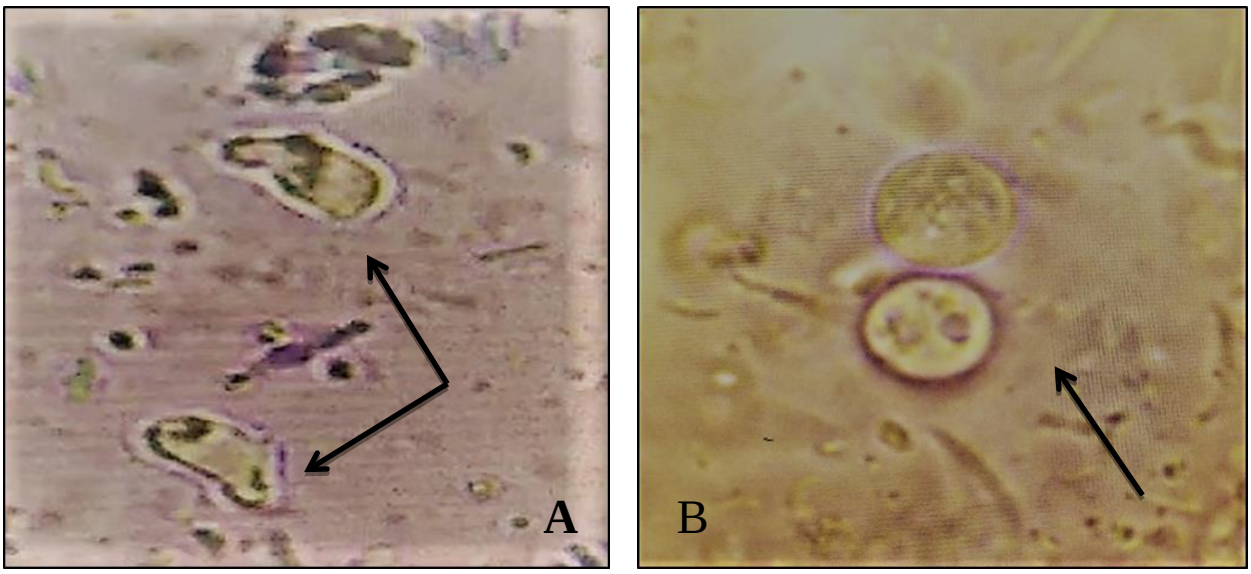

Figure 1. Microscopic examination, *E.histolytica* (A) Trophozoite stage (B) Cyst stage (400x)

Histological study

Liver:- The control group's histology study revealed increased hepatic visceral tissue and structure. The infection groups showed radiographs of the hepatic cords around the central veins and the common sinusoids between them. as well as the development of lymphatic pools at the capsular surface. Glycogen granules and fat droplets are found in the bile duct (Fig 1-A) infection groups. The presence of blood inside the central vein, as well as a clear extension of the sinusoids, were seen more than hepatic visceral tissue distributed radially for the hepatic cords around the central veins. Blood coagulation and swelling in the hepatocytes, as well as a capsular fracture, are all present in the tissue sections. (Fig 1-B) The tissue sections treated with metronidazole and Groups treated with allicin showed a clear improvement in the hepatic tissue, with no disintegration and no cell infiltration, as well as no destruction to the capsule. We also failed to observe the absence of blood congestion and the presence of hyperplasia(Fig 1-C-D). The tissue sections treated with metronidazole and allicin showed significant improvement in liver tissue, with minimal central vein congestion and good growth of hepatocytes (Fig 1-E).

Large Intestinal the control groups showed that Simple tubular (crypts of Lieberkuhn) intestinal glands extended from the surface to the muscularis mucosa in control rats, and these glands were bordered by mucous cells and absorptive cells. The lamina propria is a thin layer of loose connective tissue that is densely populated with cells (lymphocytes). The submucosa comprises loose connective tissue with blood vessels, whereas the tunica muscularis, which is a Histological investigation of post-. (Fig 2-A) infection intestinal sections of rats show trophozoites causing mucosa damage, inter-glandular epithelium degeneration,

and an increase in the number of goblet cells. Made up of strands (smooth) muscle fibers that extend upward, heavy infiltration of inflammatory cells and Severe hemorrhage. (Fig 2-B) Relative improvement was observed in the third group treated with metronidazole, with a thickening of the mucous layer, showed Simple tubular (crypts of Lieberkuhn) and a lack of inflammatory cells. (Fig 2-C) In treatment with the compound allicin, there was a decrease in inflammatory cells and a narrowing in the submucosal layer, while the serous and muscle layers appeared normal and thin. (Fig 2-D). Moreover, the groups treated allicin and metronidazole, The disappearance of the parasite phase and the improvement of the intestinal layers—figure (2- E).

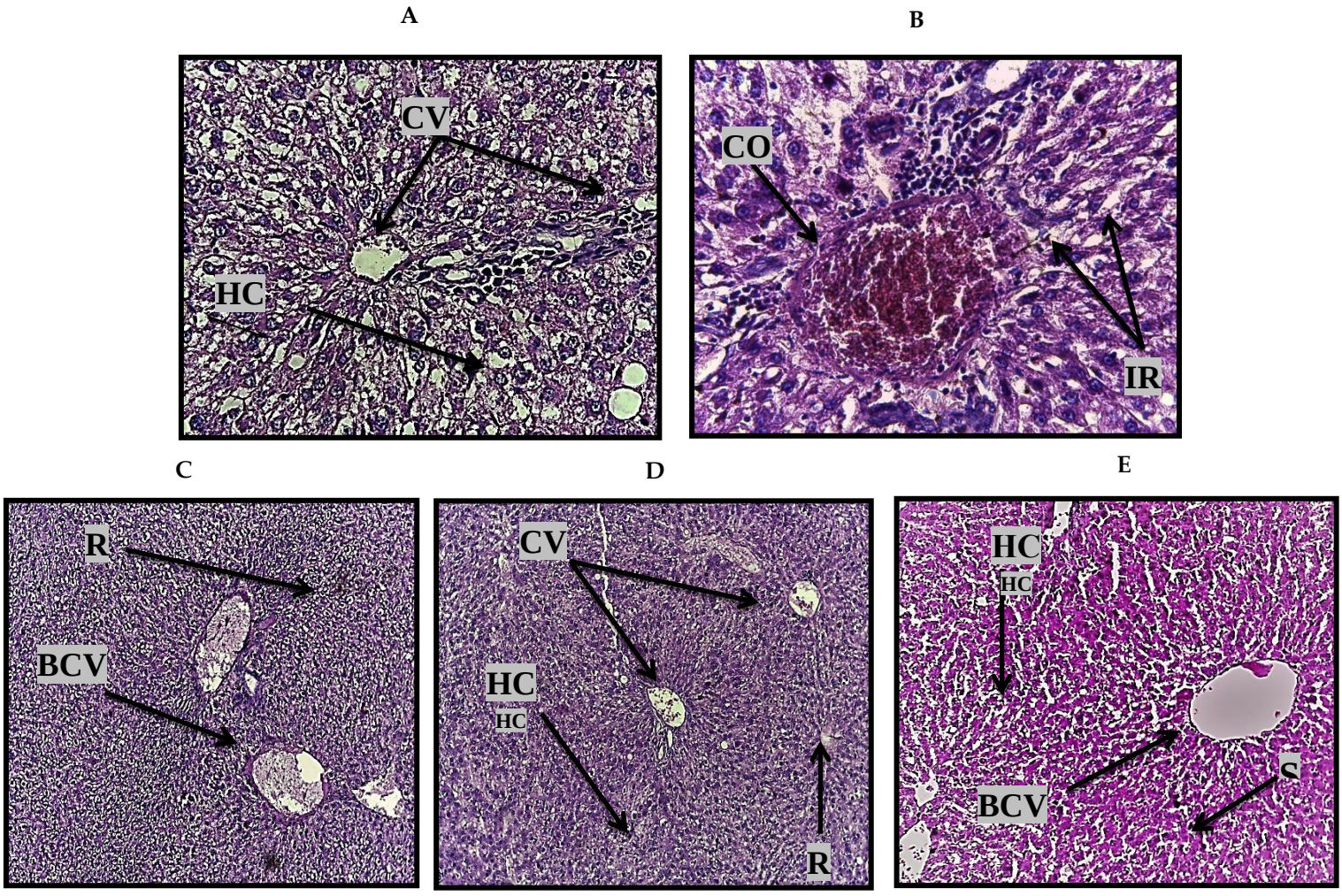

Figure 1. (A) This microphoto of liver section control Group (CV) Central Vein(HC) Hepatic Cell (H&E)(100x). (B) This microphoto of the liver section is infected with *E.histolytica* (CO) congestation (IR) irregular liver tissue (H&E)(100x). (C) This microphoto of the liver section which is infected with *E.histolytica* withtreated Metronidazole (BCV) blood Central Vein (R) regular liver tissue (H&E)(100x) (D) This microphoto of the liver section which is infected with *E.histolytica* with treated Allicin(H&E)(100x). (E) This microphoto of the liver section is infected with *E.histolytica* with treated Allicin and Metronidazole (S) Sinusoids (H&E)(100x).

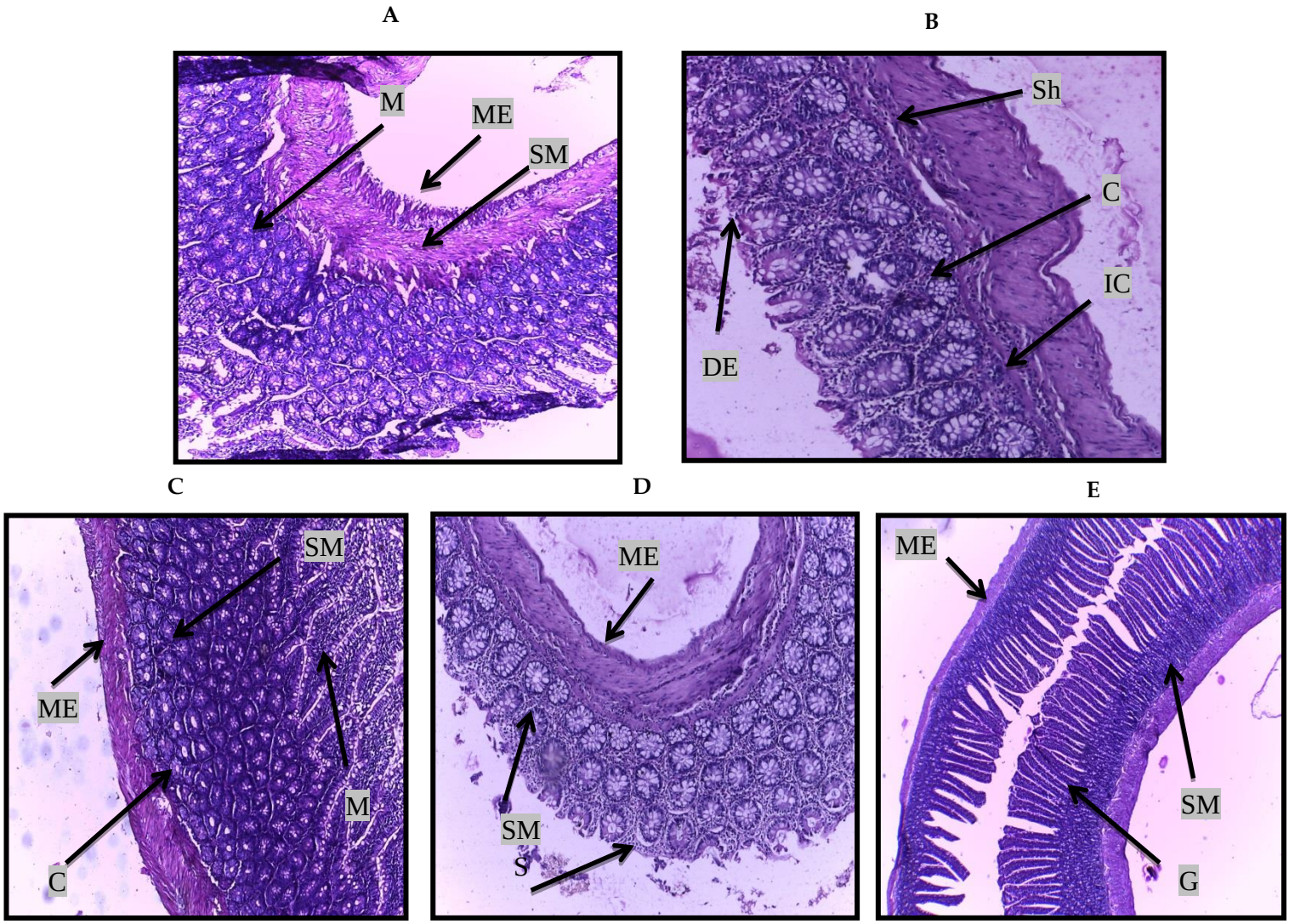

Figure 2. (A) This microphoto of the Large intestinal section control Group showed normal structure (M) mucosa surface (ST) straight crypts (SM)sup mucosa (BV) blood vesssels and (ME) muscularis externa (H&E100x). (B) infected Group showed (DE) destriction of epithelial layer,(C) intestinal crypts and (IC) inflammatory cells (H&E100x). (C) showed normal structure (H&E100x). (D) (S) Serosa Layer (E) (G) Goblet cells.

DISCUSSION

Entamoeba histolytica, a protozoan parasite that causes amebiasis, is most commonly known for its symptoms of acute diarrhea, dysentery, amebic colitis, and amebic liver abscesses. E. histolytica, the fourth most common parasite cause of human mortality, primarily affects children in impoverished nations and is spread by contaminated food and water. Most infected individuals have Entamoeba sp [6]. Following infection, E. histolytica settles in the large intestine, causing intestinal amebiasis. However, trophozoites damage the intestinal mucosa and spread to other organs in about 1% of cases, causing different types of extraintestinal amebiasis, the most common of which is amebic liver abscess (ALA) [7] ALA pathogenesis is complicated, involving host and parasite variables, as well as microenvironmental factors [8]. A strong adaptive response and protein regulation, including amebic virulence factors [9], accompany Amoebae's ability to destroy host tissue and thrive in the liver. Allicin has a strong growth inhibitory impact on Entamoeba histolytica, the main parasite that causes seasonal infections. It is responsible for more than 50 million instances of amebiasis in the gut [10]. Amoebic liver abscess formation in hamsters after intraportal inoculation

of virulent trophozoites of E.histolytica involves three steps, according to [11]: acute inflammation, abscess formation, and necrosis. Similar results were obtained in this investigation, such as sinusoidal localization of the amoebas and a generally acute inflammatory response with the production of micro-abscesses and hepatocyte damage, culminating in tissue necrosis after 4 weeks, as described by the groups mentioned above. [12] showed that amoebic molecules diffuse to the endothelium, causing necrosis in hepatocytes further away. These authors propose that cytotoxicity can result from the production of amoebic compounds that can exert harmful effects at a distance, even when trophozoites and hepatocytes are not in direct contact. Apoptotic cells were found in this investigation, and their number increased as the incubation duration increased. The presence of pyknotic nuclei and/or nuclear fragmentation, both essential factors of apoptotic cell death, was observed. This is consistent with what was said by [8]. amoeba that induced the programmed death of hepatic cells and noted that the number of apoptotic cells in the infected slices increased as the incubation time increased. ALA development causes severe destruction of the liver tissue. The attachment of trophozoites to the gut wall prevents them from transforming into cysts, but when they migrate to the large intestine, binary fission can turn them into new cysts [13]. Another pathological change caused by E. histolytica is epithelial layer degeneration and heavy infiltration of inflammatory cells into these layers. The reason for this degeneration is the ability of trophozoites to attach and lyse the epithelial layer of the intestine through the Gal/GalNAc lectin. In addition, certain enzymes, such as sialidase (N-acetylgalactosamidase) and esse, may be involved in increasing cells [14].

Histological changes discovered intestinal crypt hyperplasia, branching crypt, and degeneration area in most crypts. This feature found in our results can be explained by epithelial layer degeneration; at the beginning of the infection, a non-specific lesion can form due to glandular hyperplasia and edema [15]. Other researchers have supported the infiltration of inflammatory cells that occurred in the crypt of rats 14 and 28 days after infection due to long-term infection [16]. Without therapy, a long-term parasite infection might result in intestinal layer invasion and inflammation.

CONCLUSIONS

Our findings highlight the unique mode of action of allicin, one of the active chemicals in freshly ground garlic (*Allium sativum*) on *E. histolytica*, and allicins on inhibiting Trophozoites. Allicin, according to this finding, can be used to treat amoebic dysentery and amoebic liver abscesses.

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