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**Study of Some Physiological and Immunological
Parameters in White Rats Infected with
Entamoeba histolytica and Treated with Lectin**

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Submitted to the Council of the College of Science,
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the Degree of Master of Science in Biology**

By

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قَالَ رَبِّ اشْرَحْ لِي صَدْرِي (٢٥) وَيَسِّرْ لِي أَمْرِي (٢٦)

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Certification

I certify that the preparation of this thesis (**Study of Some Physiological and Immunological Parameters in White Rats Infected with *Entamoeba histolytica* and Treated with Lectin**)

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Dedication

I dedicate this work

To my Lord, my supporter ...

**To Prophet Muhammad and the pure infallible
Imams, my ultimate guide...**

**To my dear father my pillar of strength,
the kind heart my mother, the secret of my existence**

I will always remain grateful to them...

My dear sisters and brothers

To the martyrs of Iraq.

Huda

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Summary

The current study aimed to investigate the effects of infecting albino rats with *Entamoeba histolytica* and injecting with plant lectin on some physiological and immunological parameters (Complete blood count (CBC), Hepcidin, β -tryptase, IL-12 and IL-17, as well as studying the effect of lectin injection and *E. histolytica* infection on the histological structure of rat's livers).

This study was conducted at the University of Babylon / College of Science / Department of Biology from February 2022 to April 2022.

The experimental animals were divided into three groups, the first and second groups contained 12 animals, and the third group contained 21 animals.

The animals of the first group were injected with 3 mg/ml of plant lectin (seed wheat lectin) three times between one injection and another, one week. The second group was experimentally infected with *E. histolytica*, while the last group was infected with *E. histolytica* after being injected with lectin. 5 ml of all experimental animals were withdrawn and divided into two groups; the first group was used to perform the complete blood count (CBC), while the second group was used to obtain the serum, which was kept at $-20\text{ }^{\circ}\text{C}$ for the later use for physiological and immunological tests using ELISA technique.

The result of the present study showed that lectin injection had a significant effect on the total number of leukocytes and the percentage of granulocytes in addition to the total number of blood platelets. As for *E. histolytica* infection, it had a significant effect on the total number of leukocytes and the percentage of lymphocytes, the percentage of granulocytes, in addition to their significant effects on the total number of erythrocytes and the concentration of hemoglobin. In the third group, *E. histolytica* infection after lectin injection had a significant

effect on the total number of leukocytes, the percentage of lymphocytes, and the percentage of monocytes.

It was found from the results that lectin injection had a significant effect only on the concentration of β -tryptase, while the infection with *E. histolytica* had a significant effect on the concentration of β -tryptase and hepcidin. In the third group, *E. histolytica* after lectin injection had a significant effect on the concentration of β -tryptase only.

The result showed that the correlation among physiological and immunological parameters in a group of albino rats injected with lectin. A significant correlation was observed among the studied parameters between β -tryptase and IL-12 after a week of lectin injection, as there was a significant positive correlation between them. A significant strong positive correlation after two weeks of infection was observed in albino rats infected with *E. histolytica* group. While it was found that there were only two cases that had a significant association between them, namely, the correlation between β -tryptase and IL-12 in the first week of injection with lectin and between Heparin and IL-17 in the third week of infecting albino rats with the *E. histolytica* after injected with lectin.

It was found from the results of the present study that the injected plant lectin has a protective role against the infection of rats with *E. histolytica*. Where the time required for infection to appear in the feces of albino rats was 3 days, and 9 days rats were injected with lectin after being infected with the parasite. The period sufficient to make the infection intensity very high is 7 days, and 13 days in a group of rats infected with the parasite after being injected with lectin.

The result of the histological study shows the effect of lectins injected on the liver tissues of rats. A week after the first injection, the occurrence of damaged sinusoids with angiectasis, congestion of the central vein with fibrosis, and vein area infiltrated with mononuclear cells. While we note the presented mild

hemorrhage, revealing marked increased branching of the bile ducts, enlarged Kupffer cell, and hepatic megalocytes in the liver of rats occurs a week after the second injection of lectin. A week after the third injection with lectin, we notice the presence of irregularly arranged hepatocyte plates, separated by dilated blood sinusoids and marked dilated sinusoids associated with few inflammatory cells.

A histological study shows the effects of infection in the liver tissues of rats. It was found that the changes after a week of infection are an abnormal liver with dilation, focal congestion in the central vein, hepatocyte hypertrophy, and angiectasis. For two weeks after the infection, the changes were central vein congestion, sinusoidal endothelial subendothelial linear mononuclear cellular infiltration, central vein fibrosis with focal mononuclear infiltration, and the presence of *E. histolytica* trophozoites. The changes after three weeks of infection were the abnormal bile duct profiles, portal tract expansion with prominent fibrosis, and the presence of *E. histolytica* trophozoite.

The histological study shows the effect of *E. histolytica* infection on the liver tissues of rats after begin injected with lectin. It was found after a week of infection that the effects included extensive central vein congestion, thrombosis, sinusoidal endothelial with sub endothelial linear infiltration, and severe bile duct proliferation. While the changes after two weeks of infection were central vein congestion, central vein fibrosis with focal mononuclear cellular infiltration, sinusoidal mononuclear cellular infiltration, vacuolar degeneration hepatocytes, and hepatic megalocytes. As for the changes after three weeks of infection, they included the disturbing hepatic architecture, mononuclear cell filtration, enlarged hepatocytes, and leukocyte.

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List of Abbreviations

Abbreviation	Meaning
ANOVA	Analysis of Variance
BT LAB	Bioassay Technology Laboratory
CD	Cluster differentiation
dl	deciliter
DNA	Deoxy ribonucleic Acid
EDTA	Ethylene Diamine Tetra acetic Acid
ELISA	Enzyme linked immune sorbent assay
g	Gram
Hepc	Hepcidin
Ig	Immunoglobulin
mg	milligram
ml	milliliter

OD	Optical density
PCR	Polymerase Chain Reaction
pg	picogram
SE	Standard error
SPSS	Statistical Package for Social Science
TPS	Tryptase
μ l	microliter
μ m	Micrometer
WHO	World Health Organization

Chapter one
Introduction

1-1: Introduction

E. histolytica is pathogenic protozoa. It is the principal cause of human amoebiasis and belongs to the Entamoebidae family. It is one of the most widespread parasitic illnesses worldwide, third only to malaria and schistosomiasis (Kurt *et al.*, 2008).

E. histolytica infects over 50 million individuals worldwide each year, resulting in 40,000 to 100,000 fatalities (Kirimi, 2018). In areas with inadequate poor conditions, up to 50% of the population may be affected (Garmie, 2016).

Amoebiasis is thought to impact roughly 10% of the global population, with 90% of those affected showing no clinical symptoms (Kumari *et al.*, 2013). Amoebiasis is an invasive illness of the large intestine that can also affect the liver, lungs, pleura, pericardium, spleen, and, less commonly, the genitor-urinary tract, brain, and skin (Tillack *et al.*, 2007). *E. histolytica* migration is required for the development of amoebiasis, as it causes tissue invasion and destruction (Labruyere *et al.*, 2003).

E. histolytica infection effects on haematological parameters by increased or decreased these parameters, and that can cause anemia (Shaker and Hussein, 2016).

Lectins are glycoprotein substance that are not of immunological origin and have the ability to agglutinate cells and precipitate different types of sugars. Lectins are found in all living organisms and various methods have been used to isolate and purify them. It is used in many biological fields such as antibacterial, antiparasitic, antiviral and others (Tsaneva and Van Damme, 2020).

Hepcidin (hepatic bactericidal protein) is a peptide hormone that helps the human body maintain iron homeostasis, is a cysteine-rich peptide that was first discovered in 2000 as an antibacterial peptide in the urinary tract. Hepcidin serves a protective function against infections by removing extracellular iron from the body during infection. Hepcidin also reduces iron concentrations in duodenal

enterocytes and macrophages, as well as its transfer across the placenta (Rauf *et al.*, 2020).

β -tryptase is a subfamily of trypsin-like proteinases that are stored in the secretory granules of mast cells. Upon mast cell activation/degranulation, these enzymes, along with other mediators, are released into the extracellular medium. β -tryptases are unique in that they are active enzymes in the mast cell granules but only have extracellular action. β -tryptases appear to be involved in many mast cell-mediated allergy and inflammatory disorders. The role of β -tryptase in asthma, an inflammatory illness of the airways caused frequently by allergies, has been suggested (Fiorucci and Ascoli, 2004).

Interleukin 12 (also known as IL-12p70 or simply IL-12) is an immunoregulatory cytokine produced primarily by antigen-presenting cells. IL-12 expression regulates innate responses and defines the type of adaptive immune response after infection. IL-12 stimulates the production of interferon (IFN) and causes CD4⁺ T cells to develop into type 1 T helper (Th1) cells. IL-12 has been linked to the treatment of a variety of disorders, including viral and bacterial infections as well as cancer (Hamza *et al.*, 2010).

Because of its role in inflammatory disease, IL-17 is one of the most well-studied cytokines in immunology, the role of human IL-17 in inflammation was soon recognized. However, after the discovery of a developmentally differentiated CD4⁺ T helper subset that expresses IL-17 (the so-called Th17 lineage) and drives tissue inflammation, IL-17 became the focus of immunological investigation (Zenobia and Hajishengallis, 2015).

1-2: Aim of the study

Due to the many properties that lectins have that made them of multiple biological uses, the current study aimed to know the effect of one of the plant lectins on some physiological and immunological parameters (CBC, Hepcidin, β -tryptase, IL-12 and IL-17) in white rats experimentally infected with *E. histolytica* through: -

1-Measurement of the concentration of these parameters in albino rats injected with lectin.

2-Measurement of the concentration of same parameters in albino rats infected with *E. histolytica*.

3-Measuring variations in the concentration of same parameters in albino rats first injection with lectin and then experimentally infected with *E. histolytica*.

4-Finding the correlation between the studied parameters in all the groups.

5-Studying the effect of the above cases on histological variations in the liver of albino rats.

Chapter Two

Literature Review

2: Literature Review

2-1: *Entamoeba histolytica*

2-1-1: Historical view of the parasite

One of the most significant parts of paleoparasitology is understanding parasite history and the evolution of host/parasite relationships (Le Bailly *et al.*, 2016). James Annesley, an English physician, identified hepatic dysentery, or dysentery with hepatic abscess, in 1828, but he was unsure of the relationship between the two. An English physician serving in the Indian army, Parkes, provided a thorough account of postmortem results in persons who had most certainly died of amebiasis in 1846 (Kretschmer, 2020), and then Lamble discovered in 1855 the amoeba in the feces of one of the children with diarrhea (Dhawan, 2012).

In 1875, the Russian physician Friedrich Loesch documented the first case of amoebic dysenteric illness in a human. He described the amoeba's motility as well as the nucleus and ingested red blood cells, indicating that he was looking for the trophozoite of what is now known as *E. histolytica*. Because it appeared in the colon, he termed it *Amoeba coli*. Due to the amoeba's potential to cause tissue lysis, in 1890, William Osler explains amoebic liver abscess and colitis (Garmie, 2016).

Amebiasis was initially recognized as a separate clinical condition caused by a specific pathogen, Amoeba dysenteries, by Councilman and Lafleur in 1891. They were the first to use the terms "amebic dysentery" and "amebic abscess of the liver" which are now widely used (Dhawan, 2012). Quincke and Ros established in 1893 that cysts may survive in a moist chamber for up to 20 days and produce

dysentery when given by mouth. They also separated *E. histolytica* from *E. coli*, paving the way for the discovery of the method of amebic dysentery transmission (Lacasse, 2012). Fritz Schaudinn altered the name to *E. histolytica* in 1903 (Gachuhi, 2014). Leonard Rogers designated emetine as the first effective treatment for amoebiasis in 1912, Walker and Sellard later proved the infective cyst form of *E. histolytica*, followed by the description of the life cycle by Dobell in 1925 (Kretschmer, 2020). Sargeant and Williams demonstrated in 1978 that *E. histolytica* strains may be separated into two primary categories based on zymodemes revealed by isoenzyme electrophoresis done on cultured parasites: invasive and non-invasive (Paul *et al.*, 2007).

2-1-2: Classification of *E. histolytica*

The *E. histolytica* is classified according to Zeibig (2013).

Kingdom: Animalia

Sub kingdom: Protista

Phylum: Protozoa

Sub Phylum: Sarcomastigophora

Super Classes: Sarcodina

Class: Rhizopoda

Order: Lobosea

Family: Amoebida

Genus: *Entamoeba*

Species: *histolytica*

2-1-3: Morphology of parasite

Trophozoite, Precyst, Cyst, and Metacyst are the four phases of this parasite's life cycle as in the Figure (2-1) (Roberts and Janovy,2005).

The trophozoite has a pleomorphic shape with a diameter of 10 to 50 micrometer. It has a mucopolysaccharide-based exterior layer, as well as unidentified anionic groups and the lectin A concanavalin that produces an antibody complex that covers the cell and hides the protozoan. There are several enormous feeding vacuoles, lysosomes, and a thin endoplasmic reticulum in the endoplasm, but no mitochondria, Golgi bodies, or genuine ribosomes. In a young trophozoite, the nucleus has a two-layered membrane that makes it difficult to view. On the membranes, chromatin is structured in a regular pattern and linked to the central karyosome like the spokes of a wheel. Pores are consistently spaced inside the nuclear membrane. *E. histolytica* has intranuclear vesicles with acid phosphatase activity (John and Petri, 2006).

The trophozoite is highly motile and metabolic (MDPH, 2006). Only freshly passed specimens show motility, which is typically progressive and directed. From the refringent, clear, peripheral ectoplasm, the amoeba travels by pushing out broad, spherical pseudopodia that can exceed 100 μm in length, this system aids in the attachment of amoebae and facilitates endocytosis, which is necessary for pathogenic types. Their continual movement is fueled by the anaerobic conversion of glucose and pyruvate to ethanol (Coffie, 2017).

It is known as the non-infectious form because it is unable to survive in the environment or pass through the acidic conditions of the stomach (Stanley, 2003). From the characteristics of this form of amoeba (Dailey,1996), the existence of a central karyosome inside the nucleus is centrally located, and the feeding vacuoles

include red blood cells for different phases of digestion during the acute stage of infection (Marquardt *et al.*, 2000).

The cyst is spherical, with a diameter of 10 to 15 μm and a double membrane around it. It is resistant to harsh environmental conditions such as stomach secretions, dehydration, heat, and sunshine, and can survive outside the organism for weeks or months (Coffie, 2017). The parasite is protected from stomach enzymes, heat, and pH by the quitina material in the cyst wall, although it decomposes to a degree less than (5) and more than (40), as well as dehydration (Markell *et al.*, 1999).

The cyst has four nuclei, chromatoid bodies (ribosomal assemblies), and glycogen when it matures as in the Figure (2-1). These chromatoid bodies are refringent rods that are observable in fresh specimens and stain black with hematoxylin. RNA and DNA are found in these bodies. The glycogen vacuoles in the cytoplasm store nutrients that disappear as the cystic phase progresses, which explains why some cysts contain them and are absent absence in others (Assafa *et al.*, 2006). The immature cyst has a single nucleus that is about a third of the cyst diameter, and it may survive for seven days in the excreta in the soil at a temperature of (28-34) degrees. The cyst was also unaffected by the addition of chlorine to the water (Marquardt *et al.*, 2000).

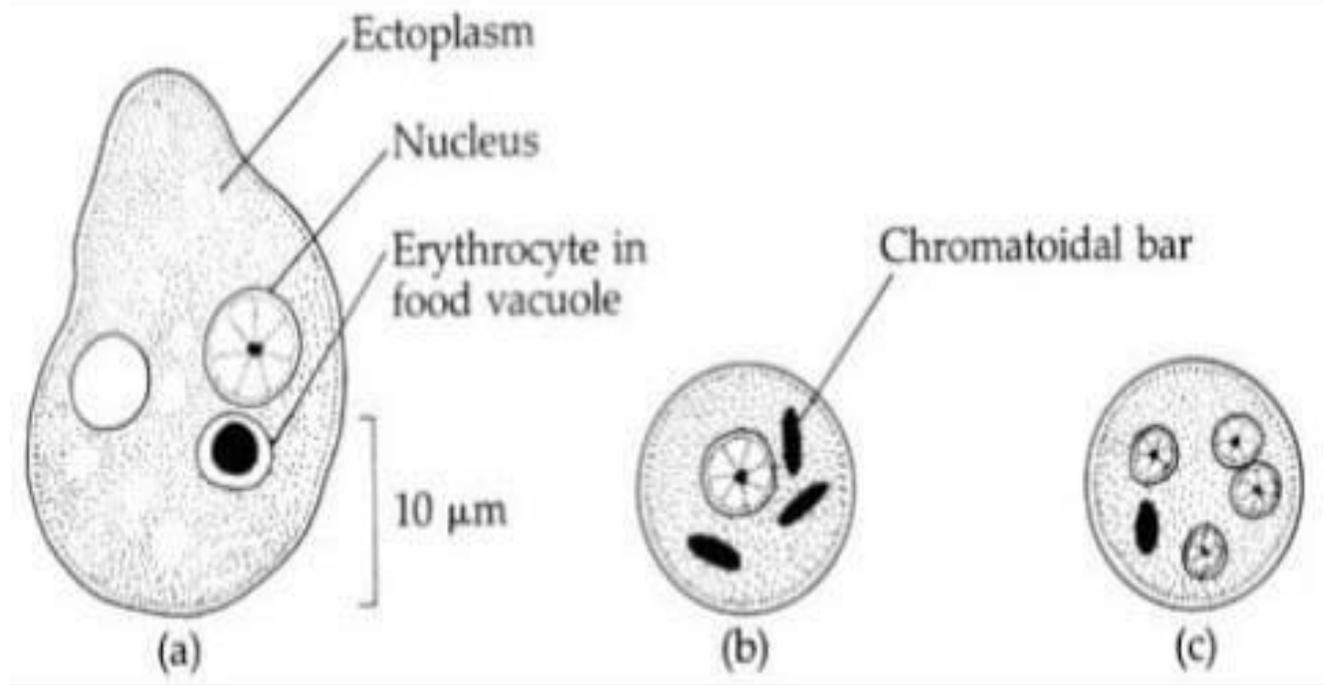


Figure (2-1): *E. histolytica*; (a) trophozoite phase, (b) immature cyst, (c) mature cyst (Chiodini *et al.*, 2003)

2-1-4: Life Cycle

The mature cysts (the infective stage) are ingested in feces-contaminated food and water (Ejaz *et al.*, 2011). Encystation occurs in the small intestines following ingestion, after which the trophozoites are released and migrate into the large intestines. The trophozoites multiply via binary fission in the large intestine, producing many cysts that are passed out in the feces Figure (2-2). The cysts have a thick wall made partially of chitin that allows them to survive in the external environment for days to weeks (Varki *et al.*, 2009).

In the case of acute diarrhea, a small number of cysts are ejected outside the body, or no cysts are present. Cysts are excreted in large quantities by parasite carriers as well as chronic cases, and the human is the primary host and source of

infection (Assafa *et al.*, 2006). They are quickly destroyed once outside the body, even if the next host ingests them. They do not survive the gastric environment since they are confined in the intestinal lumen and do not cause symptoms. Only after the trophozoites rupture the mucosal barrier and invade the colon space, producing ulceration, is a person diagnosed with amoebiasis (Ravdin and Stauffer, 2006). In some situations, trophozoites can infect the intestinal mucosa as well as other organs such as the liver, brain, and lungs (Haque *et al.*, 2003).

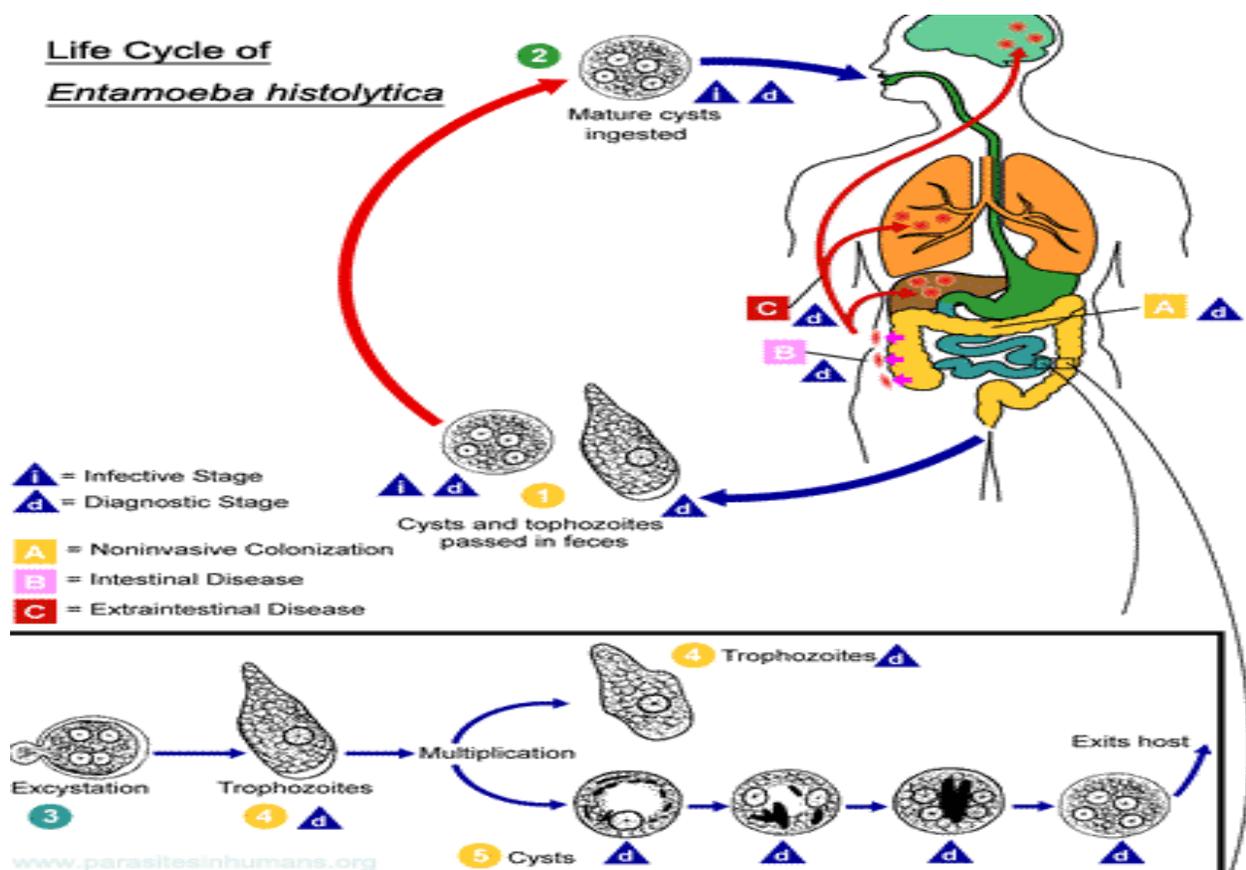


Figure (2-2): Life Cycle of *E. histolytica* (Garmie, 2016)

2-1-5: Epidemiology

2-1-5-1: Epidemiology in the world

E. histolytica is prevalent in Africa, the Indian subcontinent, the Far East, Central and South America, and other parts of the world (Samie *et al.*, 2006). This is not due to the high temperatures and humidity of these countries' tropical climates, which are known to kill *E. histolytica* (Coffie, 2017). Rather, endemic residents' inadequate personal hygiene and environmental sanitation (Hegazi *et al.*, 2013), compounded with low resources, illiteracy, and public ignorance (Ibrahim, 2008), account for the increased prevalence and incidence. Epidemics are uncommon, and only few groups have been documented from households and institutions in developed countries like the United States, where sanitation is good (ODH, 2014).

Amoebiasis is more common in immigrants and travelers from developing countries in the United States, the rate of infection in the United States and Europe is 10 %, while the rate of infection with this parasite among factory workers in India is 52% (Marquardt *et al.*, 2000).

In Africa, amoebiasis has been reported to affect up to 50% of the population (Al-Harthi and Jamoom, 2007). Only 21% of patients attending Njoro district hospital tested positive for *E. histolytica*, according to research conducted in Kenya among residents of Njoro area (Kinuthia *et al.*, 2012).

2-1-5-2: Epidemiology in the Arab world and Iraq

El-Sheikh and El-Assouli (2001) recorded the infection rate of 2.2% of this parasite by surveying of the infection of some intestinal parasites among children with acute diarrhea in Saudi Arabia, the infection rate in Egypt was 38% according

to a study by Stanly (2003). In Jordan, the rate was 31% by Dincer et al. (2017), and in Palestine found the infection rate was 28.5% by Mezeid *et al.* (2021).

In Iraq, there are several studies indicating that the rate of infection with this parasite varies from one region to another, In Basra, Al-Shahee *et al.* (2007) conducted a study on people coming to a Basra hospital, and recorded the highest rate of infection there 29.2%, and noted that the highest rate of infection was within the age group 45 years and over, reaching 31.9%, followed by the age group (1-14) years, reaching 21.5%.

Al-Hamairy *et al.* (2013) recorded through a study he conducted on the prevalence of intestinal parasites and their relationship to anemia in the village of Dulab/ Babylon, that rate of infection with *E. histolytica* was 36.7%.

In Kirkuk Hameed *et al.* (2021), the recorded rate of infection was 23.57% by the study on children ranging in age (1-14) with diarrhea from the Children's Hospital in Kirkuk and recorded the highest percentage of infection in the category (5-10) years with a percentage of (36.58%), and there were no significant differences between male and female infection, while in the different seasons of the year there was a clear difference in the percentage of infection. Where was recorded the highest rate of injuries in the summer months (June, July, August and September) at 33.33%, 45%, 33.34% and 35.7% respectively.

Many studies record the infection rate of *E. histolytica* in the different cities of Iraq, In Dhi Qar was (26.41%) and in Babil (23.37%). The percentage was lower than what was reached, in Wasit the ratio reached (47.7%), in Najaf (34.3%), in Kufa (43.29), in Maysan (76.82%), in Erbil and Dhok (46.6%), in all of Iraq (45.40%) and in Erbil (30%) (Hameed *et al.*, 2021).

2-1-6: Pathogenicity

The term "histolytica" comes from the Greek term's "tissue" and "dissolving," and it refers to the parasite's ability to degrade large amounts of tissue (Petri *et al.*, 2002). The mucus barrier (MUC2 mucin) is the first line of innate host protection in the colonic milieu, forming a bimodal layer over the single layer of epithelial cells (Johansson *et al.*, 2008). Overcoming the mucus barrier to gain entry to epithelial cells is unquestionably necessary in *E. histolytica* pathogenesis. *E. histolytica* binds to the colonic mucus layer with high-affinity through the Galactose /N- acetyl galactosamine lectin (Gal/GalNAc lectin), which targets the abundant galactose and N acetyl galactosamine residues on the O-linked sugar side chains of mucin (Chadee *et al.*, 1987).

Initial *E. histolytica* infection causes mucosal layer thickening, which may be a defense mechanism to keep *E. histolytica* from touching the intestinal epithelium. *E. histolytica* also secretes a mucin, which activates goblet cell mucin secretion, as well glycosidases and proteases that degrade mucin polymers. The amoebic Gal/GalNAc lectin binds to Gal and GalNAc residue on the surface of exposed intestinal epithelial cells (IECs) in the absence of mucin. Mucin degradation, IEC flatten, and infiltrated neutrophils are both symptoms of progressive disease (Zeibig, 2013).

Furthermore, *E. histolytica* secretory tissue prevents proximal contacts and intestinal ion transport, resulting in diarrhea (Kissoon-Singh *et al.*, 2013). Ulceration in the colon may assume the shape of a flask and pass through the portal venous system, causing damage to the liver, brain, lungs, pericardium, and other organs. Amoebae in the liver induce an inflammatory response and necrosis of

liver cells, resulting in an amoebic liver abscess (Espinosa-Cantellano and Martinez-Palomo, 2000).

2-1-7: Clinical symptoms

There are two types of *E. histolytica* infection in the large intestine: acute infection and chronic infection (Arcari *et al.*, 2000). Diarrhea with a lot of mucus and blood in the stool, abdominal pain, nausea, vomiting, weakness, bloating, and a high body temperature is the most common pathological symptoms associated with acute infection, electrolyte solutions balance, and in extreme cases, fainting and cardiovascular instability (Bansal *et al.*, 2016). Chronic intestinal infection: If left untreated, the acute phase can progress to a chronic state marked by non-bloody diarrhea and constipation with symptoms of various severity (Nowak *et al.*, 2015).

The vegetative phase attacks the tissues by its decomposing enzymes, as the infection begins with necrosis of a small region in the surface layer of the mucous membrane, generating a goblet or pellet-shaped ulcer the size of a pinhead, and subsequently grows to a diameter of one centimeter or more (Murray, 2003), as the ulcer grows larger, lymphocytes, neutrophils, macrophages, and plasma cells will congregate. The ulcerated mucous regions enlarge and get covered in a grayish-white material. Acute clinical intestinal amebiasis, amoebic colitis, and ameboma granuloma are all caused by intestine inflammation (Carranza-Rosales *et al.*, 2010).

In temperate locations, asymptomatic infections are prevalent because carriers shed millions of cysts every day. It is known as a commensal parasite in this case (WHO,2010).

Amebic liver abscess is the most common extra intestinal manifestation of amoebiasis. Approximately 50-80% of individuals with ALA will present with

symptoms within 2 to 4 weeks, with fever and constant, aching right upper quadrant pain. In up to 50% of cases, patients present more chronically with protracted diarrhea, weight loss, and abdominal pain. Cough, right-sided pleural pain, and subsequent pleural effusion may occur when the diaphragmatic surface of the liver is involved (Kantor *et al.*, 2018).

2-1-8: Diagnosis

Microscopy, polymerase chain reaction (PCR), immunofluorescence (IFA), and serological tests such as enzyme-linked immunosorbent assay (ELISA), latex agglutination, and indirect hemagglutination assay (IHA) are now applied to diagnose *E. histolytica* infection (Petri *et al.*, 2000).

Microscopy is the most common method for diagnosing *E. histolytica*. Light microscopic examination of fecal specimens using direct, concentrated, or permanently stained smears are used to identify trophozoites and cysts of *E. histolytica*. The freshly acquired stool is examined for motile trophozoites, while cysts can be seen once the stool has been preserved in suitable fixatives (Pritt and Clark, 2008).

Polymerase Chain Reaction methods such as conventional, multiplex, nested and real-time have been developed for differentiation and detection of *E. histolytica* from nonpathogenic *E. dispar* and *E. moshkovskii* (Khairnar and Parija, 2007; Al-Quraishi and Al-Sultany, 2017).

Acuna-Soto *et al.* (1993) were the first to report on the successful use of PCR in examining the epidemiology of *Entamoeba* infection. The primers were designed to amplify the extrachromosomal circular DNA, and the DNA was recovered straight from feces, eliminating the requirement to culture trophozoites.

Because of its characteristics that eliminate post-PCR analysis, real-time PCR is a new and very appealing methodology for laboratory diagnosis of infectious diseases, resulting in shorter turnaround times, a reduction in the risk of amplicon contamination of laboratory environments, and lower reagent costs (Klein, 2002). This method enables specific amplicon identification during PCR by binding to one or two fluorescence-labeled probes, allowing for continuous monitoring of amplicon (PCR product) development during the reaction (Fotedar *et al.*, 2007a; AL-SULTANY and AL QURAIISHI, 2017).

Antibodies to *E. histolytica* infections have been detected using a variety of tests. All of the other assays, except for ELISA, are either time-consuming (immunodiffusion), less sensitive and nonspecific (IHA and Latex agglutination test), or require culture and antigen preparation skills (IFA), or are expensive to perform (Fotedar *et al.*, 2007).

Molecular techniques such as the antigen-specific ELISA for *E. histolytica* or real-time PCR (Othman *et al.*, 2010), are required to correctly diagnose *E. histolytica* in clinical samples and to provide the right epidemiology of amoebiasis in endemic regions (Blessmann *et al.*, 2003).

2-1-9: Immunity

The host activates a series of immunological responses to protect against the parasite after contact with the amoeba and further invasion of the gut epithelium. (Uribe-Querol and Rosales, 2020).

Invading amoebas is first met with innate immune defenses. Acid is an effective antimicrobial agent in the stomach, although amoeba cysts are resistant to it. Because IL-8 is a strong neutrophil chemoattractant, neutrophils are the first cells of the innate immune system to invading the intestinal tract during an

amoebic infection (Moonah *et al.*, 2013). These findings indicate that amoeba-induced inflammation causes tissue damage, which facilitates amoeba invasion (Marie and Petri, 2014).

Once amoebas leave the intestine and enter the circulation, they are exposed to the complement system. Complement is an important component of innate immunity against amoeba (Snow *et al.*, 2008).

Infection with *E. histolytica* is characterized by severe inflammation and a large number of invading neutrophils (Campos-Rodriguez *et al.*, 2016). Neutrophils transfer from the bloodstream to infection sites, where they perform antimicrobial function (Mayadas *et al.*, 2014), such as phagocytosis (Neeli and Radic, 2012; Rosales and Uribe-Querol, 2017). Neutrophils appear to play a protective function against this parasite, as amoebiasis is more severe when there are less neutrophils (Estrada-Figueroa *et al.*, 2011). According to some studies, *E. histolytica* trophozoites cause neutrophil mortality (Sim *et al.*, 2007), which results in the release of neutrophil lytic products, which cause inflammation and tissue damage (Dickson-Gonzalez *et al.*, 2009; Ghosh *et al.*, 2010).

Individuals infected with *E. histolytica* can produce specific IgG antibodies, although these antibodies usually prevent invasive amebiasis or recurrence (Marie and Petri, 2014). The protective or non-protective effects of IgG subclasses generated during the immune response appear to be dependent on the amoebic infections. IgG2 antibodies are preferred by a Th1 response, whereas IgG1 antibodies are preferred by a Th2 response (Bernin *et al.*, 2014). In addition, the amoeba cysteine proteinases, which are capable of degrading IgG in a dose-dependent way, play a role in the lack of protection from IgG antibodies (Tran *et*

al., 1998). IgA antibodies detected in the intestinal lumen, on the other hand, are protective (Haque *et al.*, 2006).

2-2: Lectins

Lectin originated from the Latin "Legere", meaning select or choose, to refer to a group of proteins that show similar selectivity in their interactions with carbohydrates (Arason, 1996).

Lectins are glycoprotein that can be found in many different organisms. Because they feature at least one non-catalytic domain that binds reversibly to particular monosaccharides or oligosaccharides. They can agglutinate erythrocytes with known carbohydrate specificity. Animal lectin yields are often modest when compared to plant lectin yields, such as legume lectins. Antitumor, immunomodulatory, antifungal and anti-insect actions are all shown by lectins, which may have practical applications. Antibacterial and anti-nematode activity is demonstrated by a small number of lectins (Lam and Ng, 2011).

2-2-1: History of lectin

The lectin was first discovered in plants in the form of ricin (found in *Ricinus* extracts) and abrin (found in *Abrus precatorius* extracts), which can agglutinate blood cells and contain heterodimeric proteins with two peptide chains held together by a disulfide bridge. This ability of lectin was previously known as phyto-haemagglutinins, phytoagglutinins, or plant agglutinins because the first isolated lectin that agglutinates the erythrocyte was from a plant source (Sharon and Lis, 2004).

This term was expanded by Sharon and Lis (1972) to include all proteins of non-immune origin that can bind carbohydrates, with or without specificity for

blood type erythrocytes. In 1980, Goldstein described lectins as non-immune proteins with two or more binding sites that can reversibly interact with carbohydrates, precipitate glycoconjugates, and agglutinate animal or plant cells. Many lectins have biological activities that are based on their general features and locations in various organs (Santos *et al.*, 2014).

2-2-2: Plant lectins

Plant lectins have been researched for more than a century. Until about a decade ago, the majority of information came from biochemical, molecular, and structural studies of a few numbers of abundant lectins found in seeds and vegetative storage organs. Though the results of these research are still valid, recent advances in various areas of plant lectin research require a thorough revision of the current paradigms (Van Damme *et al.*, 2008).

The existence of lectins across all kingdoms of life, as well as the capacity of structurally different lectins to identify the same or similar carbohydrate structures, makes classifying lectins difficult. Calnexins, calreticulins, and malectins are chaperones that help proteins fold in the endoplasmic reticulum (ER) and are found in plants, fungi, and animals. Even though the majority of lectin motifs are highly different in plants and animals, many lectins in both plant and animal systems are engaged in the recognition of invaders and hence are part of the immune system (Tsaneva and Van Damme, 2020). Because of the diversity among plant lectins, can be classified to:

Based on subcellular localization

Plant lectins can be divided into two classes based on where they are found in the plant cell. All lectins generated on the ribosomes linked to the ER belong to the

first group. As a result, these lectins are carried to vacuoles, deposited in cell walls, or expelled to the extracellular area. The second category includes lectins that are produced without a signal peptide, allowing the proteins to be translated into free ribosomes in the cytoplasm. These lectins can either stay in the cytoplasm or be translocated into the nucleus after production (Lannoo and Van Damme, 2010).

Based on molecular structure

Plant lectins are a huge and diversified protein family with a wide range of molecular structures and three-dimensional folds. Mero lectin is a kind of plant lectin that has only one lectin domain. A “holo lectin” is defined as a protein that contains two or more lectin domains, whereas a “chimero lectin” is defined as a protein that contains a lectin domain linked to at least one additional protein domain. Plant lectins can be made up of numerous lectin domains with diverse carbohydrate-binding characteristics, and are referred to as "super lectins" in this situation (Peumans and Van Damme 1995).

Based on sequence

A detailed examination of the lectin sequences available from genome and transcriptome analyses shows that all plant lectins are currently known can be classified in separate families, based on the sequence of the lectin motifs and the confirmation of their carbohydrate recognition domain(s). The carbohydrate specificity of lectins is not strictly tied to the three-dimensional structure of the carbohydrate recognition domain, which is interesting (Van Damme, 2014).

Based on abundance

Lectins are found in every organ of the plant. Seeds, bark, bulbs, corms, rhizomes, and other storage tissues are high in lectins, but lectins have also been

found in roots, shoots, leaves, and flowers, but in much lesser concentrations (Tsaneva and Van Damme, 2020). It is important to note that the levels of expression of lectins vary significantly. The secretory pathway is typically used to make the highly numerous lectins, which can account for 0.1 % to 10% of total protein in seeds or vegetative tissues. Because cytoplasmic lectins are often expressed at low levels, especially in the absence of stress. These plant lectins are difficult to detect in normal growth conditions. When the plant is stressed, however, the expression of the latter group of lectins increases. As a result, these lectins are called "inducible" plant lectins (Van Damme, 2014).

2-3: Hepcidin

Hepcidin (hepatic bactericidal protein) is a cysteine-rich peptide, that was first discovered as a urinary antimicrobial peptide. It contains 25 amino acids as well as four disulfide bridges (Rauf *et al.*, 2020).

Hepcidin is mostly produced by the liver, however new research has discovered that it is also produced by macrophages, pancreatic beta cells, kidneys, and adipocytes. The contribution of extrahepatic hepcidin to the circulating pool, on the other hand, is unknown (Viatte and Vaulont, 2009).

Hepcidin is the main regulator of systemic iron homeostasis. Hepcidin regulates plasma iron content and tissue distribution of iron by restricting intestinal iron absorption, iron recycling by macrophages, and iron mobilization from hepatic stores. Hepcidin acts by inhibiting cellular iron efflux by binding to and inducing the degradation of ferroprotein, the sole known cellular iron exporter (Ganz, 2003).

Hepcidin synthesis is raised homeostatically by iron loading and reduced by anemia and hypoxia. Hepcidin levels rise during infections and inflammation, causing a decrease in serum iron levels and contributing to the development of

inflammatory anemia, most likely as a host defense mechanism to limit iron availability to invading microbes. Hepcidin deficiency, on the other hand, appears to be the ultimate cause of most cases of hemochromatosis, either as a result of mutations in the hepcidin gene or the regulators of hepcidin synthesis. The discovery of hepcidin as a pathogenic component in the majority of systemic iron diseases could open new opportunities for better diagnosis and therapy (Nemeth and Ganz, 2006).

2-4: β -Tryptase

β -Tryptase appears to be the most common type of tryptase stored in mast cell granules present in peripheral tissue that play an important role in inflammatory and allergic reactions, and it is not usually released into the bloodstream. In extreme inflammatory circumstances such as systemic anaphylaxis, however, elevated β -tryptase levels can be seen in serum (Hallgren and Pejler, 2006).

β -Tryptase is the most abundant mediator contained in mast cell granules and is a neutral serine protease. Mast cell degranulation is characterized by the release of β -tryptase from secretory granules. Mast cell β -tryptase plays a vital part in inflammation and serves as a marker of mast cell activation. The protease activated receptor type 2 is activated by tryptase. Airway homeostasis, vascular relaxation and contraction, gastrointestinal smooth muscle activity and intestinal transport, and coagulation are all influenced by this protein. The concentration of serum mast cell-tryptase is elevated in anaphylaxis and other allergic disorders. In systemic mastocytosis and other haematological disorders, it is increased (Payne and Kam, 2004).

2-5: Cytokines

Cytokines are soluble extracellular proteins or glycoproteins that act as intercellular regulators and mobilizers of cells involved in innate and adaptive inflammatory host defenses, cell growth, differentiation, cell death, angiogenesis, development, and repair processes. Although cytokines are rarely created on their own, they are almost always produced in response to harmful stimuli by practically every nucleated cell type. Cytokines work by interacting with cells that have comparable receptors (Zhang and An, 2007).

Interleukins were first named in 1981 to describe the cellular kinetics released by white blood cells that affect other white blood cells (Detrick *et al.*, 2008); however, this definition is insufficient because some of them are secreted by cells other than white blood cells. As a result, Stevens (2010) designated three features of cellular kinetics to be called interleukins, which are: -

- 1- Be genetically encoded.
- 2- It normally stimulates white blood cells.
- 3- Shows its effectiveness in inflammatory processes.

Because of the many types of interleukins that participate in the immune response, we will focus our attention on two important types:

2-5-1: IL-12

IL-12 is produced by dendritic cells, macrophages, neutrophils, and human B-lymphoblastoid cells. The interleukin-12 family which comprises IL-12, IL-23, IL-27, and IL-35, is remarkable in that it contains the only heterodimeric cytokines.

Despite having numerous structural similarities and molecular partners in common, they mediate a wide range of functional effects (Beadling and Slifka, 2006).

IL12 is a heterodimeric cytokine that is encoded by two genes: IL-12A (p35) and IL-12B (p40). A bundle of four alpha-helices makes up IL12A. There are three beta-sheet domains in IL12B (Merberg *et al.*, 1992).

The development of naive T cells into Th1 cells is aided by IL-12. It's a T cell-stimulating factor, which means it can help T cells grow and perform better. T cells and natural killer (NK) cells produce more interferon-gamma (IFN-) and tumor necrosis factor-alpha (TNF), and IL-4-mediated inhibition of IFN- is reduced. Natural killer cells and T lymphocytes both use IL-12 to carry out their functions (Vignali and Kuchroo, 2012). The cytotoxic activity of NK cells and CD8+ cytotoxic T lymphocytes is enhanced by IL-12. In NK cells (Trinchieri, 2003).

Anti-angiogenic action means that IL-12 can prevent the creation of new blood vessels. It does this by raising interferon-gamma production, which in turn increases the synthesis of a chemokine known as inducible protein-10 (IP-10). The anti-angiogenic impact is then mediated by IP-10. There has been interest in evaluating IL-12 as a potential anti-cancer medicine due to its ability to induce immune responses and anti-angiogenic action. However, it has not been found to have much activity in the tumors that have been studied so far. There is a relationship between IL-12 and the diseases psoriasis and inflammatory bowel disease that may be effective in treatment (Hamza *et al.*, 2010).

2-5-2: IL-17

IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F are the six members of the IL-17 family of inflammatory cytokines, which include IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F (Jin and Dong, 2013).

Interleukin-17 (IL-17, also known as IL-17A) is a key cytokine that links T cell activation to neutrophil mobilization and activation. As a result, IL-17 can contribute to the pathophysiology of inflammatory illnesses including psoriasis and rheumatoid arthritis by mediating protective innate immunity against pathogens (Milovanovic et al., 2020). Periodontitis, rheumatoid arthritis, and other disorders involving bone immunopathology are expected to be exacerbated by IL-17 significant pro-osteoclastogenic actions. In clinical trials for psoriasis and rheumatoid arthritis, systemic treatments with anti-IL-17 biologics have shown promising results, but their impact on the widely widespread periodontal disease has not been examined or reported. To conclusively implicate IL-17 in periodontitis and, more importantly, to establish an effective adjunctive treatment for this oral inflammatory disease, future clinical trials, preferably using locally administered IL-17 blockers, are required (Zenobia and Hajishengallis, 2015).

2-6: Complete Blood Count (CBC)

The complete blood count (CBC) is one of the most common laboratory tests performed today. It provides information about the formation of all blood cells and indicates the patient's oxygen-carrying ability through the assessment of red blood cell (RBCs) indices, hemoglobin, and hematocrit. It also offers information about the immune system through the assessment of the white blood cell (WBCs) count with differential. These tests are aid in diagnosing anemia, infection, acute hemorrhagic states, certain cancers, allergies, and immunodeficiencies as well as

monitoring for side effects of certain medicines that cause blood dyscrasias. CBC measure: red blood cells (RBCs), White blood cells (WBCs), Platelets (PLT), Hemoglobin (HGB), Hematocrit (HCT), Mean corpuscular volume (MCV). (George-Gay and Parker, 2003).

2-7: Effect of *E. histolytica* on liver tissue

During the early stages of amoebic liver abscess, there are two main components: Cellular infiltration by polymorphonuclear leukocytes and mononuclear leukocytes, and Ischemia of damaged cells due to hepatic portal vein obstruction. Gradual enlargement of the liver also occurs due to the formed abscess. An abscess is a collection of pus in a limited space resulting from the decomposition and death of cells of a specific part of the tissue due to a parasitic or bacterial infection (Ralston and Petri, 2011).

The abscess is in the form of a capsule or a cover formed by the walls of the adjacent healthy cells in order to prevent the spread of infection to other areas, as well as a space filled with pus, which is dead and decomposed white blood cells and blood cells, as a result, inflammation occurs in the place, and symptoms of inflammation appear, which include redness of the place, swelling and pain in the upper right side of the abdomen, and weight loss. The abscess may extend to the main bile duct, causing jaundice (Blazquez *et al.*, 2007).

When injecting the dysentery amoeba parasite into the liver of laboratory animals, hamsters and mice, it will lead to the killing of its cells, as some cells die by necrosis, and other cells suffer the phenomenon of programmed death (Apoptosis) which means that an Endonuclease enzyme cleaves chromatin between the bodies of histones in the DNA molecule of an infected cell, which leads to its

fragmentation, which in turn, it leads to cell death. Necrosis takes three forms, depending on the changes that occur in the nucleus. Either the nucleus appears small in size, so it is called Byknosis, or the nucleus appears in the form of small or fragmented pieces, and it is called Karuorrhesis. The third change is the disappearance and decomposition of the nucleus in the infected cell, and it is called Kariolysis (Chabuk *et al.*, 2014).

Chapter Three

Materials & Methods

3-1: Equipment's, laboratory tools, chemicals and kits used in the present study

Table (3-1): The equipment's and tools used in the present study

Equipment and tools	Company	Origin
Beaker	Lab	Germany
Centrifuge	Memmert	Germany
Class Cylinder Graduated	Lab	Germany
Compound light microscope with camera	Genex	USA
Dissection Set	Elphor	Germany
Distillator	Lab tech	Korea
EDTA tubes	Labtub	China
Electrical oven	Memmert	Germany
Electronic sensitive Balance	Sartorius	Germany
Electronic sensitive balance for animals	Denver	Canada
Eppendorf tubes	VWR International	USA
Gel tubes	Labtub	China
Hematology analyzer	Orphee	France
Hot plate	Lassco	India
Incubator	Memmert	Germany
Latex gloves	HiGeen	Jordan
Light microscope	Snitch Xsz-N107	Malaysia
Masks	Broche	P.R.C
Micropipette	Dragon	China
Micropipette tips	Citotest	China

Microplate reader	BioTek	USA
Plain tubes	Afco-Dispo	Jordan
Plastic cups		China
Refrigerator	Kiriazi	Egypt
Slides and cover slides	Citotest	China
Syringes	Josef 97	China
Water bath	Raymond A	England
Wax dispenser	Marubeni	Japan

Table (3-2): Chemicals used in the present study

Chemicals	Company	Origin
Chloroform	BDH	England
Dextrin plasticizer xylene D.P.X	Thomas Baker	India
Distilled water	Samarra	Iraq
Eosin (C ₂₀ H ₆ Br ₄ Na ₂ O ₆)	GCC	England
Ethanol	Scharlau	Spain
Formalin	BDH	England
Glacial acetic acid	BDH	England
Hematoxylin	GCC	England
Lugol's-Iodine	Merk	Germany
Mercuric oxide	Sigma	USA
Metronidazole	Samarra	Iraq
Normal saline	PIONEER	Iraq
Paraffin wax	GCC	England
Potassium alum AIK (So ₄) ₂ 12H ₂ O	Sigma	USA

Chemicals	Company	Origin
Rat Hecpidin ELISA Kit	BT LAB	China
Red mercuric oxide	Sigma	USA
Thymol crystals	GCC	England
Glycerol	Scharlau	Spain
Seeds Wheat lectin	Shaanxi Mukelya Biothchnology	China
Xylene	Scharlau	Spain

Table (3-3): Kits used in the present study

Kits	Company	Origin
Rat Hecpidin ELISA Kit	BT LAB	China
Rat tryptase ELISA Kit	BT LAB	China
Rat IL-12 ELISA Kit	BT LAB	China
Rat IL-17 ELISA Kit	BT LAB	China

3-2: Stool samples collection

Stool samples collected from patients with amoebic dysentery who suffer from mucosal bloody diarrhea and visitors to the laboratories of Babylon Hospital for Women and Children and Al Noor Hospital for Children, as samples were collected in sterile plastic bottles supplied with an airtight seal to maintain the sample's moisture and prevent its drying, it was immediately moved to the Advanced Parasitology Laboratory at the College of Science /Biology Department / The University of Babylon, as it was used in infecting laboratory albino rats as well as diagnosing samples by direct swabbing.

3-3: Samples examination

3-3-1: Macroscopic stool samples examination

The consistency, quantity, color and form of the stool gives the examiner a lot of useful information, as diarrhea resulting from the *E. histolytica* is often foul smelling and contains a lot of fecal matter, and also notes the presence of blood or mucus, or both, as their presence indicates that the person suffers from dysentery amoeba (Clark and Diamond, 2002).

3-3-2: Microscopic stool examination

The stool was microscopically examined by the Direct smear method. In this method, a drop of Normal Saline 0.9% (sodium chloride) was placed on one side of a clean, dry glass slide and another drop of Lugol's iodine dye to easily distinguish the core of the cyst, with the wooden stick, a small quantity of feces was taken and mixed in a good manner with Normal Saline and Lugol's iodine. Samples were taken from different places of the model, especially the mucous or bloods area to increase the likelihood of the parasite's emergence, then put the cover slide without causing air bubbles after removing any large particles from

the sample and then examining it with a light microscope to see trophozoite and cysts phases under magnification force 400 X (Tanyuksel and Petri, 2003).

3-4: Preparation of *E. histolytica* suspension

Where the parasite suspension was prepared, which was used in dosing rats, by mixing 200 gm of feces containing the parasite cysts with 100 ml of a Normal Saline (0.9%) and filtering the mixture through four layers of gauze to remove large feces residues from the sample and collect the filtrate in a large capacity beaker 500 ml (Chabuk, 2013).

3-4-1: Determination of the dose of *E. histolytica* cysts

Taken 50 μ l of parasite suspension by a Micropipette, which was placed on a Haematocytometer slide, and Lugol's iodine stain was added to it and examined using a light microscope and the mean number of cysts was calculated for three replicates using a fixed volume method and it was approximately 50 cysts per 50 μ l. The mean of the replicates was (50) cyst per (50) microliters. Then each rat was dosed orally with 2 ml of the parasitic suspension employing of a specialized syringe to dose the rat. The rats were left for 7 days to ensure infection and to notice changes in the rat's feces in terms of texture, color and mucus presence (Chabuk, 2013).

3-5: Preparation of lectin dose

Taken (3 mg) of wheat lectin and dissolved in (1ml) of normal saline 0.9 % and used in injecting rats by dividing the injection site into four areas of the animal's body, which are under the skin near the pelvis on the right and left sides and under the skin near the neck on the right and left sides, and this is called, repeated Injections every week for three injections only (Baintner *et al.*, 2007).

3-6: Experimental animals

This study was conducted at the University of Babylon / College of Science / Department of Biology from February 2022 to April 2022.

The present study used 45 adult female albino rats (*Rattus rattus*) obtained from the animal house of the Biology Department / College of Science at the University of Babylon, their weight ranged between (150 -170 g) and aged between 2-3 months, placed in plastic cages designated for raising rats. The floors of the cages are equipped with sawdust, which is replaced continuously to maintain hygiene. The experimental animals were dosed with metronidazole for 7 days with 20 mg/kg dose every 12 hours for the purpose of eliminating parasitic infections (Beyhan and Hokelek, 2014) and left for a week to adapt to suitable environmental conditions in terms of temperature, drinking water and aeration. The animals were left for two weeks to adapt to the experimental conditions. with the maintenance of proper hygiene and sterilization.

3-7: Experimental design

The 45 rats were classified into (3) main, each group was divided into subgroups according to the period as follows (Figure 3-1):

- Group 1 (12 rats): 3 rats were dosed with normal saline 0.9 % and considered as a control group, all remaining rats of this group were injection with lectin (first dose) after 7 days dissected control group and 3 rats of the group, then injection the remaining rats with lectin (second dose) after 14 days dissected 3 rats and injection the remaining rats with lectin (third dose) and dissected them after 21 days.
- Group 2 (12 rats): 3 rats were dosed with normal saline 0.9 % and considered as a control group, all rats of this group were infected with *E. histolytica*, after 7 days dissected control group and 3 rats of the group,

after 14 days dissected 3 rats and the remaining (3) rats dissected after 21 days.

- Group 3 (21 rats): 3 rats were dosed with normal saline 0.9 % and considered as a control group, all rats of this group were injection with lectin (first dose), after 7 days dissected control group and 3 rats of the group, then injection the remaining rats with lectin (second dose), after 14 days dissected 3 rats and injection the remaining rats with lectin (third dose) and dissected 3 rats after 21 days. After 1 week of last lectin injection, infected the remaining rats with *E. histolytica*, after 7 days of infection dissected 3 rats, after 14 days dissected 3 rats and after 21 days dissected the remaining (3) rats.

The rats were anesthetized using chloroform to dissected; blood was collected directly by heart puncture about 1 ml of fresh blood was put in an EDTA tube to measure CBC and put 4 ml in gel tubes. Allowed serum to clot for 10-20 minutes at room temperature. Centrifuged at 2000-3000 rpm for 20 minutes. Then serum was kept in Eppendroff tubes in a refrigerator until used for measuring the following immunological parameters (Hepcidin, β -tryptase, IL-12, IL-17). The liver of the rats was removed carefully and kept with formalin 10% until used in the histological study.

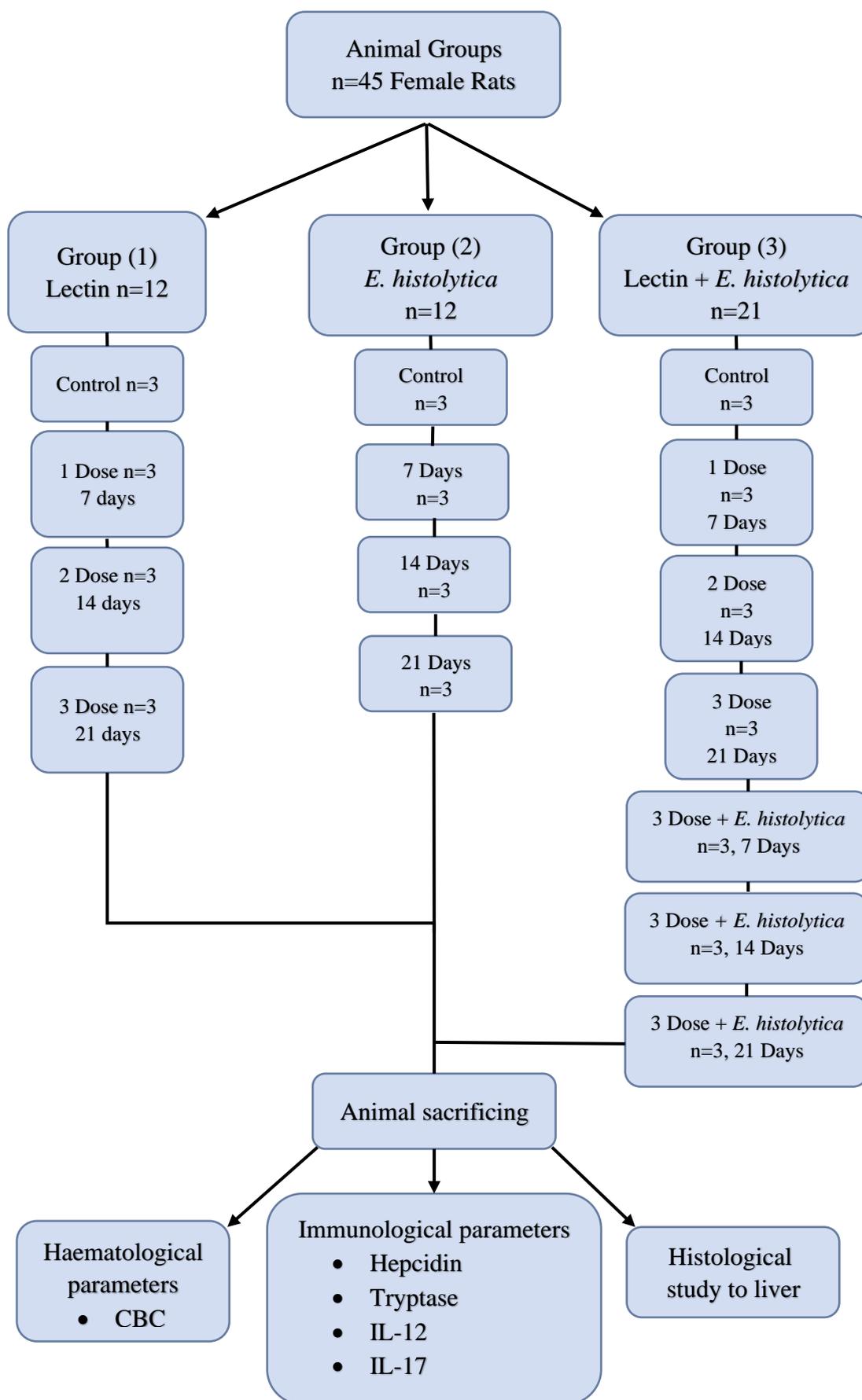


Figure (3-1): Experimental design of the present study

3-8: Hematological parameters

3-8-1: CBC measurement of blood samples

The numbers of WBCs and RBCs were calculated, the percentage of lymphocytes, monocytes and granulocytes, and HGB levels were measured, as well as the count of PLT. By using the hematology analyzer of Orphee company of France origin.

3-9: Immunological parameters

3-9-1: Measurement of serum Hepcidin

Rat Hepcidin ELISA Kit

Assay Procedure according to the kit method

- 1.** prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.
- 2.** Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.
- 3.** Add 50µl standard to standard well. **Note:** Don't add biotinylated antibody to standard well because the standard solution contains biotinylated antibody.
- 4.** Add 40µl sample to sample wells and then add 10µl anti-Hepc antibody to sample wells, then add 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.
- 5.** Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.

6. Add 50 μ l substrate solution A to each well and then add 50 μ l substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
7. Add 50 μ l Stop Solution to each well, the blue color will change into yellow immediately.
8. Determine the optical density (OD value) Figure (3-2) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

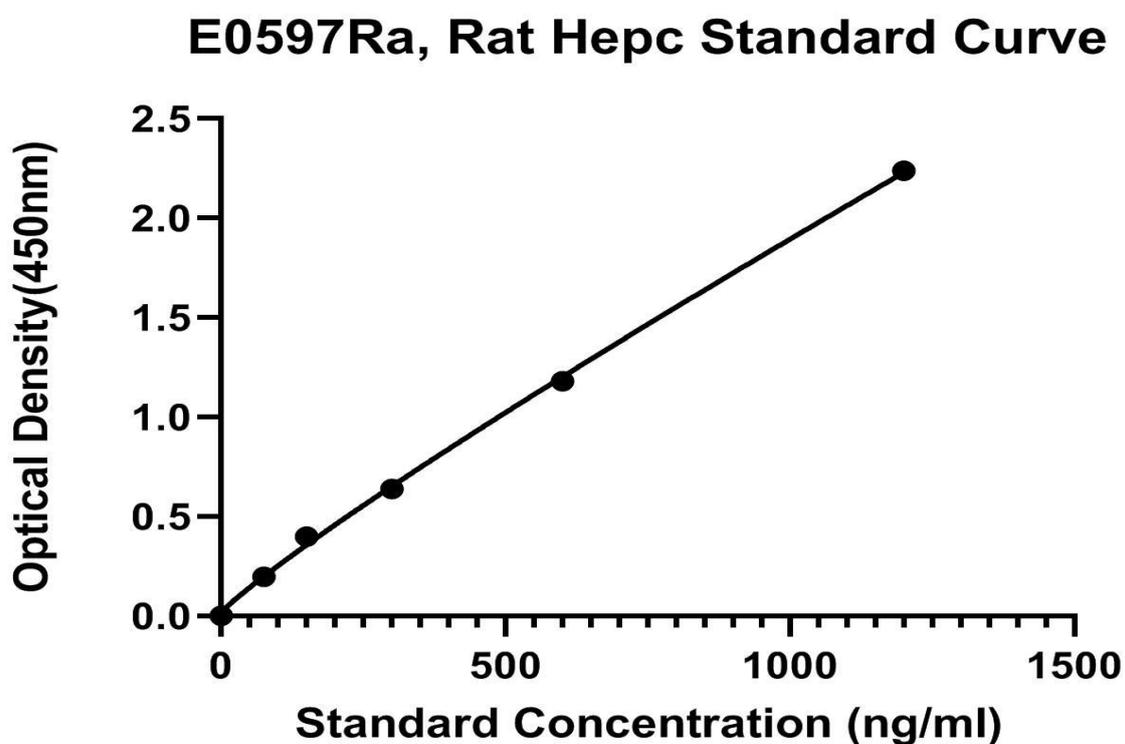


Figure (3-2): Rat Hepcidin standard curve

3-9-2: Measurement of serum β -Tryptase**Rat β -Tryptase ELISA Kit****Assay Procedure according to kit method**

1. prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.
2. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.
3. Add 50 μ l standard to standard well. **Note:** Don't add biotinylated antibody to standard well because the standard solution contains biotinylated antibody.
4. Add 40 μ l sample to sample wells and then add 10 μ l anti-TPS antibody to sample wells, then add 50 μ l streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.
5. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with 300 μ l wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.
6. Add 50 μ l substrate solution A to each well and then add 50 μ l substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
7. Add 50 μ l Stop Solution to each well, the blue color will change into yellow immediately.
8. Determine the optical density (OD value) Figure (3-3) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

E0427Ra, Rat TPS Standard Curve

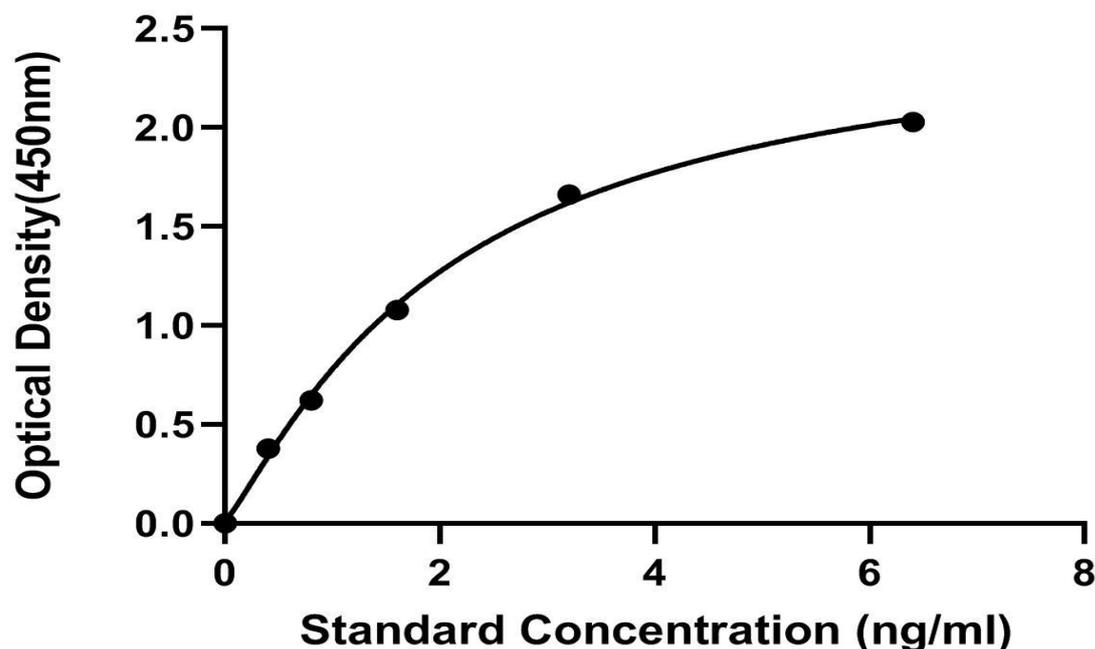


Figure (3-3): Rat β -tryptase standard curve

3-9-3: Measurement of serum IL-12

Rat IL-12 ELISA Kit

Assay Procedure according to kit method

1. prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.
2. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.
3. Add 50 μ l standard to standard well. **Note:** Don't add biotinylated antibody to standard well because the standard solution contains biotinylated antibody.
4. Add 40 μ l sample to sample wells and then add 10 μ l anti-IL-12 antibody to sample wells, then add 50 μ l streptavidin-HRP to sample wells and standard

wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.

5. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.
6. Add 50µl substrate solution A to each well and then add 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
7. Add 50µl Stop Solution to each well, the blue color will change into yellow immediately.
8. Determine the optical density (OD value) Figure (3-4) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

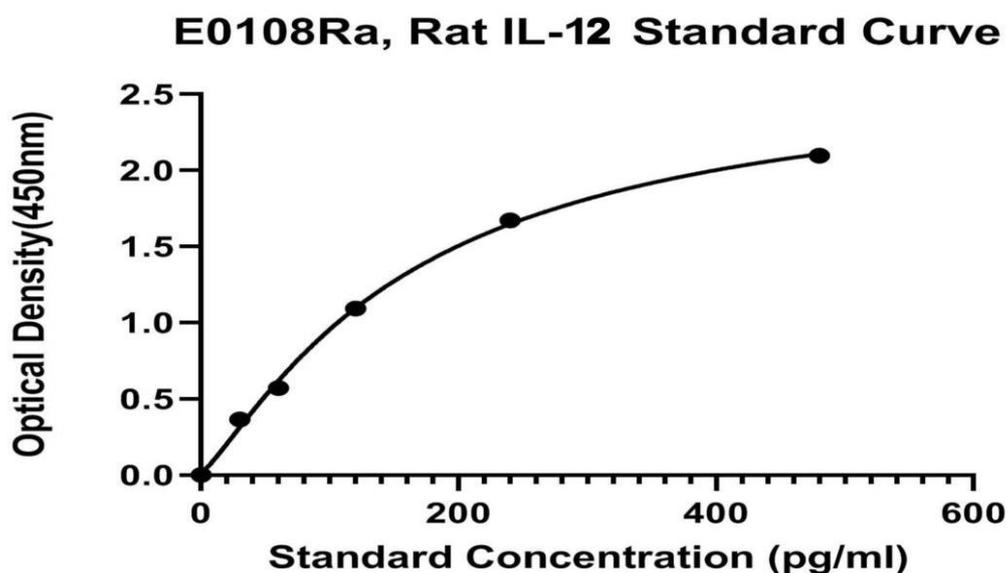


Figure (3-4): IL-12 standard curve

3-9-4: Measurement of serum IL-17**Rat IL-17 ELISA Kit****Assay Procedure according to kit method**

1. prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.
2. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.
3. Add 50µl standard to standard well. **Note:** Don't add biotinylated antibody to standard well because the standard solution contains biotinylated antibody.
4. Add 40µl sample to sample wells and then add 10µl anti-IL-17 antibody to sample wells, then add 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.
5. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.
6. Add 50µl substrate solution A to each well and then add 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
7. Add 50µl Stop Solution to each well, the blue color will change into yellow immediately.
8. Determine the optical density (OD value) Figure (3-5) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

E0115Ra, Rat IL-17 Standard Curve

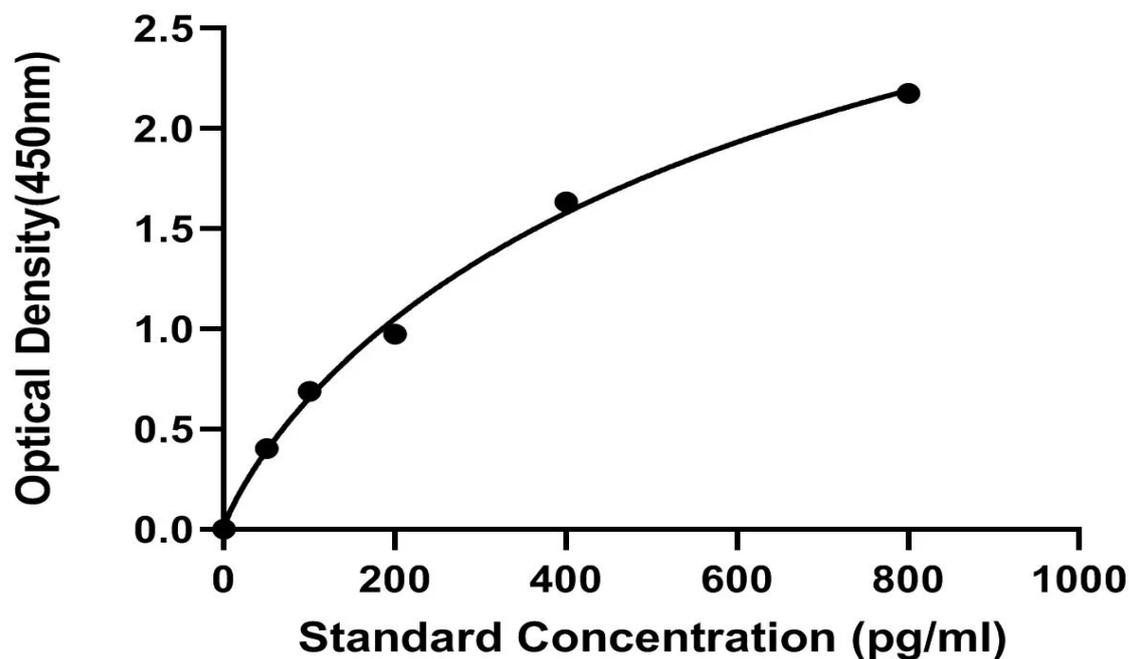


Figure (3-5): IL-17 standard curve

3-10: Histological study

3-10-1: Preparation of solution and stains

3-10-1-1: Formalin solution

Prepare a formalin solution of 10 % according to Baker and Silverton (2014), by placing one part of 25 ml of formaldehyde at a concentration of 40 % with three parts of distilled water 75 % ml in a one-liter glass tube with shaking. This solution is used to fix the tissue sections.

3-10-1-2: Hematoxylin Harris stain

This stain has been prepared according to Bancroft and Steven (2010) as follows:

- 1gm of hematoxylin powder
- 10 ml of absolute ethanol
- 20 gm of potassium alum ($KAl(SO_4)_2 \cdot 12H_2O$)

- 200 ml distilled water
- 0.5 gm of mercuric oxide
- 5 ml of glacial acetic acid

Hematoxylin powder dissolved in 10 ml of absolute ethanol. Potassium alum is dissolved in distilled water, boiled and then added to the dissolved hematoxylin. The mixture was boiled for 1/2 minutes. Then 0.5 gm of mercuric oxide was added. the solution cooled rapidly, and a few drops of glacial acetic acid was added.

3-10-1-3: Alcoholic eosin stain

It was prepared according to Bancroft and Steven (2010) by mixing 1 gm of eosin with 99 ml ethanol.

3-10-1-4: Mayer's Albumin

It was prepared according to Presnell and Schreibmaan (1997) as follow:

- 50 ml of Egg albumin
- 50 ml of Glycerol
- 1 gm of Thymol

3-11: Preparation of histological sections

Histological sections were prepared for the liver according to Bancroft *et al.* (2013) as follows:

3-11-1: Sample fixation

The samples were placed immediately after the dissection in a formalin solution (10 %) to fix them and after using this fixative, the most common histological technique, because it leaves the fixed tissues in good condition and suitable for many tissue dyes. Formalin penetrates the tissues quickly, so the appropriate timing for installation ranges from 24-48 hours.

3-11-2: Washing

The samples were washed after fixation with running water for half an hour to remove all the residues of the fixative for two things, the first to avoid over-fixing and the other to prevent the interference of the fixative residues with other chemicals used in the subsequent steps, especially in the dyeing process.

3-11-3: Dehydration

This step was done by transferring the samples into ascending concentration of ethanol as follow:

- 70% ethanol for 2 hours.
- 80% ethanol for 2 hours.
- 90% ethanol for 2 hours.
- 100% ethanol for 2 hours.

3-11-4: Clearing

The clearing process is used to remove the dehydrator agent from the samples and replace it with solutions mixed with molten paraffin wax. This step has been done by transferring the samples into a bath of xylene, two exchanges were done, one hour for each to ensure a good transparency of the tissue.

3-11-5: Infiltration

Was done by using a mix of xylene and paraffin wax (melting point 56-58 °C), and the samples were put in an electric oven at a temperature of 60 °C for 30 minutes. Then transferred to new molten wax for two stages (one hour for each stage).

3-11-6: Embedding

An electric wax dispenser was used for embedding the sections in a labeled baths of a molten paraffin wax melting point of 58 °C, two changes were performed for 2 hours for each using an embedding oven. Then the samples were

transferred to be blocked in paraffin wax using a labeled stainless-steel embedding mold (L- shaped). When the solidification of the blocks completed, the blocks were separated from the molds and were kept at the lab temperature.

3-11-7: Trimming and Sectioning

Wax molds containing the samples were trimmed using a sharp scalpel, and the samples were sectioned by using the rotary microtome at 5 μm thickness. Sections were then floated on a water bath at 50 $^{\circ}\text{C}$, then the sections were mounted on a glass slide coated with a thin layer of Mayer's albumin.

3-11-8: Staining

The hematoxylin and eosin staining procedure had been used for showing the general components of the tissues. This staining procedure had been done according to (Presnell and Schreibman, 1997) as follow:

- Dewaxing of the slides by xylene for 30 minutes.
- Rehydration of the sections with descending concentrations of ethanol (100,90,80,70 %) for 2 minutes each.
- Immersing the slides in Harris hematoxylin for 3-5 minutes.
- Rinsing the slides in running distilled water for 5 minutes for optimum bluing.
- Immersing in 1% Eosin for 1-3 minutes.
- Rising in running tap water for 3-5 minutes.
- Dehydration through ascending concentrations of ethanol (70, 80, 90, 100%) for 2 minutes each.
- Clearing for 2-3 minutes in xylene.

3-11-9: Mounting

The prepared slides were mounted with Dextrin plasticizer xylene (D.P.X), covered by a cover slip then left to dry. The slides then became ready for microscopic examination and photography.

3-12: Microscopic examination and photography

The slides were examined by using a compound light microscope to observe the histological changes in the slides of the groups compared with the slides of the control group. The tissue sections of the liver were photographed using the camera attached to the microscope.

3-13: Statistical analysis

Statistical Package for Social Science (SPSS) version 23.0 was used for statistical analysis of the data. Using the One-Way ANOVA to show the means and standard error (S.E.) and comparing the groups of rats with the control group under a significant level ($P \leq 0.05$), and using the correlation coefficient to show the linear relationships using the linear regression test for each relationship (Al-Rawi and Khalaf-Allah, 1980).

Chapter Four

Results

4: Results

4-1: The physiological and immunological study

4-1-1: The effect of lectin injection on haematological parameters

The results of the present study results as in Table (4-1) showed the effect of lectin injections on different hematological parameters (Total WBCs, Lymphocytes percentage, Monocytes percentage, Granulocytes percentage, total RBCs, Hemoglobin concentration, and total PLTs). It was observed that the total WBCs changed significantly during the periods of injection, as well as the percentage of granulocytes and the total of PLTs. The other hematological parameters were affected by an increase or decrease but not significantly.

Table (4-1): The effect of lectin injection on hematological parameters in albino rats (*R. rattus*)

Period	Hematological parameters Mean±SE						
	Total WBCs ×10 ³ /μl	Lymphocytes %	Monocytes %	Granulocytes %	Total RBCs ×10 ⁶ /μl	HGB g/dl	PLTs ×10 ³ /μl
Before injection (Control)	4.3±0.4 b	52.46±3.21 a	14.83±2.12 a	32.7±2.61 b	9.43±0.24 b	15.53±0.24 b	361.33±86.91 a
First injection (After 1 week)	8.1±0.3 c	65.06±0.71 ab	15.96±1.58 a	18.96±2.29 a	6.64±0.26 ab	11.16±0.2 ab	584.33±52.09 ab
Second injection (After 2 weeks)	7.03±0.32 c	66.7±3.6 b	16.33±2.33 a	16.96±4.59 a	5.36±1.92 a	8.73±3.3 a	773.66±115.54 b
Third injection (After 3 weeks)	5.03±0.59 b	61.07±2.35 ab	19.3±2.75 a	20.63±3.31 a	5.8±0.09 a	9.93±0.23 ab	656.66±14.74 b
Sig. level	0.000*	0.117	0.566	0.039*	0.070	0.085	0.030*
(*P ≤ 0.05)							

4-1-2: The effect of *E. histolytica* infection on haematological parameters

It was found that infection of albino rats with *E. histolytica* significantly increased or decreased all hematological parameters except the percentage of monocytes and the number of PLTs (Table 4-2).

Table (4-2): The effect of *E. histolytica* infection on hematological parameters in albino rats (*R. rattus*)

Period	Hematological parameters Mean±SE						
	Total WBCs ×10 ³ /μl	Lymphocytes %	Monocytes %	Granulocytes %	Total RBCs ×10 ⁶ /μl	HGB g/dl	PLTs ×10 ³ /μl
Before infection (control)	4.1±0.41 a	52.83±3.43 a	15.96±2.32 a	31.2±2.69 ab	9.36±0.2 c	15.53±0.37 c	360.33±87.62 a
After 1 week of infection	7.5±0.62 b	58.96±1.7 ab	16.63±1.14 a	24.4±0.95 ab	6.76±0.29 a	10.7±0.61 a	384.66±156.6 a
After 2 weeks of infection	4.36±1.14 a	51.16±6.4 a	12.36±1.39 a	36.46±7.23 b	8.5±0.71 bc	14.3±0.87 bc	479.33±37.3 a
After 3 weeks of infection	2.03±0.12 a	70.1±4.55 b	11.73±3.03 a	18.16±1.31 a	7.35±0.54 ab	11.8±1.21 ab	689.66±205.64 a
Sig. level	0.004*	0.036*	0.318	0.049*	0.021*	0.021*	0.375
(*P ≤ 0.05)							

4-1-3: The effect of *E. histolytica* infection after lectin injection on haematological parameters

Table (4-3) shows there are variations (increase or decrease) in the levels of all hematological parameters in albino rats experimentally infected with *E. histolytica* after being injected with lectin. These changes were not significant

except the total number of WBCs, Lymphocytes percentage, and Monocytes percentage.

Table (4-3): The effect of *E. histolytica* infection after lectin injection on hematological parameters in albino rats (*R. rattus*)

Period	Hematological parameters Mean±SE						
	Total WBCs ×10 ³ /μl	Lymphocytes %	Monocytes %	Granulocytes %	Total RBCs ×10 ⁶ /μl	HGB g/dl	PLTs ×10 ³ /μl
Before infection (control)	4.4±0.34 b	53.4±3.9 a	15.96±2.03 abc	30.63±2.64 b	8.9±0.27 b	14.8±0.7 b	453.59±922.01 a
First injection (After 1 week)	7.8±0.21 c	65.04±0.7 ab	15.96±1.58 abc	17.6±2.3 a	7.1±0.28 a	11.5±0.2 ab	531.33±50.3 ab
Second injection (After 2 weeks)	6.07±0.39 c	66.8±3.7 b	16.33±2.33 bc	16.2±4.1 a	5.4±1.9 a	9.1±3.4 a	653.72±110.43 b
Third injection (After 3 weeks)	5.01±0.58 b	60.06±6.06 ab	19.3±2.75 c	21.60±3.1 a	5.7±0.09 a	10.2±0.3 ab	795.72±15.63 b
After 1 week of infection	6.93±1.17 c	71.36±3.63 ab	9.93±1.13 a	18.7±2.51 a	7.06±0.46 a	11.36±0.89 a	620.33±37.25 ab
After 2 weeks of infection	7.45±0.06 c	78.26±0.68 b	10.43±0.31 ab	11.3±0.46 a	5.19±0.76 a	8.66±1.36 a	621.65±54.03 ab
After 3 weeks of infection	6.53±0.14 c	64.66±5.24 a	15.06±1.96 ab	20.26±3.29 a	6.58±0.33 a	10.83±0.67 a	632.33±19.36 b
Sig. level	0.000*	0.057*	0.035*	0.156	0.584	0.748	0.075
(*P ≤ 0.05)							

4-1-4: The effect of lectin injection on immunological parameters

It was found that the results of the current study (Table 4-4 and Figure 4-1) that studied immunological parameters were affected by significantly increased or decreased in their concentrations during the periods of lectin injection of albino rats. Statistical proved that the variations in immunological criteria concentrations during the injection periods were significant only in β -tryptase, while the other parameters were not significant.

Table (4-4): The effect of lectin injection on immunological parameters in albino rats (*R. rattus*)

Period	Immunological parameters Mean \pm SE			
	Hepcidin ng/ml	β -tryptase ng/ml	IL-12 pg/ml	IL-17 pg/ml
Before injection (control)	269.9 \pm 17.36 b	2.3 \pm 0.5 b	64.03 \pm 19.1 a	37.22 \pm 7.59 a
First injection (After 1 week)	186.41 \pm 62.05 a	0.91 \pm 0.09 a	55.26 \pm 9.55 a	69.21 \pm 20.02 b
Second injection (After 2 weeks)	145.37 \pm 9.38 a	0.57 \pm 0.03 a	61.92 \pm 10.47 a	58.77 \pm 11.69 a
Third injection (After 3 weeks)	117.97 \pm 5.94 a	1.29 \pm 0.16 a	50.7 \pm 5.86 a	39.12 \pm 2.28 a
Sig. level	0.498	0.022*	0.859	0.303
(*P \leq 0.05)				

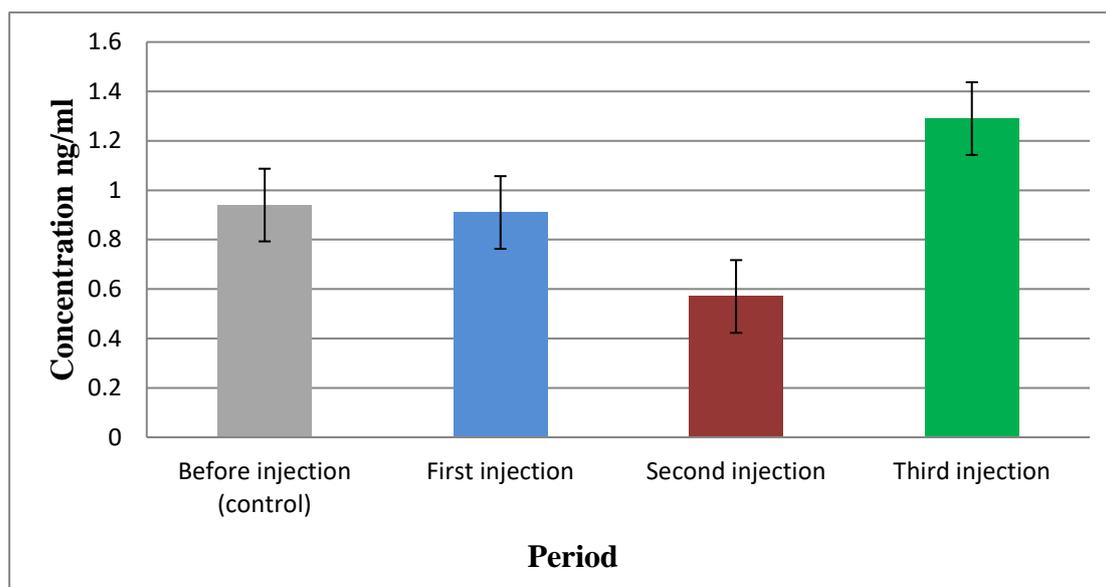


Figure (4-1): The significant effect of lectin injection on β -tryptase concentration in albino rats

4-1-5: The effect of *E. histolytica* infection on immunological parameters

It was that the effect of infecting albino rats with *E. histolytica* on the immunological was a different way, as we found that the concentration of Hepcidin began to decrease at the beginning of the infection period (after 1 week) and then began to rise, but did not reach to the level of the control concentration, while we find that the concentration of β -tryptase gradually has decreased. During the study period the difference in the concentration of both criteria was significant. The variations in the concentration of IL-12, and IL-17 were not significant (Table 4-5, Figure 4-2 and Figure 4-3).

Table (4-5): The effect of *E. histolytica* infection on immunological parameters in albino rats (*R. rattus*)

Period	Immunological parameters Mean±SE			
	Hepcidin ng/ml	β-tryptase ng/ml	IL-12 pg/ml	IL-17 pg/ml
Before injection (control)	281.2±55.15 b	2.23±0.06 b	89.29±17.78 a	41.22±9.65 a
After 1 week of infection	125.67±2.36 a	0.99±0.05 a	77.01±17.53 a	56.49±11.75 a
After 2 weeks of infection	95.21±10.07 a	0.81±0.08 a	47.89±4.67 a	30.35±5.3 a
After 3 weeks of infection	141.01±16.05 a	0.84±0.03 a	71.22±18.54 a	36.66±4.51 a
Sig. level	0.009*	0.000*	0.364	0.230
(*P ≤ 0.05)				

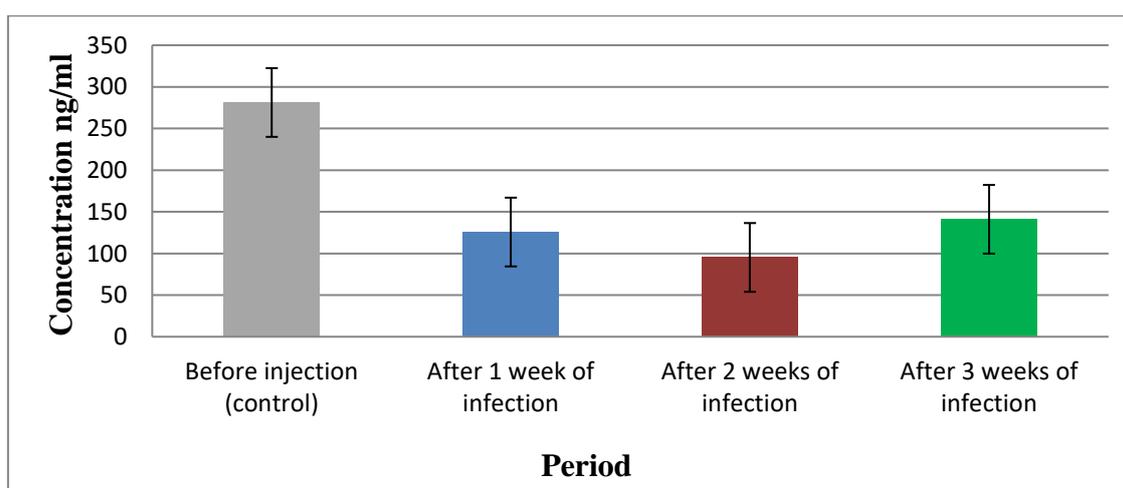


Figure (4-2): The significant effect of *E. histolytica* infection on Hepcidin concentration in albino rats

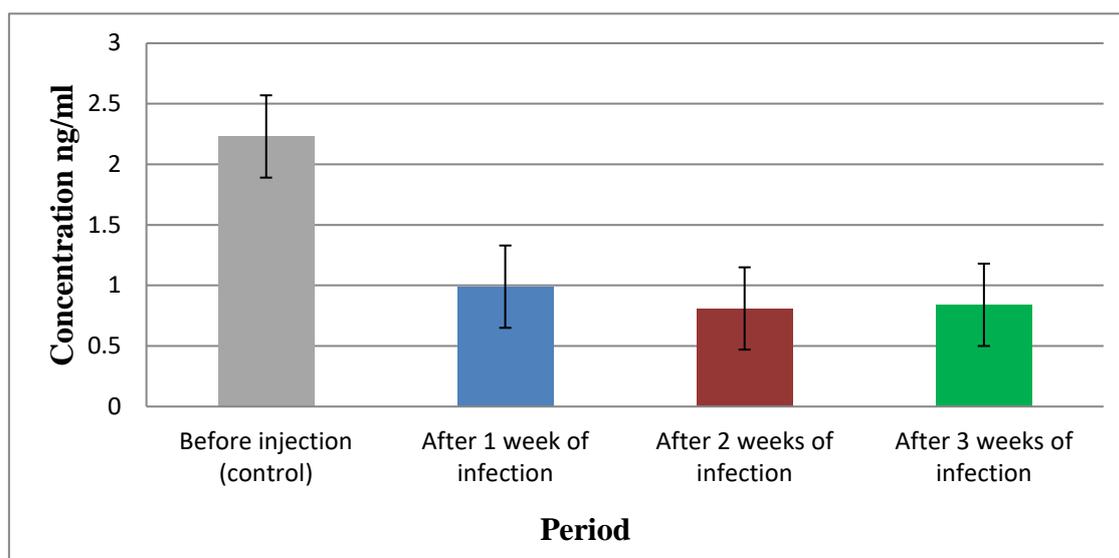


Figure (4-3): The significant effect of *E. histolytica* infection on β -tryptase concentration in albino rats

4-1-6: The effect of *E. histolytica* infection after lectin injection on immunological parameters

From the result of the current study, there was no significant change increase or decrease in the concentration of the studied parameters in a group of albino rats infected with *E. histolytica* after injection with lectin, except for the concentration of β -tryptase where the changes in its concentration during the study period were significant (Table 4-6 and Figure 4-4).

Table (4-6): The effect of *E. histolytica* infection after lectin injection on immunological parameters in albino rats (*R. rattus*)

Period	Immunological parameters Mean±SE			
	Hepcidin ng/ml	β-tryptase ng/ml	IL-12 pg/ml	IL-17 pg/ml
Before infection (control)	263.52±9.48 b	2.1±0.15 d	56.84±3.97 a	35.78±4.77 a
First injection (After 1 week)	186.41±62.05 a	0.91±0.09 ab	55.26±9.55 a	74.21±20.02 b
Second injection (After 2 weeks)	145.37±16.26 a	0.57±0.03 a	61.92±10.47 a	58.77±11.69 ab
Third injection (After 3 weeks)	117.97±5.94 a	1.29±0.16 bc	50.7±5.86 a	39.12±2.28 a
After 1 week of infection	169.44±12.57 a	2.05±0.09 d	57.54±1.43 a	28.24±0.92 a
After 2 weeks of infection	127.83±25.31 a	1.73±0.07 cd	55.08±13.53 a	44.03±5.23 ab
After 3 weeks of infection	142.43±13.83 a	1.45±0.38 bc	65.61±15.99 a	48.59±8.81 ab
Sig. level	0.612	0.000*	0.955	0.075
(*P ≤ 0.05)				

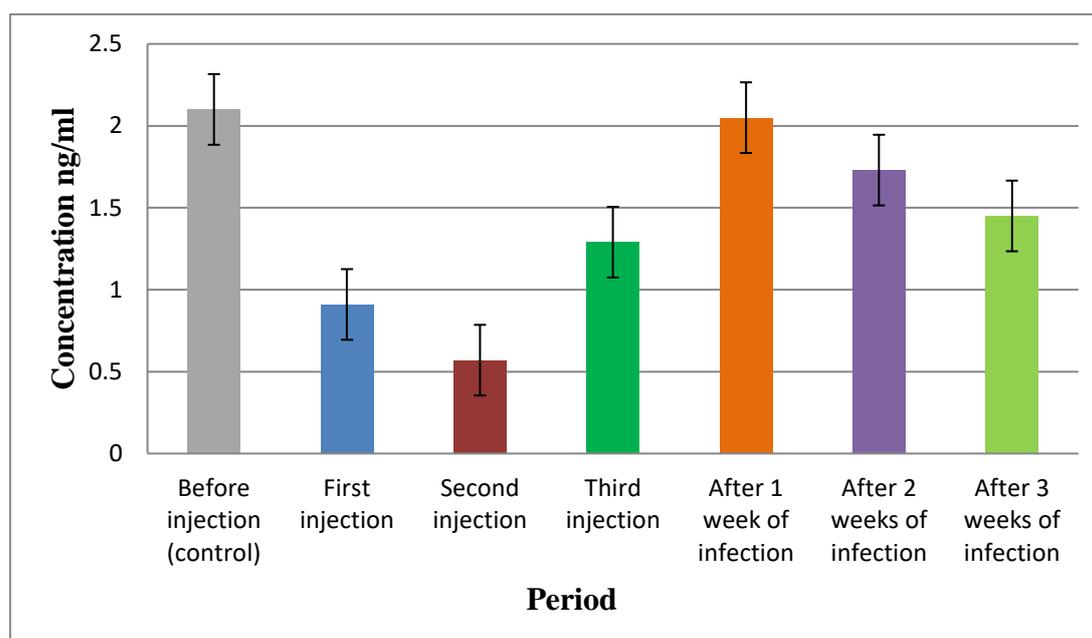


Figure (4-4): The significant effect of *E. histolytica* infection after lectin injection on β -tryptase concentration in albino rats

4-1-7: The correlation between immunological parameters in lectin injection group of albino rats

Table (4-7) shows the correlation among immunological parameters in a group of albino rats injected with lectin. A weak negative correlation was observed between Hepcidin and β -tryptase, while a strong positive correlation was found between IL-12 and IL-17 during the study period. The correlation between Hepcidin and IL-12; Hepcidin and IL-17; β -tryptase and IL-12; β -tryptase and IL-17, was variable during the study period. No significant correlation was observed among the studied parameters except between β -tryptase and IL-12 after a week of lectin injection, as there was a significant positive correlation between them.

Table (4-7): Correlation among immunological parameters in albino rats (*R. rattus*) injected with Lectin

Correlations groups	Hepcidin× β-trptase ng/ml	Hepcidin ng/ml×IL-12 pg/ml	Hepcidin ng/ml×IL17 pg/ml	β-trptase ng/ml×IL- 12 pg/ml	β-trptase ng/ml×IL- 17 pg/ml	IL-12× IL-17 pg/ml
Before injection (control)	-0.266	+0.996	+0.856	-0.313	+0.310	+0.806
First injection (After 1 week)	-0.090	-0.019	-0.409	+0.997*	+0.946	+0.920
Second injection (After 2 weeks)	-0.441	+0.661	+0.519	-0.965	-0.996	+0.985
Third injection (After 3 weeks)	-0.307	-0.427	-0.637	-0.730	-0.538	+0.969
*Correlation is significant at the $P \leq 0.05$						

4-1-8: The correlation between immunological parameters in *E. histolytica* infected groups of albino rats

Table (4-8) shows the correlation among immunological parameters in albino rats infected with *E. histolytica*. We notice a strong positive correlation between IL-12 and IL-17 in the control group, as well as in a group one week after infection, after which remain positive but weakly in the other groups during the study period. While it was found that the correlation between Hepcidin and β-tryptase was variable during the study period, where the correlation was negative and weak in the control group, then strong positive in the group after a week of infection, then positive and weak in a group after two weeks of infection, and in a group, after the third week of infection, the correlation becomes negative and strong. It was found that the correlation between Hepcidin and IL-12 was strongly positive in the control group and a weak positive in a group after the third week of infection, but in the other two groups, the correlation was strongly negative. As for the correlation between Hepcidin and IL-17, it was variable between positive and negative correlation during the duration of the experiment.

While it was found that, the correlation between β -tryptase and IL-12 was negative in the control group and in the group after a week of infection then it turned into a positive correlation in the other two groups. The correlation between β -tryptase and IL-17 was weak positive in the control group and strong positive in a group after two and three weeks of infection, and a weak negative in a group after one week of infection, where the correlation was significant in a group after two weeks of infection.

Table (4-8): Correlation between immunological parameters in albino rats (*R. rattus*) infected with *E. histolytica*

groups \ correlations	Hepcidin× β -trptase ng/ml	Hepcidin ng/ml×IL- 12 pg/ml	Hepcidin ng/ml×IL17 pg/ml	β -trptase ng/ml×IL- 12 pg/ml	β -trptase ng/ml×IL- 17 pg/ml	IL-12× IL- 17 pg/ml
Before injection (control)	-0.296	+0.834	+0.756	-0.275	+0.289	+0.812
After 1 week of infection	+0.945	-0.848	-0.393	-0.628	-0.070	+0.820
After 2 weeks of infection	+0.322	-0.704	+0.270	+0.446	+0.999*	+0.494
After 3 weeks of infection	-0.746	+0.424	-0.579	+0.287	+0.975	+0.493
*Correlation is significant at the $P \leq 0.05$						

4-1-9: The correlation between immunological parameters in *E. histolytica* infected group after lectin injection

The results in Table (4-9) show the correlation among immunological parameters in a group of albino rats infected with *E. histolytica* after injection with lectin where it found that the correlation did not take a single form, as the correlation among the criteria during period changed between negative and positive correlation. It was found that there were only two cases that had a significant association between them, namely, the correlation between β -tryptase and IL-12 in the first week of injection with lectin and between Hepcidin and IL-

17 in the third week of infecting albino rats with *E. histolytica* after injected with lectin.

Table (4-9): The correlation between immunological parameters in *E. histolytica* infected group after lectin injection

correlations groups	Hepcidin× β-trptase ng/ml	Hepcidin ng/ml×IL-12 pg/ml	Hepcidin ng/ml×IL17 pg/ml	β-trptase ng/ml×IL-12 pg/ml	β-trptase ng/ml×IL-17 pg/ml	IL-12× IL-17 pg/ml
Before injection (control)	-0.281	+0.987	+0.952	-0.322	+0.245	+0.890
First injection (After 1 week)	- 0.09	-0.017	-0.473	+0.998*	+0.986	+0.935
Second injection (After 2 weeks)	-0.468	+0.687	+0.570	-0.934	-0.991	+0.958
Third injection (After 3 weeks)	-0.327	-0.457	-0.675	-0.760	-0.566	+0.989
After 1 weeks of infection	+0.584	-0.236	+0.707	-0.927	+0.987	-0.854
After 2 weeks of infection	+0.365	+0.865	+0.283	-0.151	-0.790	+0.725
After 3 weeks of infection	+0.226	+0.020	-0.999*	+0.978	-0.271	-0.066
*Correlation is significant at the 0.05 level						

4-2: The protective role of lectin against *E. histolytica* infection in albino rats

It was found from the results of the present study (Table 4-10) show that the injected plant lectin has a protective role against the infection of albino rats with *E. histolytica*. Where the required time for infection to appear in the feces of albino rats was 3 days, while the required time for the infection to appear in the feces of albino rats injected with lectin after being infected with the parasite was 9 days. The period sufficient to make the infection intensity is very high so that the microscopic field is completely filled with the parasite phases is 7 days, while

this period in the group of albino rats infected with the parasite after being injected with lectin is 13 days but the microscopic field is not completely 100% filled.

Table (4-10): The protective role of lectin against *E. histolytica* infection in albino rats

Groups \ Days	The number of days during the infection appeared in the rats	The number of days during which infection covers all microscopic field
A group of albino rats infected with <i>E. histolytica</i>	3	7
A group of albino rats infected with <i>E. histolytica</i> after injection with lectin	9	13 but not 100%

4-3: The histological study

4-3-1: The effect of lectin injected on liver tissues of albino rats

Figure (4-6, 4-7, and 4-8) explain the effect of the number of lectins injected (a week after the first injection, a week after the second injection, and a week after the third injection, respectively) on the liver tissues of albino rats. Where we found that a week after the first injection, the occurrence of damaged sinusoids with angiectasis, congestion of the central vein with fibrosis, and vein area infiltrated with mononuclear cells (Figure 4-6) compared with control (Figure 4-5). While we note that presented mild hemorrhage, revealing marked increased branching of the bile ducts, enlarged Kupffer cell, and hepatic megalocytes in the liver of albino rats occurs a week after the second injection of lectin (Figure 4-7) compared with control (Figure 4-5). A week after the third injection with lectin, we notice the presence of irregularly arranged hepatocyte plates, separated by

dilated blood sinusoids and marked dilated sinusoids associated with few inflammatory cells (Figure 4-8) compared with control (Figure 4-5).

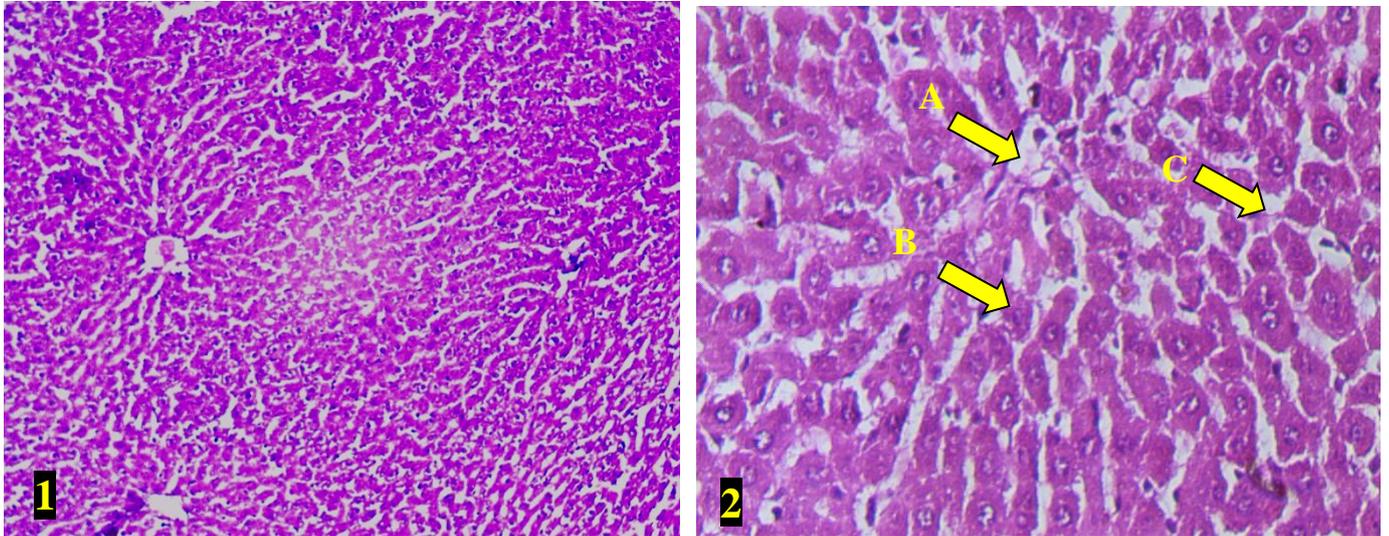


Figure (4-5): Cross section of a rat liver, (1) showed the control group demonstrating normal hepatic architectures (10x). (2) central vein (A), hepatocytes (B), and sinusoids (C) (H & E, 40x).

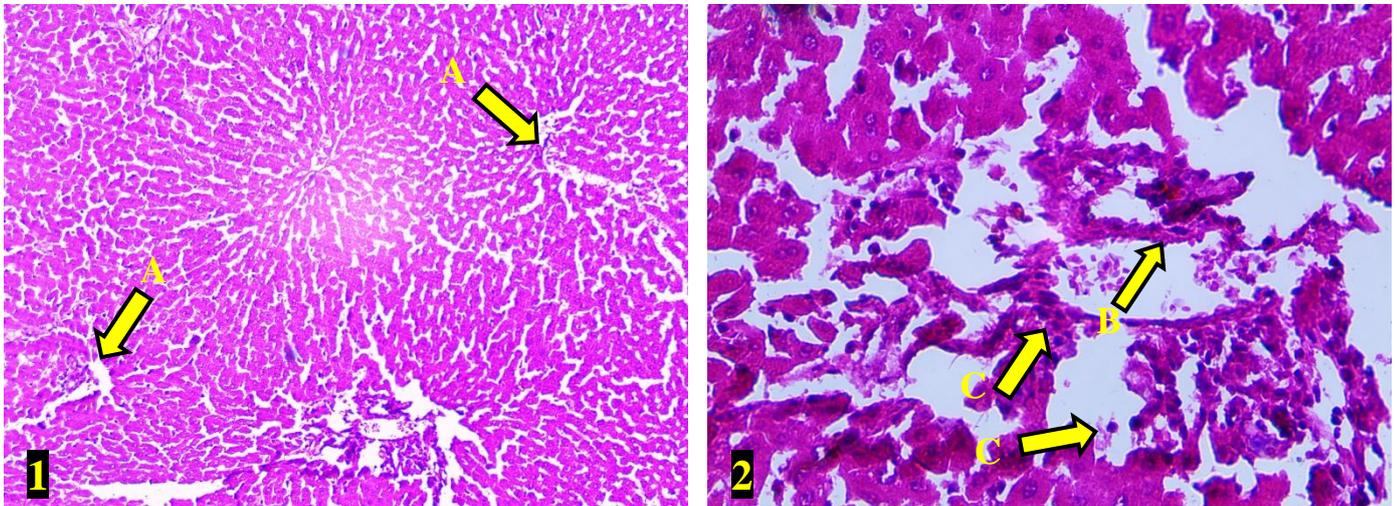


Figure (4-6): Cross section of a rat liver first injection of lectin (1) showing damaged sinusoids with angiectasis(A) (10x). (2) congestion of the central vein with fibrosis(B), and vein area infiltrated with mononuclear cells (C) (H & E,40x).

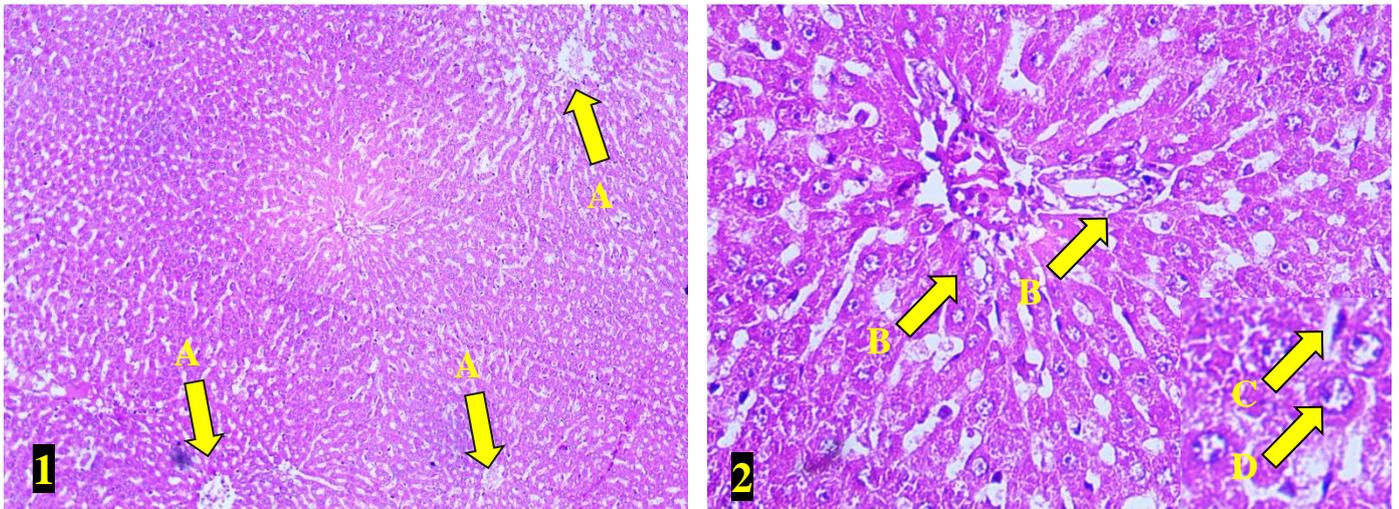


Figure (4-7): Cross section of a rat liver second injection of lectin, (1) showed presented mild hemorrhage (A) (10x). (2) revealing marked increased branching of the bile ducts (B), and enlarged kupffer cells (C), and hepatic megakaryocytes (D), (H & E, 40x).

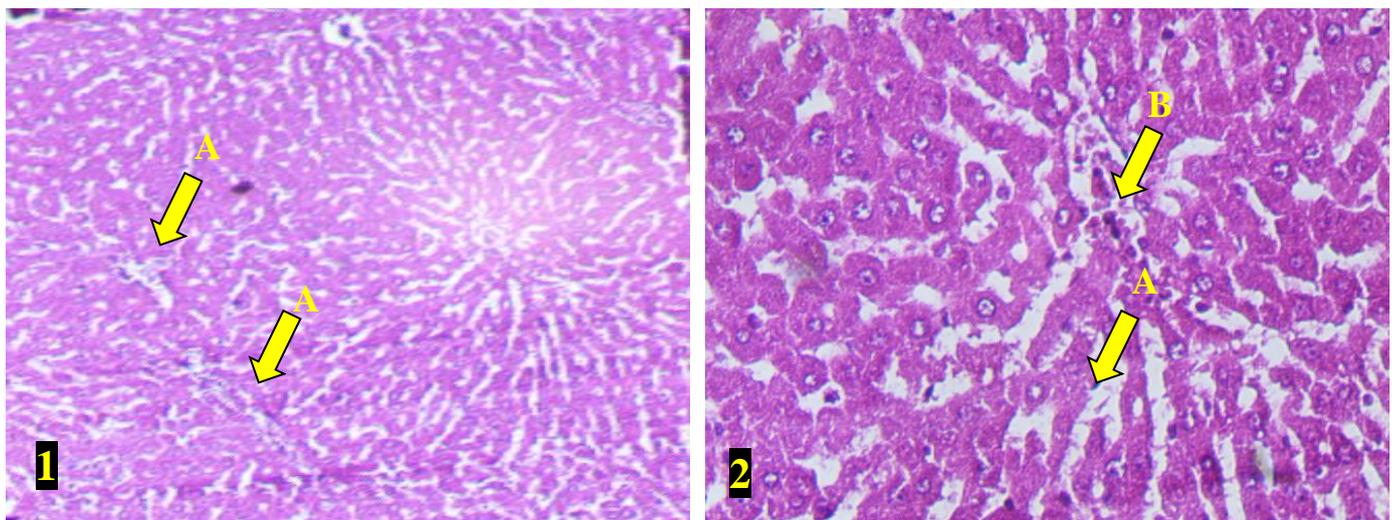


Figure (4-8): Cross section of the liver rat third injection of lectin, (1) showing irregularly arranged hepatocyte plates, separated by dilated blood sinusoids (A). (10x). (2) marked dilated sinusoids associated with few inflammatory cells (B), (H & E, 40x).

4-3-2: The effect of *E. histolytica* infected on liver tissues of albino rats

Figures (4-9 4-10, and 4-11) show the effects of infection in the liver tissues of albino rats after a week of infection. After two weeks of infection, and after three weeks of infection, respectively. It was found that the changes in the liver tissues of albino rats after a week of infection are: an abnormal liver with dilation, focal congestion in the central vein, hepatocyte hypertrophy, and angiectasis (Figure 4-9) compared with control (Figure 4-5). As for two weeks after the infection, the changes in the liver tissues were central vein congestion, sinusoidal endothelial subendothelial linear mononuclear cellular infiltration, central vein fibrosis with focal mononuclear infiltration and present of *E. histolytica* trophozoites (Figure 4-10) compared with control (Figure 4-5). The changes of liver tissues after three weeks of infection were the abnormal bile duct profiles, portal tract is expanded with prominent fibrosis and present of *E. histolytica* trophozoite (Figure 4-11) compared with control (Figure 4-5).

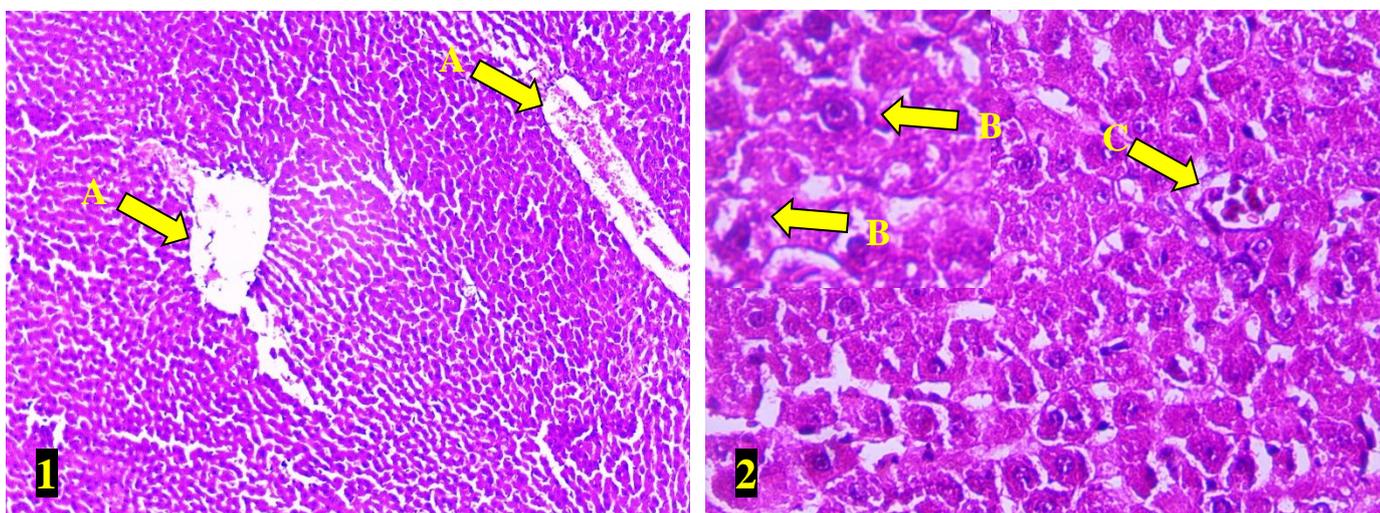


Figure (4-9): Cross section of a rat liver after week of infection with *E. histolytica*, (1) showing an abnormal liver with dilatation and focal congestion in the central vein (A). (10x). (2) hepatocyte hypertrophy (B), angiectasis (C) . (H & E, 40x).

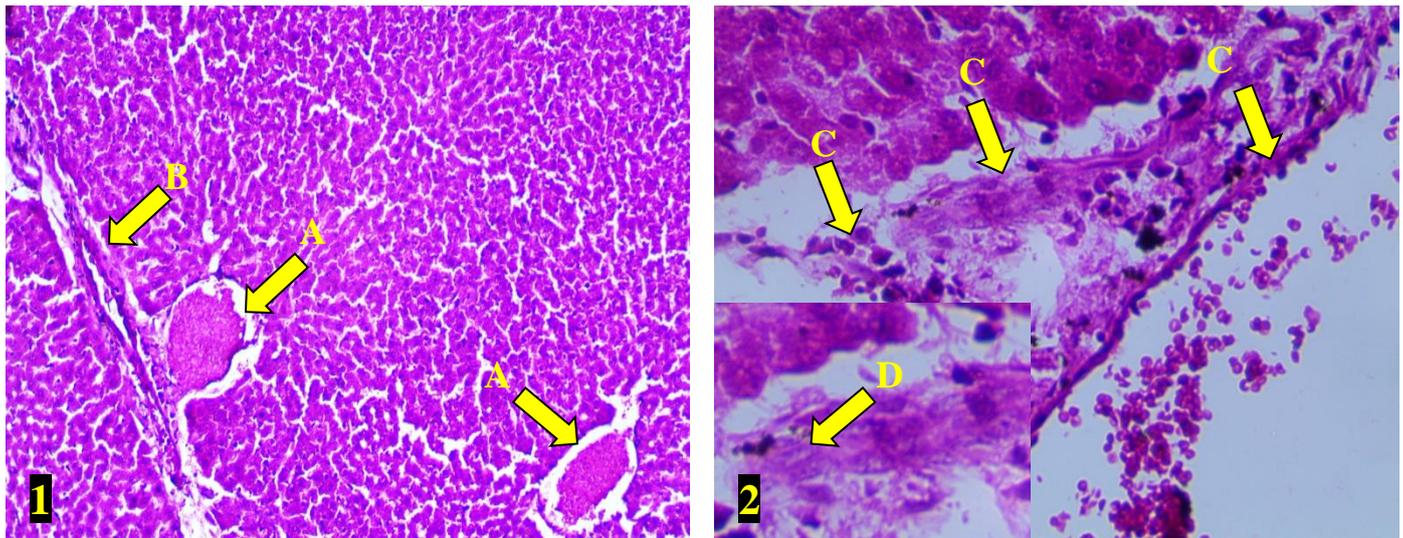


Figure (4-10): Cross section of a rat liver after two weeks of infection with *E. histolytica*, (1) showed the central vein congestion (A), (10x). (2), sinusoidal endothelial subendothelial linear mononuclear cellular infiltration (B), central vein fibrosis with focal mononuclear cellular infiltration (C), and *E. histolytica* trophozoites (D), (H & E, 40x).

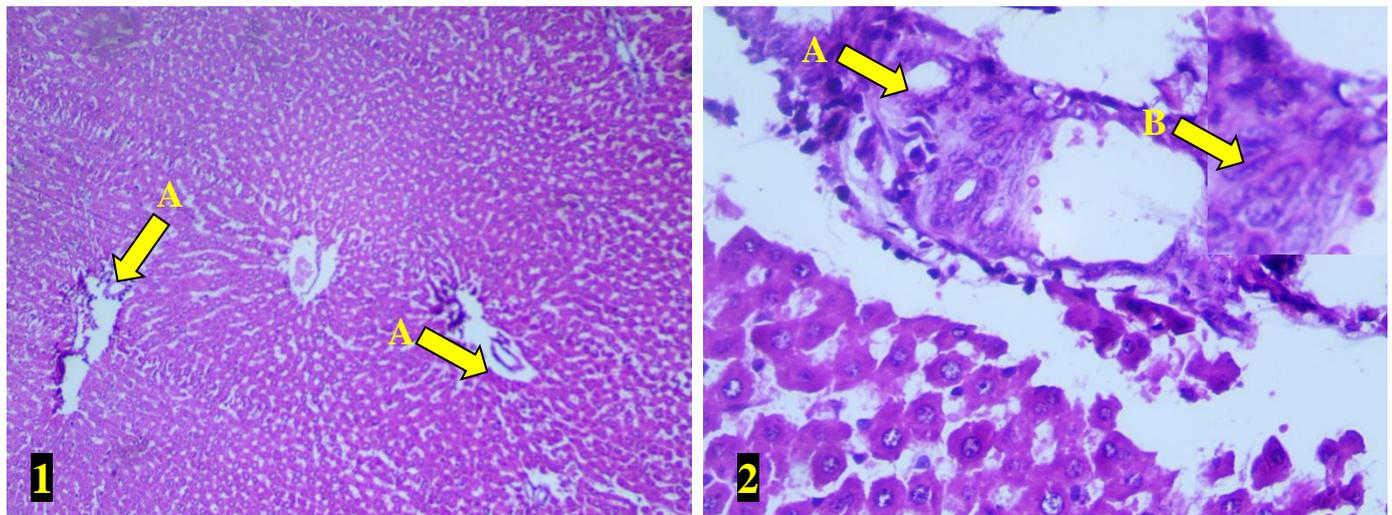


Figure (4-11): Cross section of a rat liver after three weeks of infection with *E. histolytica*, (1) showed the abnormal bile duct profiles, portal tract is expanded with prominent fibrosis (A), (10x). (2), *E. histolytica* trophozoites (B), (H & E, 40x).

4-3-3: The effect of *E. histolytica* infection on liver tissues of albino rats after lectin injected

Figures (4-12, 4-13, and 4-14) explain the effect of *E. histolytica* infection on liver tissues of albino rats after begin injected with lectin. It was found after a week of infection that the effects included extensive central vein congestion, thrombosis, sinusoidal endothelial with sub endothelial linear infiltration, and severe bile duct proliferation (Figure 4-12) compared with control (Figure 4-5). While the changes after two weeks of infection were central vein congestion, central vein fibrosis with focal mononuclear cellular infiltration, sinusoidal mononuclear cellular infiltration, vacuolar degeneration hepatocytes, and hepatic megalocytes (Figure 4-13) compared with control (Figure 4-5). As for the changes after three weeks of infection, they included the disturbed hepatic architecture, mononuclear cell filtration, enlarged hepatocytes and leukocyte.

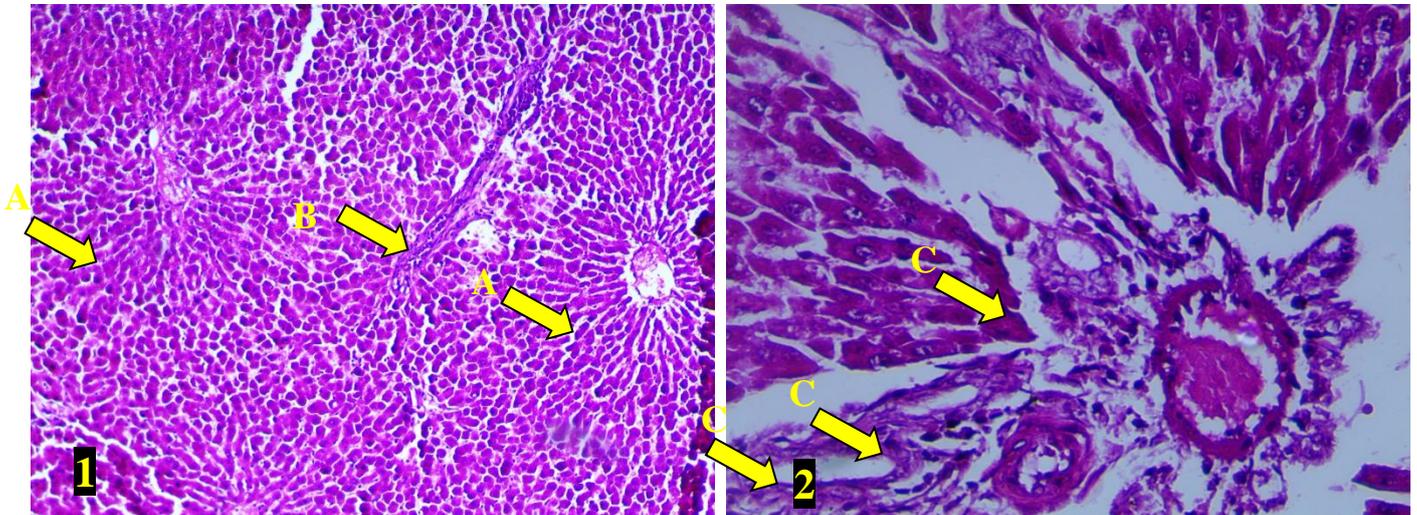


Figure (4-12): Cross section of a rat liver after a week of infection with *E. histolytica* and before that, lectin injection (1) showed the extensive central vein congestion and thrombosis(A) sinusoidal endothelitis with subendothelial linear infiltration(B), (10x). (2) severe bile duct proliferation (C), (H & E, 40x).

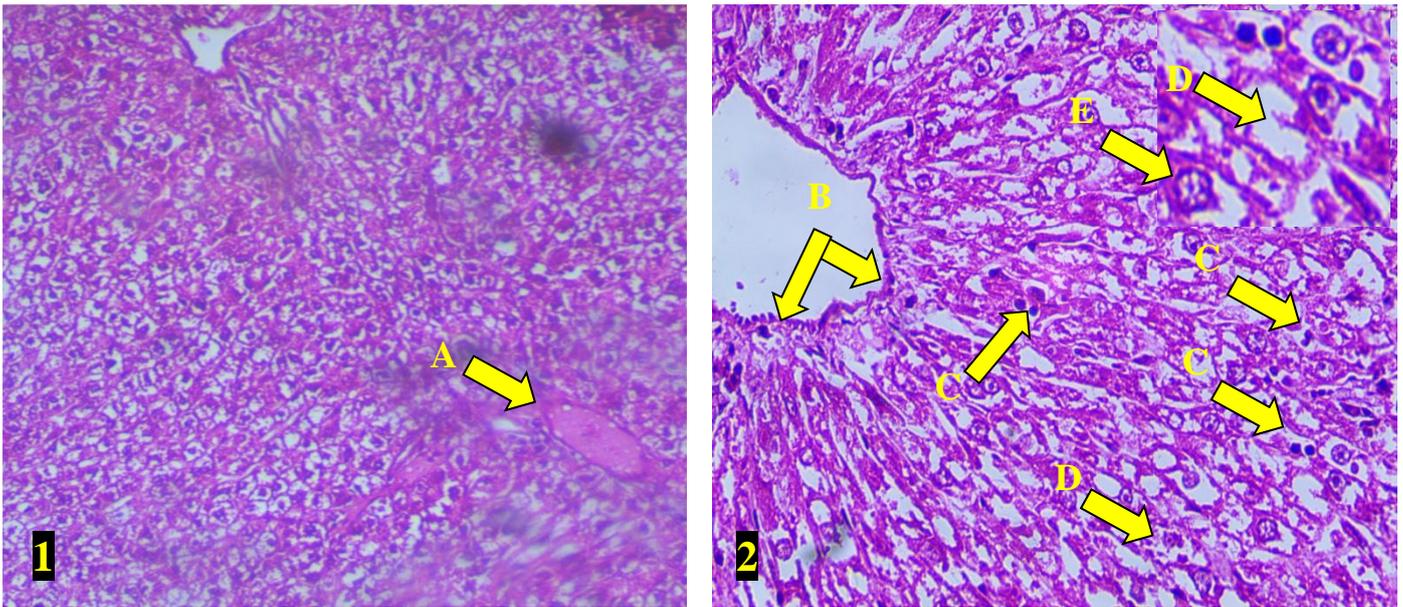


Figure (4-13): Cross section of a rat liver after two weeks of infection with *E. histolytica* and before that, lectin injection , (1) showed the central vein congestion (A), (10x) .(2) central vein fibrosis with focal mononuclear cellular infiltration(B), sinusoidal mononuclear cellular infiltration(C), vacuolar degeneration hepatocytes (D) ,and hepatic megalocytes(E), (H & E, 40x).

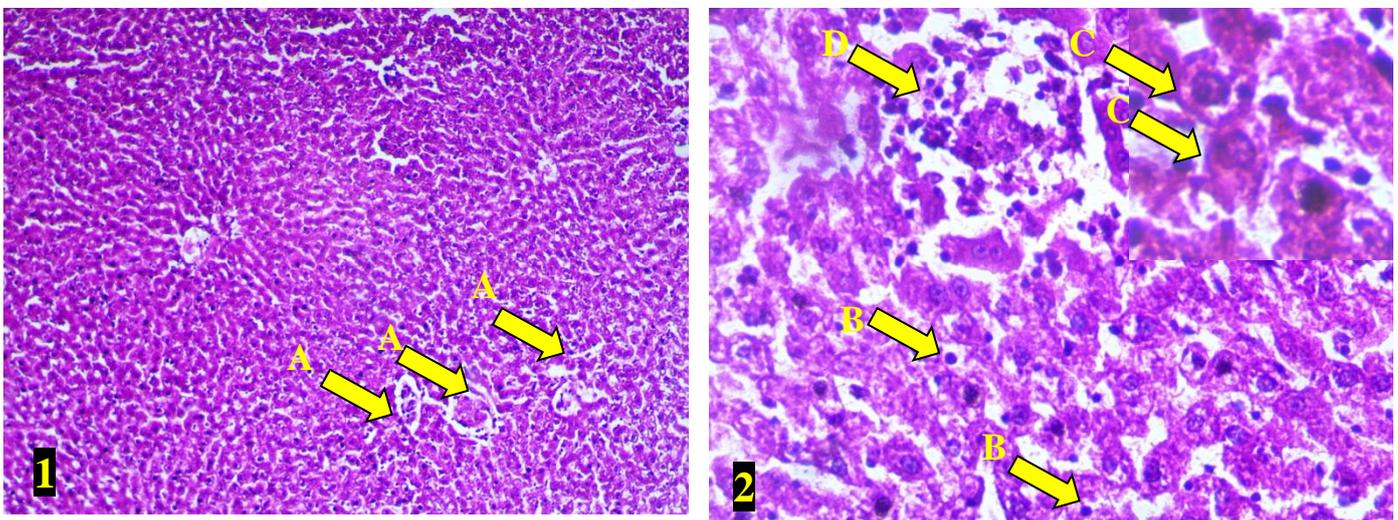


Figure (4-14): Cross section of a rat liver after three weeks of infection with *E. histolytica* and before that, lectin injection (1) showed the disturbed hepatic architecture (10x). (2) mononuclear cell infiltration (B), enlarged hepatocytes (C), and leukocyte infiltrations (D) (H & E, 40x).

Chapter Five
Discussion

5: Discussion

5-1: The physiological and immunological study

5-1-1: The effect of lectin injection on haematological parameters

The results of the present study results showed the effect of lectin injections on different hematological parameters. It was observed that total WBCs, percentage of granulocytes and the total of PLTs changed significantly during the periods of injection. The other hematological parameters were affected but not significantly Table (4-1).

Lectins proved to mediate diversified biological functions like cytotoxicity, agglutination, complement activation, innate immune response, cell-to-cell signaling, and precipitate of different types of sugars. Recently, great interest has been developed in the research and applications of lectins in agriculture and medicine due to their antiparasitic and antimicrobial potentials (Iordache *et al.*, 2015).

The effect was observed on total WBCs, which are a part of immune system that protects the body from infection. These cells circulate through the bloodstream and tissues to respond to injury or illness by attacking any unknown organisms that enter the body, infections usually cause an increased WBCs count, as well as, inflammation, cancers, asthma and allergies (Siedlinski *et al.*, 2020), and caused to an increase in the period (first and second injection).

According to Akinwande *et al.* (2004) a measurable increase in WBCs count of rats or any animal is a function of immunity or resistance to disease, while Lymphocytes, which are a type of WBCs, help the immune system remember every antigen it comes in contact with (Brodbeck *et al.*, 2005), showed an increase in the period (second injection) according to Alatorre-Cruz *et al.* (2018), because the lectin influence the initiation and regulation of lymphocyte activation and proliferation (Kilpatrick, 1999).

Total PLTs, which control blood clotting to heal a wound and stop the bleeding, PLTs count increased in some cases, such as iron deficiency, infections, cancer, bone marrow disease, spleen removal and decreased when not enough platelets are being made in the bone marrow or destroyed in the bloodstream, spleen or liver (Picker, 2011), showed an increase in the periods (second and third injection) as compared with the control group and these results agree with Alatorre-Cruz *et al.* (2018). Monocytes percentage showed no change in all periods as compared with the control group according to Willis *et al.* (2013), which is a type of WBCs, it becomes a macrophage to surround and kill microorganisms, ingest foreign material, remove dead cells, and boost immune responses (Keerthivasan *et al.*, 2021), and a decrease in all period was observed in Granulocytes percentage as to M Ezzat *et al.* (2019), which are pivotal in both innate and adaptive immune responses in their attack against infections, and it a phagocyte which can ingest foreign particles such as viruses, bacteria and other such parasites (Fleetwood *et al.*, 2005).

Total RBCs decrease in the periods (second and third injection). RBCs count decrease in the cases of anemia, cancers and blood loss while low blood oxygen level, heart and lung disease due to increase RBCs count (Anderson *et al.*, 2018), and HGB concentration decrease in the period (second injection) as compared with the control group. HGB is an important oxygen-carrying protein and plays crucial roles in establishing host resistance against pathogens and in regulating innate immune responses (Lee and Ding, 2013).

5-1-2: The effect of *E. histolytica* infection on haematological parameters

The results showed that infection of albino rats with *E. histolytica* significantly increased or decreased all hematological parameters except the percentage of monocytes and the number of PLTs (Table 4-2).

E. histolytica infection effect on haematological parameters by increased or decreased the parameters may cause anemia (Shaker and Hussein, 2016). An increase was observed in total WBCs, which are a part of immune system that protects the body from infection. These cells circulate through the bloodstream and tissues to respond to injury or illness by attacking any unknown organisms that enter the body, infections usually cause an increased WBCs count, as well as, inflammation, cancers, asthma and allergies (Siedlinski *et al.*, 2020).

In the period (after 1 week of infection) and this may be explained by the increase in the Lymphocytes percentage, which are a type of WBCs, help the immune system remember every antigen it comes in contact with (Brodbeck *et al.*, 2005).

In the period (after 3 weeks of infection) as compared with the control group. Because the infection with the pathogenic *E. histolytica* produces a marked immune response which results in the development of protective immunity or the reason for this may be due to the penetration of the vegetative stages of the parasite to the epithelial cells of the intestine as well as the hepatocytes, which leads to the response of these cells to the presence of the parasite and the secretion of cytokines which is among the chemokines that stimulate the increase in the number and migration of WBCs to the site of infection (Shaker and Hussein, 2016).

While no significant change was observed in Monocytes percentage, which is a type of WBCs. It becomes a macrophage to surround and kill microorganisms, ingest foreign material, remove dead cells, and boost immune responses (Keerthivasan *et al.*, 2021), Granulocytes percentage, which are pivotal in both innate and adaptive immune responses in their attack against infections, and it a phagocyte which can ingest foreign particles such as viruses, bacteria and other such parasites (Fleetwood *et al.*, 2005) and that agree with the result of Shlash (2016), and total PLTs, which control blood clotting to heal a wound and stop the bleeding, PLTs count increased in some cases, such as iron deficiency,

infections, cancer, bone marrow disease, spleen removal and decreased when not enough platelets are being made in the bone marrow or destroyed in the bloodstream, spleen or liver (Picker, 2011), of all periods as compared with the control group. This may suggest that *E. histolytica* activates platelets, and the degree of their activation determines their morphologic parameters. (Shaker and Hussein, 2016)

Total RBCs, which are carry oxygen from lungs to the rest of the body. Then they make the return trip, taking carbon dioxide back to lungs to be exhaled. RBCs count decrease in the cases of anemia, cancers and blood loss while low blood oxygen level, heart and lung disease due to increase RBCs count (Anderson *et al.*, 2018), and HGB concentration, which is an important oxygen-carrying protein and plays crucial roles in establishing host resistance against pathogens and in regulating innate immune responses (Lee and Ding, 2013), showed a significant decrease in the periods (after 1,3 weeks of infection) as compared with the control group. The reason may be attributed to the activity of the vegetative stages of the parasite on phagocytosis, digestion and decomposition of RBCs, and this in turn leads to a lowering of the level of hemoglobin in the blood, which causes anemia (Shlash, 2016).

5-1-3: The effect of *E. histolytica* infection after lectin injection on haematological parameters

E. histolytica infection after lectin injected change the levels of all hematological parameters. These changes were not significant except the total number of WBCs, Lymphocytes percentage, and Monocytes percentage as in Table (4-3).

Lectins proved to mediate diversified biological functions like cytotoxicity, agglutination, complement activation, innate immune response, cell-to-cell signaling, and precipitate of different types of sugars (Iordache *et al.*, 2015).

E. histolytica infection effect on haematological parameters by increased or decreased the parameters and may can cause anemia (Shaker and Hussein, 2016).

An increase in the periods (first, second injection and after 1,2 and 3 weeks of infection) was observed in total WBCs, which are a part of immune system that protects the body from infection.

These cells circulate through the bloodstream and tissues to respond to injury or illness by attacking any unknown organisms that enter the body. Infections usually cause an increased WBCs count, as well as, inflammation, cancers, asthma and allergies (Siedlinski *et al.*, 2020), as compared with the control group and Lymphocytes percentage, which are a type of WBCs, that help the immune system remember every antigen it comes in contact with (Brodbeck *et al.*, 2005), showed an increase in the period (second injection and after 2 weeks of infection). Because the injection of lectin and infection with *E. histolytica* produces a marked immune response which results in the development of protective immunity, which leads to stimulate the increase in the number and migration of WBCs to the site of infection (Shlash, 2016; Shaker and Hussein, 2016).

Total PLTs, which control blood clotting to heal a wound and stop the bleeding, PLTs count increased in some cases, such as iron deficiency, infections, cancer, bone marrow disease, spleen removal and decreased when not enough platelets are being made in the bone marrow or destroyed in the bloodstream, spleen or liver (Picker, 2011), showed an increase in the periods (second, third injection and after 3 weeks of infection) as compared with the control group according to Alatorre-Cruz *et al.* (2018). No change in all periods was observed in Monocytes percentage, which is a type of WBCs, it becomes a macrophage to surround and kill microorganisms, ingest foreign material, remove dead cells, and boost immune responses (Keerthivasan *et al.*, 2021), as compared with the control group.

While a decrease was observed in Granulocytes percentage, which are pivotal in both innate and adaptive immune responses in their attack against

infections, and it a phagocyte which can ingest foreign particles such as viruses, bacteria and other such parasites (Fleetwood *et al.*, 2005). Total RBCs, which transport oxygen from lungs to the body. RBCs count decrease in the cases of anemia, cancers and blood loss while low blood oxygen level, heart and lung disease due to increase RBCs count (Anderson *et al.*, 2018), showed a decrease in all period. HGB concentration, which is an important oxygen-carrying protein and plays crucial roles in establishing host resistance against pathogens and in regulating innate immune responses (Lee and Ding, 2013), showed a decrease in the periods (second injection and after 1,2,3 weeks of infection) as compared with the control group. The activity of the vegetative stages of the parasite on phagocytosis, digestion and decomposition of RBCs, in turn, leads to a lowering of the level of hemoglobin in the blood, which causes anemia (Shlash, 2016).

5-1-4: The effect of lectin injection on immunological parameters

The results of the current study (Table 4-4 and Figure 4-1) found that the studied immunological parameters were affected by significantly increased or decreased in their concentrations during the periods of lectin injection of albino rats. Statistics proved that the variations in immunological criteria concentrations during the injection periods were significant only in β -tryptase, while the other parameters were not significant.

Lectins proved to mediate diversified biological functions like cytotoxicity, agglutination, complement activation, innate immune response and precipitate of different types of sugars (Iordache *et al.*, 2015).

A decrease was observed in Hepcidin, which is responsible of iron homeostasis. Increases in iron levels in the plasma and iron storage stimulate the production of hepcidin, which blocks iron absorption from the diet and its further storage. Hepcidin production is suppressed in the case of iron deficiency; (Kwapisz *et al.*,2009), and an increase in IL-17 was plays essential roles in the

host immunity against infectious diseases and chronic inflammatory diseases, thus, resistant the injected lectin as a foreign body (da Silva *et al.*, 2016).

Statistics proved that the variations in immunological criteria concentrations during the injection periods were significant only in β -tryptase, which plays a vital part in inflammation and serves as a marker of mast cell activation. The concentration of serum mast cell-tryptase is elevated in anaphylaxis, other allergic disorders and other haematological disorders, it is increased (Moreno *et al.*, 2003; Payne and Kam, 2004), while the changed in IL-12 concentration, which play critical roles in multiple immune responses (Hasegawa *et al.*, 2016) were not significant.

5-1-5: The effect of *E. histolytica* infection on immunological parameters

E. histolytica infection effect on the immunological parameters was a different way. As we found that the concentration of Hepcidin began to decrease at the beginning of the infection period (after 1 week) and then began to rise, but did not reach to the level of the control concentration, while we find that the concentration of β -tryptase gradually decreased. During the study period the difference in the concentration of both criteria was significant. The variations in the concentration of IL-12, and IL-17 were not significant (Table 4-5, Figure 4-2 and Figure 4-3).

E. histolytica infection affects the large intestine, producing amebic ulcerative colitis with important inflammation (Burgess and Petri, 2016). A decrease was observed in the concentration of Hepcidin, which is responsible of iron homeostasis. Increases in iron levels in the plasma and iron storage stimulate the production of hepcidin, which blocks iron absorption from the diet and its further storage. Hepcidin production is suppressed in the case of iron deficiency; (Kwapisz *et al.*, 2009), because hepcidin levels rise during infections and

inflammation, causing a decrease in serum iron levels and contributing to the development of inflammatory anemia, most likely as a host defense mechanism to limit iron availability to invading microbes. (Nemeth and Ganz, 2006; Al-badri *et al.*, 2019).

While we find that the concentration of β -tryptase, which plays a vital part in inflammation and serves as a marker of mast cell activation. The concentration of serum mast cell-tryptase is elevated in anaphylaxis, other allergic disorders and other haematological disorders, it is decreased (Moreno *et al.*, 2003; Payne and Kam, 2004). The variations in the concentration of IL-12, which play critical roles in multiple immune responses (Hasegawa *et al.*, 2016) and IL-17, which plays essential roles in the host immunity against infectious diseases and chronic inflammatory diseases, thus, resistant the injected lectin as a foreign body (da Silva *et al.*, 2016) during the study period were not significant according to AL-Mahdawy *et al.* (2016).

5-1-6: The effect of *E. histolytica* infection after lectin injection on immunological parameters

The result of the current study showed that there was no significant change increase or decrease in the concentration of the studied parameters in a group of albino rats infected with *E. histolytica* after injection with lectin, except for the concentration of β -tryptase where the changes in its concentration during the study period were significant (Table 4-6 and Figure 4-4).

Lectins proved to mediate diversified biological functions like cytotoxicity and agglutination. (Iordache *et al.*, 2015). *E. histolytica* infection affects the large intestine, producing amebic ulcerative colitis with important inflammation (Burgess and Petri, 2016).

Hepcidin, which is responsible of iron homeostasis. Increases in iron levels in the plasma and iron storage stimulate the production of hepcidin, which blocks

iron absorption from the diet and its further storage. Hepcidin production is suppressed in the case of iron deficiency; (Kwapisz *et al.*,2009), showed a decrease in all periods because *E. histolytica* infection decrease total RBCs, that duo to anemia and serves a protective function against infections by removing extracellular iron from the body during infection (Kwapisz *et al.*,2009; Al-badri *et al.*,2019) and IL-17, which plays essential roles in the host immunity against infectious diseases and chronic inflammatory diseases, thus, resistant the injected lectin as a foreign body (da Silva *et al.*, 2016), showed an increase. While IL-12, which play critical roles in multiple immune responses (Hasegawa *et al.*, 2016), showed no change during the study period. only β -tryptase, which plays a vital part in inflammation and serves as a marker of mast cell activation. The concentration of serum mast cell-tryptase is elevated in anaphylaxis, other allergic disorders and other haematological disorders (Moreno *et al.*, 2003; Payne and Kam, 2004) and release from mast cells to bloodstream in the cases of inflammatory disorders, show a significant change in its concentration during the study period and decrease in the period (first, second, third injection and after 3 weeks of infection) and these results agree with Im *et al.* (1975) and Moreno *et al.* (2003).

5-1-7: The correlation among immunological parameters in lectin injection group of albino rats

The result of the current study shows the correlation among immunological parameters in a group of albino rats injected with lectin. A weak negative correlation was observed between Hepcidin which is the principal regulator of systemic iron homeostasis (Girelli *et al.*, 2016) and β -tryptase, which play an important role in inflammatory and allergic reactions (Theoharides *et al.*, 2012), which means the increase in Hepcidin concentration due to decrease in β -tryptase concentration, or the increase in β -tryptase concentration due to decrease in Hepcidin concentration. While a strong positive correlation was found between

IL-12 which play critical roles in multiple immune responses (Hasegawa *et al.*, 2016), and IL-17, which is a potent inflammatory cytokine capable of inducing chemokine expression and recruitment of cells to parenchymal tissue (Gaffen *et al.*, 2014).

During the study period, which refers to, the increase in IL-12 or IL-17 concentration due to increase in the other parameters. As for the correlation between Hepcidin and IL-12; Hepcidin and IL-17; β -tryptase and IL-12; β -tryptase and IL-17, it was variable because the concentration of the parameters was changed during the study period. No significant correlation was observed among the studied parameters, because the changed of the concentration of the parameters in the different periods not significant, except between β -tryptase and IL-12 after a week of lectin injection, as there was a significant positive correlation between them.

5-1-8: The correlation among immunological parameters in *E. histolytica* infected groups of albino rats

The result of the present study explains the correlation among immunological parameters in albino rats infected with *E. histolytica*. We notice a strong positive correlation between IL-12, which play critical roles in multiple immune responses (Hasegawa *et al.*, 2016), and IL-17, which is a potent inflammatory cytokine capable of inducing chemokine expression and recruitment of cells to parenchymal tissue (Gaffen *et al.*, 2014) in the control group, as well as in a group one week after infection, which means the increase in the concentration of IL-12 or IL-17 due to increase the concentration of the others parameters, after which remain positive but weakly in the others groups during the study period. While it was found that the correlation between Hepcidin which is the principal regulator of systemic iron homeostasis (Girelli *et al.*, 2016) and β -tryptase, which play an important role in inflammatory and allergic reactions (Theoharides *et al.*, 2012) was variable during the study period, that

because the changed in the parameter's concentration during the different periods, where the correlation was negative and weak in the control group, then strong positive in group after week of infection, then positive and weak in a group after two weeks of infection, and in a group after third week of infection the correlation become negative and strong. It was found that the correlation between Hepcidin and IL-12 was strong positive in the control group and a weak positive in a group after the third weeks of infection, but in the other two groups, the correlation was strong negative.

As for the correlation between Hepcidin and IL-17 it was variable between positive and negative correlation during the duration of the experiment. While it was found that the correlation between β -tryptase and IL-12 was negative in the control group and in the group after a week of infection then it turned into positive correlation in the other two groups. The correlation between β -tryptase and IL-17 was weak negative in the control group and a group after a week of infection, and then strong positive in the other two groups, where the correlation was a significant in a group after two weeks of infection.

5-1-9: The correlation among immunological parameters in *E. histolytica* infected group after lectin injection

The results of present study show that the correlation among immunological parameters in a group of albino rats infected with *E. histolytica* after injection with lectin where it was found that the correlation did not take a single form, as the correlation among the creteria during the study period changed between negative and positive correlation, the continuously changed in the concentration of the parameters caused the changed correlation form between the parameters. It was found that there were only two cases that had a significant association between them, namely, the correlation between β -tryptase, which play an important role in inflammatory and allergic reactions (Theoharides *et al.*, 2012) and IL-12, which play critical roles in multiple immune responses (Hasegawa *et*

al., 2016) in the first week of injection with lectin and between Hepcidin, which is the principal regulator of systemic iron homeostasis (Girelli *et al.*, 2016) and IL-17, which is a potent inflammatory cytokine capable of inducing chemokine expression and recruitment of cells to parenchymal tissue (Gaffen *et al.*, 2014) in the third week of infecting albino rats with *E. histolytica* after injected with lectin, because the visible changed in the concentration of the parameters in the different periods.

5-2: The protective role of lectin against *E. histolytica* infection in albino rats

The results of present study show that the infection of *E. histolytica* required 3 days to appeared in the feces, where the signs appeared in the second day included inactivity and increased symptoms on third day, represented by anorexia, increased drinking water and increased defecation. while the period sufficient to make the infection intensity very high so that the microscopic field is completely filled with the parasite phases is 7 days (Shaker *et al.*, 2018).

The time required for the infection to appear in feces of albino rats injected with lectin after being infected with the parasite was 9 days, while this period in the group of albino rats infected with the parasite after being injected with lectin is 13 days but the microscopic field is not completely 100% filled, we note the different in time to appearance the infection in the group infected with *E. histolytica* and group infected with *E. histolytica* after lectin injection, because the role of plant lectin to activate the immune system by increased the WBCs that defended against the parasite infection (Petri and Ravdin, 1991). Rios-de Alvarez *et al.*, (2012) conclude that plant lectins can have an inhibitory effect on the feeding behaviour of first stage larvae of ovine sheep gastro-intestinal nematodes in vitro.

5-3: The histological study

5-3-1: The effect of lectin injected on liver tissues of albino rats

The results of current study explain the effect of the number of lectins injected on the liver tissues of albino rats. After a week of first injection, the occurrence of damaged sinusoids is characterized by damage to small hepatic vessels affecting particularly sinusoidal endothelium. Damaged sinusoids can be associated with a partial or complete occlusion of small hepatic veins (Valla and Cazals-Hatem, 2016), with angiectasis, congestion of the central vein with fibrosis because the excessive accumulation of extracellular matrix proteins including collagen producing in the injured liver (Bataller and Brenner, 2005), and vein area infiltrated with mononuclear cells, the amoeba penetrates the liver tissue after infection, which stimulate an immune response represented by the accumulation and infiltration of inflammatory cells at the site of infection (Chabuk, 2013).

We note that presented mild hemorrhage, because of congestion of blood vessels, which is a response to the presence of the amoeba and the damage it causes within the liver tissue. This leads to an increase in the amount of blood coming to the site of injury, which leads to leakage of blood vessels (Greenstein *et al.*, 1994), revealing marked increased branching of the bile ducts, enlarged kupffer cell and hepatic megalocytes in the liver of albino rats occurs a week after the second injection of lectin. It is concluded that the megalocytes are functionally normal cells, except that they are in the process of cellular hypertrophy and are incapable of division (Svoboda *et al.*, 1971).

As for a week after the third injection with lectin, we notice the presence of irregularly arranged hepatocyte plates, because of the abnormal ability of the liver to regenerate and the formation of regenerative tissue similar to the damaged tissue. In the case of continuous damage to the liver tissue, a synchronization is

observed in the renewal of cells and the formation of the connective tissue in an abundance, and the increase in the connective tissue leads to the irregularity of the composition of the liver plates (Komori *et al.*, 2006), separated by dilated blood sinusoids and marked dilated sinusoids associated with few inflammatory cells. Hepatic sinusoidal dilatation refers to the enlargement of the hepatic capillaries. Most of the time this condition is caused by hepatic venous outflow obstruction, which results in vascular stasis and congestion of hepatic parenchyma and accumulation and infiltration of inflammatory cells (Brancatelli *et al.*, 2018).

5-3-2: The effect of *E. histolytica* infected on liver tissues of albino rats

The results of present study show the effect of *E. histolytica* infection on liver tissues for different period. After week of infection changes in the liver tissues were observed; these are an abnormal liver with dilation, which refers to the enlargement of the hepatic capillaries. Most of the time this condition is caused by hepatic venous outflow obstruction, which results in vascular stasis and congestion of hepatic parenchyma. It may be associated with hepatic sinusoidal dilatation without concurrent venous outflow obstruction (Brancatelli *et al.*, 2018).

Focal congestion in the central vein, because resultant stasis of blood within the lumen (Furlan *et al.*, 2016), angiectasis and hepatocyte hypertrophy, as a result of hyperplasia with loss of the shape and arrangement of the hepatocyte plates and the reason for this is due to the abnormal ability of the liver to regenerate, when the tissue is damaged as a result of infection with the parasite, the effectiveness of the normal division of tissue regeneration increases. which leads to volume enlargement hepatic lobe which gives abnormal liver (Komori *et al.*, 2006).

As for two weeks after the infection, the changes in the liver tissues were central vein congestion, sinusoidal endothelial subendothelial linear mononuclear cellular infiltration, after short time of infection, the amoeba penetrates the liver tissue through the hepatic sinusoids and bile ducts, which stimulate an immune response represented by the accumulation and infiltration of inflammatory cells at the site of infection (Chabuk, 2013), central vein fibrosis with focal mononuclear infiltration because the excessive accumulation of extracellular matrix proteins including collagen producing in the injured liver by portal fibroblasts, and myofibroblasts of bone marrow origin (Bataller and Brenner, 2005), and present of *E. histolytica* trophozoites, perhaps the reason for the penetration of the amoeba into the liver tissues is the flexibility of its plasma membrane, which gives it the ability to form pseudopodia, so it can change its shape and size to fit the spaces of the hepatic sinusoids and capillaries to pass through the liver tissues (Ghosh *et al.*, 2019).

The changes of liver tissues after three weeks of infection were the abnormal bile duct profiles. Gallbladder wall thickening is the most frequently noted finding; the other major finding is either extra- or intrahepatic bile duct dilatation or both. The cause of these abnormalities is unknown (Romano *et al.*, 1988). Portal tract is expanded with prominent fibrosis because excessive accumulation of extracellular matrix collagen because the transformation of stellate cells into myofibroblasts. which caused fibrosis (Bataller and Brenner, 2005) and the present of *E. histolytica* trophozoite by penetration of the amoeba into the liver tissues (Ghosh *et al.*, 2019).

5-3-3: The effect of *E. histolytica* infection on liver tissues of albino rats after lectin injected

The result of current study explains the effect of *E. histolytica* infection on liver tissues of albino rats after the injection with lectin. It was found after a week

of infection that the effects included the extensive central vein congestion because resultant stasis of blood within the lumen (Furlan *et al.*, 2016), thrombosis, sinusoidal endothelial with subendothelial linear infiltration, after short time of infection, the amoeba penetrates the liver tissue through the hepatic sinusoids and bile ducts, which stimulate an immune response represented by the accumulation and infiltration of inflammatory cells at the site of infection (Chabuk, 2013), and sever bile duct proliferation, which is a hepatic cellular reaction observed in most forms of liver disease and in a variety of experimental conditions associated with liver injury (Ryan *et al.*, 2008).

While the changes after two weeks of infection were the central vein congestion, because resultant stasis of blood within the lumen (Furlan *et al.*, 2016), central vein fibrosis with focal mononuclear cellular infiltration, because the excessive accumulation of extracellular matrix proteins including collagen producing in the injured liver by portal fibroblasts, and myofibroblasts of bone marrow origin (Bataller and Brenner, 2005), sinusoidal mononuclear cellular infiltration, because the amoeba penetrates the liver tissue after infection, which stimulate an immune response represented by the accumulation and infiltration of inflammatory cells at the site of infection (Chabuk, 2013), vacuolar degeneration hepatocytes and hepatic megalocytes, is closely related with the increase in the intrasinoidal pressure (Shibayama *et al.*, 1980).

As for the changes after three weeks of infection, they included the disturbed hepatic architecture, mononuclear cell filtration, because after infection, the amoeba penetrates the liver tissue through the hepatic sinusoids and bile ducts, which stimulate an immune response represented by the accumulation and infiltration of inflammatory cells at the site of infection (Chabuk, 2013), enlarged hepatocytes and leukocyte which are functionally normal cells, except that they are in the process of cellular hypertrophy and incapable of division (Svoboda *et al.*, 1971).

Conclusions & Recommendations

Conclusions and Recommendations

Conclusions

1. Despite the protective role of lectin against *E. histolytica* infection, it has caused some harmful effects on liver tissue.
2. The injected lectin and *E. histolytica* infection had a nearly similar effect on haematological parameters.
3. The injected lectin and *E. histolytica* infection had an obvious effect on physiological and immunological parameters.
4. It was found that each of the injected lectin and *E. histolytica* infection had a different effect on the tissues of albino rats.

Recommendations

1. Study the protective role of this lectin mixed with immune adjuvant against *E. histolytica* or other types of parasites.
2. Studying the therapeutic effect of this lectin alone or mixed with immune adjuvant against different types of parasites on the same studied criteria or others.
3. Conducting other studies on other types of lectins and studying their protective and therapeutic effects alone or mixed with immune adjuvants against different types of parasites.
4. Conducting a study on the effects of this lectin or other types of lectins on the histological structure of different organs of the body.

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الخلاصة

هدفت الدراسة الحالية إلى التحقق من آثار إصابة الجرذان البيضاء بالاميبيا الحالة للنسيج والمعاملة بالليكتين النباتي على بعض المعايير الفسيولوجية والمناعية تعداد الدم الكامل (CBC)، Hcpidin، β -tryptase، IL-12 و IL-17، وكذلك دراسة تأثير الحقن بالليكتين وعدوى الاميبيا الحالة للنسيج على التركيب النسيجي لكبد الفئران.

أجريت هذه الدراسة في جامعة بابل / كلية العلوم / قسم علوم الحياة من شباط ٢٠٢٢ إلى نيسان ٢٠٢٢.

تم تقسيم حيوانات التجربة إلى ثلاث مجموعات، المجموعة الأولى والثانية تحتوي على ١٢ حيواناً، والمجموعة الثالثة تحتوي على ٢١ حيواناً.

تم حقن حيوانات المجموعة الأولى بجرعة ٣ ملجم / مل من الليكتين النباتي (لكتين بذور القمح) ثلاث مرات بين حقنة وأخرى مدة أسبوع. المجموعة الثانية أصيبت تجريبياً بالاميبيا الحالة للنسيج بينما أصيبت المجموعة الأخيرة بالاميبيا الحالة للنسيج بعد حقنها بالليكتين. تم سحب ٥ مل من جميع حيوانات التجارب وقسمت إلى مجموعتين. تم استخدام المجموعة الأولى لإجراء تعداد الدم الكامل (CBC)، بينما تم استخدام المجموعة الثانية للحصول على المصل، والذي تم حفظه عند -٢٠ درجة مئوية لاستخدامه لاحقاً في الاختبارات الفسيولوجية والمناعية باستخدام تقنية ELISA.

أظهرت نتيجة الدراسة الحالية أن حقن الليكتين كان له تأثير معنوي على العدد الكلي للكريات البيض ونسبة الخلايا المحببة بالإضافة إلى العدد الكلي للصفائح الدموية. أما عن عدوى الاميبيا الحالة للنسيج فقد كان لها تأثير معنوي على العدد الكلي للكريات البيض ونسبة الخلايا الليمفاوية ونسبة الخلايا المحببة بالإضافة إلى تأثيرها المعنوي على العدد الكلي لكريات الدم الحمراء وتركيز الهيموجلوبين. في المجموعة الثالثة، كان لعدوى الاميبيا الحالة للنسيج بعد حقن الليكتين تأثير معنوي على العدد الكلي للكريات البيض، ونسبة الخلايا الليمفاوية، ونسبة الخلايا الوحيدة.

وجد من النتائج أن حقن الليكتين كان له تأثير معنوي فقط على تركيز β -tryptase، بينما كان للإصابة بالاميبيا الحالة للنسيج تأثير معنوي على Hcpidin و β -tryptase في المجموعة الثالثة، كان للإصابة بالاميبيا الحالة للنسيج بعد حقن الليكتين تأثير معنوي على تركيز β -tryptase فقط.

أظهرت نتائج الارتباط بين المتغيرات الفسيولوجية والمناعية في مجموعة من الجرذان البيضاء المحقونة بالليكتين وجود ارتباط معنوي بين المتغيرات المدروسة بين β -tryptase و IL-12 بعد أسبوع من حقن الليكتين، حيث كان هناك ارتباط إيجابي معنوي بينهما. لوحظ وجود ارتباط إيجابي قوي معنوي بعد أسبوعين من الإصابة في الجرذان البيضاء المصابة بمجموعة الاميبيا الحالة للنسيج في حين وجد أن

هناك حالتين فقط لهما ارتباط كبير بينهما ، وهما العلاقة بين β -tryptase و IL-12 في الأسبوع الأول من الحقن مع ليكتين وبين Hcpidin و IL-17 في الأسبوع الثالث من إصابة الجرذان البيضاء بالاميبا الحالة للنسيج بعد حقنها بالليكتين.

وجد من نتائج الدراسة الحالية أن لكتين النبات المحقون له دور وقائي ضد إصابة الجرذان بالاميبا الحالة للنسيج. حيث كان الوقت اللازم لظهور العدوى في براز الجرذان البيضاء ٣ أيام المصابة بالاميبا الحالة للنسيج، و ٩ أيام في الجرذان البيضاء المصابة بالاميبا الحالة للنسيج بعد حقنها بالليكتين. بينما الفترة الكافية لجعل شدة الإصابة عالية جداً هي ٧ أيام في الجرذان البيضاء المصابة بالاميبا الحالة للنسيج، و ١٣ يوماً في مجموعة الفئران المصابة بالطيفلي بعد حقنها بالليكتين.

أظهرت نتيجة الدراسة النسيجية تأثير الليكتين المحقون على أنسجة الكبد لدى الفئران. بعد أسبوع من الحقن الأول ، حدوث الجيوب التالفة مع توسع الأوعية ، واحتقان الوريد المركزي مع التليف ، وتسلل منطقة الوريد بخلايا وحيدة النواة. بينما نلاحظ أنه تم تقديم نزيف خفيف ، وكشف عن زيادة ملحوظة في تشعب القنوات الصفراوية ، وتضخم خلية كوبفر ، وتحدث الخلايا الضخمة الكبدية في كبد الفئران بعد أسبوع من الحقن الثاني من الليكتين. بعد أسبوع من الحقن الثالث بالليكتين ، نلاحظ وجود صفائح خلايا الكبد مرتبة بشكل غير منتظم ، مفصولة بأشباه الجيوب الدموية المتوسعة والجيوب المتوسعة المرتبطة ببعض الخلايا الالتهابية.

أظهرت الدراسة النسيجية آثار الإصابة في أنسجة كبد الفئران. حيث تم العثور على التغييرات بعد أسبوع من الإصابة هي كبد غير طبيعي مع تمدد واحتقان بؤري في الوريد المركزي وتضخم خلايا الكبد وتوسع الأوعية. لمدة أسبوعين بعد الإصابة ، كانت التغييرات عبارة عن احتقان الوريد المركزي ، تسلل خلوي خطي أحادي النواة تحت البطانية ، تليف وريدي مركزي مع تسلل أحادي النواة ، ووجود الطور الخضري للاميبا الحالة للنسيج وكانت التغييرات بعد ثلاثة أسابيع من الإصابة هي الملامح غير الطبيعية للقناة الصفراوية ، وتمدد المسلك البابي مع تليف بارز ، ووجود الطور الخضري للاميبا الحالة للنسيج.

أظهرت الدراسة النسيجية تأثير عدوى الاميبا الحالة للنسيج على أنسجة الكبد لدى الجرذان بعد حقنها بالليكتين. وجد بعد أسبوع من الإصابة أن التأثيرات شملت احتقاناً شديداً في الوريد المركزي ، تجلط الدم ، البطانية الجيبية مع تسلل خطي بطاني تحت البطاني ، وانتشار شديد في القناة الصفراوية. في حين أن التغييرات بعد أسبوعين من الإصابة كانت احتقان الوريد المركزي ، وتليف الوريد المركزي مع تسلل خلوي أحادي النواة بؤري ، وتسلل خلوي أحادي النواة جيبى ، وتنكس الخلايا الكبدية الفراغية ، والخلايا الكبدية الضخمة. أما بالنسبة للتغييرات بعد ثلاثة أسابيع من الإصابة ، فقد شملت البنية الكبدية المزعجة ، وترشيح الخلايا أحادية النواة ، وتضخم خلايا الكبد ، وكريات الدم البيضاء.



جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل – كلية العلوم
قسم علوم الحياة

دراسة بعض المتغيرات الفسيولوجية والمناعية في الجرذان البيض

المصابة بـ *Entamoeba histolytica* والمعاملة باللكتين

رسالة

مقدمة إلى مجلس كلية العلوم / جامعة بابل
كجزء من متطلبات نيل درجة ماجستير علوم في علوم الحياة

من قبل

هدى حاتم رحمن جواد
(بكالوريوس علوم حياة، ٢٠١٥)

بإشراف

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