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Study Effect of Turmeric and Pumpkin Extracts on *Entameba histolytica* in Female Rats

A Thesis
Submitted to the Council of the College of Science/ University of Babylon in Partial Fulfillment of the Requirements for the Master Degree in Science/ Biology

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وزارة التعليم العالي والبحث العلمي

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قـسـم عـلـــوم الحـيـاة

دراسة تأثير مستخلصات الكركم والقرع على اميبا الزحار في اناث الجرذان

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

﴿شَهِدَ ٱللَّهُ أَنَّهُۥ لَآ إِلَٰهَ إِلَّا هُوَ وَٱلْمَلَٰٓئِكَةُ وَأُوْلُواْ ٱلْعِلْمِ قَآئِمًۢا بِٱلْقِسْطِ ۚ لَآ إِلَٰهَ إِلَّا هُوَ ٱلْعَزِيزُ ٱلْحَكِيمُ ١٨﴾

صدق الله العظيم

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Supervisor Certification

I certify that this thesis entitled (**Study Effect of Turmeric and Pumpkin Extracts on *Entameba histolytica* in Female Rats**) was prepared under my supervision at the University of Babylon, College of Science, as a partial fulfillment of the requirements for the Master degree of science in Biology.

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Date: / /2022

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Date: / /2022

Dedication

To:

who supported me with everything he had, to lead me to the path of success…..... to my pride and the crown of my head ..... **my dear father**.

To who was credited with bringing me to this moment……. to my pure angel and the smile of my heart…..... **my dear mother**.

To the companions of the path, childhood, life partners, and beautiful memories, to those with whom i knew sincere brotherhood and true love..... **my beloved brothers.**

To my love who supported me with every step... to the joy of my heart... **my dear husband.**

And to everyone who believes that the head of wisdom the fear from Allah.

ZAINAB 2022

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Summary

The study was conducted to show the extent of prevalence of Amoebic dysentery parasite in Al-Hilla city by determining the percentage of infection among males and females and for age groups from 1 to more than 18, a total of 414 stool samples were collected from children and adults who attended some hospitals in Al-Hilla city during the period from November 2021 to March 2022.

The results showed that the total percentage of infection with *Entamoeba histolytica* was 4.5%, and there were no significant differences at the probability level ($P \leq 0.05$) in the percentages of infection between gender, where the percentages of infection between males were higher which amounted to (5.47%) compared to females which amounted to (3.75%). The results also showed that there were no significant differences between the age groups, where the highest percentage of infection was in the age group (12-18) years old, which amounted to (6.7%), while the lowest percentage was in the age group (1-5) which amounted to (1.2%).

Another series of laboratory experiments were conducted in the animal house belonging to College of Science, University of Babylon to demonstrate the effectiveness of turmeric (*Curcuma sp*.) and pumpkin (*Cucurbita sp*.) extracts in treating the infection with this parasite in experimentally infected rats after confirming that the extracts are not toxic through determining the safety of extracts concentrations. The study included (80) female rats, whose weight ranged between 250-300 g, and were divided into 15 groups (each group included 5 rats). Groups were infected with *E. histolytica*, and only one group remained uninfected which were considered a positive and negative control treatment, respectively. Feces samples were collected from some patients infected with the amoebic dysentery parasite who suffered from bloody mucous diarrhea, and those who attended the laboratories of Babel teaching hospital for

maternity and children, Al-Hilla General Teaching Hospital, and Al-Noor Hospital for Children, Babylon province.

Each rat was orally administered 2 ml of stool suspension containing the cysts of the parasite, while the last group was dosed with distilled water. After confirming the infection, different concentrations of cold and hot aqueous extract of turmeric rhizome and pumpkin seeds (100, 200, and 400 mg/ml) and metronidazole (250 and 500 mg/ml) were used to treat rats (Mus musculus) infected with the parasite at a rate of three times a day.

The results of the study showed that cold turmeric extract at a concentration of (400 mg/ml) was the best treatment among the used treatments, where it was eliminated the parasite after five days of treatment, followed by the hot turmeric extract at the same concentration where the parasite was eliminated after six days of treatment. As for pumpkin extract, it was observed that cold extract with a concentration of (400 mg/ml) eliminated the parasite after seven days of treatment, and then followed by the hot pumpkin extract (400 mg/ml), which eliminated the parasite after eight days of treatment, It was also found that metronidazole at a concentration of (500 mg/ml) is effective in treating all infected rats after five days of treatment.

Some hematological and biochemical parameters were studied, and several blood criteria were adopted, including the study of changes in the Complete blood count (CBC), represented by white blood cell counts (WBC), lymphocytes counts, Red blood cell counts, granulocytes, monocytes, Hemoglobin (HGB), mean corpuscular volume (MCV), Mean corpuscular Hemoglobin (MCH). The results showed a significant decrease in the values of (WBC, granulocytes, RBC, HGB, MCV, and MCH) for the positive control treatment and increasing it in Lymphocytes and Monocytes. As for the treatments (extracts and Metronidazole), it observed the continuation of the significant decrease in the values of (WBC, Lymphocytes, and Granulocytes) with increasing the

concentrations of the treatment (extracts and Metronidazole) and increasing it in the values of Monocytes, RBC, HGB, MCV, and MCH parameters with increasing the concentrations of the treatments (extracts and Metronidazole). The results also showed that the cold turmeric extract is the most efficient in giving levels close to normal levels.

Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and Alkaline phosphatase (ALP) were investigated as measurements related to liver function to identify the extent of influencing the liver by a parasitic infection and its treatment with plant extracts. The results showed a significant increase in (ALP, ALT, and AST) upon entering the parasite. After treatment (extracts and Metronidazole), it was observed that all treatments accessed the normal level or higher than the normal level. The results also showed that the cold turmeric extract was the most efficient in lowering the levels of the enzymes (ALP, ALT, AST) significantly by giving it levels close to the normal (control treatment), which amounted to (1.57,14.5, 25.15) IU/L, respectively compared to the rest of the treatments.

Histological examination showed vascular congestion in the kidneys and livers of infected and untreated animals. As for the small intestine, no changes were observed in the histological sections of the intestines treated with plant extracts except for the rats infected with the parasite and untreated (positive control) which showed submucosal lymphoid follicles. The results illustrated that cold turmeric extract and metronidazole have high efficiency in treating the parasite, where there were no changes in the histological sections of the liver, kidneys, and intestines compared to the hot turmeric extract and hot and cold pumpkin extracts, which showed the presence of vascular congestion and lymphocytes infiltration in the kidney and liver of rats infected with the parasite and treated with extracts

List of Contents

List of Tables

List of Figures

List of Abbreviations

Sequence	Abbr.	Meaning
1	ALT	Alanine Aminotransferase
2	ALP	Alkaline Phosphatase
3	AST	Aspartate Aminotransferase
4	CF	Complement Fixation
5	CBC	Complete Blood Count
6	CIE	Counter Immunoelectrophoresis
7	ELISA	Enzyme-Linked Immunosorbent Assay
8	H&E	Hematoxylin& Eosin
9	HGB	Hemoglobin
10	IFA	Immunofluorescence Assay
11	IgE	Immunoglobulin E
12	IgG	Immunoglobulin G
13	IHA	Indirect Haemagglutination Assay
14	IL	Interleukin
15	IU	International Units
16	MCH	Mean Corpuscular Hemoglobin
17	MCV	Mean Corpuscular Volume

18	**MPV**	**Mean Platelet Volume**
19	**LD50**	**Median Lethal Dose**
20	**PCV**	**Packed Cell Volume**
21	**PLT**	**Platelet Count**
22	**PCR**	**Polymerase Chain Reaction**
23	**RBC**	**Red Blood Cells**
24	**rpm**	**Revolutions Per Minute**
25	**TNF-α**	**Tumor Necrosis Factor Alpha**
26	**WBC**	**White Blood Cells**

Chapter One

Introduction

1. Introduction

1.1 Overview

Entamoeba histolytica is considered one of the most common parasites in the world. *E. histolytica* causes Amebiasis or Amoebic dysentery. The parasite spreads all over the world, especially in the tropics and subtropics. It spreads among the poor, and this may be due to malnutrition or unhealthy conditions. This disease is considered one of the most common parasitic diseases in the world and the number of individuals infected with amoeba worldwide is estimated to be about 40-50 million individuals annually while the WHO indicates that the amoeba parasite is responsible for the death of more than About 100,000 individuals annually, which is second only to malaria in the high death rate caused by infection with a protozoan parasite (Kantor *et al.*, 2018).

E. histolytica is a protozoa parasite capable of invading the intestinal mucosa and spreading to other organs, especially the liver, causing an amoebic liver abscess (Uribe-Querol and Rosales, 2020). Often, invasive parasitic trophozoites are accompanied by severe necrosis, due to several factors such as stimulation of inflammatory activity of host cells. the production of the enzyme - cysteine protease in trophozoites and stimulating the cellular destruction of host cells (apoptosis). Because of the importance of amoeba disease as a global health problem, Several researches tended to find a cure for patients from medicinal plants. There has been an increase in the recent interest in the therapeutic value of medicinal plants due to their effectiveness, safety and low cost compared to chemical drugs (Al-Shanawi, 2009; Faust and Guillen, 2012).

Despite the large number of people infected with *E. histolytica*, only 10% of the infected people show symptoms of the disease (Blessmann *et al*., 2003), and recently, it was agreed that everything that was previously known to be an *E.*

histolytica, in fact, includes two genetically distinct and morphologically similar types, one of which is *E. histolytica*, which is a pathogenic and invasive parasite, and an invasive parasite, while the other is an *Entamoeba dispar*, Which is considered commensally non-harmful (Al-aboody and Faris, 2014).

During its life cycle, the parasite passes through two main stages, the trophozoite stage and the cyst stage. The cyst stage represents the infective phase, where the infection by the parasite is conducted through eating food and drink contaminated with that stage (El-Dib, 2017). The trophozoite phase lives in lumen of the large intestine of the host and feeds on the mucous membrane of the large intestine and red blood cells. It secretes enzymes that degrade the mucosa which goes deep into the wall of the intestine, destroying its cells, and causing painful ulcers. Thus amoebic dysentery occurs (Muhamad, 2010). The parasite inhabits the large intestine, especially the cecum and the ends of the small intestine, causing symptomatic armoebiasis, and the infection can be transmitted to another person through water and food contaminated with the feces of infected persons containing the contagious cystic phase of the parasite (Al-aboody and Faris, 2014).

Since the traditional treatment strategy in treating amoebic dysentery is not completely clear, in addition to the side effects associated with using drugs, and with the continuation of progress in studies in a large way, The use of plant extracts has been studied, and interest has recently increased in the therapeutic value of medicinal plants, due to their effectiveness, safety and low cost.

Medicinal plants represent an alternative treatment in non-severe cases of infectious diseases, as well as they can be considered an inevitable source as new effective antibiotics, and there is no resistance against it from pathogenic strains, the most prominent of these medicinal plants are those used in local traditional medicine; Therefore, it is necessary to evaluate the possibility of using these plants

on a scientific basis to treat infectious diseases caused by common pathogens. Therefore, the researchers proceeded to extract the active substances from medicinal plants as an important source for the manufacture of some drugs.

Turmeric is a natural antibiotic, so it is used as an anti-inflammatory, oxidative, and antibacterial. It is a herbaceous rhizome plant that reproduces by rhizomes. It belongs to the *Zingiberaceae* family. East Asia is the original country of this plant and it is considered one of the important natural antibiotics (Abd El-Hack *et al*., 2021).

Pumpkin is considered one of the most important medicinal and nutritional plants, which belongs to *Cucurbitaceae* family. *(Cucurbita sp.*) is considered one of five species of *Cucurbita sp.* (Apostol *et al*., 2018). This species is abundant in South Africa. Pumpkin seeds contain essential fatty acids (palmitic, oleic, and linoleic acid) and antioxidants. These fatty acids are important for metabolism and cell growth in the body, especially in the nerves and blood vessels. Pumpkin seed powder and oil are used for their antimicrobial and antiviral benefits, where these seeds not only kill microorganisms, but also inhibit their growth, and some of the unique proteins found in pumpkin seeds have antimicrobial and antiviral properties, making these seeds useful for stopping bacterial and viral infections in the body and strengthen the immune system (Batool *et al*., 2022; Bardaa *et al*., 2016).

1.2 Aim of The Study

The study aimed to investigate the effect of pumpkin seed extract and turmeric rhizomes extract on the amebiasis parasite (*E. histolytica*) for rats infected with amoebic dysentery without side effects. Specific objectives of this project are:-

1- Studying the prevalence of the *E. histolytica* in Hilla city and identifying the causes.

2- Collecting stool samples from infected patients with *E. histolytica*.

3- Preparing a suspension of *E. histolytica*.

4- Preparation of cold and hot extracts for the turmeric and pumpkin plants.

5- Preparation of histological sections for members of infected, uninfected, and treated rats.

6- Comparison of the plant extracts with Metronidazole.

Chapter Two

Literature Review

2. Literatures Review

2.1 *Entamoeba histolytica*

2.1.1 A brief History

E.histolytica was discovered by Lösch, (1875) in a stool of patient with severe Diarrhea; Schaudinn, (1903) differentiated it from *E. coli*, which he called *histolytica* relative to dysentery and degeneration of infected tissue. Walke and Sellord, (1913) proved its pathogenic effect as well as described its life cycle and shape, where they fed volunteers in (Manila) with trophozoites and cysts phases from water contaminated with this amoeba and *E. coli*, thus distinguishing between the two types in terms of the first being pathogenic and the second non-pathogenic. The initial studies on the pathogenesis of the parasite began on dogs and cats by (Lösch, 1875; Cleveland and Sanders, 1930), in rodents and mice (Diamond *et al*., 1978), in hamsters (mattern and Keister, 1977), then in rabbits (Guirges and Shirodkir, 1982), and finally in the gerbils, which is an animal of subfamily *Gerbillinae* (Chadee and Meerovitch, 1984; 1985).

2.1.2 General Description of Parasite

Amoebiasis is considered one of the most common parasitic diseases in the world, which results from infection with *E. histolytica*, it is one of the intestinal protozoa that infect humans, and there are about 500 million infections in the world, which lead to100 thousand cases of death per year (Carrero *et al*., 2020). The parasite has several phases which are: the active phase trophozoite, pre-cyst phase, cystic phase, and meta-cyst phase (Chalmers, 2014). The diameter of the active phase ranges from (10-60) µm with an average diameter of 25 µm. A phase contains a single nucleus whose size ranges between 3-5 µm. The nucleus contains a transparent chromatin layer and a central nucleolus, while the cytoplasm consists of a transparent outer layer (ectoplasm) and an inner granular layer (endoplasm) (Kantor *et al*., 2018; Mohammad, 2019). This layer contains

several vesicles or vacuoles, and it contains red blood cells in different stages of digestion and bacteria. This phase is characterized by the presence of pseudopodia that extend in all directions, and the movement be directed forward, unlike the rest of the types of amoebas in which the movement is undirected (Luna-Nacar *et al*., 2016).

As for the pre-cyst phase, it is a circular or oval shape with a diameter of (10-20) μm, it is a transitional stage between the active phase and cyst phase, in which the pseudopods and food vacuoles disappear. Cysts are transparent, circular or somewhat oval, asymmetrical bodies with a smooth, lustrous, non-dyeable coating with a thickness of about 0.5 µm (Arredondo *et al*., 2014). The several differences in size (5-20) µm are due to the presence of strains of large and small cysts. The cytoplasm of the newly formed cyst contains vacuoles containing glycogen (animal starch) as well as cylindrical rods dyed with a bright dark dye and with rounded ends. It was reported that these bodies contain ribonucleic acid (RNA) and deoxynucleic acid (DNA), and phosphate, these bodies disappear when the cyst matures, so about half of the number of these cysts do not contain these bodies (El-Dib, 2017).

The cytoplasm contains the above-mentioned parts that represent stored nutrients. The immature cyst contains one nucleus, which is approximately one-third the diameter of the cyst. The mature cyst that causes infection contains four smaller nuclei. Therefore, it can be seen (1-4) nuclei with the same shape as the nucleus of the active phase (trophozoite) in the sacs that come out with the faeces (Bandyopadhyay *et al*., 2022). Meta-cystic phase forms when the parasite exits the excystation in the lumen of the small intestine, where it finishes with the division of its tetranuclear phase into eight nuclei, and the cytoplasm divides to form eight amoebae in the Trophozoite phase small move down to the large intestine by the intestinal current, where they attack the mucous membrane and multiply there from new (Arredondo *et al*., 2014).

2.1.3 Classification of *E. histolytica*

Parasite is classified according to (Karyakarte and Damle, 2003) into:

Kingdom: protista

Sub kingdom: protozoa

Phylum: Sarcomastigophora

Sub phylum: Sarcodina

Class: Lobosea

Order: Amoebida

Sub-order: tubulina

Family: Endamoebidae

Genus: *Entamoeba*

Species*:* *histolytica*

2.1.4 Prevalence of Parasite

E. histolytica is a widespread disease in Iraq and the world, as shown in previous studies below, where the prevalence of the disease in foreign countries such as in children under ten years of age in Indonesia, which amounted to (0.2%) (Ghulam and al Kubaissy, 2011). Despite this wide spread of species of this parasite, only 10% of *E. histolytica* infections are able to invade tissues and cause amebiasis, and the remaining 90% of infections in this genus are asymptomatic and belong to the two species *E. dispar* and *E. moshkovskii* (Solaymani *et al*., 2006).

2.1.4.1 Prevalence of The Parasite in The World

The parasite is considered one of the most widespread parasites in Asia. In Bangladesh, Alam *et al.* (2014) found a percentage of infection estimated at

about 11.1% among patients attending hospitals in the Akdeeb region (Dakar). While a study conducted by Lau *et al.* (2013) illustrated that the total infection in Malaysia was 19.5%, which is the highest, as another study in Bihar region in India showed a 14.25% infection among women in rural areas (Pandey *et al.*, 2013).

In Africa, an epidemiological study conducted by Verweij *et al.* (2003) recorded a high infection rate in Ghana, which amounted to 39.8%, and in South Africa, a similar study conducted by Samie *et al.* (2006) attempted to distinguish between the types of this genus and obtained an infection rate estimated at 18.8% for *E. histolytica* and 25.3% for *E. dispar*, while the infection rate was 12.6% in Kenya (Nguhiu *et al*., 2009), while it was the lowest in Nigeria, reaching 2.2% (Houmsou *et al.*, 2010).

A study was conducted by Kumar *et al*. (2002) on intestinal parasitic infection in the Indian state of Chennai, where 150 faeces samples were collected, and the percentage of infection with dysentery amoeba amounted to (2%). Sayyari *et al.* (2005) studied the prevalence of intestinal parasites in the Islamic Republic of Iran. In the health centres of college of Medicine, (53995) faecal samples with ages over sixty were examined, where the percentage of dysentery amoeba was about (10.9%). Haque *et al*. (2006) conducted an epidemiological study on acute diarrheal infections of amoeba dysentery among pre-school children in the poor neighborhood of Dhaka suburbs in Bangladesh, which amounted to 289 samples.

In Nepal, Tandukar *et al*. (2013) collected 1,392 stool samples (732 males and 660 females (male to female ratio 1.1:1) from school children in two government schools, two private schools and two community schools in the same area. Stool samples were examined for evidence of infection Parasitic by direct microscopy and confirmed by concentration methods (formal ether deposition technique or flotation technique using Schether's sugar solution). The prevalence of intestinal parasites was 16.7%. The highest prevalence was seen

with *Giardia lamblia* (7.4%) followed by *E. histolytica* (3.4 %) and *Cyclospora cayetanensis* (1.6%). Children aged 11-15 years and those belonging to the family of agricultural workers were most affected. The practice of hand washing and the type of drinking water also showed a significant difference.

In Pakistan, Khan *et al.* (2019) found in their study that was conducted in different hospitals in Potohar District, Punjab and Khyber Pakhtunkhwa for 356 patients, 238 (66.9%) females and 118 (33.1%) males. The mean total age was 33.4 ± 11.05 years. The seroprevalence of *E. histolytica* was 356 (73%). Blood samples were examined using enzyme-linked immunosorbent assay and serum biochemical assays. While the highest infection was recorded in Islamabad 91 (25.5%). Participants in rural areas had 2.16 times higher risk of infection compared to urban areas, while the lowest risk of infection was among those aged 50 years compared to those aged 40-49 years (P = 0.04).

Le *et al*. (2007) conducted a study to determine the relationship of anemia with intestinal parasites among school children in a poor village in Vietnam. It was found that the percentage of anemia among children infected with intestinal parasites was 25%. Anemia form a percentage amounted to (2%).

2.1.4.2 Prevalence of The Parasite in The Arab and Neighboring Countries of Iraq.

Özer *et al.* (2011) recorded a percentage of infection of 2.2% in Turkey, which is lower than what was recorded in Iran, where the percentage of infection was 4.28% (Shahbazi and Rahimi, 2013). In the Arab countries and in Egypt, the percentage of infection was 21% in a study that also included South Africa (Stauffer *et al.*, 2006). In a Palestinian study that lasted for ten years, it appeared that the percentage of infection of the parasite was 8.2-18.2% (Bdir and Adwan, 2010).

In Sudan, there were 196 cases of infection out of 246 cases examined, with a percentage of infection estimated 79% (Amir *et al.,* 2011). This is the

highest percentage among Arab countries. In Saudi Arabia, 120 cases of parasite infection were found among children and infants out of 1038 cases examined (Hegazia *et al.,* 2013). while the infection was higher in Libya amounted to 19.89% (Al-Souqi and Daw, 2013). A study was conducted in Kuwait for intestinal parasites. It appeared that the percentage of infection with dysentery amoeba was (5.9%) in relation to the samples collected, which amounted to (912) faecal samples in a health care center (AL-Nakkas *et al.,* 2006).

In Saudi Arabia, Bakhraibah (2018) mentioned through study on 188 patients (121 males and 67 females) during his visit to King Fahd Hospital in Jeddah. The disease was diagnosed in 156 patients, with a percentage of (83%) through examining the stool and watching the primary bags and larvae. It was found that the prevalence of the disease is higher among males than females.

In the United Arab Emirates, Al‑Rifai *et al.* (2020) examined stool samples microscopically and molecularly for the presence of Intestinal Parasites. A 102 workers living in workers' accommodation, they were sharing a bedroom and toilet. Fifteen types of IPs have been microscopically identified *Entamoeba* (8.1%), and Cryptosporidium species (3.5%).

2.1.4.3 Prevalence of The Parasite in Iraq

In Iraq, several epidemiological studies were conducted that showed the prevalence of parasite infection in different regions of the country, where the percentage of infection in Al-Qarya district of Basra province was 29.2% (Zuhair and AL-Maki, 2007). In a study that included the residents of Al-Khalis and Baladruz districts, Al-Qaisi and Sultan (2008) found that the percentage of infection with the parasite was 70.5%, and in the Al-Gharraf and Al-Batha districts of Dhi Qar province, the percentage of infection was 24.9% (Al-Aboudi, 2010). Adday (2009) conducted an epidemiological study of intestinal parasites and know their effect on blood parameters in Babylon province, central Iraq and the prevalence rate was (50%). The percentage of infection was 17.3%,

according to the study conducted by Al-Miqdadi and Jasim (2011), while the percentage was less than 16.6% in the study of Rahi and Mutlaq (2011) among the children of Wasit province.

Farhan (2012) recorded in a study that lasted for three years, an percentage of infection of 61.6% for patients arriving at Ramadi City Hospital, while the percentage of infection was the lowest in Qadisiyah province, where Abdullah, (2013) recorded the percentage of infection amounted to 5.6%.

In Salah al-Din, Sabry *et al*. (2021) collected 450 stool samples from patients who visited the hospital and found that the percentage of infection with the bacteria among males was 19.50% and females 14.83%. As for parasitic infections, males recorded 12.44% and females 8.71%, while with regard to infection with intestinal parasites, the total percentage of infection with *E. histolytica* was 6.88%. The group (1-10) years old recorded the highest percentage of infection with the parasite amounted to (13.23 %).

In Babylon, Al-Quraishi and Al-Saidi (2019) examined 250 watery diarrhea stool samples during the period from September 2018 till January 2019 by chromatography immune assay for patients infected with watery diarrhea (children adults, and older males and females), who attended some hospital in Babylon province as well as some primary health care and private clinics. The results showed the percentage of infection with *E. histolytica* was 8.8%. The highest percentage of infection was in the rural area 62.6% in comparison with the city was 37.4%. Also, the highest percentage of infection among males was 16.9% in comparison with females at 38%. The highest percentage of infection was 33.6% in the age group (>10) years while the lowest percentage of infection in the age group (31-40) years was 7.6%.

In Iraq, stool samples were collected from Basra Hospital for 818 people and 295 (dogs and cats) by Nassar *et al*. (2019) who showed that 32% and 51.52% of patients and animals, respectively, were infected with *E. histolytica*.

The effect of age on the infection with *E. histolytica* was studied and found that its percentage was higher at (30-40 years) 55.10%, and its percentage was low at (10-20 years) 11.33%.

In Erbil, Mahmood and Bakr (2020) found through examining stool samples microscopically for 950 patients (524 males 426 females) the percentage of prevalence of *E. histolytica* was 7.4%. The prevalence of infection in females is higher than in males and in low-income people compared to middle-income people.

2.1.5 The Life Cycle of Parasite

The first researchers who described the life cycle of the parasite in the best way is Barker and Swales (1972) who stated that *Entamoeba* has a simple life cycle and it has four distinct forms in its life cycle:

1- Active phase (Trophozoite)
2- Pre-Cyst
3- Cyst phase
4- Metacyst phase

This parasite exists in two phases during its life cycle, the active phase (trophozoite), which is the phase that causes symptoms, and the cyst phase, which may remain alive for long periods outside the host and represents the infective phase. The active phase ranges in diameter between 10-60 µm, and it has progressive rapid movement in one direction by pseudopods. The nucleus contains chromatin arranged on the nuclear membrane as well as a small centrally located karyosome, and the cytoplasm is granular, containing vacuoles filled with food and bacteria residues, and in the case of dysentery, red blood cells become visible in the cytoplasm (Roberts and Janovy, 2009).

The diameter of Cysts ranges between 12 and 15 µm, their wall is made of chitin. The maturation of the cyst includes two cycles of nuclear replication

without cell division. The cyst when present in the stool contains 1-4 nuclei, and the nucleus of this phase is similar to the nucleus of the active phase, except that it becomes smaller with each division (Roberts and Janovy, 2009).

Chromatin bodies disappear as the cyst matures, and cysts become infectious as soon as they are excreted in the faeces and remain vital for weeks or even months, depending on environmental conditions (Wiser, 2010). The life cycle of this parasite begins when the active and cystic stage's exit with faeces outside the body of the host, and infection occurs after the ingestion of the mature cyst by contaminated food, water or hands, where they pass through the acidic medium of the stomach and become active after entering the slightly neutral or alkaline medium (pH = 7-8) for the small intestine (Dey, 2009).

A tetranuclear amoeba forms inside the cyst in the small intestine, and this stage is called Metacyst, then each nucleus undergoes binary division, so the number of nuclei becomes eight, followed by the division of the cytoplasm into several parts, thus the cyst produces eight small amoebae, then cracking occurs in the wall of the cyst, the amoebae emerge, and this process is called Excystation (Arora and Arora, 2014).

In most infections, these amoebae attack the mucosal layer of the large intestine and produce a self-limiting, asymptomatic infection. In some infections, they adhere to and dissolve the epithelial tissue of the colon, thus attacking the colon, and the active phases, once they attack the intestinal epithelium, may reach sites extraintestinel, such as the liver (Haque, *et al*., 2006).

The amoeba surrounds itself with a wall made of chitin that protects it from unfavourable conditions of the external environment and this stage is called the encystation. Both the active and cystic stages pass with faeces, but the active die shortly after leaving the host, while the cysts survive for longer periods (CDC, 2010). The following is a diagram showing the life cycle of the parasite in brief:

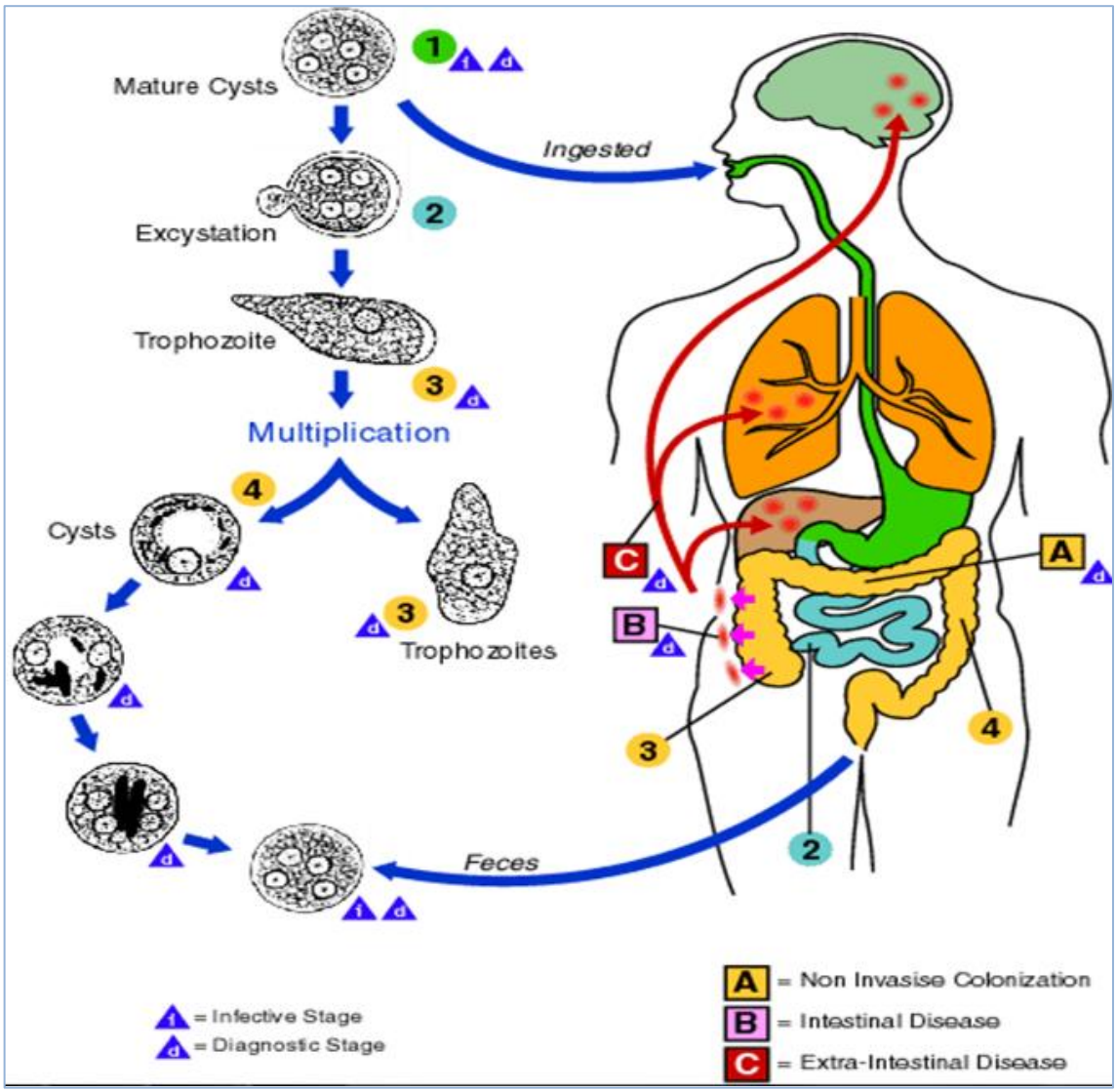


Figure (2.1): The life cycle of *E. histolytica* (Garmie, 2016).

2.1.6 Methods of The Prevalence of Parasite

The resistant cyst is formed in the lumen of the large intestine, it is then subtracted with the faeces to remain for a period until it is taken by a new host, where this phase lives for two days in the usual stool or diluted with water at 37ºC and remains alive for 9 days at 22ºC and 6 days at 0ºC, it is killed by dehydration, strong sunlight and heat, and a few cysts are subtracted outside the body in case of acute dysentery or there are no cysts in this case (Ghulam and al Kubaissy, 2011).

In chronic cases, as well as in the case of parasite carriers, cysts are frequently subtracted, and humans are considered the main host and source of

infection, while other mammals are considered important sources in this respect (Ghulam and al Kubaissy, 2011). Humans devour the cyst phases with water, vegetables and food contaminated with flies or through contaminated hands, where the mature cyst is not affected by the gastric juices and continues to move until it reaches the lower part of the small intestine, where dissolves its outer wall under the influence of the basal and neutral juices.

The four Metacyst amoebae are then released by a process called Excystation and this is called the Metacyst phase, which divide directly giving eight small active amoebae that move to down the large intestine by the intestinal current where they attack the mucous membrane and multiply there again, forming about 45 million cysts per day (Ghulam and al Kubaissy, 2011).

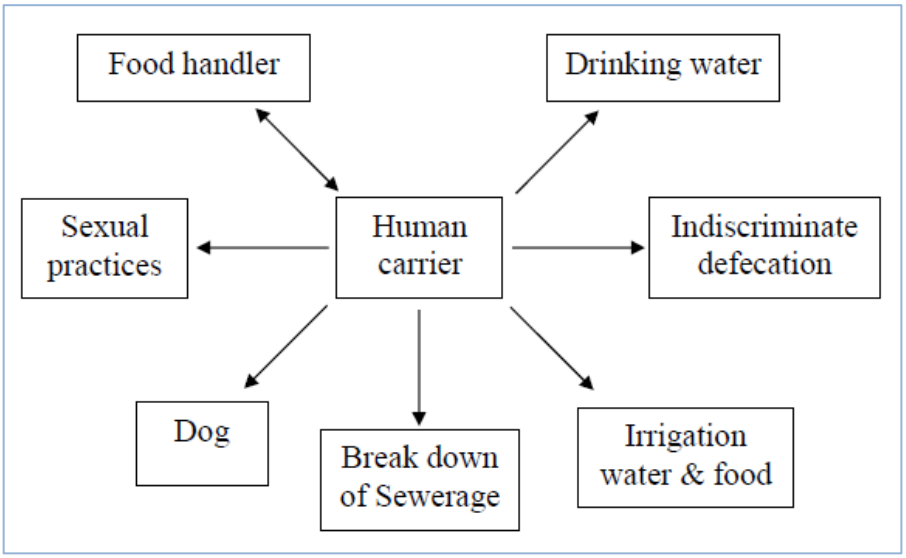


Figure (2.2): The methods of the prevalence of parasite (Ghulam and al Kubaissy, 2011).

2.1.7 Pathogenic Effect and Symptoms

2.1.7.1 Pathogenicity

The pathological effects of *Entamoeba* depend on host resistance, the severity of infection, and the state of the intestinal tract, where resistance depends on natural un-acquired immunity, nutritional status, and the free body from debilitating and infectious diseases (Ghulam and al Kubaissy, 2011; El-

Dib, 2017). The incubation period for the parasite takes between (1-4) weeks and may be as long as a year. The clinical symptoms of the disease are abdominal pain, high temperature, bloody diarrhoea, and the danger of the disease lies in children and pregnant women who suffer from malnutrition and treatment with corticosteroids (Bahrami *et al*., 2019).

The large intestine and its tortuous area are considered one of the main locations of infection due to the slow movement of the colon, which gives an opportunity for the parasite to attack the mucous layer of the intestine, and there are other locations of infection such as the ascending colon, rectum and the appendix area. Parasite penetration into intestinal tissue includes three stages: adhesion to colon cells by lectin, analysing tissue by enzymes after adhesion, and ingestion of epithelial cells and red blood cells after their death (Ghulam and al Kubaissy, 2011).

The active phase can attack tissues by means of its analytic enzymes, where the infection begins to necrosis of a small area in the surface layer of the mucous membrane, causing a capillary or circulatory ulcer that is the size of a pinhead and may access 1 cm or more in diameter. The bottom of the ulcer contains the active phase, decomposing cells and mucus. The ulcer in chronic infections extends to the deep layers, such as the submucosal layer, where the healthy mucous membrane sloughs off to reveal what is underneath, leading to the loss of the first line of defence of the body through the removal of the mucous and epithelial layer from the necrotic areas as well as the dissolution and thrombosis of capillary blood vessels, which may lead to secondary infections with bacteria that work on tissue necrosis and further destroy tissues. It may cause inflammation of the intestine, which sometimes leads to appendicitis and perforation of the intestine, with Amoeboma, and It is most often observed in the cecum and rectum (Kantor *et al*., 2018; Ghulam and al Kubaissy, 2011).

The infection spreads from the intestine via the bloodstream to the liver, causing amoebic liver abscess in around (5%) of individuals presenting with gastrointestinal symptoms that appear as a small mass of necrotic liver cells containing a viscous, pale reddish fluid resulting from decomposition of hepatocytes, red blood cells, bile juice, lipids, and others (Kumar *et al*., 2021). The percentage of infection of the right lobe of the liver in the infecters reaches about (85%), accompanied by weight loss, weakness, high temperature similar to hectic fever, pain under the right ribs, vomiting, anemia, and an increase in the number of white blood cells, with an increase in the percentage of Alkaline phosphates, jaundice, and the increase in eosinophilic white blood cells abnormally, and the infection moves through the inferior vena cava to the right side of the heart and then the lungs, causing amoebiesis pulmonary disease, then lung abscess, which occupies the second degree in importance after liver abscess, which is characterized by chills, fever, sweating, sputum, and pulmonary sclerosis, and the parasite travels from the lungs again to the left side of the heart through the systemic circulation and may reach the brain, causing brain abscess, which is a rare case (Reid-Lombardo *et al*., 2010; Ghulam and al Kubaissy, 2011; Kumar *et al*., 2021).

The amoeba can be carried in the bloodstream to the kidneys or genitals, and it may spread to other nearby organs, such as its transmission from the liver to the diaphragm, then the thoracic cavity, to the lungs, as well as from the liver to the skin, causing cutaneous amoebiasis, where the ulcer is in the form of a hard ulcer that is slow to heal, and the active phase in the tissues is multiplied by simple division, and generations of amoebas are able to attack new tissues or move to the intestinal cavity sometimes, where they become cysts and subtract with the stool. As for other infections such as ulcerative vaginitis, ulcerative colitis, the penis and the prostate gland, they are rare and the urinary tract may be infected as a result of the infection spreading from the rectal area (Reid-Lombardo *et al*., 2010; Ghulam and al Kubaissy, 2011; Kumar *et al*., 2021).

2.1.7.2 Symptoms

Asymptomatic infections are common in temperate regions, with carriers shedding millions of cysts daily. In this case, the parasite is called commensal. Acute amoebic gastroenteritis is accompanied by severe dysentery. The stool contains drops of blood, mucus, and parts of the necrotic mucosal layer, with severe abdominal pain and fever up to (37.7-38.89) °C, with weight loss. The appearance of symptoms depends on the number of ulcers, their locations and the area of the affected area. Diarrhea alternates with periods of constipation or remission of the disease (Bhagani and Cropley, 2018).

It also observes fluid loss and blood poisoning and then collapse. This is accompanied by an increase in the number of white blood cells from 7000 - 20,000 cells / mm, as well as increasing the percentage of deposition of red blood cells, enlargement of the spleen and sometimes its explosion in the abdominal cavity, as well as fever and night sweats, and this may be accompanied by vomiting and pain between the ribs in case of infection outside the intestine, especially on the right side, anemia, an increase in the percentage of Alkaline phosphates, jaundice, as well as an increase in the number eosinophilia cells abnormally (Ghulam and al Kubaissy, 2011).

2.1.8 Diagnostic Methods

Researchers in the field of parasite diagnosis have find many methods that are more accurate and reliable in diagnosing the presence of a parasite (*Entamoeba spp*.) and trying to distinguish its different types in order to obtain better results for treatment and to prevent the spread of the disease. These methods include:

2.1.8.1 Microscopic Tests

The use of a microscope remains the first method in discovering and diagnosing this genus, where it depends in the microscopic diagnosis on the size

and the presence of chromatin granules around the nuclear membrane for the nucleus of the trophic phase or the number of nuclei in the cystic phase, which requires high accuracy and great ability in diagnosis from laboratory workers, especially in the case of mixed infections (Tan *et al*., 2010).

It may sometimes resort to observing the presence of red blood cells within the trophic phase, but even this method does not help much in distinguishing between pathogenic and non-pathogenic species or strains (Parija *et al*., 2014). Rather, it is not considered accurate in the case of chronic infections, where the red blood cells are completely digested by the trophic phase. In addition, red blood cells were found ingested by non-pathogenic species (DiMiceli, 2004). A group of dyes are used in microscopic preparations such as, Giemsa, Methylene blue, Chorazole black E, Lugol's iodine Iodine-trichrome. (Tanyukse and Petri, 2003).

2.1.8.2 Cell Cultures

The culture method is considered more a research method than a diagnostic method, where it depends on the use of different types of xenic and Axenic cultures (Lian Lim and Vythilingam, 2013). Among the culture media used is starch of rice and eggs known as egg-serum medium, using horse blood serum or live cells, such as chicken embryo (Fotedar *et al*., 2007). This method of identifying species is based on the basis of distinguishing the ability and speed of growth of some species in these farms and distinguishing them using different types of enzymes such as Phosphoglucomutase, alic enzyme Hexokinase, isoenzymes, and Glucose phosphate isomerase (Tanyukse and Petri, 2003).

Their need for great effort and consumption of time are considered the most important obstacles to their use as diagnostic methods in addition to the growth of one species in more than one type of farm and the inability to limit the growth of unwanted organisms, where Blastocytis hominis grows faster than *E.*

histolytica, which gives negative results upon examination (Aguirre-Beltran, 1999).

2.1.8.3 Serological Tests

Serological tests are based in industrialized countries, where the percentage of infection with the parasite is low, where It gives positive results in 75-85% of cases and these tests depend on the presence of IgG and IgM antibodies and cannot distinguish between acute and chronic infections or old infections, where the antibodies remain in the bloodstream for long periods of time, these tests must be carried out by a specialized person (Fotedar *et al*., 2007).

Among the tests that depend on the presence of antibodies (IFA, IHA, CIE, CF, ELISA, Latex agglutination, amoebic gel diffusion test (Tanyukse and Petri, 2003). Antigen-based ELISA is the most widely used current test in diagnosing amoebiasis because it distinguishes between *E. dispa*r and *E. histolytica* and can be used by a non-specialist, where this assay uses monoclonal antibodies against Gal/GalNAc, Serine-rich, or Lysine-rich surface proteins, or against antigens including Lectin, Salivary 170-kDa, Lipophosphoglycan antigen, and others (Abd-alla *et al*., 2000).

2.1.8.4 Molecular Examinations

Molecular methods (which have become popular in use) are considered the newest way to obtain more sensitive and specific results and to avoid errors in microscopy examinations and cell cultures (Furrows *et al*., 2004). This method of diagnosis is not adopted, especially in developing countries, where the parasite is endemic because this method requires a specialist and consumes a lot of time and the difficulty of extracting DNA from excrement, and the high chance of contamination. Nested PCR, multiplex PCR, and other. (Tanyukse and Petri, 2003).

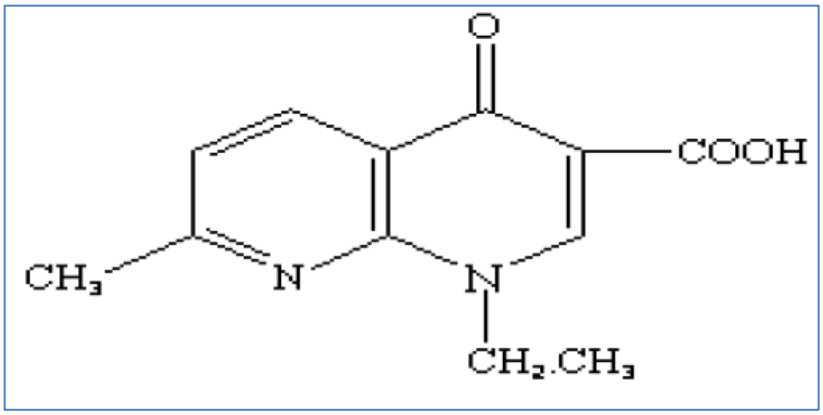
2.2 Drugs and Treatments Used Against The Parasite

2.2.1 Metronidazole

Substance name: Metronidazole

Chemical composition: 2-methyl-5-nitroimidazol-1-ethanol

Chemical Structure:



Natural form: yellow crystalline powder

Solubility: slightly soluble in ether or chloroform

Metronidazole is a medicinal drug that is used against protozoa and also against bacteria. Metronidazole is a substance known as:

1- Antiprotozoal against infection by *G. lamblia*, *E. histolytica*, *Trichomonus vaginalis*
2- Anti-bacterial: against a wide range of anaerobic bacteria including Bactriod species, *Clostridium* species, and various aerobic cocci.
3- An inhibitor of inflammatory bowel disease.

Metronidazole is rapidly and almost completely absorbed from the gastrointestinal tract and its maximum concentration in serum reaches within 1-2 hours of oral administration. Metronidazole is mainly metabolized in the liver and excreted by the kidneys, and a small amount of it is excreted through the bile. This drug has many indications for use. In addition to treating animal

protozoa, especially *G. lamblia*, it also treats colitis, inflammatory bowel disease and dysentery (Weir and Le, 2022; Ghulam and al Kubaissy, 2011).

2.2.2 The Dose

Metronidazole is used in different doses according to the disease to be treated, but what concerns us here is how to use it to treat Ambaisis and it is as follows:

Metronidazole is taken orally.

Adults: 500 mg every 8 hours for 5-10 days.

Children: 11.6-16.7 mg/kg every 8 hours for 10 days.

It is interesting that Metronidazole is not used in the presence of an active organic disease in the central nervous system, including epilepsy, and also not used if the patient has blood disorders or hypersensitivity to this drug. Metronidazole is not recommended in the case of female pregnancy, especially during the first three months Pregnancy, because it crosses the placenta, and it is also not recommended for use when breastfeeding, because it spreads in breast milk (Ghulam and al Kubaissy, 2011).

2.2.3 Side Effects

Some of the side effects of Metronidazole may appear, such as stomach and intestinal upset, nausea or vomiting, a feeling of an unpleasant metallic taste, skin rash, feeling drowsy, headache, white coating of the tongue, and low white blood cells may occur when using the drug for a long time and in large doses. Metronidazole may inhibit the metabolism of phenytoin, chemotherapy and lithium, metronidazole may potentiate the anticoagulant activity of coumarin derivatives (Ceruelos *et al*., 2019).

There are substances such as primidone and barbiturates, the metabolism of metronidazole produces a decrease in its concentration, while another

substance such as cimetidine inhibits the metabolism of metronidazole, which then leads to an increase in its concentration in the blood. It is also important to emphasize that there is evidence that continuous and long-term use at very high levels, approximately 1500, leads to the occurrence of malignant liver tumors in males and females (Ghulam and al Kubaissy, 2011).

2.3 Medicinal Plants and Their Importance

Since ancient times, humans began to use the natural pharmacy or the lands prepared by God Almighty among the thousands of blessings and bounties. The first man linked the relationship between the wild plants that cover the face of the earth between the diseases that they contract, so used these herbs or parts of them in medicine from this land or used the roots, leaves, fruits, seeds and weeds that knew during roaming and migrating. The Arabs became famous for the development of herbal medicine during the Middle Ages, and plant preparations were called Galenic preparations in relation to the scientist Galen, who tested (300) species of flowering plants for their biological effectiveness against the growth of germs in bacterial cultures, such as brother juice (Buterup), which has a significant impact on the growth of many germs, as well as garlic, tomato and cress (Shnawa, 2009).

Many of the medicinal plants and herbs currently dispersing in the world were of Sumerian origin and spread across Greece to the world. The profession of treating diseases with medicinal herbs flourished during the reign of the Assyrian king Ashurbanipal in the year (626-668) BC. This was confirmed by books and magazines that were found in his famous library (Al-Zubaidi *et al*., 2019). The Muslim Arabs were the first to open the first pharmacy to prepare medicine based on medicinal herbs in Baghdad in the eighth century AD. As for the Arab-Islamic civilization, a number of scientists and doctors appeared, and some of them dealt with diseases, symptoms and methods of treatment in medicinal plants such as Ibn al-Bitar and al-Antaki (Al-Katb, 2000).

There are many pharmacies specialized in herbal medicines, which are often of plant origin, allowing patients to use herbs, avoiding the side effects caused by chemical medicines, in addition to the fact that the active substances in plants are easy and the bodies can deal with them gently in their natural form (Qutb, 1998). This was followed by the success of pharmacists and chemists in separating the same active ingredients from plants, such as extracting morphine from the fruits of the poppy plant, and even more, as the pharmacists were able to reach the chemical composition and know the shape and molecular structure of the active ingredient (Al-Dukhani, 2003).

2.4 Effect of Plant Extracts on Microorganisms

The resistance of parasite to many antibiotics has prompted researchers to study the effect of medicinal plants and separate their active components, where medicinal plants contain special chemicals, the most important of which are: Aromatic or volatile Etherical. or Volatile oils, tannin, resin, gum, phenols, and saponins (Chanda, 2014). Several studies have been conducted to identify the inhibiting effect of many of these plants on the growth of many microorganisms that cause serious human diseases.

Shaker and Mahmood (2017) showed the effect of the alcoholic extract of green tea against *E. histolytica*, where this powder showed effectiveness in improving blood parameters through changes in their proportions, where there was a significant decrease in the level of the total number of white blood cells and granulocytes and not significant for lymphocytes, monocytes and Red blood cells also decreased hemoglobin and platelet count. Mahmood and Mohammed (2012) cleared the effect of the aqueous extract of hot pepper (Capsicum spp.) in treating *E. histolytica* in white mice, the results showed that the extract was effective in decreasing the trophic and cystic phases in mice infected with the parasite.

Ghulam and Al-Kubaisi (2011) studied the effect of the hot aqueous extract of Alhagi maurorum roots in treating mice infected with *E. histolytica* parasite. The results showed that the extract was more efficient in getting rid of the parasite and the symptoms of the disease, thus treating mice. Al- Mammury *et al*. (2020) showed the effect of aqueous extracts of two types of plants (Allium sativum and Punica granatum) on experimental infection of mice with *G. lamblia* and *E. histolytica*. The results showed that the plant extract of pomegranate at concentrations of 2000 and 3000 mg/kg succeeded effectively during a period of time less than the period required to kill this parasite using 200 mg of Metronidazole drug. The use of a concentration of 3000 mg/kg of garlic extract and a concentration of 2000 and 3000 mg/kg of pomegranate extract was effective against dysentery amoeba during a period of time less than the period required to kill this parasite by using 200 mg of Metronidazole drug.

2.5 General Description of Turmeric Plant

Turmeric is considered an important genus in the *Zingiberaceae* family, consisting of 110 species distributed in tropical Asia and the Pacific Ocean. Turmeric is a perennial tropical herbaceous plant with roots and annual leaves in the form of small tubercles that grow near the surface of the earth, and there are many species of it, the most famous of which are *Curcuma longa* and *Curcuma xanthorrhizia*. The turmeric plant is spread and found in India and Indonesia. It is also exported from these two countries to the rest of the world, including Iraq. It is used as a food in mixtures of Indian spices (condiments). It contains active substances and volatile oils at a rate ranging between 14-42%. This oil consists of about 50 compounds, the most important of which are sesquiterpene ketones, Curcuminoides and Curcmin (AL-Hendawi *et al*., 2018; Abd El-Hack *et al*., 2021). Figure (2.3) shows turmeric plant and its rhizome. One of the most important compounds of this group is Curcumin (which gives the yellow pigment). Curcumin pigment is used in food additives to dye foods such as cheese, margarine butter, sweets, fish fingers and slices due to its high ability to

bind and dye food, and it is classified by the pigment (AL-Hendawi *et al*., 2018; Abd El-Hack *et al.*, 2021).

Figure (2.3): Turmeric plant and its rhizome.

2.5.1 Classification of Turmeric Plant

Plant is classified according to (Al- Hindawi, 2018) into:

Kingdom: Plants

Division: Flowering plants

Class: Liliopsida

Subclass: *Zingiberidae*

Order: *Zingiberales*

Family: *Zingiberaceae*

Genus: Curcuma

Species: *C. longa*

2.5.2 Chemical Composition of Turmeric plant

The chemical structure of curcumin pigment is Diferuloyl methane and its chemical formula is $C_{14}H_{14}O_4$, which is soluble in hexane, alcohol, chloroform and acetic acid. The biological and chemical activity of this pigment is due to the presence of the Dieneketone system in its composition as well as the presence of CH_3 and OH groups at the edges of the chemical structure of the pigment. Figure (2.4) shows the chemical structure of curcumin pigment. Turmeric contains volatile oils at a rate ranging between 4.2-14% and this oil consists of about 50 compounds, but the most important of these compounds is a group known as SesquiterPene lacton ketones, which constitute 60% and this group is known as Turmerones (AL-Hendawi *et al*., 2018; Abd El-Hack *et al*., 2021).

Turmeric also contains another very important group known as Curcuminoids. One of the most important compounds of this group is the well-known curcumin compound, which has been separated commercially and is currently sold as a pure compound and is almost responsible for the pharmacological effects of turmeric. As well as is what gives the yellow dye that characterizes turmeric. Turmeric also contains a mixture of resin and volatile oil known as oleo-resin, as well as a fixed oil, bitter substances, protein, cellulose, neptosan, starch and minerals (AL-Hendawi *et al*., 2018; Abd El-Hack *et al*., 2021).

Turmeric is a hydroxycinnamic acid derivative containing two hydrophobic polyphenol rings with two carbonyl groups (Huang *et al*., 2015). The roots of the turmeric plant contain 6.3% proteins, 5.1% fat, 3.5% minerals, 69.4% carbohydrates, and 13.1% moisture, in addition to a group of compounds known as curcuminoids that constitute (4-3%) of the components of the root, giving the yellow colour of turmeric, which are determined by high-performance liquid chromatography (HPLC), and they are: Curcumin, which includes Curcumin-I (94%) and Curcumin-II (6%) and Curcumin-III (0.32%), in addition to

Demethoxycurcumin and Bis-demethoxycurcumin as shown in figure (2.5) (AL-Hendawi *et al*., 2018).

Curcumin is the most important compound in this group, which is responsible for the pharmacological effects of turmeric. The roots of turmeric also consist of resinous volatile oils, and it reached about 5.8% and includes the following compounds: a-Phelandrene (1%), Sabinene (0.6%), Cineol (1%), borneol (0.5%), zingiberene (25%), in addition to Sesquiterpenes that make up a proportion (53%), and this group of compounds is known as turmerones (AL-Hendawi *et al*., 2018).

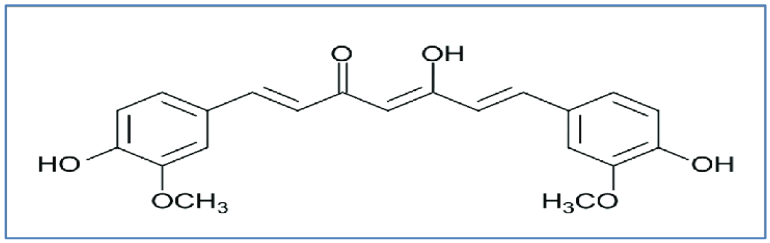
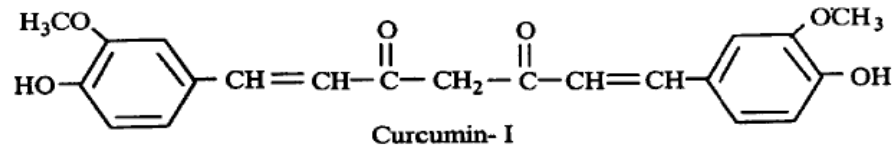
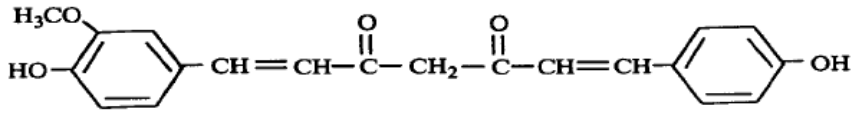


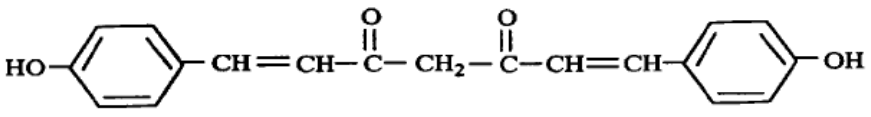
Figure (2.4): Shows the chemical structure of curcumin pigment (AL-Hendawi *et al*., 2018).



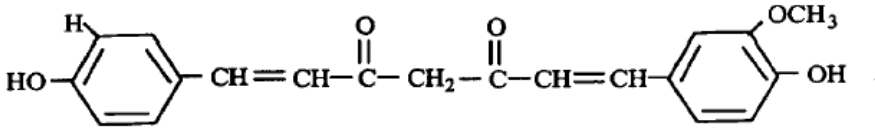
Curcumin- I



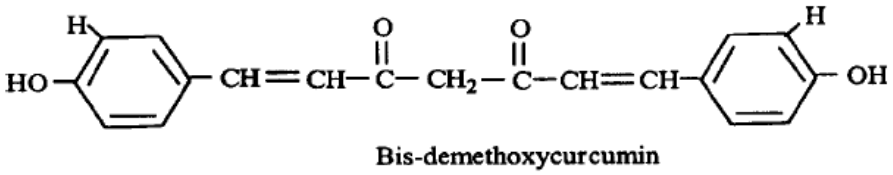
Curcumin- II



Curcumin- III



Demethoxycurcumin



Bis-demethoxycurcumin

Figure (2.5): Chemical formulas of curcuminoids (AL-Hendawi *et al*., 2018).

2.5.3 Pharmacological Properties of Turmeric Plant

Clinical studies have shown that curcumin is healthy for humans, even at large doses, but its medicinal application is extremely poor because of its limited bioavailability. Consequently, preclinical trials have stated that curcumin concentrations in plasma and target tissues are low because of their high metabolism rates. Furthermore, curcumin has been used as an anti-inflammatory agent in traditional Indian and Chinese medicines for centuries. Several studies in recent years have shown that curcumin has anticarcinogenic, antioxidant, immunomodulatory, and antiangiogenic effects (Abd El-Hack *et al.*, 2021).

It has been used to treat common inflammatory diseases, tumors, biliary diseases, anorexia, cough, topical wounds, diabetic injuries, liver disorders, rheumatism, and sinusitis. Extensive studies on the biological properties and pharmacological consequences of turmeric extracts have been conducted in recent years. Curcumin, the primary yellow biocomponent of turmeric, has anti-inflammatory, antioxidant, anticarcinogenic antitidiabetic, antibacterial, antiprotozoal, antiviral, antifibrotic, immunomodulatory, and ifungal perties Defense assessment tests showed that curcumin is tolerated well at high doses, without adverse effects. Thus, curcumin is a highly active biological material with the potential to treat different diseases in modern medicine (Abd El-Hack *et al.*, 2021).

The studies proved that turmeric has a clear effect as a treatment for rheumatism. Other studies proved that curcumin is one of the most powerful antimicrobial substances. It has also been shown to have a more powerful antioxidant effect than vitamin E. Using turmeric powder with food daily helps

protect liver and bile cells from the side effects of birth control pills. It also has a role in accelerating the healing process of wounds because of its anti-inflammatory properties. It is also used efficiently in treating cases of anaemia, where it can stimulate the production of red blood cells. It is also a tonic for immunity and mental strength and has anti-rheumatic properties, as well as being useful in cases of fever and high body temperature as it is characterized by temperature regulation in the body(Abd El-Hack *et al.,* 2021).

German authorities have proven that turmeric treats stomach glut, due to its activation of the gallbladder to secrete bile. The benefit of the turmeric plant lies in the main compound known as curcumin, which effectively contributes to reducing the formation of clots within the body, by using it in the form of turmeric capsules, or making a powder of turmeric and eating a teaspoon daily. The risk of stroke is reduced by 20% simply by eating vegetables and fruits with the three meals each day; For example, an orange a day, which contains vitamin C, reduces the risk of stroke. A recent scientific study confirmed that the turmeric plant can be used to reduce the formation of clots(Abd El-Hack *et al.*, 2021).

2.6 General Description of The Pumpkin:

Pumpkins belong to the *Cucurbitaceae* family. *Cucurbita maxima* is one of five species of *Cucurbita sp*. cultivated for pulp, flowers and seeds. This species originates in South America, where the wild form (*Andreea cucurbita*) has been known for over 4000 years. Pumpkin (*Cucurbita pepo L*.) is one of the most cultivated cucurbit crops in the world. The amount of oil found in many types of pumpkin seeds varies from 40 to 60 wt.%. The seeds are used for commercial oil extraction for food and health benefits. The unrefined pumpkin oil is of high quality for its taste, aroma, and color, which are the characteristics defining the use of pumpkin oil for salads and cold dishes (Kabutey *et al*., 2021).

Pumpkin fruits are variable in size, colour, shape and weight, having moderately hard flesh with a thick edible flesh below and a central cavity containing the seeds. Like other members of *Cucurbitaceae*, each fruit bear numerous seeds, located at its central hollow cavity, interspersed in between net like mucilaginous network. Pumpkin seed are semi-flat, feature typical ovoid shape with a conical tip while its kernels are olive-green color, sweet, buttery in texture and nutty in flavor which can be enjoyed as snack, added in desserts and in savory dishes. Generally, in order to obtain good-quality seeds, pumpkin fruit is allowed to mature completely (Devi *et al*., 2018).

Pumpkin seed oil is a thick, green oil that is produced from roasted pumpkin seeds. When used for cooking or as a salad dressing, pumpkin seed oil is generally mixed with other oils because of its robust flavor. It is used in cooking in central and eastern Europe, and, long believed to be a folk remedy for prostate problems, has in fact been shown to combat benign prostatic hyperplasia (Mathangi, 2018).

2.6.1 Classification of Pumpkin Plant

Plant is classified according to (Ferriol, 2004) into:

Kingdom: Plantae

Sub-Kingdom: Tracheophytes

Super-Division: Angiosperms

Division: Eudicots

Class: Rosids

Order: Cucurbitales

Family: *Cucurbitaceae*

Genus: Cucurbita

Species: *C. maxima*

2.6.2 The Chemical Composition of The Pumpkin Plant:

Pumpkin seeds are rich in vitamins and minerals like manganese and vitamin K, both of which are important in helping wounds heal. They also contain zinc, a mineral that helps the immune system fight bacteria and viruses. Pumpkin seeds are also an excellent source of: (Phosphorus, Magnesium, Iron, Potassium, Copper, Nutrients per Serving). A quarter-cup serving of dried pumpkin seed kernels contains (Oso and Ashafa, 2021):

- Calories: 180
- Protein: 10 g
- Fat: 16 g
- Carbohydrates: 3 g
- Fiber: 2 g
- Sugar: 0 g

Habib *et al*. (2015) analyzed the composition of pumpkin seeds cultivated in Bangladesh and obtained the following results: moisture 4.06%, ash 3.80%, crude cellulose 2.91% total lipids 36.70%, total protein 34.56%, sugar 1.08%, and %2.15starch. The oil extract content was 12% and the GLC analysis of the oil indicated that it contained 40.58% oleic acid, while the content of stearic acid, linoleic acid and palmitic acid was 27.06, 17.39, and 14.97% respectively.

2.6.3 Pharmacological Properties of Pumpkin Plant

Pumpkin is a rich source of vitamin A, being high in beta-carotene, a precursor to vitamin A. It provides substantial fiber, niacin, and lutein (important antioxidant). Pumpkin seeds have many health benefits, some of which include a good source of protein, zinc, and other vitamins, and are even lower cholesterol (Mohammed *et al.*, 2018). One gram of pumpkin seed protein contains as much tryptophan as a full glass of milk. Modern science confirms that pumpkin seeds have an impressive nutrient profile that benefits many aspects of human health. They’re a rich source of protein, unsaturated fatty

acids, vitamins, and minerals that reduce risk factors for chronic diseases, including cancer. Pumpkin seeds are a potent source of many nutrients, offering high levels of essential vitamins and minerals in a small serving. But they also are high in calories (Dar *et al*., 2017; Batool *et al*., 2022).

- **Anti-Inflammatory Effects**

Pumpkin seeds are rich in many antioxidants, which protect cells from disease-causing damage and reduce inflammation in human bodies. They're also a great source of dietary fiber, which can enhance this effect. Studies show that pumpkin seeds' anti-inflammatory abilities can help maintain good function in the liver, bladder, bowel, and joints (Dong *et al*., 2021).

- **Lower Risk of Diabetes**

Pumpkin seeds are high in magnesium, which most people don't get enough of in their diet. Magnesium content helps regulate blood sugar levels, lowering your diabetes risk. Studies show pumpkin seeds also help people with diabetes maintain blood sugar control to manage the disease (Syed *et al*., 2019).

- **Anti-Cancer Properties**

Laboratory studies showed pumpkin seeds can stop the growth of breast and prostate cancer cells. They also induce apoptosis or cancer cell death. These effects are largely attributed to pumpkin seeds' high antioxidant activity (Dong *et al*., 2021).

- **Healthy Heart Function**

The high magnesium content in pumpkin seeds helps lower and regulate blood pressure. Due to this effect, diets high in magnesium are associated with a lower risk of stroke and death from heart disease. Studies showed that the antioxidants in pumpkin seeds also increase nitric oxide levels in body. This molecule works to keep blood vessels smooth, flexible, and healthy, improving

blood flow and reducing the risk of heart and circulation problems (Dotto and Chacha, 2020).

- **Better Sleep**

Snacking on pumpkin seeds before bed may help to get a better night's rest. Pumpkin seeds are a natural source of tryptophan, an amino acid that promotes sleep. The zinc, copper, and selenium in pumpkin seeds can also affect sleep duration and quality. Finally, studies showed that magnesium can help reduce stress and anxiety, contributors to insomnia (Dotto and Chacha, 2020).

Chapter Three

Experimental Work

3. Materials and Methods

3.1 Materials

The equipment and instruments used through this study are as listed in table (3.1).

Table (3.1): The equipment and instruments used in the present study.

Instrument/Equipment	Processing company (origin)
Beakers different size	Mayamed (China)
CBC(Complete Blood Count)	Mythic 18-Vet (Germany)
Centrifuge	Hettich (Germany)
Disposable Syringes	Superestar (India)
Dissection Set	EIphor (Germany)
EDTA	Paytekht (France)
Filter Paper	Ahlstrom (USA)
Incubator	Memmert (Germany)
Jel tube	Changsha Renji Medical Equipment Co. (China)
Light microscope	Olympus(Japan)
Medicil Goss	Kahira (Egupt)
Micropipette	BOECO (Germany)
Plain tube	Levram Life Sciences Private Limited (India)
Refrigerator	Kiriazi (Egypt)
Rotary Microtome	Anglia (England)
Sensitive electric Balance	Metter (Switzerland)
Slide and cover slides	Superestar (India)
UV-spectrophotometer	Cole-Parmer, an Antylia Scientific (Germany)
Water bath	Labtech (Korea)

3.1.1: The Chemical Materials

The chemical materials used through this study are as shown in table (3.2).

Table (3.2): The utilized chemicals materials.

Material	Processing company (origin)
Canada balsam	UNIVAR(Australia)
Chloroform	BDH (England)
Ethanol	BDH (England)
Formalin(10%)	BDH (England)
Glacial Acid Acetic	BDH (England)
Methanol	BDH (England)
Metronidazole	Julphar (U.A.E)
NaCl	Riedel-De Hean (Germany)
Paraffin wax	Digboi Refineries of IndianOil (India)
Potassium Iodide	Riedel-De Hean (Germany)
TBE Solution	BDH (England)
Xylol	BDH (England)

3.1.2: The Used Stains

The stains used through this study are as shown in table (3.3).

Table (3.3): The stains utilized through this study.

Stains	Processing company (origin)
Eosin stain	Riedel-De Hean (Germany)
Hematoxylin stain	BDA-Chem..Ltd pool (England)
Lugol's-Iodine	Merk (Germany)

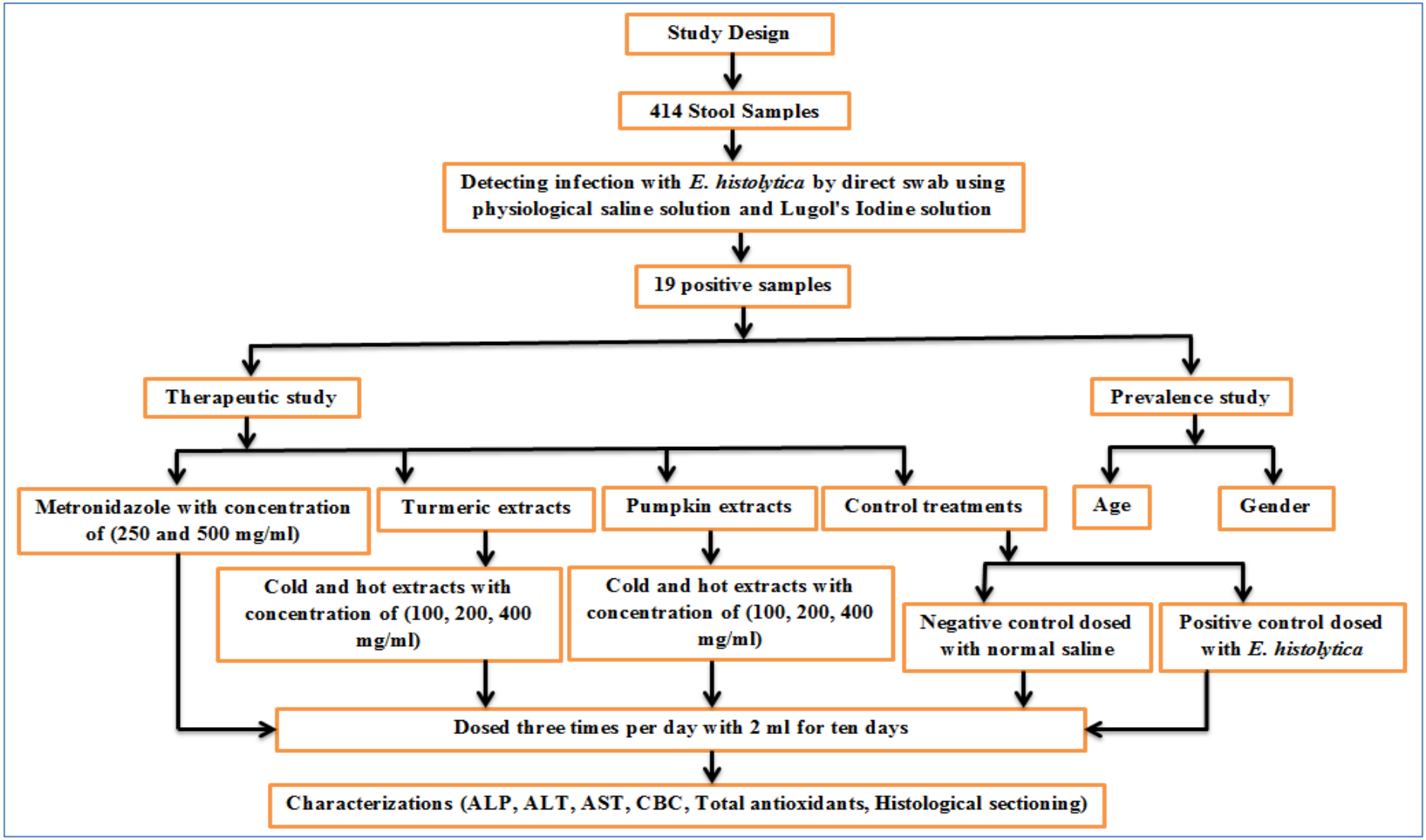
3.1.3: The List of Kits

The kits utilized through this study are as shown in table (3.4).

Table (3.4): The kits utilized through this study.

Name of kits	Processing company (origin)
Complete blood count (CBC)	Germany

3.2: Experimental Design



3.3: Methods of Preparing Solutions and Stains

3.3.1: Physiological Normal Saline Solution

The physiological normal saline solution was prepared by dissolving 0.9 g of NaCl in 100 mL distilled water in a glass flask (Alwan, 2018; Tao *et al*., 2021).

3.3.2: Formalin Solution

A formalin solution was prepared according to Alardhi and Jasem (2016) method, by adding one part (25 ml) of formaldehyde at a concentration of 40% with three parts of (75 ml) of distilled water in a 1 L glass tube with shaking. This solution is used for the purpose of fixing the tissue sections.

3.3.3: Lugol's Iodine Stain

It is consists of:

1- Iodine (5g)
2- Potassium iodide (10 g)
3- Distilled water (100 ml)

Potassium iodide was dissolved in distilled water, the iodine crystals were then slowly added and shake well. The solution was diluted with distilled water five times before use. The solutions were kept in dark flasks so that they would not be exposed to direct sunlight since they would deteriorate within three weeks (Alardhi and Jasem, 2016).

3.3.4: Harris-Hematoxylin Stain

The stain was prepared as follows Bancroft and Layton (2012):

1. A one gram of hematoxylin stain powder was dissolved in 10 ml of absolute ethyl alcohol.

2. Potassium alum [$Al_2(SO_4)_3.K_2SO_4.24H_2O$] was added previously prepared by dissolving 10 g of it in 200 ml of warm distilled water (40 ºC).
3. Mercuric oxide (5 g) was added slowly and exposed to the heat source until the purple colour is forming.
4. The colorant temperature was reduced under tap water and 2-4 ml of glacial acetic acid was added to it.

3.3.5: Alcoholic Eosin Stain (1%)

The stain was prepared by dissolving 1g of Eosin powder in 100 ml of ethyl alcohol at a concentration of 70% (Feldman and Wolfe, 2014).

3.4: The Animals of Experiment

This study was conducted in the animal house belonging to the College of Science, University of Babylon, from November 2021 to March 2022, where 110 white Swiss females Rats were used in this study, which were purchased from the animal house of the College of Science, University of Babylon, their weights ranged between 250-300 g. After making sure of their safety from infection with intestinal parasites by examining the faeces microscopically. They were distributed into groups and placed in special metal cages for raising rats, with a rate of 5 rats per cage, and their floor was spread with sawdust, which was replaced weekly to maintain clean, as well as their food consisted of a diet of grains and soybeans With 1% animal protein, the experimental animals were subjected to appropriate environmental conditions in terms of temperature, ventilation and drinking water, and the animals were left for two weeks to acclimatize with laboratory conditions.

3.5: Feces Collection

Faeces samples were collected from some patients infected with the amoebic dysentery parasite who suffered from bloody mucous diarrhoea, and those who attended the laboratories of Babel teaching hospital for maternity and

children, Al-Hilla General Teaching Hospital, and Al-Noor Hospital for Children, Babylon province. The samples were collected in sterile plastic bottles fitted with an airtight seal to maintain the sample's moisture and prevent drying, and they were transferred directly to the animal house, where they were used to infect white laboratory rats.

3.6: Stool Visual Examination

The consistency of the stool provides the examiner with useful information since the diarrhoea caused by dysentery amoeba is foul-smelling and contains a lot of fecal material. Also, attention should be paid when taking the sample to the presence of blood or mucus, because these two may indicate the presence of amoebic infection (Ghulam and al Kubaissy, 2011).

3.7: Stool Microscopic Examination

The cover of the glass slide is then placed and examined under the microscope to see the mature cyst phases, which are characterized by containing four nuclei, as well as the trophozoite phases of the parasite, where it is characterized by the presence of one nucleus with containing red blood cells, but in this way the nucleus of cyst cannot be distinguished easily, so Lugol's iodine stain is used by placing a drop of iodine solution on another glass slide, then taking a part of the stool with a wooden stick and mixing it with prolonged iodine to appear homogeneous when covered with the cover of the glass slide without causing air bubbles, then examining it with a microscope to see the trophozoite and cystic phases under the magnification power of 40 X, where the nucleus that contains the chromatin granules and the Karyosomne is stained with dark brown and the cytoplasm with yellow or light brown, and the use of iodine solution is limited to diagnosing the cysts only, while the trophozoite phases are destroyed and cannot be distinguished (Al-Torfi, 2014; Calegar *et al*., 2016).

3.8: Preparation of Plant Extracts

3.8.1: Preparation of Cold and Hot Aqueous Extracts for Turmeric and Pumpkin Plants

The Pumpkin fruits and rhizomes of turmeric were purchased from the local markets in Al-Hilla city, the Pumpkin seeds were extracted from fruits and washed, except for the rhizomes of turmeric that were cleaned and milled only. The husks were removed from the Pumpkin seeds and left to dry for a month at room temperature as shown in figure (3.1), which were then milled by an electric miller to obtain a fine powder. The samples were then placed in nylon bags and kept until extracting the active substance from them.

Figure (3.1): Step of extracting Pumpkin seeds.

A 300 g of powder was taken for each sample of the previously prepared plants and placed in glass flasks, the volume was completed to 6 L of hot distilled water (boiled at 100 ºC), with a ratio (1 g: 20 ml), mixed well, and then distributed in six beakers of 1 L per sample. The flasks were then left without

stirring for 3 hours to cool, then left for 30 minutes on a stirrer device for dissolving the powder in water. The solution was then left without moving for 3 hours, then left for 30 minutes with a shaker device. The solution in each beaker was filtered by passing it through three layers of gauze. The sediment was neglected and the filtrate was taken to separate in a centrifuge for 15 minutes (at a power of 3000 rpm). The resulting filtrate from the centrifuge was taken for each plant and placed in a glass beaker. The beakers were placed in an oven at 42 °C for 10 days to dry the hot aqueous extract and obtain the dry extract of the samples (Ali and Mohammed, 2015). As for the cold water extract of these plants, it was prepared in the same steps as the previous method, but using cold distilled water as shown in figure (3.2).

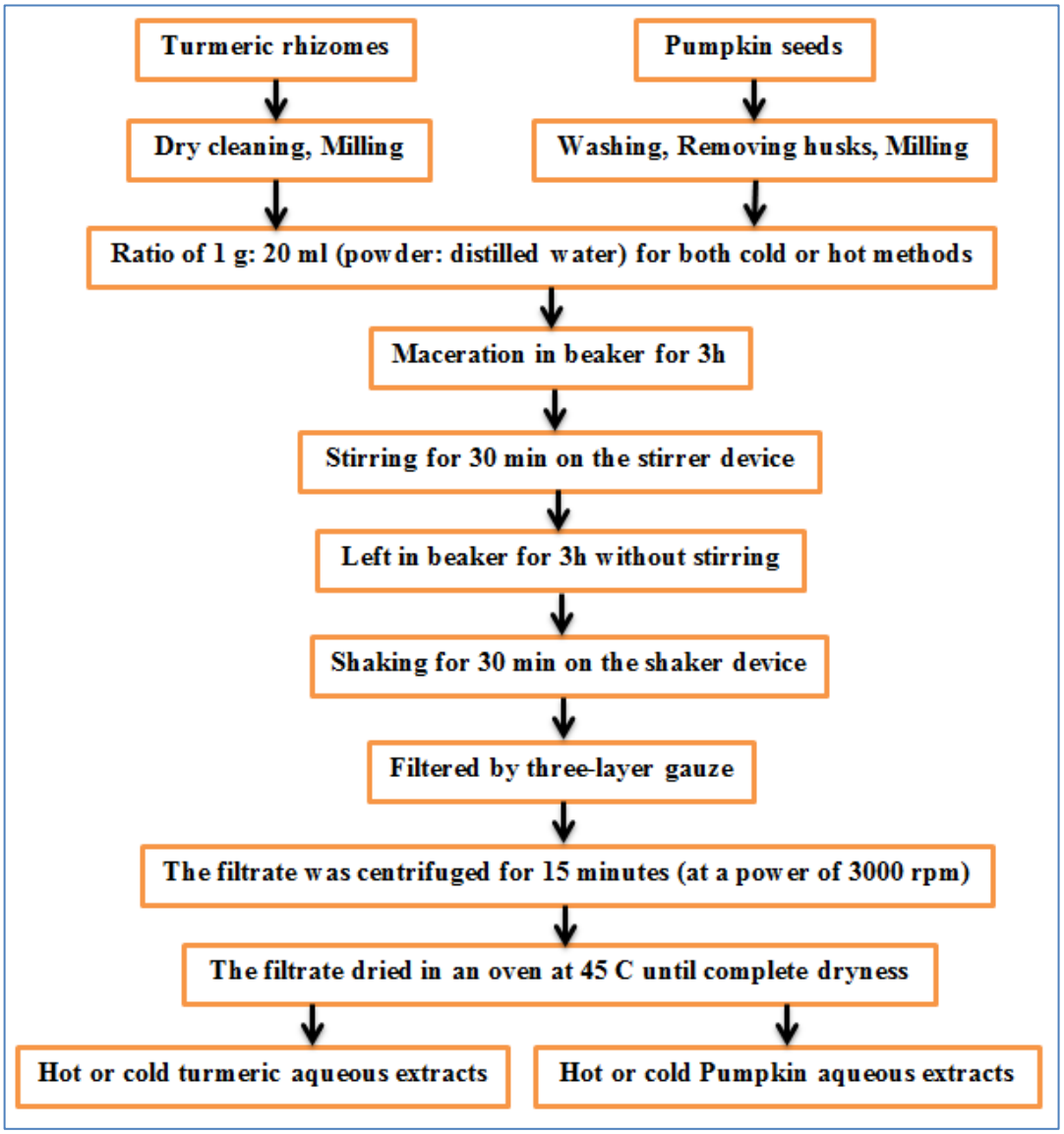


Figure (3.2): Step of preparing aqueous extracts.

3.8.2: Preparation of Concentrations of Plant Extracts

Concentrations of (100, 200, 400) mg/ml for each cold and hot plant extract were prepared by dissolving 10 g of each dry extract in 100 ml of distilled water so that the concentration of the base solution became 100 mg/ml and from which, the required concentrations were prepared, in addition to the control treatment that was prepared using distilled water only, and then kept in the refrigerator at a temperature of 4 ºC until use (Al-Taei *et al*., 2014).

3.9: Determination of The Safety of Turmeric and Pumpkin Extracts

An experiment was conducted to investigate the safety of the used concentrations for turmeric and Pumpkin extracts when dosed to rats with different weights. In this experiment, 30 animals were divided into 3 groups, each group comprising 5 animals. Different concentrations of turmeric and Pumpkin extracts were prepared. Three doses of the extracts were administered per day by oral administration to each group so that it was achieved (100, 200, 400) mg/ml and after dosing the animals were placed under observation for 24 hours during which all information related to toxicity symptoms and deaths were recorded.

3.10: Preparation of the Suspension of Amoebic Dysentery Parasite Used in Dosing Laboratory Rats

The parasite suspension used in the animal dosing was prepared by mixing 200 g of faeces with physiological salt solution (0.9%), it was filtered through four layers of gauze to remove large particles from the sample (Chabuk, 2013). Laboratory rats were orally dosed with *E. histolytica* suspension at an amount of 2 ml per animal using a modified syringe by inserting the syringe into the mouth and injecting the suspension. The negative and positive control group animals were also dosed with (distilled water and *E. histolytica*), respectively using the same method. The rats were then placed in cages that were clean and free of sawdust, the faeces were collected daily and examined to confirm the infection.

The examination continued daily and after confirming the infection, animals were dosed with plant extracts and metronidazole as follows:

- **The first group (negative control group):** five rats were administered with distilled water.

- **The second group (positive control group):** five rats were administered with *E. histolytica* suspension at a dose of 2 ml.

- **The third group (infected group):** five rats were treated with cold turmeric extract at a concentration of 100 mg/ml at a dose of 2 ml three times daily for 10 days.

- **The fourth group (infected group):** five rats were treated with cold turmeric extract at a concentration of 200 mg/ml at a dose of 2 ml three times daily for 10 days.

- **The fifth group (infected group):** five rats were treated with cold turmeric extract at a concentration of 400 mg/ml at a dose of 2 ml three times daily for 10 days.

- **The sixth group (infected group):** five rats were treated with hot turmeric extract at a concentration of 100 mg/ml at a dose of 2 ml three times daily for 10 days.

- **The seventh group (infected group):** five rats were treated with hot turmeric extract at a concentration of 200 mg/ml at a dose of 2 ml three times daily for 10 days.

- **The eighth group (infected group):** five rats were treated with hot turmeric extract at a concentration of 400 mg/ml at a dose of 2 ml three times daily for 10 days.

- **The ninth group (infected group):** five rats were treated with cold Pumpkin extract at a concentration of 100 mg/ml at a dose of 2 ml three times daily for 10 days.

- **The tenth group (infected group):** five rats were treated with cold Pumpkin extract at a concentration of 200 mg/ml at a dose of 2 ml three times daily for 10 days.

- **The eleventh group (infected group):** five rats were treated with cold Pumpkin extract at a concentration of 400 mg/ml at a dose of 2 ml three times daily for 10 days.

- **The twelfth group (infected group):** five rats were treated with hot Pumpkin extract at a concentration of 100 mg/ml at a dose of 2 ml three times daily for 10 days.

- **The thirteenth group (infected group):** five rats were treated with hot Pumpkin extract at a concentration of 200 mg/ml at a dose of 2 ml three times daily for 10 days.

- **The fourteenth group (infected group):** five rats were treated with hot Pumpkin extract at a concentration of 400 mg/ml at a dose of 2 ml three times daily for 10 days.

- **The fifteenth group (infected group):** five rats were treated with metronidazole at a concentration of 10 mg/ml (which was prepared by dissolving 250 mg of metronidazole powder in 25 ml of cold distilled water) at a dose of 2 ml three times daily for 10 days.

- **The Sixteenth group (infected group):** five rats were treated with metronidazole at a concentration of 20 mg/ml (which was prepared by dissolving 500 mg of metronidazole powder in 25 ml of cold distilled water) at a dose of 2 ml three times daily for 10 days.

3.11: Rats Anatomy

The infected rats and the dosed rats were dissected in order to conduct a histological examination on them and the samples necessary for the experiment were taken from them. They were anaesthetized with chloroform, The abdominal cavity was then opened up to the sternum bone using medical scissors as shown in figure (3.3) and blood was withdrawn directly from the heart by a heart puncture method using a sterile medical syringe with a capacity of 5 ml. After that, 2 ml of blood was placed after withdrawing into tubes containing an anticoagulant substance (EDTA) for the purpose of measuring physiological blood parameters, the other section of blood is placed in Jel tubes free of anticoagulant, where it contains a gelatinous substance that helps to increase the serum formed before the centrifugation process by leaving the samples for 30 min at room temperature and then placed in the centrifuge at a speed of 3000 rpm for 10 min in order to separate the serum. Serum was taken to measure the level of liver enzymes (ALT, AST, ALP) as well as Total Antioxidants. The required organs (liver, kidney, large intestine) were kept in formalin (10% N) for 48 hours, which was prepared for the histological sectioning process.

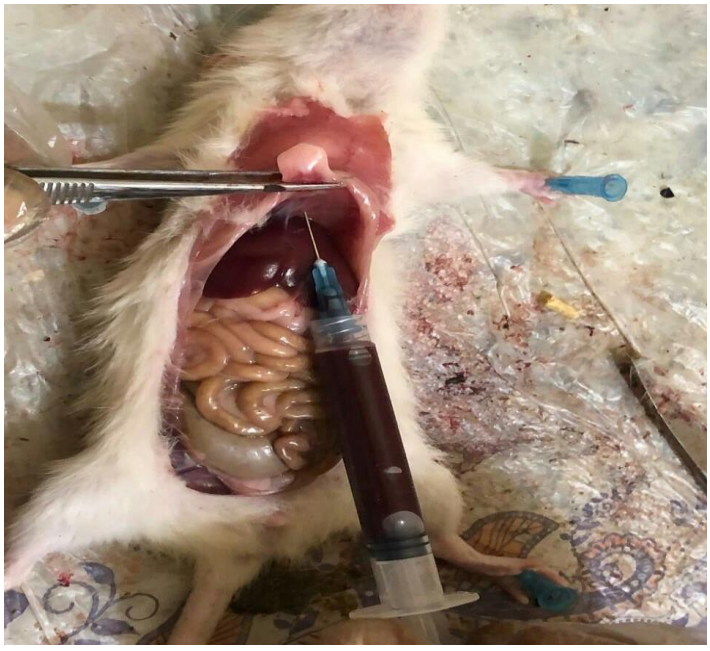


Figure (3.3): Opening cavity and withdrawing blood from the heart.

3.12: Physiological Tests

3.12.1: Testing Blood Parameters

The counts of red blood cells and white blood cells, the amount of Haemoglobin (Hb), the Packed Cell Volume (PCV), the Mean Corpuscular Volume (MCV), the Mean Corpuscular Hemoglobin (MCH), the Platelet Count (PLT), and the Mean Platelet Volume (MPV) were measured using platelets using the Complete Blood Count (CBC) device as shown in figure (3.4), where the process is conducted by placing EDTA tubes containing an anticoagulant and two milliliters of the blood sample in its designated place in the device, following the work steps found in the manual for the device. The device begins to withdraw one milliliter of the sample to calculate and measure blood parameters on its own. The process takes several minutes and then the results are printed automatically.

Figure (3.4): Complete Blood Count (CBC) device.

3.12.2: Preparation of Histological Sectioning

Histological sections were prepared using Chari *at el*. (2018) method in order to conduct a histological study on the effect of some antibiotics and the aqueous extract was used as follows:

1- Fixation

After removing the samples from the animal's body, they are fixed in a 10% formalin solution in order to maintain the cellular structure and the normal state of the tissue.

2- Washing and Dehydration

The samples were washed after fixing them with formalin in Tap water for 1 hr to get rid of formalin, it was then passed in a series of elevated ethyl alcohol with concentrations of 70%, 80%, 90% and 100% for two hours for each concentration to complete the dehydration process and remove water from the tissue.

3- Clearing and Embedding

The tissue sample, after completing the water withdrawal from it, was immersed for (30-60) minutes in xylene in two stages, and then the tissues were embedded in melted paraffin wax at a temperature of 60 ºC in two stages, two hours per each stage.

4- Blocking

The samples are placed in molds of pure melted paraffin wax. The molds are left for half an hour until the wax hardens, and then placed in the refrigerator until sectioning.

5- Sectioning Rotary

The molds were cut with a microtome, with a thickness of (5-6) microns, and each slide was transferred to a water bath at a temperature of 37 ºC to bed the slide. The tissue sections were then glued to a glass slide containing a light smear of a mixture of albumen of egg and glycerin, with a ratio (1:1).

6- Staining

The glass slides are immersed in two phases of xylene for (15-30) minutes, then they are immersed in a series of ethyl alcohol solutions with a concentration starting from 100% and then 95% for two minutes each and for a period of one minute in each of the remaining 90%, 80%, and 70% concentrations, then washed with water for two minutes, stained with hematoxylin for a period of (5-10) minutes, immersed in water, and then rinsed with (99 ml of Acid Alcohol of 70% alcohol + 1ml of HCI) and washed with tap water until It turns blue, then immersed in the eosin stain for 30 seconds, washed with water a little to get rid of the excess stain, and passed a series of alcohols with concentrations starting from 70%, 80%, 90% and 95%, at a rate of one minute for each concentration except for 100% concentration, where they are immersed for two minutes and then immersed in two stages of xylene for up to two minutes each time.

7- Mounting

Mounting was conducted using Canda Palasm and left to dry on a Hate Palate at 38°C.

8- Examination of Slides with Microscopic

Microscopic slides were examined using an Olympus Light Microscope with different magnifications to suit study requirements.

9- Photographing

After examining the samples, microscopic slides were selected that contain good tissue sections showing the clear features of the tissue, and then they were photographed using a microscope (DCE – PW1 digital camera Eyepiece).

3.13: Biochemical Tests

3.13.1: Measuring The Activity of The Enzyme ALP, ALT, AST

The level of these enzymes was measured at Al-Fadhel Institution for laboratory tests, Babylon province, Iraq using An Uv-Spectrophotometer device as shown in figure (3.5), which contains a piece of plastic (12 cm long and 1 cm wide), containing a white strip on which 33 microns of the serum to be examined, then this strip is inserted into the device, and after three minutes the device gives the level of these enzymes in the serum with a unit (IU/L).

Figure (3.5): UV-spectrophotometer device.

3.13.2: Measuring Total antioxidant

The total antioxidant was measured at Al-Fadhel Institution for laboratory tests, Babylon province, Iraq using spectrophotometer at wavelength of 450 nm according to Cuprac method (Apak et al., 2007). The principle of Cuprac method was as following

1. Copper (II) chloride solution at a concentration of 10^{-2} M was prepared from $CuCl_2.2H_2O$ by weighing 0.4262 g of it and dissolving it in H_2O and diluting it to 250 ml with water.

2. Ammonium acetate ($NH_4CH_3CO_2$) with pH = 7.0 was prepared by dissolving 19.27 g of Ammonium acetate in water and completing the volume to 250 ml.
3. Neocuproine (2,9-Dimethyl-1,10-phenanthroline) ($C_{14}H_{12}N_2$) solution at a concentration of 7.5×10^{-3} M was prepared by dissolving 0.039 g of Neocuproine in Ethanol (96%), the volume was completed to 25 ml with ethanol.
4. The standard solutions of sample antioxidants were prepared at 1.0×10^{-3} M Torolox.

3.14: Statistical Analysis

The results were statistically analyzed using the T-test,Chi-square, Anova, and correlation coefficient to find the significance for infection with the parasite under study and its relationship with different concentrations (100, 200, and 400 mg/kg), with plant extracts understudy for different days (Al-Rawi, 2000).

Chapter Four

Results

4. Results

In the current study, 110 rats were used to investigate the effect of plant extracts against the dysentery amoeba parasite between November 2021 to March 2022 in the laboratories of the Department of Biology Sciences, College of Science, University of Babylon.

4.1 Prevalence Study

4.1.1 The Total Percentage of Infection with *E.histolytica* in Al-Hilla City

During this study, 414 stool samples were examined for children and adults from one to more than 18 years old who attended hospitals affiliated to Al-Hilla city of. The total percentage of infection with *E. histolytica* in Al-Hilla city was (4.5%).

4.1.2 The Relationship of Infection by *E. histolytica* with Age

The results of the study showed that there was no difference in the percentages of infection with the parasite according to the age group. The results of the statistical analysis using the chi-square showed that there were no significant differences (p-value=0.53) between the percentages of infection according to the age groups, where the highest percentage of infection was in the age group (12-18) years old which amounted to (6.7%), while the lowest percentage of infection was in the age group (1-5) years old which amounted to 1.2% as shown in the table (4.1) and figure (1) in appendix A.

Table (4.1): The percentage of infection with *E. histolytica* according to age group.

Age (year)	Number of examined samples	Number of infected samples	Percentage of infection (%)	Chi square
1-5	83	1	1.2	P value = 0.53
6-11	112	5	4.4	
12-18	119	8	6.7	

>18	100	5	5	
Total	414	19	4.5	

* non-significant differences at P≤0.05.

4.1.3 The Relationship of Infection by *E. histolytica* with Gender

The current study showed that the percentage of infection in males was 5.4% higher than in females 3.7%. The results of the statistical analysis showed that there was no significant difference in the percentage of infection between males and females, as shown in table (4.2) and figure (2) in appendix A.

Table (4.2): The percentage of infection with *E. histolytica* according to gender.

Age (year)	**Male**			**Female**			Chi square
	Number of examined samples	Number of infected samples	Percentage of infection (%)	Number of examined samples	Number of infected samples	Percentage of infection (%)	
1-5	40	0	0	43	1	2.3	P value = 0.52
6-11	55	4	7.2	57	1	1.7	
12-18	58	5	10.4	61	3	4.9	
>18	48	2	4.1	52	3	5.7	
Total	201	11	5.47	213	8	3.75	

* non-significant differences at P≤0.05.

4.2 The Biological Study of Plant Extracts

The therapeutic effect of cold and hot aqueous extract of turmeric and pumpkin was tested by infecting rats with *E.histolytica*, which were treated with these extracts and at different concentrations.

4.2.1 Effect of Cold and Hot Aqueous Extract of Turmeric and Pumpkin and Metronidazole in Laboratory Rats:

A 110 animals were used in this experiment divided into 16 groups with 5 animals for each group. Twelve groups were used to study the effect of different concentrations (100, 200, and 400) mg/ml for both turmeric (hot and cold) and

pumpkin (hot and cold) and the thirteenth and fourteenth groups were treated with metronidazole at a concentration of (250, 500) mg/ml of body weight and the fifteenth group was the control group that was dosed with distilled water, while the sixteenth group was the positive control group that was dosed with *E. histolytica*, where the total of rats became 80 rats, while the rest of the rats were used to test the safety of turmeric and Pumpkin extracts which were 30 rats.

4.2.2 Determination of The Safety for Turmeric and Pumpkin Extracts

Table (4.3) illustrates that no deaths occurred in the rats dosed with turmeric and pumpkin extract at concentrations (100, 200, and 400) mg/ml, which were placed under observation for 24 hours, so it is a non-toxic substance and without side effects.

Table (4.3): Determination of the safety for turmeric and Pumpkin extracts for 24 hours.

Extracts	Concentrations (mg/ml)	Number of rats per group	Number of deaths
Turmeric	100	5	0
	200	5	0
	400	5	0
Pumpkin	100	5	0
	200	5	0
	400	5	0

4.2.3 Determining The Effect of Metronidazole and Extracts on The Percentage of Healing for The Rats Infected with *E.histolytica*

Table (4.4) shows the number of healed rats after being dosed with Metronidazole at concentrations (250 and 500 mg/ml) of body weight and at a dose of 2 ml within 10 days, where the table shows that the concentration of Metronidazole (500 mg/ml), the percentage of healing for animals on the fifth day was 100%, while the same percentage of healing was on the seventh day for

the concentration of Metronidazole (250 mg/ml). The results of the statistical analysis showed that there were no significant differences at the probability level of p>0.05 using the t-test, which amounted to (0.441).

Table (4.4): Effect of Metronidazole at a concentration of (250 and 500 mg/ml) on the percentage of healing for the rats infected with *E.histolytica*.

Day	Infected rats under the influence of doses					
	250 mg/ml	Number of healed rats	Percentage of healing (%)	500 mg/ml	Number of healed rats	Percentage of healing (%)
1	5	0	0	5	0	0
2	5	0	0	5	0	0
3	3	2	40	2	3	60
4	2	3	60	1	4	80
5	1	4	80	0	5	100
6	1	4	80	0	5	100
7	0	5	100	0	5	100
8	0	5	100	0	5	100
9	0	5	100	0	5	100
10	0	5	100	0	5	100

There are no significant differences at the level of probability (P≤0.05) t-test= 0.441

Table (4.5) shows the number of healed rats after being dosed with cold turmeric extract at a concentration of (100, 200 and 400 mg/ml) and with a dose of 2 ml for 10 days. The table shows with a concentration of 400 mg/ml that the percentage of healing for rats on the fifth day was 100%, while the same percentage for the concentrations (200 and 100 mg/ml) was on the days (seventh and eighth), respectively. The results of the statistical analysis showed that there were no significant differences at the probability level of p>0.05 (F= 0.984).

Table (4.5): Effect of cold turmeric extract at a concentration of (100, 200 and 400 mg/ml) on the percentage of healing for the rats infected with *E. histolytica*.

Day	Infected rats under the influence of doses								
	100 mg/ml	No. of healed rats	Percentage of healing (%)	200 mg/ml	No. of healed rats	Percentage of healing (%)	400 mg/ml	No. of healed rats	Percentage of healing (%)
1	5	0	0	5	0	0	5	0	0
2	5	0	0	5	0	0	3	2	40
3	5	0	0	4	1	20	2	3	60
4	3	2	40	3	2	40	1	4	80
5	2	3	60	3	2	60	0	5	100
6	2	3	60	2	3	80	0	5	100
7	1	4	80	0	5	100	0	5	100
8	0	5	100	0	5	100	0	5	100
9	0	5	100	0	5	100	0	5	100
10	0	5	100	0	5	100	0	5	100

There are no significant differences at the level of probability (P≤0.05) LSD= 0.387

Table (4.6) shows the number of healed rats after being dosed with hot turmeric extract at a concentration of (100, 200 and 400 mg/ml) and with a dose of 2 ml for 10 days. Where the table shows that concentration (400 mg/ml) achieved the best percentage of healing amounted to 100% on the eighth day of dosing, followed by concentration (200 mg/ml) during the eighth day, while concentration (100 mg/ml) gave complete healing on the tenth day of dosing. The results of the statistical analysis showed that there were no significant differences at the probability level of p>0.05 (F= 1.695).

Table (4.6): Effect of hot turmeric extract at a concentration of (100, 200 and 400 mg/ml) on the percentage of healing for the rats infected with *E.histolytica*.

Day	Infected rats under the influence of doses								
	100 mg/ml	No. of healed rats	Percentage of healing (%)	200 mg/ml	No. of healed rats	Percentage of healing (%)	400 mg/ml	No. of healed rats	Percentage of healing (%)
1	5	0	0	5	0	0	5	0	0
2	5	0	0	5	0	0	3	2	40

3	5	0	0	4	1	20	3	2	40
4	5	0	0	4	1	20	2	3	60
5	3	2	40	3	2	40	1	4	80
6	3	2	40	1	4	80	0	5	100
7	2	3	60	1	4	80	0	5	100
8	1	4	80	0	5	100	0	5	100
9	1	4	80	0	5	100	0	5	100
10	0	5	100	0	5	100	0	5	100

There are no significant differences at the level of probability (P≤0.05) LSD= 0.203

Table (4.7) shows the number of healed rats after being dosed with cold pumpkin extract at a concentration of (100, 200 and 400 mg/ml) and with a dose of 2 ml for 10 days. The table shows with a concentration of 400 mg/ml that the percentage of healing for rats on the seventh day was 100%, while the same percentage for the concentrations (200 and 100 mg/ml) was on the days (ninth and tenth), respectively. The results of the statistical analysis showed that there were no significant differences at the probability level of p>0.05 (F= 0.722).

Table (4.7): Effect of cold pumpkin extract at a concentration of (100, 200 and 400 mg/ml) on the percentage of healing for the rats infected with *E.histolytica*.

Day	Infected rats under the influence of doses								
	100 mg/ml	No. of healed rats	Percentage of healing (%)	200 mg/ml	No. of healed rats	Percentage of healing (%)	400 mg/ml	No. of healed rats	Percentage of healing (%)
1	5	0	0	5	0	0	5	0	0
2	5	0	0	5	0	0	5	0	0
3	5	0	0	5	0	0	4	1	20
4	5	0	0	4	1	20	3	2	40
5	3	2	40	2	3	60	1	4	80
6	3	2	40	2	3	60	0	4	80
7	2	3	60	1	4	80	0	5	100
8	1	4	80	0	4	80	0	5	100
9	0	4	80	0	5	100	0	5	100
10	0	5	100	0	5	100	0	5	100

There are no significant differences at the level of probability (P≤0.05) LSD= 0.495

Table (4.8) shows the number of healed rats after being dosed with hot pumpkin extract at a concentration of (100, 200 and 400 mg/ml) and with a dose of 2 ml for 10 days. Where the table shows that concentration (400 mg/ml) achieved the best percentage of healing amounted to 100% on the ninth day of dosing, followed by concentration (200 mg/ml) during the eighth day, while concentration (100 mg/ml) gave complete healing on the tenth day of dosing. The results of the statistical analysis showed that there were no significant differences at the probability level of p>0.05 (F= 0.670).

Table (4.8): Effect of hot pumpkin extract at a concentration of (100, 200 and 400 mg/ml) on the percentage of healing for the rats infected with *E.histolytica*.

Day	Infected rats under the influence of doses								
	100 mg/ml	No. of healed rats	Percentage of healing (%)	200 mg/ml	No. of healed rats	Percentage of healing (%)	400 mg/ml	No. of healed rats	Percentage of healing (%)
1	5	0	0	5	0	0	5	0	0
2	5	0	0	5	0	0	5	0	0
3	5	0	0	5	0	0	3	2	40
4	5	0	0	4	1	20	2	3	60
5	3	2	40	4	1	20	1	3	60
6	3	2	40	2	3	60	1	4	80
7	2	3	60	1	4	80	0	4	60
8	1	4	80	1	4	80	0	5	100
9	1	4	80	0	5	100	0	5	100
10	0	5	100	0	5	100	0	5	100

There are no significant differences at the level of probability (P≤0.05) LSD= 0.510

4.2.4 Effect of Treating with Metronidazole (250, 500 mg/ml) on The Hematological Parameters for Rats Infected with *E. histolytica*

Table (4.9) shows the effect of treating with Metronidazole extract (250, 500 mg/ml) on the (WBC, Lymphocytes, RBC, granulocytes, monocytes, HGB, MCV, and MCH) for rats infected with *E. histolytica*. The results of the table show a significant decrease in the values of (WBC, granulocytes, RBC, HGB, MCV, and MCH) for the positive control treatment, which gave (6.3,

27.46, 5.28, 9.08, 58.52, 16.27), respectively, compared to the negative control treatment, which recorded (6.58, 47.56, 6.26, 10.5, 65.56, 17.94), respectively. While it noticed an increase in the positive control treatment when entering the parasite for Lymphocytes and Monocytes, which gave (82.38, and 17.2), respectively compared to the negative treatment that was recorded (65.16, 7.18), respectively. As for the treatment with Metronidazole , it observed the continuation of the significant decrease in (WBC, Lymphocytes, and Granulocytes) with increasing the concentrations of the treatment, which achieved the lowest significant decrease at the concentration of (500mg/ml) and with a value of (3.22, 60.52, 5.78) respectively. while observed an increase in the values of Monocytes, RBC, HGB, MCV, and MCH parameters with increasing the concentrations of the extract, which achieved the highest significant increase at the concentration of (500 mg/ml) and with a value of (20.28, 5.48, 9.52, 63.52, 17.98) respectively.

Table (4.9): Effect of treating with Metronidazole (250, 500 mg/ml) on some hematological parameters for rats infected with *E.histolytica*.

Treatment Parameters	Mean ± SE				
	Negative control	**Positive control (*E. histolytica*)**	**Metronidazole (250 mg/ml)**	**Metronidazole (500 mg/ml)**	**Sig. between groups**
WBC ($\times 10^3/\mu L$)	6.58±0.16 c	6.3±0.48 C	3.30±0.13 B	3.22±0.19 B	0.000*
Lymphocytes (%)	65.16±1.58 b	82.38±1.43 C	62.92±0.14 D	60.52±0.14 C	0.000*
Monocytes (%)	7.18±0.47 b	17.2±2.14 A	18.2±0.11 A	20.28±0.23 B	0.000*
Granulocytes	47.56±9.26 c	27.46±4.82 B	12.56±0.14 A	5.78±0.1 A	0.000*
RBC ($\times 10^6/\mu L$)	6.26±0.04 c	5.28±0.07 A	5.32±0.05 A	5.48±0.08 A	0.202
HGB (g/dL)	10.5±0.14 b	9.08±0.16 A	9.41±0.13 A	9.52±0.12 A	0.687
MCV (fL)	65.56±2.56 b	58.52±0.91 A	63.02±0.15 Ab	63.52±0.12 Ab	0.100
MCH (pg)	17.94±0.31 b	16.27±0.12 A	16.09±0.14 A	17.98±0.13 B	0.000*

*P ≤ 0.05; Means having the different letters in the same raw differed significantly(*P ≤ 0.05); SE: Standard Error.

4.3 Effect of Treating with Turmeric Extract on The Hematological Parameters for Rats Infected with *E. histolytica*

Table (4.10) shows the effect of treating with cold turmeric extract (100, 200, 400) mg/ml on the (WBC, lymph, RBC, granulocytes, monocytes, HGB, MCV, and MCH) for rats infected with *E. histolytica*. The results of the table show a significant decrease in the values of (WBC, granulocytes, RBC, HGB, MCV, and MCH) for the positive control treatment, which gave (6.3, 27.46, 5.28, 9.08, 58.52, 16.27), respectively, compared to the negative control treatment, which recorded (6.58, 47.56, 6.26, 10.5, 65.56, 17.94), respectively. while it noticed an increase in the positive control treatment when entering the parasite for Lymphocytes and Monocytes, which gave (82.38, and 17.2), respectively compared to the negative treatment that was recorded (65.16, 7.18), respectively. As for the treatment with cold turmeric, it observed the continuation of the significant decrease in (WBC, Lymphocytes, and Granulocytes) with increasing the concentrations of the treatment, which achieved the lowest significant decrease at the concentration of (400 mg/ml) and with a value of (2.13, 54, 5.22) respectively. while observed an increase in the values of Monocytes, RBC, HGB, MCV, and MCH parameters with increasing the concentrations of the extract, which achieved the highest significant increase at the concentration of (400 mg/ml) and with a value of (24.7, 6.01, 9.98, 61.52, 16.98) respectively.

Table (4.10): Effect of treating with cold turmeric extract (100, 200, 400 mg/ml) on some hematological parameters for rats infected with *E. histolytica*.

Treatment / Parameters	Mean ± SE					
	Negative control	Positive control (*E.histolytica*)	Cold turmeric extract (100 mg/ml)	Cold turmeric extract (200 mg/ml)	Cold turmeric extract (400 mg/ml)	Sig.
WBC ($\times 10^3/\mu L$)	6.58±0.16 c	6.3±0.48 C	3.54±0.13 B	3.42±0.73 b	2.13±0.12 A	0.000 *
Lymphocyte	65.16±1.5	82.38±1.43	57.04±0.7	54.82±2.6	54±1.28	0.000

s (%)	8 b	C	7 A	4 a	A	*
Monocytes (%)	7.18±0.47 b	17.2±2.14 A	18.44±2.03 A	20.68±0.24 a	24.7±0.85 A	0.000 *
Granulocytes	47.56±9.26 c	27.46±4.82 b	22.06±0.17 Ab	16.94±0.35 ab	5.22±0.94 A	0.000 *
RBC ($\times 10^6/\mu L$)	6.26±0.04 c	5.28±0.07 a	5.51±0.25 Ab	5.56±0.2 abc	6.01±0.35 Bc	0.038 *
HGB (g/dL)	10.5±0.14 b	9.08±0.16 a	9.15±0.08 A	9.8±0.6 ab	9.98±0.35 Ab	0.035 *
MCV (fL)	65.56±2.56 b	58.52±0.91 a	60.02±3.17 Ab	60.62±0.22 ab	61.52±0.12 Ab	0.174
MCH (pg)	17.94±0.31 b	16.27±0.12 a	16.3±0.13 A	16.8±0.86 ab	16.98±0.15 Ab	0.071

*P ≤ 0.05; Means having the different letters in the same row differed significantly(*P ≤ 0.05); SE: Standard Error.

Table (4.11) shows the effect of treating with hot turmeric extract (100, 200, 400) mg/ml on the (WBC, lymph, RBC, granulocytes, monocytes, HGB, MCV, and MCH) for rats infected with *E. histolytica*. The results of the table show a significant decrease in the values of (WBC, granulocytes, RBC, HGB, MCV, and MCH) for the positive control treatment, which gave (6.3, 27.46, 5.28, 9.08, 58.52, 16.27), respectively, compared to the negative control treatment, which recorded (6.58, 47.56, 6.26, 10.5, 65.56, 17.94), respectively. while noticed an increase in the positive control treatment when entering the parasite for Lymphocytes and Monocytes, which gave (82.38, and 17.2), respectively compared to the negative treatment that was recorded (65.16, 7.18), respectively. As for the treatment with hot turmeric, it observed the continuation of the significant decrease in (WBC, Lymphocytes, and Granulocytes) with increasing the concentrations of the treatment, which achieved the lowest significant decrease at the concentration of (400 mg/ml) and with a value of (2.14, 64.61, 6.77) respectively. while observed an increase in the values of Monocytes, RBC, HGB, MCV, and MCH parameters with increasing the concentrations of the extract, which achieved the highest significant increase at

the concentration of (400 mg/ml) and with a value of (23, 6.73, 11.8, 62.75, 17.7) respectively.

Table (4.11): Effect of treating with hot turmeric extract (100,200,400 mg/ml) on some hematological parameters for rats infected with *E. histolytica*.

Treatment / Parameters	Mean ± SE					
	Negative control	Positive control (*E.histolytica*)	Hot turmeric extract (100 mg/ml)	Hot turmeric extract (200 mg/ml)	Hot turmeric extract (400 mg/ml)	Sig.
WBC ($\times10^3/\mu L$)	6.58±0.16 c	6.3±0.48 c	5.36±0.42 C	2.52±0.19 ab	2.14±0.16 A	0.000 *
Lymphocytes (%)	65.16±1.58 b	82.38±1.43 c	82.08±0.42 D	73.06±2.68 c	64.61±1.05 B	0.000 *
Monocytes (%)	7.18±0.47 b	17.2±2.14 a	17.74±0.13 A	20.8±0.48 bc	23±0.32 B	0.000 *
Granulocytes	47.56±9.26 c	27.46±4.82 b	18.58±0.43 B	12.3±2.38 b	6.77±0.17 B	0.000 *
RBC ($\times10^6/\mu L$)	6.26±0.04 c	5.28±0.07 a	5.55±0.14 A	5.61±0.12 c	6.73±0.27 b	0.000 *
HGB (g/dL)	10.5±0.14 b	9.08±0.16 a	10.02±0.54 B	10.46±0.14 b	11.08±0.21 a	0.000 *
MCV (fL)	65.56±2.56 b	58.52±0.91 a	58.4±0.13 A	62.24±0.42 abc	62.75±1.14 bc	0.012 *
MCH (pg)	17.94±0.31 b	16.27±0.12 a	16.52±0.14 A	16.9±0.55 b	17.7±0.14 b	0.000 *

*P ≤ 0.05; Means having the different letters in the same row differed significantly(*P ≤ 0.05); SE: Standard Error.

4.4 Effect of Treating with Pumpkin Extract on The Hematological Parameters for Rats Infected with *E. histolytica*

Table (4.12) shows the effect of treating with cold pumpkin extract (100, 200, 400mg/ml) on the (WBC, lymph, RBC, granulocytes, monocytes, HGB, MCV, and MCH) for rats infected with *E. histolytica*. The results of the table show a significant decrease in the values of (WBC, granulocytes, RBC, HGB, MCV, and MCH) for the positive control treatment, which gave (6.3, 27.46, 5.28, 9.08, 58.52, 16.27), respectively, compared to the negative control treatment, which recorded (6.58, 47.56, 6.26, 10.5, 65.56, 17.94), respectively.

While it noticed an increase in the positive control treatment when entering the parasite for Lymphocytes and Monocytes, which gave (82.38, and 17.2), respectively compared to the negative treatment that was recorded (65.16, 7.18), respectively. As for the treatment with cold pumpkin, it observed the continuation of the significant decrease in (WBC, Lymphocytes, and Granulocytes) with increasing the concentrations of the treatment, which achieved the lowest significant decrease at the concentration of (400 mg/ml) and with a value of (3.9, 67.38, 9.46) respectively. while observed an increase in the values of Monocytes, RBC, HGB, MCV, and MCH parameters with increasing the concentrations of the extract, which achieved the highest significant increase at the concentration of (400 mg/ml) and with a value of (18.08, 8.06, 10.9, 64.82, 17.48) respectively.

Table (4.12): Effect of treating with cold pumpkin extract (100, 200, 400 mg/ml) on some hematological parameters for rats infected with *E. histolytica*.

Treatment / Parameters	Mean ± SE					
	Negative control	**Positive control (*E.histolytica*)**	**Cold pumpkin extract (100 mg/ml)**	**Cold pumpkin extract (200 mg/ml)**	**Cold pumpkin extract (400 mg/ml)**	**Sig. between groups**
WBC ($\times 10^3/\mu L$)	6.58±0.16 c	6.3±0.48 c	4.78±0.13 A	4.08±0.42 a	3.9±0.74 a	0.381
Lymphocytes (%)	65.16±1.58 b	82.38±1.43 c	81.42±0.36 C	80.78±2.32 c	67.38±0.96 b	0.000 *
Monocytes (%)	7.18±0.47 b	17.2±2.14 a	17.54±0.12 A	17.60±0.11 a	18.08±0.97 b	0.000 *
Granulocytes	47.56±9.26 c	27.46±4.82 b	17.78±2.17 A	10.68±0.15 a	9.46±2.44 a	0.000 *
RBC ($\times 10^6/\mu L$)	6.26±0.04 c	5.28±0.07 a	5.38±0.39 A	6.02±0.15 a	8.06±8.82 a	0.432
HGB (g/dL)	10.5±0.14 b	9.08±0.16 a	9.66±0.63 A	9.94±0.17 b	10.9±0.18 b	0.000 *
MCV (fL)	65.56±2.56 b	58.52±0.91 a	59.9±0.16 A	60.96±0.88 ab	64.82±1.55 bc	0.026 *
MCH (pg)	17.94±0.31 b	16.27±0.12 a	16.40±0.15 A	17.12±0.44 a	17.48±29.63 a	0.463

*P ≤ 0.05; Means having the different letters in the same row differed significantly(*P ≤ 0.05); SE: Standard Error.

Table (4.13) shows the effect of treating with hot pumpkin extract (100, 200, 400mg/ml) on the (WBC, lymph, RBC, granulocytes, monocytes, HGB, MCV, and MCH) for rats infected with *E. histolytica*. The results of the table show a significant decrease in the values of (WBC, granulocytes, RBC, HGB, MCV, and MCH) for the positive control treatment, which gave (6.3, 27.46, 5.28, 9.08, 58.52, 16.27), respectively, compared to the negative control treatment, which recorded (6.58, 47.56, 6.26, 10.5, 65.56, 17.94), respectively. While it noticed an increase in the positive control treatment when entering the parasite for Lymphocytes and Monocytes, which gave (82.38, and 17.2), respectively compared to the negative treatment that was recorded (65.16, 7.18), respectively. As for the treatment with hot pumpkin, it observed the continuation of the significant decrease in (WBC, Lymphocytes, and Granulocytes) with increasing the concentrations of the treatment, which achieved the lowest significant decrease at the concentration of (400 mg/ml) and with a value of (3.26, 64.42, 14.04) respectively. while observed an increase in the values of Monocytes, RBC, HGB, MCV, and MCH parameters with increasing the concentrations of the extract, which achieved the highest significant increase at the concentration of (400 mg/ml) and with a value of (18.1, 5.94, 9.5, 60.88, 18.54) respectively.

Table (4.13): Effect of treating with hot pumpkin extract (100,200,400 mg/ml) on some hematological parameters for rats infected with *E. histolytica*.

Treatment / Parameters	Mean ± SE					
	Negative control	**Positive control (*E.histolytica*)**	**Hot pumpkin extract (100 mg/ml)**	**Hot pumpkin extract (200 mg/ml)**	**Hot pumpkin extract (400 mg/ml)**	**Sig. between groups**
WBC ($\times 10^3/\mu L$)	6.58±0.16 c	6.3±0.48 c	4.38±0.12 A	3.98±0.26 a	3.26±0.41 a	0.311
Lymphocytes (%)	65.16±1.58 b	82.38±1.43 c	71.58±1.41 C	68.82±0.23 c	64.42±0.15 b	0.000*
Monocytes (%)	7.18±0.47 b	17.2±2.14 a	17.4±0.14 Ab	17.52±0.13 a	18.1±0.17 b	0.000*

Granulocytes	47.56±9.26 c	27.46±4.82 b	22.44±0.16 A	20.86±0.17 a	14.04±1.36 a	0.000*
RBC ($\times 10^6$/µL)	6.26±0.04 c	5.28±0.07 a	5.82 ±0.05 A	5.83±0.64 a	5.94±0.25 a	0.130
HGB (g/dL)	10.5±0.14 b	9.08±0.16 a	9.18±0.43 Ab	9.26±0.59 bc	9.5±0.14 a	0.004*
MCV (fL)	65.56±2.56 b	58.52±0.91 a	59.54±0.16 A	59.56±1.85 a	60.88±0.22 a	0.040*
MCH (pg)	17.94±0.31 b	16.27±0.12 a	16.32±0.2 A	16.40±0.19 a	18.54±0.72 a	0.003*

*P ≤ 0.05; Means having the different letters in the same row differed significantly(*P ≤ 0.05); SE: Standard Error.

Table (4.14) and figure (3) in appendix (A) show a comparison between the values of haematological parameters and the therapeutic treatments of rats infected with *E. histolytica*. The results showed that the treatment with cold turmeric extract at a concentration of (400) was significantly superior to the rest of the treatments by giving it the lowest values for WBC, Lymphocytes and Granulocytes and the best values Close to normal values for Monocytes, RBC, HGB, MCV.

Table (4.14): The relationship between hematological parameters and the therapeutic treatments of rats infected with *E. histolytica*.

Parameters / Treatment	WBC	Lymphocytes	Monocytes	Granulocytes	RBC	HGB	MCV	MCH
Negative control	6.58	65.16	7.18	47.56	6.26	10.5	65.56	17.94
Positive control	6.3	82.38	17.2	27.46	5.28	9.08	58.52	16.27
Cold turmeric extract (100 mg/ml)	3.54	57.04	18.44	22.06	5.51	9.15	60.02	16.3
Cold turmeric extract (200 mg/ml)	3.42	34.82	20.68	16.94	5.56	9.8	60.62	16.8
Cold turmeric extract (400 mg/ml)	2.13	54	24.7	5.22	6.01	9.98	61.52	16.98
Hot turmeric extract (100 mg/ml)	5.36	82.08	17.74	18.58	5.55	10.02	58.4	16.52
Hot turmeric extract (200 mg/ml)	2.52	73.06	20.8	12.3	5.61	10.46	62.24	16.9
Hot turmeric extract (400 mg/ml)	2.14	64.61	23	6.77	6.73	11.08	62.75	17.7
Cold pumpkin extract (100 mg/ml)	4.78	81.42	17.54	17.78	5.38	9.66	59.9	16.4
Cold pumpkin extract (200 mg/ml)	4.08	80.78	17.6	10.68	6.02	9.94	60.96	17.12
Cold pumpkin extract (400 mg/ml)	3.9	67.38	18.08	9.46	8.06	10.9	64.84	17.48
Hot pumpkin extract (100 mg/ml)	4.38	71.58	17.4	22.44	5.82	9.18	59.54	16.32
Hot pumpkin extract (200 mg/ml)	3.98	68.82	17.52	20.86	5.83	9.26	59.56	16.4
Hot pumpkin extract (400 mg/ml)	3.26	64.42	18.1	14.04	5.94	9.5	60.88	18.54
Metronidazole (250 mg/ml)	3.3	62.92	18.2	12.56	5.32	9.41	63.02	16.09
Metronidazole (500 mg/ml)	3.2	60.52	20.28	5.78	5.48	9.52	63.52	17.98

4.5 Biochemical Parameters:

4.5.1 Effect of Treating with Metronidazole on AST, ALP, ALT, and Total Antioxidant Enzymes in The Blood Serum of Rats Infected with *E. histolytica*.

Table (4.15) illustrates that there is a significant increase in the concentrations of AST, ALP, and ALT, when the parasite enters the body of the rat, as shown in the values of the positive control treatment, which amounted to (3.86, 37.52, 90.41), respectively compared to the negative control treatment, which amounted to (1.5, 13.92, 26.02), respectively. Metronidazole had a significant effect in reducing the activity of these enzymes close to normal, so the activity of AST and ALT ALP enzymes, when treated with concentration (500 mg/ml) of Metronidazole, equalled (1.63, 13.01, 24.98), respectively, which is close to the values in the negative control treatment (uninfected). As for treating with Metronidazole at a concentration of (250 mg/ml), it decreased significantly, which amounted to (2.55, 27.04, 30.29), respectively compared to the negative control treatment but it did not reach the normal state, and this indicates that the increase in the concentration of the treatment increases the decrease in these concentrations to reach the normal state.

As for total antioxidant enzymes, the results showed a significant decrease in their values when the parasite entered the body of the rat (positive control), which amounted to (817.8) compared to the negative control treatment, which amounted to (981.55), while it observed a gradual increase in the values of total antioxidant enzymes when treating with the drug, which reached the highest values when treating with metronidazole at a concentration of (500 mg/ml) amounted to (943.47), which is close to the normal values of the negative control.

Table (4.15): Effect of treating with metronidazole on AST, ALP, ALT, and total antioxidant enzymes in the blood serum of rats infected with *E. histolytica*.

Treatment / Parameters	Mean ± SE				
	Negative control	Positive control (*E. histolytica*)	Metronidazole (250 mg/ml)	Metronidazole (500 mg/ml)	Sig. between groups
ALP	1.5±0.11 a	3.86±0.18 c	2.55±0.1 c	1.63±0.12 a	0.000*
ALT	13.92±4.03 a	37.52±0.11 c	27.04±0.15 b	13.01±0.05 c	0.000*
AST	26.02±12.39 a	90.41±0.29 d	30.29±0.1 c	24.98±0.05 a	0.000*
Total antioxidant	981.55±12.38 b	817.8±3.12 a	939.66±9.91 b	943.47±5.81 b	0.000*

*P ≤ 0.05; Means having the different letters in the same row differed significantly(*P ≤ 0.05); SE: Standard Error

4.5.2 Effect of Treating with Turmeric Extract on AST, ALP, ALT, and Total Antioxidant Enzymes in The Blood Serum of Rats Infected with *E. histolytica*.

Table (4.16) illustrates that there is a significant increase in the concentrations of AST, ALP, and ALT, when the parasite enters the body of the rat, as shown in the values of the positive control treatment, which amounted to (3.86, 37.52, 90.41), respectively compared to the negative control treatment, which amounted to (1.5, 13.92, 26.02), respectively. Turmeric extract had a significant effect in reducing the activity of these enzymes close to normal, so the activity of AST and ALT ALP enzymes, when treated with a concentration (400 mg/ml) of cold turmeric extract, equalled (1.57, 14.5, 25.15), respectively, which is close to the values in the negative control treatment (uninfected). As for treating with cold turmeric extract at a concentration of (100, 200) mg/ml, it decreased significantly, which amounted to (2.69, 28.97, 80.08, and 1.97, 18.16, 58.51), respectively compared to the negative control treatment but it did not reach the normal state, and this indicates that the increase in the concentration of the treatment increases the decrease in these concentrations to reach the normal state. As for total antioxidant enzymes, the results showed a significant decrease

in their values when the parasite entered the body of the rat (positive control), which amounted to (817.8) compared to the negative control treatment, which amounted to (981.55), while it observed a gradual increase in the values of total antioxidant enzymes when treating with the extract, which reached the highest values when treating with cold turmeric extract at a concentration of (400 mg/ml) amounted to (978.85), which is close to the normal values of the negative control.

Table (4.16): Effect of treating with cold turmeric extract at a concentration of (100, 200 and 400 mg/ml) on AST, ALP, ALT, and total antioxidant enzymes in the blood serum of rats infected with *E. histolytica*.

Treatment Parameters	Mean ± SE					
	Negative control	Positive control (*E.histolytica*)	Cold turmeric extract (100 mg/ml)	Cold turmeric extract (200 mg/ml)	Cold turmeric extract (400 mg/ml)	Sig. between groups
ALP	1.5±0.11 a	3.86±0.18 c	2.69±0.25 B	1.97±0.2 ab	1.57±0.53 a	0.000*
ALT	13.92±4.03 a	37.52±0.11 c	28.97±4.78 Bc	18.16±2.23 bc	14.5±2.05 b	0.001*
AST	26.02±12.39 a	90.41±0.29 d	80.08±5.32 Cd	58.51±12.73 ab	25.15±8.12 ab	0.000*
Total antioxidant	981.55±12.38 b	817.8±3.12 a	856.49±26.85 A	931.3±5.81 b	978.85±34.46 b	0.000*

*P ≤ 0.05; Means having the different letters in the same row differed significantly(*P ≤ 0.05); SE: Standard Error

Table (4.17) illustrates that there is a significant increase in the concentrations of AST, ALP, and ALT, when the parasite enters the body of the rat, as shown in the values of the positive control treatment, which amounted to (3.86, 37.52, 90.41), respectively compared to the negative control treatment, which amounted to (1.5, 13.92, 26.02), respectively. Turmeric extract had a significant effect in reducing the activity of these enzymes close to normal, so the activity of AST and ALT ALP enzymes, when treated with a concentration (400 mg/ml) of hot turmeric extract, equalled (1.65,15.6, 29.62), respectively, which is close to the values in the negative control treatment (uninfected). As for treating with cold turmeric extract at a concentration of (100, 200) mg/ml, it decreased significantly, which amounted to (2.49, 30.50, 65.16, and 2.03, 22.37,

30.59), respectively compared to the negative control treatment but it did not reach the normal state, and this indicates that the increase in the concentration of the treatment increases the decrease in these concentrations to reach the normal state. As for total antioxidant enzymes, the results showed a significant decrease in their values when the parasite entered the body of the rat (positive control), which amounted to (817.8) compared to the negative control treatment, which amounted to (981.55), while it observed a gradual increase in the values of total antioxidant enzymes when treating with the extract, which reached the highest values when treating with hot turmeric extract at a concentration of (400 mg/ml) amounted to (1059.05), which is close to the normal values of the negative control.

Table (4.17): Effect of treating with hot turmeric extract at a concentration of (100, 200 and 400 mg/ml) on AST, ALP, ALT, and total antioxidant enzymes in the blood serum of rats infected with *E. histolytica*.

Treatment Parameters	Mean ± SE					
	Negative control	Positive control (*E.histolytica*)	Hot turmeric extract (100 mg/ml)	Hot turmeric extract (200 mg/ml)	Hot turmeric extract (400 mg/ml)	Sig. between groups
ALP	1.5±0.11 a	3.86±0.18 c	2.49±0.07 cd	2.03±0.34 bc	1.65±0.05 b	0.000*
ALT	13.92±4.03 a	37.52±0.11 c	30.50±0.26 c	22.37±0.62 b	15.6±1.97 b	0.000*
AST	26.02±12.39 a	90.41±0.29 d	65.16±0.008 b	30.59±5.94 b	29.62±1.71 ab	0.025*
Total antioxidant	981.55±12.38 b	817.8±3.12 a	906.49±46.91 a	1001.89±10.14 b	1059.05±5.05 b	0.000*

*P ≤ 0.05; Means having the different letters in the same row differed significantly(*P ≤ 0.05); SE: Standard Error.

4.5.3 Effect of Treating with Pumpkin Extract on AST, ALP, ALT, and Total Antioxidant Enzymes in The Blood Serum of Rats Infected with *E. histolytica*.

Table (4.18) illustrates that there is a significant increase in the concentrations of AST, ALP, and ALT, when the parasite enters the body of the rat, as shown in the values of the positive control treatment, which amounted to (3.86, 37.52, 90.41), respectively compared to the negative control treatment,

which amounted to (1.5, 13.92, 26.02), respectively. Pumpkin extract had a significant effect in reducing the activity of these enzymes close to normal, so the activity of AST and ALT ALP enzymes, when treated with a concentration (400 mg/ml) of cold pumpkin extract, equalled (1,53, 15.18, 25.78), respectively, which is close to the values in the negative control treatment (uninfected). As for treating with cold pumpkin extract at a concentration of (100, 200) mg/ml, it decreased significantly, which amounted to (3.01, 31.85, 45.23, and 1.94, 23.54, 33.97), respectively compared to the negative control treatment but it did not reach the normal state, and this indicates that the increase in the concentration of the treatment increases the decrease in these concentrations to reach the normal state. As for total antioxidant enzymes, the results showed a significant decrease in their values when the parasite entered the body of the rat (positive control), which amounted to (817.8) compared to the negative control treatment, which amounted to (981.55), while it observed a gradual increase in the values of total antioxidant enzymes when treating with the extract, which reached the highest values when treating with cold pumpkin extract at a concentration of (400 mg/ml) amounted to (1020.31), which is close to the normal values of the negative control.

Table (4.18): Effect of treating with cold pumpkin extract at a concentration of (100, 200 and 400 mg/ml) on AST, ALP, ALT, and total antioxidant enzymes in the blood serum of rats infected with *E. histolytica*.

Treatment Parameters	Mean ± SE					
	Negative control	Positive control (*E.histolytica*)	Cold pumpkin extract (100 mg/ml)	Cold pumpkin extract (200 mg/ml)	Cold pumpkin extract (400 mg/ml)	Sig. between groups
ALP	1.5±0.11 a	3.86±0.18 c	3.01±0.08 c	1.94±0.31 b	1.53±0.15 a	0.000*
ALT	13.92±4.03 a	37.52±0.11 c	31.85±0.42 c	23.54±0.4 b	15.18±2.61 a	0.000*
AST	26.02±12.39 a	90.41±0.29 d	45.23±0.3 bc	33.97±0.14 abc	25.78±0.26 A	0.011*
Total antioxidant	981.55±12.38 b	817.8±3.12 a	924.9±3.89 b	950.57±10.83 bc	1020.31±12.4 d	0.000*

*P ≤ 0.05; Means having the different letters in the same row differed significantly(*P ≤ 0.05); SE: Standard Error

Table (4.19) illustrates that there is a significant increase in the concentrations of AST, ALP, and ALT, when the parasite enters the body of the rat, as shown in the values of the positive control treatment, which amounted to (3.86, 37.52, 90.41), respectively compared to the negative control treatment, which amounted to (1.5, 13.92, 26.02), respectively. Pumpkin extract had a significant effect in reducing the activity of these enzymes close to normal, so the activity of AST and ALT ALP enzymes, when treated with a concentration (400 mg/ml) of hot pumpkin extract, equalled (1.57, 16.18, 30.2), respectively, which is close to the values in the negative control treatment (uninfected). As for treating with hot pumpkin extract at a concentration of (100, 200) mg/ml, it decreased significantly, which amounted to (2.32, 36.26, 65.8, and 2.19, 29.23, 42.24), respectively compared to the negative control treatment but it did not reach the normal state, and this indicates that the increase in the concentration of the treatment increases the decrease in these concentrations to reach the normal state.

As for total antioxidant enzymes, the results showed a significant decrease in their values when the parasite entered the body of the rat (positive control), which amounted to (817.8) compared to the negative control treatment, which amounted to (981.55), while it observed a gradual increase in the values of total antioxidant enzymes when treating with the extract, which reached the highest values when treating with hot pumpkin extract at a concentration of (400 mg/ml) amounted to (1011.72), which is close to the normal values of the negative control.

Table (4.19): Effect of treating with hot pumpkin extract at a concentration of (100, 200 and 400 mg/ml) on AST, ALP, ALT, and total antioxidant enzymes in the blood serum of rats infected with *E. histolytica*.

Treatment Parameters	Mean ± SE					
	Negative control	Positive control (*E.histolytica*)	Hot pumpkin extract (100 mg/ml)	Hot pumpkin extract (200 mg/ml)	Hot pumpkin extract (400 mg/ml)	Sig. between groups
ALP	1.5±0.11 a	3.86±0.18 c	2.32±0.18 b	2.19±0.16 b	1.57±0.1 a	0.000*

ALT	13.92±4.03 a	37.52±0.11 c	36.26±0.12 c	29.23±4.3 b	16.18±14.61 c	0.000*
AST	26.02±12.39 a	90.41±0.29 d	65.8±4.48 b	42.24±0.13 a	30.2±2.67 a	0.022*
Total antioxidant	981.55±12.38 b	817.8±3.12 a	837.07±3.51 a	957.05±30.34 b	1011.72±7.61 b	0.000*

*P ≤ 0.05; Means having the different letters in the same row differed significantly(*P ≤ 0.05); SE: Standard Error

Table (4.20) shows the presence of a highly significant positive correlation between ALP and ALT at the negative control treatment (untreated) which amounted to (+0.945) at the probability level (0.05), in addition to highly significant positive correlations for rats infected with *E. histolytica* and treated with hot turmeric extract at a concentration of (400 mg/ml) with a value of (+0.881) as shown in apendix A (figure 21), and the treatment with hot pumpkin extract at a concentration of (100 mg/ml) with a value of (+0.942) as shown in apendix A (figure 25), and the treatment with metronidazole at a concentration of (250 mg/ml) with a value of (+0.948) as shown in apendix A (figure 29).

The results showed highly significant negative correlations between ALP and ALT for rats infected with *E. histolytica* and treated with cold turmeric extract at concentrations (200 and 400 mg/ml) which their values were (-0.963 and -0.908), respectively and at the probability level (0.01 and 0.05), respectively, as shown in apendix A (figures 9 and 15).

The results of the same table show that there are highly significant negative correlations between ALP and AST at the probability level (0.05) for rats infected with *E. histolytica* and treated with cold turmeric extract at concentrations (400 mg/ml) with a value of (-0.931) as shown in apendix A (figure 16) and the negative control treatment with a value of (-0.934) as shown in apendix A (figure 4). In addition to a highly significant negative correlation at the probability level (0.01) for rats infected with *E. histolytica* and treated with cold turmeric extract at concentrations (200 mg/ml) with a value of (-0.968) as shown in apendix A (figure 10).

The results showed that there were highly significant positive correlations between ALP and Anti-oxidant at the probability level (0.05) for rats infected with *E. histolytica* and treated with cold turmeric extract at a concentration (400 mg/ml) with a value of (+0.916) as shown in apendix A (figure 17) and cold pumpkin extract at concentrations of (100 and 400 mg/ml) with values of (+0.881 and +0.898), respectively as shown in apendix A (figures 23 and 24), and hot pumpkin extract at a concentration of (400 mg/ml) with a value of (+0.889) as shown in apendix A (figure 27). In addition to a highly significant positive correlation at the probability level (0.01) for treatment with cold turmeric extract at a concentration of (200 mg/ml) with a value of (+0.981) as shown in apendix A (figure 11).

The results of the table also showed significant positive correlations between ALTand AST at the probability level (0.01) for rats infected with *E. histolytica* and treated with cold turmeric extract at concentrations of (200 and 400 mg/ml) with values of (+0.999 and +0.997) respectively as shown in apendix A (figures 12 and 18) and treatment of hot pumpkin extract at a concentration of (400 mg/ml) with a value of (+0.987) as shown in apendix A (figure 28). It also shows the presence of highly significant negative correlations at the probability level (0.01 and 0.05) for the positive and negative control treatments, respectively, with values of (-1.00 and -0.951) as shown in apendix A (figures 5 and 7). In addition to a positive correlation at the probability level (0.05) for treatment with cold turmeric extract at a concentration of (100 mg/ml) with a value of (+0.894) as shown in apendix A (figure 8).

The table also shows the presence of highly significant positive correlations between ALT and Anti-oxidant at the probability level (0.01) for rats infected with *E. histolytica* and treated with hot turmeric and hot pumpkin extracts at concentrations of (400 and 200), respectively, with values of (+0.998 and +0.989) as shown in apendix A (figures 22 and 26) and Highly negative correlations at the probability level (0.05) for cold and hot turmeric treatments at

concentrations of (200 and 400) and the negative control treatment with values of (-0.892, -0.935 and -0.879), respectively as shown in apendix A (figures 13, 19, and 6).

The results showed highly significant negative correlations between AST and Anti-oxidant at the probability level (0.05) for rats infected with *E. histolytica* and treated with cold turmeric extract at concentrations of (200 and 400) with values of(-0.902 and -0.958) as shown in apendix A (figures 14 and 20).

Table (4.20): Correlation between plant extracts and chemical paramiters of rats infected with *E.histolytica*.

Correlations / Treatment	ALP ×ALT	ALP×AST	ALP×Anti-oxidant	ALT×AST	ALT×Anti-oxidant	AST× Anti-oxidant
Negative control	+0.945*	-0.934*	-0.788	-0.951*	-0.879*	+0.688
Positive control (*E.histolytica*)	-0.605	+0.613	+0.856	-1.00**	-0.324	+0.335
Cold turmeric extract (100 mg/ml)	+0.806	+0.637	+0.541	+0.894*	+0.809	+0.538
Cold turmeric extract (200 mg/ml)	-0.963**	-0.968**	+0.981**	+0.999**	-0.892*	-0.902*
Cold turmeric extract (400 mg/ml)	-0.908*	-0.931*	+0.916*	+0.997**	-0.935*	-0.958*
Hot turmeric extract (100 mg/ml)	+0.851	-0.215	-0.482	+0.079	-0.485	+0.492
Hot turmeric extract (200 mg/ml)	-0.452	+0.278	-0.446	+0.604	+0.661	+0.350
Hot turmeric extract (400 mg/ml)	+0.881*	-0.877	+0.866	-0.584	+0.998**	-0.575
Cold pumpkin extract (100 mg/ml)	+0.508	+0.550	+0.881*	+0.711	+0.472	+0.762
Cold pumpkin extract (200 mg/ml)	-0.727	+0.695	+0.112	-0.073	-0.196	-0.067
Cold pumpkin extract (400 mg/ml)	+0.313	+0.097	+0.898*	-0.435	+0.498	+0.047
Hot pumpkin extract (100 mg/ml)	+0.942*	+0.599	+0.779	+0.819	+0.711	+0.429
Hot pumpkin extract (200 mg/ml)	+0.450	+0.155	+0.434	+0.750	+0.989**	+0.836
Hot pumpkin extract (400 mg/ml)	-0.866	-0.831	+0.889*	+0.987**	-0.543	-0.489
Metronidazole (250 mg/ml)	+0.948*	-0. 235	+0.098	-0.106	+0.101	+0.714
Metronidazole (500 mg/ml)	+0.235	+0.544	+0.837	-0.186	+0.703	+0.296

** Correlation is significant at the 0.01 level , *Correlation is significant at the 0.05 level

4.6 Histopathological study

4.6.1 Effect of Metronidazole and plant extracts on the histological sections

4.6.1.1 Kidney

Microscopic examination of the histological sections for the kidneys of rats infected with the parasite and untreated showed a change in the histological structure of the kidney, where was observed the presence of vascular congestion as shown in figure (4.1). As for rats infected with the parasite and treated with hot turmeric extract (400 mg/ml) and hot pumpkin extract (400 mg/ml), they also showed vascular congestion in their histological sections of kidneys as shown in figures (4.2, 4.3).

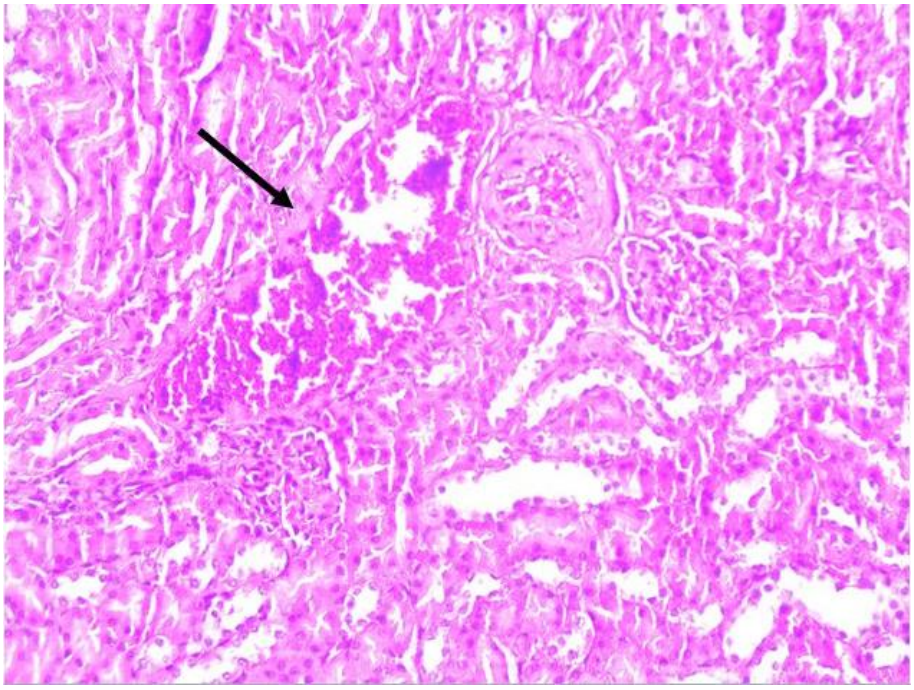


Figure (4.1): A cross-section of the kidney tissue for one of the rats infected with parasite and untreated, in which vascular congestion has appeared (black arrow),H&E,X20.

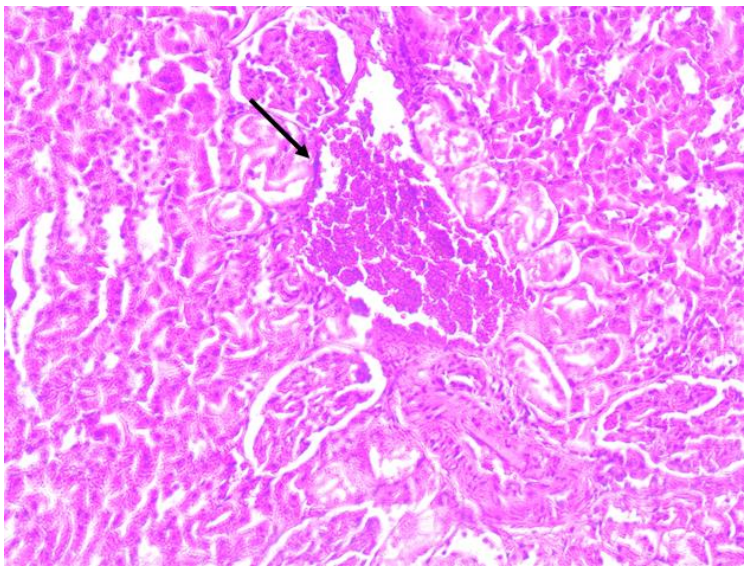


Figure (4.2): A cross-section of the kidney tissue for one of the rats infected with parasite and treated with hot turmeric extract (400 mg/ml), in which a vascular congestion was observed(black arrow),H&E,X20.

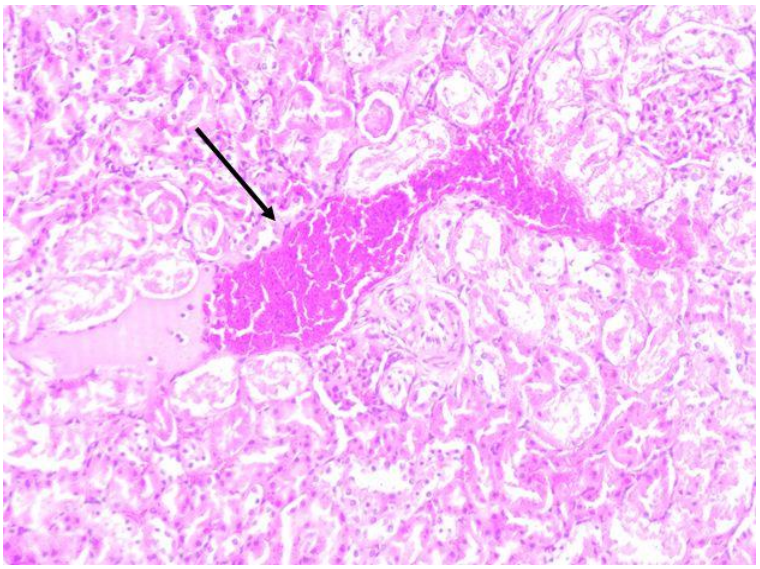


Figure (4.3): A cross-section of the kidney tissue for one of the rats infected with parasite and treated with hot pumpkin extract (400 mg/ml), in which a vascular congestion was observed (black arrow),H&E,X20.

4.6.1.2 Liver

Microscopic examination of the histological sections of the liver of rats infected with the parasite and untreated showed a change in the histological structure of the liver, where was observed the presence of vascular congestion as shown in Figure (4.4). As for rats infected with the parasite and treated with hot turmeric

extract (400 mg/ml), hot and cold pumpkin extract (400 mg/ml), it was also observed vascular congestion in the liver of rats treated with hot pumpkin extract (400 mg/ml) as shown in figure (4.5), while mild congestion and mild lymphocytic infiltrate (figure 4.6) and vascular congestion and lymphocytic infiltration (figure 4.7) in the liver of rats treated with hot turmeric extract (400 mg/ml) and cold pumpkin extract (400 mg/ml), respectively.

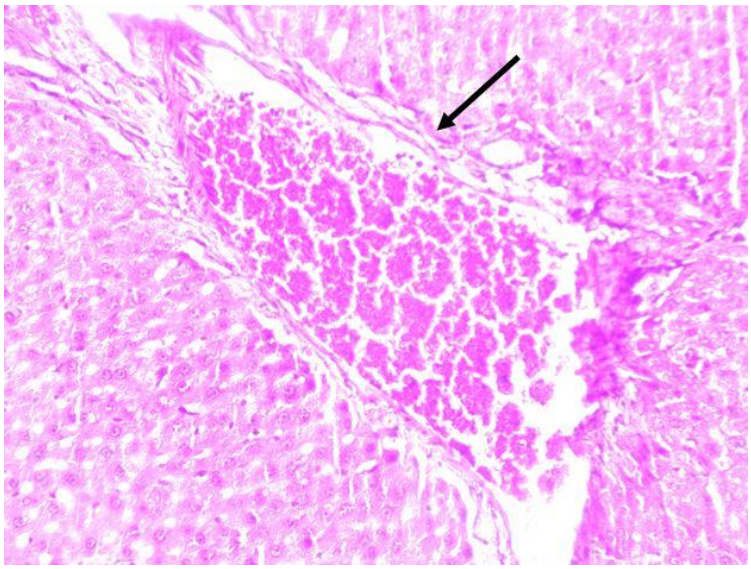


Figure (4.4): A cross-section of the liver tissue for one of the rats infected with the parasite and untreated, in which vascular congestion has appeared(black arrow),H&E,X20.

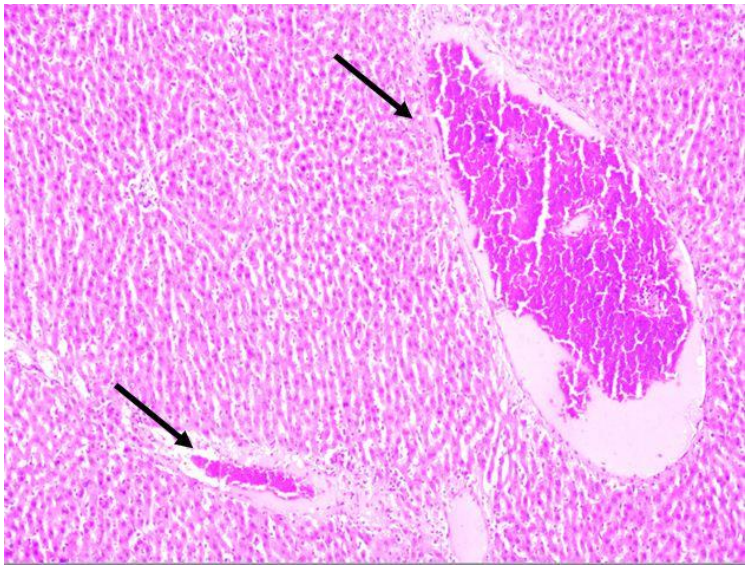


Figure (4.5): A cross-section of the liver tissue for one of the rats infected with parasite and treated with hot pumpkin extract (400 mg/ml), in which a vascular congestion was observed (black arrow),H&E,X10.

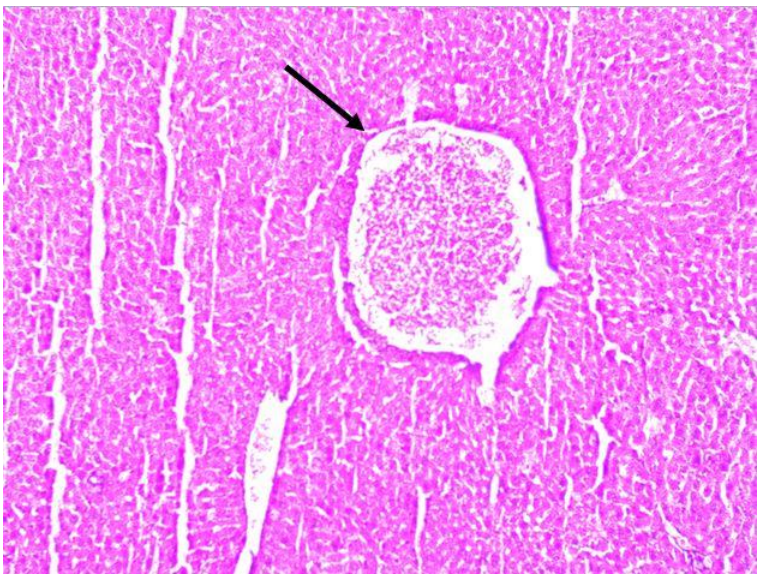


Figure (4.6): A cross-section of the liver tissue for one of the rats infected with parasite and treated with hot turmeric extract (400 mg/ml), in which a mild congestion and mild lymphocytic infiltrate was observed (black arrow),H&E,X10.

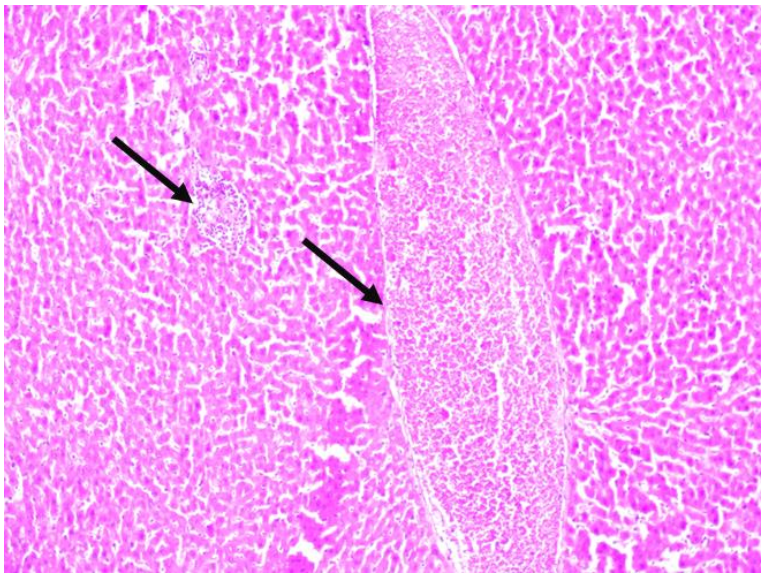


Figure (4.7): A cross-section of the liver tissue for one of the rats infected with parasite and teated with cold pumpkin extract (400 mg/ml), in which vascular congestion and lymphocytic infiltration was observed (black arrow),H&E,X10.

4.6.1.3 Small Intestine (Bowel)

Microscopic examination of the histological sections of the bowel in all treatments showed that there were no observed histological changes in it as shown in appendix (A) (figure 30) except for the rats infected with the parasite

and untreated (positive control) which showed submucosal lymphoid follicles as shown in figure (4.8).

As for the treatments of Metronidazole (250 and 500 mg/ml) and cold turmeric extract (400 mg/ml), they did not show any significant changes in histological sections of all members (kidney, liver and bowel) as shown in the appendix (A) (figure 30).

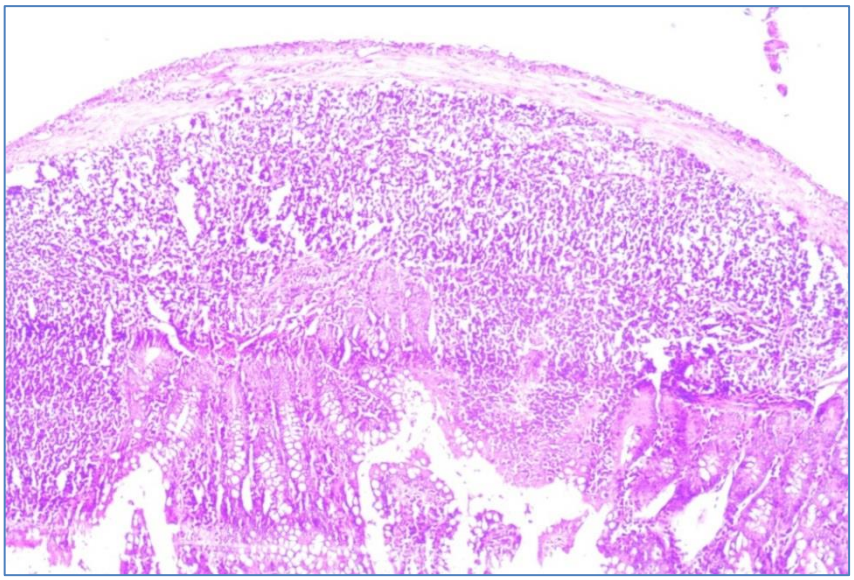


Figure (4.8): A cross-section of the Bowel tissue for one of the rats infected with the parasite and untreated, in which submucosal lymphoid follicles, H&E,X10.

Chapter Five

Discussion

5. Discussion

Human infection with parasitic diseases is a global problem, but it is more frequent in developing and poor countries, where the polluted environment, unhealthy lifestyle, and unavailable sewage systems, in addition to the use of water and food from polluted sources.

Infection with *E. histolytica* is considered one of the global problems (Elizondo-Luévano *et al*., 2018) because it causes death. Some medical drugs are used to treat the disease, but they cause many symptoms and side effects on the patient such as diarrhea, metallic flavor, loss of appetite, and nausea due to doses and long-term treatment (Martínez-Castillo *et al*., 2018).

Therefore, the current global trends are heading towards medicinal herbal treatment to get rid of the effects caused by chemotherapy (Al-Saadi, 2006). Also, the use of medicinal herbs is increasing in many countries of the world as an alternative to chemotherapy, where plant extracts are characterized as a mixture of many biologically active compounds that all contribute to giving the appropriate effect in treatment (Rice, 2014). Medicinal herbs are characterized as naturally occurring substances, so they are safe to use and free from danger affecting the body (Ashar and Dobs, 2004). Herbs have been used since ancient times for medicinal purposes, where human was guided to them by virtue of their experiences with them and by virtue of the intellectual faculty that God (Glory be to Him) distinguished by it over all other creatures (Al-Saadi, 2006).

5.1 Prevalence Study

5.1.1 The Total Percentage of Infection with *E. histolytica* in Al-Hilla City

The results of the current study showed that the percentage of infection with *E. histolytica* in Al-Hilla city was (4.5%). The survey studies related to the prevalence of a parasite varied, where it was observed that the percentages of

infection with this parasite in some studies were similar and different from the current study.

Al-Durie and Al-Nasiri (2020) showed that the percentage of infection in the city of Al-Rifai, Dhi Qar province amounted to 20.46%. Oliewi and Al-Hamairy (2016) indicated that the percentage of infection with *E. histolytica* in Babel Maternity and Children Hospital and Marjan Specialized Hospital for Internal Diseases and Heart in Babel province amounted to 26.4%. Al-Aboody and Al-Rekabi, (2015) indicated that the percentage of infection among patients at Bint Al-Huda Maternity Hospital and Al-Hussein Teaching Hospital in Nasiriyah city was 97%.

Alwan (2018) recorded that the percentage of infections for the patient of Al-Sadr General Hospital in Maysan province amounted to (76.82%). Zaki (2022) indicated in the study on the patients of Ibn al-Athir Teaching Hospital for Children in Mosul for the years 2019 and 2020, which amounted to (13.2% and 15.7%), respectively. The study of Al-Salehy and Mohammad, (2020) showed that the percentage of infection with *E. histolytica* for patients who come to Al-Mousawi hospital and Bint Al-Huda teaching hospital for children and women amounted to (27%). Al-Husseini *et al*. (2016) showed that the percentage of infection with *E. histolytica* in stool samples of children in Baquba city amounted to (50.4%).

Mahmood and Bakr (2020) indicated in their study that the percentage of infection in Erbil city was (6%). Sabry *et al*. (2021) explained when they examined stool samples in Salah al-Din General Hospital, the percentage of infection amounted to (6.88%). According to Nassar *et al*. (2019), when examining patient samples in Basrah Hospital, the percentage of infection amounted to 32%. The percentage of infection in Samarra General Hospital was (12.8%) as shown in a study by Bazzaz *et al*. (2017) on the stool of hospital attending. Al-Qaisi and Al-Sultan (2008) explained in their study in the districts

of Al-Khalis and Baladruz that the percentage of infection amounted to (70.5 %).

Kadir *et al*. (2018) showed when they examined stool samples in Tikrit province that the percentage of infection was (9.3%). Ibrahim (2012) recorded that the percentage of infection for children visiting Al Kadhimiya Technical Hospital in Baghdad was (9.80%). The study by Kadir *et al*. (2013) on the visitors of Kirkuk General Hospital and primary health care centers in Kirkuk city showed that the percentage of infection was (6.38%).

Obaid (2014) showed through it study on the patients of Hawija General Hospital in Kirkuk province that the percentage of infection was (40.5%). Shariff *et al*. (2011) showed the total percentage of infection with *E. histolytica* for patients visiting Chibayish dispensary in Nasiriyah city amounted to (49.5%). Al-Azawi, (2009) indicated that the percentage of total infection among children who attended Abu Ghraib General Hospital was (32.5%).

The reason for the difference in the percentage of infections recorded in the current study compared to the studies mentioned previously is due to several reasons, including the similarity of the environmental and climatic conditions of the country in general, in addition to the different regions and the different time period covered by the study and the difference in the size of the sample and the difference in ages for which the study was concerned. All these factors can explain the reasons for the discrepancy in the findings of previous studies.

5.1.2 The Relationship of Infection with *E. histolytica* with Age

Table (4.1) and figure (1) in appendix (A) shows that the percentage of infection was more common in the age group (12-18 years) with a percentage of 6.7%. The age group (6-11 years) recorded a percentage of infection amounted to 4.4% with no significant difference. The study recorded a slight, non-significant decrease in the percentage of infection at the age group (18 years old)

with a percentage of 5%, while the lowest percentage of infection was in the age group (1-5 years) at a percentage of 1.2%.

The high percentage of infection with parasites in young age groups may be due to the lack of commitment of these ages to general hygiene, such as eating fruits and vegetables without washing and not washing and sterilizing hands before food and after defecation. Also, children who are highly mobile and active are poor in personal hygiene. In addition, children, especially in rural areas, often play with animals such as cats, dogs and livestock, which are important sources of human infection (Buret *et al*., 1990) in addition to not completing treatment courses and weakening the immune system, and this was confirmed by (Park, 1981).

As for the high percentage of infection in the age group (more than 18 years), it can be attributed to their exposure to chronic diseases more than others, which can weaken the immune system with age, in addition to the inability of some of them to observe the rules of personal hygiene.

As for the low percentage of infection with *E. histolytica* in children under the age of 5 years, this can be explained by the fact that this group includes infants and children older than 1.5 years (non-infant). Infants are greatly under the care of mothers, in addition to the simplicity of foods, which are well selected by the parents. On the other hand, breastfeeding has a great role in protecting the child from infection

As for children older than 1.5 years (non-infant), Children at the present time, with the advent of technology, have become preferring home technological games such as phones and tablets, in addition to the entry of Coronavirus epidemic and spreading it, forced mothers to prevent children from going out and playing in the street, in addition to the mothers keenness to sterilize and forcing and monitoring children at these ages to adhere to hygiene.

5.1.3 The Relationship of Infection with *E. histolytica* with Gender

The results of the statistical analysis showed that there were no significant differences in the percentages of infection between males and females in the current study as shown in table (4.2) and figure (2) in appendix A, where it was noted that the percentage of infection was higher in males compared to females.

This can be explained on the basis of the behavior of males in dealing with environmental pollutants surrounding them, and some jobs and self-employment expose many of them to multiple pathogens, including infection with the parasite *E. histolytica*. The reason may be due to immune or genetic factors linked to gender, and this is consistent with (Salman, 2002).

The results of the study agreed with (Al-Hussuny *et al*., 2016), which indicated that the percentage of infection in males was 25.7%, compared to 24.2% in females, and with Obaid (2014) in Kirkuk. The results of the study also agreed with some global survey studies of *E. histolytica*. Klein (2004) showed that there are several factors that contribute to the difference in percentage of infection between the genders, including the rate of exposure to pollutants, social behavior and the environment. Ibrahim (2012) also indicated that the percentage of infection with *E. histolytica* in Al-Kadhimiya is higher in males compared to females.

The reason is due to the behavior of males in dealing with the environment around them due to working conditions abroad. While the results of the study did not agree with what was mentioned by Al-Azawi (2009) and the study by Al-Saqur *et al*. (2017), these studies indicated that there are significant differences in infection between the genders.

5.2 Determination of The Safety for Turmeric and Pumpkin Extracts

The acute toxicity test was used to determine the safety of turmeric and pumpkin extracts, where different doses ranged from 100-400 mg/ml of animal

body weight, with studying the percentage of mortality, which is an indicator of toxicity, in addition to studying behavioral and physiological changes. It appeared through the results shown in Table (4.3) that the turmeric and pumpkin extracts have no toxic effects or side effects. They are safe substances that did not cause any fatalities for animals (rats) that were fed with the mentioned substances and with using a high dose orally.

These results are in agreement with (Govind, 2011) who tested the Median lethal dose of turmeric (Curcuma longa) on female albino rats using three concentrations of turmeric extract (250, 500, and 1000 mg/kg body weight) where no mortality was found in any group of mice up to 48 h. It also agrees with the results of (Cruz *et al*., 2006) who tested the toxicity of pumpkin extract at a concentration (5000 mg/kg) on mice, where it was found that the aqueous-alcoholic extract of *C. maxima* seeds, at a dose of (5000 mg/kg), represents a great safety margin, where it is free of acute toxicity. Serum levels of alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and hematological parameters showed no changes.

5.3 Determining The Effect of Metronidazole and Extracts on The Percentage of Healing for The Rats Infected with *E. histolytica*

In table (4.4), it was found through the study that Metronidazole had a clear effect in eliminating the dysentery amoeba parasite within five days at a concentration (500 mg/ ml). The reason is that Metronidazole affects fermentation-stimulating enzymes such as pyruvate decarboxylase and lactate dehydrogenase, which leads to the elimination of the process of building raw materials inside the parasite's body (Samuelson, 1999; Al-Kubaisi, 2007).

Studies have also shown that Metronidazole affects the nucleoside phosphorylase enzyme, which in turn binds to a second enzyme called thioredoxin reductase, which leads to the modification of the protein synthesis process and its transformation into an inhibitory enzyme (Leistsch *et al*., 2007).

Metronidazole also works on its binding to (DNA), which leads to the inhibition of the chain of nucleic acid formation (Vidakovic *et al*., 2003). Through the results of the table, it was observed that the percentage of complete recovery of animals with a concentration of 250 was relatively late, and the reason may be attributed to the small amount of treatment carried out to the parasite's body, which is confirmed by an increase in the cure rate by increasing the concentration of the drug.

It was found through the study that the treatment of infected rats with a concentration of (400 mg/ml) of cold turmeric extract proved its efficiency and therapeutic ability to eliminate the parasite inside the living body completely and before the end of the treatment period. This may be attributed to the components of the extract from effective compounds such as tannins and phenolic compounds (Vo *et al*., 2021), which are characterized by their ability to synthesize proteins found in the cell membrane or inside the living cell when osmosis through the membrane and the formation of hydrogen bonds between free and multiple phenolic hydroxyl groups and nitrogenous compounds or proteins, thus inhibiting some of the necessary enzymes in the organism (Reed, 1995; Covington 1997).

Guseva (1953) also referred to the ability of tannins to bind with proteins inside the organism, which prevents their decomposition, thus impeding the metabolic processes related to nitrogen and amino acids, which are essential in the continuation of the vitality of the organism. Most studies have confirmed the effect of cold turmeric extract on many intestinal parasites inside the body as well as outside the body (Al-Jubouri, 2003; 2005). Through the results, it was observed that the percentage of animals healed at a concentration of (100 mg/ml) was relatively low, and the reason may be due to the low amount of the active substance that was carried into the parasite's body, which is confirmed by an increase in the percentage of healing by increasing the concentration of the extract.

5.4 Effect of Plant Extracts (Turmeric and Pumpkin) and Metronidazole on Hematological Parameters:

Table (4.9-4.13) shows a significant decrease in the number of white blood cells after the parasite entered for the positive control compared to the negative control. The reason for the decrease that occurred after the entry of the parasite may be attributed to the fact that the white blood cells are stimulated quickly when the antigen (parasite) enters, which leads to the migration of large numbers of them to the site of the antigen's presence due to the secretions that attract them, which led to a decrease in the number of the parasite present in the first hours. For this reason, we observe a decrease in the number of white blood cells that can kill and destroy foreign and pathogenic bodies, where the particles resulting from the destruction can stimulate T-cells, thus stimulating the acquired immune system (Shakir and Mahmood, 2017), and we note that it continues to decrease significantly after treatment with plant extracts.

As for the effect of these extracts on the total number of granular white blood cells, the results of the table (4.9-4.13) showed a highly significant decrease in white blood granular cells (acid, basal, and neutral) of animals infected with the parasite (positive control). The reason for the decrease that occurs in the white granular cells in the blood after entering the parasite is the migration of these cells to the sites of the presence of the parasite in the body tissues, which leads to their decrease in order to eliminate it by the process of phagocytosis (Shakir and Mahmood, 2017).

The reason for the decrease that occurs in the white granular cells in the blood after entering the parasite is the migration of these cells to the sites of the presence of the parasite in the tissues of the body, which leads to their decrease in order to eliminate it by the phagocytosis process. Therefore, this process is considered an important part of the myeloid phagocytic system, and the most important of these cells that contribute to the phagocytosis process is the

neutrophil, and the feature of inflammation is the resorption of cells (Bellanti, 2013). As for eosinophils, they have a major role in the immune response against parasitic infections by migrating to the sites of infection due to eosinophil attracting factors, which leads to a severe reaction that removes the granulation of mast cells (Shakir and Mahmood, 2017).

These cells also attack the parasite by binding them to the immunoglobulins (IgG, IgE), which causes the process of losing their granules and releasing their contents (Bellanti, 2013). The eosinophil cells destroy the parasites through their yeasts, which affect the outer wall of the parasite, which leads to its destruction, which helps the eosinophil cells in their work are mast cells, where the immunoglobulin (IgE) binds with the receptors located on the surface of the mast cells upon exposure to the parasitic antigen and works to attract the eosinophil cells to the site of infection. As for basophil cells, they work to induce inflammation at the site of antigen localization, and their work is similar to that of mast cells (Brock *et al*., 2003).

The current study agrees with Al-Abadi (2005) and differs with Al-Mozan (2011) that the migration of neutrophils and monocytes from the bloodstream to the tissue is controlled by the expression of adhesion molecules on the vascular endothelial cells. This mechanism is regulated by the mediators of acute inflammation, including tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-1. After the secretion of these two kinetics, the expression of adhesion molecules with receptors on the surfaces of neutrophils and monocytes increases, and the infiltration of neutrophils and monocytes from the bloodstream into the tissue increases. In addition, these two kinetics (IL-1, TNF) stimulate the bone marrow to produce more neutrophils and monocytes (Richard, 2009; Kaushansky, 2016).

As for the effect of the extracts on the number of lymphocytes, the results of the study show that the number of lymphocytes increased significantly for the

parasite group, compared with the negative control as shown in Table (4.9-4.13). After treatment with extracts, it observed a decrease in cells. The reason for the low numbers of lymphocytes around the blood vessels is that these cells do not have the ability to phagocytosis, but they have a specific ability to move amoeboid and in most circumstances exit and reach outside the blood vessel, but near it. Therefore, the role of lymphocytes in inflammation is to contribute to the immune response, where they lead a cycle in the secretion of toxic substances and the formation of antibodies that have a direct and effective effect against the parasite (Bellanti, 2013). This result is consistent with (Tachibana *et al*., 2004) and differs with (Al-Abadi, 2005).

As for monocytes, tables (4.9-4.13) indicate a significant increase in their numbers in animals infected with the parasite and their continued increase after treatment with the extracts. This change in the number of white blood cells may be attributed to the effective therapeutic effect of the plant extracts and the drug because they contain active substances such as alkaloids, flavonoids, steroids, electrolytes, terpenes, tannins, phenolic compounds, coumarins, soaps, quinones, amino acids, and proteins (Vo *et al*., 2021; Dar *et al*., 2015).

Flavonoids are considered substances that have many biological properties, they are antioxidants that reduce the chance of cancer, microbes, viruses, and infections, and they have the ability to prevent blood clotting (Russo *et al*., 2000; Havsteen, 2002). Chinese medicine used raw extracts due to the anti-inflammatory ability of flavonoids.

As for the effect of the extracts on RBC, it observed a significant decrease in the parasite group compared to the negative control. This result is in agreement with (Al-Abadi, 2005; Al-Mozan, 2011). This is due to the fact that the parasite depends on its nutrition on devouring red blood cells, which causes a decrease in their numbers among those infected with intestinal amebiasis (Packers, 2002). In addition, diarrhea caused by infection with the parasite

causes fluid loss, in addition to the exit of blood with the excrement caused by infection with the parasite, which leads to anemia. After treatment with the extracts, it was observed an increase in the number of red blood cells. The reason is that these extracts had an effect on the mitotic index of bone marrow cells, which had a stimulating effect on bone marrow cells, where these extracts affected the stromal cells in the bone marrow by stimulating them to release a number of cellular mediators necessary for the division and differentiation of stem cells into different types of blood cells.

As for hemoglobin, the results of the study showed a significant decrease in the percentage of hemoglobin for the positive control group compared to the negative control. These results agreed with (Al-Douri, 2009; Al-Jubouri, 2009) and differed with (Al-Mamouri, 1997; Shaneen, 2005; Al-Keez, 2011). The cause of anemia in the rats in this study may be due to malnutrition, where rats tend to eat less food. This result may be due to the fact that this parasite causes digestive upset, it also releases the trophozoite motile feeding stage, which attaches to the villi of the intestine and sucks chime from the villi (Russo *et al*., 2000) and secretes proteolytic enzymes that dissolve host tissues and host cells and engulf RBCs (Al-Abadi, 2005).

In addition, infection with *E. histolytica* leads to necrosis of the intestinal mucosa causing damage and degeneration of the sites of absorption of necessary substances, along with the bleeding associated with this process. Infection with intestinal parasites has an important effect on blood values (Packers, 2002). For these, intestinal parasites are closely linked to the development of anemia because they cause malabsorption, nutritional deficiencies, and blood loss in the gastrointestinal tract (Knupp *et al*., 1988). After treatment, it was observed a rise in hemoglobin and its return to near-normal levels.

As for the MCH and MCV, it was observed through the tables a significant decrease in the MCH and MCV of the positive control group compared to the negative control. Significant changes in the values of MCV and MCH can indicate the presence of macrocytic anemia. This type of microcytic anemia can be caused by iron deficiency and the body's inability to absorb iron, which can be caused by a digestive disorder caused by parasites. This result is in agreement with (Darlan *et al*., 2018) who reported that intestinal parasitic infection in general and protozoa infection affect the values of hemoglobin, MCV, and MCH, leading to anemia. After treatment, it was observed an increase in the values of MCV and MCH and their return to values close to normal.

The results of the study agreed with Mehdi *et al*. (2019, 2020, and 2021) in their studies on *Tamarindus indica* extract, *Z. mauritiana* extract, and *Reseda sphenocleoides* leaves extracts, respectively, where the results of their studies showed a significant increase at $P \leq 0.05$ in the number of red blood cells (RBC) and hemoglobin (Hb), the mean cell volume (MCV) and red cell distribution width (RDW) in the groups treated with extracts compared with metronidazole and the control group during the treatment phases and a significant decrease at $P \leq 0.05$ in granulocytes (GR) when compared with the control groups. While the current study differs with him in white blood cell counts (WBC), lymphocyte counts, monocytes, and Mean corpuscular hemoglobin (MCH). It also agrees with Shaker and Hussein (2016) in their study on 320 patients, where the results showed a significant decrease ($P \leq 0.05$) in the number of red blood cells, hemoglobin level, and MCV in patients infected with *E. histolytica* (positive control) while lymphocytes increased significantly ($P \leq 0.05$) in the same treatment (positive control).

5.5 Effect of Treating with Turmeric Extract on AST, ALP, ALT, and Total Antioxidant Enzymes in The Blood Serum of Rats Infected with *E. histolytica*.

This part of the study aims to investigate the effect of ALP, ALT, and AST enzymes in rats after experimental infection with *E. histolytica* parasite and to measure the amount of variation in the values of these enzymes when treated with plant extracts and the drug and then compare all these measurements with the positive and negative control. An increase in the levels of ALP, ALT, and AST enzymes in the serum occurs when severe necrosis occurs in the heart muscle tissue and in the case of chronic liver diseases such as tissue damage and cirrhosis (Wong *et al*., 2004). However, the effectiveness of these enzymes returns to the normal level after repairing damaged liver tissues due to the presence of the parasite in them (Rueda *et al*., 1995).

The results of the study showed that AST, ALT, and ALP enzyme tests were increased in all infected rats (*E. histolytica*) as compared to the control group, which indicates that *E. histolytica* leads to histological lesions in the liver such as apoptosis death of cells as well as changes in biochemical parameters (ALP, AST, ALT) (Chabuk *et al*., 2014). Al-Kubaissi, (2002) also observed a high level of ALP enzyme concentration, which amounted to 90% of cases, along with a high level of AST and ALT enzyme in the serum of patients with dysentery. Mahmood and Mohamed (2012) show different lesions in liver of mice that administrated with *E. histolytica* including necrosis and degeneration of hepatocytes with infiltration of lymphocytes

Also, Hussein *et al*. (2013) indicated that *E. histolytica* leads to various lesions in the liver including liver abscess, hepatocyte necrosis, hepatocyte hyperplasia, and lymphocyte filtration. Various liver lesions may be due to the ability of the parasites to invade the intestines to other organs such as the liver and kidneys and lead to degenerative changes (Ali and Alattar, 2018). The

observed levels of liver enzymes increased directly with the incubation time, and it was also observed that high levels of these enzymes in the blood serum led to increased numbers of damaged cells during the apoptosis process, probably due to the fact that these enzymes are predominantly found within the cells of the liver. But when the liver is infected for any reason, these enzymes leak into the bloodstream.

These enzymes are usually contained mostly within liver cells and to a lesser extent in muscle cells. If the liver is infected or damaged, the liver cells pour these enzymes into the blood, which raises the levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and indicates liver disease, while ALP is a substance found in the bile ducts of the liver, intestines, and bones. Damage or blockage of the bile ducts may cause ALP levels to rise. These tests can provide a range of information about a range of pathological processes (Dufour, 2001). This is consistent with (Al-Kubaissi, 2002) who observed the high level of ALP enzyme concentration reached 90% of the cases with the high level of AST and ALT enzyme in the serum of patients with dysentery. This result is consistent with (Pluta, 2008) who showed very high levels of liver enzymes in the serum of patients infected with the parasite.

Al-Ghanimi (2013) also showed an increase in liver levels in mice infected with the *Giardia lamblia* parasite. But this is inconsistent with what has been recorded by Fernandez *et al*. (2009) who found that liver function tests in patients with dysentery were normal, with the exception of ALP enzyme which is observed increasing it. The results of this study concluded that *E. histolytica* caused histological changes in organs, especially the liver, such as apoptosis of cells as well as changes in biochemical parameters (ALP, AST, ALT).

Treatment with plant extracts or drugs had an effect in returning these enzymes to a level close to normal (Al-Tikriti, 2004; Al-Mayah *et al*., 2012). The results of the current study showed a high level of these enzymes in the

untreated positive control group. The results are in agreement with (Rueda *et al*., 1995; Al-Haditi *et al*., 2009; Al-Dabbagh, 2010; Muchtaromah *et al*., 2011; Kassim, 2012; Mero and Abdullah, 2012). This elevation is explained on the basis of the extent of blockage in the hepatic ducts and then collecting within the liver (Rueda *et al*., 1995; Muchtaromah *et al*., 2011). This increase is also a sensitive indicator of the infiltration of inflammatory cells in the liver as a result of infection (Premvati, 1980).

Turmeric is considered one of the most widely used and well-documented medicinal plants in the world. Turmeric contains phenolic compounds such as flavonoids. Phenols play an important role against Entamoeba species, where phenols inhibit parasite enzymes by oxidizing compounds. Curcumin has antioxidant properties, so it observed an increase in total antioxidants by increasing concentrations, where it provides effective protection against damage caused by free radicals by reducing oxidative stress, and also reduces the generation of NO (Nitric Oxide) molecules. The beneficial effects of curcumin derive from its ability to reduce increased cellular peroxisome levels.

Pumpkin extract caused a decrease in ALP, AST, and ALT activities to normal levels which may be a result of plasma membrane stabilization as well as repair of streptozotocin-induced hepatic tissue damage (Baldi *et al*., 2010; Sharma *et al*., 2013). Pumpkin seeds contain a high content of phenols, beta-carotene, vitamin C, and alpha-tocopherol, which are effective antioxidants that protect against oxidation of cell membranes, maintain a high level of antioxidant enzymes and prevent the formation of free radicals that cause damage to cell membranes, thus maintaining the concentration of hepatic enzymes within normal limits (Kulczyński *et al*., 2020; Abed and Alkalby, 2021).

Antioxidant supplementation may protect protein structures, prevent reactive oxygen species inactivation, and stabilize cell membranes, which may be responsible for this protective and favorable effect of ascorbic acid and α-

tocopherol (Rayman, 2000; Schröder *et al*., 2001; Lodhi *et al*., 2011). The natural plant components found in pumpkin can improve the liver against alcohol-induced liver toxicity and oxidative stress (Oboh, 2005). Sayed and Aal (2014) showed that treating male rats with pumpkin reduced the toxic effect of azathioprine on the liver and this may be due to the high content of beta-carotene. The antioxidant and protective effect of pumpkin may be due to the properties of polyphenols and beta-carotene which have been shown to be powerful antioxidants and protective actions against cell infection (Nwanna and Oboh, 2007).

5.6 Histopathological Study

5.6.1 Effect of Treatment with Metronidazole, Turmeric (Cold and Hot) Extract at a Concentration of (400 mg/ml), and Pumpkin (Cold And Hot) Extract at a Concentration of (400 mg/ml) on The Liver, Kidneys, and Small Intestines of Rats Infected with *E. histolytica*.

Through the examination, it was observed that there were no changes in the histological structure of the liver, kidneys, and small intestines treated with cold turmeric extract at a concentration of (400 mg/ml). The reason for this may be due to the effect of the active substances contained in the extract such as alkaloids, resins, tannic acid, and Lydic acid, which led to stopping the effect While these active substances had no significant effect on the histological structure of the liver, kidneys, and intestines, which enhances the therapeutic efficiency of the parasite.

The liver is considered the main organ in which drugs and medicines are metabolized, and this is why it was used to determine the toxic effect of plant extract and metronidazole. Liver cells are compromised by many toxins and drugs. The results of Macías-Pérez *et al*. (2019) study showed the efficacy of curcumin treatment against hepatic amebiasis because this compound has a great ability to resist inflammation. Curcumin treatment significantly reduced acute

liver damage caused by *E. histolytica* and completely prevented liver damage caused by this parasite within 7 days. They have also shown that curcumin protects liver tissue in both the early and late stages of liver infection.

Several reports indicate that curcumin protects the liver from hepatotoxic compounds, such as carbon tetrachloride, alcohol, and paracetamol (Varatharajalu *et al*., 2016; Granados-Castro *et al*., 2016). Furthermore, curcumin's antibacterial, antiviral, and antifungal activity has been reported (Zorofchian *et al*., 2014). Similarly, curcumin has been shown to possess antiparasitic activity against malaria and *G. lamblia* (Ali *et al*, 2017; Gutiérrez-Gutiérrez *et al*., 2017).

Microscopic examination of the histological sections of the kidneys of rats infected with the parasite and untreated showed a change in the histological structure of the kidney, where was observed the presence of vascular congestion as shown in figure (4.1). These changes may be attributed to the length of the infection period and its severity, where the kidney filters the blood containing the metabolites and toxins of the parasite, so the accumulation of these substances may justify the damage to the kidney tissue. As for the infiltration of inflammatory cells, it may be the result of the immune response to infection with the parasite.

Although pumpkin seed oil contains a high percentage of phenols, beta-carotene, vitamin C, and alpha-tocopherol, which are effective antioxidants that protect against peroxidation of cell membranes, maintain a high level of antioxidant enzymes and prevent the formation of free radicals that cause damage to cell membranes and it contains many vitamins and active substances as mentioned in the previous chapters. However, these existing damages in the organs (liver, kidneys, and small intestines) may be due to the fact that the duration of treatment and the amount of the dose is insufficient to complete the

pumpkin repair of the affected organs, and this is what our results showed through the percentages of healing.

As for metronidazole, it has eliminated the parasite and worked on repairing tissues (liver, kidneys, and small intestines), so no damage appeared on the tissues. However, metronidazole has side effects, as we explained previously, such as feeling dizziness, headache and nausea, loss of appetite, diarrhea or constipation, irritation in the digestive system, the appearance of skin rashes, dry mouth, and feeling the need to drink large quantities of water, and sometimes it results in the feeling of having an undesirable taste in the mouth that looks like metal. This medicine may darken the urine, but this is not a concern because it will disappear once you stop taking the medicine. moreover. More serious reactions are rare and occur only during prolonged treatment and include stomatitis, candidiasis (Dusengeyezu and Kadima, 2016).

Conclusions & Recommendations

Conclusions

From the results of the current work, the following can be concluded:

1. There are no significant differences in the total percentages of infection with parasite between the concentrations of turmeric extracts (100, 200, 400 mg/ml) and pumpkin extracts (100, 200, 400 mg/ml). The current study showed the high efficiency of the turmeric plant extract (cold extract at a concentration of 400 mg/ml) in treating cases of parasite infection within a reasonable period of time.
2. The results of the histological study show the presence of pathological effects on the tissues of the liver, kidneys, and intestines in the groups of rats infected with the parasite and the group of animals infected with the parasite and treated with pumpkin extract, while there were no effects on the tissues of infected rats and treated with turmeric extract, as well as no effects on the tissues of infected rats and treated with metronidazole.

Recommendations

Based on the results obtained in the current study, the following are recommended:

1- Conducting subsequent studies on diagnosing the type of parasite using PCR, ELISA, and serological methods.
2- Conducting subsequent studies on the side effects of turmeric and pumpkin plants on human health when used to treat parasites.
3- Encouraging researchers to continue working in the field of using natural materials as an alternative treatment to manufactured drugs in order to avoid their side effects.

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Appendices

Appendix A

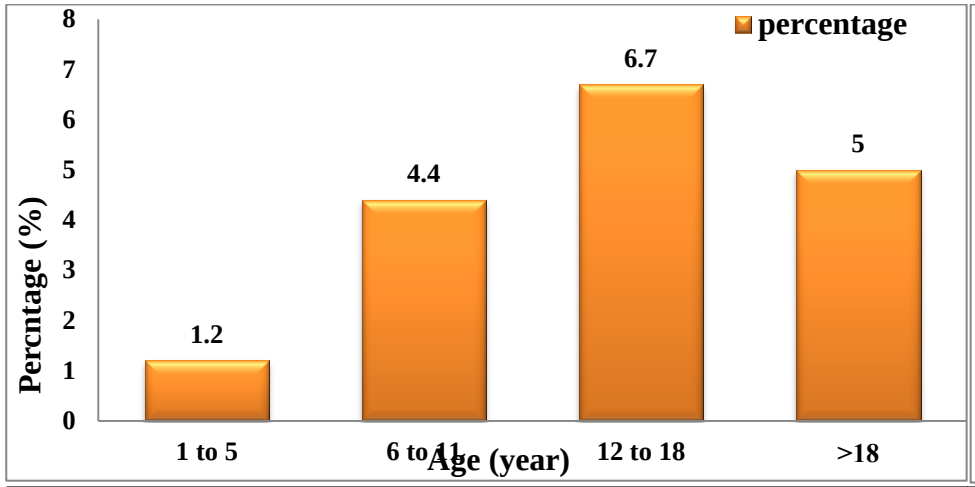


Figure 1: The percentage of infection with *E. histolytica* according to age group.

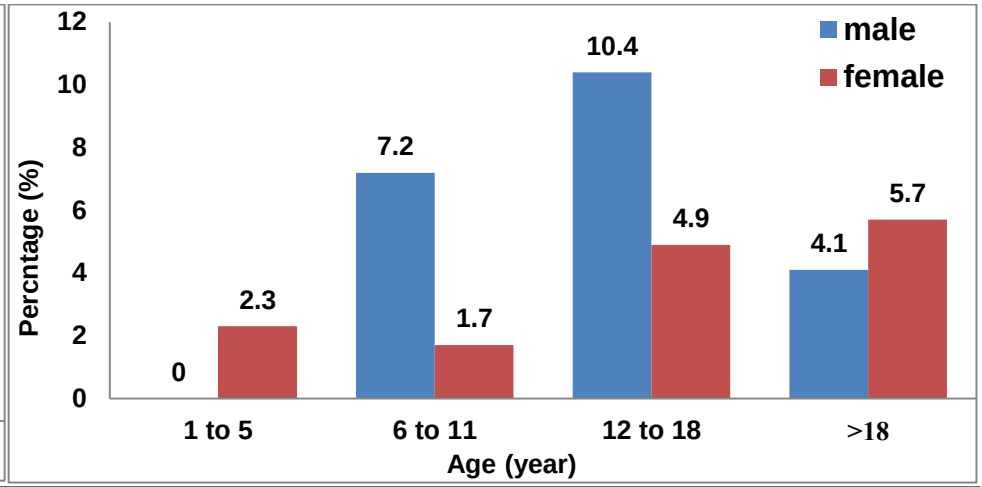
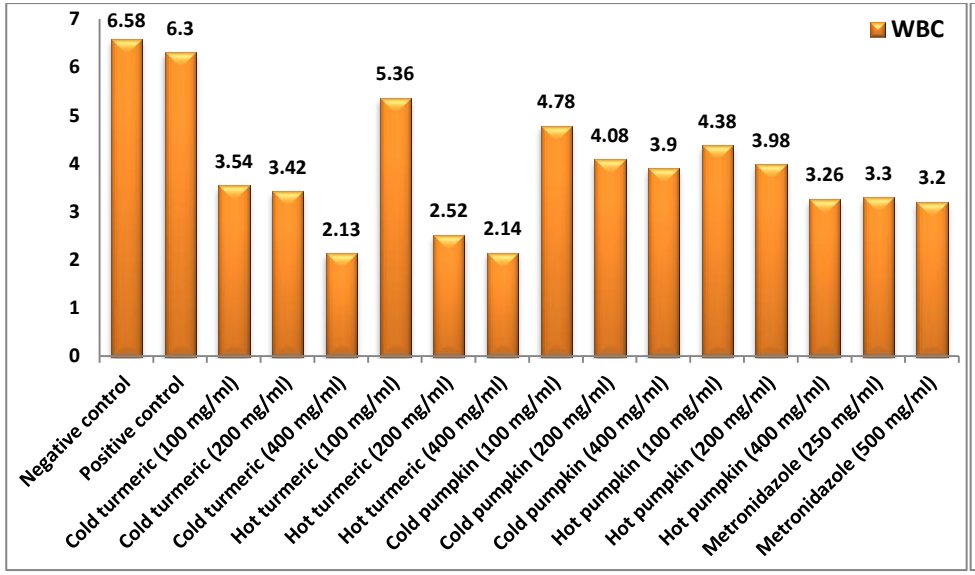
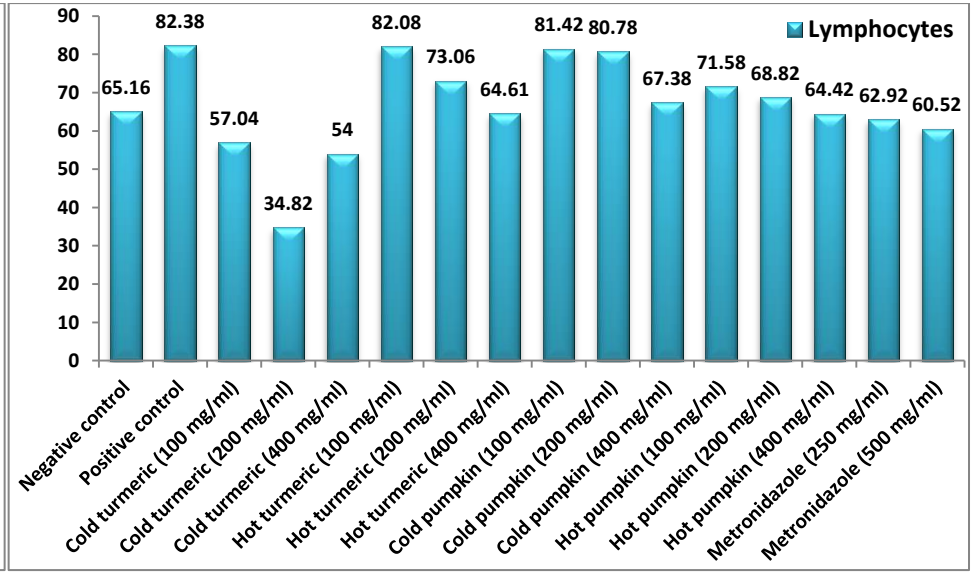
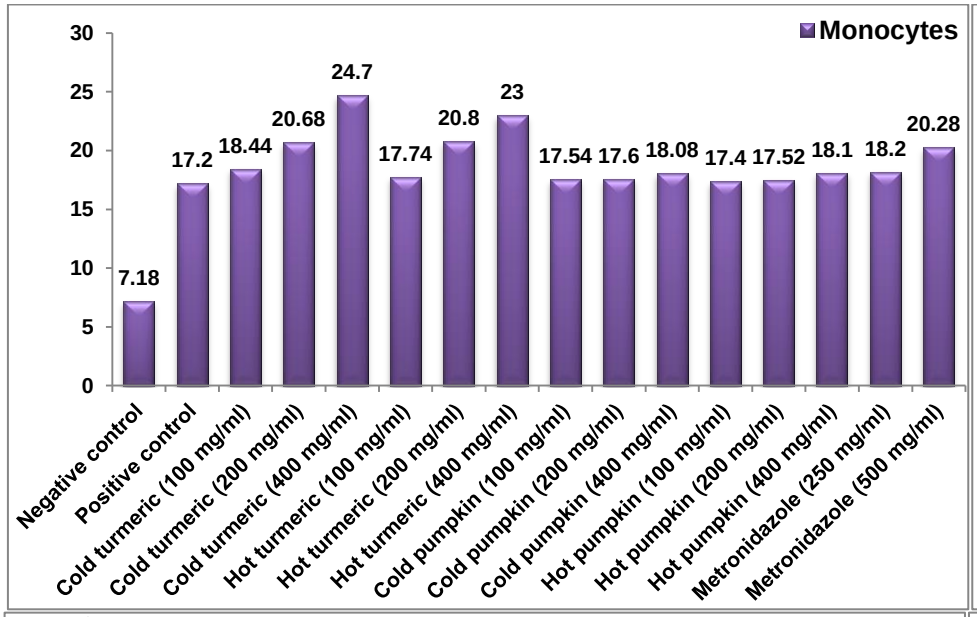


Figure 2: The percentage of infection with *E. histolytica* according to gender.






Monocytes
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Negative control
Positive control
Cold turmeric (100 mg/ml)
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Hot turmeric (100 mg/ml)
Hot turmeric (200 mg/ml)
Hot turmeric (400 mg/ml)
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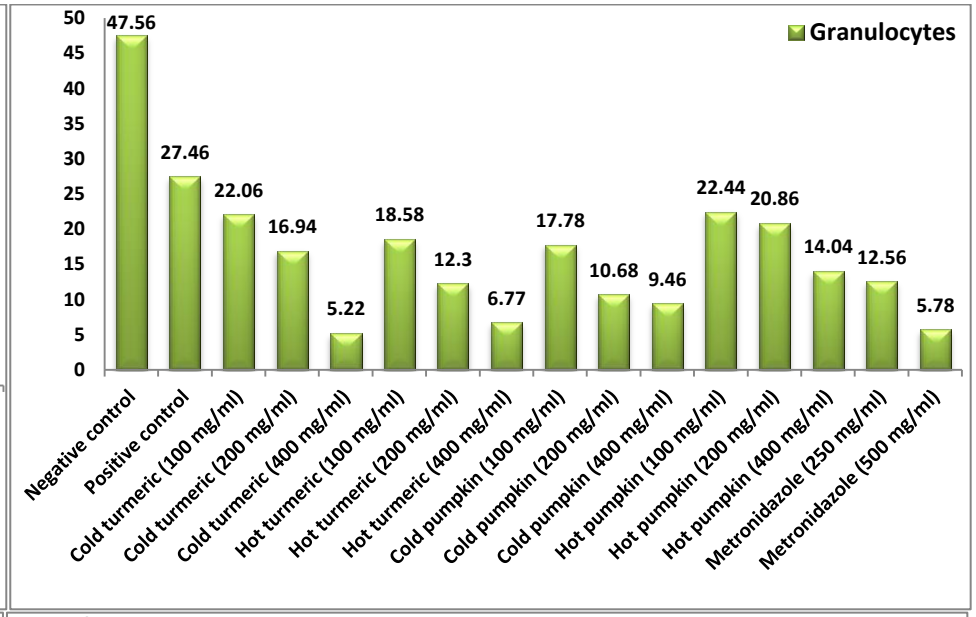
Cold pumpkin (200 mg/ml)
Cold pumpkin (400 mg/ml)
Hot pumpkin (100 mg/ml)
Hot pumpkin (200 mg/ml)
Hot pumpkin (400 mg/ml)
Metronidazole (250 mg/ml)
Metronidazole (500 mg/ml)


Granulocytes
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17.78
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Negative control
Positive control
Cold turmeric (100 mg/ml)
Cold turmeric (200 mg/ml)
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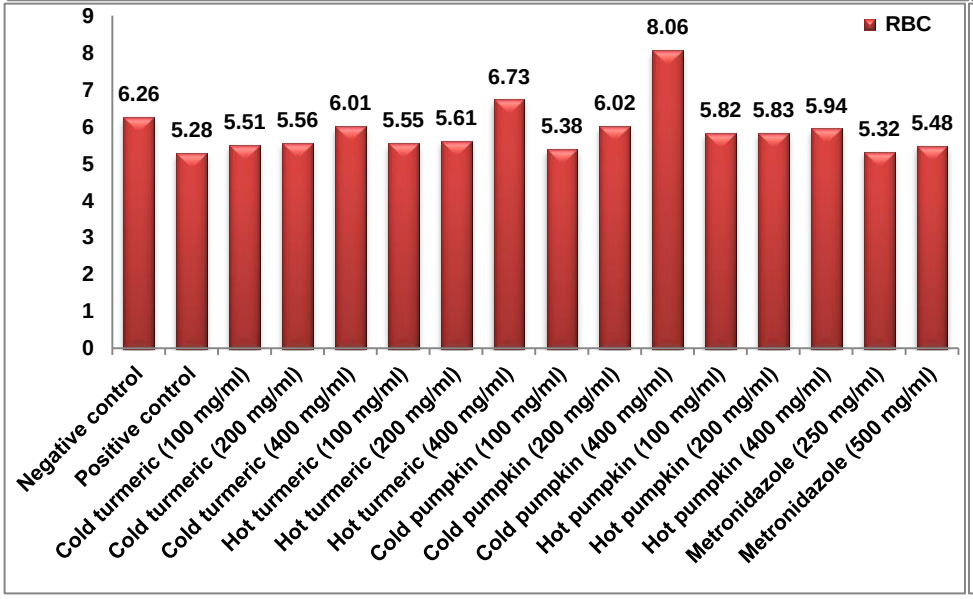
Hot pumpkin (400 mg/ml)
Metronidazole (250 mg/ml)
Metronidazole (500 mg/ml)


RBC
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5.51
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5.61
6.73
5.38
6.02
8.06
5.82
5.83
5.94
5.32
5.48
Negative control
Positive control
Cold turmeric (100 mg/ml)
Cold turmeric (200 mg/ml)
Cold turmeric (400 mg/ml)
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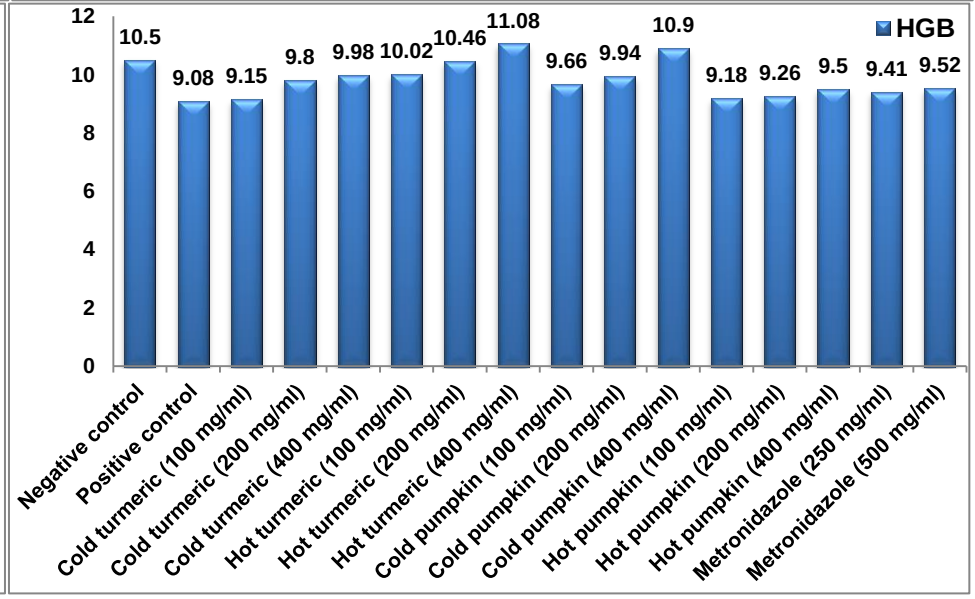
Hot turmeric (400 mg/ml)
Cold pumpkin (100 mg/ml)
Cold pumpkin (200 mg/ml)
Cold pumpkin (400 mg/ml)
Hot pumpkin (100 mg/ml)
Hot pumpkin (200 mg/ml)
Hot pumpkin (400 mg/ml)
Metronidazole (250 mg/ml)
Metronidazole (500 mg/ml)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9.08
9.15
9.8
9.98
10.02
10.46
11.08
9.66
9.94
10.9
9.18
9.26
9.5
9.41
9.52
Negative control
Positive control
Cold turmeric (100 mg/ml)
Cold turmeric (200 mg/ml)
Cold turmeric (400 mg/ml)
Hot turmeric (100 mg/ml)
Hot turmeric (200 mg/ml)
Hot turmeric (400 mg/ml)
Cold pumpkin (100 mg/ml)
Cold pumpkin (200 mg/ml)
Cold pumpkin (400 mg/ml)
Hot pumpkin (100 mg/ml)
Hot pumpkin (200 mg/ml)
Hot pumpkin (400 mg/ml)
Metronidazole (250 mg/ml)
Metronidazole (500 mg/ml)

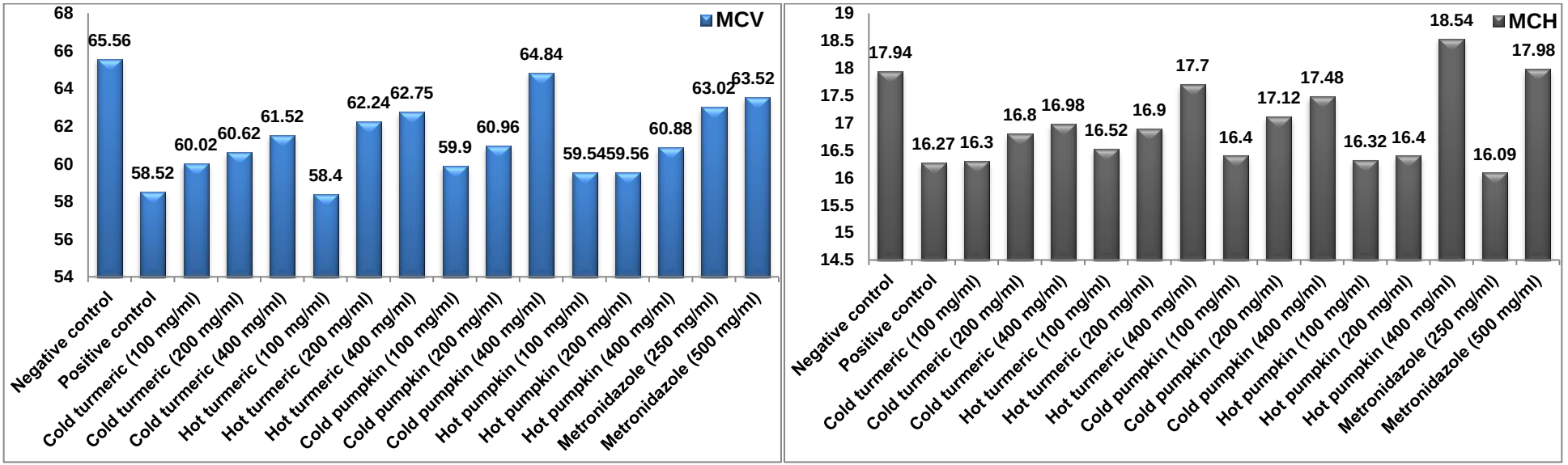


Figure 3: The relationship between hematological parameters and the therapeutic treatments of rats infected with *E. histolytica*.

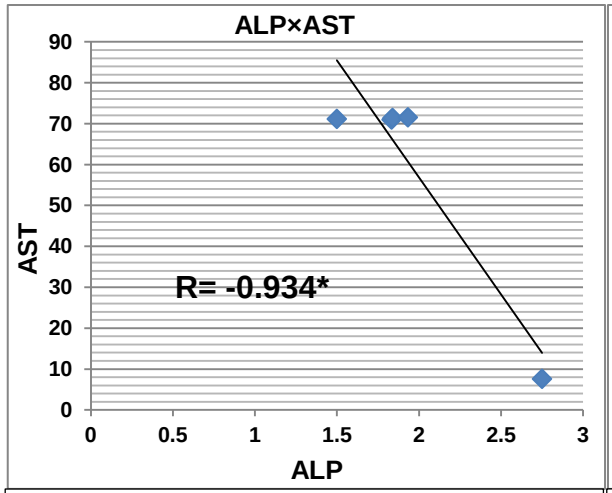


Figure 4: The significant negative Correlation between ALP and AST for the rats uninfected with parasite.

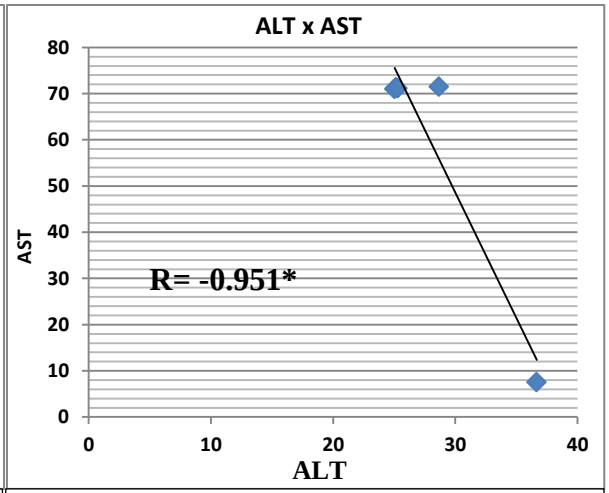


Figure 5: The significant negative Correlation between ALT and AST for the rats uninfected with parasite.

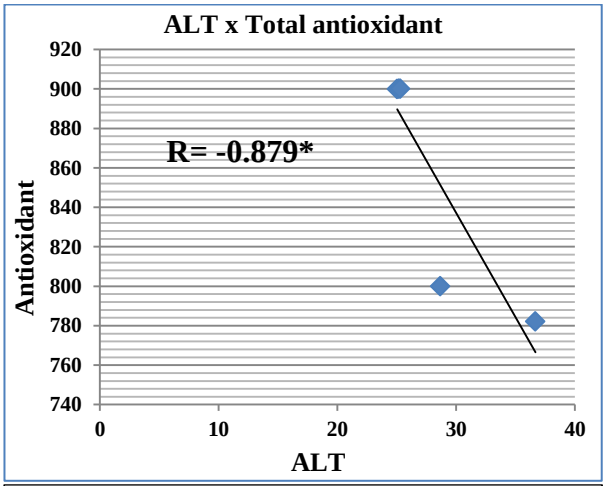


Figure 6: The significant negative Correlation between ALT and Total antioxidant for the rats uninfected with parasite.

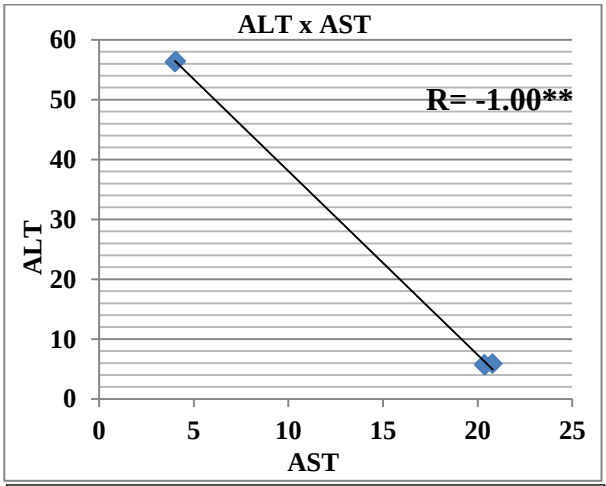


Figure 7: The significant negative Correlation between ALT and AST for the rats infected with *E. histolytica* and untreated.

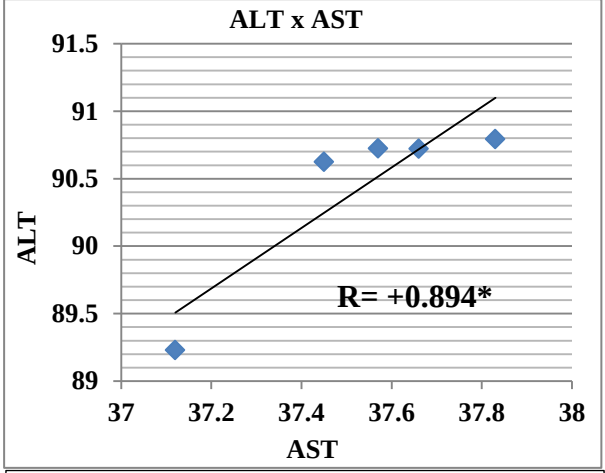


Figure 8: The significant positive Correlation between ALT and AST for the rats infected with parasite and dosed with cold turmeric extract (100 mg/ml).

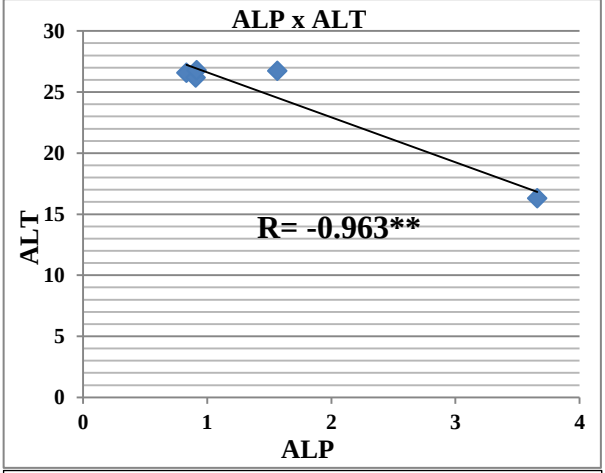


Figure 9: The significant negative Correlation between ALP and ALT for the rats infected with parasite and dosed with cold turmeric extract (200 mg/ml).

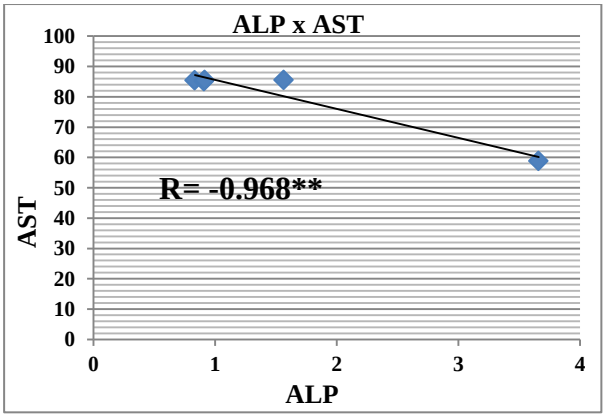


Figure 10: The significant negative Correlation between ALP and AST for the rats infected with parasite and dosed with cold turmeric extract (200 mg/ml).

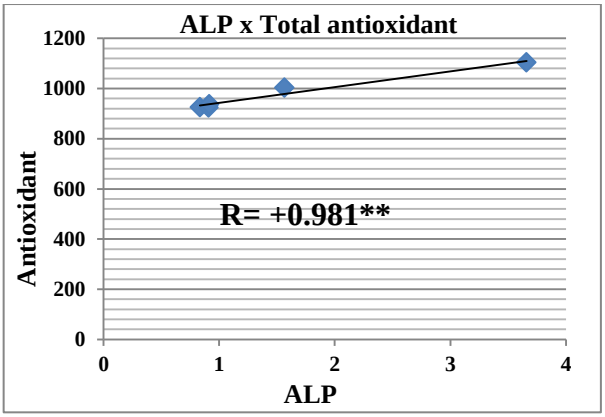


Figure 11: The significant positive Correlation between ALP and Total antioxidant for the rats infected with parasite and dosed with cold turmeric extract (200 mg/ml).

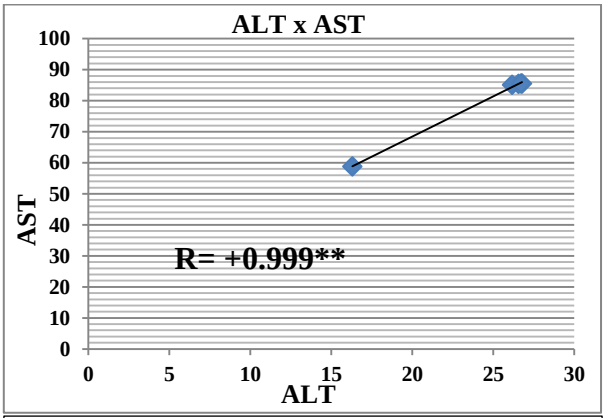


Figure 12: The significant positive Correlation between ALT and AST for the rats infected with parasite and dosed with cold turmeric extract (200 mg/ml).

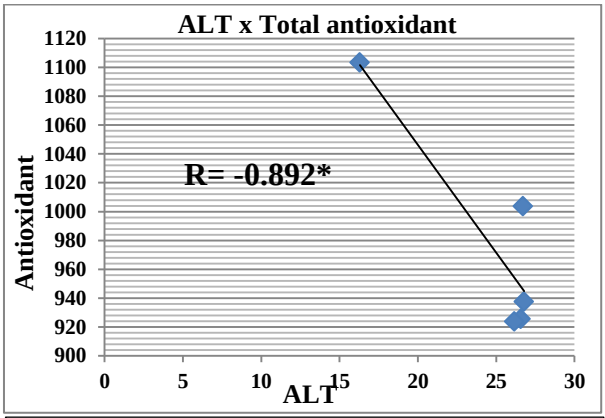


Figure 13: The significant negative Correlation between ALT and Total antioxidant for the rats infected with parasite and dosed with cold turmeric extract (200 mg/ml).

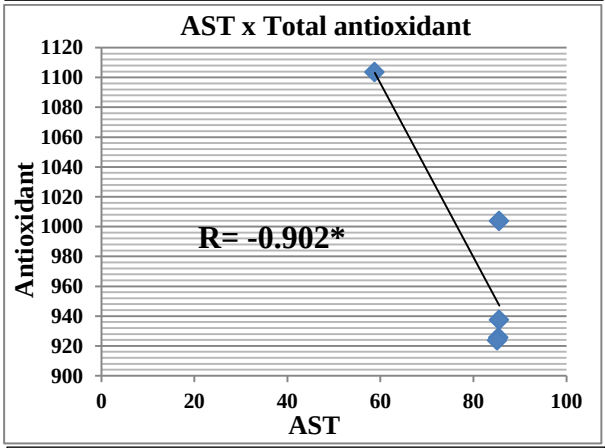


Figure 14: The significant negative Correlation between AST and total antioxidant for the rats infected with parasite and dosed with cold turmeric extract (200 mg/ml).

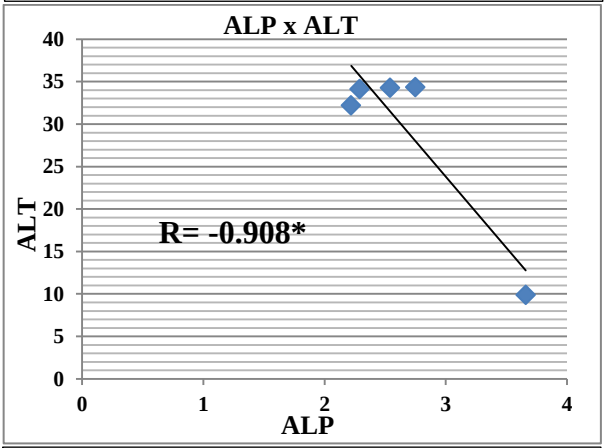


Figure 15: The significant negative Correlation between ALP and ALT for the rats infected with parasite and dosed with cold turmeric extract (400 mg/ml).

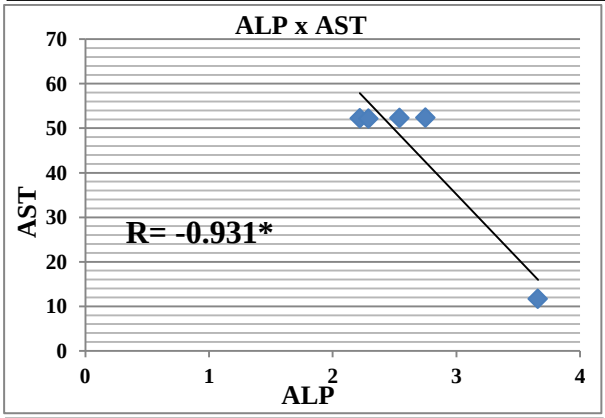


Figure 16: The significant negative Correlation between ALP and AST for the rats infected with parasite and dosed with cold turmeric extract (400 mg/ml).

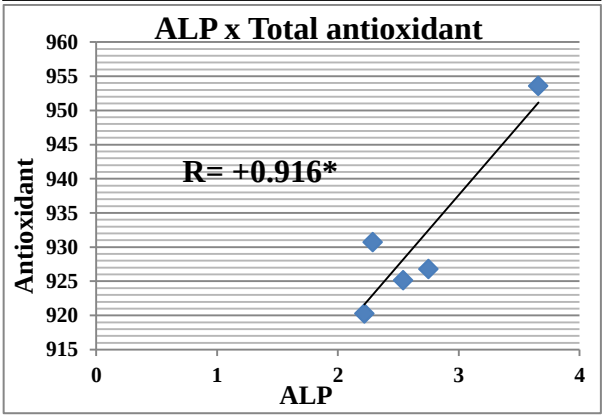


Figure 17: The significant positive Correlation between ALP and total antioxidant for the rats infected with parasite and dosed with cold turmeric extract (400 mg/ml).

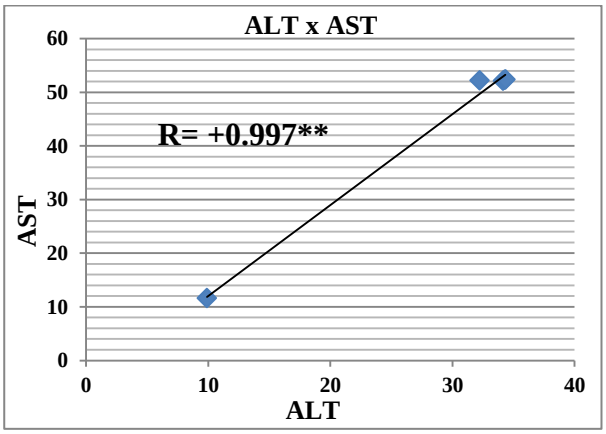


Figure 18: The significant positive Correlation between ALT and AST for the rats infected with parasite and dosed with cold turmeric extract (400 mg/ml).

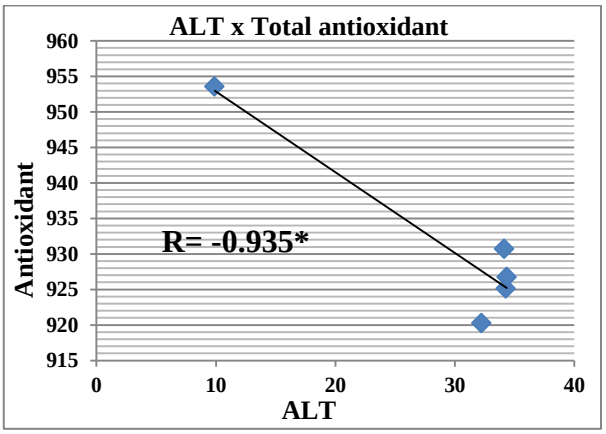


Figure 19: The significant negative Correlation between ALT and total antioxidant for the rats infected with parasite and dosed with cold turmeric extract (400 mg/ml).

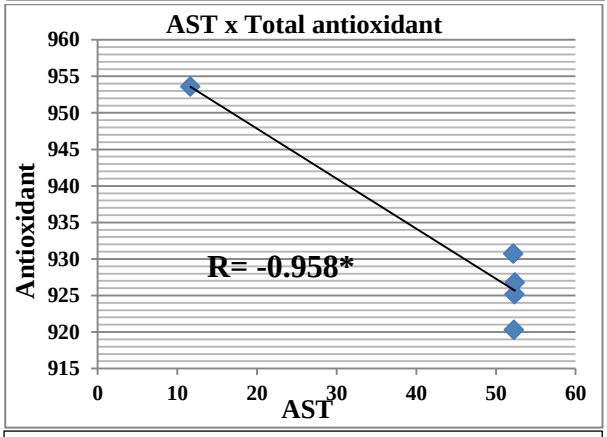


Figure 20: The significant negative Correlation between AST and total antioxidant for the rats infected with parasite and dosed with cold turmeric extract (400 mg/ml).

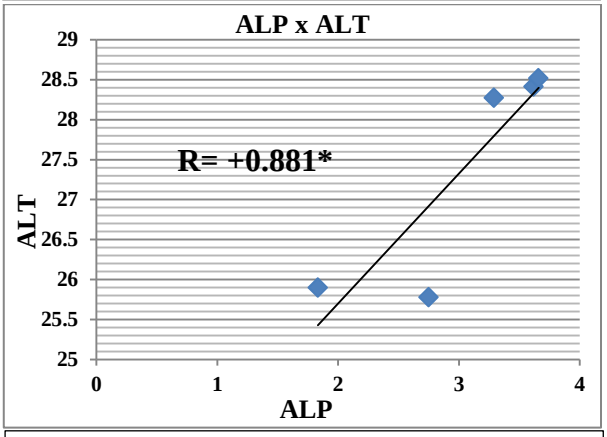


Figure 21: The significant positive Correlation between ALP and ALT for the rats infected with parasite and dosed with hot turmeric extract (400 mg/ml).

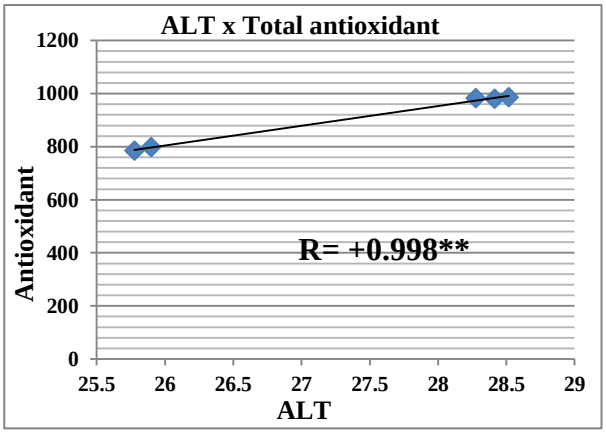


Figure 22: The significant positive Correlation between ALT and total antioxidant for the rats infected with parasite and dosed with hot turmeric extract (400 mg/ml).

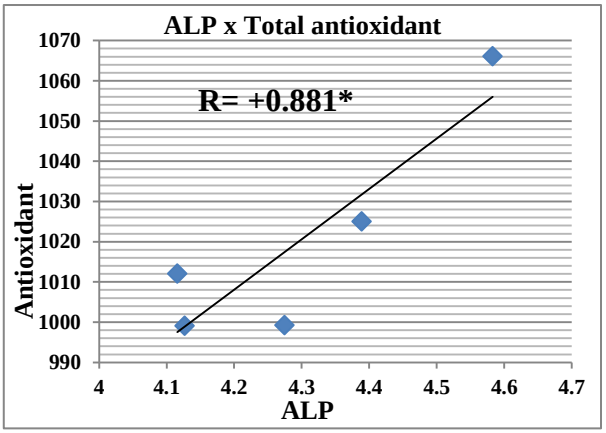


Figure 23: The significant positive Correlation between ALP and total antioxidant for the rats infected with parasite and dosed with cold pumpkin extract (100 mg/ml).

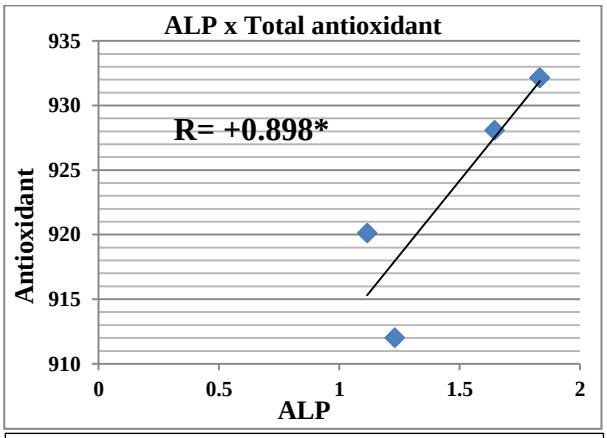


Figure 24: The significant positive Correlation between ALP and total antioxidant for the rats infected with parasite and dosed with cold pumpkin extract (400 mg/ml).

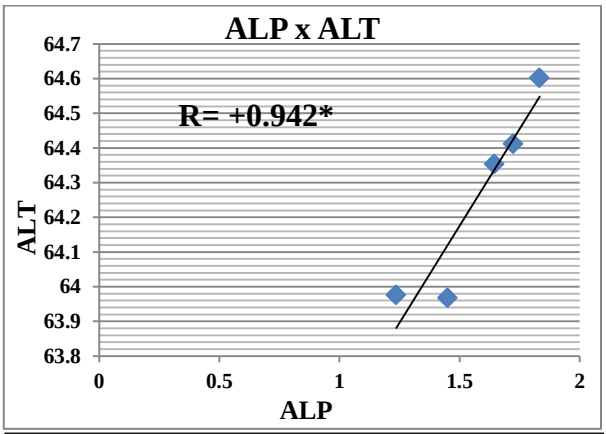


Figure 25: The significant positive Correlation between ALP and ALT for the rats infected with parasite and dosed with hot pumpkin extract (100 mg/ml).

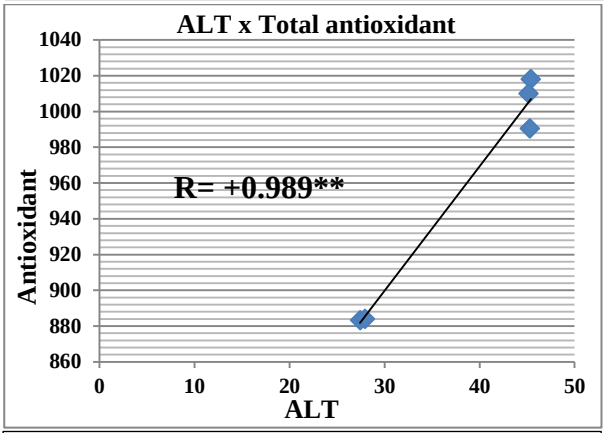


Figure 26: The significant positive Correlation between ALP and total antioxidant for the rats infected with parasite and dosed with hot pumpkin extract (200 mg/ml).

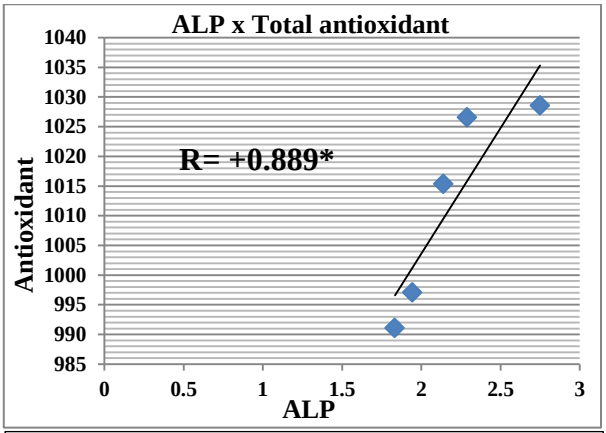


Figure 27: The significant positive Correlation between ALP and total antioxidant for the rats infected with parasite and dosed with hot pumpkin extract (400 mg/ml).

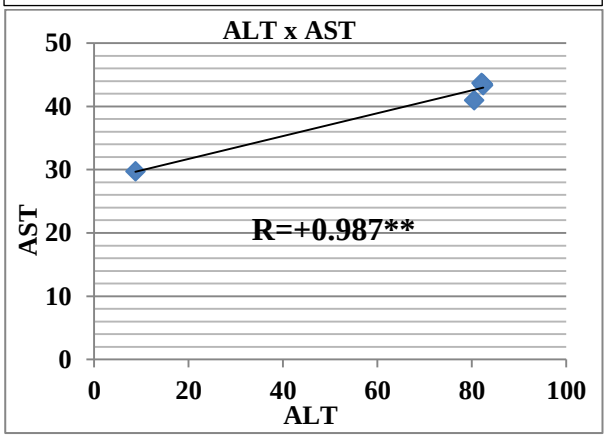


Figure 28: The significant positive Correlation between ALT and AST for the rats infected with parasite and dosed with hot pumpkin extract (400 mg/ml).

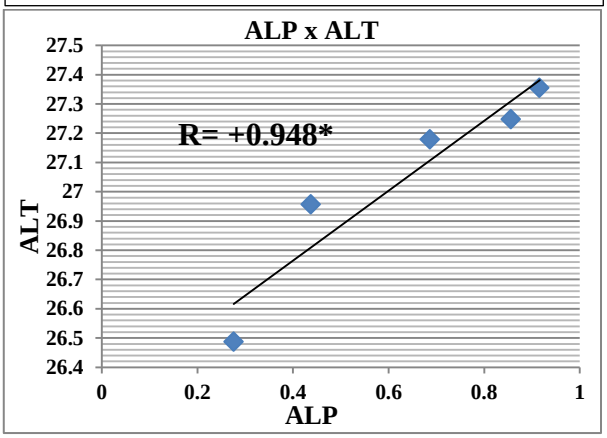
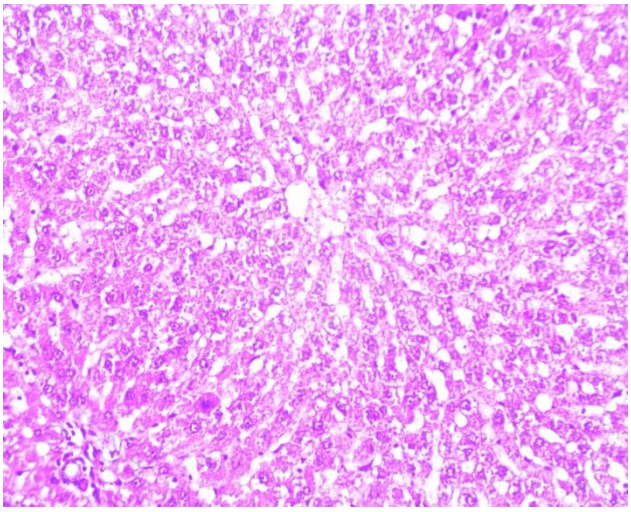
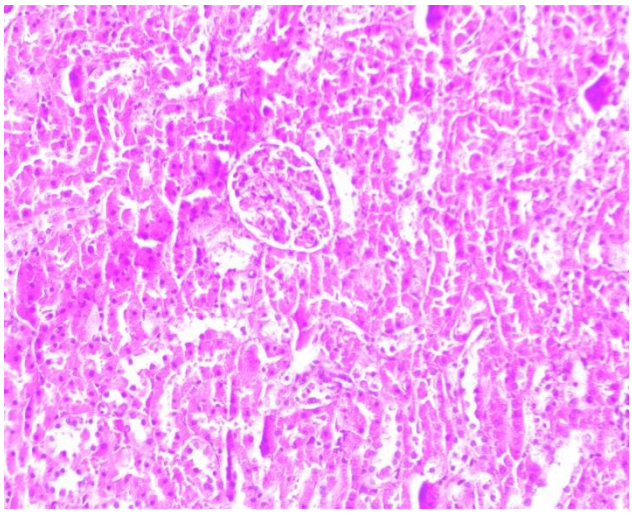


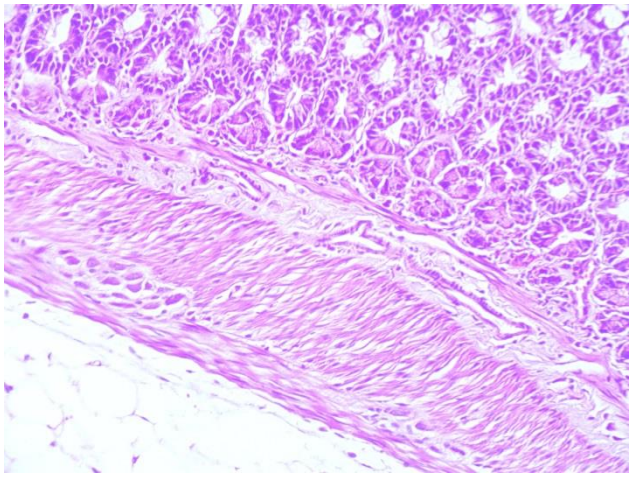
Figure 29: The significant positive Correlation between ALP and ALT for the rats infected with the parasite and dosed with Metronidazole (250 mg/ml).



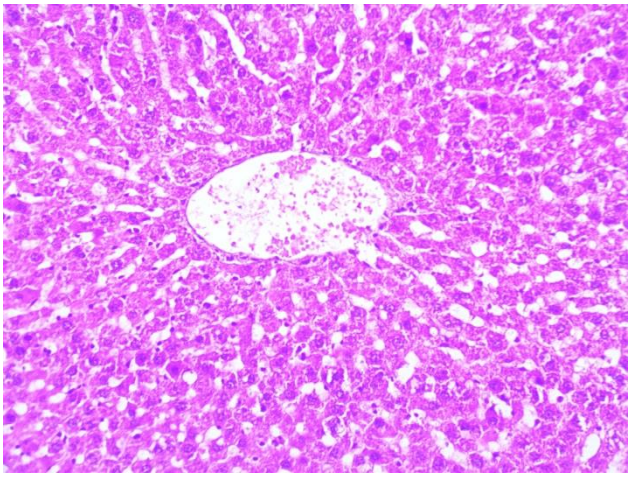
Liver for metronidazole (250 mg/ml) treatment



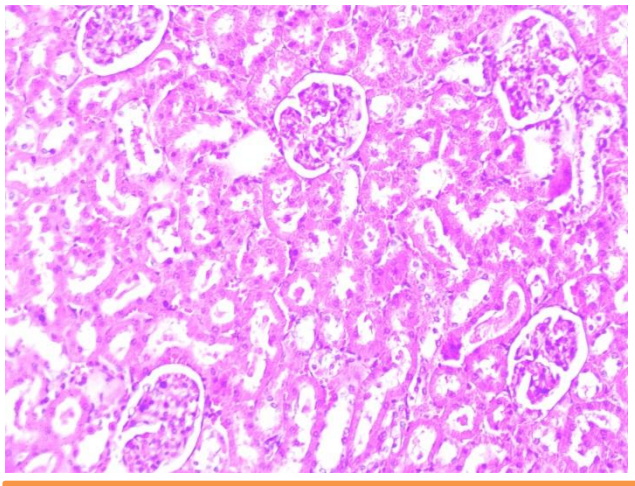
Kidney for metronidazole (250 mg/ml) treatment



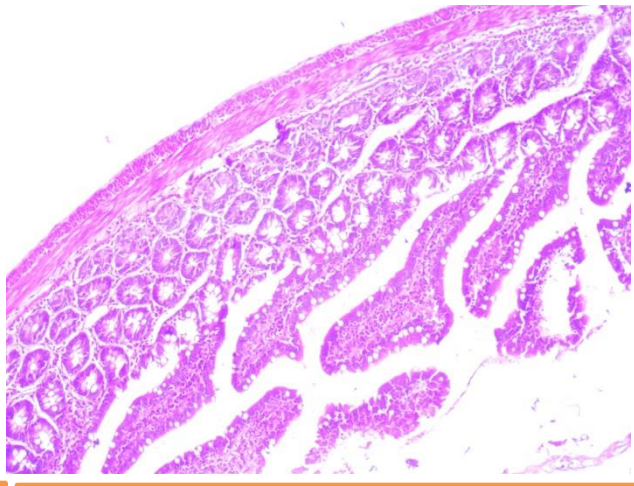
Bowel for metronidazole (250 mg/ml) treatment



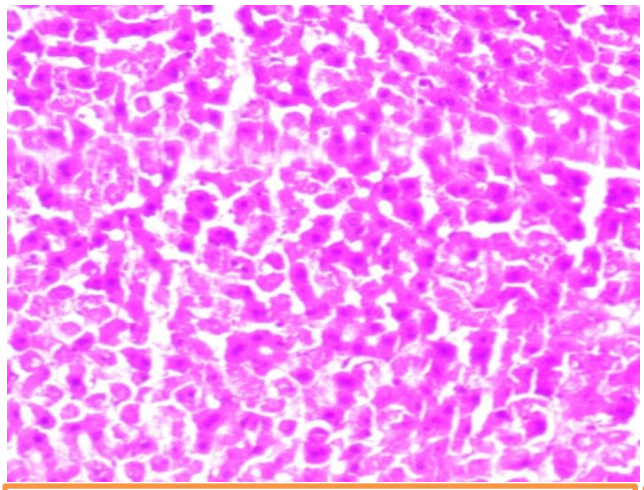
Liver for metronidazole (500 mg/ml) treatment



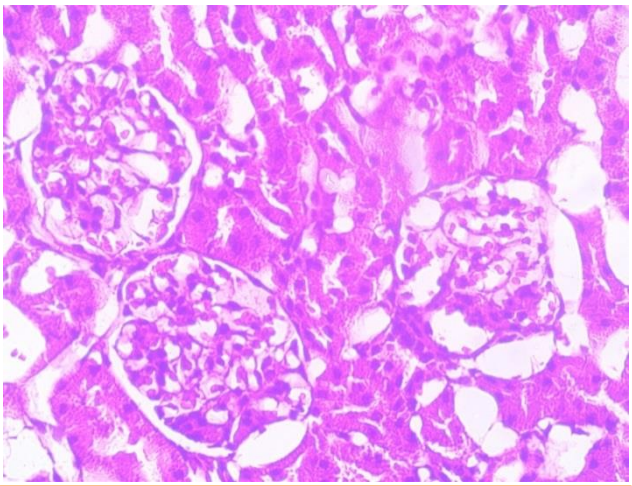
Kidney for metronidazole (500 mg/ml) treatment



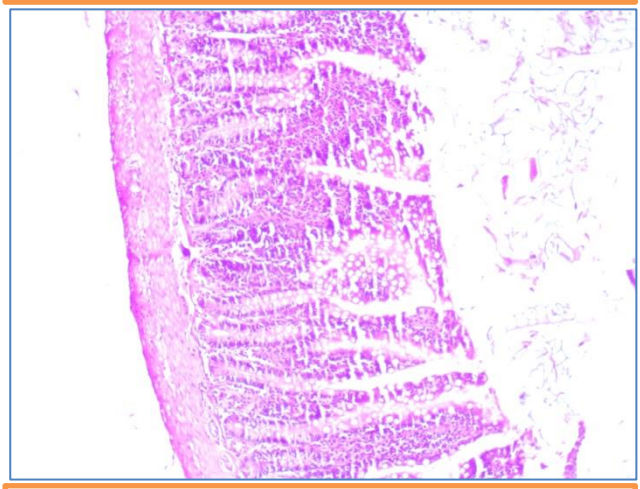
Bowel for metronidazole (500 mg/ml) treatment



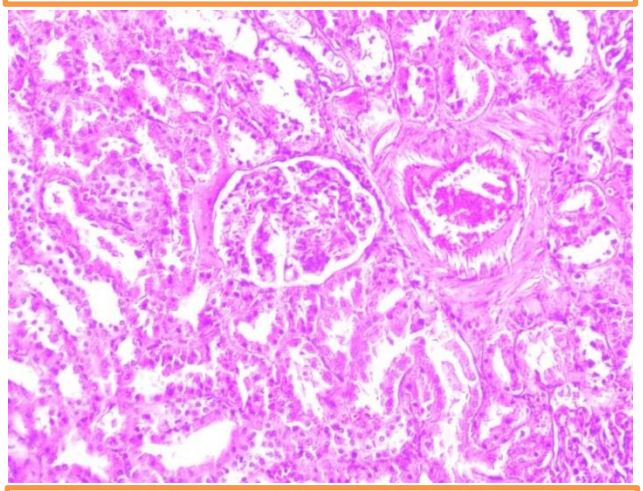
Liver for cold turmeric extract (400 mg/ml) treatment



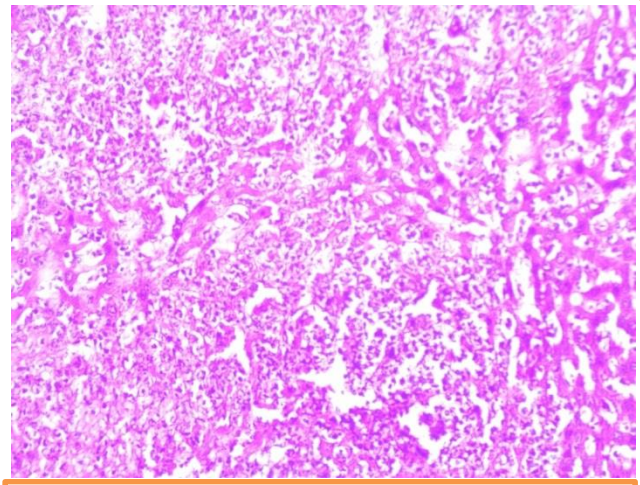
Kidney for cold turmeric extract (400 mg/ml) treatment



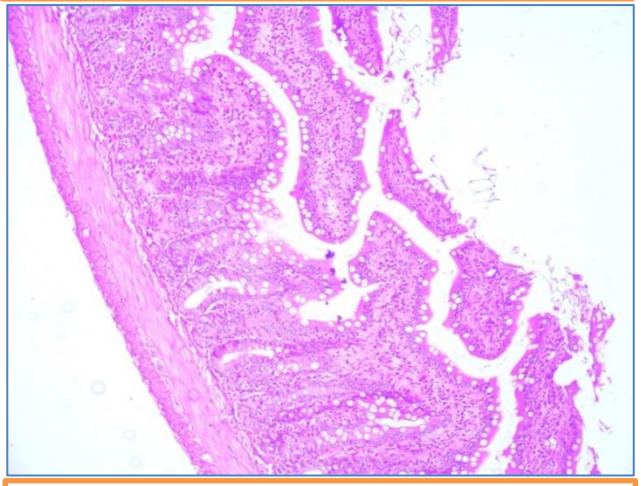
Bowel for cold turmeric extract (400 mg/ml) treatment



Kidney for uninfected rats



Liver for uninfected rats

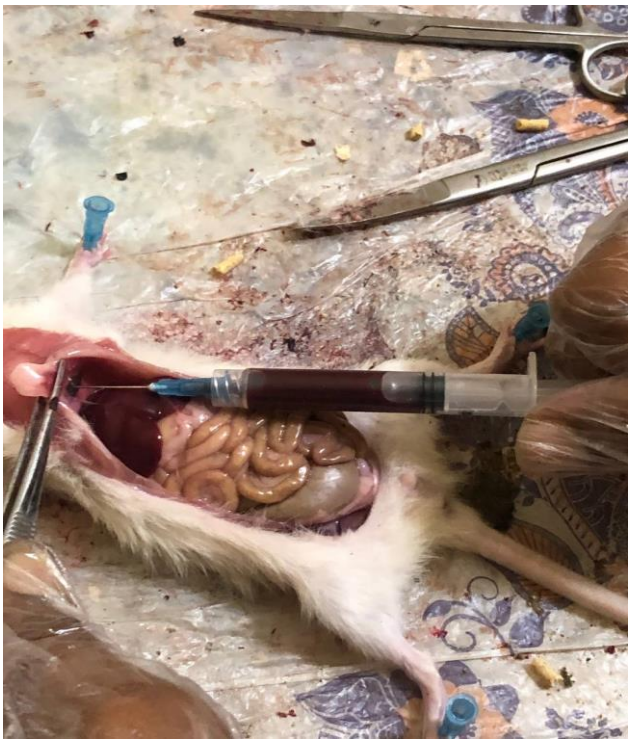


Bowel for uninfected rats

Figure 30: Histological sections of metronidazole (250 and 500 mg/ml) and cold turmeric extract (400 mg/ml).

Appendix B

Some figures of the laboratory aspect for the study



الخلاصة

الخلاصة:

اجريت الدراسة لبيان مدى انتشار طفيلي الزحار الاميبي في مدينة الحلة من خلال تحديد نسبة الاصابة بين الذكور والاناث وللفئات العمرية من سنة الى 18 سنة فاكثر اذ تم جمع 414 عينة براز من الاطفال والبالغين المراجعين لبعض المراكز الصحية والمستشفيات في مدينة الحلة خلال المدة من تشرين الاول 2021 الى اذار 2022 .

بينت النتائج ان نسبة الاصابة الاجمالية بطفيلي الزحار الاميبي هي 4.5% وسجلت عدم وجود فروق معنوية عند مستوى دلالة ($P\leq0.05$) في نسب الاصابة بين الجنس اذ كانت الذكور اعلى (5.47%) مقارنة مع الاناث (3.75%). اظهرت النتائج عدم وجود فروق معنوية بين الفئات العمرية وكانت اعلى نسبة في الفئة العمرية 12-18 اذ بلغت 6.7% بينما اقل نسبة في الفئة العمرية من 1-5 بنسبة (1.2%).

اجريت سلسلة اخرى من التجارب المختبرية في البيت الحيواني التابع لكلية العلوم/ جامعة بابل لبيان فعالية مستخلصات نباتي الكركم والقرع في علاج الاصابة بهذا الطفيلي في الجرذان المخمجة تجريبيا بعد التأكد من أن المستخلصات ليس لها تأثير سامي وذلك بتحديد سلامة تراكيز المستخلصات. اشتملت الدراسة على (80) أنثى من الجرذان تراوحت أوزانها ما بين 250-300 غم ، وتم تقسيمها إلى 15 مجموعة (كل مجموعة ضمت 5 فئران). تم اصابة المجموعات بالاميبا الحالة للنسيج، وبقيت مجموعة واحدة فقط غير مصابة والتي اعتبرت معاملة تحكم إيجابية. تم جمع عينات البراز من بعض المرضى المصابين بطفيلي الزحار الأميبي الذين يعانون من إسهال مخاطي دموي، الذين حضروا الى مختبرات مستشفى بابل التعليمي للولادة والأطفال ، ومستشفى الحلة التعليمي العام ، ومستشفى النور للأطفال في محافظة بابل.

تم تجريع كل جرذ عن طريق الفم 2 مل من عالق البراز الحاوي على اكياس الطفيل بينما جرعت المجموعة الأخيرة بالماء المقطر فقط. بعد التأكد من الإصابة ، تم استخدام تركيزات مختلفة من المستخلص المائي البارد والحار لجذور الكركم وبذور القرع (100 و 200 و 400 ملغم / مل) والميترونيدازول (250 و 500 ملغم / مل) لعلاج الجرذان المختبرية المصابة بالطفيلي بمعدل ثلاث مرات في اليوم.

أظهرت نتائج الدراسة أن مستخلص الكركم البارد بتركيز (400 ملغم / مل) كان أفضل علاج بين العلاجات المستخدمة ، حيث تم القضاء على الطفيل بعد خمسة أيام من العلاج ، يليه مستخلص الكركم الحار بنفس التركيز حيث تم القضاء على الطفيل بعد ستة أيام من العلاج. أما بالنسبة لمستخلص القرع فقد

الخلاصة

لوحظ أن المستخلص البارد بتركيز (400 مجم / مل) يقضي على الطفيل بعد سبعة أيام من العلاج ثم يتبعه مستخلص القرع الحار (400 مجم / مل) والذي يقضي على الطفيل بعد ثمانية أيام من العلاج ، كما وجد أن الميترونيدازول بتركيز (500 مجم / مل) فعال في علاج جميع الجرذان المصابة بعد خمسة أيام من العلاج.

تمت دراسة بعض المتغيرات الدموية والكيمياء الحيوية ، واعتماد العديد من معايير الدم ، بما في ذلك دراسة التغيرات في تعداد الدم الكامل (CBC) و المتمثل في تعداد خلايا الدم البيضاء (WBC) و تعداد الخلايا الليمفاوية و تعداد خلايا الدم الحمراء و الخلايا المحببة و الخلايا الوحيدة و الهيموغلوبين (HGB) و متوسط حجم الجسم (MCV) و متوسط الهيموغلوبين العضلي (MCH). أظهرت النتائج انخفاضًا معنويًا في قيم (WBC، granulocytes، RBC، HGB، MCV MCH) لمعاملة التحكم الإيجابي وزيادتها في الخلايا الليمفاوية والوحيدة. أما بالنسبة للمعالجات (المستخلصات والميترونيدازول) فقد لوحظ استمرار الانخفاض المعنوي في قيم (WBC، Lymphocytes، Granulocytes) مع زيادة تراكيز العلاج (المستخلصات والميترونيدازول) وزيادتها في قيم الخلايا الوحيدة ومعلمات ال RBC و HGB و MCV و MCH مع زيادة تركيزات العلاجات (المستخلصات والميترونيدازول). كما أظهرت النتائج أن مستخلص الكركم البارد هو الأكثر فعالية في إعطاء مستويات قريبة من المستويات الطبيعية.

تم قياس إنزيمات الكبد (ALP و ALT و AST) كقياسات متعلقة بوظيفة الكبد لتحديد مدى تأثير العدوى الطفيلية على الكبد ومعالجته بالمستخلصات النباتية. أظهرت النتائج زيادة معنوية في (ALP وALT و AST) عند دخول الطفيل. بعد العلاج (المستخلصات والميترونيدازول) لوحظ أن جميع العلاجات وصلت إلى المستوى الطبيعي أو أعلى من المستوى الطبيعي. كما أظهرت النتائج أن مستخلص الكركم البارد كان الأكثر فاعلية في خفض مستويات الإنزيمات (ALP و ALT و AST) بشكل كبير بإعطائه مستويات قريبة من المعدل الطبيعي (معاملة التحكم) والتي بلغت (1.57 و 14.5و 25.15)، على التوالي مقارنة ببقية العلاجات.

أظهر الفحص النسيجي وجود احتقان في الأوعية الدموية للكلى والكبد للحيوانات المصابة وغير المعالجة. أما بالنسبة للأمعاء الدقيقة فلم تلاحظ أي تغيرات في المقاطع النسيجية للأمعاء المعالجة بالمستخلصات النباتية باستثناء الجرذان المصابة بالطفيلي وغير المعالجة (معاملة السيطرة الموجبة) والتي أظهرت ارتشاح الخلايا اللمفية تحت المخاطية. وأوضحت النتائج أن مستخلص الكركم البارد والميترونيدازول لهما كفاءة عالية في علاج الطفيلي ، حيث لم تحدث تغيرات في المقاطع النسيجية للكبد والكلى والأمعاء مقارنة بمستخلص الكركم الحار ومستخلصات القرع الحارة والباردة مما اظهر وجود

الخلاصة

احتقان في الأوعية الدموية وارتشاح الخلايا الليمفاوية في الكلى والكبد للجرذان المصابة بالطفيلي والمعالجة بالمستخلصات