A Study of Some Physiological and Immunological Parameters in Albino Rats Experimentally Infected with Entamoeba Histolytica and Treated with Lectin

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Abstract

The current study aimed to investigate the effects of infecting albino rats with E. histolytica and injecting with plant lectin on some physiological and immunological parameters (Hepcidin, βtryptase, IL-12, IL-17). The experimental animals were divided into three groups, the first and second groups contained 12 animals, and the third group contained 21 animals. The animals of the first group were injected with 3 mg/ml of plant lectin (seed wheat lectin) three times between one injection and another, one week. The second group was experimentally infected with E. histolytica, while the last group was infected with E. histolytica after being injected with lectin. 5 ml of all experimental animals were withdrawn and divided into two groups; the first group was used to perform the complete blood count (CBC), while the second group was used to obtain the serum, which was kept at -20 c° for the later use for physiological and immunological tests using ELISA technique The result showed that lectin injection had a significant effect on the total numbers of leukocytes and the percentage of granulocytes in addition to the total number of blood platelets. As for E. histolytica infection, it had a significant effect on the total number of leukocytes and the percentage of lymphocytes, the percentage of granulocytes, in addition to their significant effects on the total number of erythrocytes and the concentration of hemoglobin. In the third group, E. histolytica infection after lectin injection had a significant effect on the total numbers of leukocytes, the percentage of lymphocytes and the percentage of monocytes. It was found from the results that lectin injection had a significant effect only on the concentration of β -tryptase, while the infection with E. histolytica had a significant effect on the concentration of β-tryptase and hepcidin. In the third group, E. histolytica after lectin injection had a significant effect on the concentration of β -tryptase only. We concluded from the results of the current study that lectin injection, E. histolytica infection, and both together, did not significantly affected the concentration of IL-12 and IL-17, while they significantly affected the concentration of β -tryptase only in all groups.

Keywords: Entamoeba histolytica. Lectin, Albino Rats, physiological Parameters, immunological Parameters.

1. Introduction

Entamoeba histolytica is pathogenic protozoa. It is the principal cause of human amoebiasis and belongs to the Entamoebidae family. It is one of the most widespread parasitic illnesses worldwide, third only to malaria and schistosomiasis (Kurt et al., 2008). E. histolytica infects over 50 million individuals worldwide each year, resulting in 40,000 to 100,000 fatalities (Kirimi, 2018). In areas with inadequate poor conditions, up to 50% of the population may be affected (Garmie, 2016). Amoebiasis is thought to impact roughly 10% of the global population, with 90% of those affected showing no clinical symptoms (Kumari et al., 2013). Amoebiasis is an invasive illness of the large intestine that can also affect the liver, lungs, pleura, pericardium, spleen, and, less commonly, the genitor-urinary tract, brain, and skin (Tillack et al., 2007). E. histolytica migration is required for the development of amoebiasis, as it causes tissue invasion and destruction (Labruyere et al., 2003). E. histolytica infection effect on haematological parameters by increased total White Blood Cells and Lymphocytes percentage, and decrease total Red Blood Cells and Hemoglobin concentration, that can cause anemia (Shaker and Hussein, 2016).

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

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

Received: 05.10.22, Revised: 16.11.22, Accepted: 21.12.22

macrophages, as well as its transfer across the placenta (Rauf et al., 2020). β -tryptase is a subfamily of trypsin-like proteinases that are stored in the secretory granules of mast cells. Upon mast cell activation/degranulation, these enzymes, along with other mediators, are released into the extracellular medium. β -tryptases are unique in that they are active enzymes in the mast cell granules but only have extracellular action. β -tryptases appear to be involved in many masts cell-mediated allergy and inflammatory disorders. The role of β -tryptase in asthma, an inflammatory illness of the airways caused frequently by allergies, has been suggested (Fiorucci and Ascoli, 2004).

Interleukin 12 (also known as IL-12p70 or simply IL-12) is an immunoregulatory cytokine produced primarily by antigen-presenting cells. expression regulates innate responses and defines the type of adaptive immune response after infection. IL-12 stimulates the production of interferon (IFN) and causes CD4+ T cells to develop into type 1 T helper (Th1) cells. IL-12 has been linked to the treatment of a variety of disorders, including viral and bacterial infections as well as cancer (Hamza et al., 2010). Because of its role in inflammatory disease, IL-17 is one of the most well-studied cytokines in immunology, the role of human IL-17 in inflammation was soon recognized. However, after the discovery of a developmentally differentiated CD4+ T helper subset that expresses IL-17 (the socalled Th17 lineage) and drives tissue inflammation, IL-17 became the focus of immunological investigation (Zenobia and Hajishengallis, 2015).

The present research aimed to study the effect of one of the plant lectins on some physiological and immunological parameters (CBC, Hepcidin, β -tryptase, IL-12 and IL-17) in albino rats experimentally infected with E. histolytica.

2. Materials and Methods

Stool Samples Collection

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

Experimental Animals

This study was conducted at the University of Babylon / College of Science / Department of Biology from February 2022 to April 2022. The present study used 45 adult female albino rats (Rattus rattus) obtained from the animal house of the Biology Department / College of Science at the

University of Babylon, their weight ranged between (150 -170 g) and placed in plastic cages designated for raising rats. The floors of the cages are equipped with sawdust, which is replaced continuously to maintain hygiene. The experimental animals were dosed with metronidazole for 7 days with 20 mg/kg dose every 12 hours for the purpose of eliminating parasitic infections (Beyhan and Hokelek, 2014) and left for a week to adapt to suitable environmental conditions in terms of temperature, drinking water and aeration. The animals were left for two weeks to adapt to the experimental conditions. with the maintenance of proper hygiene and sterilization.

Samples examination

Macroscopic Stool Samples Examination

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

Microscopic Stool Examination

The stool was microscopically examined by the Direct smear method. In this method, a drop of Normal Saline 0.9% (sodium chloride) was placed on one side of a clean, dry glass slide and another drop of Lugol's iodine dye to easily distinguish the core of the cyst, with the wooden stick, a small quantity of feces was taken and mixed in a good manner with Normal Saline and Lugol's iodine. Samples were taken from different places of the model, especially the mucous or bloods area to increase the likelihood of the parasite's emergence, then put the cover slide without causing air bubbles after removing any large particles from the sample and then examining it with a light microscope to see trophozoite and cysts phases under magnification force 400 X (Tanyuksel and Petri, 2003).

Preparation of E. histolytica Suspension

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

Determination of the dose of E. histolytica cysts

Taken 50µl of parasite suspension by a Micropipette, which was placed on a Haematocytometer slide, and Lugol's iodine stain was added to it and examined using a light microscope and the mean number of cysts was calculated for three replicates using a fixed volume method and it was approximately 50 cysts per 50µl. The mean of the replicates was (50) cyst per (50) microliters, equivalent to 1x103 cyst/milliliter. Then each rat was dosed orally with 2 ml of the

parasitic suspension employing of a specialized syringe to dose the rat. The rats were left for 7 days to ensure infection and to notice changes in the rat's feces in terms of texture, color and mucus presence (Chabuk, 2013).

Preparation of lectin dose

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

Experimental design

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

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

Group 2 (12 rats): 3 rats were dosed with normal saline 0.9 % and considered as a control group, all rats of this group were infected with E. histolytica, after 7 days dissected control group and 3 rats of the group, after 14 days dissected 3 rats and the remaining (3) rats dissected after 21 days.

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

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

Hematological parameters

CBC measurement of blood samples

The numbers of WBCs and RBCs were calculated,

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

Immunological Parameters

Hepcidin, β -tryptase, IL-12 and IL-17 were measured by using rat serum according to the kits procedure of Bioassay Technology Laboratory (BT LAB) of China company.

Statistical analysis

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

3. Results and Discussion

The Effect of Lectin injection on Haematological Parameters

The results of the present study results as in Table (1) showed the effect of lectin injections on different parameters hematological (Total WBCs, Lymphocytes percentage, Monocytes percentage, Granulocytes percentage, total RBCs, Hemoglobin concentration, and total PLT), where it was observed that the total WBCs changed significantly during the periods of injection, as well as the percentage of granulocytes and the total number of PLTs. As for the other hematological parameters, they were affected by an increase or decrease, but not significantly. Lectins proved to mediate diversified biological functions like cytotoxicity, agglutination, complement activation, cell-to-cell and hostpathogen communications, innate response, cell-to-cell signalling, and precipitate different types of sugars Recently, great interest has been developed for the research and applications of lectins in agriculture and medicine due to their antiparasitic and antimicrobial potentials (lordache et al., 2015), the effect was observed on total WBCs and caused to a significant increase of the period (first and second injection). According to Akinwande et al. (2004) a measurable increase in WBCs count of rats or any animal is a function of immunity or while to disease, Lymphocytes resistance percentage showed a significant increase in the period (second injection) (Alatorre-Cruz et al., 2018) because the lectin influence the initiation and regulation of lymphocyte activation and proliferation (Kilpatrick, 1999) and total PLT of the periods (second and third injection) as compared with the control group (Alatorre-Cruz et al., 2018). Monocytes percentage showed no significant change in all periods as compared with the control group (Willis et al., 2013), and a significant decrease in all period was observed in Granulocytes percentage (M Ezzat et al., 2019), Total RBCs in the periods (second and third injection) and HGB concentration of the period

(second injection) as compared with the control group.

Table (1): The effect of Lectin injection on Hematological Parameters in Albino Rats (R. rattus)							
	Hematological parameters Mean±SE						
Period	Total WBC	otal WBCLymphocytesMonocytesGranulocytesTotal RBC HGB					
	×10³/μl	%	%	%	×106/μl	g/dl	×10³/µl
Before injection (Control)	4.3±0.4	52.46±3.21	14.83±2.12	32.7±2.61	9.43±0.24	15.53±0.24	361.33±86.91
Before Injection (Control)	b	а	а	b	b	Ь	а
First injection(After 1 week)	8.1±0.3	65.06±0.71	15.96±1.58	18.96±2.29	6.64±0.26	11.16±0.2	584.33±52.09
Thist injection(After Tweek)	С	ab	а	а	ab	ab	ab
Second injection (After 2 weeks)	7.03±0.32	66.7±3.6	16.33±2.33	16.96±4.59	5.36±1.92	8.73±3.3	773.66±115.54
	С	b	а	а	а	а	b
Third injection (After 3 weeks)	5.03±0.59	61.07±2.35	19.3±2.75	20.63±3.31	5.8±0.09	9.93±0.23	656.66±14.74
	b	ab	а	а	а	ab	b
Sig. level	0.000*	0.117	0.566	0.039*	0.070	0.085	0.030*
$(*P \le 0.05)$							

The Effect of *E. histolytica* infection on Haematological Parameters

It was found that infection of albino rats with E. histolytica significantly increased or decreased all hematological parameters excepts percentage of monocytes and number of PLTs (Table 2). A significant increase was observed in total WBCs in the period (after 1 week of infection) and this may be explained by the increase in the Lymphocytes percentage in the period (after 3 weeks of infection) as compared with the control group. Because the infection with the pathogenic E. histolytica produces a marked immune response which results in the development of protective immunity or the reason for this may be due to the penetration of the vegetative stages of the parasite to the epithelial cells of the intestine as well as the hepatocytes, which leads to the response of these cells to the presence of the parasite and the secretion of cytokines which is among the chemokines that stimulate the increase in the number and migration of WBCs to the site of infection (Shaker and Hussein, 2016). While no significant change was observed in Monocytes percentage, Granulocytes percentage (Shlash, 2016) and total PLT of all periods as compared with the control group. This may suggest that E. histolytica activates platelets, and the degree of their activation determines their morphologic parameters. (Shaker and Hussein, 2016).

Total RBCs and HGB concentration showed a significant decrease in the periods (after 1,3 weeks of infection) as compared with the control group. The reason may be attributed to the activity of the vegetative stages of the parasite on phagocytosis, digestion and decomposition of RBCs, and this in turn leads to a lowering of the level of hemoglobin in the blood, which causes anemia (Shlash, 2016).

Table (1): The effect of Lectin injection on Hematological Parameters in Albino Rats (R. rattus)							
Hematological parameters Mean±SE							
Period	Total WBC *10³/µl	Lymphocytes %	Monocytes %	Granulocytes %	Total RBC *10 ⁶ /µl	HGB g/dl	PLT *10³/µl
Before infection (control)	4.1±0.41	52.83±3.43	15.96±2.32	31.2±2.69	9.36±0.2	15.53±0.37	360.33±87.62
before injection (control)	а	а	а	ab	С	С	а
After 1 week of infection	7.5±0.62	58.96±1.7	16.63±1.14	24.4±0.95	6.76±0.29	10.7±0.61	384.66±156.6
After I week of infection	b	ab	а	ab	а	а	а
After 2 weeks of infection	4.36±1.14	51.16±6.4	12.36±1.39	36.46±7.23	8.5±0.71	14.3±0.87	479.33±37.3
	а	а	а	b	bc	bc	а
After 3 weeks of infection	2.03±0.12	70.1±4.55	11.73±3.03	18.16±1.31	7.35±0.54	11.8±1.21	689.66±205.64
	а	b	а	а	ab	ab	а
Sig. level	0.004*	0.036*	0.318	0.049*	0.021*	0.021*	0.375
(*P ≤ 0.05)							

The Effect of *E. histolytica* infection after Lectin injection on Haematological Parameters

As shown in the result of present study (Table 3) there are variations (increase or decrease) in the levels of all hematological parameters in albino rats experimentally infected with *E. histolytica* after being injected with lectin. These changes were not

significant except in the total of number of WBCs, Lymphocytes percentage and Monocytes percentage, while the differences in other criteria were not significant. A significant increase in the periods (first, second injection and after 1,2 and 3 weeks of infection) was observed in total WBCs as compared with the control group and Lymphocytes percentage showed a significant increase in the period (second injection and after 2 weeks of infection). Because the injection of lectin and

infection with *E. histolytica* produces a marked immune response which results in the development of protective immunity, which leads to stimulate the increase in the number and migration of WBCs to the site of infection (Shlash, 2016; Shaker and Hussein, 2016). Total PLT showed a significant increase in the periods (second, third injection and after 3 weeks of infection) as compared with the control group (Alatorre-Cruz *et al.*, 2018). No significant change in all periods was observed in Monocytes percentage

as compared with the control group. While a significant decrease was observed in Granulocytes percentage and total RBCs in all period and HGB concentration in the periods (second injection and after 1,2,3 weeks of infection) as compared with the control group. Because the activity of the vegetative stages of the parasite on phagocytosis, digestion and decomposition of RBCs, and this in turn leads to a lowering of the level of hemoglobin in the blood, which causes anemia (Shlash, 2016).

Table (3): The effect of E. histolytica infection after Lectin injection on Hematological Parameters in Albino Rats (R. rattus)

Rais (R. Tattus)								
	Hematological parameters Mean±SE							
Period	Total WBC *103/µl	Lymphocytes %		Granulocyt es %	Total RBC *106/µl	HGB g/dl	PLT *103/µl	
Before infection (control)	4.4±0.34	53.4±3.9	15.96±2.03	30.63±2.64	8.9±0.27	14.8±0.7	453.59±922.01	
	b	a	abc	b	b	b	a	
First injection (After 1 week)	7.8±0.21 c	65.04±0.7 ab	15.96±1.58 abc	17.6±2.3	7.1±0.28 a	11.5±0.2 ab	531.33±50.3 ab	
Second injection (After 2 weeks)	6.07±0.39	66.8±3.7	16.33±2.33	16.2±4.1	5.4±1.9	9.1±3.4	653.72±110.43	
	c	b	bc	a	a	a	b	
Third injection (After 3 weeks)	5.01±0.58	60.06±6.06	19.3±2.75	21.60±3.1	5.7±0.09	10.2±0.3	795.72±15.63	
	b	ab	c	a	a	ab	b	
After 1 week of infection	6.93±1.17	71.36±3.63	9.93±1.13	18.7±2.51	7.06±0.46	11.36±0.89	620.33±37.25	
	c	ab	a	a	a	a	ab	
After 2 weeks of infection	7.45±0.06	78.26±0.68	10.43±0.31	11.3±0.46	5.19±0.76	8.66±1.36	621.65±54.03	
	c	b	ab	a	a	a	ab	
After 3 weeks of infection	6.53±0.14	64.66±5.24	15.06±1.96	20.26±3.29	6.58±0.33	10.83±0.67	632.33±19.36	
	c	a	ab	a	a	a	b	
Sig. level								
(*P ≤ 0.05)								

The Effect of Lectin injection on Physiological and Immunological Parameters

It was found that results of the current study (Table 4) that studied immunological parameters were affected by significantly increased or decreased in their concentrations during the periods of lectin injection of albino rats. Statical proved that the variations in immunological criteria concentrations during the injection periods were significant only in β -tryptase, while the other parameters were not significant. A significant decrease was observed in Hepcidin was responsible of iron homeostasis (Kwapisz et al.,2009), and a significant increase in IL-

17 was plays essential roles in the host immunity against infectious diseases and chronic inflammatory diseases, thus, resistant the injected lectin as a foreign body (da Silva et al., 2016). Statical proved that the variations in immunological criteria concentrations during the injection periods were significant only in β -tryptase, which plays a vital part in inflammation and serves as a marker of mast cell activation. The concentration of serum mast cell-tryptase is elevated in anaphylaxis, other allergic disorders and other haematological disorders, it is increased (Moreno et al., 2003; Payne and Kam, 2004), while the changed in IL-12 concentration were not significant.

Table (4): The effect of Lectin injection	on Physiological and Immunological Parameters in Albino Rats
	(R. rattus)

(N. Tattus)								
Period	Immunological parameters Mean±SE							
renod	Hepcidin Pg/ml	β -tryptase Pg/ml	IL-12 Pg/ml	IL-17 Pg/ml				
Before injection (control)	269.9±17.36 b	2.3±0.5 B	64.03±19.1 a	37.22±7.59 a				
Before Injection (control)	207.7±17.30 D	2.3±0.3 D	04.03±17.1 a					
First injection (After 1 week)	186.41±62.05 a	0.91±.0.09 A	55.26±9.55 a	69.21±20.02b				
Second injection (After 2 weeks)	145.37±9.38 a	0.57±0.03 A	61.92±10.47 a	58.77±11.69 a				
Second injection (After 2 weeks)	143.37 ± 7.30 a	0.37±0.03 A	01.72±10.47 a					
Third injection (After 3 weeks)	117.97±5.94 a	1.29±0.16 A	50.7±5.86 a	39.12±2.28 a				
Third injection (After 5 weeks)	117.77±3.74 d	1.29±0.10 A	50.7±5.00 a					
Sig. level	0.498	0.022*	0.859	0.303				
(*P ≤ 0.05)								

The Effect of *E. histolytica* infection on Physiological and Immunological Parameters

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

were not significant. A significant decrease was observed in the concentration of Hepcidin, because hepcidin levels rise during infections and inflammation, causing a decrease in serum iron levels and contributing to the development of inflammatory anemia, most likely as a host defense mechanism to limit iron availability to invading microbes. (Nemeth and Ganz, 2006; Al-badri et al.,2019). while we find that the concentration of β -tryptase which plays important role in asthma and an inflammatory, decreased. (Im et al., 1975). The variations in the concentration of IL-12 and IL-17 during the study period were not significant (AL-Mahdawy et al., 2016).

Table (5): The effect of <i>E. histolytica</i> infection on Physiological and Immunological Parameters in Albino Rats (<i>R. rattus</i>)							
	Immunological parameters Mean±SE						
Period	Hepcidin Pg/ml	β-tryptase pg/ml	IL-12 Pg/ml	IL-17 Pg/ml			
Before injection (control)	281.2±55.15 b	2.23±0.06 B	89.29±17.78 a	41.22±9.65 a			
* * * * * * * * * * * * * * * * * * * *			07.27±17.70 a				
After 1 week of infection	125.67±2.36 a	0.99±0.05 A	77.01±17.53 a	56.49±11.75 a			
After 2 weeks of infection	95.21±10.07 a	0.81±0.08 A	47.89±4.67 a	30.35±5.3 a			
After 2 weeks of infection			47.07±4.07 a				
After 3 weeks of infection	141.01±16.05 a	0.84±0.03 A	71.22±18.54 a	36.66±4.51 a			
After 5 weeks of infection			/1.22±10.54 a				
Sig. level	0.009*	0.000*	0.364	0.230			
(*P ≤ 0.05)							

The Effect of *E. histolytica* infection after Lectin injection on Physiological and Immunological Parameters

From the result of current study, there were a significant change increased or decreased in the concentration of the studied parameters in group of albino rats infected with *E. histolytica* after injection with lectin, except for the concentration of β -tryptase, where the changes in its concentration during the study period were not significant (Table 6). Where Hepcidin showed a decrease in all periods because *E. histolytica* infection decrease total RBCs, that due to anemia and serves a protective function against infections by removing extracellular iron from

the body during infection (Kwapisz et al.,2009; Albadri et al.,2019) and IL-17 showed an increase in , IL-17 was plays essential roles in the host immunity against infectious diseases and chronic inflammatory diseases, thus, resistant the injected lectin and E. histolytica as a foreign body (da Silva et al., 2016). While IL-12 showed no significant change during the study period. only β -tryptase, which release from mast cells to bloodstream in the cases of inflammatory disorders, show a significant change in its concentration during the study period and decrease in the period (first, second, third injection and after 3 weeks of infection) (Im et al., 1975; Moreno et al., 2003).

Table (6): The effect of E. histolytica infection after Lectin injection on Physiological and Immunological								
Parameters in Albino Rats (R. rattus)								
Period	Immunological parameters Mean±SE							
renod	Hepcidin Pg/ml	β-tryptase pg/ml	IL-12 Pg/ml	IL-17 Pg/ml				
Before infection (control)	263.52±9.48 b	2.1±0.15 d	56.84±3.97 a	35.78±4.77 a				
First injection (After 1 week)	186.41±62.05 a	0.91±0.09 ab	55.26±9.55 a	74.21±20.02 b				
Second injection (After 2 weeks)	145.37±16.26 a 0.57±0.03 a 61.92±10.47 a 58.77±11.69 ab							
Third injection (After 3 weeks)	117.97±5.94 a	1.29±0.16 bc	50.7±5.86 a	39.12±2.28 a				
After 1 week of infection	169.44±12.57 a	2.05±0.09 d	57.54±1.43 a	28.24±0.92 a				
After 2 weeks of infection	127.83±25.31 a	1.73±0.07 cd	55.08±13.53 a	44.03±5.23 ab				
After 3 weeks of infection	142.43±13.83 a	1.45±0.38 bc	65.61±15.99 a	48.59±8.81 ab				
Sig. level	0.612	0.000*	0.955	0.075				
$(*P \le 0.05)$								

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