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RESEARCH ARTICLE

Biochemical Dynamics of Acanthamoeba Infection: Hematological and Hormonal, Impacts in Laboratory Mice

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ABSTRACT

The study aimed to investigate the most important biochemical, hematological, and histological changes in the liver of mice infected with the Acanthamoeba parasite, as well as its effect on insulin hormone. Parasite infections were established in laboratory mice, and biochemical, hematological, and histological changes were investigated 1st, 2nd, and 4th weeks after infection, and compared to the results of the control group. Blood results showed a significant decrease in WBC counts and a significant increase in platelets in the second and fourth weeks of infection. Cholesterol and triglyceride results showed a significant increase in the second week, with non-significant differences in light and heavy lipoproteins and bilirubin. A significant increase in C-reactive protein and the liver enzymes AST, ALT, and LDH starting from the first week of infection until the fourth week of infection, while total protein and albumin concentrations did not show a significant difference throughout the infection period. Histological examination in the second week from infection revealed hepatic cell degeneration, enlargement of the sinusoids, infiltration of inflammatory cells, and progressed to congestion of the central portal vein, extensive necrosis, infiltration of inflammatory cells, especially eosinophils, around the vein in the fourth week from infection and the presence of the vegetative and cystic phases. Our results didn't show a significant difference in the levels of the insulin hormone all over the period of infection. In conclusion, this study revealed a harmful effect of Acanthamoeba on liver tissue and its function

INTRODUCTION

The one but yet dangerous amoeba known as Acanthamoeba operates as an independent living organism that would take advantage of the chance to cause fatal infections that concern the central nervous system. The health encroachments that occur as a result of Granulomatous amebic encephalitis (GAE) are commonly known as 'these conditions' [1]. It is amoeba cyst cystic maely of anarityrce that can cause some health issues. Amoebic keratitis (AK), an eye affliction that has been associated with contact lenses for its development (AK), is another such problem [2]. Using a cutaneous acanthamoebiasis (the wide-range of amoebic skin diseases representing a significant type of amoeba diseases) as example, Acanthamoeba causes this condition [3]. The infection can occusinosally to the bones and lungs at times.

Acanthamoeba as a pathogen causing more sickness and death among humans has made diagnostics inadequate and treatment options not uniform. Additionally, its resistance to treatment and transmission of micro-organisms have contributed to the emergence of a more damaging amoeba [6]. Basically, the amoeba species about its host and the host's immune system, together with the amount of agent the host is infected with may determine the severity of the infection [7].

Possibly, the findings of empirical studies demonstrated an association between parasite infection and the host. For instance, studies of mice in the laboratory are of incredible significance in investigating the Human Alzheimer's Disease Disease relationship [8]. Data collected result from elevating blood serum indicators of renal function such as urea, creatinine and other ionic components associated with this disease, also includes liver enzymes and others lipid levels [9, 10]. The possibility of disregarding the normal biological processes might thus lead to the occurrence of precocious disease while the impact on the everyday organism is hard to evaluate. This job is going to study how does acanthamoeba parasite alters physiological factors, with a particular focus on blood-biochemical indicating signs. The inter-relatedness of the onset of the infections to the time that they can be diagnosed in the laboratory has been hindered by the fact that the earier the time the condition is detected and for proper medical treatment to be started, then the chances of occurrence of systemic infections will be avoided. Thus this study through the sequence of analysis of any key markers of the liver will demonstrate the Acanthamoeba parasite's potential to produce corresponding pathophysiological changes in the host, which is more or less the liver.

MATERIALS AND METHODS

Isolation of Acanthamoeba parasite

Subjects at Basrah Teaching Hospital and Al-Sadr Teaching Hospital who were suspected of having an Acanthamoeba keratitis infection provided specimens under the direction letter No. 333, dated 12/19/2022, from the Basrah Health Department (Training and Human Development Center and Research Committee). Using a sterile cotton swab, material from the lower conjunctiva was collected to acquire eye samples. The swab was moved from side to side in a circular manner. The samples were then examined using a light microscope [11].

Cultivation media

Acanthamoeba was cultivated on Peptone Yeast Glucose Agar (PYGA) medium that said medium contains proteose peptone (0.75% w/v), yeast extract (0.75% w/v), glucose (1.5% w/v), and 1% agar. Aliquots of sterile aqueous solutions of sodium hydroxide (NaOH) at the initial concentration of 1mg per milliliter were added to the culture medium to obtain the final pH in the 6.5–6.6 range. Furthermore, antibiotic penicillin was valued at 0.1 mg/ml. After the media were sterilized, they were kept at an optimal medium temperature of 30 C to 32 oC for storage. Providing mass cultures for the organism both in its vegetative and cyst forms was one of the focuses in the research. It was the NNA agar medium that we used to deprive the parasite of nutrients. By mixing of 15 grams of agar (from the product Sigma-Aldrich) in the hot water of 1000 ml (121°C for 15 minutes), we sterilized needed solution for Nano-Neuronium Arrays (NNA).

Examination and staining technique

Utilizing a wet suspension method, two drops (100 µl) of Acanthamoeba suspension were spread out on a glass slide, and the sample was covered with the slide [13] in accordance with the previously described phenotypic features [14]. Using the wet suspension staining technique, a drop of eosin and methylene blue dye was added to a glass slide, the cover slide was fixed over the drop, and the samples were examined under an optical microscope at a magnification of 40 or 100. The dye was prepared beforehand in accordance with the manufacturer's instructions.

Cells viability test

The vitality of *Acanthamoeba* cells was examined using Trypan Blue dye at a concentration of 0.4% and a ratio of 1:1 according to the method of [15].

Counting of Acanthamoeba

After twice rinsing the culture with sterile Phosphate Buffered Saline (PBS) solution (17.8 g Na2HPO, 4.2 g KH2PO4, 80 g NaCl, 2 gm KCl), the amoeba cells were removed from the culture media and the centrifuge was run for five minutes at 2000 rpm. Following [16], the Acanthamoeba cells were counted with a hemocytometer and inspected under an optical microscope. A series of dilutions (2×104, 6×106, and 12×104) of cells per milliliter of PBS solution were then made to determine the right number of cells to infect laboratory mice [17].

Experiment animals

This investigation was carried out using fifty Balb/C (Mus musculus) laboratory mice, weighing between 20 and 30 grams and with ages ranging from 6 to 12 weeks. There were four groups of mice created. Fifteen mice were slaughtered in the first group one week after contracting Acanthamoeba, and another fifteen mice were sacrificed in the second group two weeks later. Fifteen mice from the third group were put to death four weeks after infection. Five mice made comprised the fourth group, which served as a control group at the same time. The experimental mice were housed in 20*18*25 cm customized plastic cages with 12 hours of light and darkness, a regulated temperature of 25±3) oC, and an environment free of sickness. Throughout the trial, they were given water and a healthy meal.

Experimental infection with Acanthamoeba

The experimental infection of mice with the *Acanthamoeba* parasite was induced orally with a dose of $6*10^4$ cells of *Acanthamoeba*/ml/mouse. The parasite cultures were washed with sterile water and then centrifuged at a speed of 5000 rpm for 10 minutes. The sediment was then washed with sterile water and centrifuged again and finally, the number of cells in the sediment was counted using a Haematocytometer.

Blood sample collection and anatomical study

After the end of the specified infection period, blood was drawn from the heart. The laboratory animals were dissected one week, two, and four weeks after the experimental infection. Blood (0.7-1.5 ml) was drawn from these mice directly from the heart and then kept inside two types of laboratory tubes, the first one containing an anticoagulant to conduct blood tests, and the second one does not contain an anticoagulant to separate the blood serum using a centrifuge at a speed of 2000 rpm and for 15 minutes to perform biochemical tests.

Blood tests

Blood tests were conducted in the Biovet Lab (Basrah Province, Al-Mashreq, Iraq) after taking the blood samples inside test tubes containing EDTA anticoagulant to analyze the blood image, which included (the number of red and white blood cells and the number of platelets) using an automatic blood analyzer (Exigo800, Sewissland).

Biochemical analysis

Biochemical tests were conducted in the Biovet Lab (Basrah Province, Al-Mashreq, Iraq). The mandatory device was used for automatic chemical analysis. The device contains 54 holes numbered from 1 to 54. The model was placed in each hole, and the detector was placed in a special holder next to the holes to measure biochemical parameters. The serum biochemical parameters evaluated by this device included (total protein, albumin, cholesterol, triglycerides LDL, HDL, AST, ALT, LDH,

TBILC, and CRP). In addition, the levels of the hormones insulin and aldosterone were measured in the infected mice and the control group using an ELISA device using a special kit (Biotech, Germany).

Histological examination

Statistical examination

Data were collected from three replicates for each experiment, summed to determine the mean ± standard error (S.E.M.), and analyzed statistically using SPSS version 25 by ANOVA test among the experimental groups. P values less than 0.05 (P value < 0.05) were considered statistically significant (SPSS Science, Chicago, IL, USA).

RESULTS

Figure 1. Laboratory mice four weeks after infection with *Acanthamoeba*

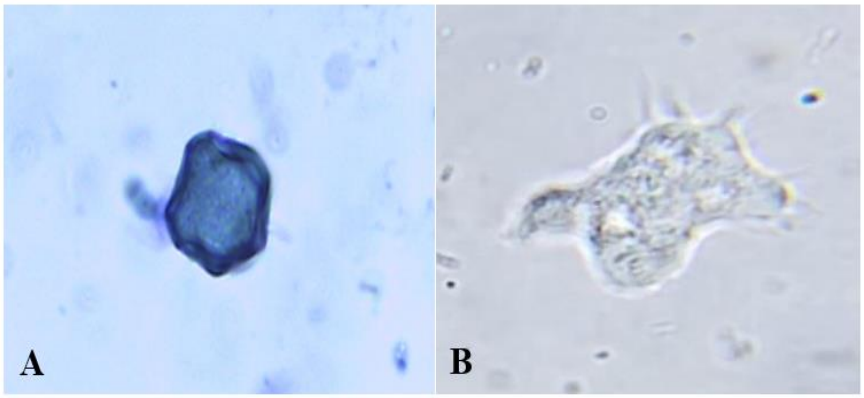

Figure 2. (A) Living cyst appeared blue when stained with trypan blue (magnification 100 x) .(B) Living trophozoiet without stain (magnification 100 x).

The effect of the parasite on the hematological parameters of white blood cells and platelets

In the present investigation, the number of white blood cells decreased significantly ($p<0.05$) in the second and fourth weeks of infection (3.02±0.58 and 4.04±1.19) in comparison to the control group's 6.63±0.90, but there was no significant difference in the first week (5.18±1.09) between the two groups (Figure 3). Regarding platelet counts, there was no discernible variation between the

experimental group (330.50±22.20) and the experimental group (291.00±18.86) during the first week of infection. However, as Figure 4 illustrates, there was a significant increase ($p<0.05$) in the experimental group (651.80±143.41 and 648.14±121.73) during the second and fourth weeks of infection.

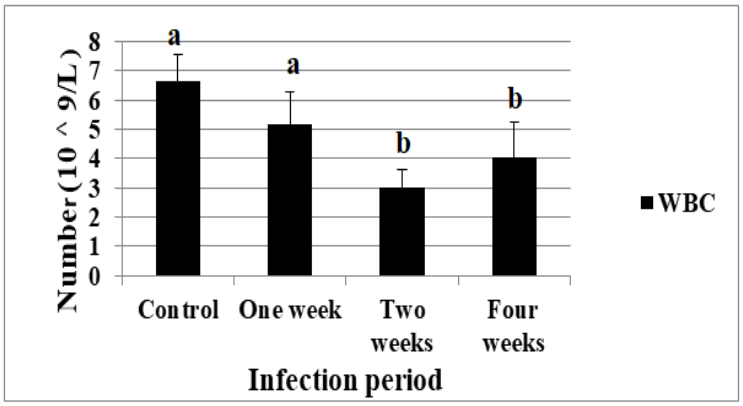

Figure 3. White blood cell counts (10^9/L) in laboratory mice one, two, and four weeks after infection with *Acanthamoeba*. The difference in letters within one group is statistically significant, with a significant difference at the p<0.05 level.

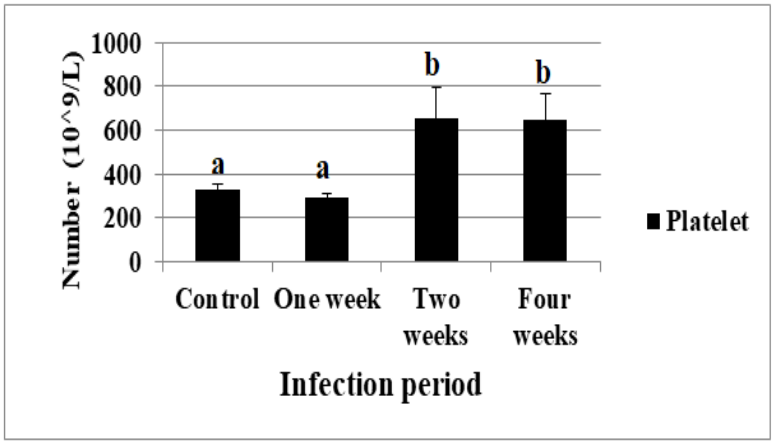

Figure 4. Platelets counts (10^9/L) in laboratory mice one, two, and four weeks after infection with *Acanthamoeba*. The difference in letters within one group is statistically significant, with a significant difference at the p<0.05 level

Effect of the parasite on biochemical parameters

The effect of the parasite on cholesterol and triglyceride concentrations

The present investigation found that the concentrations of triglycerides and cholesterol in the first and fourth weeks of infection (101.50±9.66 and 92.05±9.28) and in the second week of infection (168.64±9.78 and 188.94±22.79, respectively) were not significantly different from those in the control group (114.16±17.24 and 98.84±16.99, respectively). However, there was a significant increase ($p<0.05$) observed in the second week of infection (168.64±9.78 and 188.94±22.79, respectively, in comparison to the control group (Figure 5).

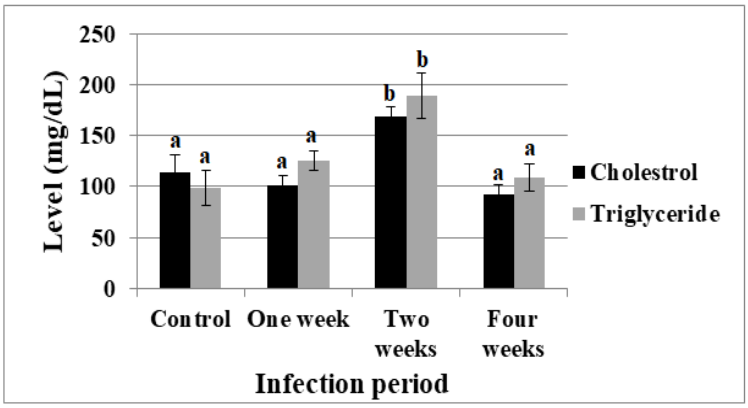

Figure 5. Laboratory mice's serum cholesterol and triglyceride levels (mg/dL) at one, two, and four weeks post-Acanthamoeba infection. A significant difference at the p<0.05 level indicates the variation in letters within the group.

The effect of the parasite on the concentrations of light and heavy fat proteins (LDL and HDL)

It was noted in the study that there were no significant differences in the level of LDL in all stages of the infection (19.45±2.70, 31.06±8.87, and 30.36±9.88), respectively, compared to the control group 23.76±5.84 Figure (6). Also, no significant differences were observed in the level of HDL in all stages of the infection (39.25±7.24, 42.39±6.02, and 42.58±5.99), respectively, compared with the control group 54.84±3.81 (Figure 6).

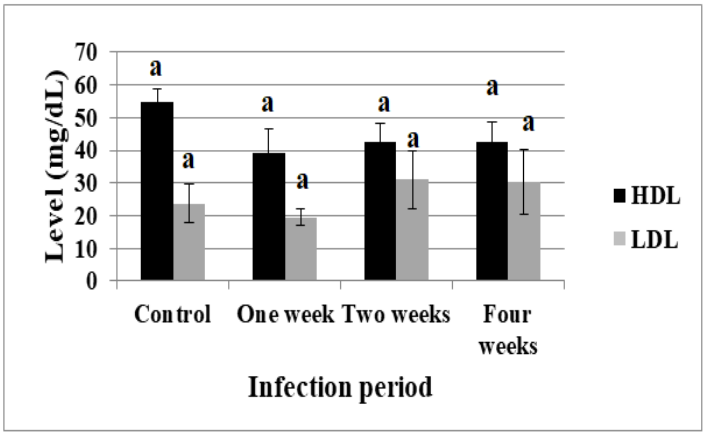

Figure 6. HDL and LDL level (mg/dL) in laboratory mice one, two, and four weeks after infection with *Acanthamoeba*. The difference in letters within group is indicated with a significant difference at the p<0.05.

The effect of the parasite on the concentrations of bilirubin and C-reactive protein

The study showed that there were no significant differences in total bilirubin levels in all stages of the infection during the first, second, and fourth weeks (0.77±0.18, 1.57±0.38, and 0.81±0.13), respectively, compared with the control group, 0.89±0.13 (Figure 7). While a significant increase (p<0.05) was observed in the concentration of CRP at all stages of parasite infection (4.59±0.52, 4.26±0.68, and 4.69±0.39), respectively, compared to the control group 1.28±0.19 (Figure 7).

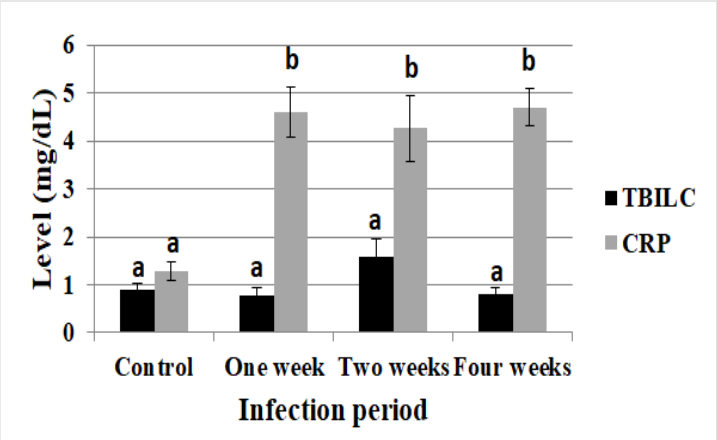

Figure 7. TBILC and CRP level (mg/dL) in laboratory mice one, two, and four weeks after infection with *Acanthamoeba*. The difference in letters within group is indicated with a significant difference at the p<0.05.

The effect of the parasite on the concentrations of liver enzymes ALT and AST

As for the AST and Alt concentration, it has been observed that there is a moral increase (P <0.05) at its levels in the first, second and fourth week of the parasite (278.22 ± 34.80, 215.11 ± 21.56, 224.00 ± 27.94) and (344.22 ± 64.00, 197.00 ± 20.86, 302.81 ±48.18) respectively compared to the control group (40.44±6.75 and 37.55±7.43) respectively, (Figure 8).

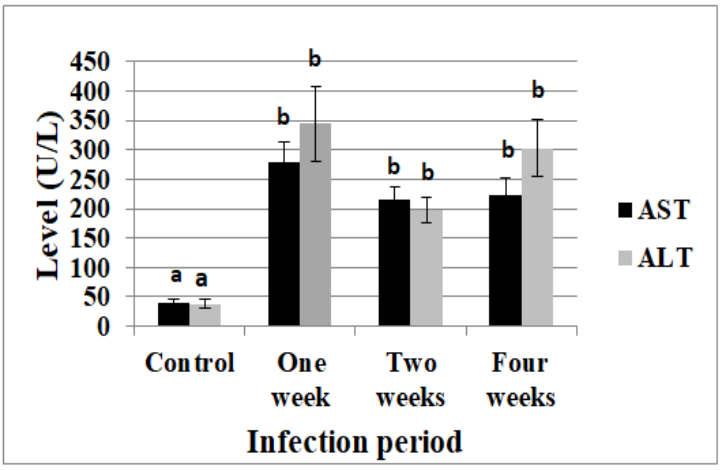

Figure 8. AST and ALT level (U/L) in laboratory mice one, two, and four weeks after infection with *Acanthamoeba*. The difference in letters within group is indicated with a significant difference at the p<0.05.

The effect of the parasite on the concentrations of the enzyme Lactic Dehydrogenase (LDH)

The study showed a significant increase (p<0.05) in the concentration of LDH in the first week of infection, 517.88±61.52, compared to the control group, 268.44±34.23, and a significant (p<0.05) gradual increase in the second and fourth weeks of infection (978.55±91.19 and 1134.54). ±144.43) respectively compared to the control group (Figure 9).

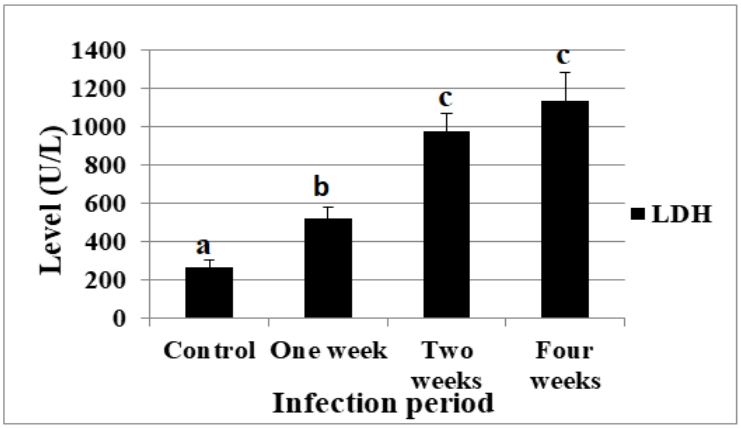

Figure 9. LDH(U/L) enzyme levels in laboratory mice one, two, and four weeks after infection with *Acanthamoeba*. The difference in letters within one group is statistically significant, with a significant difference at the p<0.05 level.

The effect of the parasite on total protein and albumin concentrations

It was also noted from the results that there were no significant differences in the concentrations of total protein and albumin at all stages of parasite infection in the first, second, and fourth weeks (3.84±0.63, 3.81±0.71, and 5.90±0.54) and (1.82±0.31, 2.14±0.48, and 3.42±0.35) respectively compared with the control group (4.73±0.62 and 2.26±0.43), as shown in Figure 10.

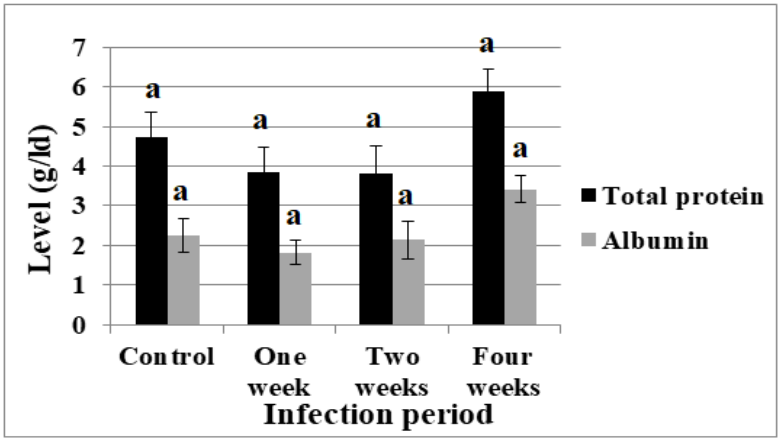

Figure 10. Total protein and albumin levels (g/dL) in laboratory mice one, two, and four weeks after infection with *Acanthamoeba*. The difference in letters within one group is statistically significant, with a significant difference at the p<0.05 level.

The effect of the parasite on insulin

The present study demonstrated that there were no statistically significant variations in insulin hormone levels across all stages of infection with the parasite, including the first, second, and fourth weeks (0.29±0.03, 0.27±0.03, and 0.19±0.01), when compared to the control group (0.23±0.02) as depicted in Figure 11.

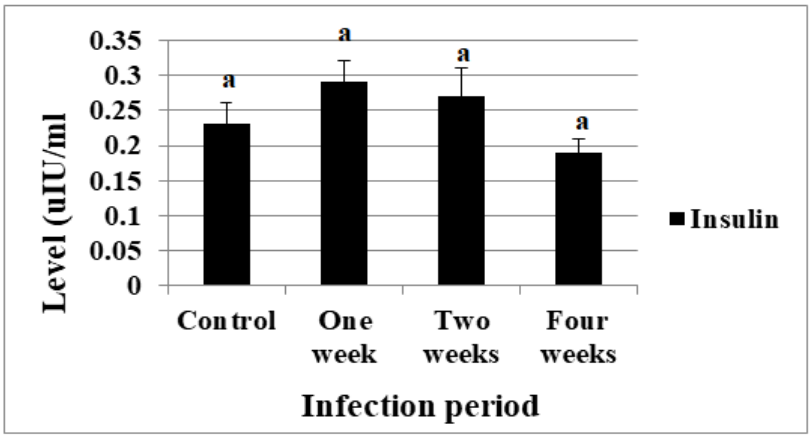

Figure 11. Insulin levels (uIU/ml) in laboratory mice one, two, and four weeks after infection with *Acanthamoeba*. The difference in letters within one group is statistically significant, with a significant difference at the p<0.05 level.

Histological changes in the liver of mice infected with the parasite

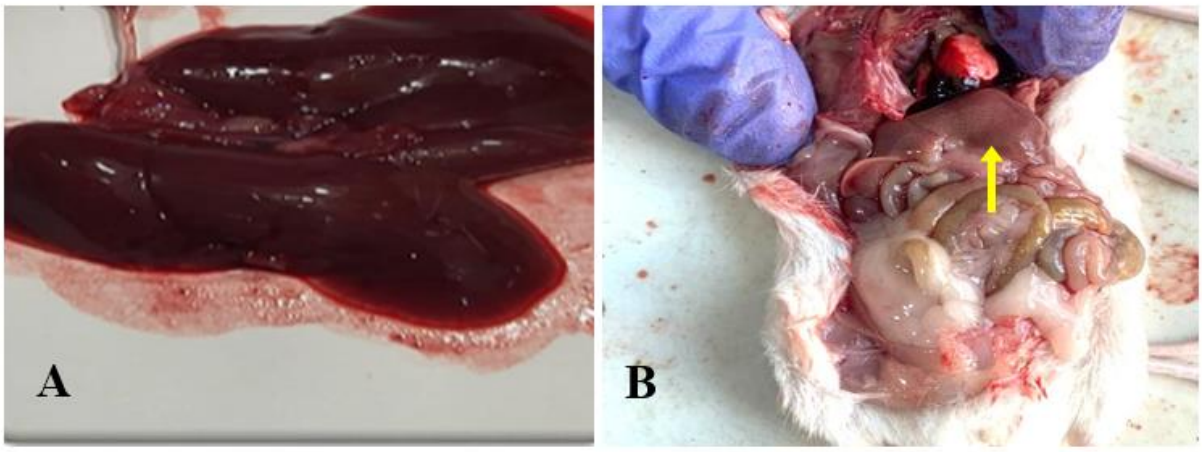

Figure 12. (A) Mouse liver within in control group (B) Mouse liver with necrosis after four weeks from induced infection with *Acanthamoeba*

Histological sections of the livers of the control group showed that the liver tissue consists of a central vein surrounded by hepatic cells that are arranged in the form of radial cords extending from the center to the periphery, as in the Figure 13.

Microscopic examination of tissue sections taken from the livers of animals in the second group, after the first week of infection with the parasite, showed the occurrence of blood congestion and the accumulation of inflammatory cells, with the appearance of the vegetative and cystic phases, as in the Figure 14. The results of histological sections of the liver after the second week of infection with the parasite showed hepatic cell degeneration, enlargement of the sinusoids, infiltration of inflammatory cells, and the presence of the vegetative and cystic phases, as in Figure 15. Histological examinations four weeks after the parasitic infection of the mice showed congestion of the central portal vein,

extensive necrosis, infiltration of inflammatory cells, especially eosinophils, around the vein, and the presence of cystic and vegetative stages, as in the Figure 16.

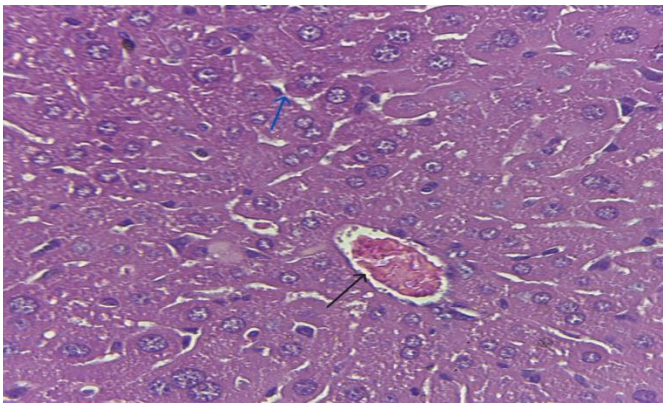

Figure 13. The Histological section of the liver of the control group mice shows normal hepatic cell cords H&E stain. 400X.

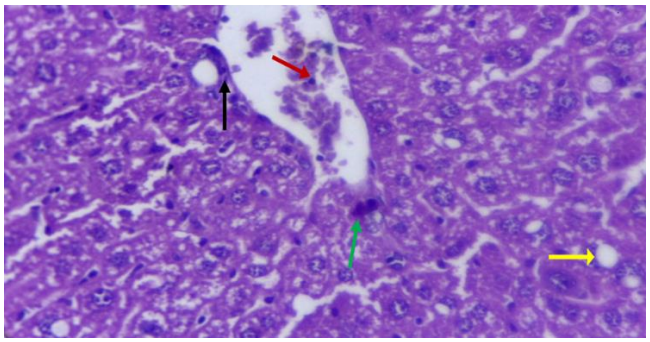

Figure 14. Histological section of the liver of mice after the first week of infection with Acanthamoeba vascular cuffing of inflammatory cells (black arrow), the presence of ruptures between hepatocytes, with trophozoite (green arrow) and cyst (red arrow) H&E stain. 400X .

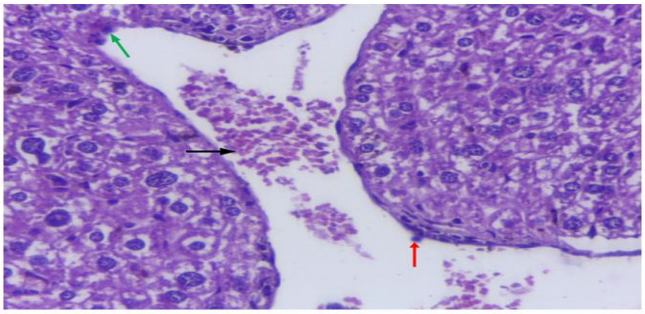

Figure 15. Histological section of the liver of mice after the second week of infection with Acanthamoeba congested blood vessels (black arrow) with trophozoite (green arrow) and cyst (red arrow) H&E stain. 400X

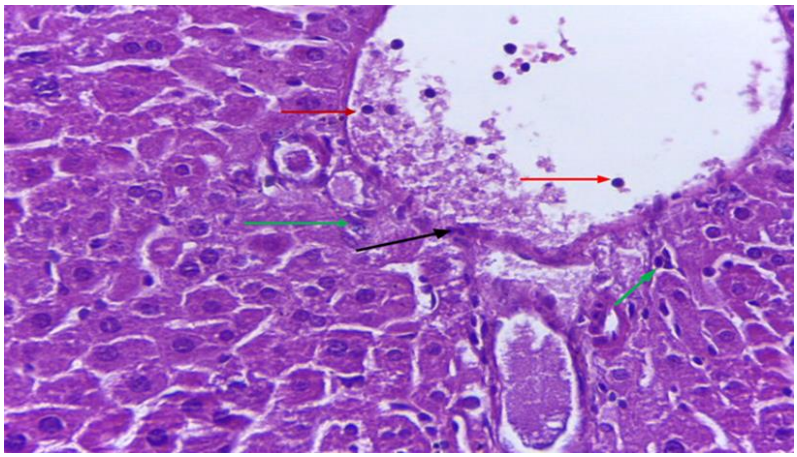

Figure 16. Histological section of the liver after the fourth week of infection with Acanthamoeba necrotic thrombosis appears surrounding the central vein (black arrow) with spread trophozoite (green arrow) and cyst (red arrow) H&E stain. 400X

DISCUSSION

Many human disorders that impact the liver, lungs, kidneys, heart, eyes, brain, and other organs are significantly influenced by Acanthamoeba. Infections in the skin, diaphragm, adrenal gland, pancreas, prostate, lymph nodes, and bone marrow are among the other bodily regions where it also causes infections. The highly virulent pathogenic strains of Acanthamoeba are responsible for its pathogenicity and pose a serious danger to human health, especially in those with weakened immune systems [19]. Similar results on Acanthamoeba's ability to infiltrate organs, survival potential, ability to elude the innate immune system, and ability to cause infection were found in the current study.

Also, in the control group, they had significantly smaller white blood cells count than the ones of experimental groups were observed by the current study. Parasitic infections induce inflammation, thereby shrinking the number of red blood cells as their proteins migrate to a surrounding tissue rather than blood vessels due to a protective immune reaction [20]. This result is in line with another research [21] documented a cell count decrease which have resulted from and supposedly as the consequence of some physiological alterations in the blood of a seven-month old child diagnosed with amoebic pneumonia. The current study found a significant increase in platelet count in all of the groups as well as the control group when compared to the lab. The dots arouse when hemorrhagic patches develop in infection laboratory mice and rupture of the impaired vessels happens. This is through the parasite itself and the response of the tissue that is observed in the tissue, thus bleeding of the tissue captured within the parasite to occur, eventually, leading to the rise in the count. Anemia is the result of malaria infection that creates a decrease in cellular hemoglobin which is responsible for carrying oxygen within erythrocytes. The platelets which are a clotting agent that curtails the bleeding stop the bleeding as well as help with the repair of the tissues damaged by parasites. The current studies strengthen the findings of the previous work [22] that have shown that infections or chronic conditions may cause the count of platelets to rise, however, the level may be seen to return to normal following recovery from the infection or the chronic condition. So, the disease is regulated by allowing thrombopoietin cytokines to be at the desired level in the bloodstream after these stimulates the process of platelet production. Cholesterol plays a crucial role in cellular immunity, since its reduction impairs the function of lymphocytes and macrophages, making it easier for infections, particularly pneumonia, to develop [23]. The present investigation showed that throughout the first and fourth weeks of infection, cholesterol and triglyceride levels remained

within the normal range, similar to the control group. However, a notable elevation in these values was seen during the second week of infection.

This study resembled the research conducted by [24], as it demonstrated a correlation between *Acanthamoeba* infection and alterations in triglyceride metabolism within the host. The study observed that levels of triglycerides were elevated in mice infected with *Acanthamoeba*. Furthermore, mice infected with the parasite and possessing normal immunity exhibited a substantial increase in triglyceride levels, surpassing those of infected and immunosuppressed mice. Elevated triglyceride levels in the body may be attributed to certain liver or kidney infections [25]. This finding aligns with the present research, which validates the existence of liver and kidney infections. [26] suggest that elevated triglyceride levels in dogs with babesiosis are linked to heightened liver enzyme activity or the immunological response of the host.

The restoration of triglyceride levels to baseline in the fourth week of infection may suggest that autoimmunity has assumed dominance over the parasite's defensive mechanisms, therefore diminishing its capacity to inflict more harm on the liver and kidneys. As the duration of infection lengthens, it might result in chronic infections.

Throughout the study, there were no notable variations in the levels of HDL and LDL at any point - one, two, and four weeks - when compared to the control group. This finding aligns with the research conducted by [27], which affirms that the proportions of high-density and low-density lipoproteins remain stable in mice infected with the parasite. Additionally, this includes the presence of innate immunity.

The present investigation validated that LDH levels exhibited a notable rise during the first week of *Acanthamoeba* infection, and this escalation further intensified during the second week, ultimately reaching elevated levels by the fourth week of infection, in comparison to the control group. LDH levels in the bloodstream increase due to tissue degradation, leading to elevated levels in many medical conditions such as hematological disorders, malignancies, fibrotic tissue, hepatic ailments, cardiac insufficiency, and numerous respiratory illnesses. Elevated levels of LDH indicate widespread organ damage, with particular impact on liver and heart function [28].

The research conducted by [29] found that elevated LDH levels in the blood serum had detrimental effects on individuals with immunodeficiency.

The present research showed consistently high concentration of total bilirubin at all-time point measurements (1st, 2nd and 4th weeks). The key purpose of the bilateral procedure is to enumerate the presence of such diseases as GI tract disorders and bloodstream disorders [30]. It is the fact that the contribution from either blocked bile ducts in the liver or gallbladder is uniformly constant that is responsible for not having any more than beginning stage fibrosis of the liver. The blood plasma level of CRP, a liver protein, demonstrated the sharpest upward trend among all parameters in the course of the study. The presence of a high number of CRP is an indicator of associated health problems which range from acute to chronic infections; such as bacterial or viral infections since this protein is synthesized by liver at the time of acute inflammatory process and transports the bloodstream to various organs of the body by means of fibrinogen. Thus, it allows the conjunction of the subjective and objective data and establishes an opinion of an experienced doctor. Such infections as abscesses and severe infections have a tendency to become long connection, with pus formation noted repeatedly in 30 % of cases [30].

Research has revealed that the latter is a significant note related to liver tissue architecture damage. The enzymes of the liver function in the energy metabolism, managing moving of the amino acids around the body, and found in many tissues, such as the brain, the heart muscle and the liver [31]. Leak of ALT and AST enzymes is expected to occur as these are bound to the inside of the hepatocytes, this could increase their activities outside of their cells [32]. The results stated that both ALT and AST

enzymes at all stages had elevated levels, differed significantly from the control group. Similar concentrations of ALT and AST were consistent with the results from [32] who isolated immune-compromised mice as well as organisms from the Acanthamoeba genus caused amoebic infections. The current analysis showed that the amounts of albumin and total protein were stable, which is similar with the results of the research by [10], indicating that their percentages were stable. Since the total protein level test offers information on kidney function and is part of routine testing to evaluate general health and identify renal illnesses and anomalies, its stability may be linked to the kidneys performing their functions. The lack of effect on albumin levels shown in this investigation implies that kidney and liver infections have not yet progressed to a point where they would materially alter albumin levels. It also suggests that liver diseases, including cirrhosis or chronic liver disease, have not advanced to the point where ascites, or the buildup of fluid in the abdomen, has become common. The underlying cause of this incidence is often linked to liver failure. There is only one source from which albumin is produced [33], and a shortage of albumin in the circulation is caused by nephrotic illness, a chronic kidney infection [34].

The current study concluded that the insulin hormone remained at normal levels since there was no discernible variation from the control group. Insulin is utilized as a diagnostic tool to find aberrant blood sugar levels that may indicate the presence of insulinemia, metabolic syndrome, or blood problems [35]. The steady amounts of insulin observed in the first, second, and fourth weeks of the experiment indicate that the pancreas was unharmed. There are two reasons why this phenomenon could occur. One is that the parasite's ability to damage the pancreas has been diminished and inflammation has been suppressed by the immune system [36]. The alternative is that the parasite's harm hasn't yet increased to the point where it may spread sickness [37]. This study, which is the first to examine how the Acanthamoeba parasite affects blood insulin levels in lab mice, definitively shows that insulin levels were constant during the whole trial.

Regarding the histological alterations in the liver of laboratory mice infected with the Acanthamoeba parasite, the findings of this investigation demonstrated a discernible progression in the degree of infection in the liver from the initial to the fourth week of infection, exhibiting a build-up of inflammatory cells, blood congestion, and the emergence of the vegetative and cystic phases. Beginning in the second week, there was a noticeable deterioration of the hepatic cells, growth of the sinusoids, infiltration of inflammatory cells, and the existence of two stages of the parasite, all of which were linked to the hydrolytic enzymes of the parasite. Since all Acanthamoeba strains contain efficient hydrolytic enzymes and play a significant role in the parasite's pathogenicity, this has been verified by [36]. The hepatic portal vein was congested in the four-week group, and there was widespread necrosis along with the infiltration of inflammatory cells, particularly eosinophil cells around the central vein. This is unmistakable proof of a parasitic infection and the propagation of the parasite in all of its phases. It is also in line with research [38] that found the parasite may infiltrate blood arteries, the central vein, and sinusoids. Furthermore, in laboratory mice infected with Acanthamoeba, [38] demonstrated the parasite's capacity to induce necrosis in the liver parenchyma, resulting in significant bleeding from the necrosis of cells and blood vessels. The present study's advanced Acanthamoeba-infected animals demonstrated both the presence of defense cell clusters in the liver tissue and the extensive parasite infection inside the liver lobules, which resulted in necrosis in the liver cells.

CONCLUSION

Acanthamoeba parasite causes clear changes in the tissue structure of the liver and affects the liver enzymes AST, ALT, and LDH and combined with an increase in CPR levels in the blood and revealed hematological changes in white blood cells and platelets.

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CONFLICT OF INTEREST

In our study, there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

SH. A, S.K: Perceived and designed the study. S.A: Performed the experiments. S.A., SH. A, S.K: Wrote the manuscript. S.A., SH. A, S.K Analyzed the data.

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