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The therapeutic role of *Aloe vera* on some hematological and biochemical tests in rats infected with the *Entamoeba histolytica* parasite experimentally

Shahad Saad Daham

Tikrit University, College of Education for Pure Sciences

***shahd.saad@tu.edu.iq**

I. Abstract:

The current study was conducted on 10-week-old male albino rats weighing 250-280 grams. These rats were housed in special cages at the animal house of the College of Veterinary Medicine, Tikrit University, from the beginning of October 2025 until November 2025. They were divided into three groups: a control group, a group experimentally infected with the *Entamoeba histolytica* and a group infected and treated with an aqueous extract of *Aloe vera*. Statistical analysis showed a significant increase in white blood cells in the group infected with the parasite 14.25 ± 2.30 compared to the control group 6.73 ±1.52. Blood tests showed a significant decrease in hemoglobin levels 8.68 ±1.70 g/dl and packed cell volume 36.13 ± 0.70% in the group infected with the parasite compared to the control group 12.60 ± 0.54 g/dl, 39.70 ± 1.01% respectively. The infected and extract-treated groups did not show significant differences in white blood cells, hemoglobin, and red blood cell volume 12.13 ± 0.98 g/dl, 11.09 ± 1.24 g/dl and 37.10 ± 0.39%, respectively. The results showed a significant increase in liver enzymes (AST, ALT, ALP) in the parasite-infected group 61.13±28.69 ng/dl, 3839.00 ±930.04 pg/ml, 81.5 ±11.19 ng/ml respectively compared to the control group 2.03 ± 1.07 ng/ml, 376.4 ± 277.7 pg/ml and 21.62 ± 9.17 ng/ml respectively. The infected and treated groups showed no significant differences in liver enzymes (AST, ALT, and ALP) 26.84 ± 9.78 ng/ml, 2363 ± 888.9 pg/ml and 58.25 ± 3.99 ng/ml, respectively. However, a significant increase in kidney function tests (urea and creatinine) was observed in the infected group 53.88 ±16.18 mg/dl, 3.69 ±13.1 mg/dl, respectively compared to the control group 21.63 ± 5.24 mg/dl and 0.53 ± 0.17 mg/dl, respectively. No significant differences were observed in the levels of urea and creatinine in the infected group. The parasite and the treatment with the extract 2.5 ± 0.77 mg/dl and 42 ±13.05 mg/dl, respectively indicate the efficiency of the aqueous extract of the *Aloe vera* plant in eliminating the *E. histolytica*

Key word *E. histolytica, Aloe vera* **,** Liver enzyme, Renal function

II. Introduction:

Entamoeba histolytica is an intestinal parasite that causes amoebiasis in humans, which is responsible for approximately 100,000 deaths annually and is a major cause of death (1). *E. histolytica* is most prevalent in subtropical and tropical regions of the world. It is linked to unfavorable health conditions and economic and social conditions(2**).** The parasite is present in human feces in several stages: the trophozoite stage, the precystic stage, the cystic stage, and the precystic stage. It causes symptoms such as bloody diarrhea, intestinal cramps, fever, and fatigue (3**).** The parasite is transmitted to humans and causes diseases through the consumption of food and drink contaminated with parasite

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cysts (4**).** Damage to the epithelial cell layer lining the large intestine in humans includes the destruction of tissue cells, resulting in bloody diarrhea(5). *Aloe vera* is a well-known medicinal plant containing numerous active compounds such as amino acids, polysaccharides, enzymes, and vitamins. These compounds contribute to antioxidant, anti-inflammatory, and immune-modulating effects (6). *Aloe vera* also contains active compounds such as flavonoids, tannins, saponins, sterols, resins, terpenoids, and anthrones (7**).** It has played a role in medical practices; it was used in horde medicine as far back as 2000 years ago and is a vital ingredient in many food products. Its effectiveness and versatility make it a must-have in any natural therapy **(**8). It is still widely used in many modern societies, where countries like Africa, China, and India rely on it as an essential component of their traditional medicine (9).

III. Materials and Methods:

2-1-Laboratory animals:

This study was conducted on male albino rats aged 10 weeks and weighing 250-280 grams. These rats were placed in suitable cages and lined with wood shavings taken from the animal house of the College of Veterinary Medicine/Tikrit University. Throughout the study period, the rats were placed under laboratory conditions in terms of the degree and availability of ventilation, temperature, and appropriate lighting and were provided with the prepared feed and water until the end of the experiment.

2-2-Parasite isolation:

The parasite was isolated from stool samples of individuals with bloody diarrhea who were diagnosed with *Entamoeba histolytica* using a microscope, as follows: 1.Take 1.5 g of stool, add distilled water, and filter through several layers of gauze. Then, collect the filtrate and add distilled water to it.

2- Centrifuge the tubes at 6000 rpm for 6 minutes.

3- Collect the precipitate and discard the filtrate. Repeat the process until the filtrate becomes a clear solution.

4- Add 2 ml of zinc sulfate to the precipitate. Centrifuge the tubes at 6000 rpm for 6 minutes. Discard the supernatant and collect the precipitate.

5- Add 2 ml of zinc sulfate to the precipitate and store at -20°C (10).

2-3- Determination of infection does

A 0.1 ml drop of the amoebic suspension was taken and placed on a haemocytometer slide using a micropipette. The number of amoebic cysts in one drop was counted to determine the infection dose in mice according to the following equation: 200 x (number of cysts)/(0.2). The amoebic suspension was kept at -20°C until use (10).

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2-4- Experimental Design:

The animals were divided into three groups, each consisting of eight rats of similar weights, as follows:

1- **Group One: Negative Control Group (G1**): This group consisted of eight rats that were given normal physiological saline for 14 days.

2**- Group Two: Positive Control Group (G2):** This group consisted of eight rats that were given a suspension of amoebic cysts (6000) using an 18-gauge curved needle. The needle was inserted orally, and the liquid containing the parasite cysts was pushed directly into the stomach for 10 days. The negative control rats were given physiological saline in the same manner.

The negative control rats were given physiological saline in the same way. Two weeks after infection, the rats were placed in clean cages. The presence of parasite cysts in the feces of the infected rats was investigated experimentally by preparing several fecal smears on glass slides and examining them under a microscope to observe the parasite and its various stages. After confirming infection, blood was drawn from the rats 6 ml and 3 ml was placed in EDTA tubes for hematological analysis, and 3 ml was placed in gel tubes for biochemical analysis.

3-**Group Three: The Infected and Treated Group (G3**): It included 8 rats infected with the *Entamoeba histolytica* parasite, and they were given an aqueous extract of *Aloe vera* at a dose of 0.02 mg/kg daily for 14 days. After confirming the infection of the animals, they were given the plant extract. After the end of the specified period of the experiment, blood was drawn from the rats 6 ml and 3 ml were placed in EDTA tubes for hematological tests and 3 ml in gel tubes for biochemical tests.

2-5-Collection of *Aloe vera* plant samples:

Aloe vera leaves were obtained from nurseries in Tikrit and classified according to (11). The leaves were washed with distilled water to remove dust and dirt, then left to air dry. After that, they were cut into small pieces and dried in the oven for 24 hours at a temperature of 45°C.

2-6-Preparation of *Aloe vera* Gel Extracts:

1-Crude *Aloe vera* Gel Extract:

The crude extract of *Aloe vera* gel was obtained from the leaves as follows:

The *Aoe vera* leaves were cut lengthwise into two equal halves. The gel was then extracted using a tablespoon and placed in an electric blender for 3 minutes. It was then filtered through four layers of cheesecloth. The sediment was collected, and the filtrate was discarded.

2-Preparation of Aqueous *Aloe vera* Gel Extract:

500 grams of crude gel were collected as in the previous step. This was then mixed with 1000 ml of distilled water (aqueous extract) using an electric blender for 3 minutes. The mixture was left to stand

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for 12 hours, with continuous stirring using an electric shaker. It was then placed in an incubator at 37°C until it became a dry powder. The powder was then collected in opaque bottles and stored in the refrigerator until use (12).

2-7-Heamatological analysis:

Haematological analysis was performed in central laboratories at Tikrit University using an Automatic Haematology Analyser (CBC Mindray BC-3000 Plus) as described by the manufacturer.

2-8-Biochemical tests:

1.Measurement of AST enzyme concentration in rat serum: The enzyme concentration was measured using an ELISA analyser with a specialised kit from Bioditech, a Korean company.

2.Measurement of ALT enzyme concentration in rat serum: The enzyme concentration was measured using an ELISA analyser with a specialised kit from Bioditech, a Korean company.

3.Measurement of ALK.P enzyme concentration in rat serum: The enzyme concentration was measured using an ELISA analyser with a specialised kit from Bioditech, a Korean company.

4-Measurement of urea concentration in rat serum: The urea concentration was measured using a spectrophotometer with a specialised kit from Biolab, a French company.

5.Measurement of creatinine concentration in rat serum: The creatinine concentration was measured using a spectrophotometer with a specialised kit from Biolab, a French company.

6.Measurement of uric acid concentration in rat serum: The uric acid concentration was measured using a spectrophotometer with a specialised kit from Biolab, a French company.

2-9 - Statistical Analysis:

The data were analyzed using the statistical software (Minitab) on a computer, and the statistical difference between the three experimental groups was analyzed using one-way analysis of variance (ANOVA).

3-Results and Discussion:

3-1-Effect of infection with the *Entamoeba histolytica* parasite on blood tests in rats treated with an aqueous extract of *Aloe vera* and comparison with the control group:

1-Measurement of White blood cells (WBC) in rat blood:

The results of the current study showed a significant increase in the concentration of white blood cells, reaching 14.25 ±2.30 in the group infected with the *Entamoeba histolytica* parasite experimentally, compared to the control group, which reached 6.73 ± 1.52 Significant differences were

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recorded at a probability level of $p \leq 0.001$ between the group infected with the parasite and the control group. However, after using the aqueous extract of the *Aloe vera* plant, the results showed no significant differences in white blood cells in the infected group, which reached 12.13±0.98 (Table 1).

2-**Measurement of Hemoglobin (Hb) in Rat Blood:**

The results of the current study showed a significant decrease in hemoglobin (Hb) concentration in rats infected with the amoeba *Entamoeba histolytica* parasite 8.68 ± 1.70 g/dl compared to the control group 12.60 ± 0.54 g/dl. Significant differences were recorded at a probability level of $p \leq 0.001$ between the infected and control groups. No significant differences were observed after the use of an aqueous extract of *Aloe vera* in the infected group with a decrease of 11.09 ± 1.24 g/dl (Table1).

3-Measurement of Packed Red Cell Volume (PCV) in Rat Blood:

This current study observed a significant decrease in PCV concentration of 36.13 ± 0.70% in the rat group infected with the *Entamoeba histolytica* parasite experimentally compared to the control group 39.70 ±1.01% in the rats. The results of the statistical analysis showed significant differences at the probability level of $p \leq 0.001$ between the infected group and the control group. The results did not show significant differences after using the aqueous extract of the *Aloe vera* plant for the infected group, as it reached 37.10 ± 0.39% (Table 1).

Table (1) shows the blood tests in the group infected with the *Entamoeba histolytica* parasite and treated with an aqueous extract of the *Aloe vera* plant and compares them with the control group.

Blood tests / Groups	Mean ± S.D		
	W.B.C $\times 10^9$/L	**Hb g/dl**	**P.C.V %**
Negative Control	6.73 ± 1.52 **A**	12.60 ± 0.54 **A**	39.70 ± 1.01 **A**
Positive Control	14.25 ± 2.30 **B**	8.68 ± 1.70 **B**	36.13 ± 0.70 **B**
Infection and treatment group	12.13 ± 0.98 **C**	11.09 ±1.24 **C**	37.10 ± 0.39 **C**

M: mean **S.D:** standard deviation

***The different letters indicate significant differences at a probability level of ($p \leq 0.001$) between the rates of the different groups**

The results of the current study are consistent with those of researchers (13) who demonstrated a significant increase in white blood cells in the group experimentally infected with the *Entamoeba histolytica* parasite compared to the control group. An increase in white blood cell count is a normal consequence of parasitic infection. This increase is attributed to an inflammatory immune response in

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the intestinal mucosa and sub mucosa. Therefore, the elevated white blood cell count is synchronous with the heightened immune response during parasitic infection. White blood cells secrete toxic by products of the lysis of the *Entamoeba histolytica* trophozoite through adhesion and lysis mechanisms of the pathogen (15). These results also align with the findings of researchers (13) who reported that *E. histolytica* parasites cause a decrease in hemoglobin (Hb) and red blood cells (RBC), indicating anemia. This may be due to the ability of *Entamoeba histolytica* to destroy the plasma membranes of red blood cells, leading to hemolysis and a corresponding decrease in red blood cell count. The effect is directly proportional to hemoglobin (Hb) concentration and compressed red blood cell (PCV) volume, or it may result from a digestive disorder caused by the *E. histolytica* parasite. It was demonstrated (14) that the *Entamoeba histolytica* can overcome the body's defenses and destroy red blood cell membranes, thus reducing their number (which means a decrease in hemoglobin (Hb) and PCV volume). The results showed that *Aloe vera* extract plays an effective role in improving the hematological changes associated with parasitic infection, indicating its potential as a therapeutic or adjunctive agent in reducing the negative effects of infection on blood components. The results of this study are consistent with those of (13), who demonstrated in their study that an extract of the leaves of the *Ziziphus mauritiana* plant has the efficacy to improve some blood parameters in rats infected with the *Entamoeba histolytica* parasite by modifying the proportions of blood components without any apparent negative effects on the studied hematological tests.

3-2- Effect of parasite infection on liver enzymes in rats treated with an aqueous extract of *Aloe vera* and comparison with the control group:

1-Measurement of Aspartate aminotransferase (AST) concentration in rat serum:

This study recorded a significant increase ($p \leq 0.001$) in the average concentration of the liver enzyme AST in rats infected with the *Entamoeba histolytica* parasite 61.13 ± 28.69 ng/ml compared to the control group 2.03 ±1.07 ng/ml. After using an aqueous extract of *Aloe vera*, no significant differences were observed between the infected and treated groups, with the concentration remaining at 26.84 ± 9.78 ng/ml (Table 2).

2-Measurement of Alanine Aminotransferase (ALT) Concentration in Rat Serum:

The results of the study showed a significant increase ($p \leq 0.001$) in the average concentration of ALT enzyme in the rat group infected with *Entamoeba histolytica* 3839.00 ± 930.04 pg/ml compared to the uninfected control group 376.4 ± 277.7 pg/ml. Statistical analysis revealed significant differences between the infected and control groups. However, no significant differences were found after the use of an aqueous extract of *Aloe vera* in the infected group, with a concentration of 2363 ± 888.9 pg/ml (Table 2).

3- Measurement of Alkaline Phosphatase (ALP) Concentration in Rat Serum:

The results of the current study showed a significant increase ($p \leq 0.001$) in the average ALP concentration of 81.5 ± 11.19 ng/ml in rats infected with the *Entamoeba histolytica* parasite compared to the control group 21.62 ± 9.17 ng/ml. The results also showed no significant differences after administering the aqueous extract of *Aloe vera* to the infected rats 58.25 ±3.99 ng/ml, (Table 2).

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Table (2) Effect of infection with the *Entamoeba histolytica* parasite on liver enzymes in rats and comparison with the control group

Enzymes / Groups	Mean ± S.D		
	AST ng /ml	**ALT pg /ml**	**ALP ng /ml**
Negative Control	2.03 ± 1.07 **A**	376.4 ± 277.7 **A**	21.62 ± 9.17 **A**
Positive Control	61.13 ± 28.69 **B**	3839.00 ± 930.04 **B**	81.5 ± 11.19 **B**
Infection and treatment group	26.84 ± 9.78 **C**	2363 ± 888.9 **C**	58.25 ± 3.99 **C**

***The different letters indicate significant differences at a probability level of ($p \leq 0.001$) between the rates of the different groups**

The results of this study are consistent with (18), which indicated a significant increase in the levels of liver enzymes (AST, ALT) in mice infected with the parasite compared to the control group. They are also consistent with (19), which indicated a significant increase in the liver enzymes (AST, ALT) in the group experimentally infected with the *Entamoeba histolytica* parasite. The reason for the increase in the concentration of AST and ALT is that these enzymes are secreted and remain in the liver. When the liver is infected with the *E. histolytica* parasite, the liver cells release these enzymes into the blood, which raises the levels of AST and ALT in it, indicating liver disease. This increase in AST and ALT causes the deterioration of liver cell tissue that occurs during the migration of the parasites (20). The AST enzyme is found in various body tissues, particularly skeletal muscle, kidneys, liver, and heart. The ALT enzyme is specialized for liver cell activity. Any damage to liver cells due to parasite invasion leads to the release of these enzymes into the bloodstream, thus increasing their concentration. This explains the increased activity of both ALT and AST enzymes in groups infected with *E. histolytica* compared to the control group. The results of the current study are consistent with study (21), which showed a significant increase in alkaline phosphatase (ALP) levels in mice infected with the *E. histolytica* before treatment compared to the control group. ALP is an enzyme found in the bile ducts of the liver and in bones and intestines and is an indicator of liver diseases. High levels of ALP can result in bile duct obstruction due to parasite infection. These tests can reveal various details related to a range of diseases (22). The results showed that *Aloe vera* extract has an effective role in improving liver enzymes in the group infected with the parasite, which demonstrates its potential as an effective and safe treatment for improving liver enzymes. The results of the study are consistent with the study (19) as the results showed a difference in the average concentration of liver enzymes AST and ALT in rats infected with *E. histolytica* and treated with concentrations of 3.75 and 5 cm^3 of gold nanoparticles.

3-3- Effect of *Entamoeba histolytica* infection on kidney function in rats treated with an aqueous extract of *Aloe vera* and compared with the control group:

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1-Measurement of urea concentration in rat serum:

The results of the study showed a significant increase in the average urea concentration in the rat group infected with *Entamoeba histolytica* at 53.88 ± 16.18 mg/dl compared to the uninfected control group at 21.63 ± 5.24 mg/dl. Statistical analysis showed significant differences at a probability level of $p \leq 0.001$ between the infected and control groups. However, after administering the aqueous extract of *Aloe vera* to the infected group, no significant differences were observed, with an average of 2.55 ± 0.77 mg/dl (Table 3).

2-Measurement of creatinine concentration in rat serum:

This study recorded a significant increase ($p \leq 0.001$) in creatinine concentration in the group of rats infected with the *E.histolytica* parasite 3.69 ± 1.13 mg/dl compared to the control group 0.53 ± 0.17 mg/dl. No significant differences were observed after treating the infected group with an aqueous extract of *Aloe vera*, it reached 42 ±13.05 mg/dl (Table 3).

Table (3) Effect of parasite infection on kidney function in rats compared with the control group

Enzymes / Groups	Mean ± S.D	
	Urea mg/dl	Creatinine mg/dl
Negative Control	21.63 ± 5.24 **A**	0.53 ± 0.17 **A**
Positive Control	53.88 ± 16. 18 **B**	3.69 ± 1 .13 **B**
Infection and treatment group	2.55 ± 0.77 **C**	42 ± 13.05 **C**

***The different letters indicate significant differences at a probability level of ($p \leq 0.001$) between the rates of the different groups**

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The study results are consistent with those of researchers (23) who observed elevated urea and creatinine levels in individuals infected with *E. histolytica* compared to healthy individuals. The increased blood urea levels may be attributed to protein breakdown due to the disease's impact on the kidneys, leading to increased urea concentration. One explanation is that urea is the end product of protein metabolism (24). The significant increases in urea and creatinine levels demonstrate the detrimental effects of the infection on kidney function. The results also showed that the aqueous extract of *Aloe vera* played an effective role in improving kidney function in the group infected with the parasite. The findings of the current study are consistent with previous studies conducted on other medicinal plants, such as chamomile flower extract. Researchers (25) observed that the use of chamomile flower extract at a concentration of 50 and 100 mg/kg in the group treated with the *E. histolytica* suspension did not produce significant changes ($p \leq 0.05$) in kidney function compared to the control group.

4-Conclusions:

Experimentally infection with the *Entamoeba histolytica* parasite in rats affected some blood tests, causing a significant decrease in hemoglobin (Hb) and red blood cell volume (PCV), while showing a significant increase ($p \leq 0.001$) in total white blood cell count, liver enzymes (AST, ALT, ALK), and kidney function tests (urea and creatinine). However, after using an aqueous extract of *Aloe vera*, the results showed no significant differences in blood parameters or liver and kidney function tests. It is concluded that the aqueous extract of *Aloe vera* has therapeutic efficacy against *E. histolytica* in male rats. These results underscore the importance of assessing organ function indicators when developing effective diagnostic and treatment plans.

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