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Article

Protective Role of Ethanolic Alcoholic Extract of *Boswellia carterii* Against Some Physiological and Histological Disorders Induced by Amoebic Infection Experimentally

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ABSTRACT
The current study was designed to prospect the protective effect of alcoholic extract of *Boswellia carterii* in some physiological parameters and histopathological changes caused by amoebic infection experimentally of female rats; thirty-two adult female rats were used. It was divided into four groups: the first group received physiological saline as the control group, the second group was infected experimentally with amoeba (*Entamoeba histolytica*), which was administrated (1 mL/Amoebic suspension /Animal /day) for 10 days, and the third and fourth groups treated with alcoholic extract of plant at (1 mL /animal/day) for one and two months respectively after amoebic infection was ten days. The results indicated a significant decrease ($P \leq 0.05$) in body weight and liver enzymes (ALT, AST, ALP) of the amoebic group compared with the control group. In contrast, a significant increase ($P \leq 0.05$) in these parameters occurred by using alcohol extract of the plant compared with the infected group by amoeba. Also, the phagocytosis factor and kidney functions (urea and creatinine) were increased significantly ($P \leq 0.05$) when amoebic infection compared with the control group. That alcoholic extract caused a significant decline ($P \leq 0.05$) for natural levels in these parameters compared with the amoebic group. On the other hand, the present results reported that administering an alcoholic extract of *Boswellia carterii* caused improvement in the histopathological damage of the kidney and liver, which resulted from amoebic infection experimentally by *Entamoeba histolytica*. These histological changes in the kidney comprised fibrosis, inflammation, structural changes in glomeruli as absence, shrinkage and death of glomeruli, enlargement of Bowman's space, congestion and hemorrhage. While in livers, that included infiltration of inflammatory cells, enlargement of the sinusoid, congestion, and severe hemorrhage.
Keywords: *Boswellia carterii*, Liver Enzymes, Kidney

INTRODUCTION

Amoebic dysentery is one of the important, largely common diseases that result from infection by a unicellular parasite (Entamoeba histolytica); the genus Entamoeba is composed of six species that were established in the intestinal lumen of humans, but other species reside in the oral cavity, among of them, Entamoeba histolytica which considered only pathological species [1]. It infects

humans and animals, causing pathological lesions that lead to death [2]. The pathological effects of infection involved intestinal and histological damages (extraintestinal abscesses) as heart, lung, liver and brain abscesses [3]. Also, there are acute and chronic amoebiasis. Acute infection was characterized by diarrhea, fatigue, cramping, ulcers, abdominal pain, and blood and mucous in stool; the ability of Entamoeba for infection was upon severeness of infection, host resistance and condition of the digestive tract [4].

Boswellia is a medical plant, a deciduous tree that belongs to Burseraceae. It includes about 23 species that are widespread throughout the world. The plant produces a natural material called olibanum or frankincense, a milk-like resin that stiffens later to orange-brown gum resin [5, 6]. Plant extracts such as Boswellia species were used for natural therapies and food preservation; they had antibacterial activity, anti-leukotriene, anti-inflammatory, anti-viral, and anti-fungal, and they were used as a fixative substance in soap and perfumes [7]. Also, Boswellia was used previously to treat many diseases such as tumors, inflammation ulcers, cough and dysentery [8]. It involves some phytochemical substances such as phenols, terpenes, boswellic acids and essential oils in its chemical composition; these are responsible for antibacterial activity [9].

MATERIALS AND METHODS

Preparation of Amoebic Suspension

Amoeba suspension was prepared by diluting infected stool containing cysts; 1:10 was the percentage of dilution by distilled water. It had been filtered, 2 mL of filtrate was taken, and 2 mL of normal saline (NaCl 0.9%) was added. The suspension was centrifuged at 2500 cycles/minute for 5 minutes. That resulted in suspension, which consisted of cysts used for infection of rats [10].

Preparation of Killed Yeast Suspension

The suspension of killed yeast was prepared for phagocytosis study using Saccharomyces cerevisiae, and 10 grams of yeast was suspended in 150 ml sterilized normal saline. The suspension was put in a water bath for boiling for one hour, and two layers of gauze filtrated it. The suspension was put in a test tube and kept at (-20 °C). When used, the suspension was dissolved in a water bath at (37 °C) and washed twice with normal saline.

Preparation of Alcoholic extract of Boswellia carterii

The concentration 95% of ethanol alcohol was used according to [11]. The method included 100 grams of plant powder was dissolving in 200 mL of ethanolic alcohol (1:2). These mixed and put in a shaker incubator at (28 °C) for 24 h. Then, the mixture was filtrated by a Buckner funnel with filter paper type Wattman No.1 . The infiltrate was collected, the alcohol was vaporized, and it was dried at 40 °C in the electric oven.

Animals and Designed of Experiment

Thirty-two adult female rats of Rattus norvegicus were (2-3) months old and weighed (250-270) grams. Rats were obtained and housed in the animal house of the biology department /College of Education for Pure Sciences / University of Thi-Qar under standard conditions [12]. It was divided randomly into four groups.

Each group contains eight rats (n=8) as follows: The first group (Control group) in which rats treated with 1 mL normal saline (0.9 % NaCl); the second group (Infected group) in which animals infected by an amoeba (Entamoeba histolytica) these administrated (1 mL / Amoebic suspension / Animal/day) for 10 days. The third group infected by amoebic suspension (1 mL/animal/day) and treated with alcoholic extract of Boswellia carterii at (1 mL /animal/day) for one month, while the fourth group in which rats infected by amoebic suspension (1 mL/animal/day) and treated with alcoholic extract of Boswellia carterii at (1 mL /animal/day) for two months. All animals in the third and fourth groups were given the aqueous extract after the occurrence of amoebic infection in rats.

Confirmation of Amoebic infection

For confirmation of amoeba present after administration of amoebic suspension, the method of [13], which included one drop of normal saline (0.9%NaCl), was put on the first edge of the slide, and another drop of Lugol's iodine was put on the second edge of the slide. A few stools were mixed with a wooden stick with normal saline and iodine, and then a cover slide was used without an air bubble.

Body Weight

The body weight of animals in all groups was measured using an electronic balance. It was used to compare the control group and other groups.

Study of Phagocytosis

The phagocytes present in blood can swallow cells of killed yeast; the phagocytosis was studied according to [14]; it included mixing 0.025 mL blood with 0.05 mL yeast suspension and 0.025 mL Hanks balanced salt solution in a test tube. These were incubated at (37 °C) for 30 minutes then blood smears were prepared. Wright's stain stained the slides; after staining, they were washed with tap water and then examined by light microscope as follows:

Phagocytosis factor (%) = (Count of phagocytosed / Total count of phagocytes) ×100

Biochemical Parameters

The biochemical parameters measured in the current study included kidney functions and liver enzymes. These parameters required serum to measure it. Sera were obtained by separating blood collected in tubes without anticoagulant (EDTA); it was separated by centrifuge at 3000 rpm for 10 minutes and stored at (-20°C). Methods 15 and 16 were used to measure kidney functions (urea and creatinine) using urea and creatinine kits. The liver enzymes, which included Alanine Transaminase (ALT), Aspartate Transaminase (AST) and Alkaline phosphatase (ALP), were estimated according to [17] for the first enzymes. In contrast, the third enzyme (ALP) was determined according to [18].

Histological Study

The method of [19] was used for the preparation of the histological section. The liver and kidney were separated after the scarification of animals. It was fixed with 10 % formalin for 48 hours, then washed with tap water to eliminate residues of the fixation solution. The ethyl alcohol was used for dehydration, cleared by xylene, and paraffin wax was used for embedding. Five microns thick serial section was obtained by rotary microtome. The sections were stained by

routine dyes hematoxylin and eosin (H&E). The final step was histological reading by microscopic examination under a light microscope.

Statistical Analysis

The data were analyzed statistically by using SPSS (Version 21). All the values appeared in tables as mean ± standard deviation. Significance at (P≤0.05) was used to compare these means by LSD.

RESULTS

Body Weight, Phagocytosis factor

Table 1 included results of body weight and phagocytosis factor. It indicated a significant decrease (p≤0.05) in body weight of the amebic group (infected group) compared with a control group, and two groups of plant extract, a significant increase (p≤0.05) was observed in body weight of groups which treated with the alcoholic extract (one and two months) compared with the amoebic group.

A significant increase (p≤0.05) occurred in the phagocytosis factor of the amebic group compared with a control group, and two groups of plant extract, a significant decrease (p≤0.05) were present in the phagocytosis factor of groups treated with alcoholic extract for one and two months compared with the amoebic group. This is increasingly linked to the ability of phagocytes to cell death caused by the presence of amoeba in the body and the response of the immune system to defense by phagocytosis. This is accepted by [24] who point to phagocytosis occurring by parasitic infection, and it is an indicator of the action of phagocytes against pathogenic agents.

Group	Body weight (gram)	Phagocytosis factor
Control group	261.51 ± 2.14 [a]	**51.45±1.77 [c]**
Infected group (Amoebic group)	242.92 ±1.45 [d]	**66.78±3.62 [a]**
Alcoholic extract (one month)	255.22± 2.62 [c]	**53.26 ± 1.45 [b]**
Alcoholic extract (Two months)	260.67 ± 1.38 [a]	**50.98± 0.91 [c]**
LSD	0.95	**1.06**

Table 1. Effect of ethanolic alcohol extract of the plant in body weight and Phagocytosis factor (n=8) (Mean ± Standard deviation) Different letters indicate a significant difference (P≤ 0.05) among groups

Biochemical parameters

The parameters of kidney functions and liver enzymes were found in Table 2: a significant increase (P≤0.05) in urea concentration and creatinine in the amoebic group compared with a control group and with alcoholic extract groups, while a significant decrease (P≤0.05) was present in urea and creatinine of groups that administrated alcoholic extract compared with an infected group (amoebic

group). Also, table 2 demonstrated a significant decrease (P≤0.05) in liver enzymes (ALT, AST and ALP) of the amoebic group compared with the control group. At the same time, the treatment by alcoholic extract of a plant at one and two months led to a significant increase (P≤0.05) in these liver enzymes of the amoebic group.

Group	Urea (mg/dL)	Creatinine (mg/dL)	ALT IU/L)(	AST IU/L)(	ALP IU/L)(
Control group	46.32± 0.81 [d]	0.75± 0.00 [d]	25.54± 0.68 [a]	24.89± 1.15 [a]	**146.26± 1.89 [a]**
Infected group (Amoebic group)	60.76± 2.10 [a]	0.88± 0.01 [a]	10.02± 0.72 [d]	11.91± 0.67 [d]	**91.50± 0.79 [d]**
Alcoholic extract (one month)	52.40± 1.46 [b]	0.79± 0.00 [b]	20.10± 1.34 [c]	19.91± 0.88 [c]	**103.12± 2.06 [c]**
Alcoholic extract (Two months)	48.72± 0.83 [c]	0.76± 0.01 [c]	23.95± 1.19 [b]	22.80± 1.22 [b]	**141.67± 1.21 [b]**
L.S.D.	0.68	0.00	0.50	0.49	**0.76**

Table 2. Effect of ethanolic alcohol extract of the plant on kidney functions and liver enzymes (n=8) (Mean ± Standard deviation).Different letters indicate a significant difference (P≤ 0.05) among groups

Histopathological Changes

Histological examination of the presently studied organs (kidneys and livers) referred to many changes in the infected group by amoeba, such as histological damages in the kidneys, including inflammation, fibrosis, structural changes in glomeruli as absence, shrinkage and death of glomeruli, enlargement of Bowman's space, congestion and hemorrhage. In livers, infections cause infiltration of inflammatory cells, enlargement of sinusoids, congestion, and severe hemorrhage. While the current results observed that the administration of ethanolic alcohol extract of Boswellia carterii at one and two months led to amelioration, these histological effects are as follows (Figure 1-16).

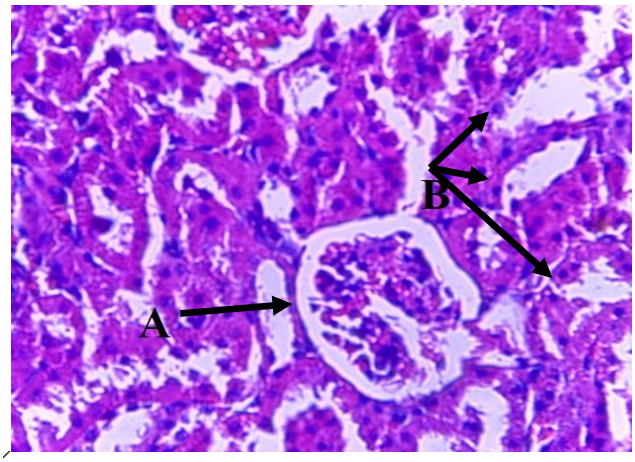

Figure 1. Section in kidney of control group showing glomerulus (A) renal tubules (B) (H&E) (100 X) .

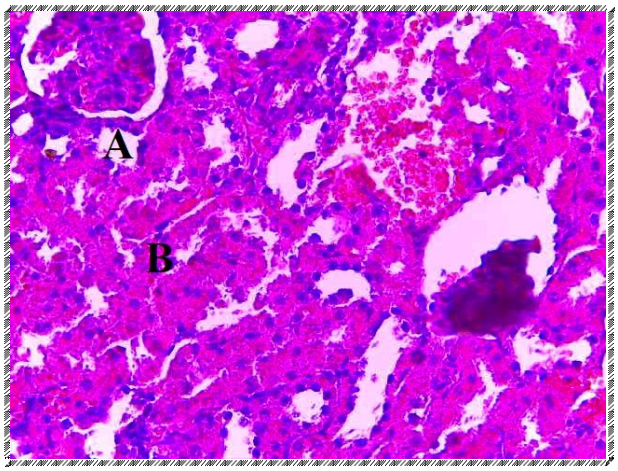

Figure 2. Section in kidney of amoebic group showing sever hemorrhage (A) death of glomerulus (B) (H&E) (100 X) .

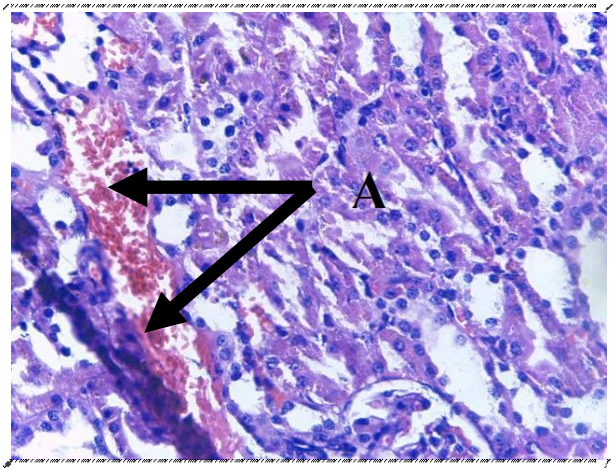

Figure 3. Section in kidney of amoebic group showing large congestion (A) (H&E) (100 X) .

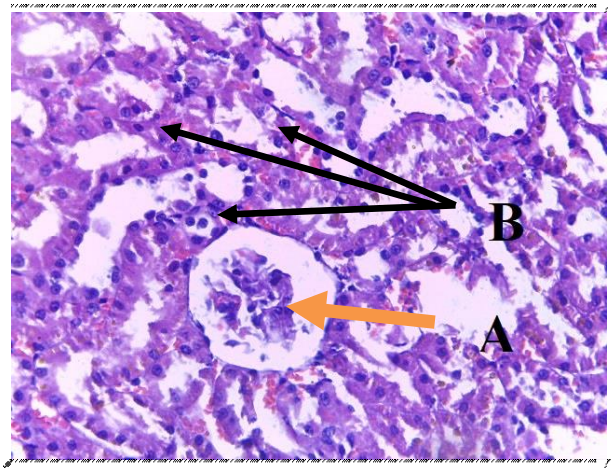

Figure 4. Section in kidney of amoebic group showing enlargement of Bowman's space (A) hemorrhage (B) (H&E) (100X).

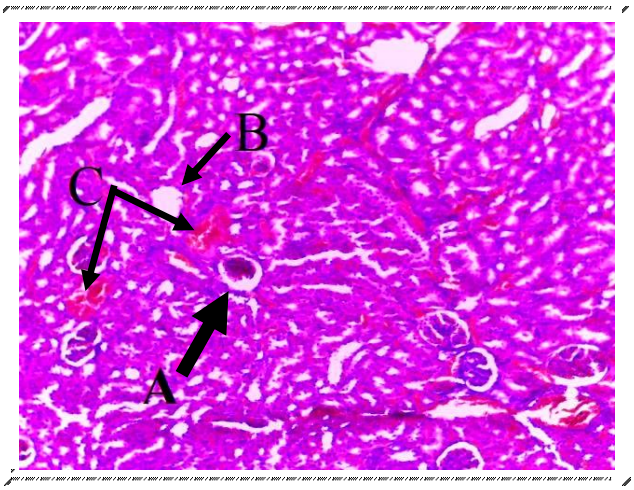

Figure 5. Section in kidney of amoebic group showing atrophy of glomerulus (A) absence (B) hemorrhage (C) (H&E) (100X).

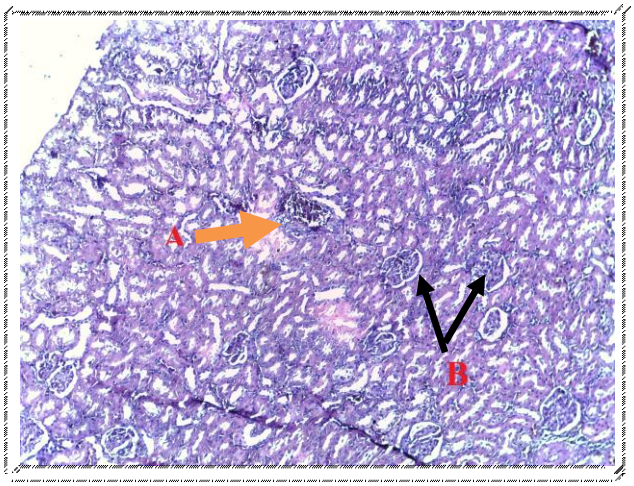

Figure 6. Section in kidney of alcoholic extract (One month) group showing simple congestion (A) natural glomerulus (B) (H&E) (100 X) .

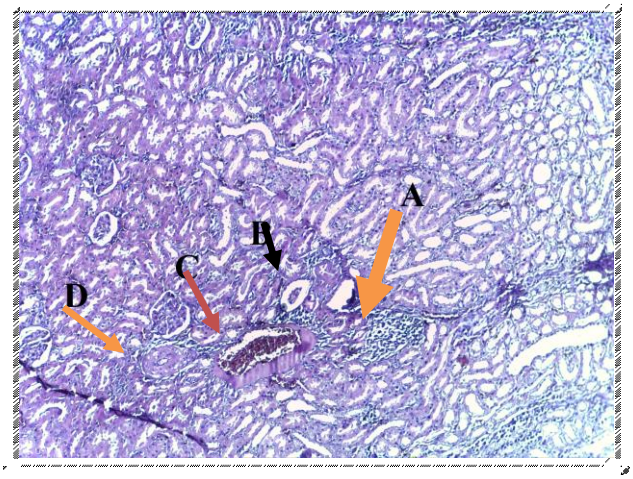

Figure 7. Section in the kidney of alcoholic extract (One month) group showing infiltration of inflammatory cells (A) shrinkage of glomerulus (B) simple congestion (C) fibrosis (D) (H&E) (100 X) .

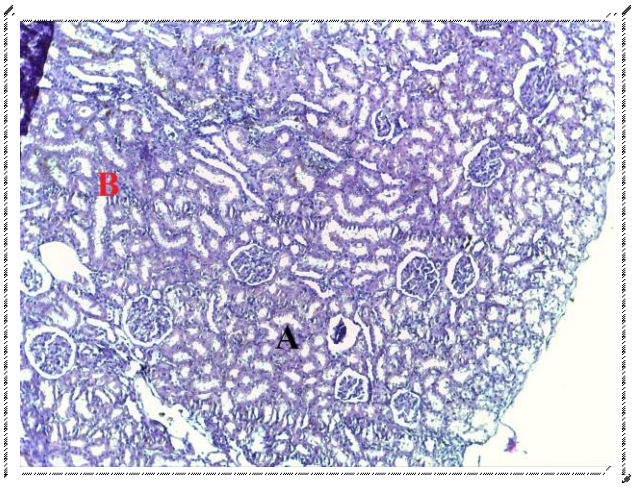

Figure 8. Section in the kidney of alcoholic extract (Two month) group showing natural numbers of glomerulus (A) infiltration of inflammatory cells (B) (H&E) (100 X) .

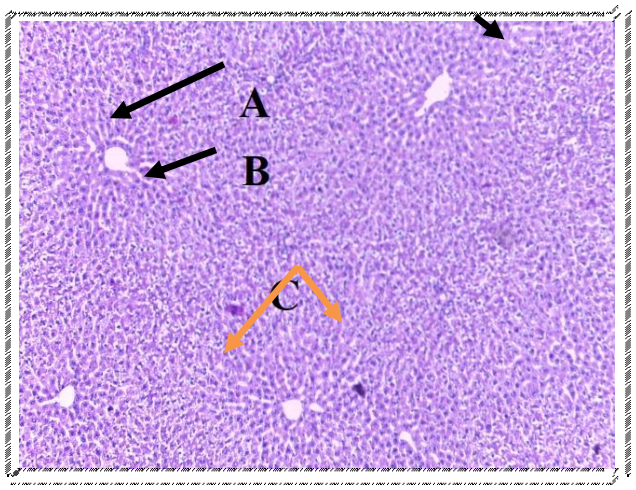

Figure 9. Section in the liver of control group showing central vein (A) hepatocytes (B) sinusoids (C) (H&E) (100 X)

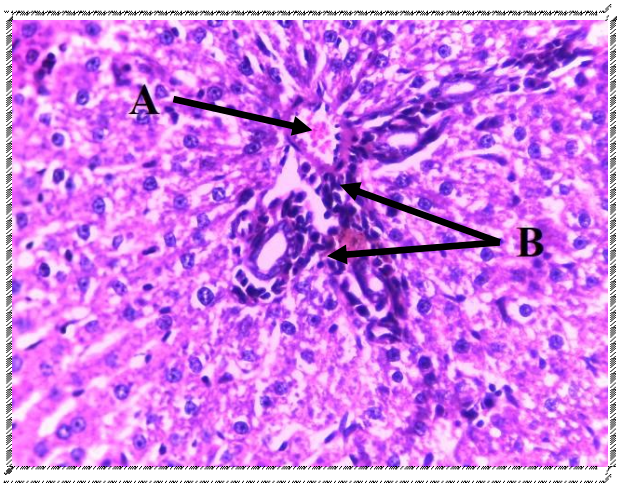

Figure 10. Section in the liver of amoebic group showing congestion (A) sever infiltration of inflammatory cells (B) (H&E) (100 X).

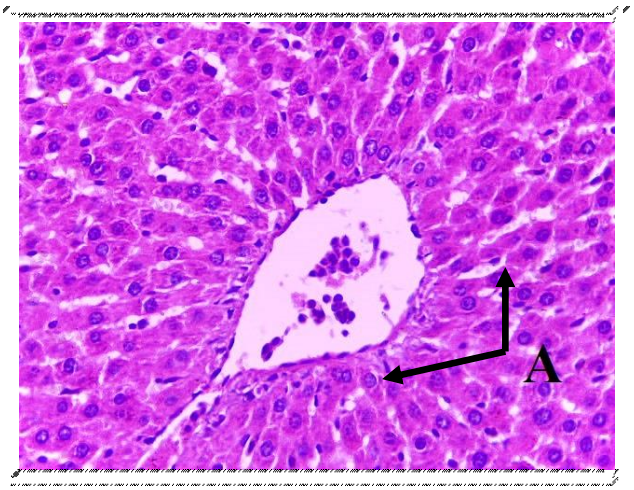

Figure 11. Section in the kidney of energy beverage group showing enlargement of sinusoids (A) (H&E) (100 X)

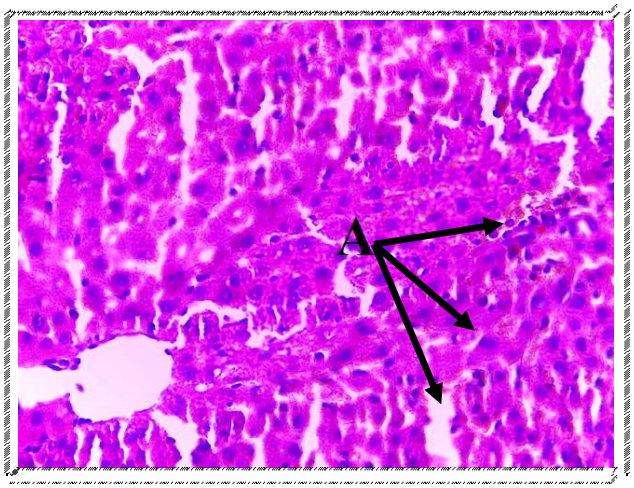

Figure 12. Section in the kidney of amoebic group showing bleeding (A) (H&E) (100 X).

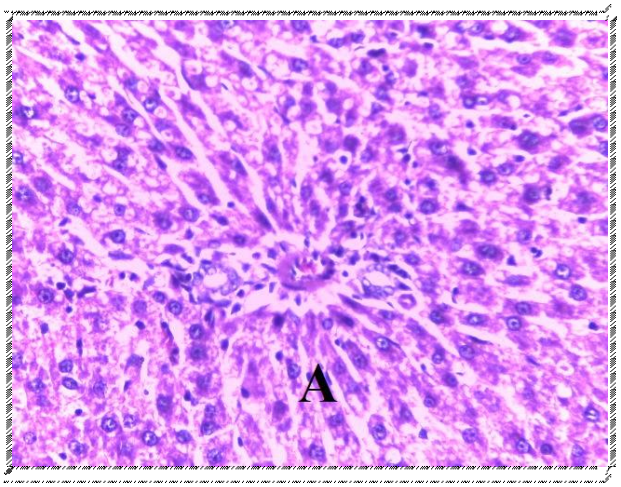

Figure 13. Section in the liver of alcoholic extract (One month) group showing simple infiltration of inflammatory cells (A) (H&E) (100X) .

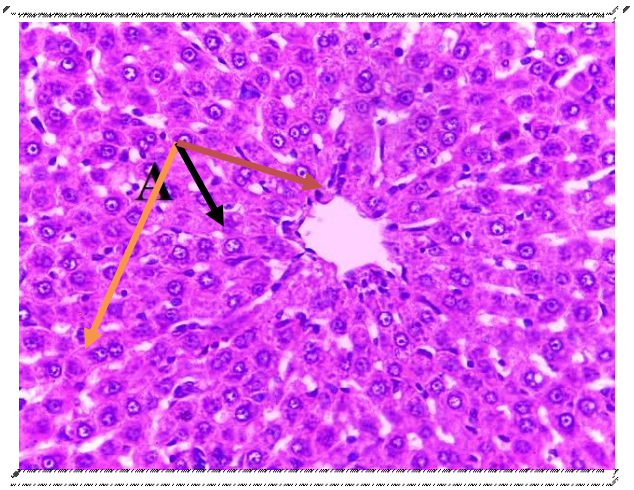

Figure 14). Section in the liver of alcoholic extract (Two month) group showing natural structure of liver (A) (H&E) (100X).

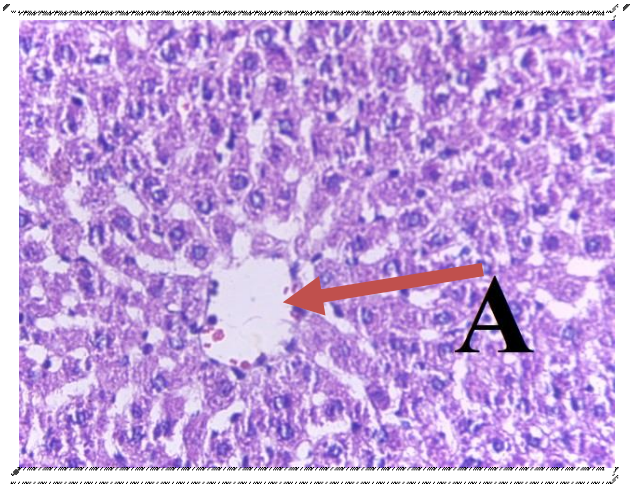

Figure 15. Section in the liver of alcoholic extract (Two month) group showing simple hemorrhage (A) (H&E) (100X)

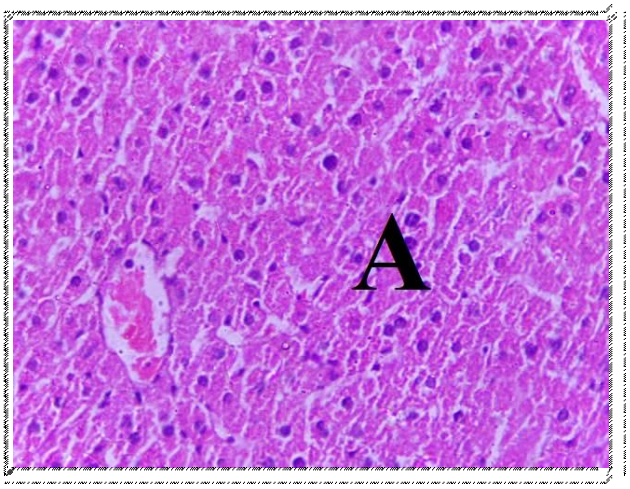

Figure 16. Section in the liver of alcoholic extract (Two month) group showing simple congestion in central vein (A) (H&E) (100X).

DISCUSSION

The decline in body weight may belong to malnutrition, and frequent diarrhea was observed in the infected group by amoeba; these effects cause a diminution of nutritional elements and lowing of absorption, subsequently, a decline in body weight. Twenty showed malnutrition of the host caused by *Entamoeba histolotica*. This result agrees with [21] who found diarrhea and dysentery, which are symptoms of amoebic infection. While the elevated body weight in groups that received an alcoholic extract of Boswellia was observed, this result agrees with [22], who recorded a raising of body weight of broiler by the addition of olibanum to diets; this does not agree with [23], who stated a non-significant increase in body weight.

These findings agree with [25], who revealed changes in biochemical parameters in liver enzymes and increasing urea and creatinine resulting from diabetic disease and the possibility of Boswellia with other plant mixtures in its treatment. Elevated urea, creatinine and reduction of liver enzymes in the amoebic group may be back to the effect of the amoebic present in the kidney and liver, causing structural disturbances of these organs led to disorders in their function; this corresponds to [26] who stated that defects in the structure of kidney cause accretion of urea and creatinine. By administration of alcoholic extract of the plant, the function of kidney and liver enzymes was at natural levels; this may be associated with the important role of plant extract in the recovery of natural structure in the kidney and liver, which reflects positively on the functions of these organs; this result accepted with [26] who demonstrated the function of organs linked to its integrity.

These histological changes may be correlated to the ability of amoeba (*Entamoeba histolytica*) ability to penetrate the mucous membranes of the intestine and access different body organs by the blood. This agrees with [27], [28] who reported parasitic infection for the intestine arrived in other organs such as the liver, kidney, brain and spleen, leading to histological changes. Also, previous results were accepted by [29], who explained damages in the host's liver by Entamoeba histolytica. Inflammation appeared in the kidney and liver may be regarded to the production of interleukins, [30] indicated inflammation by E. histolytica by activated NF-KB and production of IL-IB1 from epithelial cells, which causes an influx of inflammatory cells to the mucous layer, led to tissue damage. Also, a Histological examination of the kidney showed fibrosis, inflammation, structural changes in glomeruli as absence, shrinkage and death of glomeruli, enlargement of Bowman's space, congestion and hemorrhage. These damages may be due to the effect of amoebic infection by E. histolytica, this agreement with [31] who mention damages by cell death resulted from the invasion of amoeba and reproduction, or these damages to correlation demoralization of renal cells action and aggregation of inflammatory cells and apoptosis. The same author reported the breakdown of kidney tissue by-products of the appearance of inflammatory reactions and, subsequently, inflammation. While the decline of damages was observed when treated with plant extract, this may be regarded as the use of the alcoholic extract of Boswellia against amoebic infection; this agrees with [32], which refers to the use of Boswellia species for the treatment of different diseases. Amelioration of inflammation by plant extract accepted with [33], [34] who reported anti-inflammatory ability of Boswellia species due to the presence of phytochemical substances. The therapeutic role of the plant may be related to tannin, carbohydrates, glycosides, tanning materials and resins; this

agrees with [35], who noted an active substance called tannin against amoebic infection *by E. histolytica*.

CONCLUSIONS

The current study was to detect the protective effects of alcoholic extract of *Boswellia carterii* on physiological and histological changes in female rats infected by amoebic experimentally.

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