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EFFECT OF AQUEOUS AND ALCOHOLIC EXTRACTS OF *ALOE VERA* ON RATS EXPERIMENTALLY INFECTED WITH *ENTAMOEBA HISTOLYTIA* AND INDUCED DIABETES MELLITUS

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Abstract:

The current study was designed to investigate the effect of aqueous and alcoholic extracts of *Aloe vera* plant on the tissues of white rats of the type albino, aged 6 weeks, and weighing 250–280 grams, which were taken from the animal house of the College of Veterinary Medicine/Tikrit University, and these rats were placed in special cages at home. The animals were spread with sawdust with care for the cleanliness of the cages, and the animals were placed throughout the study period under appropriate laboratory conditions in terms of ventilation, temperature, and adequate lighting. They were provided with water and the prepared ration continuously and in sufficient quantities until the end of the experiment. The microscopic examination of tissue sections taken from rats infected with the amoeba parasite The tissues of patients with newly diagnosed diabetes have pathological changes in the large intestine (colon) and the liver, as it was noted that the intestinal mucosa contains the degeneration of a number of epithelial cells, covering the intestinal mucosa with the degeneration of a number of those cells. In the liver, the liver was observed in the positive control group and the presence of histological changes It was observed around the liver tissue in which the capsule was sloughed off and the liver parenchyma spread in which the hypertrophy of the liver cells spread and the cytoplasm lost most of its pigment with Some of the nuclei of hepatocytes thickened, and hepatocytes appeared compact as a single mass

Keywords:

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterized by elevated blood sugar levels resulting from defects in insulin secretion, insulin action, or both (1). Diabetes contributes to premature deaths worldwide. The number of people with diabetes is increasing due to growth. The prevalence of diabetes among adults will increase from 8.8% in 2015 to 10.4% in the year 2040 (2). The World Health Organization estimated that 422 million people worldwide were suffering from diabetes in In 2014 (3), people with diabetes were more likely to develop gastrointestinal diseases than those without diabetes (4). The *aloe vera* plant is one of the most widely used and diverse plants in the treatment of many diseases. This plant has been used medicinally to treat a variety of diseases such as mild fever, wounds, burns, digestive disorders, diabetes, and cancer, as well as various skin diseases. It is also used in the pharmaceutical industry as it is used in the manufacture of topical ointments and gels (5).

MATERIALS AND METHODS

Microscopic Examination of stool samples

1-Direct Wet Mount Method

The faecal samples were examined by preparing a wet direct swab to investigate the infectious stages of the parasite by taking a quantity of faeces with wooden sticks special for this purpose and from different areas of the sample and placing them on clean glass slides with a drop of normal saline on one end of the glass slide and a drop of Logule solution Iodin 1% on the other end of the slide and mixing them together well, then putting the cover of the slide on and examining the samples under the light microscope under magnification powers of 10% and 40% (6).

2- Concentration method

A- Flotation technique by sucrose solution

A medium with a density greater than the density of the primary bags and the eggs of worms is used, so they float to the top of the medium. Mix 2 g of faeces in a sufficient amount of distilled water for 10 ml with wooden sticks to

release the bags from the faeces, then filter the mixture through four layers of gauze and then transfer the filtrate to test tubes. It is placed in the centrifuge for 5 minutes at a speed of 1000 rpm and the supernatant is removed from the test tubes. Then a little distilled water (3 ml) is added to dissolve the precipitated part, and the tube is filled with water and returned to the centrifuge. The scavenger was neglected and a saturated sucrose solution was added to the precipitate. Then the test tubes were returned to the centrifuge at the same speed and duration and the supernatant was carefully withdrawn with a fine pipette and placed on a glass slide with a drop of iodine-locker solution and examined under the microscope (7).

Aloe vera sample collection

Aloe vera plant

Aloe vera leaves were collected from local markets and classified according to (8). The plants were cleaned of dust, washed with water well, and dried at room temperature.

Preparation of aloe vera gel extracts

1- Raw extract of *aloe vera* gel

He obtained the crude extract of *aloe vera* gel from the leaves according to method (9) and as follows:

Aloe vera leaves were collected, washed with distilled water, dried and cut longitudinally into two equal halves. Then the gel was extracted by a tablespoon. The gel was placed in an electric mixer for one minute, then filtered by eight layers of gauze. The sediment was taken and the filter was neglected.

2- Preparation of the aqueous and alcoholic extract of *aloe vera* gel

Collect 400 grams of crude jelly as in the previous step, then mix the crude jelly with 1000 milliliters of distilled water (aqueous extract) or 100 milliliters of 70% ethyl alcohol (alcoholic extract) using an electric mixer for one minute. Leave the mixture for 12 hours with constant stirring. Using an electric vibrator, dry the powder completely and place it in an incubator at 37°C until it becomes a dry powder, then collect the powder in tight and dark bottles and store it in the refrigerator until use.

Determination of drugs does

Determination of the effective dose of aqueous and alcoholic extracts of *Aloe vera*

This step represents a pilot study to determine the most effective and optimal dose of the aqueous and alcoholic extract of the *aloe vera* plant that lowers blood glucose. The healthy animals were randomly divided into 7 groups, each group included 3 animals, and they were divided as follows:

The first group (control group): was given distilled water only.

The second group: was dosed with aqueous extract of *aloe vera* at a concentration of 0.02 mg/kg.

The third group: was dosed with aqueous extract of *aloe vera* at a concentration of 0.07 mg/kg.

The fourth group was dosed with aqueous extract of *aloe vera* at a concentration of 0.1 mg/kg.

The fifth group was dosed with alcoholic extract of *aloe vera* at a concentration of 0.02 mg/kg.

The sixth group: they were dosed with alcoholic extract of *aloe vera* at a concentration of 0.07 mg/kg.

The seventh group: it was administered with alcoholic extract of *aloe vera* plant at a concentration of 0.1 mg/kg, and each animal was administered orally. After 24 hours, blood samples were drawn from the eye socket by capillary tubes and glucose concentration was measured using a glucose meter. In light of that, the most effective and most effective dose was selected. The effect of the *aloe vera* plant was 0.02 mg/kg.

Parasite Isolation

The parasite was isolated from faecal samples obtained from patients with diarrhea, and the presence of the parasite was confirmed by direct microscopy and the following:

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1- Take 2 g of faeces and add distilled water to it and filter it through four layers of gauze, then take the filtrate and add distilled water to it.
2- The tubes were placed in the centrifuge at a speed of 2500 rpm for 5 minutes.
3- Neglect the filtrate and take the sediment, then repeat the process until the filtrate becomes clear.
The tubes were centrifuged at 2500 rpm for 5 minutes before the float was removed and the precipitate was collected.
The precipitate was treated with 5-2 mL of zinc sulfate and stored at -20°C (10).

Determination of infection does

A drop of 0.1 ml was taken from the filtrate and placed on a hemocytometer slide, and the number of bags was calculated in one drop in order to determine the infection dose according to the following equation: (200 x (number of cysts)/(0.2) and the suspension was stored at -20°C until use (10).

Dosing and injecting rats

Laboratory rats were dosed with amoeba suspension (8000) orally via sacs using an 18-gauge hooked needle by inserting the needle through the mouth into the esophagus and then the stomach and pushing the liquid containing the sacs directly into the stomach. In the same way, the negative control animals were dosed with physiological saline as shown in Figure (1).

Figure (1) Dosing laboratory animals

Experimental diabetes mellitus was induced in male white rats by subcutaneous injection of Alloxan, which was prepared at the time of injection at a dose of 150 mg/kg of body weight (11) after starving the animals for 24 hours before inducing diabetes in them (12) as in the figure (1)

Figure 2: Injection of laboratory animals

Immediately after the injection, she was provided with food and glucose solution (5%) with drinking water for 24 hours to prevent a sharp drop in blood sugar level as a result of the breakdown of pancreatic beta cells and the release of large amounts of insulin, which works to lower the level of sugar in the blood (13) and for a period of two weeks after infection. Rats were placed in clean cages free of sawdust, and parasite bags were investigated in the stools of infected animals by preparing several swabs of infected rats' stools on a glass slide and examining them under a microscope and watching the parasite. Diabetes was also confirmed by examining sugar with a glucose meter. The blood sugar of infected rats ranged in the range of 360–500, and after confirming the infection, the animals were dosed with plant extracts. After 10 days of taking the rats for treatment, their stools were examined. Several swabs were prepared from the infected rats' stools on a glass slide and examined under a microscope to investigate the presence of the parasite or its possible stages, and then the arithmetic mean of the number of feeding and accumulating stages of the parasite was calculated for each group of rats. Infected rats before treatment and after 15 days of taking the treatment. The percentage of parasite activity was calculated for each of the infected groups of rats (positive control) and groups of rats after 15 days of taking the treatment, according to the following equation:

(number of anaplastic or fed instars after treatment/number of ingested or fed instars after treatment) x 100 (14).

After the expiry of the specified period of the experiment, the rats were killed after being anesthetized by chloroform for a few seconds, and the abdominal region was opened longitudinally using dissection scissors as in Figure (3). Formalin was diluted 10% for 24 hours, after which it was preserved in 70% alcohol to study the histological changes during the period of infection, which lasted for two months from the beginning of September until the month of November.

Figure 3: Anatomy of laboratory animals

Design of Experiment

The animals were divided into (6) groups, each group included 6 animals of close weight, as follows:
The first group (negative control group (G1)): included 6 rats that were dosed with normal physiological solution.The second group (positive control group (G2)): included 18 rats injected with alloxan once at a dose of 0.04 mg/kg and given amoebic suspension for 10 days.
(The third group, the infected and treated group (G3)): It included 6 rats with diabetes induced by alloxan and *Entamoeba* parasite, and they were dosed with aqueous extract at a dose of 0.02 mg / kg per day for 15 days.
(The fourth group, the infected and treated group (G4)): It included 6 rats with diabetes induced by alloxan and *Entamoeba* parasite, and they were dosed with alcoholic extract at a dose of 0.02 mg/kg daily for 15 days.
(Group Fifth (G5)): It included 6 uninfected rats that were dosed with aqueous extract at a dose of 0.02 mg/kg for 15 days.
(Group Six (G6)): 6 uninfected rats were dosed with alcoholic extract at a dose of 0.02 mg/kg for 15 days.

Histological study

The tissue sections were prepared according to the method (15):

1-Fixation: The organs (liver and large intestine) of the experiment groups were fixed in 10% formalin between 12-24 hours to avoid tissue autolysis, as the sections were placed in special glass bottles containing the fixation medium.

2-Dehydration: The samples were passed with ascending concentrations of ethyl alcohol 50%,70%,90%,100% for an hour for each concentration for the purpose of withdrawing water from the samples and preparing them to receive the leaching substance.

3-Clearing: Use xylene in this step, due to its feature in removing alcohol from tissues and making them more transparent, as samples are placed in it for 30 minutes and according to the type of tissue.

4-Infiltration: The samples were immersed in paraffin wax with a melting point of 60 °C for an hour to saturate the tissues with wax and this depends on the type of tissue and the size of the sample.

5- Embbeding: The samples were buried with paraffin wax by pouring a little wax and quietly into special L-shape molds. Then the tissue sample was fixed in the mold with hot forceps before use, and then the tissue was immersed in wax by pouring it once to avoid the occurrence of air bubbles. Then the molds were left to harden at a temperature laboratory temperature and then kept in the refrigerator for the time of cutting using a rotary microtome.

6- Tissue Sectioning The sections were cut with a rotary microtome in the form of a strip of slices with a thickness of 5 micrometers. The sections were transferred to a water bath to brush the sections at a temperature of 40 °C for several minutes, then they were loaded onto the glass slides and left to dry on a hot plate at a temperature of 37 °C.

Hemotoxylin and Eosin staining

Sections were stained with hematoxylin-eosin stain according to method (16):

1- Dewaxing paraffin wax was removed from the sections by placing them in xylene for 15 minutes to get rid of the remaining traces of wax.

2-The sections were hydrationed by immersing them in decreasing concentrations of ethyl alcohol (100%, 90%, 70%) for 5 minutes for each concentration.

3- The sections were stained by immersion in hematoxylin dye for 5 minutes.

4- The sections were washed with tap water.

5-The sections were immersed in Acid Alcohol twice to clarify them Differentiated.

6- The sections were washed with running tap water until the color of the core became blue.

7- Sections were stained with eosin for 15 seconds (two quick covers).

8- The sections were pricked by passing them with ascending concentrations (70%, 90%, 100%) of ethyl alcohol for 30 minutes at each concentration for the purpose of removing water from the tissue.

9- Sections were sanded with xylene for 5 minutes to remove alcohol from the tissue. This is done by transferring the sample to xylene and twice for each time for half an hour to ensure that leaching occurs.

10- The cover slide was affixed by placing drops of D.P.X (Distrene plastizer xylene) on the sample with little pressure and left to dry, then examined under the light microscope, and the results showed the color of the nucleus in blue and the cytoplasm in pink. Imaging and diagnosis: Histological sections were diagnosed using a light microscope for the purpose of detecting possible changes in the tissue structures of the studied samples and all treated experimental groups and comparing them with the control group. A digital camera was used for imaging for the purpose of tissue photography, and the images were printed using a color printer to diagnose the situation and were read by a histologist.

RESULTS AND DISCUSSIONS:

The histological changes of the group of rats injected with alloxan and dosed with the tissue amoeba parasite were observed by microscopic examination of rat feces after the tenth day of infection. When comparing the histological sections in the negative control group (parasite infection and untreated diabetes mellitus) and the positive control

group (parasite infection and untreated diabetes mellitus), The infiltration of a number of blood cells was observed in the primary plate between the intestinal mucosal glands than in the figure (5) compared with the negative control group. If the microscopic examination of tissues did not reveal the intestines in the rats of this group, it was because the intestinal mucosa contained the epithelial cells lining the intestinal wall, and it was composed of uncle cells. The primary lamina of the intestinal mucosa is simple epithelial friendly, with the proliferation of large numbers of mucosal goblet cells between the epithelial cells and in the intestinal glands, as well as the proliferation of large numbers of white blood cells.

As for the liver in the negative and positive control groups, it is shown in Figures (6) and (7), respectively, that the liver in the positive control group was observed to have histological changes, as it was observed around the liver tissue in which the sloughing of the capsule and the liver board spread, hyperplasia of hepatocytes and the loss of cytoplasm for most of its pigment. With the thickening of some nuclei of hepatocytes and the appearance of compacted liver cells as a single mass compared to the negative control group, which seemed normal, where the central vein continuous with the blood sinusoids at its edges appeared in the liver tissue. Furthermore, the container on the spherical nuclei dark pigment is large in size.

The current histopathological study of sections taken from different parts of the intestines of orally infected rats showed that infection and penetration of the active phases of the intestinal tissues cause different histological effects, and this study agrees with the results (17) when studying the tissue amoeba parasite on mice, where he observed histopathological changes in some organs of animals infected with the parasite were represented by glandular hyperplasia of goblet cells, infiltration, cellulitis, and erosion of the heads of the villi in the intestine. As for the liver tissue, necrosis of liver cells, infiltration of inflammatory cells, and congestion of blood vessels were observed.

There are indications that the immune response stimulated by infection with the parasite may be related to the overall picture of the pathological lesions. It is known that the parasitic protozoa stimulate the antibody immune response and the cellular immune response. Cellular immunity is directed more towards intracellular parasites and sero antibodies directed against protozoan surface antigens may coat the antigen and facilitate opsonization, agglutinate the antigen, or may inhibit its immobilization. Antibodies with complement and cytotoxic cells may kill these organisms (18).

Microscopic examination of tissue sections taken from the intestines of infected rats treated with aqueous extract of aloe vera plant showed that the surface of the mucosa in the intestine contained epithelial cells covering the intestinal wall from the side of the intestinal lumen with the spread of large numbers of mucosal goblet cells among the epithelial cells, and it was noted that these goblet cells were contained within the lamina The primary cells lined the intestinal glands, and the goblet cells contained many dye-free mucous droplets, and the interstitial tissue between the glands was seen to contain infiltration of numbers of white blood cells as in figure (8).

Figure (9) shows the liver of infected rats treated with aqueous extract of the aloe vera plant, where the central vein appeared in the center of the hepatic lobule, in which there was a small blood clot surrounded by some white blood cells. It was observed that the blood sinusoids between the rows of cells in which some of the coffer cells and the sinusoids are continuous with the central vein. These results are consistent with (17) through his study of the effect of the aqueous extract of Saad plant on the tissue amoeba parasite, where he observed the intestinal villi appeared correctly with the observation of lymph nodular accumulation within the main lamina of the intestine. There are clearly defined boundaries.

Figure (10) shows the intestine of infected rats treated with the alcoholic extract of the *aloe vera* plant, where it was observed that the intestinal mucosa contained many mucous goblet cells and mucous droplets in the cytoplasm of those cells in addition to the epithelial columnar cells facing the intestinal cavity, and the base plate was filled with inflammatory white blood cells. The disassembled intestinal glands, where a few goblet cells appeared, with the spread of white blood cells to the epithelial layer and the sub mucosal layer from the bottom. Figure (11) illustrates a histological section of an infected rat liver treated with an alcoholic extract of *aloe vera* plant, where the portal region of the liver was observed to contain a branch. The portal vein containing red blood cells, the bile duct branch, the hepatic artery branch, and those vessels and ducts are surrounded by infiltration of some white blood cells, surrounded by groups of hepatocytes from the outside and compacted with each other, including some blood sinusoids, which contain cover cells. This may be attributed to the effective therapeutic effect of plant extracts because they contain active substances such as phenols, alkaloids, glycosides, saponins, tannins, carbohydrates, proteins, amino acids, fats, vitamins and minerals (19) that may weaken the parasite inside the intestine, thus inhibiting The parasite is prevented from dividing and multiplying inside the intestine, and thus the parasite does not continue to cause pathogenicity.

The results did not show any side effects for the aqueous and alcoholic extracts of *aloe vera* at a concentration of 0.02 mg/kg. Figure (12) shows the intestines of rats treated with aqueous extract of *aloe vera*, where the villi appeared in a compact manner, and those villi were covered with simple columnar epithelial cells with some goblet cells, and the primary lamina on the intestinal glands contained large numbers of goblet cells, and their lumen was filled with large mucus droplets, and the interstitial tissue between the glands was noted to contain white blood cells.

As for Figure (13), it shows a rat liver treated with aqueous extract of *aloe vera,* where the portal area on the portal vein is excessive in size and hypercongested with blood with the presence of bile duct branches and hepatic artery branches with the infiltration of large numbers of white blood cells with the appearance of a number of liver cells and hyperinflation with the presence of ruptures in the cytoplasm and the thickening of some nuclei.

Figure (14) shows a rat intestine treated with the alcoholic extract of *aloe vera*, where the mucosa of the intestine was lined in a fusiform manner with mucous cells that contained mucus droplets in the cytoplasm with other cells secreting yeast, and the interstitial tissue between the glands contained infiltration of large numbers of white blood cells and macrophages extending to the sub mucosal layer. The smooth muscle bundles have some disintegration of their fibers, especially in the outer part of the muscle.

As for Figure (15) it shows a rat liver treated with the alcoholic extract of *aloe vera* plant, where the branches of the central vein contained some red blood cells with the walls of the vein connected with the blood sinusoids extending from the edges of the hepatic lobule and those surrounded by hepatocytes multi-ribbed together and arranged in the form of beehives, and each cell contained a large central nucleus of dark pigment, Coffer cells found in the lumen of the sinusoidal ducts.

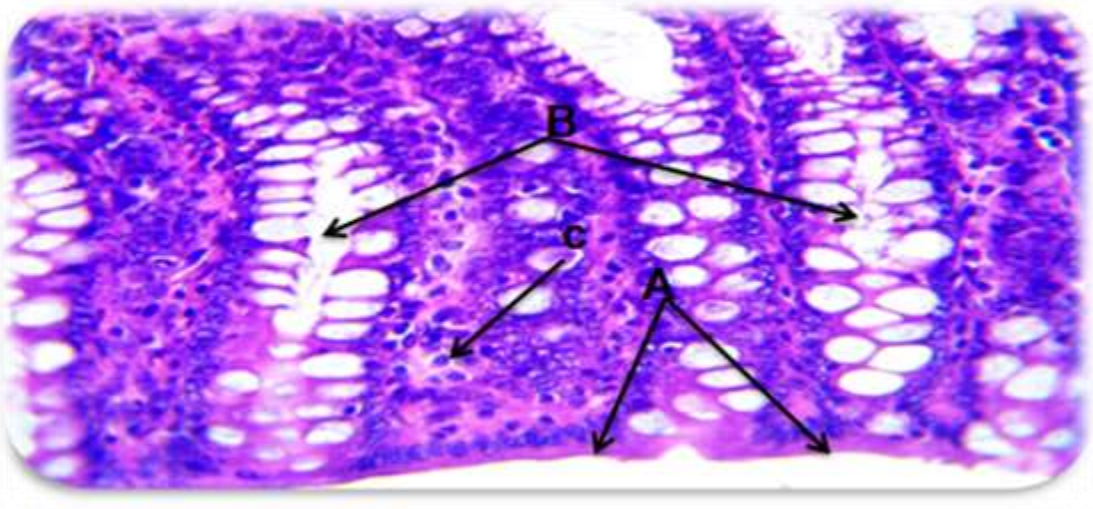

Figure (4) A section of an uninfected rat intestine showing the intestinal mucosa and simple epithelial cells (A) of the intestinal glands, with mucosal goblet cells (B), interstitial tissue, and white blood cells (C) (40 XH2E).

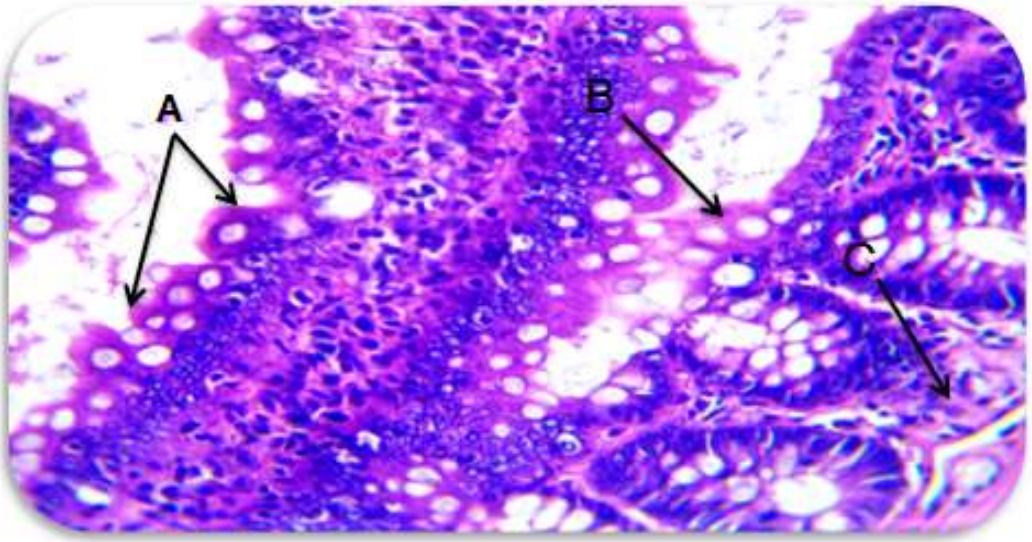

Figure (5) A section of an alloxan-injected rat intestine cultured with amoeba tissue shows the mucosa of the intestine containing degeneration of the epithelial cells lining the intestine (c) (40 XH2E)..

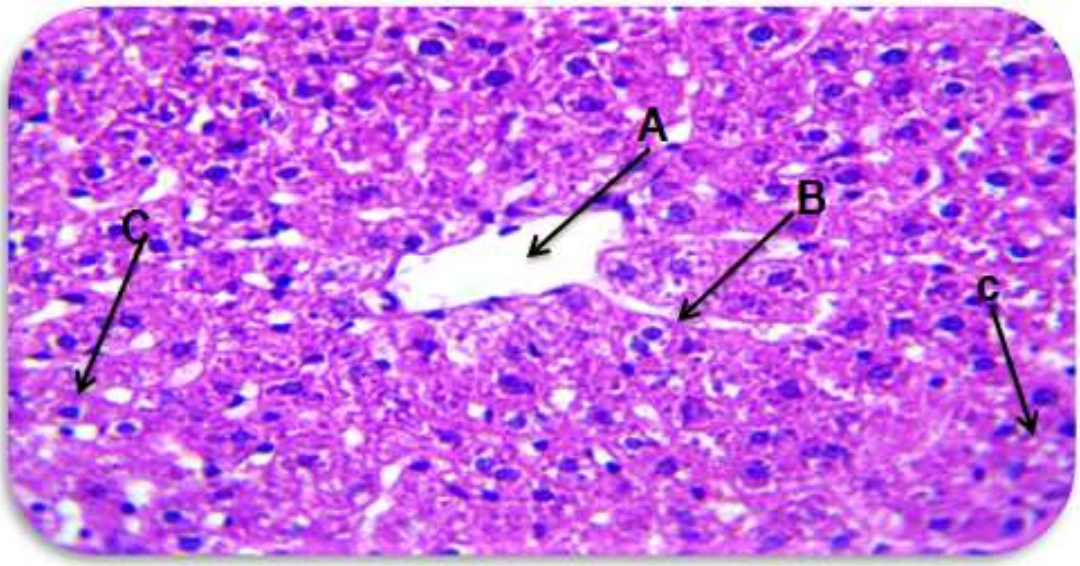

Figure 6: A section of an uninfected rat liver showing the central vein (A) sinusoidal (B) hepatocyte rows (C) (40 X H2E)..

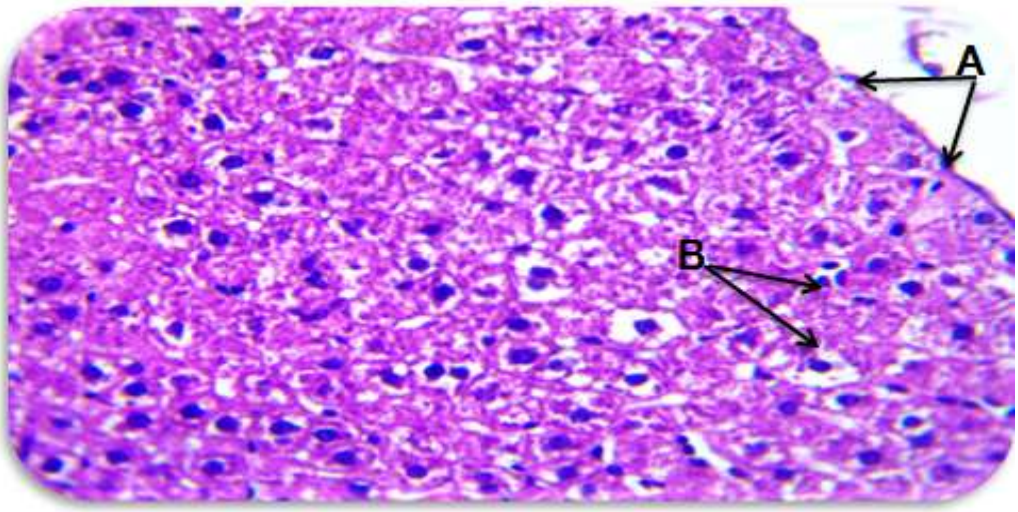

Figure (7) A section of a rat liver injected with aloxan and cultured with amoeba tissue shows circumference of liver tissue and capsular degeneration (A), hepatocyte hyperplasia (B) with loss of cytoplasmic pigment(40 XH2E).

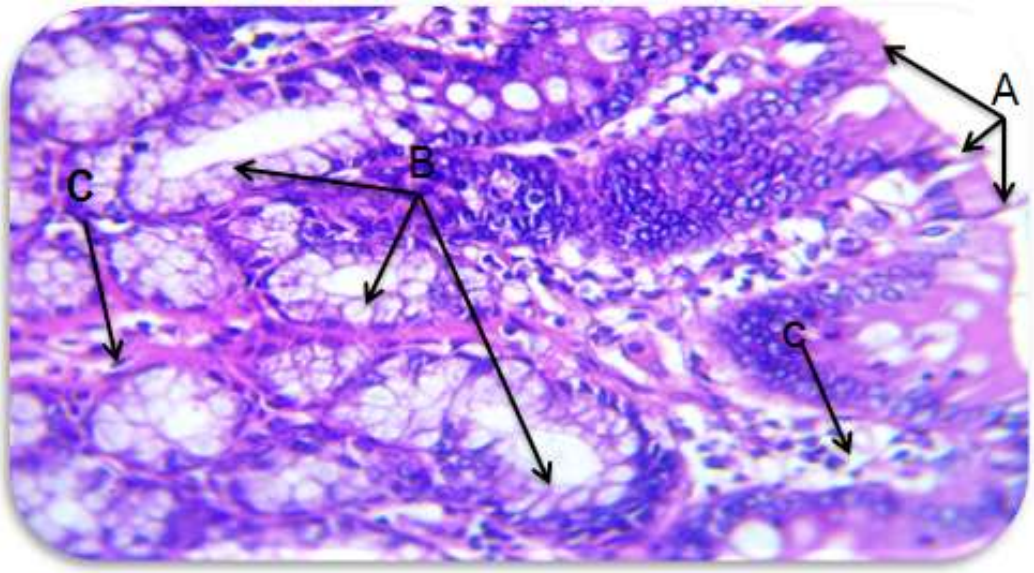

Figure (8) a section of infected rat intestine treated with aqueous extract of *aloe vera* plant showing the mucosa of the intestine covered with simple columnar cells (A) The main plate, which contains the intestinal glands containing mucous cells (B), white blood cells in the interstitial tissue (C) (40 XH2E)..

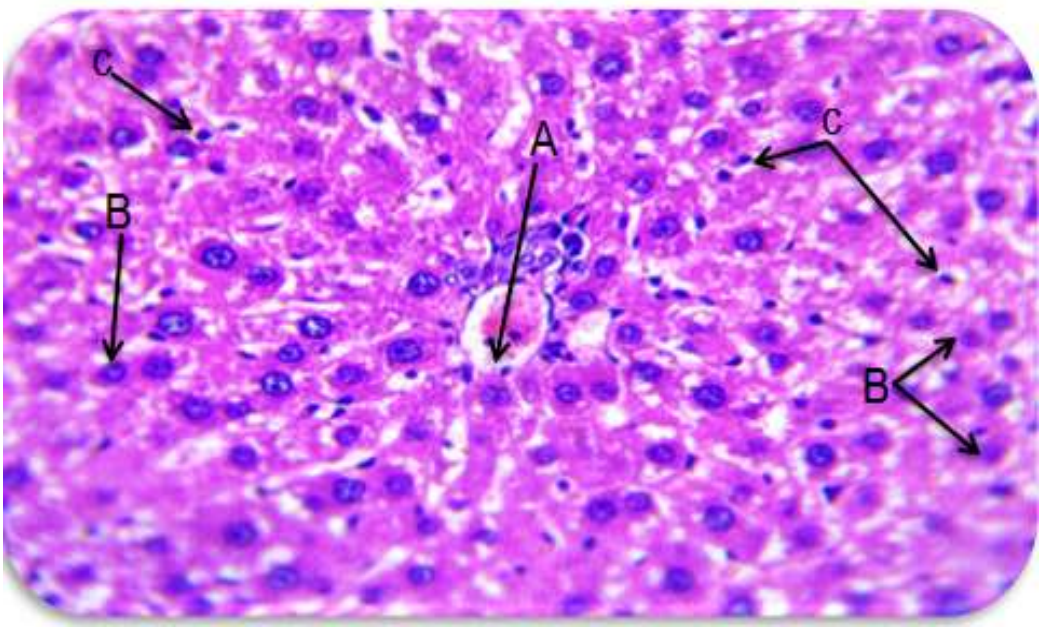

Figure (9) is a section of an infected rat liver treated with aqueous extract of aloe vera plant showing the central vein (A) containing a blood clot, rows of hepatocytes (B), blood sinusoids, and covering (C) cells(40 XH2E)..

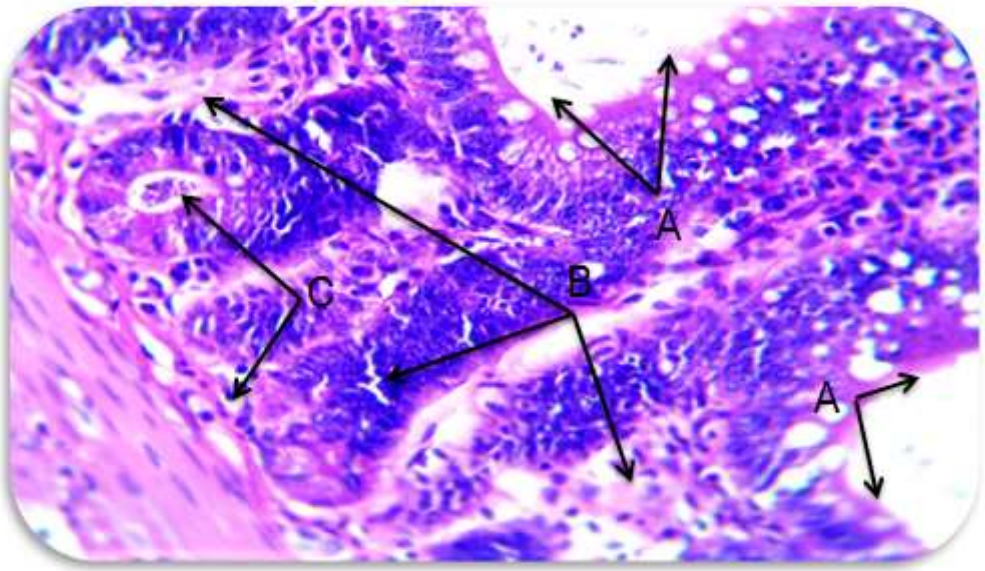

Figure (10): a section of infected rat intestine treated with alcoholic extract of *aloe vera* plant showing the mucosa of the intestine with mucus droplets scattered on the surface of the epithelium (A) disassembled intestinal glands (B) proliferation of white blood cells (C) in the base plate and sub mucosa (40 XH2E).

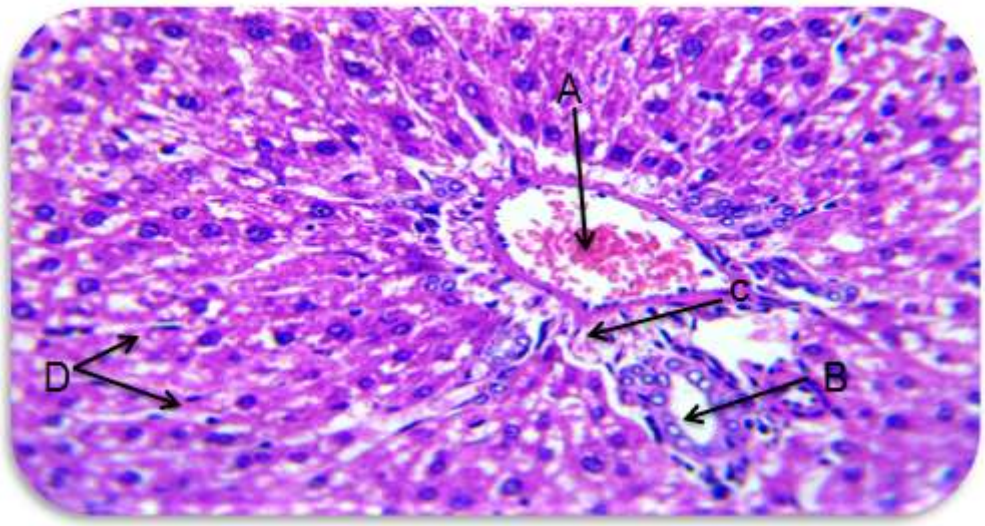

Figure (11) Section of a rat liver treated with alcoholic extract of *aloe vera* plant showing the hepatic portal area, portal vein and red blood cell hyperemia (A) bile duct branch (B) white blood cell infiltration (C) hepatocyte agglutination (D) (40 XH2E)..

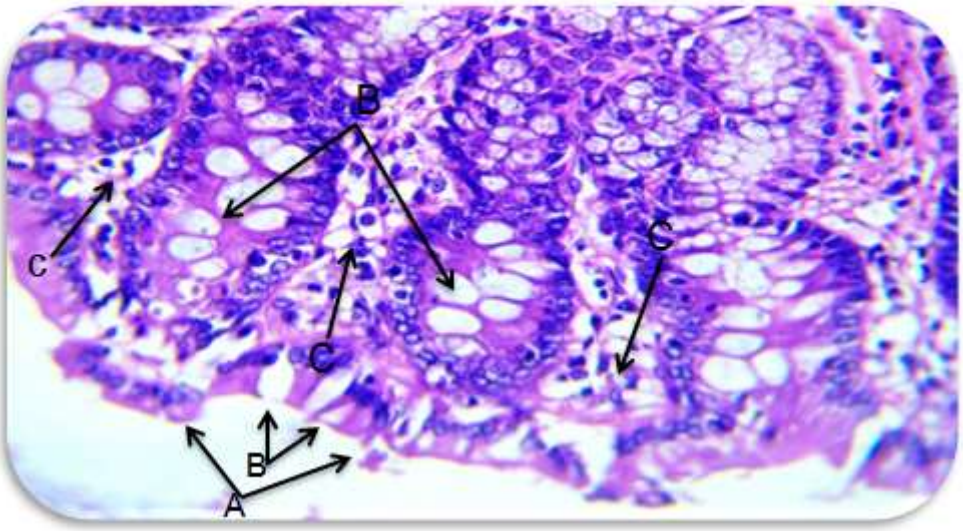

Figure (12) A section of a rat intestine treated with aqueous extract of *aloe vera* shows the agglutination of the intestinal villi, which are covered with simple columnar cells (A), mucus-secreting goblet cells (B), and white blood cells between the intestinal glands (C) in the primary lamina (40 XH2E)..

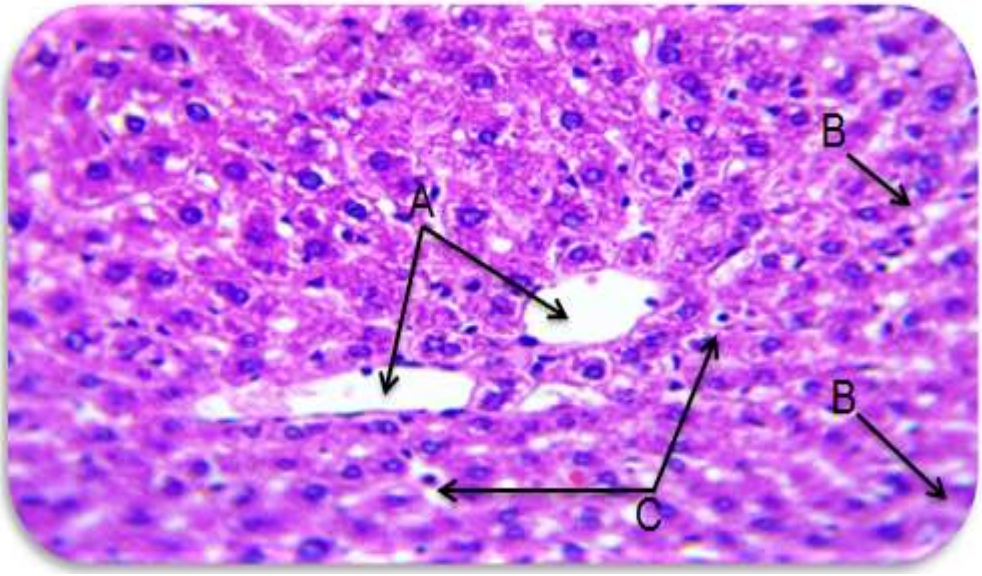

Figure (13) A section of a rat liver treated with *aloe vera* plant aqueous extract shows the central vein branch (A) devoid of blood, rows of hepatocytes (B), sinusoidal ducts with cover cells (B), sinusoidal ducts (C), and cover cells (C) (40 XH2E)..

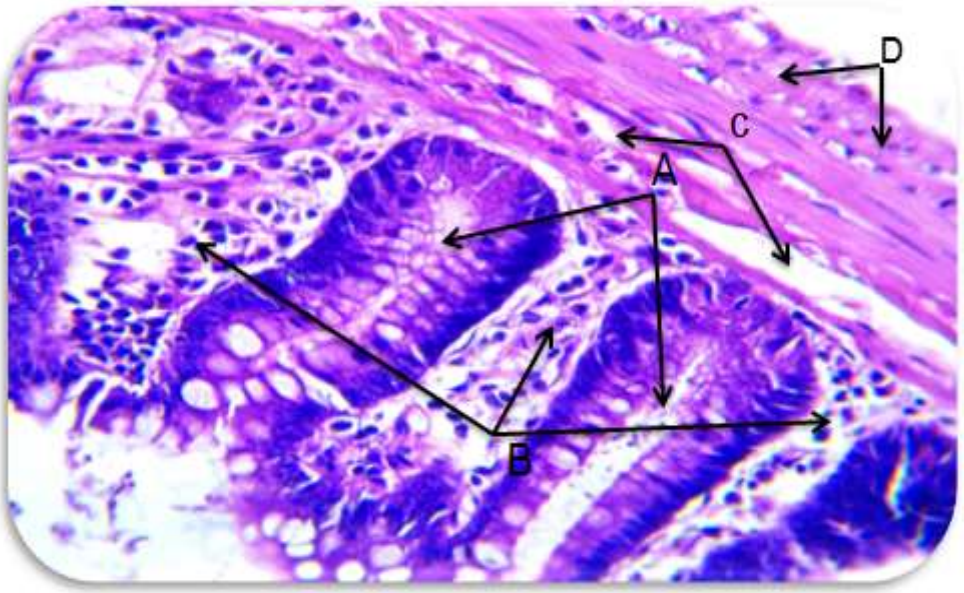

Figure (14) A section of rat intestine treated with alcoholic extract of *aloe vera* plant shows the mucosa of the intestine, the intestinal glands (A) extending to the depth of the base plate, infiltration of large numbers of white blood cells (B) in the interstitial and sub mucosal tissue (C), necrosis of smooth muscle fibers (D) (40 XH2E)..

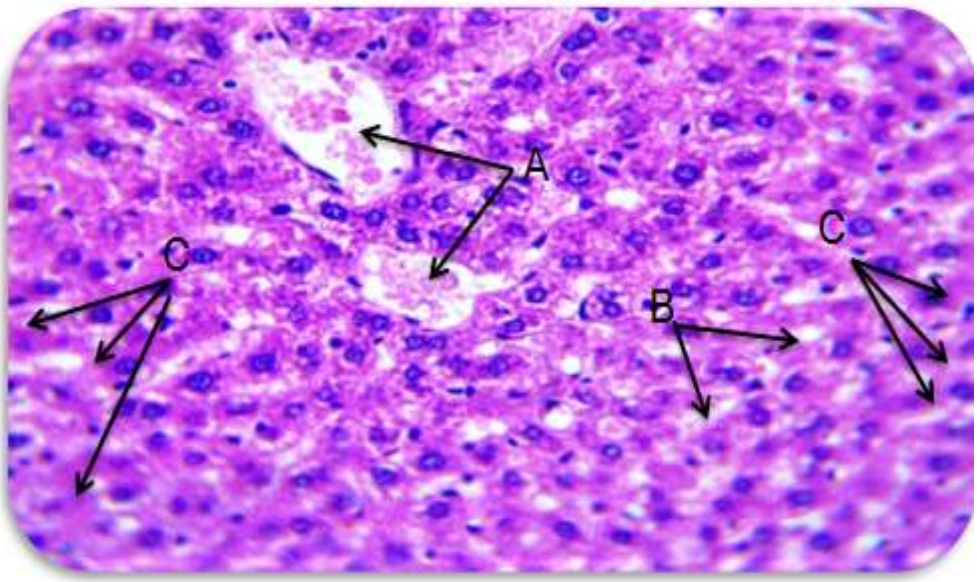

Figure (15): a section of a rat liver treated with alcoholic extract of *aloe vera* plant showing liver tissue, central vein branch (A), blood sinusoids continuous with the central vein (B), rows of hepatocytes in the form of hives (C) (40 XH2E)..

CONCLUSIONS:

The experimental development of diabetes mellitus in male rats infected with the tissue amoeba parasite led to disturbances in a number of physiological and histological variables that were studied. In comparison to the positive control group, the addition of 0.02 mg/kg of *aloe vera* aqueous and alcoholic extract had a positive effect on reducing the number of egg sacs excreted with the feces and repairing damaged tissues.

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تأثير المستخلصات المائية والكحولية لنبات الصبار على الجرذان الخمجة تجريبيا بطفيل اميبا الحالة للنسيج وداء السكري المستحدث

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الخلاصة:

صممت الدراسة الحالية لبحث تأثير المستخلص المائي والكحولي لنبات الصبار (الالوفيرا) في انسجة الجرذان البيض من النوع albino بعمر6 اسابيع ووزن (250-280) غرام والتي أخذت من البيت الحيواني التابع لكلية الطب البيطري/ جامعة تكريت , ووضعت هذه الجرذان في اقفاص خاصة في البيت الحيواني وفرشت بنشارة الخشب مع العناية بنظافة الأقفاص , ووضعت الحيوانات طوال مدة الدراسة تحت ظروف مختبرية مناسبة من حيث التهوية ودرجة حرارة وتوفر اضاءة مناسبة وزودت بالماء والعليقة المحضرة بشكل مستمر وبكميات كافية لحين انتهاء التجربة , اوضح الفحص المجهري للمقاطع النسيجية المأخوذة من الجرذان المخمجة بطفيل اميبا الحالة للنسيج والمصابة بداء السكري المستحدث حصول تغيرات نسيجية مرضية في الامعاء الغليظة (القولون) والكبد اذ لوحظ ان مخاطية الأمعاء احتوت على تنكس لأعداد من الخلايا الظهارية المغلفة لمخاطية الأمعاء مع انسلاخ اعداد من تلك الخلايا اما في الكبد فقد لوحظ الكبد في مجموعة السيطرة الموجبة وجود تغيرات نسجية اذ لوحظ محيط نسيج الكبد فيه انسلاخ للمحفظة ومتن الكبد انتشر فيه فرط تضخم خلايا الكبد وفقدان السايتوبلازم لمعظم صبغته مع تغلظ بعض انوية الخلايا الكبدية وظهرت خلايا الكبد متراصة ككتلة واحدة.