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Effect of zinc oxide nanoparticles of peach seed extract on liver structure in female c/bulb strain albino mice experimentally infected with *Leishmania donovani*

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Abstract
This investigation was performed from 1/11/2022 to 1/3/2023, where zinc oxide nanoparticles were prepared from peach seed extract to obtain different concentrations (10-15 kg / g). In the in vivo study, 36 female mice were used. The animals were put into 6 groups of 6 female albino mice of the c/Bulb strain, aged between 8 and 10 weeks and weighing 25 and 35 grams. The first group (negative control) was injected subcutaneously with normal saline. Mice of the second group were subcutaneously injected with *L. donovani*(positive control), the third group were injected with *L. donovani*and nano-extract of peach plant seeds (10 mg/kg), the fourth group mice were injected with *L. donovani*and nano-extract of peach plant seeds (15 mg/kg), while the fifth group was given.They were injected with peach seed nano-extract (10 mg/kg), while the sixth group was treated with peach seed nano-extract (15 mg/kg). Histological liver slices were created to study and detect histological alterations compared to control groups.The histological evaluation of mice organs revealed pathological abnormalities in the groups treated with Leishmania for each liver part. The pathological changes of the liver are represented by: In the presence of inflammation and enlargement of hepatocytes, necrosis of the cell membranes, contraction of the glomerulus, and expansion of the space capsule.The histological analysis of the groups that received treatments containing 10–150 mg/kg of nano-peach seed extract for each liver revealed the protective effect of the extract, which is demonstrated by the preservation of the normal histological structure and no change to the normal histological structure.

Introduction:
Leishmaniasis is one of the most significant vector-borne illnesses affecting people and several species of the protozoan parasite Leishmania cause it.Leishmaniasis is one of the neglected tropical diseases posing a medical challenge in 98 countries, with a yearly death rate of approximately 50,000 people. Between 12 and 15 million individuals are infected, with an additional 350 million at risk[1]. Each year, 1.5-2 million new leishmaniasis cases are recorded, with 1-1.5 million cases of cutaneous leishmaniasis and 500,000 cases of visceral leishmaniasis.The intermediate host and vector in the life cycle is a sandfly of the species Phlebotomus. A female sandfly is the main carrier of the disease[7] . Chronic fever, weight loss, and hepatosplenomegaly are typical symptoms, and a blood test will reveal pancytopenia. Without treatment, VL often nearly results in death. After seemingly successful treatment for VL caused by L. donovani, a persistent skin rash known as post-kala-azar dermal leishmaniasis (PKDL) may appear. While PKDL affects 25% to 50% of patients in Sudan, it is significantly less frequent and occurs later after therapy in the Indian subcontinent [2].Because of its high effectiveness and therapeutic index against microorganisms, nanotechnology is a viable therapeutic technique. Using nanoparticles as innovative biomaterials to achieve this feat is receiving global interest. Nanoparticles have the potential to become an essential viable treatment alternative for treating drug-resistant illnesses[4]. They are now considered feasible antibacterial alternatives and/or supplements[13]. Researchers are very interested in metal and metal oxide nanoparticles because they have the most promise out of all the nanoparticles. They have different effects on different pathogens that are resistant to multiple drugs. Silver nanoparticles (AgNps) and gold nanoparticles are the most researched metal nanoparticles (AuNps) [3].The goal of this study is designed to be another source of finding drug alternatives by using nanocomposites extracted from natural plants. Furthermore, Diagnosis of the therapeutic ability of the peach seed nanoparticle extract to reduce the pathological changes produced by the parasite by studying the anatomical and histological changes[5].

Materials and Methods

Experimental Animals Prepare: This experiment was conducted on female albino mice of the c/Bulb strain (8-10) weeks old and weighed (25-35) grams. These mice were procured and bred at an animal facility. These mice were housed at a temperature of (25 ˚C)and light-controlled laboratory environment.It was divided into 12 hours of darkness and 12 hours of light. Then we left the Animals for 14 days to acclimatisation with each other before the start of the experiment Cages made of Cage Plastic, 8 animals per cage, and these cages have a cover In the shape of a metal clip, it is equipped with a bottle to put water and a place to put food and dimensions (5.27 cm long-10 cm wide-12 cm high) The floor was covered with sawdust wood that was replaced once every two days to maintain cleanliness and was given during the work period Appropriate amounts of locally manufactured and integrated rations and water that are changed daily for some time Experience[6].

Design of study: Use 36 female mice. There were 12 mice in the experiment, split evenly across 6 groups. Mice in Group G2 (positive control) were given L. donovani Injections subcutaneously, while mice in Group G1 (negative control) received normal saline. The G3 mice were injected subcutaneously with the peach seed Zinc oxide nanoparticles (15 mg/kg .).The G4 was injected with peach seed Zinc oxide nanoparticles (10mg/kg) .G5 were injected with peach seed Zinc oxide nanoparticles (15 mg/kg) and infected with the *L donovani*. The G6 was injected with peach seed Zinc oxide nanoparticles (10 mg/kg) and infected with *L donovani*. Autopsies were done once after 6 weeks of infection induction, and the totals were compared with the control group[8].

Animal anatomy: The weights of the animals (mice) to be dissected were taken and then placed in a sealed container. It contains pieces of cotton saturated with chloroform, considered an anaesthetic, for 3 minutes, after which it is proven. The animal was placed in the autopsy dish, and the abdomen and chest were sterilised with ethyl alcohol (70%). The skin was opened, And then the muscles, after which the liver and intestine were removed. It was divided into small pieces, from which he made impressions on glass slides and dyed them with Kimsa dye. The other was cultivated on the culture media of the parasite to ascertain the presence of the parasite In the infected and treated groups as well. Then the remaining pieces were preserved with ethyl alcohol at a concentration of 70% to prepare histological sections and observe changes under the microscope[9].

Prepare dose of parasite: The promastigotes were grown in NNN media at a temperature of 26±1˚C. Promastigotes were collected from a parasite culture in the stationary growth phase, washed in locks' solution by centrifuging at 1500 rpm for ten minutes, and then the supernatant was removed with Pasteur pipettes while the sediment was resuspended in the right size locks' solution (approximately 5 ml)[11]. Every 72–96 hours, stationary phase promastigotes were subcultured in fresh media. Hemocytometer counts were used to determining the promastigote concentration, which was then adjusted to 1.2 x 106 promastigote cells per ml. This counts the number of living things in 16 tiny corner squares. N x 10 x 1000 x 20 (N = the number of cells counted, 10 = the number of cells in lmm3, 1000 = the number of cells in l ml, 20 = dilution factor) is the formula for the total number of cells per ml[10].

Preparation of Nanomaterial and Experimental Design: A concentration of 150 mg/kg of zinc oxide nanoparticles powder was prepared by dissolving 750 mg of this powder in 20ml of saline solution Sodium 9.0% Chloride to prepare a solution ready for injection in a tightly closed tube, then the tube was placed in a magnetic vibrator Magnetic stirrer for 30 minutes in order to mix the material well[23], after that, the tube was placed in a device Processor Liquid Ultrasonic Sonicator for 15 minutes to homogenise the solution Well and to prevent the occurrence of agglomerations, and before each injection, the solution tube was placed in the vibrator magnetically for 15 minutes in order to mix the material and prevent its agglomeration, after which the animals were injected into a cavity Intraperitoneally with a special syringe for insulin glaucoma, with 1.0 ml of a solution of this substance, once a day, and during two periods of time[12].

Biosynthesis of zinc oxide nanoparticles with alcoholic extract of peach seeds

To make zinc oxide nanoparticles, (6 g) of peach seed powder was mixed with (100 ml) of distilled water in a 500 ml glass flask. Then, (0.1 g) of ZnO was added (NO3) 2 nitrate was added to each (100 ml) of the

solution, and diluted ammonia was added to change the pH of the solution to pH=7. The mixture was stirred constantly with a magnetic stirrer (200 revolutions per minute) for 24 hours at (37°C), until the colour changed from dark to dark. Conqueror [14]. Whatman (No.1) filter paper was then used to filter the solution. The impurities were then separated by centrifugation (4500 revolutions per minute) for 30 minutes. After removing the precipitate, it was completely twice rinsed with distilled water and dried in an oven at (50 ° C) to produce powdered zinc oxide nanoparticles[23].

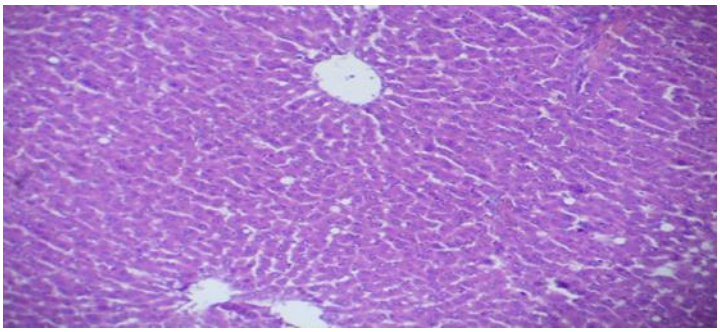

Figure (1): A cross-section of liver tissue from a control group displaying the central vein and hepatocytes in their typical form (1) showing the central vein (10x). (2) (40x) (H&E)

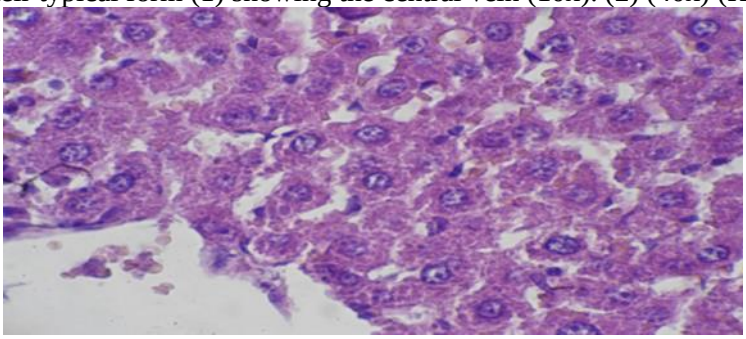

Figure (2):A cross-section of the liver tissue from the group that received Leishmania parasite and nano-extract at a dose of 10 mg/kg reveals the presence of simple necrosis and some inflammatory cells surrounding the major vein. (400x) (H&E) (H&E)

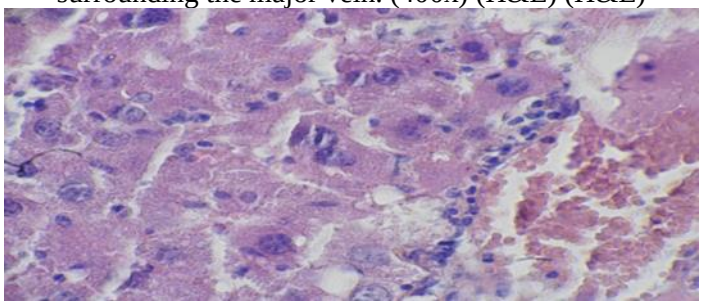

Figure (3):A cross-section of the liver tissue from the group that received nano-extract and Leishmania parasite treatment at a dose of 15 mg/kg reveals the presence of simple necrosis and some inflammatory cells surrounding the major vein. (400x) (H&E) (H&E)

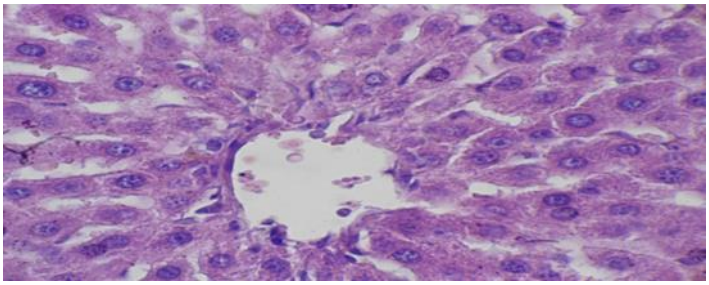

Figure (4): Cross-sections of liver tissue from the group treated with nano-extract peach seed displaying the normal organisation of liver cells and the central vein. (400x) (H&E) (H&E)

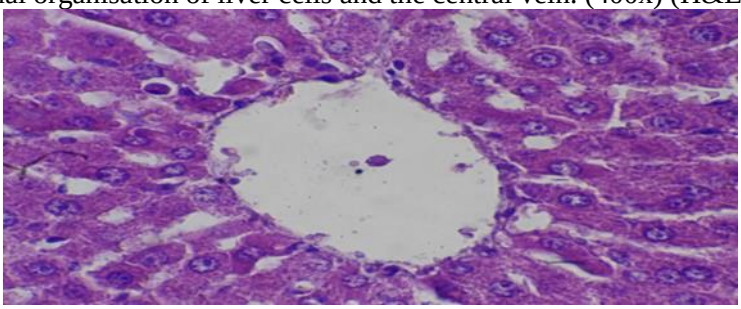

Figure (5): A positive group cross-section of liver tissue revealing L.donovania in hepatocytes and some inflammatory cells surrounding the central vein and necrosis (10x). (2) (400x) (H&E) (H&E)

Discussion:

One-fifth of an adult's total weight is made up of the liver, making it the largest gland and organ in the body. Location of the Liver The sickle ligament divides the right upper abdominal quadrant into two lobes, the bigger on the right and the smaller on the left.This lies a little bit below the diaphragm and to the right of the stomach[15].The gastrohepatic ligament connects the liver to the digestive system and the stomach to the left hepatic lobe via neurovascular structures like the hepatic vagus nerve. The liver is the main pathway for oxygenated and deoxygenated blood from the duodenum and portal structures to the heart because they are related to the hepatoduodenal ligament and portal liver[7]. It was seen that the liver's principal veins, hepatic cords, and sinusoids all had their normal histological structure. Animal models are also an important tool in this field [26]. DENA and Ccl4 have been shown to develop HCC (Hepato Cellular Carcinoma) in rats. Several investigations have revealed that apricot and other fruit seeds are hazardous, as seen by abnormally increased liver chemistry tests. These results concurred with research done previously[16].

In contrast, they proposed amygdalin as a potential hepatic fibrosis prevention method. ALT (Alanine Amino Transferase) is a more specific indicator of liver disease than AST (Aspartate Transaminase) (Aspartate transaminase)[25].Orally given amygdalin generates a considerable quantity of cyanide, despite its toxicity and efficacy in animal models being carefully investigated. A recent study found that the liver function markers AST, ALT, and ALP, as well as the tumour marker AFP (Alpha-fetoprotein) and reactive oxygen species (ROS) as measured by MDA, increased significantly in rats treated with DENA and Ccl4, suggesting liver damage, tumour incidence, and progression. This is consistent with prior research that found a significant increase in liver function throughout the initiation and course of hepatocarcinogenesis [17]. These results pertain to the development of HCC in rats given DENA and Ccl4. Previous research have suggested that DENA and Ccl4 may generate HCC in mice, therefore this result is in line with those findings[18].A strong neurotoxin produces reactive oxygen species (ROS), impairing vital organs such as the kidney, liver, brain, and heart. In rats fed DENA and Ccl4, an increase in the apoptotic markers cytochrome-c and caspase-3 suggested gene disruption and aberrant DNA damage. The induction of apoptosis by genotoxic DENA at high concentrations during hepatocarcinogenesis is a plausible explanation.The body's defence strategy is eliminating damaged DNA-containing cells to inhibit tumour

cell formation but not multiplication. Amygdalin can be transformed to hydrocyanic acid by complex emulsion enzymes, including -D-glucosidase (present in meals) and bacteria from the colon and small intestine.Boiling water causes amygdalin to epimerase, despite its ability to break down glucose, benzaldehyde, and hydrocyanic acid without enzymes.[19].Moreover, oxidative stress develops if free radicals' negative effects are not countered. A recent study discovered that amygdalin is poisonous when taken orally but not when given intravenously; however, the method of action and hazardous levels have yet to be validated. Repeated oral dosages of amygdalin were shown to be hazardous, which was ascribed to a varied intestinal consortium [20].In contrast to the typical morphological patterns found in the control and other amygdalin-treated groups, histological investigation of selected tissues indicated substantial degenerative alterations in the liver of rats given a high dosage of amygdalin.This liver damage investigation saw vascular congestion, Kupffer cell activation, and substantial cytoplasmic vacuolisation. Orally given cyanide caused histopathological liver damage in rats. Nevertheless, cyanide compound exposure resulted in minor hepatocellular damage in goats[21].

Effects of *Leishmania donovani* on the Histological Structure of Liver

Visceral leishmaniasis causes Kupffer cells, hepatocytes, Ito cells, portal tracts, sinusoids, and hepatic veins. 44% of Kupffer cells had parasites and hypertrophy. Hepatocytes displayed ballooning degeneration [24].There was no correlation between the parasite load, the inflammatory response in the samples, and the liver changes. Ballooning degeneration was still seen in some treated samples after the parasites had been eradicated and the inflammation had abated. The origins of this shift's pathology are obscure. The lack of detectable parasites does not exclude immunological damage to the hepatocytes, especially near the lobular periphery.

The inflammatory cellular reaction affects the portal tracts comprising parasitised macrophages, lymphocytes, and plasma cells. This reaction can be widespread or localised inside the lobule's sinusoid[23]. Although the parasites vanished following treatment, in 77% of instances, the inflammatory response persisted or even worsened. The only dead mice exhibited significant fatty alterations. Another mouse that passed away from visceral leishmaniasis and had a fatty liver was previously observed. Visceral leishmaniasis patients with significant liver fatty changes have a bad prognosis.Compact epithelioid granulomas and Langhans' big cells are not present. In contrast, cutaneous leishmaniasis is associated with a delayed hypersensitivity reaction, as shown by a positive leishmanin test and well-developed, compact granulomas[22].

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