ORIGINAL ARTICLE

CONTENTS

Investigating the impact of zinc oxide nanoparticles derived from the alcoholic extract of *Origanum majorana* leaves on the histological morphology of *Leishmania donovania* infected albino rats' livers

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ABSTRACT

Aim: The impacts of zinc oxide nano-extract from *Origanum majorana* leaves on the liver histological morphology of rats were investigated in the current study.
Materials and Methods: There were forty-two rats that are rats are divided into six groups of seven animals. Performing the experiment, 0.5 mg of normal saline was given to the first group, T1. Additionally, a 10 mg/kg nano-extract of *Origanum majorana* leaves was given to the second group, T2. A nano-extract solution containing 15 mg/kg of *Origanum majorana* leaves extract was injected to the third group, T3. A nano-extract derived from the seeds of *Origanum majorana* leaves of 10 mg/kg in combination with *L. donovania* was injected to the fourth group, T4. A nano-extract from *Origanum majorana* leaves at a concentration of 15 mg/kg in conjunction with *L. donovania* was injected to the fifth group, T5. Finally, *L. donovania* alone was injected for 29 days to the sixth group, T6.
Results: Considerable changes were noticed in the nucleus and fib. Moreover, the findings revealed that there are several changes in their liver's structure like sinusoidal dilatation, prescience of specific inflammatory cells surrounding the central vein, and prescience of simple necrosis and bleeding in *L. donovani*-infected tissue.
Conclusions: The histological changes on the liver treated with Nano-extract (zinc oxide) of the *Origanum majorana* leaves at different concentrations was employed for treating the histological shifts and reducing the damage caused by the parasite. Thus, effectiveness of the nano-extract is effective in reducing the influences and resisting the parasite.

KEY WORDS: zinc oxide, *Origanum majorana* leaves, *Leishmania donovania,* rats' livers

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INTRODUCTION

One of the large organ and gland in the human body is liver which held almost 2% of the adult's total weight. It occurs in the upper right quadrant of the abdomen exactly below the diaphragm. It is created of a larger right lobe and a smaller left lobe by the sickle ligament [1]. The gastrohepatic ligament connecting the stomach to the left hepatic lobe and the liver to the digestive system includes neurovascular components such the hepatic branch of the vagus nerve. The hepatoduodenal ligament and portal liver link the duodenum and portal structures, making the liver a major conduit for oxygenated and deoxygenated blood to the heart. In addition, the transverse colon may sometimes be seen in close proximity to or immediately contacting the right lobe of the intestinal tract [2, 3]. Presently, nanoparticles (NPs) are being employed in medical fields for the diagnosis and treatment of numerous diseases, including cancer and autoimmune disorders [4]. However, their application in medical fields is severely restricted due to the detrimental impacts they have on healthy cells and organs. Nanoparticle size, surface area, shape, dispersion, and protein corona effects also affect their safety and toxicity [5]. Interdisciplinary cancer nanobiotechnology, which combines engineering, science, and medicine, is an expanding field with numerous applications. The cancer treatment strategy offered is comprehensive and unique, including preventive measures, personalized therapy interventions, early diagnosis, prediction, and medication [6].

AIM

The impacts of zinc oxide nano-extract from *Origanum majorana* leaves on the liver histological morphology of rats were investigated in the current study.

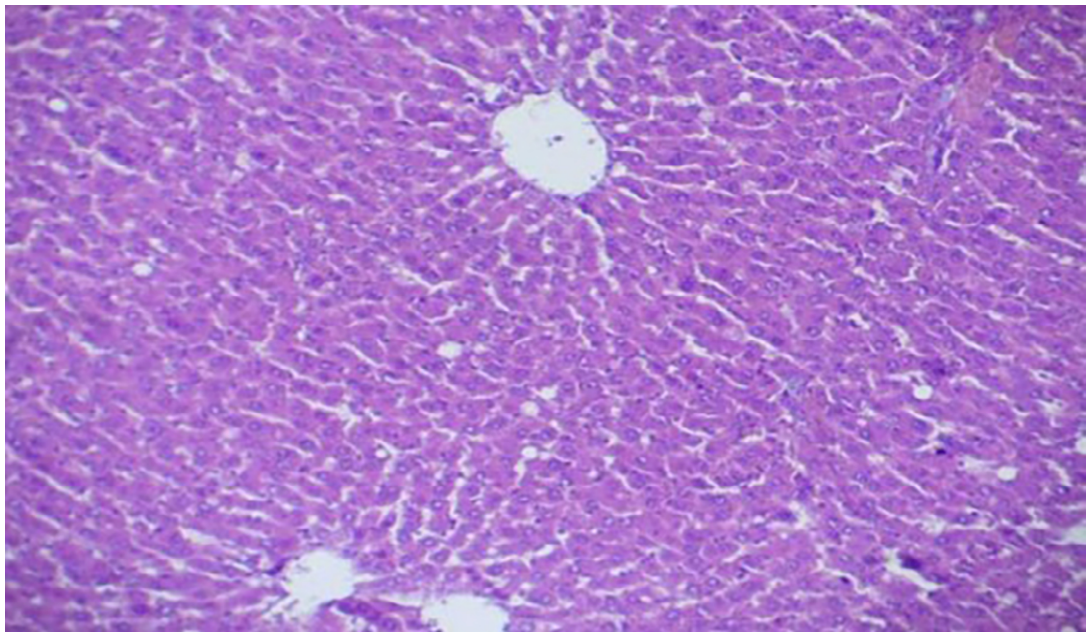

Fig. 1. A liver tissue cross-section in control group displaying the normal structure of hepatocytes and the central vein (100x) (H&E)
Source: Auhor's own study

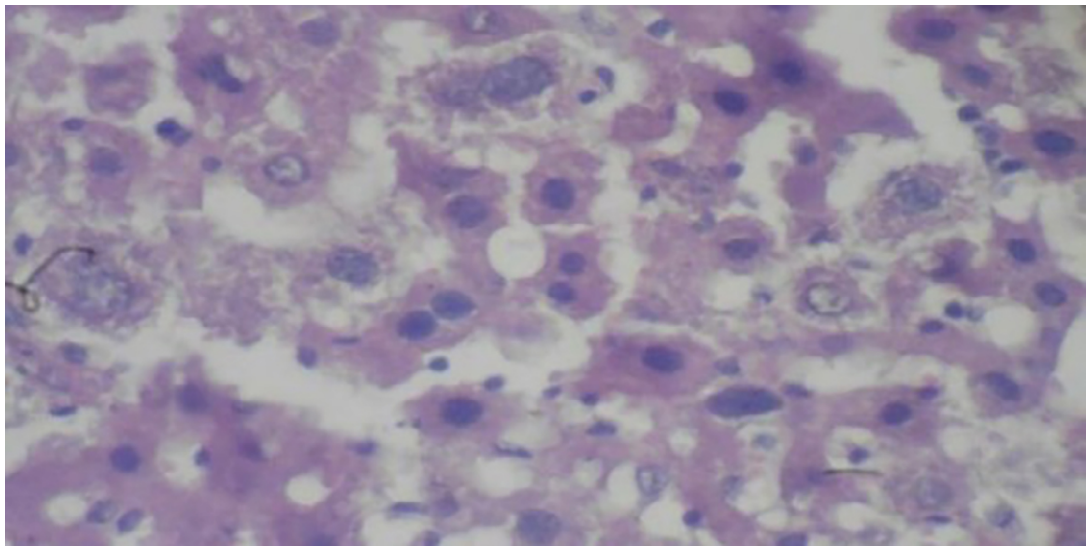

Fig. 2. A liver tissue cross-section of the rat in the experimental group that was treated with a nano-extract derived from the seeds of *Origanum majorana* at a concentration of 10 ml/kg. The hepatocytes exhibit normal appearance, the hepatic fibers demonstrate regularity, and histological changes are absent (400x H&E stain)
Source: Auhor's own study

MATERIALS AND METHODS

Methods for producing an alcoholic extract from powdered *Origanum majorana* leaves. The methanolic extract of the seeds obtained from the leaves of *Origanum majorana* was prepared, with certain modifications, in accordance with the micro-FTIR method [7]. The process was modified to produce nanoparticles (zinc oxide) from methanolic *Origanum majorana* leaves extract [7]. The research employed thirty adult male white Albino rats of the type Rattus, weighing between 250 and 300 g and aged between 6 and 10 weeks. Plastic boxes with iron clips on top that held water bottles were used to keep the animals. Cages are always being cleaned and have trash on top of them. A natural pellet meal is supplied, and the animals are kept in acceptable laboratory settings with 12 hours of light and 12 hours of darkness at 20-25°C. The animals were left for 14 days prior to the commencement of the experiment in order to facilitate adaptation. Throughout this time, they were supplied with water and a formulated diet [8]. The Institutional Animal Care's Central Ethics Committee Biology conducted the first evaluation, authorization, and acceptance of the procedures for the use of live animals in research. The study was conducted from January 2021 to March 2022. The rodents were categorized into the following six groups. The control group (T1) consists of 7 animals. The rats were given 0.5 mg of normal saline intravenously every time they were tested. The second group (T2) comprised 7 animals that were injected with a 10 mg/kg nano-extract of *Origanum majorana leaves*. Group T3, consisting of 7 animals, was administered a nano-extract solution containing 15 mg/kg of *Origanum majorana* leaf extract. Group T4, consisting of 7 animals, was injected with a nano-extract derived from the leaves

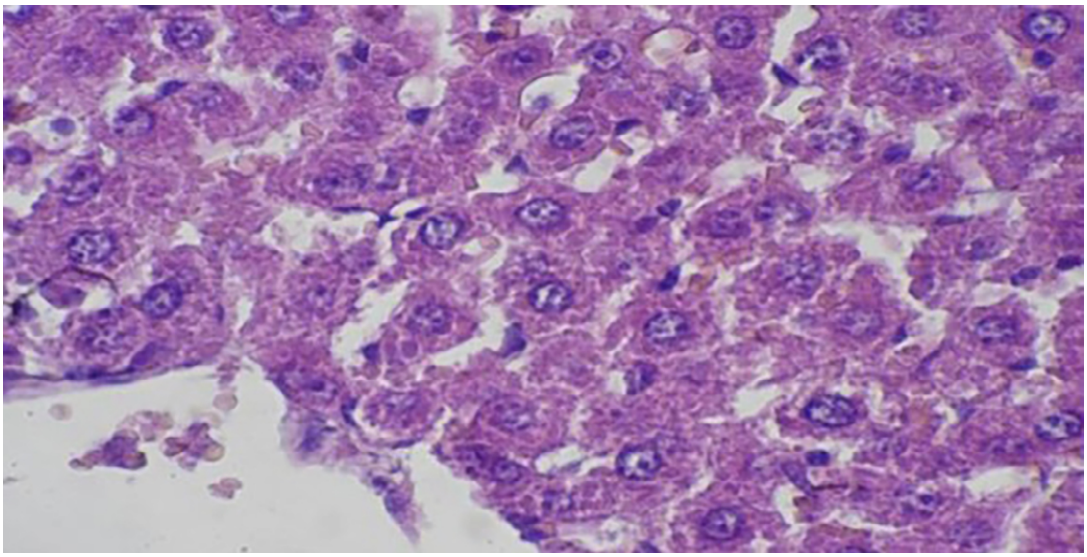

Fig. 3. A cut through the liver tissue of a rat in the group that was given a nano-extract made from *Origanum majorana* seeds at a 15 ml/kg dose showed the following: There are a few binuclear cells among the liver nerves and hepatocytes (400x H&E), which give them their unique shape and pattern
Source: Auhor's own study

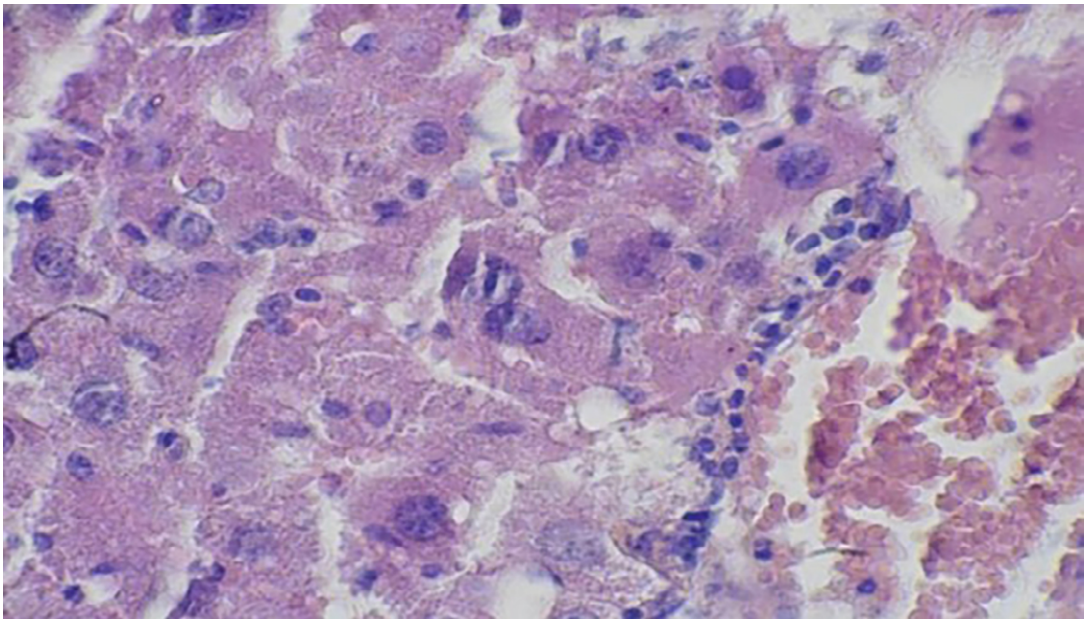

Fig. 4. A liver tissue cross-section from a rat in the *Origanum majorana* leaf treatment group (10 mg/kg plus *L. donovania*) demonstrates a central vein with noticeable hepatocyte necrosis (H&E X400)
Source: Auhor's own study

of *Origanum majorana* at a concentration of 10 mg/kg in conjunction with *L. donovania*. Group T5, consisting of 7 animals, was administered a nano-extract derived solely from the leaves of Origanum majorana at a concentration of 15 mg/kg in conjunction with *L. donovania*. Group T6, consisting of 7 animals, that were injected solely with *L. donovania* as the positive control group. All of the animals from the beginning of the experiment were slaughtered once the first trial ended. To perform an autopsy, the animals were fastened to a dissection plate constructed from cork and firmly fastened with staples. The purpose of the autopsy was to assess the impact of different doses 10 and 15 mg/kg of nano-zinc oxide derived from *Origanum majorana* leaves seed extract on the liver. The animals were rendered unconscious by injecting a mixture of 20 mg of xylazine and 10 mg of ketamine [9]. The promastigotes of the *L. donovani* parasite were cultured and activated in Novy-MacNeal-Nicolle (NNN) media. After that, it was put into RPMI-1640 medium together with 1% of the antibiotic (penicillin and streptomycin) and 10% FBS serum. Very sterile, and after 72 hours, put in a refrigerated incubator at 26°C, the right temperature for the parasite's flagellated stage of development [10]. In order to confirm that the parasite's promastigote stage was growing, the previously incubated culture medium was checked by taking a prepared slide with a 40x lens for microscopic details after a drop of the media was applied to it. When it was confirmed that the flagellar stage of the parasite had appeared, 0.5 ml of the isolate was transferred [11]. After collecting the promastigotes, they were centrifuged at 1500 rpm for 10 minutes to wash them in locks' solution. The pellet was then resuspended in approximately 5 ml of lock solution and the supernatant was removed using a Pasteur

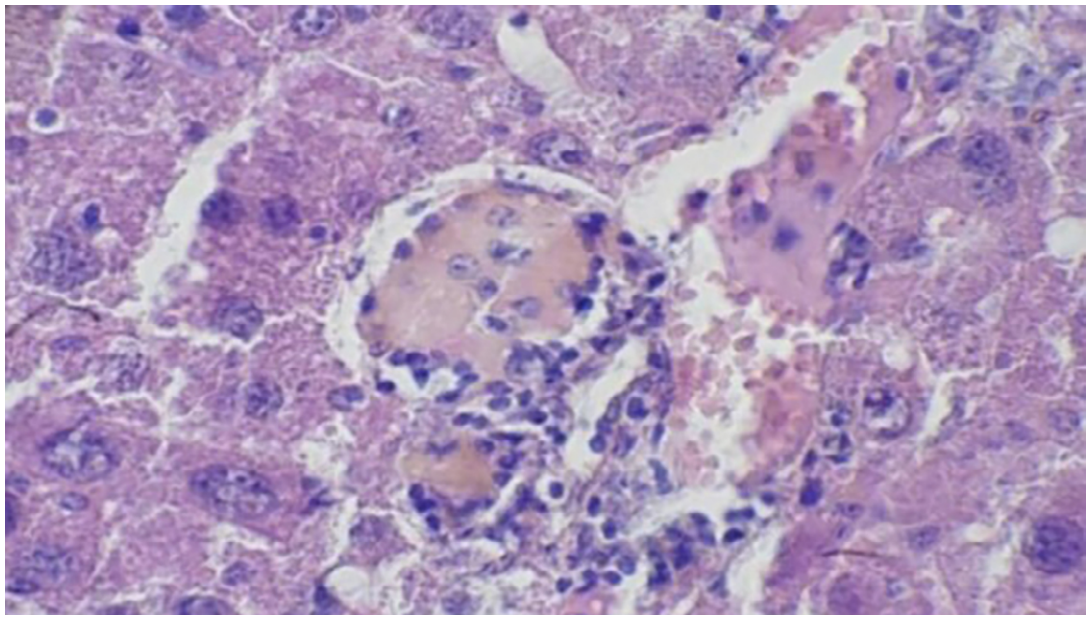

Fig. 5. A rat's liver tissue cross-section from the group that was given 15 mg/kg of *Origanum majorana* leaves along with *L. donovania* shows that inflammatory cells have started to appear (H&E X400)
Source: Auhor's own study

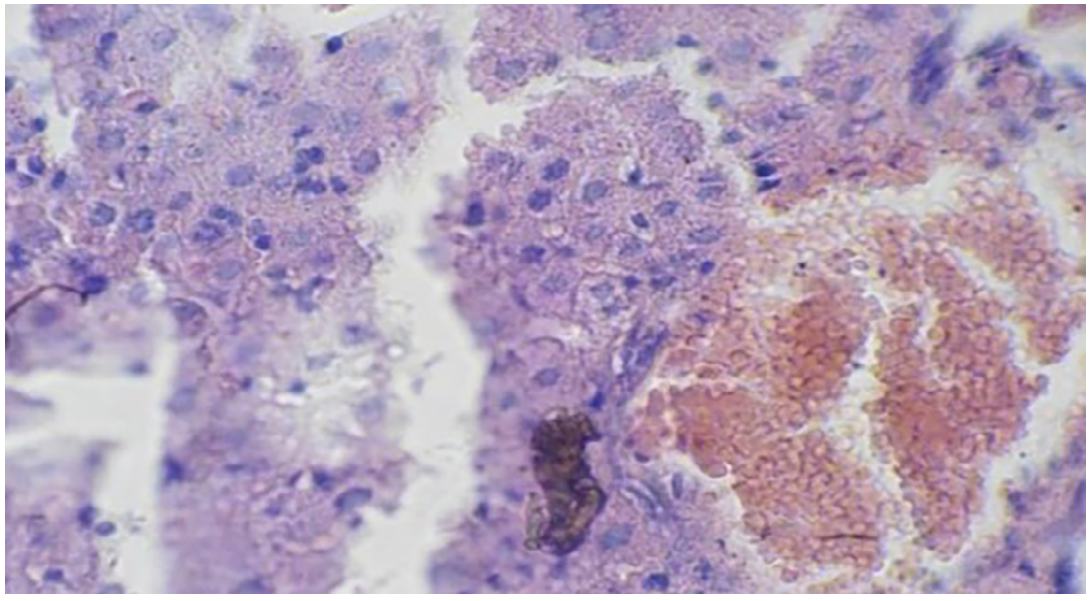

Fig. 6. A liver tissue cross-section from a rat infected with *L. donovania* that shows the presence of inflammatory cells and bleeding (H&E X400)
Source: Auhor's own study

pipette. Using a hemocytometer, the concentration was adjusted to 1.2106 parasite promastigote cells based on the number of promastigotes per ml. The following is a list of the animals found in each of the 16 little corner squares. The total number of cells per ml = N x 10 x 1000 x 20 where N = number of cells counted, 10 = number of cells in l mm^3, 1000 = number of cells in l ml, 20 is the dilution factor.

RESULTS

In figure 1, microscopic inspection of the control group's livers showed normal liver structure and no hepatocyte histological alterations. As can be seen in figure 2, when animals were given a nano-extract of *Origanum majorana* leaves at a dosage of 10 mg/kg, their liver cells had a normal structure, regular arrangement of liver tissue, and no histological changes. As seen in figure 3 show, that after receiving of a 15 mg/kg nano-extract of *Origanum majorana* leaves *via* injection, the livers of the animals exhibited typical hepatic cord morphology, hepatocytes with their characteristic configuration, and the development of certain binuclear cells. Figure 4 demonstrates that upon administering a nano-extract derived from *Origanum majorana* leaves at a dose of 10 mg/kg together with *L. donovani*, the livers of the mice had a central vein with significant hepatocyte necrosis. As seen in figure 5, inflammatory cells invaded the livers of mice that were administered a 15 mg/kg dosage of *Origanum majorana* leaf nano-extract together with *L. donovani*. Figure 6 shows that the livers of mice injected with *L. donovani* exhibited the presence of atypical cells, bleeding, and inflammatory cells.

DISCUSSION

Nanotechnology is an efficient treatment method because of its high efficacy and therapeutic index against germs. The use of novel biomaterials like nanoparticles to accomplish this accomplishment is generating curiosity all over the world. For the treatment of diseases that are resistant to medication, nanoparticles have the potential to become a very important viable alternative [12]. This is consistent with a close study on the effects of the inhibitory nano-extract. Nanotechnology is a brand-new, enabling technology that has the potential to lead to a wide range of innovative uses and better technologies for biological and biomedical applications. Nanotechnology is one of the factors contributing to the increased interest [13]. The liver, central veins, hepatic cords, and sinusoids are histologically normal. Animal models are also useful. Some studies show a toxicity concern after apricot and other fruit seed ingestion, as seen by increased liver chemistry tests. These findings corroborated a prior study [14, 15]. In contrast, Shaibah et al. [16] proposed amygdalin as a potential preventative measure against hepatic fibrosis. A higher concentration of serum ALT (alanine aminotransferase) than AST (aspartate aminotransferase) signifies liver injury with greater specificity [17]. Even though amygdalin's toxicity and effectiveness in animal models have been extensively studied, oral administration releases much cyanide [18]. The conversion of amygdalin to hydrocyanic acid can occur through the activity of emulsion complex enzymes [19], which include β-D-glucosidase, a compound present in foods and small intestine and colon microflora [20]. Amygdalin may undergo hydrolysis in the absence of enzyme catalysis [21]. Benzaldehyde, glucose, and hydrocyanic acid are the byproducts of its hydrolysis in water. Amygdalin, on the other hand, goes through epimerization when it's heated. Cyanide is a very toxic substance that may cause harm to essential organs such as the kidneys, liver, brain, and heart by producing reactive oxygen species (ROS). In addition, the failure to reduce free radical damage leads to oxidative stress [22]. Recent research has revealed that amygdalin is toxic when ingested orally but not when administered intravenously; however, its mechanism of action and potentially lethal concentrations remain unknown [23]. It is possible that an eclectic gut is connected with the toxic effects caused by many oral dosages of amygdalin [24]. Histopathological examination of particular tissues showed severe liver degeneration in rats given high-dose amygdalin. In contrast, the control and amygdalin-treated livers had typical morphology. In the current investigation, cytoplasmic vacuolization, Kupffer cell activation, and vascular congestion were indicative of liver injury. Similar histological damage was seen in the rats' livers after oral cyanide treatment [25]. Nonetheless, goats' exposure to cyanide chemicals resulted in minor hepatic degenerative alterations [26].

CONCLUSIONS

The extract of zinc oxide nanaoextract was more efficient in inhibiting the amastigote phase of *L. donovani* in rats and did not show any side effects on the histological structure of spleen. The current study was conducted to determine the effect of zinc oxide nanoparticles from *Origanum majorana* leaves extract at different concentrations (10-15 kg/g).

REFERENCES

1. Sherif R Z Abdel-Misih, Mark Bloomston. Liver anatomy. Surg Clin North Am. 2010 Aug; 90(4): 643-53. doi: 10.1016/j.suc.2010.04.017.
2. Abdul-Jabbar ZS, Mohammed JA. Study of Histological Changes in the Bones of Front and Hind Limbs of White Rat Treated with Ibuprofen and Lepidium Sativum. Revista Geintec-Gestao Inovacao e Tecnologias. 2021;11(2):1988-2003. doi:10.47059/revistageintec.v11i2.1813. DOI
3. Zhou C, Qian L, Ma H, et al. Enhancement of amygdalin activated with β-d-glycosidase on HepG2 cells proliferation and apoptosis. Carbohydr Polym. 2012;90(1):516–523. doi: 10.1016/j.carbpol.2012.05.073. DOI
4. Upadhyaya H, Shome S, Sarma R, Tewari S, Bhattacharya MK, Panda SK. Green synthesis, characterization and antibacterial activity of ZnO nanoparticles. Am J Plant Sci. 2018;9:1279-1291. doi: 10.4236/ajps.2018.96094. DOI
5. Sukayna JM, Jabbar AA. Effect Addition of the Extract Nigella sativa on the Histological and Physiological Changes of the Domestic Chicken Experimental Infected with Eimeria maxima. J Pharm Sci Res. 2018;10(8):1934-1938.
6. Ahmed AJA, Saleem A, Sukayna JM. Immune Response in Pregnant Women Infected with Acute Vaginal Abscess Caused by Staphylococcus Aureus and Trichomonas Vaginalis. JCDR. 2018; I-12(6): DC51-DC55. doi: 10.7860/JCDR/2018/35653.11643. DOI
7. Chen Y, Zou C, Mastalerz M, Hu S, Gasaway C, Tao X. Applications of micro-Fourier transform infrared spectroscopy (FTIR) in the geological sciences – a review. Int J Mol Sci. 2015;16:30223-30250. doi: 10.3390/ijms161226227. DOI
8. Chang YS, Lin CL, Lee CW, Lin HC, Wu YT, Shih YH. Exercise Normalized the Hippocampal Renin-Angiotensin System and Restored Spatial Memory Function, Neurogenesis, and Blood-Brain Barrier Permeability in the 2K1C-Hypertensive Mouse. Int J Mol Sci. 2022; 23(10): 5531. doi: 10.3390/ijms23105531. DOI

9. Sukayna JM, Saleem KA, Jabbar AM. Effect of Magnetized Water on Reducing the Histological and Physiological Effects of Experimental Infection with Ascaridia galli in Domestic Chicken. JGPT. 2018;10(01):97-103.
10. Khodir SA, Faried MA, Abd-Elhafiz HI, Sweed EM. Sitagliptin Attenuates the Cognitive Deficits in L-Methionine-Induced Vascular Dementia in Rats. Biomed Res Int. 2022 Feb 27; 2022: 7222590. doi: 10.1155/2022/7222590. DOI
11. Blaheta RA, Nelson K, Haferkamp A, Juengel E. Amygdalin, quackery or cure? Phytomedicine. 2016;23(4):367-376. doi: 10.1016/j.phymed.2016.02.004. DOI
12. Savic IM, Nikolic VD, Savic-Gajic IM, Nikolic LB, Ibric SR, Gajic DG. Optimization of technological procedure for amygdalin isolation from plum seeds (Pruni domesticae semen). Front Plant Sci. 2015;6:276. doi 10.3389/fpls.2015.00276.
13. Mostafa OAA, Ibrahim F, Borai E. Protective effects of hesperidin in cyclophosphamide-induced parotid toxicity in rats. Sci Rep. 2023 Jan 4;13(1):158. doi: 10.1038/s41598-022-26881-w. DOI
14. Varma RS. Health concerns of various nanoparticles: a review of their in vitro and in vivo toxicity. Nanomaterials (Basel). 2018 Sep; 8(9): 634. doi: 10.3390/nano8090634. DOI
15. Abar ES, Alaridhi JA. Study of the Effect of Aqueous Extract of (Ginger) Zingiberofficinale rosco in the Histological Structure of prostate gland of white male rabbits Oryctolagus cuniculus. Plant Arch. 2019;19(Suppl 1):293-298.
16. Shaibah HS, Elsify AK, Medhat TM, Rezk HM, El-Sherbiny M. Histopathological and immunohistochemical study of the protective effect of triptorelin on the neurocytes of the hippocampus and the cerebral cortex of male albino rats after short-term exposure to cyclophosphamide. J Microsc Ultrastruct. 2016(Jul-Sep);4(3):123-132. doi: 10.1016/j.jmau.2015.12.002.
17. Mushattat SJ, Alaridi JA. Effect of cold water extract Zingiber officinale on the histological changes of the Experimental infection of domestic chickens with Ascaridia galii. J Pharmaceut Sci Res. 2018;12(1):186-190.
18. Abdel-Hafez SMN, Rifaai RA, Abdelzaher WY. Possible protective effect of royal jelly against cyclophosphamide induced prostatic damage in male albino rats; a biochemical, histological and immuno-histo-chemical study. Biomed Pharmacother. 2017 Jun;90:15-23. doi: 10.1016/j.biopha.2017.03.020. DOI
19. Ibrahim HM, Zommara MA, Elnaggar ME. Ameliorating effect of selenium nanoparticles on cyclophosphamide-induced hippocampal neurotoxicity in male rats: light, electron microscopic and immunohistochemical study. Folia Morphol (Warsz). 2021; 80(4): 806-819. doi: 10.5603/FM.a2020.0117 DOI
20. Jabbar AM, Methak AA. The Histological structure of Thyroid gland and the relationship between the hyperthyroidism and total protein, albumin, globulin, liver enzymes and some minerals deficiency. IJPRIF. 2016;9(8):189-196.
21. Jabbar EM, Noor MH. The Effect of Cirprofloxacin (CPX) on the Histological Structure of Albino Rabbit Ovary. JGPT. 2018;10(03):498-508.
22. Kadiri HE, Asagba SO. The chronic effects of cyanide on oxidative stress indices in the domestic chicken (Gallus domesticus L). J Basic Appl Zool. 2019;80(1):30. doi 10.1186/s41936-019-0098-y.
23. Mushattat SJ, Beshboosh NN, Mehdi LA, Hassan AB. Effect of Toxoplasma gondii on Some Physiological Indicators in Women's Patients with Diabetic Mellitus Type 2. J Pharmaceut Res Int. 2021; 33(46B): 61-65. doi: 10.9734/jpri/2021/v33i46B32915. DOI
24. Cigni A, Faedda R, Atzeni MM, et al. Hormonal strategies for fertility preservation in patients receiving cyclophosphamide to treat glomerulonephritis: a nonrandomized trial and review of the literature. Am J Kidney Dis. 2008 Nov;52(5):887-96. doi: 10.1053/j.ajkd.2008.06.017. DOI
25. Ibrahim KM, Darwish SF, Mantawy EM, El-Demerdash E. Molecular mechanisms underlying cyclophosphamide-induced cognitive impairment and strategies for neuroprotection in preclinical models. Mol Cell Biochem. 2023. doi: 10.1007/s11010-023-04805-0. DOI
26. AL-Aameli MH, Al-Qazwini YM, Mohammed JA. Histological Investigation Of The Effects Of Cinnamon Extract On Skin Of Male Sheep Affected By Mange. Sys Rev Pharm. 2020;11(12):380-386.

Ethical approval
This study was approved by the Animal Care Committee at the University of Kufa, Iraq (2020/No.20660)

CONFLICT OF INTEREST
The Authors declare no conflict of interest

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