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# Histopathological and immunohistochemical studies on the effects of silver oxide nanoparticles (AgNPs) on male rats' liver

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## Abstract

**Background:** Silver oxide nanoparticles (AgNPs) have many uses as antiviral materials that cause diseases, they are considered AgNPs to destroy bacteria, they can also be considered as antidotes for many serious diseases such as cancer and viruses**,** on the creation and characterization of silver oxide nanoparticles (AgNPs) and investigate the impact of varying doses of AgNPs on the antioxidant defense system, as well as the histology and immunohistochemistry of male rats liver. **Methods:** There were fifty male rats (150–170 g) utilized. The animals were split up into five groups, each with ten rats treated for three weeks, the first group served as the control, while groups II, III, IV, and V received oral treatment with AgNPs at doses of 1/150 LD50, 1/100 LD50, 1/50 LD50, and 1/25 LD50 mg/kg BW/day. After the trial, liver and blood samples were taken to look at various factors. **Results:** Silver nanoparticles (AgNPs) were prepared at different concentrations, used in laboratory animals, and characterized using different instruments. treatment with silver nanoparticles (AgNPs) in different concentrations Changes in histopathology and immunohistochemistry were noted, confirming that biochemical disruptions were caused by silver nanoparticles (AgNPs) in distinct amounts in the liver of rats. **Conclusion:** It is evident that silver nanoparticles (AgNPs) Produced significant negative effects in rat liver tissue and p53 protein in a dose-dependent way.

**Keywords:** Silver oxide nanoparticles (AgNPs), P53, Eosin.

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## Introduction

Nanoparticles (NPs) have developed and gained great importance worldwide due to their high properties, such as their small size and high percentage of surface atoms. Over the past two decades, previous studies in the field of biotechnology have led to the manufacture of nanomaterials at very low prices that are beneficial to society and the environment. Numerous investigations in the domains of science, engineering, and biotechnology have been carried out during the previous 20 years to build affordable

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and environmentally friendly nanomaterials **[1]**.

Nanoparticles (NPs) have unique qualities (e.g., electrical, optical, quantum mechanical, etc.) that may be used in a range of methods because of their physicochemical characteristics, which include tiny size, large surface area, and high reactivity **[1,2].** The use of nanocomposites produces toxic effects on liver tissue, and our study aims to study the toxic effect of silver oxide nanoparticles (AgNPs) on the liver of male rats and to know the changes in tissue composition and immunohistochemistry **[3].** In recent years, there has been a wide interest and development in the field of nanotechnology, specifically in science and technology, silver oxide nanoparticles (AgNPs) have shown a very high interest in all journals, especially medical ones **[4].** It has been proven through previous references that silver oxide nanoparticles (AgNPs) have many effects on diabetics and the thyroid gland in rats **[5].** Numerous newly developed SnO2 NPs have the potential to be used in a range of technical applications, some of which may be environmental in nature. This might lead to their discharge into the atmosphere and contact with relevant native biota, such as bacteria. This is concerning since NPs' physico-chemical characteristics—such as their size and shape—which enable their novel uses may also make them extremely active in biological systems and hazardous **[6].** Reactive oxygen species (ROS) generated by nanoparticles are a major cause of tissue and cell damage **[7].** Overproduction of ROS may lead to oxidative stress, which interferes with cells' ability to carry out their normal physiological redox-regulated functions. Damage to DNA, abnormal cell signaling, altered motility, cytotoxicity, apoptosis, and the onset of cancer can result from this. ROS have been linked to several stages of the carcinogenesis process, such as epigenetic modifications, structural DNA damage, and changes in proteins and lipids **[8].** Studies have shown a correlation between occupational exposure to nanomaterials and elevated levels of DNA damage (8-OHdG) **[9,10].** Our study aims to study the toxicity caused by AgNPs at different concentrations on tissues and immune tissue chemistry in the liver of male rats.

## Material and Method

### Chemicals

Each chemical employed in this study is of the caliber of an analytical reagent, and it was used precisely as given.

### Materials

1. Silver nanoparticles (AgNPs) were used at different concentrations.

### Animals and Experimental Design

Fifty male Albino Wistar rats weighing 150-170g. The guidelines for handling laboratory animals were followed while handling the animals. The rats were kept in wire cages with stainless steel bottoms, with a temperature of 22 ± 2oC and a relative humidity of 40–60%. They were also given unrestricted access to a pellet meal that consisted of 20% casein, 15% corn oil, 55% corn starch, 5% mixture of salts, and 5% vitamin-starched starch. The animals were given the test drugs following the subsequent experimental methodology:

- Gp I (control): Control rats were given distilled water orally every day for three weeks using a ball-tipped, curved intubation needle.
- Gp II (AgNPs): For three weeks, rats were given a daily dosage of 1/150 LD50 mg/kg BW by orally.
- Gp III (AgNPs): For three weeks, rats were given a daily dosage of 1/100 LD50 mg/kg BW by orally.
- Gp IV (AgNPs): For three weeks, rats were given a daily dosage of 1/50 LD50 mg/kg BW by orally.
- Group V (AgNPs): For three weeks, rats were given a daily dosage of 1/25 LD50 mg/kg BW by orally.

### Tissue samples

Rats were sacrificed, and the liver was taken out right away and cleaned with cold saline. The liver was then weighed and cleaned using a 0.9% cooled saline solution. Using a Potter-Elvehjem type homogenizer, tissues were chopped and homogenized (10% w/v) in ice-cold sodium phosphate buffer (0.01 M, pH 7.4) containing 1.15% KCl. The homogenates were centrifuged for 20 minutes at 4°C at 10,000 xg. The resulting supernatants were utilized to analyze several biochemical parameters, free radicals, and enzyme activity.

### Histopathological studies

Liver specimens were preserved for 48 hours in 10% neutral buffered formalin. Liver sections underwent trimming, washing, dehydration, and bedding in paraffin. They were then sliced into sections seven microns thick and stained with hematoxylin and eosin according to **[11,12].**

### P53 immunohistochemical

The liver segment's apoptotic p53 protein expression was determined through the P53 antibody (dilution 1:200 DAKO Japan Co, Ltd., Tokyo, Japan) and the ABC (Elite-ABC, Vector Laboratories) technique according to **[13,14,15].**

## RESULTS

### Liver histopathology

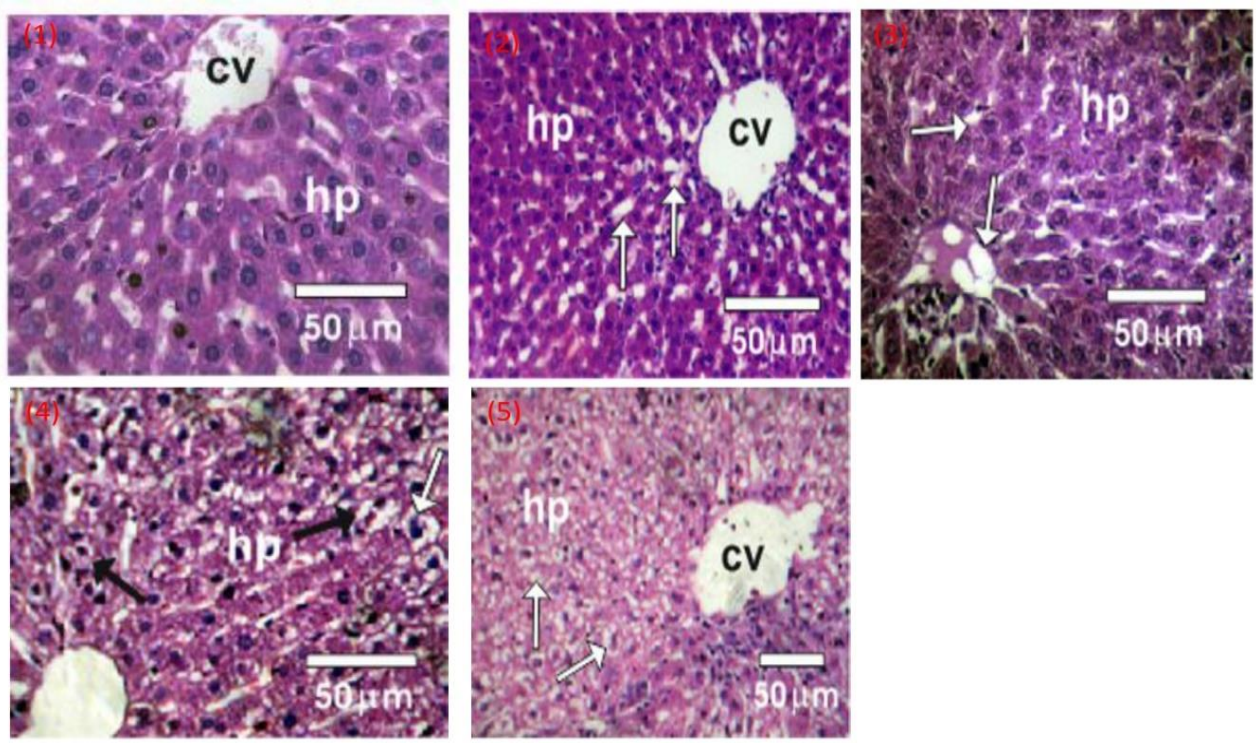


**Figure** 1. (1) Photomicrograph of rat Liver sections in control rats group stained with Haematoxylin & Eosin and revealed normal structure of hepatocytes with normal central veins (CV). (2) Liver sections after the administration of silver nanoparticles (AgNPs) at a dose of 1/150 LD50 revealed moderate atrophy (arrows), an increase in the number of Kupffer cells, congested central vein, and blood sinusoids. (3) Photomicrographs of rat liver sections stained with Haematoxylin & Eosin. Liver sections after the administration of silver nanoparticles (AgNPs) at a dose of 1/100 LD50 revealed moderate atrophy (arrows), an increase in the number of Kupffer cells, congested central vein, and blood sinusoids. (4) Liver sections after the administration of a dose of 1/50 LD50 of Silver nanoparticles (AgNPs) revealed marked degeneration of hepatic cords, fatty changes, focal necrosis, pyknotic nuclei, increased incidence of vacuolar degeneration and diffused hepatic necrosis with cellular infiltration. (5) Liver sections after the administration of a dose of 1/25 LD50 of Silver nanoparticles (AgNPs) revealed marked degeneration of hepatic cords, fatty changes, focal necrosis, pyknotic nuclei, increased incidence of vacuolar degeneration and diffused hepatic necrosis with cellular infiltration.

**P53 immunohistochemical changes in rat liver**

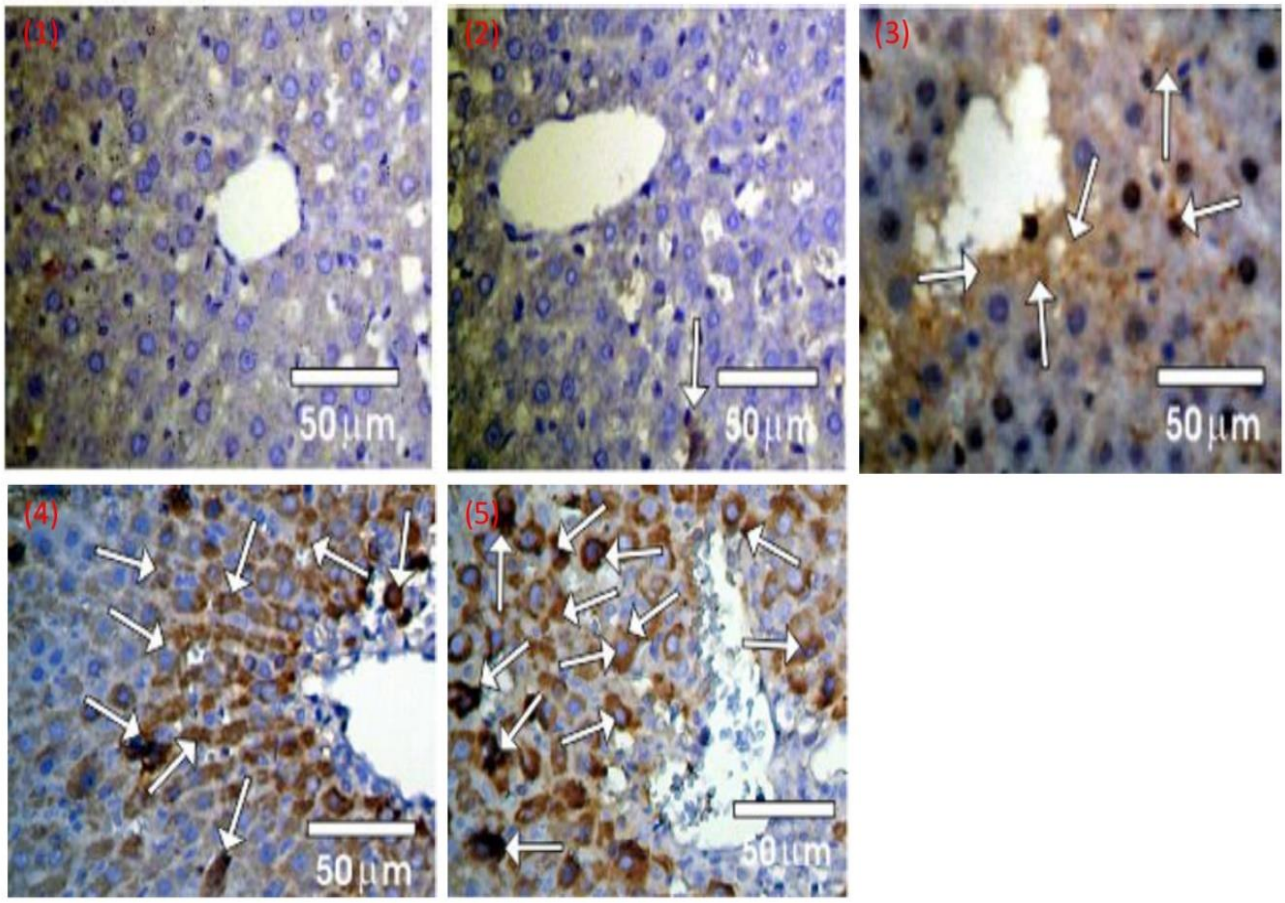


**Figure** 2. (1) Photomicrograph of rat liver section in control group stained with P53-ir showed negative or faint positive reaction for P53. (2). Liver sections with 1/150 LD50 Silver nanoparticles (AgNPs) revealed faint positive reactions for P53 immunoreactivity. (3). Liver sections with 1/100 Silver nanoparticles (AgNPs) revealed mild to moderate positive reactions for P53 immunoreactivity in hepatocytes (arrows). (4) Liver sections with 1/50 LD50 Silver nanoparticles (AgNPs) revealed strong positive reactions for P53 immunoreactivity in hepatocytes (arrows). (5). Liver sections with 1/25 LD50 Silver nanoparticles (AgNPs) revealed strong positive reactions for P53 immunoreactivity in hepatocytes (arrows).

## Discussion

Certain nanoparticles, such as silver nanoparticles (AgNPs) and zinc oxide (ZnO), are utilized in items like auto-cleaning glasses and tiles, according to earlier research. Because of their tiny size, nanoparticles may thus pass through cell membranes and disrupt vital cell processes. According to recent research, when mice are given nanoparticles, they accumulate in several organs, such as the brain, testis, liver, and kidney (16). The control rats' hepatic parenchyma was made up of several hepatic lobules divided by very thin connective tissue septa containing a trio of portals. Hepatic cords spreading outward from the center of each hepatic lobule encircled the thin-walled central vein. The hepatic portal vein, a hepatic artery branch, and a bile ductile are all located in the portal region. When Silver nanoparticles (AgNPs) with 1/150 LD50 concentrations were administered, liver sections showed normal hepatocyte structure with only mild atrophy and degeneration along with an increase in Kupffer cell count. Conversely, liver sections treated

with silver nanoparticles (AgNPs) with 1/100 LD50 concentration showed moderate atrophy and an increase in Kupffer cell count, localized mononuclear cellular infiltration, blood sinusoids and central vein congestion, and apoptotic cell death. High doses of silver nanoparticles (AgNPs) (1/50 LD50 and 1/25 LD50 concentrations) caused perivascular round cell infiltration, which is linked to membrane changes in endothelial lining cells that show periportal fibrosis, significant hepatic cord degeneration, and a moderate increase in Kupffer cell count, fat alterations, localized necrosis, pyknotic nuclei, a higher frequency of vacuolar degeneration, and disseminated hepatic necrosis with macrophage and lymphocyte infiltration. Organogenesis, a process of targeted cell induction and differentiation, and maturation, a process by which an organ reaches full functional maturity, are the two processes that give rise to an organ's development **[17].**

In the liver sections under control, there was very little apoptotic cell incidence and very little cytoplasmic p53 expression. As silver nanoparticles (AgNPs) at varying doses were administered to liver hepatocytes, there was a notable rise in the expression of cytoplasmic p53 as compared to the control group. P53 immunoreactivity in liver sections treated with 1/150 LD50 and 1/100 LD50 Silver nanoparticles (AgNPs) showed modest to moderate positive responses, similar to those in control groups. Additionally, P53 immunoreactivity was seen in somewhat positive ways in the renal tubules on kidney sections treated with 1/150 LD50 and 1/100 LD50 Silver nanoparticles (AgNPs). On the other hand, the liver sections treated with 1/50 LD50 and 1/25 LD50 Silver nanoparticles (AgNPs) showed substantial positive responses for P53 immunoreactivity. In the current investigation, rat liver silver nanoparticles (AgNPs) P53 intensity was noticeably higher than in the control group. P53 expressions significantly rise with increasing concentrations of silver nanoparticles (AgNPs) because cells that are exposed to external damaging stimuli activate the control of these genes' expression. The present findings concur with **[18,19,20].** Additionally, exposing cells to nanoparticles may experience DNA damage, repairable oxidative stress or undergo apoptosis by force, all of which might change a cell's ability to proliferate, differentiate, or communicate with other cells **[21,22,23].** In reaction to various types of ultrafine particles, nanoparticles can cause changes in gene expression and cell signaling, as well as inflammation, cytokine release, cytoskeletal alterations, altered vesicular trafficking, oxidative stress, and apoptosis **[24,25].** The study's findings indicated that silver nanoparticles (AgNPs) could cause hepatocytes to undergo apoptosis by increasing the production of ROS inside cells.

## Conclusion

It is evident that silver nanoparticles (AgNPs) Produced significant negative effects in rat liver tissue and p53 protein in a dose-dependent way.

## Conflicting Interests:

The research, writing, and publishing of this paper do not present any possible conflicts of interest for the authors.

## Ethical approval:

The study design was approved by the Institutional Ethical Committee for Animal Care and Use (code: IACUC-SCI-TU-0241).

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## References

1) Ullah, Z., Gul, F., Iqbal, J., Abbasi, B. A., Kanwal, S., Chalgham, W., ... & Mahmood, T. (2023). Biogenic synthesis of multifunctional silver oxide nanoparticles (Ag2ONPs) using parieteria alsinaefolia delile aqueous extract and assessment of their diverse biological applications. Microorganisms, 11(4), 1069.

2)Jin, W.X., Ma, S.Y., Tie, Z.Z., Li, W.Q., Luo, J., Cheng, L., Xu, X.L., Wang, T.T., Jiang, X.H., Mao, Y.Z. (2015). Synthesis of hierarchical $SnO_2$ nanoflowers with enhanced acetic acid gas sensing properties. Appl Surf Sci. 353:71-78.

3) Abdel Aal, S. M., Mohammed, M. Z., Abdelrahman, A. A., Samy, W., Abdelaal, G. M., Deraz, R. H., & Abdelrahman, S. A. (2023). Histological and biochemical evaluation of the effects of silver nanoparticles (AgNps) versus titanium dioxide nanoparticles (TiO2NPs) on rat parotid gland. Ultrastructural Pathology, 47(4), 339-363.

4) Sulaiman, F. A., & Adeyemi, O. S. (2010). Changes in haematological indices and protein concentrations in Trypanosoma brucei infected rats treated with homidium chloride and diminazene aceturate. EXCLI journal, 9, 39.

5) Adeyemi, O. S., & Akanji, M. A. (2012). Psidium guajava leaf extract: effects on rat serum homeostasis and tissue morphology. Comparative Clinical Pathology, 21, 401-407.

6) Gebreslassie, Y. T., & Gebretnsae, H. G. (2021). Green and cost-effective synthesis of tin oxide nanoparticles: a review on the synthesis methodologies, mechanism of formation, and their potential applications. Nanoscale research letters, 16(1), 97.

7)Ab Kadir, R., Li, Z., Sadek, A.Z., Abdul Rani, R., Zoolfakar, A.S., Field, M.R., Ou, J.Z., Chrimes, A.F., Kalantar-zadeh, K. (2014). Electrospun granular hollow $SnO_2$ nanofibers hydrogen gas sensors operating at low temperatures. J. Phys. Chem. C 118: 3129-3139.

8)Suresh, A.K., Pelletier, D.A., Doktycz, M.J. (2013). Relating nanomaterial properties and microbial toxicity. Nanoscale 5: 463-474.

9)Yen, H. J., Hsu, S.H., and Tsai, C.L. (2009). Cytotoxicity and immunological response of gold and silver nanoparticles of different sizes. Small. 5:1553–1561.

10)Yin, J.J., Liu, J., Ehrenshaft, M., Roberts, J.E., Fu, P.P., Mason, R.P. and Zhao, B. (2012). Phototoxicity of nano titanium dioxides in HaCaT keratinocytes generation of reactive oxygen species and cell damage. Toxicology and applied pharmacology, 263(1), pp.81-88.

11)Daniela, P., Hana, B., Jana, K., Vladimir, Z., Tomas, N., Zdenka, F., Stepanka, V., Jaroslav, S., Nadezda, Z., Petr, K., Martin, K., Jaroslav, B., Sergey, Z. (2015). Raman microspectroscopy of exhaled breath condensate and urine in workers exposed to fine and nano TiO2 particles: a cross-sectional study. J Breath Res. 9: 036008.

12) Hassan, A. A., Adil, H., Alyasiri, T., Alsayed, R., Makia, R., Kadhom, M., ... & Yousif, E. (2024). Nickel oxide nanoparticles with ginger extract: An environmentally sustainable method for antibacterial applications. Results in Chemistry, 101617.

13) Hasan, A. F., Alankooshi, A. A., Abbood, A. S., Dulimi, A. G., Mohammed Al-Khuzaay, H., Elsaedy, E. A. & Tousson, E. (2023). Impact of B-Glucan Against Ehrlich Ascites Carcinoma Induced Renal Toxicity in Mice. OnLine Journal of Biological Sciences, 23(1), 103-108. https://doi.org/10.3844/ojbsci.2023.103.108.

14) Alankooshi, A. A., Alankooshi , A. A., Hasan, A. F., Tousson, E., El-Atrsh, A. &

Mohamed, T. M. (2023). Impact of Coriander Seeds Extract Against Thyroidectomy Induced Testicular Damage and DNA Replication in Male Rats. OnLine Journal of Biological Sciences, 23(2), 193-201. https://doi.org/10.3844/ojbsci.2023.193.201.

15) Hasan A. F, Hameed H. M, Tousson E, Massoud A, Atta F, Youssef H, Hussein Y. Role of Oral Supplementation of Damiana (Turnera diffusa) Reduces the Renal Toxicity, Apoptosis and DNA Damage Associated with Amitriptyline Administration in Rats. Biomed Pharmacol J 2022;15(3).

16) Aljezani, H., Ali, I. Cytotoxicity assessment of silver nanoparticles on liver and spleen of females rats. *Journal of Medical and Life Science*, 2022; 4(3): 53-60. doi: 10.21608/jmals.2022.257163

17) Hameed, H. M., Hasan, A. F., Razooki, Z. H., Tousson, E. & Fatoh, S. A. (2023). Orlistat Induce Renal Toxicity, DNA Damage, and Apoptosis in Normal and Obese Female Rats. OnLine Journal of Biological Sciences, 23(1), 25-32. https://doi.org/10.3844/ojbsci.2023.25.32 .

18) Ezz A. M. M, ALheeti O. N, Hasan A. F, Zaki S, Tabl G. A. Anti-Diabetic Effects of Pomegranate Peel Extract and L-Carnitine on Streptozotocin Induced Diabetes in Rats. Biomed Pharmacol J 2023;16(3).

19) Hasan, A.F., Hameed, H.M., Hadid, M.A.,and Tousson, E. (2024). Impact of Chia (Salvia hispanica) Seeds Extract on Ehrlich Ascites Model Induced Kidney Toxicity in Female Mice. Asian Journal of Dairy and Food Research. DOI: 10.18805/ ajdfr.DRF-397.

20- Ciftci, H., TÜRK, M., TAMER, U., Karahan, S. and Menemen, Y., (2013). Silver nanoparticles: cytotoxic, apoptotic, and necrotic effects on MCF-7 cells. Turkish Journal of Biology. 37(5):573-581.

21)Eustaquio, T. and Leary, J.F. (2012). Single-cell nanotoxicity assays of superparamagnetic iron oxide nanoparticles. Nanotoxicity: Methods and Protocols, pp.69-85.

22) Hasan, A. F., Mutar, T. F., Tousson, E. M. & Felemban, S. G. (2021). Therapeutic Effects of Turnera diffusa Extract Against Amitriptyline-Induced Toxic Hepatic Inflammation. OnLine Journal of Biological Sciences, 21(2), 395-408. https://doi.org/10.3844/ojbsci.2021.395.408

23) Hasan, A. F., Jasim, N. A., Abid, A. T., & Tousson, E. (2024). Role of Salvia hispanica seeds extract on Ehrlich ascites model induced liver damage in female mice. Journal of Bioscience and Applied Research, 10(2), 161-169.

24) Alyasiri, T., Alchalabi, S., & AlMayaly, I. (2018). In vitro and in vivo antioxidant effect of Spirulina platensis against lead induced toxicity in rats.Future of Food: Journal on Food, Agriculture and Society, 2018, 6(1), pp. 66–7

25) Hasan, A. F., Alankooshi, A. A., Modher, M. N., El-Naggar, S. A., El-Wahsh, H. M., El-Bagoury, A. E., ... & Kabil, D. I. (2024). Artemisia Annua Extract Ameliorates Hepato-Renal Dysfunctions in Obese Rats. Opera Medica et Physiologica, 11(2), 47-65.