Histological and Immunohistochemical changes induced by exposure to different doses of silver nanoparticles in Liver and Lungs of adult Albino rat

Dina Mohammed Salah[a], Abeer FareedAbd El-Naeem[a*],Zahraa Mohamed Ismael[a]

[a]Human Anatomy & Embryology Department, Faculty of Medicine, Sohag University, Sohag, Egypt

Abstract

Background: Nowadays there are varied manipulations of the silver nanoparticles (AgNPs) in educative and manufacturing topics**;** they are employed in surgical implements, orthopedic equipment, tools of family planning and catheters. They are also handled in drug conveyance, antiviral, germicidal and anticancer mediators.

Objectives: Study the changes that occurred due todifferent dosages of silver nanoparticles in the liver and lungs of adult albino rats.

Materials and methods: Silver nanoparticle solution was consumed. A total of 30 adult male albino rats were alienated to three equivalent groups; group I (control): divided into 2 subgroups, negative and positive with 5 rats each, group II (AgNPs low dose) which was intraperitoneally injected with AgNPs in a dose of 2 mg/kg once daily for 4 weeks, group III (AgNPs high dose): rats were injected intraperitoneally with AgNPs in a dose of 4 mg/kg body weight once daily for 4 weeks. 24 hours after the last dose, rats were anesthetized and dissected, where the lungs and livers were taken and prepared for hematoxylin and eosin (H&E) and immunohistochemical study.

Results: Histological and immunohistochemical investigations showed the destructive and inflammatory effects of low dose of silver nanoparticle in the liver and lung tissues of adult rats and in larger doses these toxic effects were more extensive.

Conclusion: The toxicity of silver nanoparticles caused dose-related deteriorating alterations in the liver and lung tissues.

Keyword: AgNPs; TNF-α; Liver; Lung.

DOI: 10.21608/SVUIJM.2025.345727.2054

***Correspondence:** abeerfareed167@gmail.com

Received: 10 December,2024.

Revised: 20 January, 2025.

Accepted: 25 January, 2025.

Published: 28 January, 2025

Cite this article as Dina Mohammed Salah, Abeer FareedAbd El-Naeem,Zahraa Mohamed Ismael.(2025). Histological and Immunohistochemical changes induced by exposure to different doses of silver nanoparticles in Liver and Lungs of adult Albino rat. *SVU-International Journal of Medical Sciences*. Vol.8, Issue 1, pp: 170-184.

Introduction

Nanotechnology is well executed in many scopes that utilize nanoparticles in synthesis of food and medical appliances. The peculiarity of Nanoparticles (Nps) is from their sizes which is among 1-100 nm, minimum one proportion, also their physical and chemical features can be modulated leading to increase their interactivity with biological tissues (**Formoso et al.,2016).**

Nanoparticles of silver (AgNPs) are largely exploited in industry and medicine, they are employed in damp wipes, shampoo and air sanitizer spray. In medical appliances, they are manipulated in manufacture of surgical device, drains, orthopedic equipages, tools of family planning and catheters. They are handled also in drug conveyance, antiviral, germicidal and anticancer mediators **(Wong and Liu 2010; Schluesener and Schluesener HJ, 2013; Zhang et al., 2016).**

The probable harmfulness of the nanomaterials is from its raising usage, as some types of NPs have the proportions to pass some barriers of vital organs as the brain, kidney and liver that dedicate various toxic effects **(Ahamed et al.,2010 ;Ruolan et al., 2018).**

Aim of the work was to study the histological and immunohistochemical changes made by dissimilar doses of nanomaterials of silver in liver and lungs of adult rats .

Materials and methods

Chemical and preparation: nanoparticles of silver citrated covered emulsion from Nano-Tech Egypt for Photo-electronics (6th of October Country).

Provision of AgNPs formulation of 5x10-3 mol dm-3 of nanospheres of silver was in agreement with protocol of Turkevich the citrate diminution was done. Silver nitrate about 0.0850 gram further to 100 ml of two-fold refined water. Then, 25ml of the average suspension was augmented to 100 ml of duple refined water. The emulsion reheated till it initiates to bustle. To the hot emulsion, 5ml of 1% sodium citrate was added with energetic blending. Heat up till the color changed to yellow. Heat up for extra 15 minutes then remove the emulsion and mixed furthermore 15 minutes. The AgNPs emulsion was accomplished to 125 ml by duple refined water and stored at 4°c (**Turkevich et al., 1951).**

Animal and treatments: About 30 adult male rats weighting (200-250gm) were utilized, they were conveyed from the animal household of Sohag faculty of medicine, they were be nurtured under the traditional conditions of feeding, temperature and light-dark proportion.

Ethical consideration: Ethical issue as consider dealing with experimental animal design were taken in each step of the research. Approval of the The Institutional Animal Care and Use Committee (IACUC) of Sohag University was taken with number 5-12-4-2024-02.

Groups: The rats were separated into 3 equivalent groups:

- Group I: It was composed of 10 adult male albino rats, subdivided into 2 equal subgroups:
  - Ia (Negative control): included five rats, kept without treatment.
  - Ib (positive control): included five rats, ingested with saline .
- Group II :(AgNPs low dose) rats were AgNPs injected intraperitoneal, a dosage of 2 mg/kg via insulin inject one shot every-day for 4 wks (**El Mahdy et al., 2014)**.
- Group III (high dose AgNPs): rats were AgNPs injected intraperitoneal, a dosage of 4 mg/kg via insulin inject one shot every-day for 4 wks **(El Mahdy et al.,2014).**

Scarification was done 24 hours after the final dose, they were be sedated by admixture of Ketamine (90 mg/kg) and

Xylazine (10 mg/kg), the livers and lungs are taken and prepared for study.

The histological alterations in the liver and lung will be examined by:

1. Light microscopy: The samples were fixed in bouin solution for 24 hours and then was washed in alcohol 70% then placed in paraffin, fragmented to slices 0.5-1cm and stained with (H&E) to be examined **(Bancroft and Gamble, 2008).**

A score for hemorrhage, was done on sections ×40 objective, and scored from 0 - 3: 0 (normal) ,1 (mild) <.25%, 2 (moderate) 25-50%, and 3 (severe) >75% **(Özcan et al., 2005).**

2. Immunohistochemical test methods: Boiling specimens were in 10mm citrate shield (AP.9003) for 10 minutes at PH 6 for antigen retrieval, after 1 hour it cultivated with Tumor necrosis factor (TNF) antibody **(Dabbs, 2002),** Mayer's Haematoxylin (TA060-MH) stain were applied in counterstain the sections. The reaction of brown discoloration represented positivity.

3. Morphometric analysis: The following measures were taken:

a. Diameter of central vein: on sections × 400 magnification and measured as the widest distance between two points on the edges.

b. Area percent of TNF on sections ×200 magnification**.**

c. Score for hemorrhage: scaled from 0 (normal), 1 (mild)<.25%, 2 (moderate) 25-50%, and 3 (severe) >75%

All these measures were taken from 5 different sections, with 5 measures from each one using image J software (version 1.51k, Wayne Rasband, National Institutes of Health, and USA).

Statistical analysis

The mean ±standard deviation was stated to whole data by using SPSS (Statistical Package for the Social Sciences) version 16, variables and p value equated by ANOVA (Analysis of Variance) if it was statistically significant, a post-hoc test was then done to identify variation between each two groups, it was considered significant when below 0.05.

Results

Light microscopic results (Hematoxylin & Eosin)

1. Liver

A. control group: staining sections showed conserved histological construction of the liver lobule as; the cords of hepatocytes surrounding the central vein and had a vesicular round nucleus and a pronounced nucleolus. Sinusoids of liver were appeared among the hepatocytes **(Fig. 1 a&b)**.

B. Group II (nanoparticles of silver low dose): Liver lobules had disrupted histologic construction. The central vein lumen was distended. Hepatic cords disintegrated with cytoplasmic vacuolations, pyknosis of their nuclei and dilated sinusoids **(Fig. 2 a&b).**

C. Group III (nanoparticles of silver high dose): The liver lobule shows a bothered histological construction. There was severe distension and congestion of central vein lumen with more widespread bleeding in it. Hepatocytes looked disintegrated with manifest vacuolation. Blood sinusoids looked expanded. Liver parenchyma offered inflammatory cell infiltrates **(Fig. 3 a,b).**

2. Lung

Stained sections of the control lung (group I) of an adult albino rat showing normal spongy lung construction with thin-walled alveoli, intact alveolar sacs, thin interalveolar septum with intact blood vessels and intact bronchioles lined by folded epithelium formed of ciliated columnar cells and surrounded by a thin outer musculosa **(Fig. 4 a,b).**

Group II (low dose of silver nanoparticles) exhibited thickened alveolar walls, some cellular infiltrates, loss of normal structure of alveolar sacs, thickened bronchial walls and dilated blood vessels with some hemorrhagic exudates **(Fig. 5 a,b).**

Group III (high dose of silver nanoparticles) presented apparent alteration in lung tissue with damage of normal outlines of the alveoli. Some alveolar sacs are collapsed. Marked cellular infiltrates, congested blood vessels and destructed bronchioles were noted. Some sections showed thickened interalveolar septum, many collapsed sacs and multiple areas of hemorrhages **(Fig. 6 a,b).**

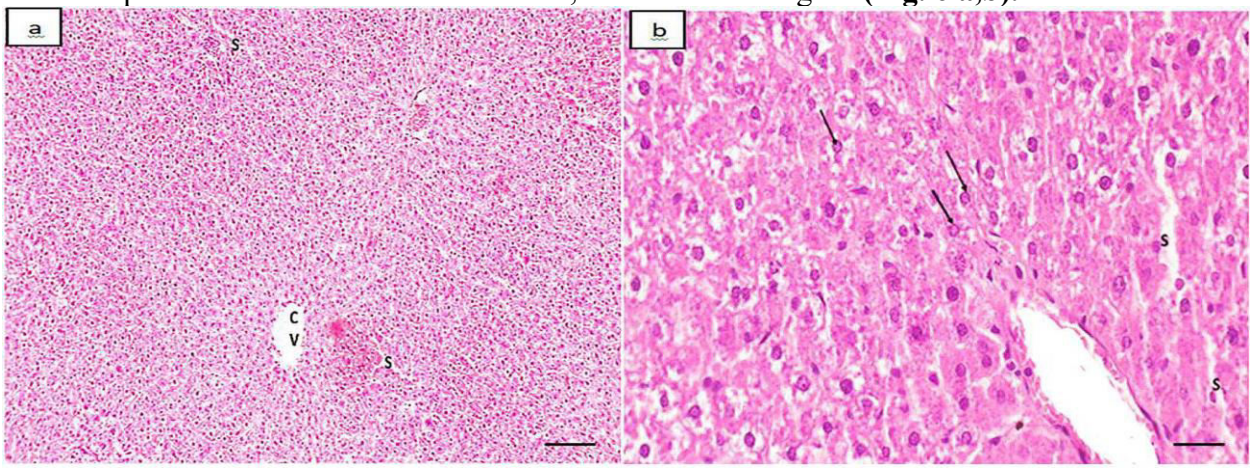

Fig.1. a) Aphotomicrograph of the liver group I rat presenting central venule (**CV**) bounded threads of liver cells & sinusoids (**S**). b) Higher magnification of the previous photo presenting central venule (**CV**), the liver cells (**arrow**) have vesicular circular nuclei and pronounced nucleoles ,normal liver sinusoids (**S**). H&E (x100, scale bar=100µm &x400, scale bar=25 µm, respectively).

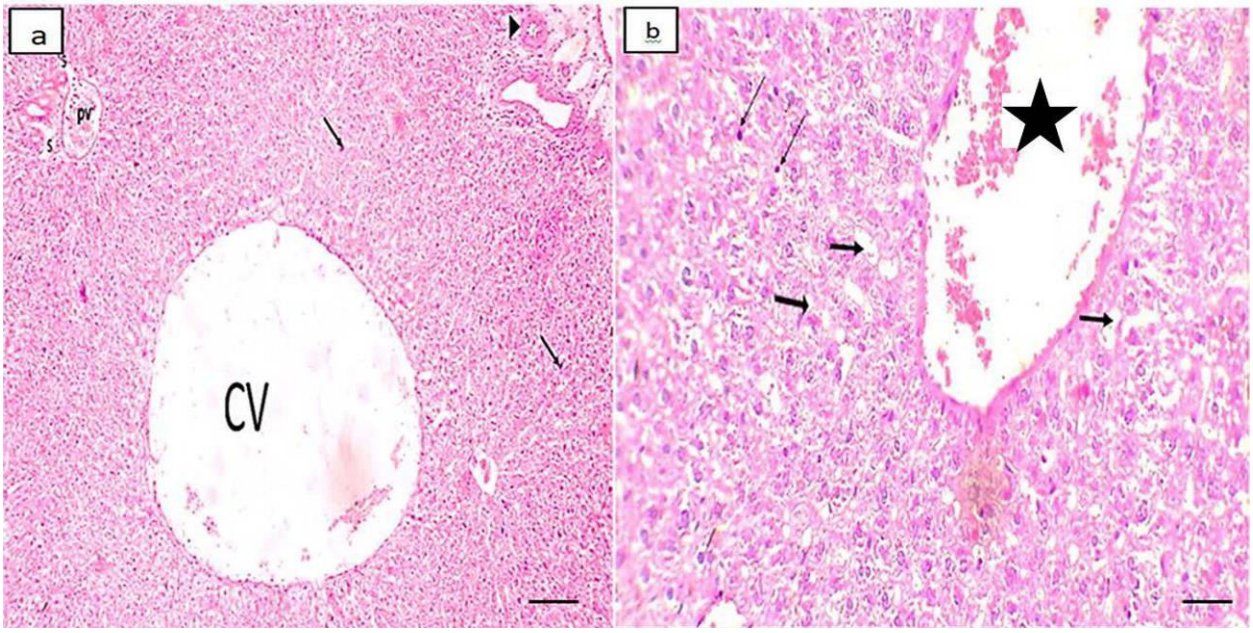

Fig. 2. a) A photomicrograph of the liver tissue of group II rat presenting distension and bleeding of the central vein (**CV**). Normal hepatic artery **(arrow head**), a small but severely congested hepatic portal vein (pv) surrounded by dilated sinusoid (**s**) was seen, some of hepatic cells showed pyknotic nuclei **(thin arrow**). **b)** A higher magnification of the photo presenting distended hemorrhagic central vein (**star)**. Liver cells showed cytoplasmic vacuoles (**thick arrow**) and pyknosis (**thin arrow**). H&E (x100, scale bar=100µm & x400, 25 µm, scale bar=, respectively).

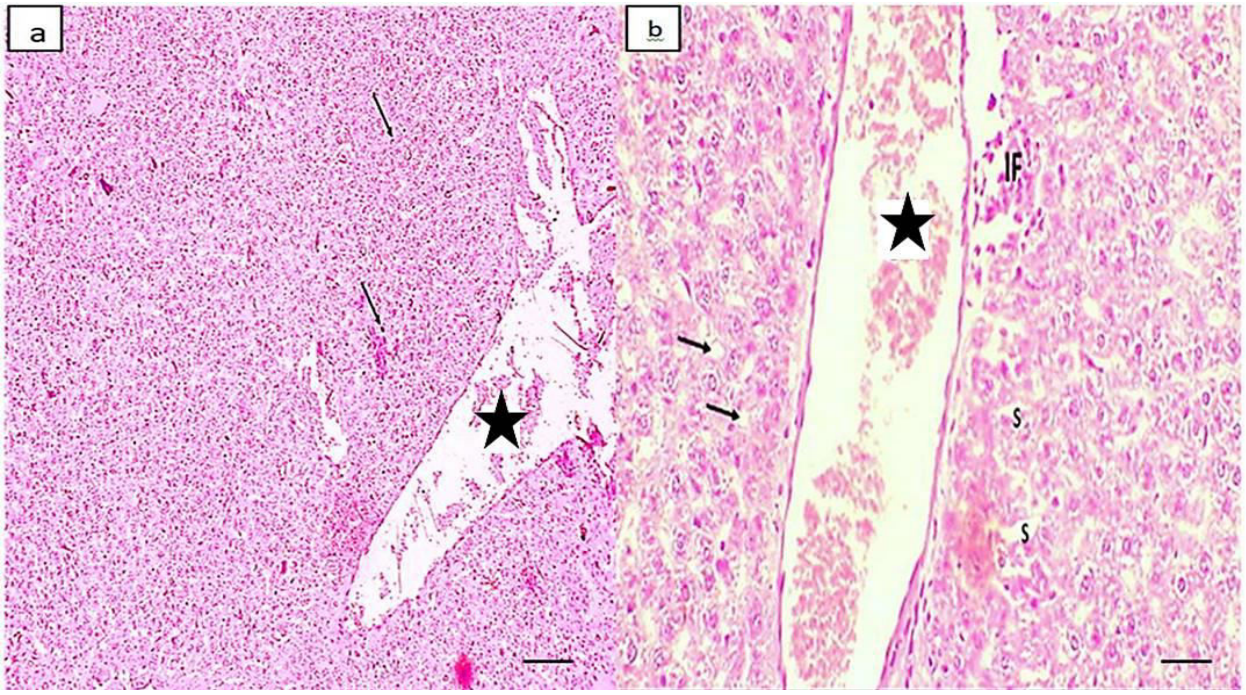

Fig. 3. a) A photomicrograph of liver of group III presenting a marked distension of the central vein with more extensive bleeding (star). Some hepatocytes show pyknotic nuclei (black arrow). **b)** Higher magnification exhibits a spacious distension of the central venule with more widespread bleeding (star). Hepatic cells are deteriorated with vacuolations (black arrow),liver parenchyma displays inflammatory cell infiltrates (IF), sinusoids are expanded (S). H&E (x100, scale bar=100µm& x400, scale bar= 25 µm , respectively,).

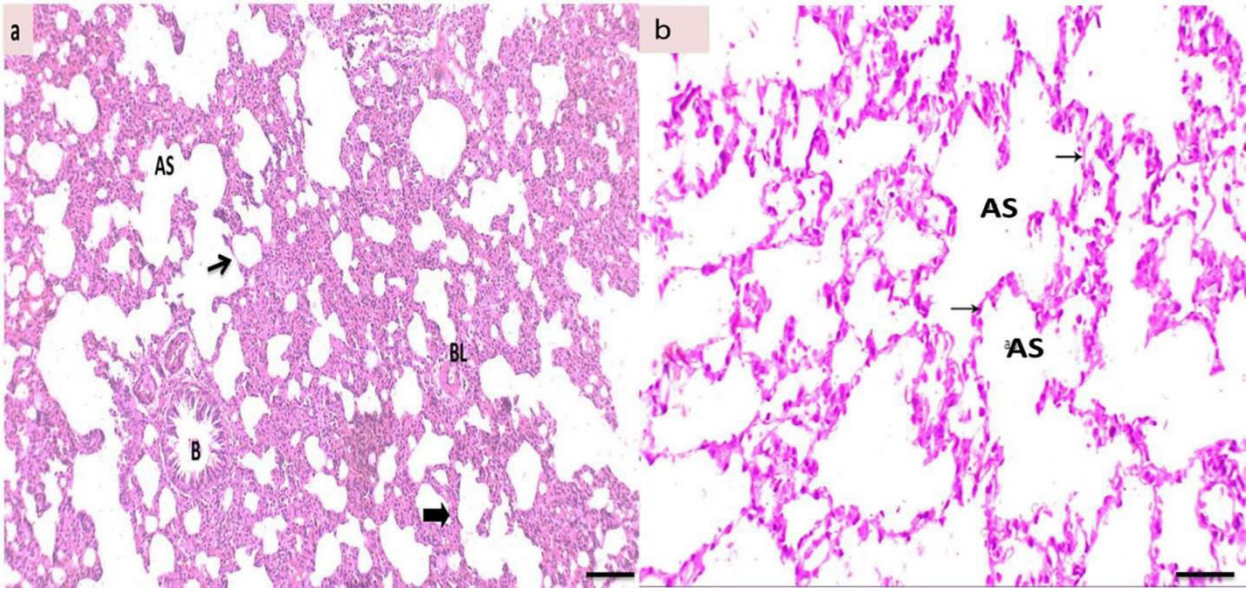

Fig.4. a) a micrograph of the control lung (group I) of an adult rat: showing normal lung construction with thin-walled alveoli (thin arrow), intact alveolar sacs (AS), thin interalveolar septum (thick arrow), bronchioles (B), and blood vessels (BL). b): a higher amplification of the previous picture showing normal lung tissue, alveolar sacs (AS), thin-walled alveolar walls (thin arrow). H&E (X 100, scale bar=100µm & X 200, scale bar=50 µm, respectively).

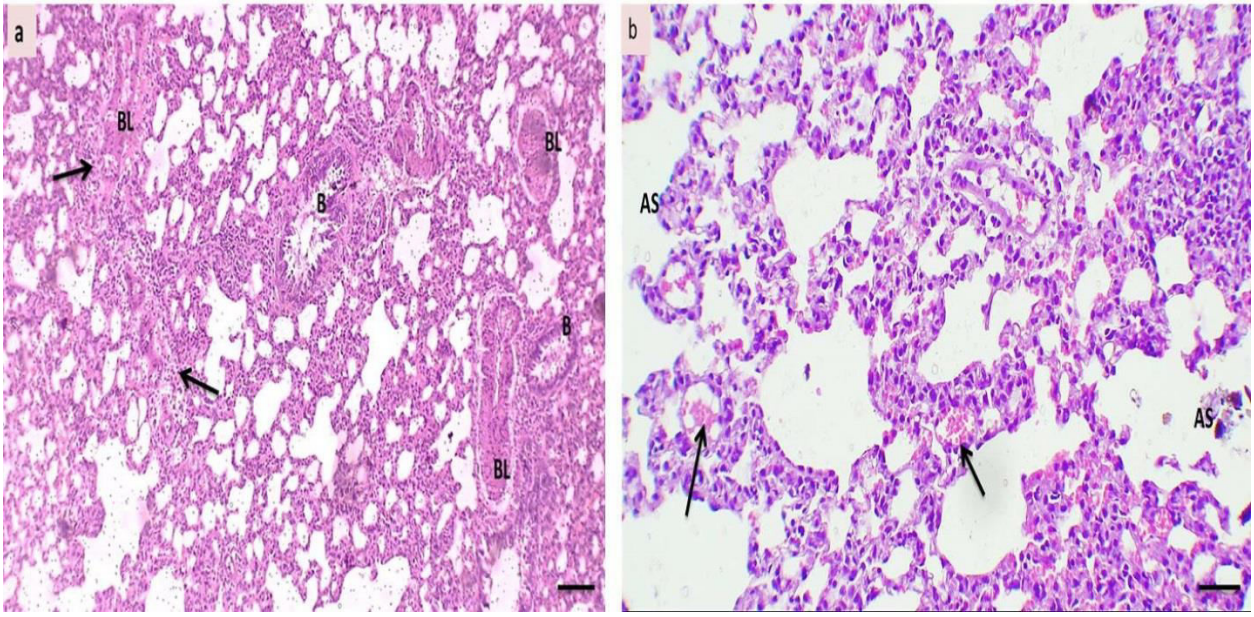

Fig. 5. a) A photomicrograph of a cross section of the lung of adult albino rat had low dose of silver nanoparticles (group II) shows disturbed hemorrhagic bronchioles(B) , many inflammatory cellular infiltrates (arrows), thickened dilated blood vessels (BL). b) a higher amplification of the lung tissue showing disturbed lung tissue with abnormal alveolar sacs (AS), some hemorrhagic exudate were seen (arrows). H&E (X100, scale bar=100µm & X200 scale bar=50 µm, respectively).

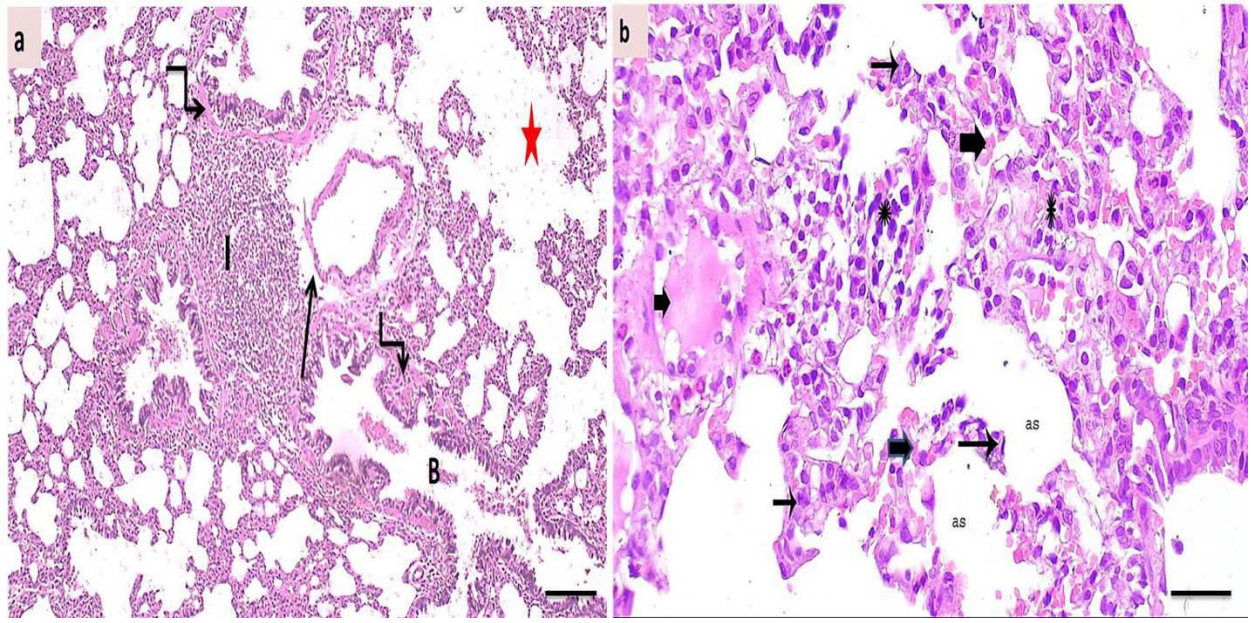

Fig. 6. a photomicrograph of the lung of adult rat had high dose of silver nanoparticles (group III): **a)** showing obvious alteration in lung tissue with loss of normal outlines of the alveoli (star), many inflammatory cells (I), destructed bronchioles with obvious dilatation and hemorrhage (B), per bronchial edema was seen(arrow), congested muscles were seen also(elbow arrow) . **b)** higher magnification showing many destructed alveolar sacs (as), thickened interalveolar septum(arrow), bleeding in the alveoli (thick arrow)and many cellular infiltrates(stars) H&E (X100, scale bar=100µm & X200, scale bar=50 µm,respectively) .

Light microscopic results (Immunohistochemical evaluation)

1. liver

The control group presented negative immune reactions for TNF- α **(Fig. 7).**

Group II (low dose of silver nanoparticles) exposed moderate immune reactions for TNF-α in the form of brown discoloration in the hepatocytes (**Fig. 8**).

Group III (high dose of silver nanoparticles) exposed strong positive immune reactions for TNF-α in the form of massive brown discoloration in the hepatocytes and around portal vein (**Fig. 9**). The mean differences in immunoreactivity of TNF-α of surface areas between the three groups were revealed in **(Table 1, Fig.10).**

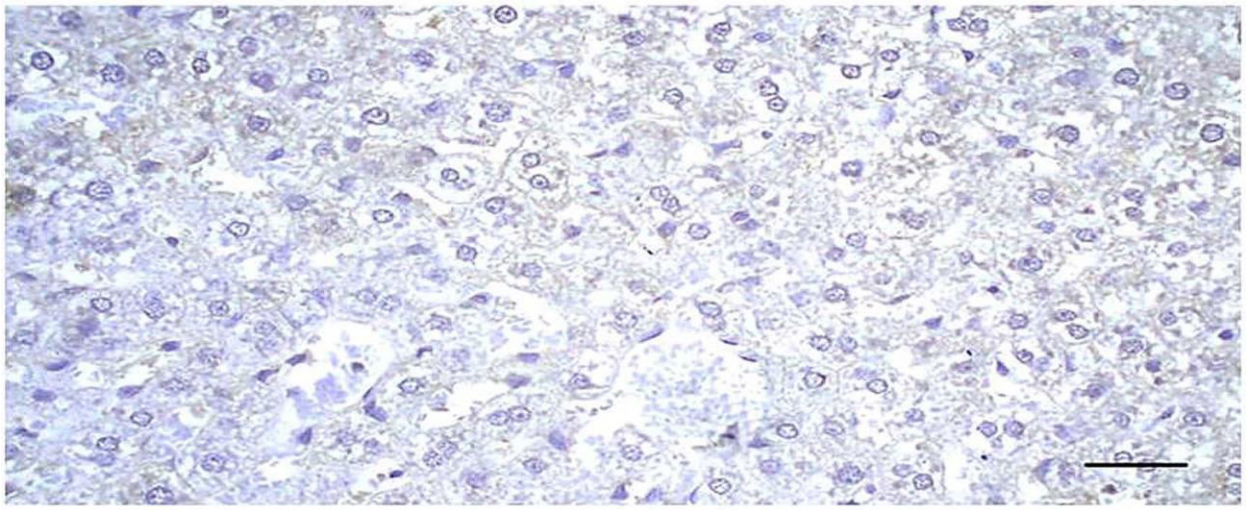

Fig. 7. A micrograph of control rat liver revealing negative immune reactions for TNF- α. (TNF-α immune stain, ×200, scale bar=50µm).

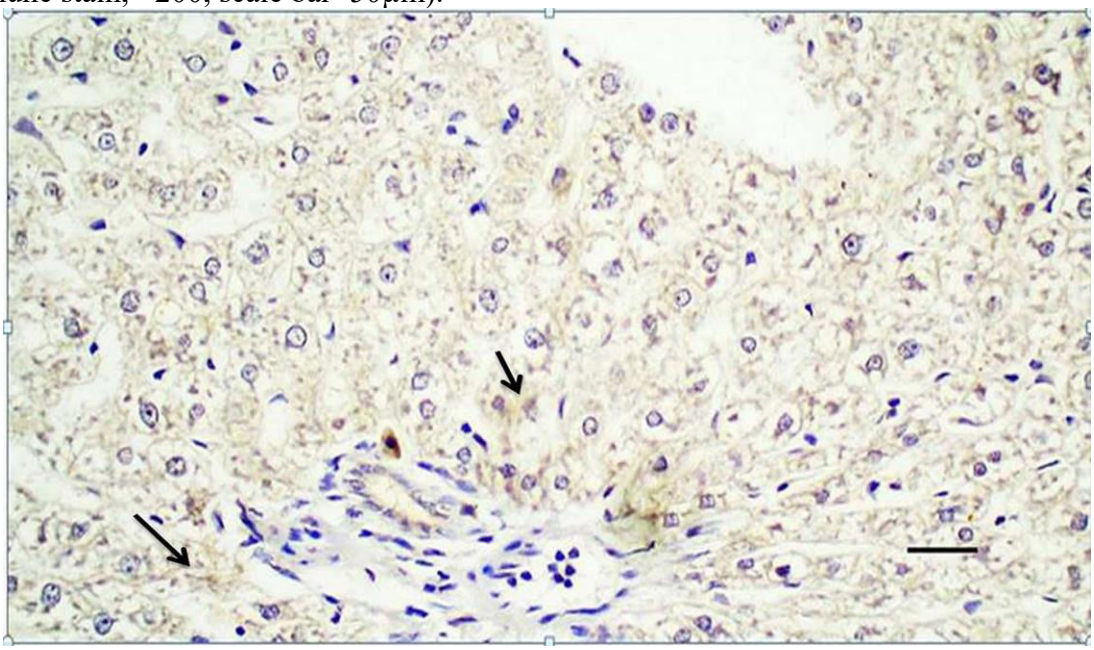

Fig. 8. A micrograph of the liver of rat group II revealing moderate positive cytoplasmic immune reactions for TNF-α in the heptocytes (arrows)(TNF-α immune stain, ×200,scale bar=50µm).

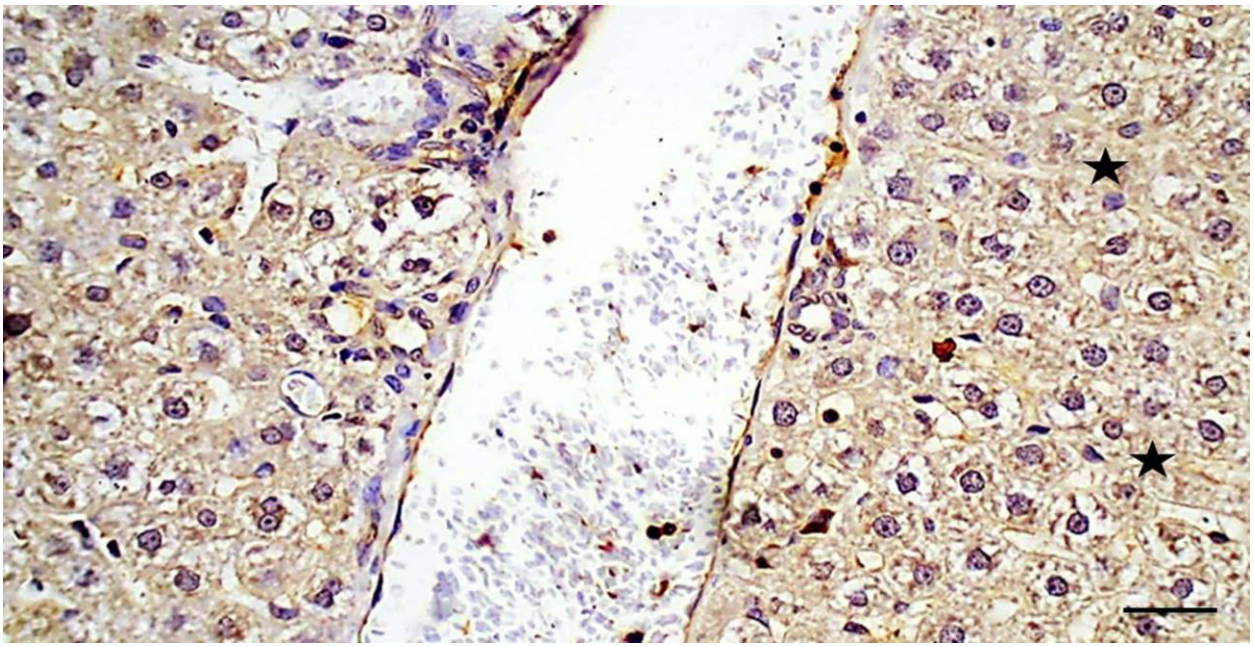

Fig. 9. A micrograph of the rat liver group III exhibiting intense positive cytoplasmic immune responses for TNF-α in the hepatocytes (stars) and around portal vein (TNFα immune stain, ×200, scale bar=50µm).

Table 1. Diameter of central vein of liver in different groups

Variables	Control	Group II	Group III	P value
Mean ±SD of diameter of CV	90.7± 78.911 pixel	441.33±82.75940 pixel	510.26±122.46135 pixel	**P1 0.000*** P2 0.04***

* SD: standard deviation; * Significant variance at p value < 0.05 ** Highly significant at p value < 0.00; P1: evaluates amongst group I& II; P2: evaluates amongst group II & III

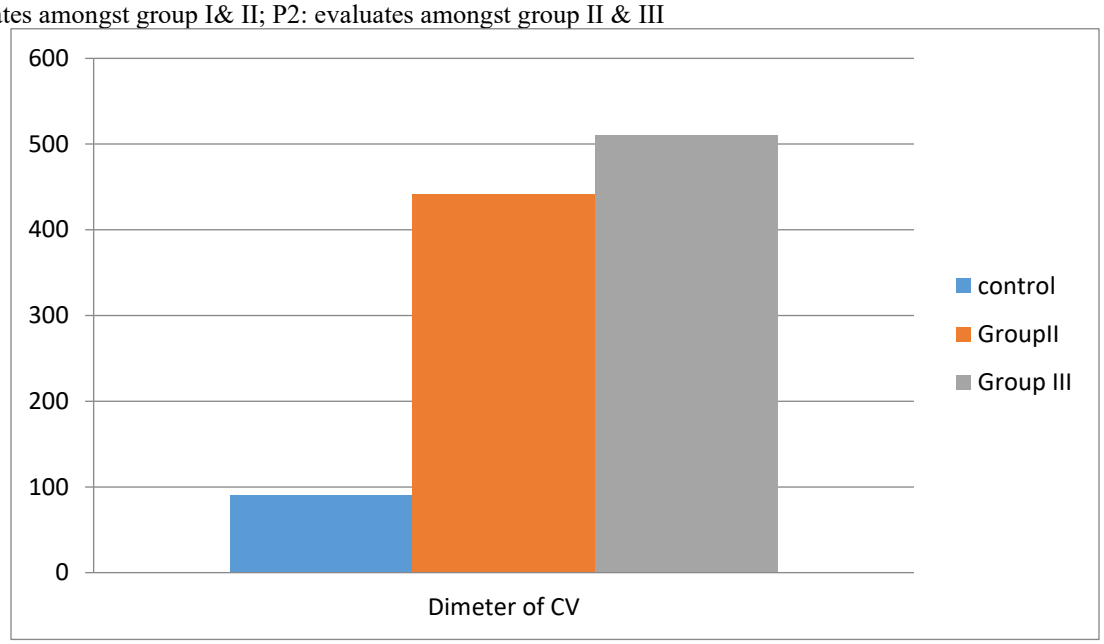

Fig.10. Diameter of CV in all groups

2. Lung

The control group showed minimal reaction for TNF- α in the lung parenchyma **(Fig. 11).**

Group II (had low dose of silver nanoparticles) showed a moderate immunoreaction of TNF-α in the form of brown discoloration in parenchymatous lung tissues **(Fig. 12).**

Group III (had high dose of silver nanoparticles) showed a strong immunohistochemical reaction of the lung parenchyma to TNF-α **(Fig. 13).**

The mean differences in immunoreaction to the TNF-α of surface areas between the three groups were revealed in **(Table.2, Fig.14).**

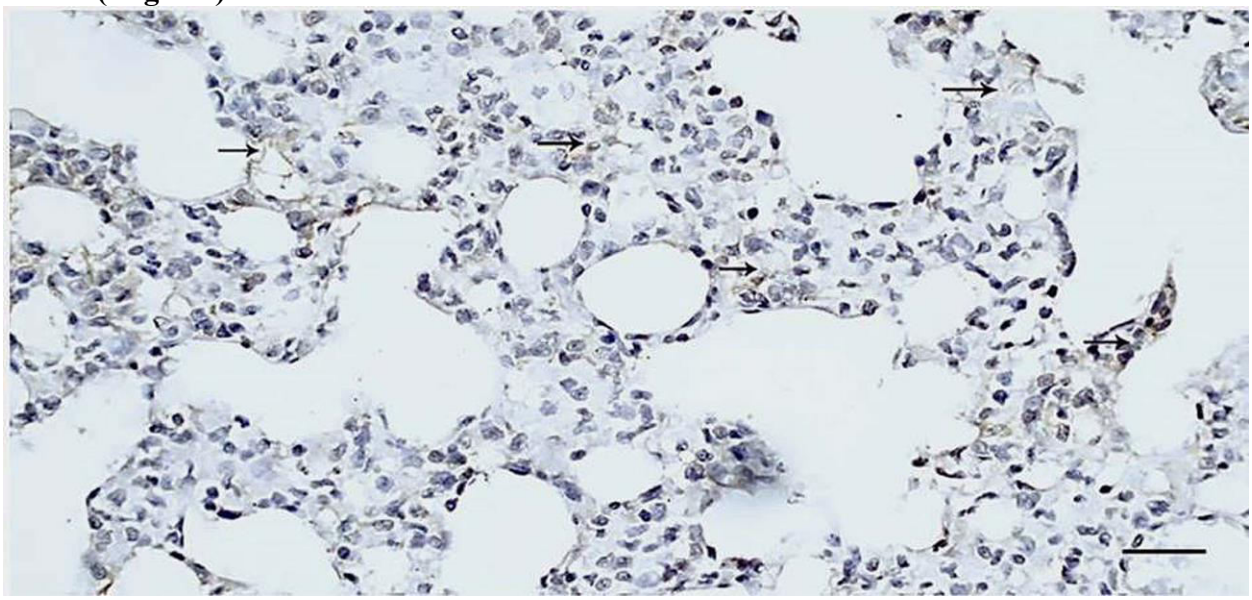

Fig. 11. a photomicrograph of the control lung of a rat presenting minimal reaction for TNF- α in the lung parenchyma (arrows). (TNF-α immune stain, × 200, scale bar=50µm).

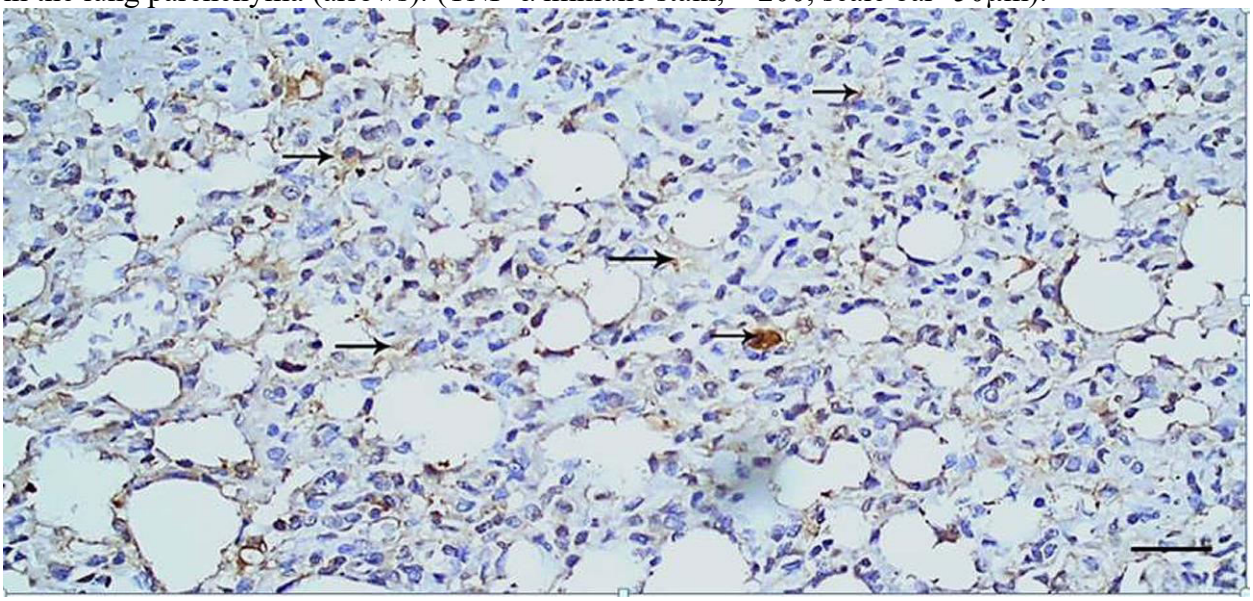

Fig . 12. a photomicrograph of a lung rat (group II) exposing a moderate immunoreaction of TNF-α in parenchymatous lung tissues (arrows). (TNF-α immune stain, ×200, scale bar=50µm).

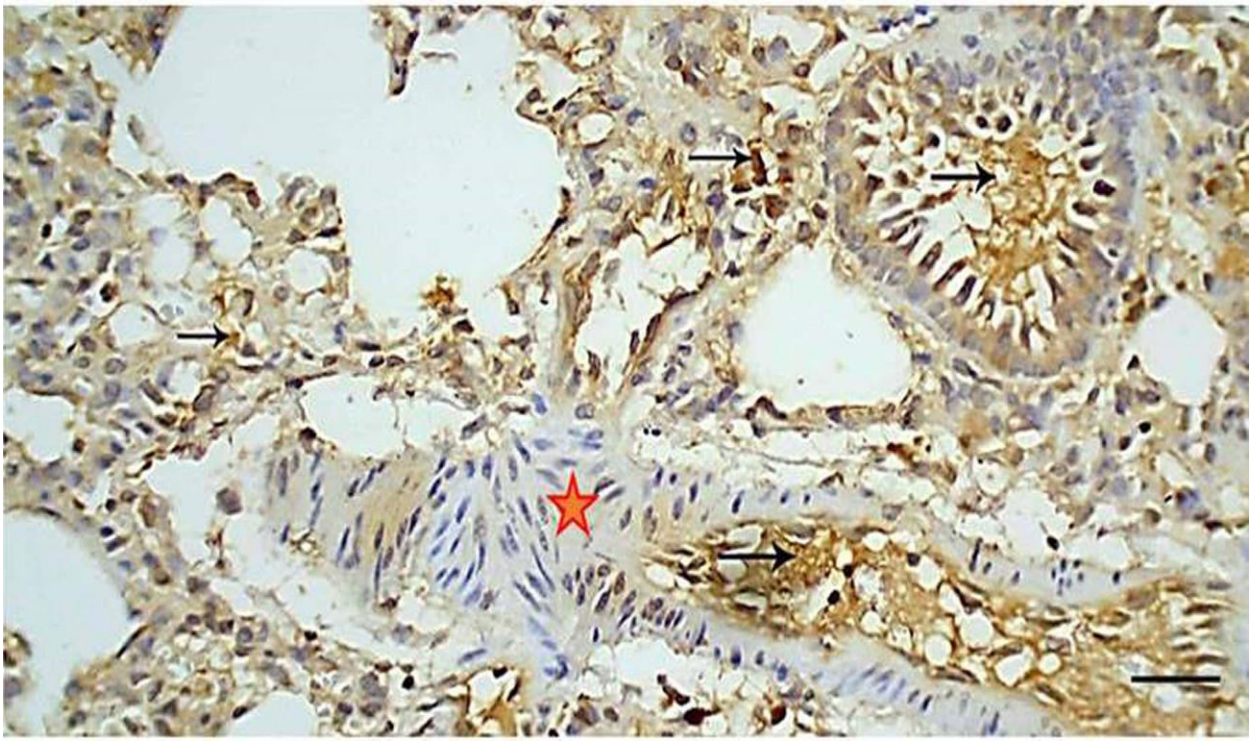

Fig . 13. a micrograph of a rat lung (group III) exposing a strong immunohistochemical reaction of the lung parenchyma to TNF-α(arrows) ,notice inflammatory infiltration(stars) . (TNF-α immune stain, ×200, scale bar=50µm)

Morphometric results

1. Diameter of central vein (Table.1, Fig.10): The diameter of central vein increased significantly in group II, then increased more in group III

2. Area percentage of TNF α (Table.2, Fig.14): There was significant increase in area percent of TNF α in the liver and lung in group III than group II, and group II than the control group

Table 2. Mean of area percentage of TNF α in the three groups in liver and lung.

Variables	Control	Group II	Group III	P value
Mean ±SD Area % TNF α in liver	7.11±3.60	11.40 ± 4.30	24.25 ± 9.47	**P1 0.02* P2 0.000****
Mean± SD Area % TNF α in lung	6.88±2.32	10.26±3.5	23.57±6.27	**P1 0.01* P2 0.000****

* SD: standard deviation; * Significant variance at p value < 0.05 ** Highly significant at p value < 0.00; P1: evaluates amongst group II& I; P2: evaluates amongst group II & III

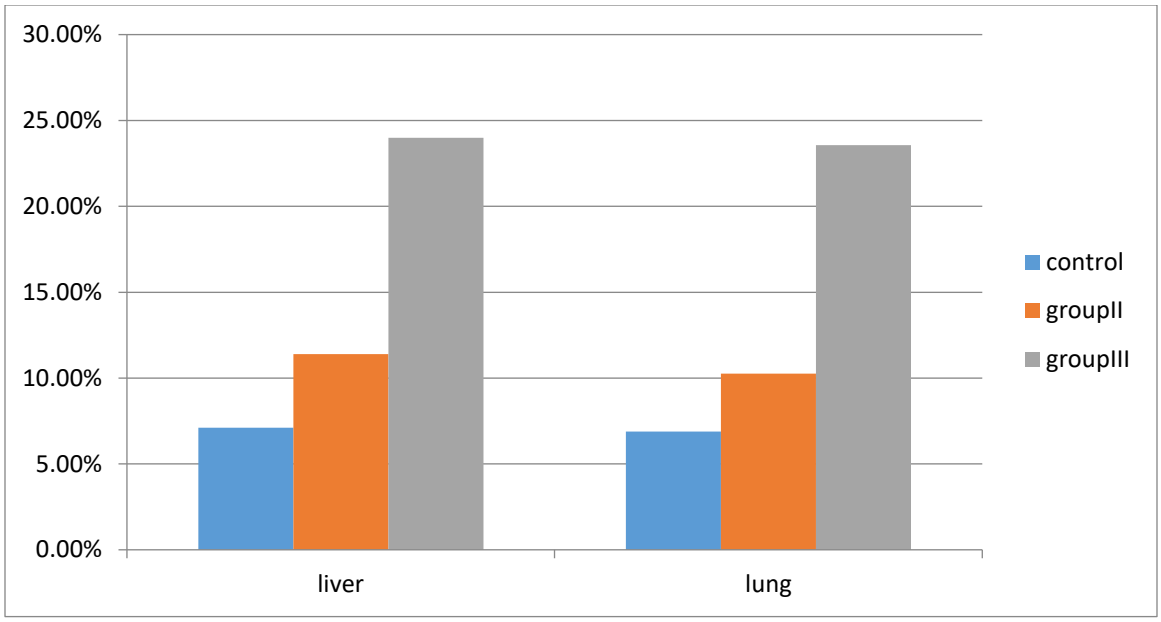

Fig.14. Percent area of immunoreaction to TNFα in liver and lung architecture in all groups.

3. Score for portal vein hemorrhage (Table.3, Fig.15): There was non-significant increase in portal hemorrhage in group III than group II but highly significant increase of both treated groups than the control group.

Table 3. Score for portal vein hemorrhage in all groups

	Control	Group II	Group III	P value
Score of hemorrhage	.14 ±.117	1.59 ± .26	1.860 ±. 323	**P1 0.001**** **P2 0.05**

* SD: standard deviation; * Significant variance at p value < 0.05 ** Highly significant at p value < 0.00; P1: evaluates amongst group I& II; P2: evaluates amongst group II & III

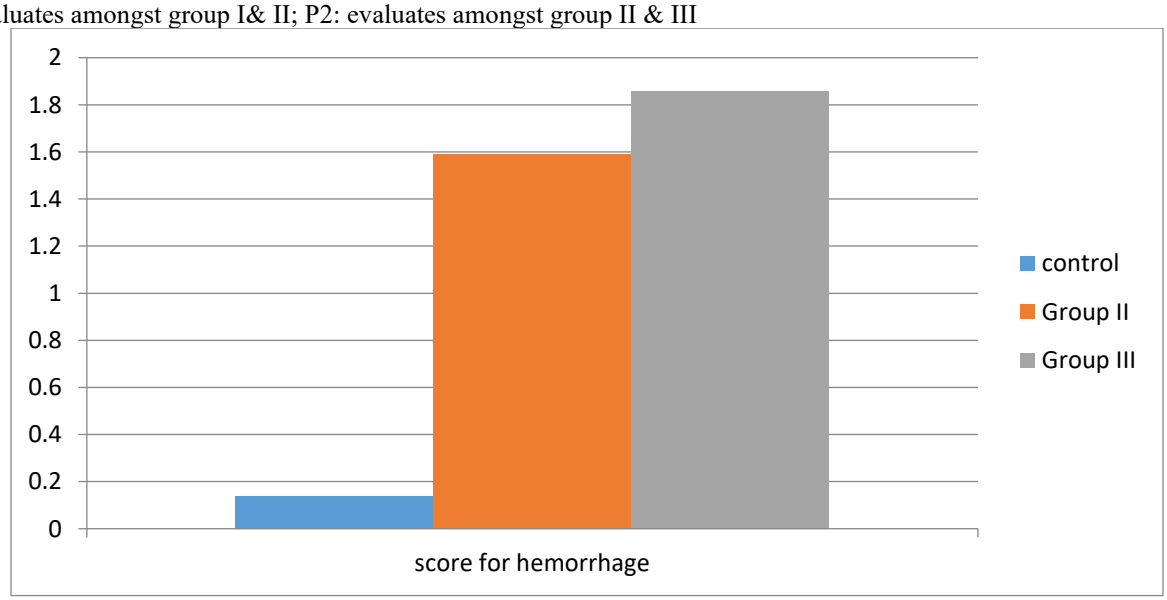

Fig.15. Score for portal vein hemorrhage in all groups

Discussion

Nanoparticles of silver Ag-NPs attracted excessive scientific interest due to their properties, even though countless alarms provoked regarding their probable harmfulness to assorted tissues proved by **Roda et al. (2017).**

Barkalina et al. (2014) denoted that the widespread use of silver nanomaterials may result in their release into the environment as it can enter our bodies through various routes, such as breathing, gastrointestinal tract, skin contact and via the placenta.

In our investigational study, we elected intraperitoneal injection of Ag-NPs because it avoids absorption, and the peritoneum has a further of blood and lymphatic vessels so it is a speedy path to distribute Ag-NPs to the blood flow proved by **Garcia et al. (2014).**

In this study by hematoxylin and eosin-stained segments of the liver tissues of group ll and group lll that had low dose and high dose of nanoparticles of silver respectively we found some changes in normal histology in group ll as dilatation and congestion of central vein with blood in its lumen, hepatocytes seemed disintegrated. Liver sinusoids were enlarged, these changes are more obvious in group lll which exposed sever dilatation and congestion of central venule, hepatocytes appeared disintegrated with obvious vacuolation, blood sinusoids appeared dilated &liver parenchyma presented inflammatory cell infiltrates.

Ansar et al. (2017) perceived that the liver and spleen were the main site of gathering the administered AgNPs, also described that intraperitoneal injection of 5 mg/kg AgNPs in rats increased AST and ALT activities and histopathologic alterations in the liver.

Moradi-Sardareh et al. (2018) proved that pests treated with dissimilar doses of AgNPs (1, 0.5 and 0.25 mg/kg) intraperitoneally for nine days revealed histopathological revolutions in the liver, brain, and further organs, also **Ragab et al.(2022)** stated that nanoparticles of silver influence structural alters in a dose related endometrial trials of female rats and cautiously given to females to avoid its hazards.

The diameter of central vein showed highly significant increase with increasing dose of Ag-NPs , portal vein hemorrhage score also increased with increasing dose of Ag-NPs which showed the relation between the dose and markers of destruction.

This was accepted with **Baraaj (2021)** who studied the effect of 2 doses of Ag-NPs and evaluated increase in histological and biochemical markers of destruction which were dose dependent.

Hematoxylin and eosin stained segments of lung tissue of the group ll and group lll we found some changes in normal histology in group ll as destructed lung tissue, thickened alveolar walls, some cellular infiltrates with thickened bronchial walls but these changes are marked in group lll as loss of normal outlines of the alveoli, some alveolar sacs are collapsed, marked cellular infiltrates, congested blood vessels and destructed bronchioles, thickened interalveolar septum, many collapsed sacs and multiple areas of hemorrhages.

In suggestion with **Ma et al. (2020**) that denoted AgNPs demonstration caused ultra-histopathological fluctuations in the lung tissue, persuaded mitochondrial injury, oxidative stress aggravation, and amplified mitochondrial lethality.

Setyawati et al. (2013) denoted about the cellular infiltrates caused by revelation to Ag NPs was due to their affection on the cell membrane permeability and endothelial cell damage caused in leakage of numerous inflammatory cells.

(**Wen et al., 2017)** energized our study and clarified the occurrence of cellular

infiltration other studies have demonstrated similar results to ours , confirming cellular infiltrates in multiple organs had high doses of nanoparticles of silver. **(Pani et al., 2015**)reported destruction in liver tissue and approved its toxicity as our study said.

In contrast **Pourhamzeh et al. (2016)** were discovered that if the 28 -day experimental rat AGNP oral administration was administered at a different dose, it did not show significant changes in serum AST and ALT levels. These consequences may due to the custom of higher nanoparticles (78.59 nm) than those consumed in the investigational researches.

In this study by immune-histological studies we found that group II (low dose AgNPs) showed a moderate immunoreaction of TNF-α in parenchymatous lung tissues & in liver tissue as well while in group III (high dose AgNPs) exposed a strong immunohistochemical reaction to TNF-α in both lung and liver tissues, with high significant increase which was dose dependent.

This in concord with **Ferdous et al. (2021)** who observed that histological signal of inflammation in the lung, spleen, liver and kidney caused by the AgNPs and detected significant intensification in indicators of cellular stress as (TNF-α) and interleukins, also Park, 2010 stated that the causes of increase inflammatory signals after administration of AgPNs is due to its phagocytosis that elicits TNF-α secretion, high levels of TNF-α lead to cell loss.

Yahya et al. (2024) approved with immunohistochemical study the dose dependent destruction of AgNPs on the liver tissue . **Altwaijry et al. (2024)** approved the destructive effect of AgNPs on the lung with immunohistochemical markers which was accepted also with ours.

Yang et al. (2017) denoted that the mechanism of silver nanoparticle toxicity triggered via initiation of oxidative stress, which triggering cellular alterations as DNA destruction, formation of cytokines and death of cells**.**

Al-Bishri et al. (2018) reported that the noxious matters of AgNPs were dose-relevant, several studies accomplished that mitochondrial cytotoxicity resultant of the cumulative dose and concentration of AgNPs, causing severe impairment of the mitochondria, leakage of the membranes, inflame augmentation and death of cells.

Yahya et al.(2024) approved with immunohistochemical study the dose dependent destruction of AgNPs on the liver tissue . **Altwaijry et al. (2024)** approved the destructive effect of AgNPs on the lung with immunohistochemical markers which was accepted also with ours.

Limitations of the study: The research need blood investigations to evaluate the effect of AgNPs on liver and lung functions.

Conclusion

The liver and lung tissues underwent dose-related deterioration due to the toxicity of silver nanoparticles. It is best to avoid high doses of AgNPs, however, further long-term clinical studies in humans are required to substantiate the results attained from animal considerations.

References

- **Ahamed M, Alsalhi MS, Siddiqui MK. (2010).** Silver nanoparticle applications and human health. Clinica Chimica Acta , 411 (23–24): 1841–1848**.**
- **Al-Bishri WM. (2018).** Toxicity study of gold and silver nanoparticles on experimental animals. Pharmacophore, 9 (1):48-55.
- **Altwaijry N, lmasryT , Alotaibi B, Saleh A, Tossoun E, Alodhayani A, et al.,(2021).** Potential therapeutic effects of avenanthramide-C against lung toxicity caused by silver nanoparticles

injection in rats. Pakistan Journal of Pharmaceutical Sciences,34(1.SUP):337-343.

- **Ansar S, Alshehri SM, Abudawood M, Hamed SS, Ahamad T. (2017).** Antioxidant and hepatoprotective role of selenium against silver nanoparticles. International Journal of Nanomedicine, 12:7789-7797.
- **Baraaj AH. (2021).** Histological, Biochemical and DNA Changes in the Liver of Male Albino Rats Treated with Silver Nanoparticles . Nano Biomedicine and Engineering, 13:(1**):20-26**
- **Bancroft, J. and M. Gamble, 2008.**Theory and practice of histological techniques.6th Ed., Elsevier health sciences, pp: 126-127.
- **Barkalina N, Charalambous C, Jones C, Coward K. (2014)**. Nanotechnology in reproductive medicine: Emerging applications of nanomaterials, 10:921-938.
- **Dabbs, 2002.** Diagnostic immunohistochemistry. Churchill Livingstone. 2nd Ed.
- **El Mahdy MM, Salah Eldinb TA, Aly HS, Mohammed FF, Shaalan MI. (2014).** Evaluation of hepatotoxic and genotoxic potential of silver nanoparticles in albino rats. Experimental and Toxicologic Pathology, 67:21–29.
- **Ferdous Z, Al-Salam S, Yuvaraju P, Ali B, Nemmar A. (2021).** Remote effects and biodistribution of pulmonary instilled silver nanoparticles in mice, NanoImpact, 22: 2452-0748.
- **Formoso P, Muzzalupo R, Tavano L, De Filpo G, Pasquale Nicoletta F. (2016).** Nanotechnology for the environment and medicine. Mini Reviews in Medicinal Chemistry, 16(8): 668-675. 2.
- **Garcia TX, Costa GMJ, França LR, Hofmann MC. (2014).** Sub-acute intravenous administration of silver nanoparticles in male mice alters Leydig cell function and testosterone levels. Reproductive Toxicology, 45:59–70.
- **Ma W, He S, Ma H, Jiang H, Yan N, Zhu L, Bang JJ, Li PA, Jia S. (2020).** Silver Nanoparticle Exposure Causes Pulmonary Structural Damage and Mitochondrial Dynamic Imbalance in the Rat: Protective Effects of Sodium Selenite. International Journal of Nanomedicine, 30 (15): 633-645.
- **Moradi-Sardareh H, Basir HRG, Hassan ZM, Davoudi M, Amidi F. (2018).** The Expression of Caspase-3 in the Liver Tissues of Male Rats. Avicenna Journal of Medical Biochemistry, 4(2): e35557.
- **Özcan A, Korkmaz A, Oter S, Coskun O. (2005).** Contribution of flavonoid antioxidants to the preventive effect of MESNA in cyclophosphamide-induced cystitis in rats. Archives of Toxicology, 79: 461– 465.
- **Pani JP, Mishra SP, More RS, Singh S, Singh R. (2015).** Hepatotoxic effect of characterized silver nanoparticle. International Journal of Therapeutic Applications, 29:13-18.
- **Park EJ. (2010).** Silver nanoparticles induce cytotoxicity by a Trojan-horse type mechanism. Toxicology in Vitro, 24:872–878.
- **Pourhamzeh M, Mahmoudian ZG, Saidijam M, Asari MJ, Alizadeh Z. (2016).** The effect of silver nanoparticles on the biochemical parameters of liver function in serum, and the expression of caspase-3 in the liver tissues of male rats. Avicenna Journal of Medical Biochemistry, 4(2): e35557.
- **Ragab A, Ragab M, Tawfik S. (2022).** Histological and Immunohistochemical Alterations Induced by Exposure to

Different Doses of Silver Nanoparticles in the Endometrium of Adult Albino Rat, 45(3):756-773.

- **Roda E , Barni S, Milzani A, Dalle-Donne I, Colombo G, Coccini T. (2017).** Single Silver nanoparticle instillation induced early and persisting moderate cortical damage in rat kidneys. International Journal of Molecular Sciences, 18 (2):1-15.
- **Ruolan W, Bin S, Junrong W, Yanli Z, Aijie C, Longquan S. (2018).** Potential adverse effects of nanoparticles on the reproductive system .International Journal of Nanomedicine , (13): 8487–8506.
- **Schluesener JK, Schluesener HJ. (2013).** Nano silver application and novel aspects of toxicology. Archives of toxicology, 87 (4): 569-567.
- **Setyawati MI, Tay CY, Chia SL, Go H, Fang W, Neo MJ, Xie JP. (2013).** Titanium dioxide nanomaterials cause endothelial cells leakiness by disrupting the homophilic interaction of VE – cadherin. Nature communications, 4: 1673.
- **Turkevich J, Stevenson PC, Hillier A. (1951).** Study of the nucleation and growth processes in the synthesis of colloidal gold. Discussions of the Faraday Society, 11: 55-75.
- **Wen H, Dan M, Yang Y, Lyu J, Shao A, Cheng X. (2017).** Acute toxicity and genotoxicity of silver nanoparticle in rats. Plos one, 12 (9):1-16.
- **Wong KK, Liu X. (2010).** Silver nanoparticles- the real silver bullets in clinical medicine. RSC Medicinal Chemistry, 1(2):125-131.
- **Yahya A , Alsadee SA , Obaid WA , Hameed OM , Hasan AF.(2024).** Histopathological and immunohistochemical studies on the effects of silver oxide nanoparticles (AgNPs) on male rats' liver. Journal of Bioscience and Applied Research, 10(3): 392-398.
- **Yang L, Kuang H, Zhang W, Aguilar ZP, Wei H, Xu H. (2017).** Comparisons of the biodistribution and toxicological examinations after repeated intravenous administration of silver and gold nanoparticles in mice. Scientific Reports, 7 (3303):1-12. 59.
- **Zhang C, Hu Z, Deng B. (2016).** Silver nanoparticles in aquatic environment. Physicochemical behaviour and antimicrobial mechanisms. water research, 88: 420-427.