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Research Article

Histopathological alternation of the liver and spleen of mice treated with Ag nanoparticles

Suzan Ibrahim BAJILAN*, Asmehan Adnan ALNAQEEB, Salwa Ghazi TURKI

Department of Basic Science, College of Nursing, University of Baghdad, Baghdad, Iraq.
**Email: suzanibrahim1977@gmail.com*

Abstract: Ag Nanoparticles are recently among the most utilized nanomaterials due to their unique characteristics. These characteristics make them desirable to numerous industries but, also may contribute to their potential toxicity. The toxicity of colloidal Ag NPs solution on the liver and spleen of mice was studied. This solution was administrated with two concentrations of 0.5 and, 1ppm to females by intraperitoneal injection and another over two periods of 30 and 45 days. Twenty-five female mice weighing about 25-30g were divided into five groups, a control group (GC), G1 was injected with 0.5ppm of AgNPs for 30 days, G2 was injected with 1ppm for 30 days, G3 was injected with 0.5ppm for 45 days and G4 group injected with 1ppm for 45 days. Animals were then sacrificed and their liver and spleen were removed, preserved, processed, sectioned, and stained. Slides were examined with Lieca fluorescent microscope. The results revealed tangible histological changes in the liver and spleen in the G1 group. However severe deteriorations (hemorrhage, fibrosis, necrosis, and deposition of amyloid protein in hepatic vein) were observed in the rest. In conclusion, Ag Nps caused severe damage to the architecture of the liver and spleen even when utilized with less concentration.

Keywords: Ag nanoparticles, Hemorrhage, Fibrosis, Amyloid protein.

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Introduction

Nanoparticles (NPs) can be defined as an object with a diameter of less than 100 nanometers. Owing to their size being tiny, their surface area is large, with specific physical/chemical characteristics. NPs are nowadays broadly investigated in many research laboratories across the world (Paunovic et al. 2017). Ag NPs are applied widely in textiles/fabrics, and food technology because of their unique biological and chemical properties (Elkhawass et al. 2015). Products being manufactured comprising Ag-NPs might include drugs, wound dressings, cosmetics, clothing, bedding, purification of water, washing machines, humidifiers, and deodorants (Lee et al. 2013). They are also incorporated into many commercial products such as furniture, household appliances, refrigerators, cosmetics, and even children's toys (Pantic 2014). Moreover, Ag NPs are widely utilized in healthcare and medical sectors, because of their antimicrobial against viruses, bacteria, and fungi (Elkhawass et al. 2016). Other biological activities of Ag NPs include helping the healing of bone and repairing wounds, improving the vaccine's immunogenicity, and effects as anti-diabetic (Xu et al. 2020).

Ag NPs can enter the body via inhalation, ingestion, and contact with skin or genitourinary tract and deposited in vital organs i.e., liver, brain, or kidneys. Such NPs might damage or change cellular functions by passing membranes of cells and then interacting with bio-molecules resulting in protein and DNA damage (Adeyemi & Adewumi 2014). Despite the growing applications for products containing Ag NPs, there is slight data regarding its side effects and potential toxicity. *In vitro* evidence supports that Ag NPs induce strong cytotoxicity and

pro-inflammatory effects in a broad spectrum of cells (Pourhamzeh et al. 2016). Biomedical studies also exhibited that Ag NPs are able to arrive at blood stream and stored in diverse body organs and tissues rendering such tissues and organs vulnerable to destruction and changed physiological processes (Park et al. 2010). In fact, the sizes of nano-particles are extra toxic compared to other-sized particles (Ashajyothi & Chandrakanth 2019).

The liver is a fascinating organ with a high capacity for regenerative and complex actions. Its location as strategic as related to the supply of food through the portal vein, and the hepatocyte's unique patterns of protein and gene expression, allow it to act as a biochemical defense versus toxic compounds arriving in the digestive tract and as an absorbed food ingredients repressor (Mahmoudian et al. 2016). Ag NPs transmit to the bloodstream passing after any route of exposure and accumulating in the target liver organ (Pani et al. 2015). Extreme Ag NPs deposition/accumulation in the liver caused definite contrary effects, including noticeable pathological fluctuations in bile-duct hyperplasia, morphology of organs, and inflammation of cell infiltration. Meanwhile, the accumulation of these metallic NPs in the liver leads to hepatic cell interaction and changes their structure and function (Yao et al. 2019).

The liver and spleen are the most exposed organs to nano-materials due to *in vivo* studies dealt with Ag NPs toxicity and bio-distribution in mice and rats, confirmed that the administration of these particles by ingestion, inhalation, i.p or i.v. injections are consequently perceived in blood and lead to toxicity in numerous organs, including the liver, lung, kidney, brain, and intestine (Ferdous & Nemmar 2020). The effect of i.p injection of Ag NPs was studied by Elkhawass et al. (2015) and the results showed that the spleen and liver were the chief target of Ag accumulation organs with 20 and 50 nm doses. The prevalence of phagocytic cells in the reticuloendothelial system of these organs enhances the accumulation of nanomaterials (Ashajyothi & Chandrakanth 2019). Biochemical liver enzymes' changes such as aspartate transferase (AST) and alanine transferase (ALT) besides histo-pathological liver alterations were also studied by Pourhamzeh et al. (2016). Wen et al. (2017) also reported a high accumulation of Ag NPs in the immune system organs, kidneys and liver could be the greatest affected organs by an Ag NPs acute i.v dose. Therefore, the liver in particular is among the major organs for the accumulation of Ag NPs (Pourhamzeh et al. 2016). This study was designed as a continuous to preceding work by Bajilan et al. (2019) that took the effect of i.p. injection of Ag NPs on the ovaries. In this study, the toxicity of colloidal Ag NPs solution on the liver and spleen of mice was studied.

Materials and Methods

Animals: Mature female mice (25) with 20-25g weight were obtained from Al-Nahrain University/ High Institute of Infertility Diagnosis and Assisted Reproductive Technology animal house. They were kept under standard temperature conditions of 25-28°C and 12 light-dark h cycles throughout the experiment period. In addition to the control (GC) group, animals were distributed into four groups (n = 5) based on the Ag NPs concentration solution (0.5 and 1ppm) and the period of 30 and 45 days. These treatments were as follows: G1: injected with 0.5ppm Ag NPs solution for 30 days, G2: injected with 1ppm Ag NPs for 30 days, G3: injected with 0.5ppm Ag NPs solution for 45 days, and G4: injected with 1ppm Ag NPs solution for 45 days. All the animals were treated by i.p twice a week during the experiment. At every experiment termination, animals were sacrificed, organs were isolated and fixed in Bouns solution for 24 hours, then were replaced by ethanol (70%). Processing of these organs was achieved according to Bancroft & Stevens (2010) for histological study.

Ag NPs solution: Ag NPs colloid solution was provided by the Iranian Company of Nano-parspanda at 4000ppm concentration and 50-100 nanometer size. The solution characteristics were confirmed at

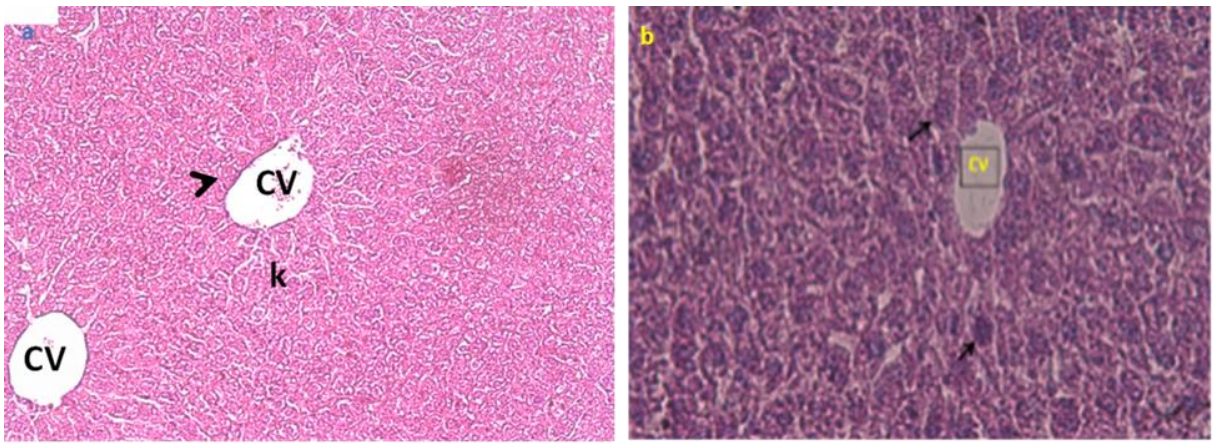

Fig.1. Section of the liver of (a) control group GC revealing: Hepatic cells (arrows) aligned around the central vein. With the presence of Kupffer cells (K) at the margin of the central vein. Objective 10X/0.22, and (b) hepatic cells in the G1 group were distorted, the central vein (HV) was shrunk with hydropic degeneration of hepatocytes, Kupffer cells (head arrows) (H&E, 20X/0.30).

the Chemistry Department, College of Science, Baghdad University using a Scanning Probe Microscope (SPM). This solution was activated with the usage of an ultrasound sonicator at the Department of Physics, Science College, Baghdad University every 2 weeks. In the current work, 0.5 and 1ppm concentrations were done from the stock, based on the following formula: C1 V1= C2 V2 (Skoog et al. 2004).

Histological study: The organs being fixed were routinely processed, embedded in paraffin, sectioned, de-paraffinized, and rehydrated utilizing the techniques as standard (Bancroft & Stevens 2010). Then, slides were stained by routine stains of haematoxylin and eosin. Inverted fluorescent microscopic (Leica) was used to examine the histological sections of the liver and spleen.

Results

This study was designed as a contagious to a previous study by the same author (Bajilan et al. 2019). Two concentrations of colloidal Ag NPs solution (0.5 and 1ppm) were applied with two periods of treatment (30 and 45 days) to find out the histopathological changes in the liver and spleen of female mice. Liver sections from the GC revealed normal structure as hepatic, the vein being central, hepatocyte and Kupffer cells around the central vein (Fig. 1A). The architecture of most hepatocytes was distorted in the G1 group where hydropic degeneration was seen in most cells and there was a shrinking in the central vein (Fig. 1b).

Section of the liver from the G2 group exhibited serious damage to the structure of the hepatocytes. There was congestion within the dilated central vein with infiltration of neutrophils which indicates the presence of inflammation as a result of oxidative stress from xenobiotics. Some necrotic spots appeared in the stroma (Fig. 2a). Whereas, the section from the liver of the G3 group exhibited severe damage. A large dilated hepatic vein appeared with infiltration of neutrophils and deposition of amorphous proteinaceous amyloid within it. Most hepatocytes in this section revealed pyknosis in their nuclei which indicate necrosis. There were some fatty changes near the hepatic vein. Two giant cells appeared in this section. These multinucleated cells form by macrophage fusion and presumably contribute to the debris removal from tissues (Fig. 2b). Section of the liver from the G4 group is represented in Figure 2d, where the hepatic vein is seriously damaged with infiltration of white blood cells and hemorrhage. A number of necrotic spots appeared at the margin of the hepatic vein, and a number of Kupfer cells appeared in the stroma of the organ and at the margin of the hepatic vein.

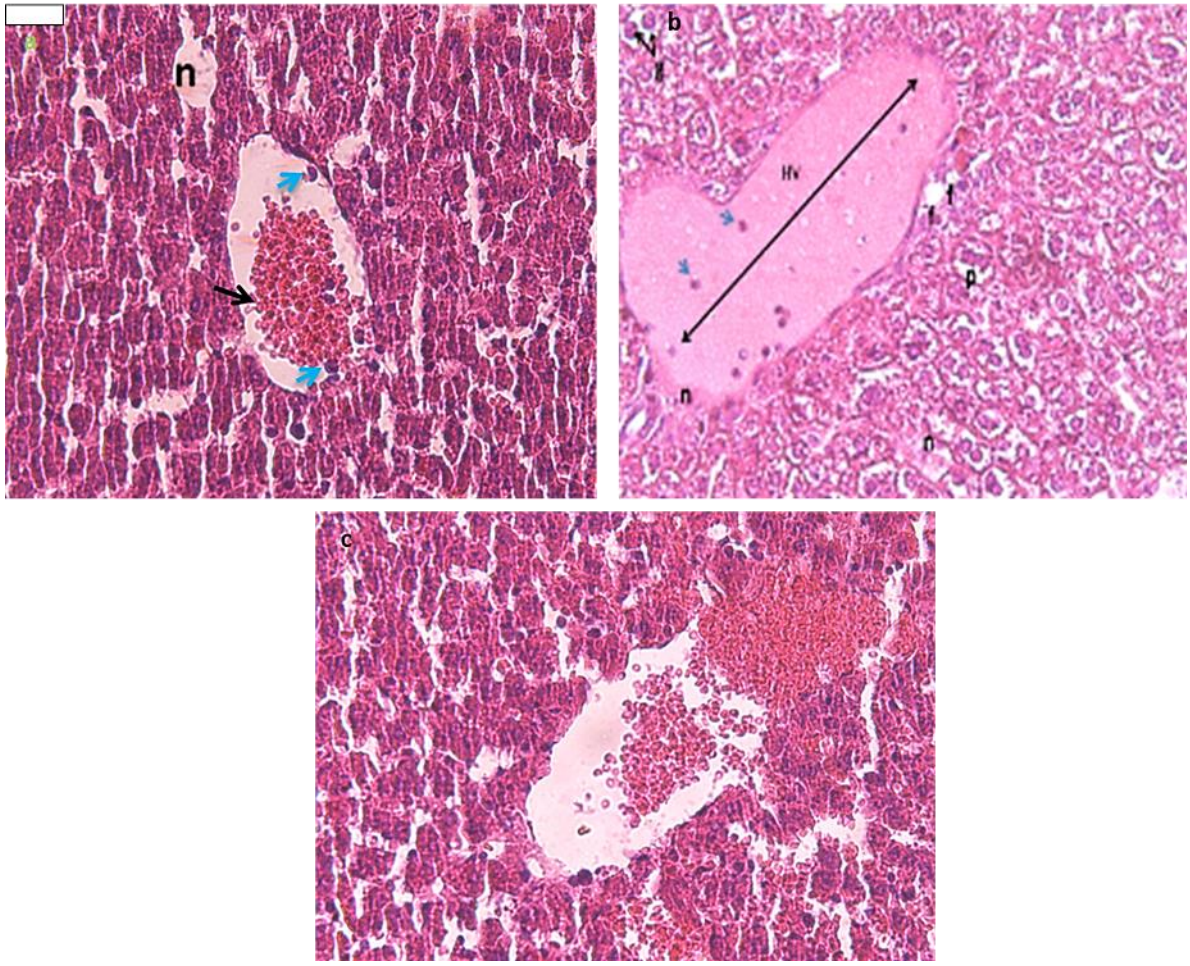

Fig.2. Sections in the liver of a mouse from the c-G2 group: most hepatocytes at this section are seriously damaged, congested (c) dilated hepatic vein with infiltration of neutrophils (arrows). d-Hepatocytes in this section from the G3 group are vacuolated with pyknosis (p) in their nuclei, fatty changes (f) are seen in cells near the dilated hepatic vein (HV), infiltration of neutrophils within the hepatic vein(arrows), large amyloid protein deposits within the hepatic vein (double head arrow). necrosis (n)at the lining of the hepatic vein and some hepatocytes. e- Section in the liver from the G4 group showing: a dilated damaged hepatic vein with infiltration of neutrophils in the center and hemorrhage (h), some necrotic spots appeared (n). Kupfer cells (k) were seen at the margins of the hepatic vein and in the stroma (H&E, 20x/30).

The spleen of the GC shows white pulp which is composed primarily of lymphocytes. The red pulp forms the bulk of the splenic parenchyma (Fig. 3a). An expansion in the white pulp areas was seen in the spleen of the G1 group, and the red pulp area was also increased (Fig. 3b). The red pulp area was clearly expanded in the G2 group which indicates extramedullary hematopoiesis (EMH) while the white pulp seemed shrunk. Fibrosis is characterized by increased deposition of collagenous stroma in the spleen. Some necrotic spots appeared in the stromal tissue of the organ. Fibrosis can occur as a reparative process following injury (toxicity) or inflammation (Fig. 3a).

The white pulp was involute in the spleen of the G2 group. The fibrosis appeared in the parenchymal tissue which was also obvious in the splenic capsule in some sections indicating necrosis. An ill-defined diffutilized red pulp area (Fig. 4c). While the section of spleen from the G3 group revealed vacuolated cells and shrunk nuclei in the red pulp, and the white pulp was also involute, fibrosis appeared in the subcapsular area and in the capsule. Megakaryocytes appeared in this section indicating extramedullary hematopoiesis (EMH), misfolded insoluble amyloid protein was deposited in splenic vessels in this

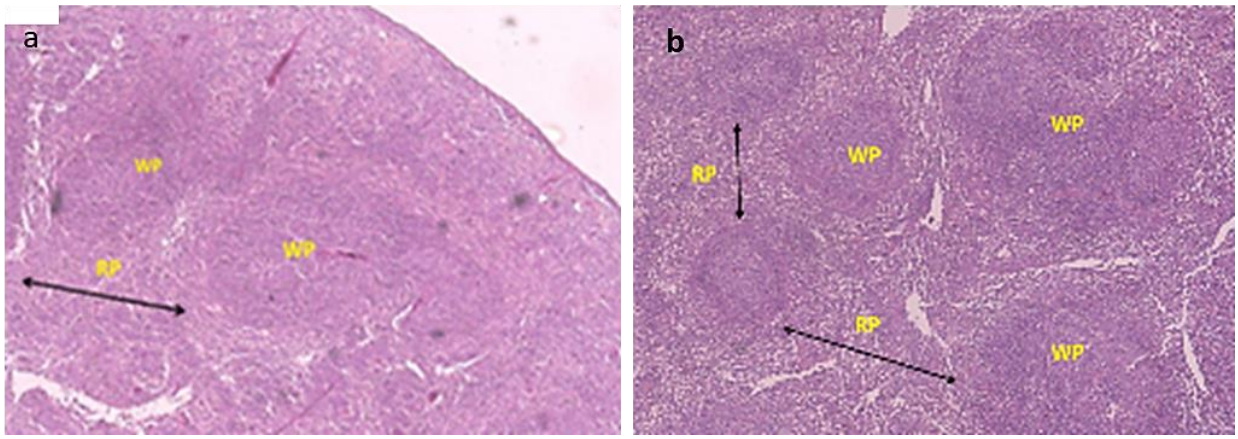

Fig.3. (a) Sections in the spleen of female mice, a- control group showing the with pulp (WP), the red pulp (RP), and (b) G1 group showing an increase in white pulp area (WP), expansion of the red pulp area (RP). (H&E, 10x, 0.22).

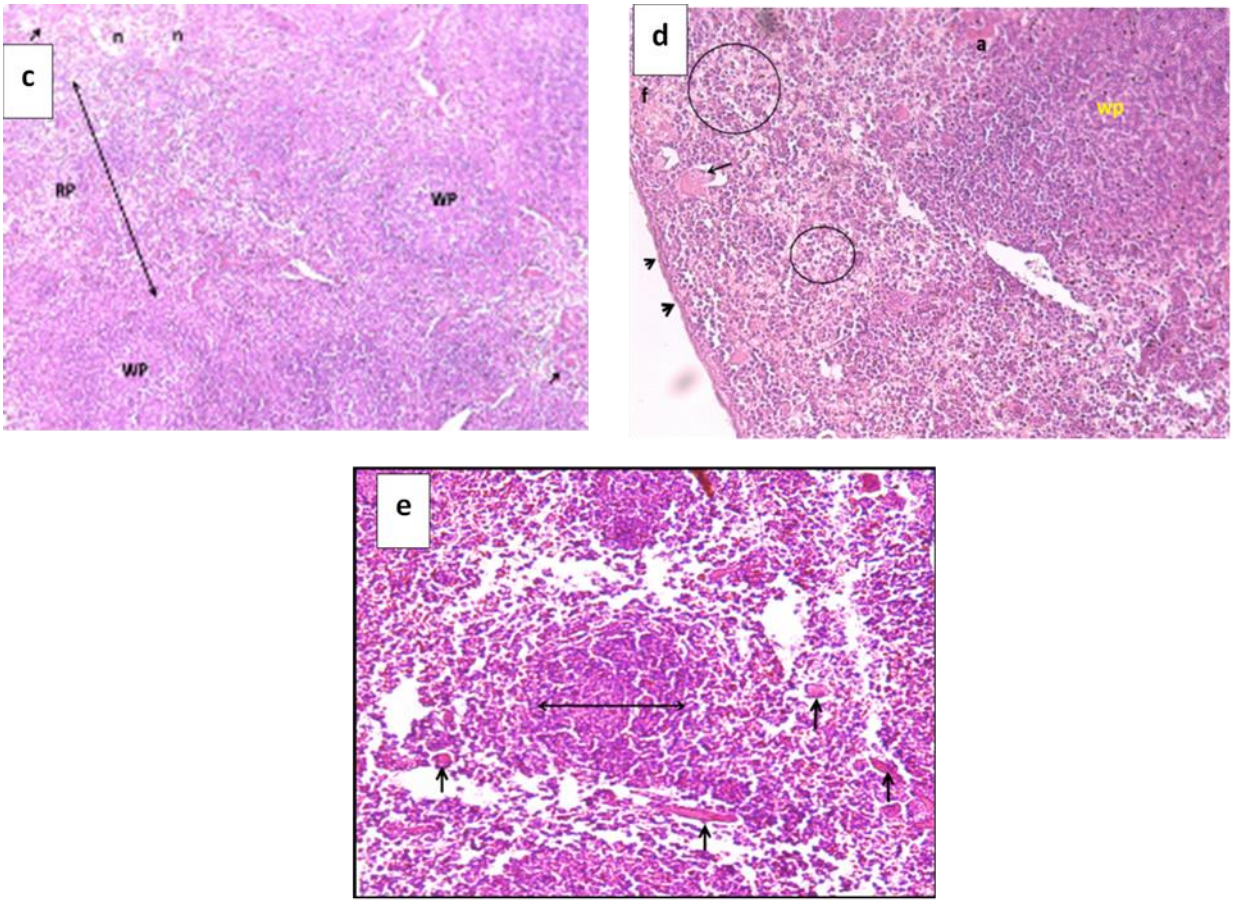

Fig.4. Section in spleen of a mouse c- group involuted white pulp (WP), ill-defined diffused red pulp (double head arrow), fibrosis (arrow) some necrotic (n) spots. The section in the spleen of a treated mouse from d- the G3 group most cells in the red pulp are with vacuolated cytoplasm and shrunk nuclei (circles), fibrosis (f) appeared at some spots, and in the capsule (head arrow), megakaryocyte (arrow), amyloid protein (a) deposits in the splenic vessel. e-spleen of a mouse from the G4 group, the architecture of the spleen is seriously damaged, with shrunk white pulp (double head arrow), and deposition of amyloid in more than a spot in the red pulp (arrow). (H&E, 20x/0.30).

section which appeared as a hyaline pink color (Fig. 4d). The structure of the spleen is seriously damaged in the G4 group. There was a shrinking in the white pulp area and amyloid protein was deposited in more

than the area of the red pulp (Fig. 4e).

Discussion

After the huge nano-technology application, there is a rising trend regarding the synthesis, design, and engineered (NPs) utilized in diverse areas, including cosmetics, medicine, electronics, coating, paints, bioremediation, and the food industry (Ibrahim et al. 2018). Today, Ag NPs are one of the most commonly used nano-sized compounds in industry, manufacturing, and biomedicine (Paunovic et al. 2017). The liver is the principal detoxification organ in the body and it has the ability for detoxification, storage of glycogen, and protein synthesis secretion (Yao et al. 2019). In the current work, remarkable histopathological changes were found in the liver of the G1 group and disorganized hepatic architecture was obvious as a result of hydropic degeneration. These results were in line with Sarhan & Hussien (2014) as they found that intraperitoneal injection of Ag NPs (2000mg/kg) for two successive days caused swollen hepatic cells and narrowing of the sinusoidal lumen. In addition, Ramadi et al. (2016) observed confirmation of hepatocyte necrosis as dose-dependent in the liver of mice treated with a composite of Ag: Cu: B (dose range 1-20 mg/kg), upsurge in sinusoidal Kupffer cells, lobular granulomas, and abscess formation foci that were most definite at 24 h after NP administration. It was noted by Elkhawass et al. (2018) that Ag NPs reveal a 6-fold or greater solubility in water than other forms of Ag and more than 100-fold greater release of Ag^+ than that from other metallic Ag forms. This accumulation of Ag in its ionic form is toxic both for cell DNA and organelles and may induce various signal pathways leading to ROS creation, apoptosis, or necrosis (Paunovic et al. 2017).

The observation of Kupffer cell aggregation in hepatic tissue is in line with the findings of Kim et al. (2010), who tested the oral Ag NPs toxicity (56nm) for 90 days in rats. The histopathological results of the liver revealed the presence of numerous foci of inflammatory cell infiltration particularly around the central vein and sinusoids on hepatic lobules. The result also agreed with the work of Yousuf et al. (2022) who tested the oral toxicity (0.5ml) of Ag NPs solution at a concentration of 25ppm for 14 days. Histo-pathological alteration of the liver showed a congested and dilated central vein which, indicates the presence of inflammation and vacuolated ballooned hepatocytes. Macrophages of the liver are specified in the foreign NPs internalization, playing a vital part in its organism destination, as they are involved in their uptake and trafficking *in vivo* (Colino et al. 2020). Furthermore, Korani et al. (2015) noted that Ag NPs have the ability to inhibit the activities of numerous cytochrome P450 (CYP) enzymes in accordance to elevation in liver biomarkers i.e., AST, ALT, and GGT, these alterations in return induce histo-pathological changes at the same organ.

There were discrepancies in the toxicological changes in the liver at the current work. Hydropic degeneration of hepatocytes and fatty changes in some hepatic cells also was observed; giant cells appeared in some areas. These are attributed to variations in the Ag NPs size that were utilized and the duration of exposure (Pourhamzeh et al. 2016). The size of Ag NPs utilized as the colloidal solution in these experiments ranged between 50-100nm. Destruction in the endothelium of hepatic vein congestion and hemorrhage was in line with the findings of Wen et al. (2017). that the kidney and liver were the greatest affected organs through acute Ag NPs intravenous dose. Liver cells also showed degeneration, necrosis, and hemorrhage (Wen et al. 2017).

With regard to the presence of giant cells, it is known that metallic NPs ingoing inside the body or liver cells prompt inflammation (Yao et al. 2019). As a result, multi-nucleated giant cells (MNGs) form by macrophage fusion and are presumably contributing to the debris removal from cells. These cells form in diverse inflammatory diseases and are specified for complement-mediated phagocytosis and large target destruction (Milde et al. 2015). Deposition of

amyloid was observed in the liver of the G3 group. The serum amyloid A (SAA) is secreted predominantly by liver hepatocytes and produced also by a diversity of tissues and cells. In cases of regulating inflammation, immunity, and lipid metabolism, oral administration and subcutaneous amyloid-enhancing factor injection from various species of animals with Ag nitrate able to induce AA amyloidosis in mice (Lin et al. 2021).

The extra-medullary hematopoiesis (EMH) was observed in the spleen of G3. The spleen is a hematopoietic organ in mice. During embryonic development, hematopoietic stem cells (HSC) migrate and reside in both the spleen and bone marrow throughout the mouse's life. In addition, extramedullary hematopoiesis occurs in the spleen in pathological conditions (Iseki et al. 2008) Nevertheless, there was hemorrhage in some sections as a result of toxicity or inflammation. Fibrosis appeared in the splenic parenchyma in addition to the capsule. These results agreed with the findings of Mazen et al. (2017) who reported the effect of different doses of Ag NPs administrated to rats by intraperitoneal injection for 28 days. Their results revealed some vacuolation and degeneration in the spleen white pulp for the group treated with 100mg/Kg of Ag NPs. In this work, congested splenic sinuses were seen in the red pulp which was in agreement with our observation. Besides, Sutomo et al. (2022) pointed out that Ag NPss cause spleen toxicity in mice at different concentrations (0.00082, 0.0052, 0.074, 0.22, 0.67, 2.0, and 6.0mg/kg with a size of 20 and100nm. The histopathological features showed inflammation and brownish color degradation of red blood cells, and lymphocyte reduction in splenic tissue.

Megakaryocyte was observed in the spleen of the G3 group due to the EMH. This observation was in accordance with Mazen et al. (2017) and Hassan et al. (2019). This feature is observed commonly in rodents as normal splenic red pulp components. It occurs more frequently in females compared to males and in mice compared to rats. A variety of conditions may cause an increase in the number of hematopoietic cells such as hematotoxic insult, infection, and hemorrhage. EMH may include an increased number of erythroid precursors, myeloid precursors, and megakaryocytes (National Toxicology Program 2013). The involution in white pulp seen in our results also are consistence with the findings of Mazen et al. (2017) and Hassan et al. (2019).

In conclusion, tangible histopathological changes were seen with a concentration of 0.5ppm but, a longer duration of treatment caused chronic inflammation in the liver and spleen. This might be attributed to the release of more Ag ions from the NPs. Ag NPs using their specific properties can freely pass through the cell membrane after which they are converted to Ag ions. These ions when accumulated are toxic both for cell DNA and organelles and may induce various signal pathways leading to ROS creation, apoptosis, or necrosis.

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References

Adeyemi, O.S. & Adewumi I. 2014. Biochemical evaluation of Ag NPss in Wistar Rats. International Scholarly Research Notices 2014: 1-8.

Ashajyothi, C. & Chandrakanth, R.K. 2019. A pilot toxicology study of biogenic Ag NPss: *In Vivo* by intraperitoneal and intravenous infusion routes in Rats. Journal of Experimental Nanoscience 14(1): 89-106.

Bancroft, J.D. & Stevens, A.S. 2010. *Theory and Practice of histological Techniques*. 2nd ed. Churchil Livingstone. Edinburgh. London. 250 p.

Bajilan, S.I.; Abdul-Sada, W.N. & Neamah, A.M. 2019. Ag NPs cause some physiological changes in ovaries of mice treated with human chorionic gonadotropin (hCG). Journal of International Pharmaceutical Research 45(5): 462-470.

Colino, C.I.; Lanao, J.M. & Gutierreze- Millan, C. 2020. Targeting hepatic macrophages by therapeutic NPss.

Frontiers in Immunology 11: 1-17.

Elkhawass, E.A.; Mohallal, M.E. & Soliman. M.F.M. 2015. Acute toxicity of different sizes of Ag NPss intraperitonally injected in Balb/C mice using two toxicological methods. International Journal of Pharmaceutical Sciences 7(1): 94-99.

Hassana, O.A.; Saadb, A.H. and and Hamouda, A.H. 2019. Ag NPss induced multiole organ toxicity in mice. Egyptian Journal of Forensic Sciences and Applied Toxicology 19(4): 31-46.

Ibrahim, K.E.; Al-Mutary, M.G.; Bakhiet, A.O. & Khan, H.A. 2018. Histopathology of the Liver, Kidney, and Spleen of Mice Exposed to Gold NPss. Molecules 23(1848): 1-14.

Iseki, A.; Morita, Y.; Nakauchi, H. & Ema, H. 2008. Hematopoietic stem cells in the mouse spleen. Blood 112(11): 2421.

Kim, Y.S.; Song, M.Y. & Park, J.D. 2010. Subchronic oral toxicity of Ag NPss. Particle and Fiber Toxicology 7(20): 1-11.

Korani, M.; Ghazizadeh, E.; Korani, S.; Hami, Z. & Bardbori, A.M. 2015. Effects of Ag NPss on human health. European Journal of Nanomedicine 7(1): 51-62.

Lee, T-Y.; Liu, M-S.; Huang, L-J.; Lue, S-I.; Lin, L-C.; Kwan, L-A. & Yang, R-C. 2013. Bioenergetic failure correlates with autophagy and apoptosis in rat liver following Ag NPs intraperitoneal administration. Particle and Fibre Toxicology 10: 40.

Lin, X.; Watanabe, K.; Kuragano, M. & Tokuraku, K. 2021. Aggregation of mouse serum amyloid A protein was promoted by amyloid-enhancing factors with the more genetically homologous serum amyloid A. International Journal of Molecular Sciences 22: 1-12.

Mazen, N.F.; Saleh, E.Z.; Mahmoud, A.A. & Shaalan, A.A. 2017. Histological and immunohistochemical study on the potential toxicity of sliver NPss on the structure of the spleen in adult male albino rats.

Polish Journal of Environmental Studies 32(2): 991-997.

Milde, R.; Ritter, J.; Tennent, G.A.; Pepys, M.B. Verschoor, A. & Helming, L. 2015. Multinucleated giant cells are specialized for complement-mediated phagocytosis and large target destruction. Cell Reports 13: 1937-1948.

National Toxicology Program. NTP - Nonneoplastic Lesion Atlas. 2013. Spleen – Extramedullary Hematopoiesis. Available at: https://ntp.niehs.nih.gov/atlas/nnl/immune-system/spleen/ExtramedullaryHematopoiesis.

Pantic, I. 2014. Application of Ag NPss in Experimental Physiology and Clinical Medicine: Current Status and Future Prospects. Reviews on Advanced Materials Science 37: 15-19.

Park, E-J.; Yi, J. Kim, Y.; Choi, K. & Park, K. 2010. Ag NPss induce cytotoxicity by a Trojan-horse type mechanism. Toxicology in Vitro 24: 872-878.

Paunovic, J.; Vucevic, D.; Radosavljevic, T.; Pantic, S.; Nikolovski, D.; Dugalic, S. & Pantic, I. 2017. Effects of metallic NPss on physiological liver functions. Reviews on Advanced Materials Science 49: 123-12.

Pourhamzeh, M.; Mahmoudian, Z.G.; Saidijam, M.; Asari, M.J & Alizadeh, Z. 2016. The Effect of Ag NPss 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.

Ramadi, K.B.; Mohamed, Y.A. & Al-Sbiei, M.A. 2016. Acute systemic exposure to Ag-based NPss induces hepatotoxicity and NLRP3-dependent inflammation. Nanotoxicology 10(8): 1-46.

Sarhan, O.M.M. & Hussein, R.M.2014. Effects of intraperitoneally injected Ag NPss on histological structures and blood parameters in the albino rat. International Journal of Nanomedicine 9: 1505-1517.

Skoog, D.A., West, D.A., Holler, F.J. and Crouch, S.R. 2004. Fundamental of analytical chemistry. 4th ed. Thomson Learning Inc. *USA:* 15-16.

Sutomo, D.F.I.; Ahsani, D.N. & Fidianingsih, I. 2022. Toxicity of NPss on the spleen in animal studies: A scoping review. Jurnal Ilmu Kefarmasian Indonesia. 20(2): 1-11.

Wen, H.; Dan, M.; Yang, Y.; Lyu, J.; Shao, A.; Cheng, X.; Chen, L. & Xu, L.2017. Acute toxicity and genotoxicity of Ag NPs in rats. Plos One 0185554.

Xu, L.; Wang, Y.Y.; Huang, J.; Chen, C-Y.; Wang, Z-X. & Xie, H.2020. Ag NPss: Synthesis, medical applications and biosafety. Theranostics 10(20): 1.

Yao, Y.; Zang, Y.; Meng, J.Q. & Zhang, T.T. 2019. The toxicity of metallic NPss on liver: The subcellular damages, mechanisms, and outcomes. International Journal of Nanomedicine 14: 8787-8804.

Yousof, S.M.; Erfan, H.; Hosny, M.M.; Shehata, S.A. & El-Sayed, K. 2022. Subacute toxic effects of Ag NPss oral administration and withdrawal on the structure and function of adult Albino Rats' hepatic tissue. Saudi Journal of Biological Sciences 29:3 890-3898.