International Journal of Pharmaceutical and Bio-Medical Science

ISSN(print): 2767-827X, ISSN(online): 2767-830X

Volume 04 Issue 12 December 2024

Page No : 998-1003

DOI: https://doi.org/10.47191/ijpbms/v4-i12-12, Impact Factor:7.792

Effect Toxicity of Silver Nanoparticles on Biochemical Markers and Oxidative Stress *in Adult Male Wistar Rats*

Prof. Dr. Najlaa Abdulameer Ali Al-Dahhan[1], Prof. Dr. Hawraa Abdulameer Ali Al-Dahhan[2]

[1]Department of Basic Science, College of Dentistry, University of Kufa, Al-Najaf, Iraq.

[2]College of Science, University of Kufa, Al-Najaf, Iraq.

ABSTRACT

Background: The assessment the health risks of silver nanoparticles(AgNPs) has become extremely important due to increase their use in all fields i.e., health,industry ,commerce etc.

Objectives: To assess the toxicity of oral AgNPs by assessing the activity of liver enzymes; AST, ALT, GGT,and ALP.Also,assess induction of ROS and the concentration of LHP in male Wistar rats.

Materials and Methods: The study included 60 adult male Wistar rats, divided into five groups in each group 12 rats, groups 1 to 4 were orally exposed to AgNPs,once daily for one week at doses of 10,35,75 and 100 mg/kg,Bwt. The fifth group included acontrol group.Venous blood was collected after 24 hours of end the experiment. Serum TP,TBil and Alb were measured using an autoanalyzer.The chromatic method was used to measure serum ALT,AST,ALP ,and GGT levels according to the instructions of trade kits were obtained from Sigma-Aldrich. LHP kits purchased from Calbiochem**.**ROS production were estimate according to Lawler et al.method **Results:** No significant changes were observed in serumTP, TBil, and Alb levels between rats groups that orally exposed to AgNPs and the control group($P>0.05$).Increased serum ALT,AST,ALP, GGT levels,induction of ROS and increased concentration of LHP in rats groups that orally exposed to AgNPs with increasing dose concentration of AgNPs compared with control group($p<0.05$).The highest two doses(75 and100)mg/kg showed increase statistically significant in levels of enzymes,LHP concentration,and induction ROS in rats groups that orally exposed to AgNPs compared to other and controls ($p<0.05$).The results from DLS showed agglomeration of AgNPs more than their base volume and the potential value of Zeta AgNPs was-35mV.Histopathological examination show occurrence of central vein damage,hepato cellular vacuolation, necrosis and Pycknotic in livers of rats after exposed to AgNPs.

Conclusions: Depending on these findings,it can be said that the short-term administration of high doses from silver nanoparticles causes increased serum enzymes activity,increased LHPconcentration,oxidative stress, stimulation of ROS and organ toxicity.

KEYWORD: *Silvernanoparticles; aminotransferases; alkalinephosphatase; lipid hydroperoxide; reactive oxygen species.*

ARTICLE DETAILS

Published On:
23 December 2024

Available on:
https://ijpbms.com/

INTRODUCTION

Nanotechnology is concerned with the production of engineering nanoparticles (Hussain and Schlager,2009). AgNPs are used in food industries,kitchen appliances, packaging materials, household appliances and medical fields especially in wound care products(Bartlomiejczyk et al.,2013; Munger et al.,2014). AgNPs have anti-bacterial and anti-microbial properties(Xu et al.,2012;Kulthong et al., 2012;Pourhamzeh etal.,2016).In recent years,its applications have increased dramatically in various medicines,laboratory evidence,cosmetics, etc.AgNPs induce effective cellular toxicity and pro-inflammatory effects(Dziendzikowska etal.,2012). AgNPs stimulate production of ROS and release of inflammatory cytokines(Xu etal.,2012).ROS is continually generated in living systems during biological reactions, in turn,occurs reduction of endogenous or exogenous antioxidants(Goodarzi et al.,2014; Mohammadi et al.,2015).Excessive production of ROS can lead to DNA damage and programmed cell death (Xu et al.,2012). Programmed cell death begins with the activation of the caspase chain, which is one group of cysteine proteins found in cells as inactive enzymes (Eckle etal.,2004). ROS plays a beneficial and harmful role in biological reactions,so it has been proposed as a potential toxic mechanism.It has harmful effects by disrupting the functions of some enzymes by oxidizing amino acids in proteins, oxidation of cofactors in their structure, oxidation of polyunsaturated fatty acids in fats and then DNA damage (Tampa et al.,2018). Permanent exposure of AgNPs generates the ability to interact with biological tissues and produce ROS in large quantities.The production of free radicals and lipid hydroperoxide(LHP) is related to the toxicity of nanomaterials (Murrayet al.,1988). Consequently, injury and cell death can occur as a result of oxidative damage to the polyunsaturated fatty acid known as LHP(Gutteridge andQuinlan,1983;Halliwell,1984).The oxidative damage to lipid membranes can be assessed by estimating the quantities of LHP(De Zwart et al.,1999).

Liver cells and tissues are targets of attack by various of ROS and lipid peroxide, which are associated with the electron transport chain in metabolism and possibly hepatotoxicity. This study was conducted to evaluate the effect the toxicity of AgNPs on biochemical parameters and liver tissue in adult male Wistar rats.Therefore,our findings are extremely important to assess health risks due to the increased use of new nanoscale products in daily life. Therefore, findings are important for assessing health risks due to the increased use of new nanoproducts in in daily life.

MATERIAL AND METHODS

AgNPs were obtained in water at a volume (10) nm, AgNPs were suspended directly in deionized water (DI water) and dispersed by ultrasound vibration (100W,30KHz) for 30 minutes to produce four concentrations different at 10 , 35,75 and 100 mg/kg.

The study included 60 adult male Wistar rats, ranging in age from 3-4 months, and weighing between 200 ± 25 g. The temperature was 250°C and the light (12 hours light / 12 hours darkness). It was supplied with food from food establishments, and water is drinking water used by citizens in the city of Najaf. Rats adapted to the laboratory environment within 10 days, rats were randomly divided into 5 groups, 12 rats in each group. rats were orally exposed to AgNPs daily using Gavage for one week, according to the method (Park et al.,2010). Groups from 1to4 received dosesin different concentrations from the orally AgNPs(10, 35, 75 and 100 mg/kg), respectively using special needles, one dose per 24 hours for one week. Each rat, in the group received seven doses at 24-hour intervals.The fifth group, serving as a control group, received only deionized distilled water. AgNPs were diluted with deionized water. Laboratory animals were cared for according to international laws and principles relating to animal use in biomedical research according to (Giles,1987).Blood samples were collected after end the experiment, and allowed to coagulate for 45 minutes at room temperature. After coagulation,serum was centrifuged at 1500 x g for 10min.Serum total protein(TP), totalbilirubin(TBil)and albumin(Alb) were measured using an autoanalyzer (Hitachi 7180, Hitachi,Japan).The chromatic method was used to measure serum aspartate transaminas(AST),alanine transaminase(ALT),gamma glutamyltranspeptidase(GGT) and alkaline phosphatase(ALP) levels according to the instructions of trade kits were obtained from Sigma-Aldrich (St.Louis,MO,USA). lipid hydroperoxide(LHP) kits purchased from Calbiochem (La,Jolla,CA,USA), acording to Anita et al.(2015)method.The mixture was incubated for (5) min and absorption was recorded for each sample using optical spectrophotometer(2800,Unico spectrophotometer,USA)at awavelength(500)nm.Where lipid hydro-peroxides were directly measured which use oxidation and reduction reactions with iron ions, and the resulting hydrocarboxides are highly unstable and interact easily with ferric ions,use calbiochem and the resulting ferric ions are discovered using chromogen ion thiocyanate. Fig.1. The standard curve for the examination of Lipid hydro peroxides (LPO) shows where the y-axis represents the absorbance at(500)nm while the x-axis represents the concentrations of the reference standard(nmol).ROS production were estimate according to Lawler et al.(2003)method,Use hydrogen peroxide(30% H_2O_2), to evaluate the probe interactivity. The AgNPs sample was analyzed by DLS (dynamic light scattering), to understand the state of particle dispersion when placed in a DI water (deionized). After stupefying the rats using diethyl ether and dying, the livers were separated and preserved in 10% formalin. Liver tissue was prepared according to common histological methods and preserved with paraffin blocks. The blocks were cut to a diameter of 4-5 µm, and these put on glassy slides, then cleaned of paraffin and watered. To be ready for the microscopy, the slides were stained with the Haematoxylin-Eosin (H&E) method,according to Humason(1967)method.

STATISTICAL METHODS.

All data were presented as a standard mean error (M±SEM).Data were subjected to statistical analysis using Graph pad Prism 5(GraphPad Soft ware Inc.,San Diego, CA) and significant difference between groups was done by student`s t- test with a *P-value* of <0.05 was considered statistically significant.

RESULTS

No significant changes were observed in serumTP, TBil, and Alb levels between rats groups that orally exposed to AgNPs and the control group($P>0.05$)Table 1.Increased serum ALT,AST,ALP,GGTlevels,induction of ROS and increased concentration of LHP in rats groups that orally exposed to AgNPs with increasing dose concentration of AgNPs compared with control group($p<0.05$) Table1. The highest two doses(75and100) mg/kg showed increase statistically significant in levels of enzymes,LHP concentration, and induction ROS in rats groups that orally exposed to AgNPs compared to other and controls ($p<0.05$)Table1.The results from DLS showed agglomeration of AgNPs more than their base volume and the potential value of Zeta AgNPs was -35 mV. The Ag^{+} ions release were shown in Fig. 2. Histopathological examination show occurrence of central vein damage,hepato cellular vacuolation, necrosis and Pycknotic in livers of rats after exposed to AgNPs Fig.3.

DISCUSSION

Use of AgNPs is more effective compared to larger molecules.Several studies have pointed to the toxicity of nanoparticles (Munger et al.,2014), which stimulate oxidative stress in liver cells (Xu etal.,2012),DNA damage in testicular cells (Asare et al.,2012),and Programmed cell death in heLa cells(Miura and Shinohara,2009).In this study,no significant changes were observed in serumTP, TBil, and Alb levels between rats groups that orally exposed to AgNPs and the control group($P>0.05$)Table 1.

The enzymes are inside the liver cells, and an increase in the levels of these enzymes is an indication of problems or damage in the liver(Gavanji et al.,2014;Parang and Moghadamnia,2018). However,aminotransferase are the most sensitive enzymes, and to check for liver damage their activity is the first evaluated. Liver function were assessed in rats by measuring the activity of serum enzymes(ALT,AST,ALP and GGT)after administration with different doses ofAgNPs. The vital features of hepatotoxins and oxidative stress were evaluated by measuring ROS and LHP concentrations.The results of the study showed an increase in the activity of liver enzymes (ALT, AST, ALP and GGT), the induction of ROS and increased concentration of LHP with increased exposure to AgNPs in treated rats groups compared to the control group Table 1. The size and exposure period of the nanoparticles play an important role in variation their toxicity. The increased in serum enzymes activities is a strong indicator of inflammation and liver cell damage, impaired kidney function, and cholesterol behavior, etc. On the other hand, any obstruction or defect that occurs in the liver includes the biliary drainage system and this imbalance leads to a significant increase in the activity of alkaline phosphatase.These results were consistent with previous reports(Anita et al.,2015;Kim et al.,2008;Parang and Moghadamnia,2018).Akradi et al.(2012)indicated that nanoparticles create free radicals and oxidative stress, and through the oxidative stress mechanism free radicals attack living tissues, causing damage to tissues and organs.While,Ling song etal.(2012) confirmed that AgNPs cause damage to cell membranes and reduce the activity of superoxide dismutases(SOD)and glutathione peroxidase(GPX). These studies are consistent with the results of our current study which indicated an increase in the production of reactive oxygen species in the cellular environment with an increase in the concentration of nanomaterials doses given to rats (Anita et al.,2015;Gavanji et al.,2014).

Park etal.(2010)reported high levels of ALP and AST in rats after treatment with different concentrations(0.25,0.5and 1)mg/kg from AgNPs and at a volume 42nm.

Contrary to our results, Pourhamzeh et al.(2016)indicated that using different concentrations of AgNPs for a period of 28 days had no effects on liver function, i.e. it did not cause major disorders in rats liver cells($P>0.05$), but did lead to early stages of apoptosis.Also,Kulthong et al.(2012)noted that treating rats with 180 nm of AgNPs at doses of 50, 100, 250, 500 and 1000 mg/ kg/ day for two weeks did not have a significant effect of altering serum ALT and AST levels.AST, ALT,and ALP are present in hepatocytes under normal conditions, and after cell damage,they are released into the serum(Geho et al.,2006).Gavanji et al.(2014) they indicated that spherical AgNPs with a diameter of 4 nm were unable to influence and alter the activity of enzymes. We observed that 10 mg/kg of AgNPs spherical increased the activity of rats liver enzymes Table 1.

The highest two doses(75 and 100) mg/kg of AgNPs showed a statistically significant increase in enzymes levels, concentration LHP and induction of ROS in the exposed rats groups compared to the other groups andcontrols ($p<0.05$)Table1.This indicates to DNA damage, as increased ROS in the cellular environment lead to damage, and large amounts of H_2O_2 play an important role in DNA damage and oxidation of cellular proteins. These results are consistent with other studies (Anita etal.,2015;Gavanji et al.,2014).

High levels of enzymes do not necessarily cause damage to liver cells,however, large amounts of AgNPs are given to rats over 100 mg/kgBwt.AgNPs often cause hyperactivity, weight loss, altered liver enzymes, altered neurotransmitter levels, enlarged heart, reduced immunity and then death (Anita et al.,2015;Hadrup and Lam ,2014).

The levels of LHPs were measured to determine the role of oxidative stress in toxicity resulting from AgNPs in liver cells. The results showed an increase in the levels of LHPs with an increased dose of AgNPs.However,the highest doses(75and 100)mg / kg showed a statistically significant increase in the LHP concentration in groups exposed to AgNPs compared to the other groups and controls($P<0.05$)Table 1.These results are consistent with previous studies (Wu and Zhou,2013;Anreddy et al.,2013). It is important to distinguish the toxic effects of AgNPs and dissolved Ag ions. AgNPs and released ions are easily bind to proteins and DNAwithin the cell, causing their destruction.The amount of silver ions released during dispersion was quantified, and the toxicity may also arise from aqueous silver ions. Therefore, if the ionic silver (colloidal silver) emitted from the surface of the particles was responsible for the toxicity of AgNPs,then it is possible that smaller particles would be responsible compared to the larger particles due to the larger surface area per unit of weight.Several studies support this interpretation(Anita etal.,2015;Pratsinis etal.,2013) and several reports have indicated that smaller nanoparticles are more toxic than larger particles (Park etal.,2010;Gavanji et al.,2014). Silver and nanoparticles bind to living organic tissues and cause poisoning, cellular damage, cell activation and production of ROS which is a source of inflammation and programmed cell death (Xia et al.,2006).

AgNPs enter the bloodstream and collect in the liver, spleen, lung, kidney and brain etc. Fig.3. Shown histopathological characterization(H & E Staining 200 X) of liver in adult male Wistar rats exposed to AgNPs at different concentrations(10,35,75 and 100)mg/kg for one week and control group. central vein and normal hepatocytes were visible in the control group treated with deionized water only.Observed occurrence of central vein damage,hepato cellular vacuolation, necrosis and Pycknotic in livers of rats after exposed to AgNPs.These results are consistent with other studies(Parangand Moghadamnia,2018; Ajobola et al.,2019). Agglomerations of AgNPs may reduce surface area. AgNPs were given to rats caused damage in liver tissues, because the liver was center of deposition of those particles, and this led to changes in the serum enzymes activities. The effect of nanoparticles depends on their size, diameter and shape(Anita et al.,2015; Pourhamzeh etal.,2016). AgNPs which rats were exposed accumulate in liver ,and are absorbed through the mesenteric vein through the portal system and then distributed into the liver tissue. This fact is confirmed by previous studies(Anita et al.,2015;Pourhamzeh etal.,2016;Gavanji etal.,2014). Park et al.(2010) observed accumulation of AgNPs throughout the liver,lung,brain, kidney and testis after orally exposed to 1 mg/kg for 14 days at sizes less than 100 nm, whereas the larger sizes (323)nm of AgNPs were not detected in those tissues.These studies are consistent with our current results, as the levels of specific parameters increased with increasing doses of nanoparticles (Moudgi and Robert ,2006; Gavanji et al.,2014;Anita et al.,2015).

CONCLUSIONS

High and short-term doses of AgNPs cause liver toxicity , increase oxidative stress and DNA damage.AgNPs have harmful effects on a wide range of cells, human health and the environment. The high toxicity of nanomaterials and especially AgNPs does not mean that they are banned from biomedical applications. On the contrary, further studies are carried out to determine its toxicity to the living body and its environmental and occupational hazards.More detailed studies with smaller AgNPs and longer exposed periods are necessary to assess their direct effects.

ACKNOWLEDGMENTS

We thank all the staff at the animal house, Colleges of Veterinary Medicine and Agriculture / University of Baghdad, for their support and assistance.

REFERENCES

I. Ajobola,I.O.;Adenike,S. F.; Afolabi,S. A.; Toba,A. A.; Olayinka,A. J.; Adeiza, O.D.; Damilare,R.;Filibus,M.R.andStephen,A.O.(2019).Cypermethrin and chlorpyrifos raises serum urea level and causes abnormal sperm morphology in Wistar rats.J.Open Access;9(3):3969 – 3973.

II. Akradi,L.;Sohrabi-Haghdoost,I.and Djeddi, A.N.(2012).Histopathologic and apoptotic effect of nanosilver in liver of broiler chickens.African Journal of Biotechnology;11(22): 6207-6211.

III. Anita, K. p.;Diahanna, H.and Paul, B.T.(2015). Silver nanoparticle induced oxidative stressdependent toxicity in sprague-dawley rats. mol cell biochem.;399(0):257–268.

IV. Anreddy, R.N.; Yellu, N.R. and Devarakonda, K.R.(2013). Oxidative biomarkers to assess the nanoparticleinduced oxidative stress. Methods Mol Biol.;1028:205–219.

V. Asare, N.; Instanes, C.; Sandberg,W.J.;Refsnes, M.;Schwarze, P.and Kruszewski, M.(2012). Cytotoxic and genotoxic effects of silver nanoparticles in testicular cells. Toxicology.;291(1-3):65–72.

VI. Bartlomiejczyk, T.; Lankoff, A.; Kruszewski, M.and Szumiel,I.(2013). Silver nanoparticles – allies or adversaries?. Ann Agric Environ Med.;20(1):48–54.

VII. De Zwart, L.L.; Meerman, J.H.;Commandeur ,J.N.andVermeulen, N.P.(1999). Biomarkers of free radical damage applications in experimental animals and in humans. Free Radic Biol Med Jan.; 26(1–2):202–26.

VIII. Dziendzikowska, K.; Gromadzka-Ostrowska, J.; Lankoff, A.; Oczkowski,M.; Krawczynska, A.and Chwastowska, J .(2012). Time-dependent biodistribution and excretion of silver nanoparticles in male Wistar rats. J Appl Toxicol.;32(11):920–8.

IX. Eckle, V.S.; Buchmann, A.; Bursch, W.;Schulte-Hermann, R. and Schwarz,M. (2004). Immunohistochemical detection of activated caspases in apoptotic hepatocytes in rat liver.Toxicol Pathol.;32(1):9–15.

X. Gavanji, S.; Sana Sayedipour,S.; Doostmohammadi,M. and Larki,B.(2014). The Effect of different Concentrations of Silver Nanoparticles on Enzyme Activity and Liver Tissue of Adult Male Wistar Rats in-vivo Condition. International J Scientific Research in Knowledge; 2(4):182-188.

XI. Geho, D.H.; Jones, C.D.; Petricoin, E.F.and Liotta,L.A.(2006). Nanoparticles: potential biomarker harvesters.Curr Opin Chem Biol.;10(1):56–61.

XII. Giles,A.R. (1987).Guidelines for the use of animals in biomedical research.Thromb Haemost.; 58(4):1078–1084.

XIII. Goodarzi ,M.T.; Tootoonchi, A.S.; Karimi ,J.and Abbasi Oshaghi, E. (2014).Anti-diabetic effects of aqueous extracts of three Iranian medicinal plants in type 2 diabetic rats induced by high fructose diet. Avi J Med Biochem.;1:7–13.

XIV. Mohammadi ,A.;Vafaei, S.A.; Moradi, M.N.; Ahmadi, M.; Pourjafar, M.and Oshaghi, E.A.(2015). Combination of ezetimibe and garlic reduces serum lipids and intestinal niemann-pick C1-like 1 expression more effectively in hypercholesterolemic mice. Avicenna J Med Biochem.;3(1).

XV. Gutteridge, J.M.C.and Quinlan, G.J. (1983).Malondialdehyde formation from lipid peroxides in thiobarbituric acid test. The role of lipid radicals, iron salts and metal chelator. J Appl Biochem.; 5:293–299.

XVI. Halliwell,B.(1984). Oxygen radicals:A common sense look at their nature and medical importance. Med Biol.;62:71–77.

XVII. Murray, R.K.; Granner, D.K.; Mayes, P.A. and Rodwell, V.W.(1988). Harper’s Biochemistry. 21. Englewood Cliffs, NJ: Prentice Hall:138-139.

XVIII. Hadrup, N.and Lam ,H.R.(2014). Oral toxicity of silver ions, silver nanoparticles and colloidal silver-areview. Regulatory Toxicol and Pharmacol.;68:1–7.

XIX. Humason,G. L.(1967). Animal Tissue Techniques.2nd edn.,W. H. Freeman Company, San Francisco. Biochemical Assays. Munger, M.A.; Radwanski, P.; Hadlock, G.C.; Stoddard, G.; Shaaban, A.and Falconer, J. (2014). In vivo human time-exposure study of orally dosed commercial silver nanoparticles.Nanomedicine.;10(1):1–9.

XX. Hussain, S.M.and Schlager,J.J.(2009). Safety evaluation of silver nanoparticles: inhalation model for chronic exposure.Toxicol Sci.;108(2):223–4.

XXI. Moudgi ,B.M.and Robert, S.M. (2006).Designing asterategies for safety evaluation of nanomaterials.Partnano-interface in a microfluidic chip to probe livingVI.Characteri zation of nanoscale particles for cells:challenges and perspectives. Toxicological Science USA;103:6419-6424.

XXII. Kim, Y.S.; Kim JS, Cho HS, Rha DS, Kim JM, Park J.D.(2008). Twentyeight-day oral toxicity, genotoxicity, and gender-related tissue distribution of silver nanoparticles in Sprague-Dawley rats. Inhal Toxicol.;20(6):575–83.

XXIII. Kulthong, K.; Maniratanachote, R.; Kobayashi ,Y.; Fukami, T.and Yokoi ,T. (2012).Effects of silver nanoparticles on rat hepatic cytochrome P450 enzyme activity. Xenobiotica.; 42(9):854–62.

XXIV. Lawler, J.M.; Song, W.and Demaree, S.R.(2003). Hindlimb unloading increases oxidative stress and disrupt antioxidant capacity in skeletal muscle.Free Radical Biol Med.; 35:9–16.

XXV. Ling song, X.; Li, B.and Xu, K. (2012). Citotoxicity of watersoluble Mpeg-SH-coated silver nanoparticles in HL-7702 cells.Cell boiltoxico;l(28):225-237.

XXVI. Miura, N.and Shinohara, Y.(2009).Cytotoxic effect and apoptosis inductionby silver nanoparticles in HeLa cells.Biochem Biophys Res Commun.;390(3):733–7.

XXVII. Parang, Z.and Moghadamnia,D.(2018). Effects of silver nanoparticles on the functional tests of liver and its histological changes in adult male rats.J.Nanomed Res.;3(3):146-153.

XXVIII. Park, E.J.; Bae, E.; Yi, J.; Kim,Y.; Choi, K.; Lee, S.H.; Yoon, J.; Lee, B.C.and Park , K.(2010). Repeated-dose toxicity and inflammatory responses in mice by oral administration of silver nanoparticles.Environ Toxicol Pharmacol.; 30(2):162–168.

XXIX. Pourhamzeh,M.; Gholami,Z. M.; Saidijam,M.; Javad,M. A. and Alizadeh,Z.(2016). he 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 J Med Biochem.;4(2):35557.

XXX. Pratsinis,A.;Hervells,P.;Leroux,J.C.;Pratsinis,S.E. and Sotiriou,G.A.(2013). Toxicity of silver nanoparticles in macrophages. Small.;9(15):2576–2584.

XXXI. Tampa,M.; Mitran,M.I.;Mitran,C.I.;Sarbu ,M.I.; Matei,C.; Nicolae,I.; Caruntu,A.; Tocut,S.M.; Popa,M.I.;Caruntu ,C.and Georgescu,S.R.(2018). Mediators of Inflammation- A Potential Source of Biomarkers in Oral Squamous Cell Carcinoma, J. Immunology Research, Article ID 1061780, 12 pages.

XXXII. Wu, Y.and Zhou,Q.(2013). Silver nanoparticles cause oxidative damage and histological changes in medaka (Oryzias latipes) after 14 days of exposure.Environ Toxicol Chem.;32(1):165–173.

XXXIII. Xia,T.;Kovochich, M.; Brant, J.; Hotze, M.; Sempf, J.; Oberley, T.;Sioutas, C.;Yeh, J.I.;Wiesner,M.R. and Nei, A.E.(2006). Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm.Nano Lett.; 6:1794–1807.

XXXIV. Xu, L.; Li, X.;Takemura, T.;Hanagata, N.;Wu, G.and Chou, L.L. (2012). Genotoxicity and molecular response of silver nanoparticle (NP)-based hydrogel. J Nanobiotechnology;10:16.

Table 1. Effect different concentrations from AgNPs for one week on the levels of biochemical parameters, lipid hydroperoxides and reactive oxygen species in liver of adult male Wistar rats and control.

Parameters	Control(n=12) Mean± SD	Ag-NPs mg/Kg Bwt.(n=48) Mean ± SD			
	0 mg/Kg	**10 mg/Kg**	**35 mg/Kg**	**75 mg/Kg**	**100 mg/Kg**
T Bil mg/dL	0.42 ± 0.07	0.40 ± 0.08	0.32 ± 0.06	0.42 ± 0.07	0.42 ± 0.06
Alb mg/dL	4.14 ± 0.03	4.55 ± 0.03	4.01 ± 0.18	4.00 ± 0.18	4.08 ± 0.25
TP mg/dL	4.32 ± 0.40	7.22 ± 0.23	5.35 ± 0.46	5.35 ± 0.88	4.88 ± 0.47
ALT IU/I	0.187 ± 0.04	0.488 ± 0.06	0.522 ± 0.05	0.675 ± 0.07*	0.787 ± 0.08*
AST IU/I	0.343± 0.023	0.422 ± 0.03	0.664 ± 0.064	0.755 ± 0.027*	0.886 ± 0.045*
ALP IU/I	0.135 ± 0.056	0.258 ± 0.065	0.344 ± 0.047	0.426 ± 0.042*	0.542 ± 0.058*
GGT IU/I	1.55±0.22	1.75±0.24	1.34±0.11	1.34±0.12*	1.62±0.31*
LHP Mm	7.89 ± 3.06	24.8 ± 2.10	25.8 ± 3.84	35.78 ± 3.89*	60.89 ± 4.76*
ROS	10.75± 3.03	21.43 ± 3.74	23.76 ± 4.46	35.65 ± 5.42*	38.77 ±9.78*

Bwt,body weight.Data represents (mean ± SD). Statistical significance (p<0.05) is depicted as (*).

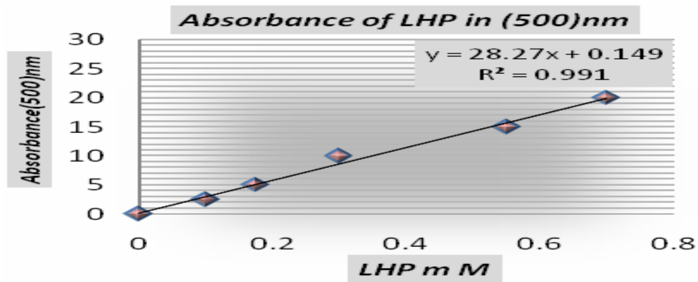

Fig.1. Standard curve forStandard curve for Lipid hydro peroxides (LPO) at 500 nm .

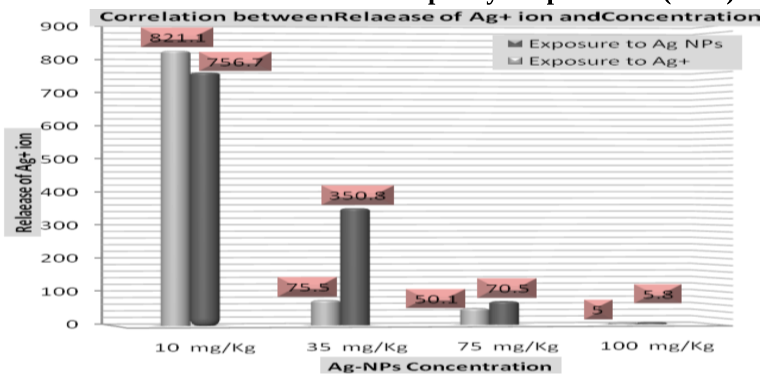

Fig.2. Average release of (Ag^+) ions mg/Kg) to DI water for 1 week.

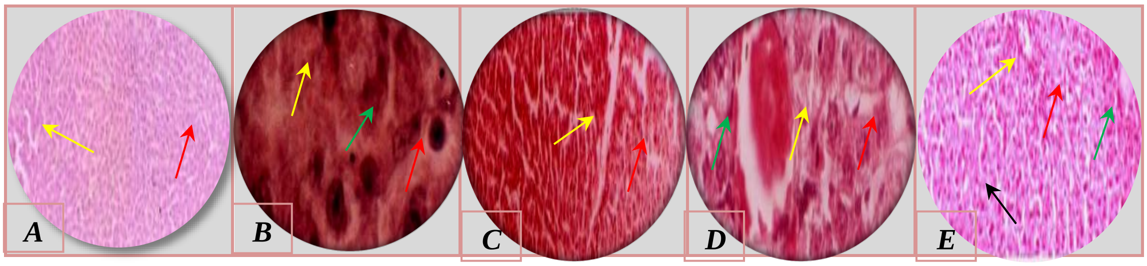

Fig.3. Histopathological Characterization(H & E Staining 200 X) of liver in adult male Wistar rats exposed to AgNPs; A:Negative Control Deionized water(red arrow:Central vein,yellow arrow: Hepatocytes). B:10 mg/kg exposed liver(red arrow:Central vein damage, yellow arrow:Hepato cellular vacuolation, green arrow:Necrosis). C: 35 mg/kg (red arrow:Central vein damage and yellow arrow: Necrosis).D:75mg/kg (red arrow:Central vein damage, yellow arrow:Hepato cellular vacuolation and green arrow:Pycknotic). E:100 mg/kg (red arrow:Central veindamage,yellow arrow:Hepato cellular vacuolation, green arrow :Necrosis and black arrow:Pycknotic).