Role of Chitosan Nanoparticles in Improving Hepatic and Renal Toxicity Induced by Silver Nanoparticles Coated by Fe₃O₄ in Rats

Hadeer ElBadry [a*], **Afaf El-Atrash** [a], **Somaya Abdelhalim** [a]
and Ehab Tousson [a]

[a] *Zoology Department, Faculty of Science, Tanta University, Tanta, Egypt.*

Authors' contributions

. This work was carried out in collaboration among all authors. Authors AEA, SA and ET designed the study, performed the statistical analysis, and wrote the protocol. Author HE wrote the first draft of the manuscript, managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: Silver nanoparticles (Ag NPs) are an important class of nanomaterials used as antimicrobial agents for a wide range of medical and industrial applications.Current study was performed to study the therapeutic effects of chitosan nanoparticles extract towards the treatments with Ag NPs in rat induced kidney and liver damage.
Study Design: A total of 60 male adult albino rats were equally divided into six groups (G1, Control group; G2, chitosan group; G3, Ag NPs group as acute toxicity; G4, acute Ag NPs+Chitosan group; G5, Ag NPs group as chronic toxicity; G6, chronic Ag NPs+Chitosan).
Results: Current results revealed that; a significant increase in the levels of serum ALT, AST, ALP, urea, creatinine, sodium, potassium, chloride ions and MDA in liver and kidney tissues after treatments with Ag NPs (in case of acute and chronic toxicity) as compared to control group. In contrast; a significant decrease in serum albumin, total proteins, calcium ions, SOD, catalase and GSH in liver and kidney tissues after treatments with Ag NPs as compared to control groups. Treatment of Ag NPs with Chitosan nanoparticles (Ch NPs) improved this change in liver and kidney functions as compared to Ag NPs.

Corresponding author: E-mail: helbadry13@gmail.com;

Conclusion: These findings suggested that the misuse of silver nanoparticles may contribute to continuous hepatic and renal damage. This shows that the desired dose of Ag NPs can safely be used with Chitosan in improving hepatic and renal damage in toxic group in young rats.

1. INTRODUCTION

Nanotechnology refers to the branch of science and engineering dedicated to materials, having dimensions in the order of 100th of nm or less [1]. Metal NPs have gained more attention and play a major role in day by day due to its vast of area of application like development of biosensors etc [2-7].

Ag NPs are an important class of nanomaterials used as antimicrobial agents for a wide range of medical and industrial applications [4,8]. Currently, there is also an effort to incorporate silver nanoparticles into a wide range of medical devices, including bone cement, surgical instruments, surgical masks, etc. Moreover, it has also been shown that ionic silver, in the right quantities, is suitable in treating wounds [9]. Studies have found that the biological effects of AgNPs depend on the different surface charges of their coatings, which can affect the interaction of AgNPs with living systems [10].

Chitosan is a cationic natural polymer and it is a natural biodegradable polymer which is obtained by partial N-deacetylation of chitin. Chitin is widely found in the shells of insects, shrimp, crab, crawfish, lobster, mollusks, insect exoskeleton and other crustacean shells [11-14]. Chitosan nanoparticles have reduced the pharmacological toxicity and it demonstrated higher antimicrobial, antioxidant, and anticancer capacities [15].

No sufficient information present about the toxic effect of acute and chronic Ag NPs on kidney and liver. Accordingly, current study were performed to study the therapeutic effects of chitosan nanoparticles extract towards the treatments with Ag NPs in rat induced kidney and liver damage.

2. MATERIALS AND METHODS

Silver nano particles (Ag NPs) with a particle size less than 100 nm and a 99.9% trace metal basis was purchased from Sigma-Aldrich Chemicals, Cairo, Egypt.

Chitosan nanoparticles (Ch NPs) and the dose of chitosan nanoparticles was 140 mg/kg BW (dissolved in distilled water) <30 nm particle size were bought from nano-tech Company (Nanotech Egypt).

2.1 Experimental Design

The experiments were performed on 60 male albino rats weighing 150 ±10g and of 9-10 week's age.The rats were divided into six groups (10 animals each).

1st group is control rat group, while 2nd group is chitosan (140 mg/Kg body weight/ day) rat group. On the other hand 3rd group is AgNPs coated by Fe_3O_4 (100 mg/Kg body weight/ day) rat group for 1 weeks as acute treatments, while 4th group is treated AgNPs coated by Fe3O4 for one week and then treated with chitosan for two weeks. 5th group is treated rats with AgNPs coated by Fe_3O_4 (100 mg/Kg body weight/ day) rat group for 8 weeksas chronic treatments, while 6th group is treated AgNPs coated by Fe_3O_4 for 8 weeks and then treated with chitosan for two weeks.

At the end of the experimental period, animals fasted overnight and blood samples were individually collected from the eyes by retroorbital puncture using blood capillary tubes without heparin as per requirement under mild ether anaesthesia for clinical chemistry examinations. Blood samples were incubated at room temperature for 10 minutes and left to clot then centrifuged at 3000 r.p.m for 10 min and the serum was collected, serum was separated and kept in clean stopper plastic vial at –80ºC until the analysis of serum parameters [16].

2.2 Serum Liver and Kidney Functions Biomarkers

Serum albumin, total proteins, aspartate transaminase (AST), alanine transaminase (ALT) and serum alkaline phosphatase (ALP) activities were assessed in the sera as per Tousson et al. [17], while Serum urea and creatinine were determined in the mouse sera according to [18]. The approach proposed by AbdEldaim et al. [19] was followed to measure the levels of serum

electrolytes (Potassium, sodium, calcium and chloride ions) using commercial kits (Sensa core electrolyte, India).

2.3 Determination of Antioxidant Parameters

Malondialdehyde (MDA) were measured in kidney and liver homogenate using the method of Saggu et al. [20]. Reduced glutathione (GSH) content was measured after reaction with 5, 5′-dithiobis-(2-nitrobenzoic acid) using the method of Ellman [21]. Superoxide dismutase activity (SOD; EC 1.15.1.1) was determined according to Misra and Fridovich [22]. The assay procedure involves the inhibition of epinephrine auto-oxidation in an alkaline medium to adrenochrome, which is markedly inhibited by the presence of SOD. The enzyme catalase (CAT; EC 1.11.1.6) converts H_2O_2 into water. The CAT activity was measured spectrophotometrically at 240 nm by calculating the rate of degradation of H_2O_2, the substrate of the enzyme [23].

2.4 Statistical Analysis

Data were expressed as mean values ± SE and statistical analysis was performed using the unpaired t-test to assess significant differences among treatment groups. The criterion for statistical significance was set at $p<0.05$ for the biochemical data. All statistical analyses were performed using SPSS statistical version 21 software package (SPSS® Inc., USA).

3. RESULTS

3.1 Liver Function

Table (1) revealed that; a significant increase in the level of ALT, AST and ALP in the treated rats with Ag NPs (G3 & G5) as compared control group (G1) and Ch-NPs (G2). In contrast; a significant decrease in the level of albumin and total proteins in G3 and G5 as compared G1 and G2. On the other hand; treatment of acute and chronic Ag NPs with chitosan(G4 & G6) revealed a significant decrease in the level of ALT, AST and ALP and significant increase in the level of albumin and total proteins as compared to treated rats with Ag NPs (G3&G5).

3.2 Kidney Function

Table (2) revealed that; a significant increase in the level of urea, creatinine, sodium ions, potassium ions and chloride ions in the treated rats with Ag NPs (G3 & G5) as compared with control (G1) and Ch-NPs (G2) groups. In contrast; a significant decrease in the level of calcium ions in G3 and G5 as compared G1 and G2. On the other hand; treatment of acute and chronic Ag NPs with chitosan(G4 & G6) revealed a significant decrease in the level of urea, creatinine, sodium ions, potassium ions and chloride ions while it revealed a significant increase in the level of calcium ions as compared to treated rats with Ag NPs (G3&G5).

Table 1. Changes in liver functions in different groups

	G1	G2	G3	G4	G5	G6
ALT(U/L)	24.8#±1.11	31.0#±1.82	72.4*±4.27	40.2#*±2.08	89.2*±4.53	28.4#±4.19
AST (U/L)	136.4#±3.81	138.8#±1.16	200.0*±4.11	159.2#*±6.18	194.6*±3.47	139.8#±7.37
ALP (U/L)	83.6#±1.21	81.4#±2.84	106.6*±2.46	92.6#±1.03	113.2*±4.07	76.0#±3.70
T. protein (g/dl)	5.89#±0.17	6.11#±0.06	4.17*±0.04	5.98#±0.07	3.73*±0.19	5.94#±0.10
Alb (g/dl)	4.74#±0.08	4.94#±0.06	3.20*±0.21	4.82*±0.14	3.58*±0.17	4.8#±0.115

Values are expressed as means±SE; n=10 for each treatment group; (*) & (#) significant 0.05 compared to control (G1) and to AgNPs groups (G3&G5) respectively

Table 2. Changes in kidney functions in different groups

	G1	G2	G3	G4	G5	G6
Urea(mg/dl)	31.6#±1.69	32.2#±2.06	42.0*±2.53	37.0#*±1.58	45.8*±1.59	33.6#±1.5
Creat(mg/dl)	0.47#±0.42	0.526#±0.018	0.77#±0.03	0.6#±0.01	4.82*±1.05	1.35#*±0.41
Uric acid (mg/dl)	3.62#±0.07	3.93#±0.12	3.32*±0.07	3.5#±0.14	3.33*±0.08	3.61#±0.14
K+ (mEq/L)	3.99#±0.14	3.44#±0.08	5.95*±0.13	4.37#±0.19	6.05*±0.09	4.97*±0.98
Na+ (mEq/L)	134.2#±1.33	135.7#±0.61	148.5*±2.65	133.7#±0.58	150.1*±1.59	142.5#*±1.76
ca++ (mEq/L)	1.25#±0.02	1.23#±0.01	1.09*±0.03	1.182#*±0.02	1.12*±0.11	1.17#*±0.02
cl- (mEq/L))	101.6#±0.58	100.6#±0.7	113.5*±1.05	106.3#*±0.77	118*±1.93	110.9#*±1.1

Values are expressed as means±SE; n=10 for each treatment group; (*) & (#) significant 0.05 compared to control (G1) and to AgNPs groups (G3&G5) respectively

3.3 Oxidative Stress and Antioxidant Parameters

In liver: Table (3) revealed that; a significant increase in the level of lipid peroxidation (MDA) in the treated rats with Ag NPs (G3 & G5) as compared with control (G1) and Ch-NPs (G2) groups. In contrast; a significant decrease in the level of calcium ions in G3 and G5 as compared G1 and G2. On the other hand; treatment of acute and chronic Ag NPs with chitosan(G4 & G6) revealed a significant decrease in the level of urea, creatinine, sodium ions, potassium ions and chloride ions while it revealed a significant increase in the level of calcium ions as compared to treated rats with Ag NPs (G3&G5).

Table (3) revealed that; a significant rise in the level of in the treated rats with Ag NPs (G3) as acute toxicity and (G5) as chronic toxicity compared with control (G1) and Ch-NPs (G2) groups. In contrast; a significant decline in the level of superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) in liver tissues in treated rats with Ag NPs (G3) and (G5) as compared control (G1) and Ch-NPs (G2) groups. Likewise; treatment of Ag NPs with Ch-NPs (G4) and (G6) revealed a significant reduction in the level lipid peroxidation (MDA) in liver tissues as compared to treated rats with Ag NP (G3 and G5). Also; treatment of Ag NPs with Ch-NPs (G4 and G6) revealed significant increase in the level of superoxidedismutase (SOD), catalase (CAT) and glutathione (GSH) as compared with treated rats with Ag NPs (G3&G5).

In kidney: Table (4) reveled that; a significant increase in the level of lipid peroxidation (MDA) in kidney tissues in the treated rats with Ag NPs (G3) as acute toxicity and (G5) as chronic toxicity compared with control (G1) and Ch-NPs (G2) groups. In contrast; a significant decline in the level of superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) in kidney tissues in treated rats with Ag NPs (G3 and G5) as compared control (G1) and Ch-NPs (G2) groups.Likewise; treatment of Ag NPs with Ch-NPs (G4) and (G6) revealed a significant reduction in the level lipid peroxidation (MDA) as compared to treated rats with Ag NP (G3) and (G5). Also; treatment of Ag NPs with Ch-NPs (G4) and (G6) revealed significant increase in the level of superoxidedismutase (SOD), catalase (CAT) and glutathione (GSH) as compared with treated rats with Ag NPs (G3&G5).

4. DISCUSSION

Ag NPs are widely used in medicine and drug delivery device due to antibacterial properties [24], very important to understand the behavior of Ag NPs in vivo. The objective of this study is to evaluate the role of Ch NPs in treatments of Ag NPs induced liver and kidney toxicity. Pervious study revealed that the accumulation of NPs in livers caused remarkable hepatic toxicity [25]. Many studies have demonstrated that exposure of silver nanoparticles may lead to clear accumulation in various organs including liver, as well as the kidneys, testes, lungs, and brain [26]. The most important way to contact it, especially in the gastrointestinal tract, is in colloidal form [27]. Cytotoxicity is a direct outcome due to oxidation stress caused by Ag NPs and release of Ag ions. As liver organ is able to actively remove compounds from the blood and transform those to chemical forms that can easily be excreted, so silver nanoparticles might have impacted on the liver, as a major organ of detoxification.

Current results revealed that; Ag NPs induced elevations in AST, ALT, and ALP, and exhaustions in total protein and albumin suggesting liver dysfunction. The increase in liver enzymes may be due to the free radicals released from the nanosilver particles when attacking hepatocytes and releasing ALT stored in them and entering into the blood serum.

The hepatocytic inflammation in liver tissue of the current study is consistent with Lee et al., [26] study on rat liver following Nano silver administration,according to our studyHepatic function is evaluated by measuring AST and ALT. The results of our investigation are consistent with other studies e.gCheraghi et al., [28] with Nano silver on these enzymes showing elevation of hepatic enzymes so that AST level in serum was elevated in male and female mice as compared to the control. The levels of liver function enzymes (including ALT, AST, and ALP) were elevated when 40 mg/kg of Ag NPs were injected [29].

Significant elevations of creatinine, and urea levels were seen, indicating disruptive changes in liver and kidney function. Previous studies have shown that metal nanoparticles alter the levels of various biochemical markers indicating changes in composition of serum enzyme levels [30], suggesting hepatocellular injury, hepatic inflammation, and impairment of kidney function

Table 3. Changes antioxidant parameters (MDA, Catalase, GSH and SOD) levels in different experimental groups in liver

	G1	G2	G3	G4	G5	G6
SOD (U/mg tissue)	$13.07^{\#}\pm0.21$	$12.23^{\#}\pm0.24$	$4.18^{*}\pm0.1$	$6.85^{*}\pm0.31$	$5.16^{*}\pm0.11$	$8.44^{\#*} \pm 0.16$
CAT (mmol/min/gm/tissue)	$88.79^{\#}\pm0.48$	$96.36^{\#}\pm1.37$	$42.57^{*}\pm0.64$	$73.13^{\#*}\pm0.99$	$52.28^{*}\pm1.21$	$68.47^{*} \pm 0.63$
MDA (nmol/gm tissue)	$149.9^{\#}\pm1.31$	$157.6^{\#}\pm1.13$	$287.8^{*}\pm2.46$	$249.3^{*}\pm2.5$	$257.2^{*}\pm2.9$	$215.2^{\#*}\pm 2.08$
GSH (mmol/gm tissue)	$13.2^{\#}\pm0.15$	$11.5^{\#}1\pm0.36$	$5.809^{*}\pm0.14$	$8.107^{*}\pm0.1$	$6.64^{*}\pm0.1$	$9.03^{\#*} \pm 0.1$

Values are expressed as means±SE; n=10 for each treatment group; (*) & (#) significant 0.05 compared to control (G1) and to AgNPs groups (G3&G5) respectively

Table 4. Changes antioxidant parameters (MDA, Catalase, GSH and SOD) levels in Kidney tissues in different experimental groups

Kidney	G1	G2	G3	G4	G5	G6
SOD (U/mg tissue)	$19.65^{\#}\pm0.29$	$22.46^{\#}\pm0.27$	$7.2^{*}\pm0.27$	$12.99^{\#*}\pm0.19$	$9.76^{*}\pm0.25$	$14.69^{\#}\pm0.48$
CAT (mmol/min/gm/tissue)	$88.1^{\#}\pm0.89$	$101.7^{\#}\pm1.34$	$59.53^{*}\pm0.85$	$71.37^{*}\pm1.21$	$62.07^{*}\pm1.33$	$76.96^{\#*}\pm0.84$
MDA (nmol/gm tissue)	$73.03^{\#}\pm0.82$	$63.24^{\#}\pm1.65$	$147.1^{*}\pm2.92$	$115.8^{\#*}\pm1.47$	$123^{*}\pm1.75$	$87.66^{\#}\pm3.3$
GSH(mmol/gm tissue)	$9.0^{\#} \pm 0.2$	$8.8^{\#}\pm0.16$	$5.14^{*}\pm0.06$	$6.41^{*}\pm0.15$	$5.728^{*}\pm0.24$	$7.307^{\#*}\pm0.17$

Values are expressed as means±SE; n=10 for each treatment group; (*) & (#) significant 0.05 compared to control (G1) and to AgNPs groups (G3&G5) respectively

[30,31].Metal NPs like Ag NPs, prefer to release ions that could cause toxic effects, such as disruption of cytomembrane integrity, improper protein function, DNA damage and cell apoptosis [32]. We observed a significant decrease in GSH level activity as common antioxidants in hepatocytes and a significant increase in MDA level (lipid peroxidation marker) in the liver of the treated group offspring. Our results suggest that the ability of antioxidant defense was probably depressed in the liver tissues which in turn caused lipid peroxidation [33]. Some in-vitro studies have reported ROS generation, decrease in GSH, and lipid peroxidation in many cell lines [34].

Ag NPs causes alterations in the biochemical parameters electrolytes (Na+), (Cl+) and (K+) ions in the rats according with Stensberg et al. [35] who reported that Ag NPs has led to kidney injury and significant changes in the renal function in rats through an increase in the levels of potassium and chloride ions and decreased sodium and calcium ions**.** According to our results the presence of chitosan in treated group enhance liver and kidney functions and ameliorative the levels of calcium ions, chloride ions, potassium ions, and sodium ions in the rats. The protective effect of Chitosan has been documented in several reports which suggested that chitosan counteract free radicals through its antioxidant properties and/or to the ability to curb lipid accumulation through its antilipidemic property [36].

5. CONCLUSION

These findings suggested that the misuse of silver nanoparticles may contribute to continuous hepatic and renal damage. This shows that the desired dose of Ag NPs can safely be used with chitosan nanoparticles in improving hepatic and renal damage in toxic group in young rats.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Knoll W, Fritzsche W. Editorial: Nanoparticles for biotechnology applications. IEE Proceedings - Nanobiotechnology, 2005;152(1):1.
2. Elango G, Roopan SM. Green synthesis, spectroscopic investigation and photocatalytic activity of lead nanoparticles. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2015;139:367-73.
3. Altwaijry N, El-Masry TA, Alotaibi B, Tousson E, Saleh A. Therapeutic effects of rocket seeds (*Eruca sativa L.*) against testicular toxicity and oxidative stress caused by silver nanoparticles injection in rats. Environmental toxicology. 2020;35 (9):952-60.
4. Altwaijry N, El-Masry TA, Alotaibi BS, Tousson E, Alodhayani AA, El-Morshedy K, Elmaghed NA, Sayed AE, Saleh A. Potential therapeutic effects of avenanthramide-C against lung toxicity caused by silver nanoparticles injection in rats. Pak. J. Pharm. Sci. 2021;34(1):337-43.
5. Alotaibi B, Tousson E, El-Masry TA, Altwaijry N, Saleh A. Ehrlich ascites carcinoma as model for studying the cardiac protective effects of curcumin nanoparticles against cardiac damage in female mice. Environmental Toxicology. 2021;36(1):105-13.
6. Alotaibi B, El-Masry TA, Tousson E, Alarfaj SJ, Saleh A. Therapeutic effect of rocket seeds (*Eruca sativa* L.) against hydroxyapatite nanoparticles injection induced cardiac toxicity in rats. Pak. J. Pharm. Sci. 2020;334(Suppl):1839-1845.
7. El-Masry TA, Altwaijry N, Alotaibi B, Tousson E, Alboghdadly A, Saleh A. Chicory (*Cichoriumintybus L.)* extract ameliorates hydroxyapatite nanoparticles induced kidney damage in rats. Pak. J. Pharm. Sci. 2020;33(3):1251-60.
8. Recordati C, De Maglie M, Bianchessi S, Argentiere S, Cella C, Mattiello S, Cubadda F, Aureli F, D'Amato M, Raggi A, Lenardi C. Tissue distribution and acute toxicity of silver after single intravenous administration in mice: nano-specific and

size-dependent effects. Particle and fibre toxicology. 2015;13(1):1-7.

9. Woodmansey EJ, Roberts CD. Appropriate use of dressings containing nanocrystalline silver to support antimicrobial stewardship in wounds. International Wound Journal. 2018;15 (6):1025-32.

10. Alashmouni S, El-Atrash A, Kandeel M, Tousson E. Role of Oats in Ameliorating Hepatic and Renal Toxicity Induced by Acute Lead Nanoparticles in Male Rats. Asian Journal of Research in Biochemistry. 2020;7(2):38-45.

11. Zvezdova D. Synthesis and characterization of chitosan from marine sources in Black Sea. НаучниТрудовеНаРусенскияУниверсите т. 2010;49(9):65-9.

12. Xiong Y, Luo B, Chen G, Cai J, Jiang Q, Gu B, Wang X. CuS@ Corn Stalk/Chitin Composite Hydrogel for Photodegradation and Antibacterial. Polymers. 2019; 11(9):1393.

13. Nottagh S, Hesari J, Peighambardoust SH, Rezaei-Mokarram R, Jafarizadeh-Malmiri H. Effectiveness of edible coating based on chitosan and Natamycin on biological, physico-chemical and organoleptic attributes of Iranian ultra-filtrated cheese. Biologia. 2020;75(4):605-11.

14. Sivaramakrishna D, Bhuvanachandra B, Mallakuntla MK, Das SN, Ramakrishna B, Podile AR. Pretreatment with KOH and KOH-urea enhanced hydrolysis of α-chitin by an endo-chitinase from Enterobacter cloacae subsp. cloacae. Carbohydrate polymers. 2020;235:115952.

15. Kim S. Competitive biological activities of chitosan and its derivatives: antimicrobial, antioxidant, anticancer, and anti-inflammatory activities. International journal of polymer science; 2018.

16. Oyouni AA, Saggu S, Tousson E, Rehman H. Immunosuppressant drug tacrolimus induced mitochondrial nephrotoxicity, modified PCNA and Bcl-2 expression attenuated by Ocimumbasilicum L. in CD1 mice. Toxicology reports. 2018;5: 687-94.

17. Tousson E, El-Moghazy M, Massoud A, El-Atrash A, Sweef O, Akel A. Physiological and biochemical changes after boldenone injection in adult rabbits. Toxicology and industrial health. 2016;32 (1):177-82.

18. Salama AF, Tousson E, Ibrahim W, Hussein WM. Biochemical and histopathological studies of the PTU-induced hypothyroid rat kidney with reference to the ameliorating role of folic acid. Toxicology and industrial health. 2013;29(7):600-8.

19. AbdEldaim MA, Tousson E, El Sayed IE, Awd WM. Ameliorative effects of Saussurealappa root aqueous extract against Ethephon-induced reproductive toxicity in male rats. Environmental toxicology. 2019;34(2):150-9.

20. Saggu S, Sakeran MI, Zidan N, Tousson E, Mohan A, Rehman H. Ameliorating effect of chicory (*Chichoriumintybus L.)* fruit extract against 4-tert-octylphenol induced liver injury and oxidative stress in male rats. Food and chemical toxicology. 2014;72:138-46.

21. Ellman GL. Tissue sulfhydryl groups. Archives of biochemistry and biophysics. 1959;82(1):70-7.

22. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological chemistry. 1972;247(10):3170-5.

23. Aebi H. Catalase in vitro. Methods in Enzymology. 3rd Ed, Philadelphia: Lippincott-Raven Publishers. 1984;105: 121–126.

24. Shakir AA. silver nanoparticles as antimicrobial against salmonella typhi, proteus mirabilis and bacillus cereus as a model of gram–negative and gram–positive bacteria. world journal of pharmaceutical research. 2016;5(10):49-55.

25. Heydrnejad MS, Samani RJ, Aghaeivanda S. Toxic effects of silver nanoparticles on liver and some hematological parameters in male and female mice (Mus musculus). Biological trace element research. 2015; 165(2):153-8.

26. Lee TY, Liu MS, Huang LJ, Lue SI, Lin LC, Kwan AL, Yang RC. Bioenergetic failure correlates with autophagy and apoptosis in rat liver following silver nanoparticle intraperitoneal administration. Particle and fibre toxicology. 2013;10(1): 1-3.

27. Chang AL, Khosravi V, Egbert B. A case of argyria after colloidal silver ingestion. Journal of cutaneous pathology. 2006;33 (12):809-11.

28. Cheraghi J, Hosseini E, Hoshmandfar R, Sahraei R, Farmany A. In vivo effect of Silver Nanoparticles on serum ALT, AST and ALP activity in male and female mice. Advances in Environmental Biology. 2013; 116-23.

29. Tiwari DK, Jin T, Behari J. Dose-dependent in-vivo toxicity assessment of silver nanoparticle in Wistar rats. Toxicology mechanisms and methods. 2011;21(1):13-24.

30. Kim S, Choi JE, Choi J, Chung KH, Park K, Yi J, Ryu DY. Oxidative stress-dependent toxicity of silver nanoparticles in human hepatoma cells. Toxicology in vitro. 2009;23(6):1076-84.

31. Loeb WF, Quimby FW, editors. The Clinical Chemistry of Laboratory Animals. 2nd ed. 1999 Philadelphia, PA, USA: Taylor & Francis; 1999.

32. De Matteis V, Malvindi MA, Galeone A, Brunetti V, De Luca E, Kote S, Kshirsagar P, Sabella S, Bardi G, Pompa PP. Negligible particle-specific toxicity mechanism of silver nanoparticles: the role of Ag+ ion release in the cytosol. Nanomedicine: Nanotechnology, Biology and Medicine. 2015;11(3):731-9.

33. Fatemi M, Moshtaghian J, Ghaedi K. Effects of silver nanoparticle on the developing liver of rat pups after maternal exposure. Iranian journal of pharmaceutical research: IJPR. 2017; 16(2):685.

34. Hussain SM, Hess KL, Gearhart JM, Geiss KT, Schlager JJ. In vitro toxicity of nanoparticles in BRL 3A rat liver cells. Toxicology in vitro. 2005;19(7):975-83.

35. Stensberg MC, Madangopal R, Yale G, Wei Q, Ochoa-Acuña H, Wei A, Mclamore ES, Rickus J, Porterfield DM, Sepúlveda MS. Silver nanoparticle-specific mitotoxicity in Daphnia magna. Nanotoxicology. 2014;8(8):833-42.

36. Sivakumar R, Rajesh R, Buddhan S, Jeyakumar R, Rajaprabhu D, Ganesan B, An R. Antilipidemic effect of chitosan against experimentally induced myocardial infarction in rats. Journal of Cell and Animal Biology. 2007;1(4):071-7.