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Biological synthesis of silver nanoparticles using *Citrus sinesis* seeds: Effects on hepatic and renal functional integrities and antioxidant activities.

***Fatoki J. O.[1], Badmus J. A.[2], Ajilore B. S.[1], Faokunla O.[3], Alabi S. O.[4]**

Abstract

Objective: This work reports the possible toxicological effects of AgNPs on the liver and kidney. Additionally, it also beamed its searchlight on its effects on the antioxidant defense mechanism in male Wistar rats.

Method: Male Wistar rats (n=28) were used for the study. Control animals (n=7) were exposed to only drinking ware *ad-libitum* for 12 weeks. The remaining 21 rats were randomized into 3 groups of 7 animals each and were exposed to 50, 150 and 250 kg/mg body weight AgNPs biosynthesized from *Citrus sinensisi* for the same period after which blood and liver were removed from the rats and analyzed spectrophotometrically.

Results: A non-significant reduction of plasma ALT, AST, ?GT and ALP characterized the effects of AgNPs in the animal tested. Similarly, AgNPs significantly depleted the plasma creatinine and urea level. The exposure also up-regulated the activities/concentration of antioxidant markers. Malondialdehyde concentration was also significantly depleted by AgNPs.

Conclusion: The findings from this study revealed that biologically synthesized AgNPs induced no toxicological potential on hepatic and renal structural and functional integrity. Meanwhile, it enhanced the activities and concentration of antioxidant markers

Keywords: Nanoparticles; Silver Nanoparticles; hepatic and renal functions; antioxidants.

***Corresponding author**
Fatoki J. O.
ORCID-NO: http://orcid.org/0000-0003-3246-9172
Email: john.fatoki@uniosun.edu.ng

[1]Department of Medical Biochemistry, College of Health Sciences, Osun State University, Osogbo, Nigeria.
[2]Department of Biochemistry, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.
[3]Department of Biochemistry, Federal University of Lokoja, Lokoja, Nigeria.
[4]Department of Science Laboratory Technology, Federal Polytechnic, Offa, Nigeria

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Synthèse biologique de nanoparticules d'argent à l'aide de graines de *citrus sinesis*: effets sur l'intégrité fonctionnelle hepatique et renale et sur les activités antioxydantes

***Fatoki J. O.[1], Badmus J.A.[2], Ajilore B.S.[1], Faokunla O.[3], Alabi S.O.[4]**

Résumé

Objectif de l'étude: Ce travail rapporte les effets toxicologiques possibles des AgNPs sur le foie et le rein. De plus, il a également braqué son projecteur sur ses effets sur le mécanisme de défense antioxydant chez les rats Wistar mâles.

Méthode de l'étude : Des rats mâles Wistar (n = 28) ont été utilisés pour l'étude. Les animaux témoins (n = 7) ont été exposés uniquement à de la vaisselle à boire *ad libitum* pendant 12 semaines. Les 21 rats restants ont été randomisés en 3 groupes de 7 animaux chacun et ont été exposés à 50, 150 et 250 kg/mg de poids corporel AgNPs biosynthétisés à partir de *Citrus sinensisi* pendant la même période, après quoi le sang et le foie ont été prélevés sur les rats et analysés par spectrophotométrie.

Résultats de l'étude: Une réduction non significative des plasma ALT, AST, ?GT et ALP a caractérisé les effets des AgNPs chez l'animal testé. De même, les AgNPs ont considérablement appauvri le taux plasmatique de créatinine et d'urée. L'exposition a également régulé à la hausse les activités/concentration des marqueurs antioxydants. La concentration de malondialdéhyde a également été significativement appauvrie par les AgNPs.

Conclusion : Les résultats de cette étude ont révélé que les AgNPs synthétisés biologiquement n'induisaient aucun potentiel toxicologique sur l'intégrité structurale et fonctionnelle hépatique et rénale. Pendant ce temps, il a amélioré les activités et la concentration des marqueurs antioxydants

Mots-clés : Nanoparticules, nanoparticules d'argent, fonctions hépatique et rénale, antioxydants

***Corresponding author**
Fatoki J. O.
ORCID-NO: http://orcid.org/0000-0003-3246-9172
Email: john.fatoki@uniosun.edu.ng

[1]Department of Medical Biochemistry, College of Health Sciences, Osun State University, Osogbo, Nigeria.
[2]Department of Biochemistry, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.
[3]Department of Biochemistry, Federal University of Lokoja, Lokoja, Nigeria.
[4]Department of Science Laboratory Technology, Federal Polytechnic, Offa, Nigeria

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INTRODUCTION

Nanotechnology is a new research field that involves the design, synthesis, and utilization of particles ranging in size from around one to a hundred nanometers (1). The synthesis of metal nanoparticles draws an amassed curiosity as a result of their novel and different features as compared with those of the macroscopic phase. This had allowed pretty applications in several fields such as antimicrobials (2), medicine, biotechnology, optics, microelectronics, catalysis, information storage, and energy conversion (3). Examples of materials that have been widely use in the synthesis of nanoparticles include metals such as silver (Ag), gold (Au), platinium (Pt), cerium (Ce), zinc (Zn), and palladium (Pd) (4). Silver nanoparticles (AgNPs) are well known, it is one of the most commonly used nanomaterials because of its beneficial roles (5). It has a very high surface area, very small size (<20 nm), and a high dispersion (6). Experimental data from previous studies have shown that AgNPs possessed cytotoxic, anti-oxidative, antidiabetic, antiseptic, and antibacterial properties (7,8,9,10). As a result of the broad-spectrum antimicrobial potential of AgNPs (11), it has been widely incorporated into several products such as antimicrobial coating materials for medical devices used for the sterilization of surfaces, air, and textiles, wound dressing materials, and domestic antiseptic sprays (12).

Although physical and chemical procedures are more popular and widely used for the synthesis of nanoparticles, these processes make use of numerous toxic chemicals as reducing agents, which are capable of causing environmental toxicity and non-biodegradable end products. As a result of this, it becomes very important to urgently develop a biosynthetic process that is eco-friendly and reduces the use of toxic chemicals.

Therefore, nanoparticles synthesized from biological sources such as medicinal plants are currently of great scientific interest (13). The biological synthetic method for the synthesis of AgNPs is beneficial over physicochemical techniques. This is because the method is simple for mass production, environmental friendly and economical (14). AgNPs biologically synthesized can be utilized safely for several therapeutic application and it is biocompatible. Nowadays, many materials from medicinal plants, fruits, weeds, mushrooms, herbs, and spice, as well as herbal remedies are being used for the synthesis of nanoparticles (15,16,17,18).

Orange, *Citrus sinensisi* (L) is mainly characterized for its sweet taste (19). It is extensively processed mainly to obtain its natural juices and pulps. This process, however, generates waste such as seed and peels which is about 50% of the whole fruit (20). The seed of *C. sinensis* (L) have been previously shown to be a good source of oils that are rich in carotenoids, phenolic compounds, tocopherols, and phytosterols (21,22), all of which are very potent antioxidants and act to prevent the incidence of oxidative stress and the associated degenerative diseases. Meanwhile, nanomaterials have been shown to modulate improvement in antioxidant activities of various biomolecules (23). For instance, it had been reported that the antioxidant activities of Kinnow fruits (24), 3,6-dihydroxyflavonone (25), and tomatoes (26) were all enhanced when embedded in nanocarriers.

Although, we have previously reported the disruption of lipid metabolic dynamics by subchronic exposure to AgNPs (27), however, to the best of our knowledge there seems to be no information in the literature on the antioxidants and toxicological potentials of AgNPs biosynthesized using *Citrus sinensisi* (L) seed extract, the present study is aimed at achieving this.

MATERIALS AND METHODS

Sample collection

Citrus sinesis (Oranges) was purchased from Aradaa market, Ogbomoso, southwestern Nigeria. The oranges were split open to obtain the seeds. The seeds were washed thoroughly, cut into small pieces (approximately 0.5cm^2), and were air-dried for 7 days at room temperature. The dried seeds were then ground using an electric grinder.

Biosynthesis of AgNPs

The AgNPs used for the present study were synthesized biologically using the *Citrus sinesis* seed extract as describe by Lateef *et al*., (28). Briefly, about 5g of the *Citrus sinesis* dried seed powder was suspended in 100ml distilled water. The suspension is then heated in the water bath at 60°C for 1 h to obtain the extract. The extract was then filtered with Whatman filtered paper. The filtrate was then centrifuged at 4000 rpm for 20 minutes to obtain the crude extracts.

About 10 ml of the extract was reacted with 400 ml of 1 mM silver nitrate ($AgNO_3$) solution in a reaction vessel. This was allowed to stand for 30 minutes at room temperature. The

formation of AgNPs was observed visually for color change of the reaction mixture. The AgNPs solution obtained was transferred into an amber bottle to stop further reaction, after which it was thereafter centrifuged at 12000 rpm for 1 h to obtain a solid residue, which was then allowed to dry at room temperature to obtain a dried powder AgNPs.

Chemicals

All the chemicals used were of pure grade and were procured from Sigma-Aldrich, Missouri, U.S.A and British Drug House (BDH) Chemicals Limited, Poole, England.

Animal treatment

Twenty-eight (28) adult male Wistar rats (120-150 g) were purchased from Mac Temmy farm, Ogbomoso, and were kept in a compartmentalized cages and were housed in the animal house of the Department of Biochemistry, LAUTECH under a conditions of uniform temperature (25 ± 2°C), humidity and 12 hour light-dark cycle. They were allowed unlimited access to food and drinking water *ad libitum*.
After two weeks acclimation period, the animals were randomly grouped into four of seven animals each. Group 1 animals (control) were not exposed to AgNPs. Groups 2, 3, and 4 animals were exposed to 50, 150, and 250 mg/kg body weight AgNPs via oral gavage, once daily for 12 weeks. At the end of the administration, blood samples were collected into heparinized tubes, under mild ether anesthesia via cardiac puncture after an overnight fast. Furthermore, liver was excised from the animals for biochemical studies. The blood samples were centrifuged for 5 minutes at 5000 rpm to obtain plasma. All samples were kept at -20°C until analysed.

Biochemical analyses

Determination of hepatic transaminases: Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities were assayed in the plasma using the commercially available LAB KIT reagent kits according to the procedure described by Reitman and Frankel (29). Similarly, the Plasma level of gamma glutamyl transferase (γ-GT) was assayed in the plasma by using the reconstituted diagnostic reagent kit manufactured by LAB KIT Limited United Kingdom, following standard method Szasz (30).

Determination of Alkaline phosphatase: The plasma activity of alkaline Phosphatase (ALP) was assayed by the optimized standard method as recommended by Deutsche Gesellschaft Fur Klinische Chemie (31)

Determination of creatinine: Creatinine concentration was determined using RANDOX diagnostic kit following the procedure described by Bartel et al. (32).

Determination of urea: Urea concentration was determined with the same diagnostic kit as described by the Urease-Berthelot method (33).

Determination of superoxide dismutase (SOD): SOD activity was quantified using Fortress diagnostic kit which employed Arthur method (34) as previously described by Fatoki, et al. (35).

Determination of glutathione peroxidase (GPx): GPx activity was evaluated using the method of Rotruck and coworkers (36).

Determination of reduced glutathione (GSH): The procedure described by Beutler et al. (37) was employed in evaluating the level of GSH in the liver homogenate.

Determination of glutathione-s-transferase (GST): The hepatic activity of GST was assayed by the method of Habig and Jakoby (38). In brief, the reaction medium contained 0.1 M phosphate buffer (pH 6.8) and 20 mM CNDB. This medium was pre-incubated at 37°C for 5 minutes. The reaction was initiated by the addition of the sample (0.03 ml) and the absorbance was monitored at 340 nm for 3 minutes at 30 seconds intervals.

Determination of hepatic and erythrocyte malondialdehyde (MDA) levels: Lipid peroxidation (LPO) was determined by quantifying thiobarbituric reactive oxygen species (TBARS) in form of MDA using the method described by Varshney and Kale (39).

Determination of total proteins: Total Protein concentration was quantified using LAB KIT diagnostic kit following the Biuret method described by Gornall *et al*. (40).

RESULTS

Table 1 depicts the initial, final, and liver weights of animals exposed to 50, 150, and 250 mg/kg bw AgNPs relative to the the control group. The terminal bodyweight of the animals

increased in an hormetic manner, that is, the lowest dosage of 50 mg/kg bw produced the highest increase in the body weight (48%) whereas, the highest dosage of 250 mg/kg bw led to 40% increase in body weight as against 72.36% increase observed in the control group. Similarly, the treatment also caused an increase in percentage relative liver weight when compared with the control group. As shown, exposure to 50, 150, and 250 mg/kg bw AgNPs resulted in a 3.19%, 3.67%, and 3.00% increase in the relative hepatic weight respectively as compared with a 2.97% increase observed in the control group.

The extent of alterations in hepatic transaminases induced by 50, 150, and 250 mg/kg bw AgNPs is depicted in figure 1. Repeated oral administration of the various doses of AgNPs resulted in a non-significant ($p>0.05$) reduction in the amount of the hepatic transaminases (ALT, AST, and ?GT) available in the plasma. The only exception to this is the dose of 250 mg/kg bw AgNPs which induced a significant ($p<0.05$) decrease in the concentration of the ALT in the plasma. The decrease in the concentration of hepatic ALT and AST in the plasma is dose-dependent. Meanwhile, the three doses of AgNPs decreased the amount of ?GT in the plasma from 7.22 U/L in the control group to 6.97 U/L, 5.77 U/L, and 5.84 U/L by 50, 150, and 250 mg/kg bw respectively.

The mean values of investigated plasma ALP concentration of the rats exposed to 50, 150, and 250 mg/kg bw AgNPs are illustrated in figure 2. Exposure to the three doses of the AgNPs resulted in a non-significant ($p>0.05$) depletion of the enzyme in the plasma of the exposed animals.

As illustrated in figure 3, 50 mg/kg bw AgNPs reduced the plasma concentration of creatinine from 30.83 µmol/L in the control group to 26.40 µmol/L. Similarly, other doses of 150 and 250 mg/kg bw reduced the concentration to 30.53 µmol/L and 29.00 µmol/L respectively.

As presented in figure 4, exposure to 50 and 150 mg/kg bw AgNPs orally induced a significant decrease ($p<0.05$) in the plasma concentration of urea. For instance, 50 mg/kg bw depleted the plasma urea by 40% while 150 mg/kg bw resulted in 21% depletion. 250 mg/kg bw AgNPs however showed no significant ($p>0.05$) on the plasma urea concentration.

As depicted in figure 5, exposure of experimental rats to 50, 150, and 250 mg/kg bw AgNPs orally, led to a dose-dependent increase ($p<0.05$) in the plasma activity of SOD. The three doses increased the activity of the enzyme from 4.38 U/mg protein in the control to 6.18 U/mg protein, 7.32 U/mg protein, and 8.90 U/mg protein respectively.

As shown in figure 6, experimental doses of AgNPs significantly ($p<0.05$) increased the activity of the glutathione peroxidase in the plasma. The magnitude of this increase in the plasma activity of glutathione peroxidase is about 3-fold in the groups that received 50 and 250 mg/kg bw AgNPs and about 2-fold in the group that was exposed to 150 mg/kg bw AgNPs.

Figure 7 illustrates the effects of AgNPs on the level of reduced glutathione in the plasma of the animals exposed to 50,150 and 250 mg/kg bw AgNPs orally. This exposure significantly increased ($p<0.05$) the concentration of reduced glutathione in the plasma in a dose-dependent manner.

The mean values of glutathione S – transferase activity in the plasma of rats exposed to AgNPs are as illustrated in figure 8. The exposure induced a significant increase ($p<0.05$) in the activity of the enzyme. For instance, glutathione s – transferase activity was increased from 2.70 per mg/protein in the control group to 3.54 per mg/protein in the group exposed to 50 mg/kg bw, 8.15 per mg/protein in the group exposed to 150 mg/kg bw and 5.07 per mg/protein in the group that was exposed to 250 mg/kg bw.

The hepatic and erythrocyte malondialdehyde contents are shown in figure 9. At all doses tested, AgNPs exposure for 12 weeks significantly depleted ($p<0.05$) the MDA levels in both the liver and red blood cell in a dose-dependent manner. For instance, 50, 150, 250 mg/kg bw AgNPs caused 1.45-. 1.61-and 2.36-folds depletion of the hepatic MDA respectively, meanwhile, exposure to the same doses of AgNPs reduced the erythrocyte MDA level by 22%, 33%, and 38% respectively.

The total protein in the plasma of the experimental rats exposed to 50, 150, and 250 mg/kg bw as shown in figure 10 indicate that AgNPs at all the doses tested significantly up-regulated ($p<0.05$) the total protein contents in the plasma in a dose-dependent manner. An initial elevation of total protein content occurred at 50 mg/kg bw AgNPs (1.40-fold), this elevation progressed further at 150 mg/kg bw (1.77-fold) and climaxed at 250 mg/kg bw (2.63-fold).

DISCUSSION

To maintain regular homeostasis in the body, many enzymes act as a catalyst in specific biochemical reactions. Alteration in the levels of

these enzymes in the system could be an indication of a particular organ or tissue damage (41). ALT, AST, and ?-GT are mainly found in the liver and some other extra-hepatic tissues such as the heart, muscle, and so on. The level of these enzymes is a valuable maker that is used in the diagnosis of hepatic injury. The increased level of these enzymes in circulation as a result of continuous release from the liver is an indication of liver damage (42). As such, the level of these enzymes in the blood is directly related to the magnitude of liver damage (43). Our findings in the present study indicated that various doses of AgNPs used in this study, although caused alteration in the plasma contents of these enzymes, at all doses, tested these changes in the plasma concentration are not statistically significant ($p<0.05$). This suggests that AgNPs synthesized from the seed of *C. sinensis* (L) have no damaging effect on the structural and membrane integrity of the hepatocytes.

ALP is a hydrolase enzyme that dephosphorylates various proteins and nucleotides (43). It an important marker of hepatobiliary injury, its level negatively correlates with the potency of the bile ducts. It is widely used as a maker of cholestatic liver injury. Similarly, Bhudhisawasdi, *et al*. (44) reported high plasma ALP activity in patients with liver injury. As observed for ALT, AST and ?-GT, AgNPs synthesized from *C. sinensis* (L) seed have no significant effect on the plasma concentration of ALP. This further confirmed that unlike AgNPs synthesized from chemical sources, AgNPs biologically synthesized from *C. sinensis* (L) seed may have no negative effect on the structure and function of the liver cells.

Creatinine is a non-protein nitrogenous (NPN) waste product, produced as a by-product from the catabolism of creatine and phosphocreatine. Creatinine can serve as a marker for renal function (45). Most of the creatinine found in the body system are from the muscle; therefore, the plasma creatinine concentration mainly is influenced by the muscle mass and is less affected by the diet and therefore, a very suitable marker of kidney function. The determination of plasma creatinine concentrations therefore indicates the glomerulus filtration capacity, that is, the glomerular filtration rate (GFR.). In kidney disorder, GFR is decreased, creatinine clearance through the kidney is therefore compromised with the attendant up-regulation of the plasma creatinine level (46,47). In the present study administration of various doses of AgNPs for three months impacted no significant effect on the plasma creatinine concentration when compared with the animals in the control group. This result then implies that the AgNPs synthesized from the seed of *C. sinensis* (L) do not perturb the GFR and as such posed no danger for renal function.

Urea also known as blood urea nitrogen (BUN) is a product of protein metabolism. It is also considered a nonprotein nitrogenous substance (NPN) waste product. Normally, ammonia is released, when the amino acids derived from the breakdown of protein are deaminated. The ammonia is then converted to urea. The plasma concentration of urea is therefore dependent on the following; firstly, protein intake, secondly, the capacity to catabolize protein, and thirdly, the capacity of the kidney to adequately excrete urea from the body system. Urea accounts for up to 80% to 90% of the NPNs excreted by the body (*48)*. The body's dependency on the kidney to excrete urea makes it a useful analyte to appraise the renal function (49). Lowered plasma urea concentrations as observed following exposure to 50 and 150 mg/kg bw AgNPs in this study may be suggested to implies an AgNPs-induced enhancement of renal excretion function. Thus, it can be hypothesized that AgNPs synthesized from biological sources have the potential to enhance renal function and posed no harmful effect for the kidney.

Free radicals such as hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\cdot}OH$), superoxide radicals ($O_2^{\cdot-}$), and singlet oxygen ($^1O_2$) are usually generated as by-products of metabolism in the biological system (50). They are mainly produced by the mitochondria during both physiological and pathological conditions (51). The continuous production by the mitochondria will expectedly increase the cellular level of reactive oxygen species (ROS). When there is an increased cellular level of ROS, the ROS will start by showing harmful effects on important cellular macromolecules such as nucleic acid, lipids, and protein (52), Meanwhile, cells employ antioxidant defense systems that are both enzymatic and non-enzymatic to counteract the effects of ROS and save themselves from the ROS-induced cellular damage (53). This endogenous antioxidant system acts by converting these dangerous ROS to non-toxic products (54). In a situation where there is an imbalance between the production of free radicals and the antioxidant defense system, the result is oxidative stress. It had been shown by a large body of evidence that oxidative stress can

be responsible, with various degrees of importance, in the onset and/or progression of myriads of diseases such as cancer, diabetes, atherosclerosis, cardiovascular disease, and metabolic disorder (55).

Increased concentration of MDA; which is a product of free radicals attack on cells and an important marker of lipid peroxidation indicates oxidative stress-induced tissue damage. It is produced following an attack on tissue fatty acids by free radicals. In this study, exposure to biologically synthesized AgNPs significantly decreased the hepatic and erythrocyte MDA levels with the concomitant significant increase in the activities and concentrations of both enzymatic and non-enzymatic antioxidants. Specifically, exposure to AgNPs significantly increased the activities of SOD, GPx, and GST. The exposure also significantly increased the concentration of GSH. This observation from our study suggests that the AgNPs synthesized from the seed of *C. sinensis* (L) exhibited antioxidant activities and/or enhanced the antioxidant defensive mechanism of the exposed animals, indicating that the AgNPs are capable of preventing the onset or progression of oxidative stress and associated disorders. This is evident in the significant reduction in MDA level and enhanced antioxidant defense system observed in our study.

The above is not surprising as the observation is in total agreement with the observation from previous studies. For instance, oil extracted from citrus seed had been shown to be rich in phytochemicals (such as carotenoids, phenolic compounds, α-tocopherol, etc) and antioxidant activity (56,57,58). Similarly, it had also been shown that AgNPs biosynthesized from various plant parts such as seed and fruits enhanced the phytochemical and antioxidant composition of such plant materials (59).

SOD is considered the first-line defense against the injurious effects of ROS in synergy with catalase (CAT) (60). SOD converts ROS to H_2O_2; the H_2O_2 is in turn converted to water and molecular oxygen by CAT. Although the activity of CAT was not assayed in this study, the up-regulation of SOD activity is an indication of an enhanced enzymatic antioxidant system against continued production of ROS by the mitochondria.

At the cellular level, GSH, GST, and GPx act in the detoxification of both xenobiotic and endobiotic compounds by participating in the GSH redox cycle, where they aid the conversion of lipid peroxides and H_2O_2 to other non-toxic products (61). In this study, AgNPs caused a marked increase in the GSH concentration and an enhanced GST and GPx activities. This increase in the GSH status could be attributed to a decreased oxidative stress burden, likely to be as a result of reduced cellular level of the toxic ROS that could have hitherto induced lipid oxidation. This speculation is confirmed by the significant decline in the hepatic and erythrocyte MDA level of the exposed animals when compared with the control group.

CONCLUSION

We have studied the effects of various concentrations of AgNPs biosynthesized from the seed of *C. sinensis* (L) on some markers of hepatic and renal toxicity as well as on antioxidant activities. The data obtained from the present study suggested that AgNPs biosynthesized from the seed of *C. sinensis* (L) poised no harm on hepatic and renal function and cellular integrity and it also boosted the system antioxidant capacity.

Conflict of interest: The authors declare no conflict of interest.

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Table 1. Body and organ weight and weight change in rats orally exposed to AgNPs for 12 weeks.

Groups	Initial body weight (g)	Final body weight (g)	Weight change (%)	Liver weight (g)	Relative liver weight (%)
Control	123 ± 8.30[a]	212 ± 12.56[a]	72.36	6.3 ± 1.00[a]	2.97
50 mg/kg bw	125 ± 14.32[a]	185 ± 10.99[a]	48	5.9 ± 0.31[a]	3.19
150 mg/kg bw	130 ± 20.19a	188 ±24.60[a]	44.62	6.9 ± 1.60[a]	3.67
250 mg/kg bw	150 ± 9.90[b]	210 ± 21.23[a]	40.00	6.3 ± 1.00[a]	3.00

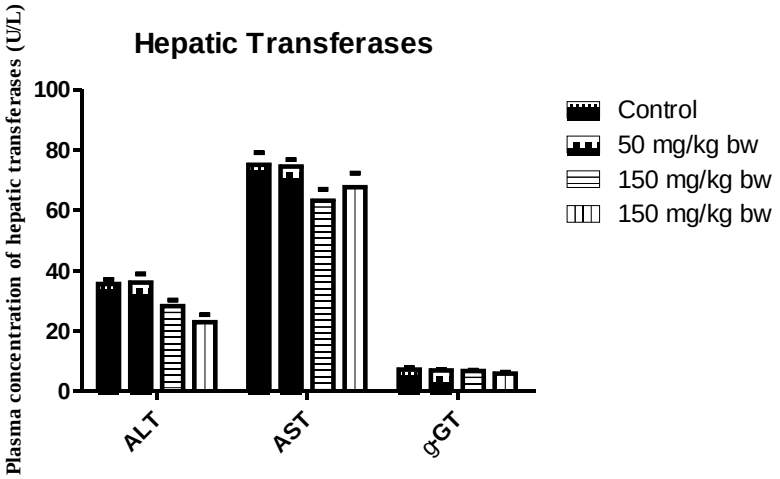

Figure 1: Plasma Concentration of hepatic transaminases in rats orally exposed to AgNPs for 12 weeks.

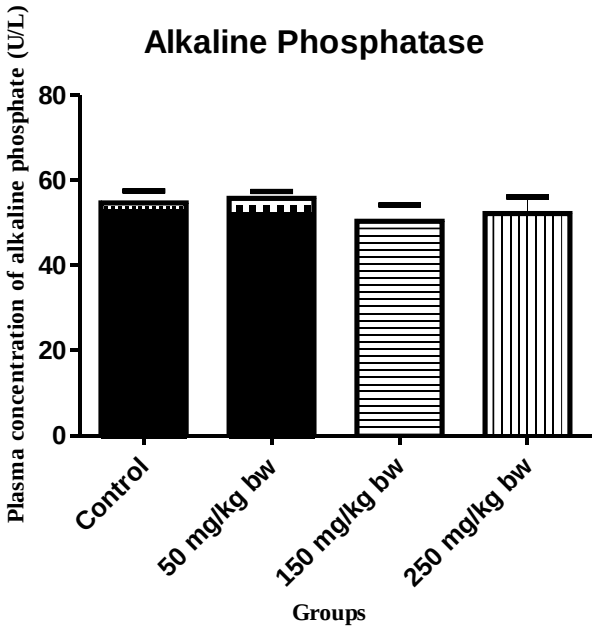

Figure 2: Plasma Concentration of alkaline phosphatase in rats orally exposed to AgNPs for 12 weeks.

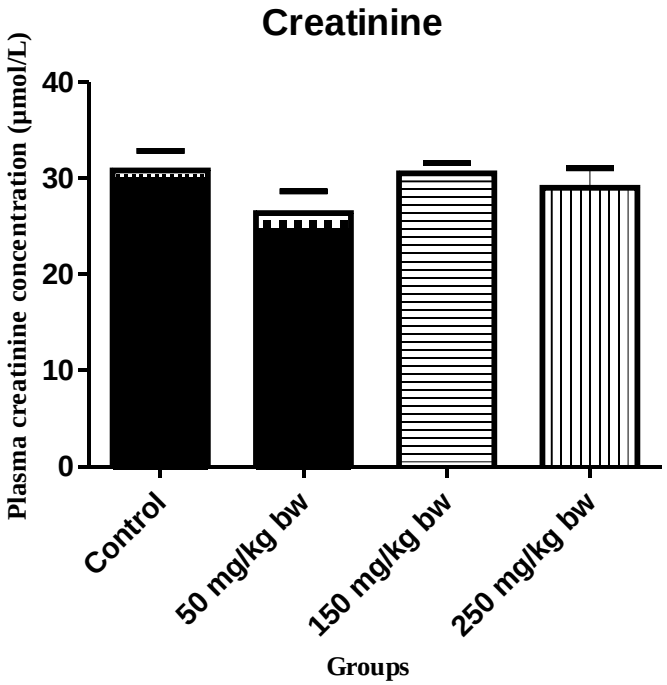

Figure 3: Plasma Concentration of creatinine in rats orally exposed to AgNPs for 12 weeks.

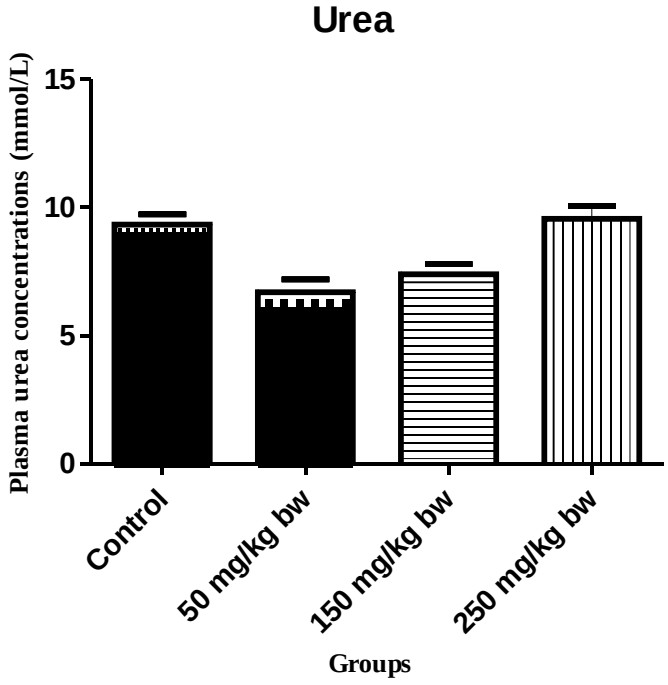

Figure 4: Plasma Concentration of urea in rats orally exposed to AgNPs for 12 weeks.

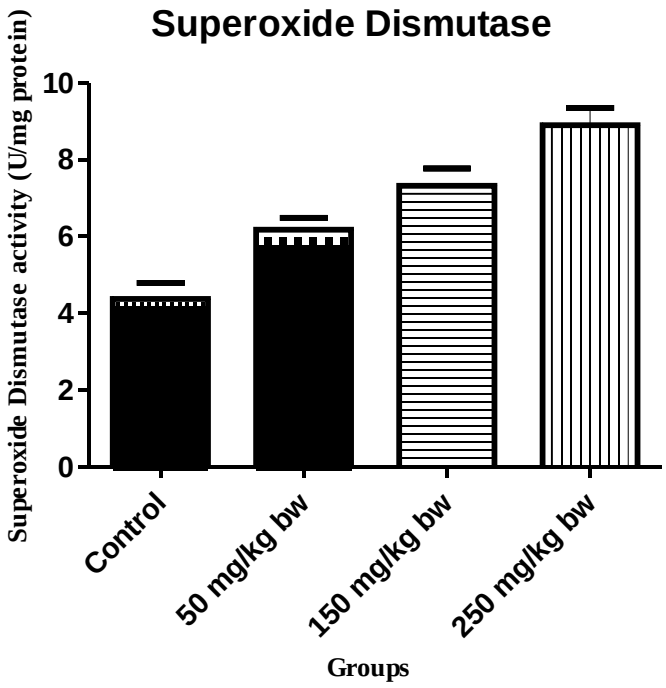

Figure 5: Superoxide dismutase activities in rats orally exposed to AgNPs for 12 weeks.

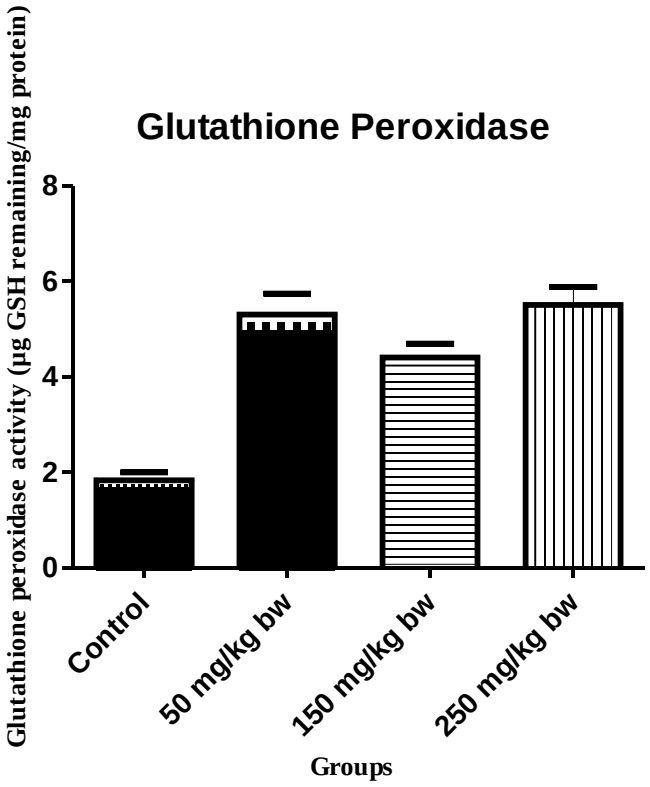

Figure 6: Glutathione peroxidase activities in rats orally exposed to AgNPs for 12 weeks.

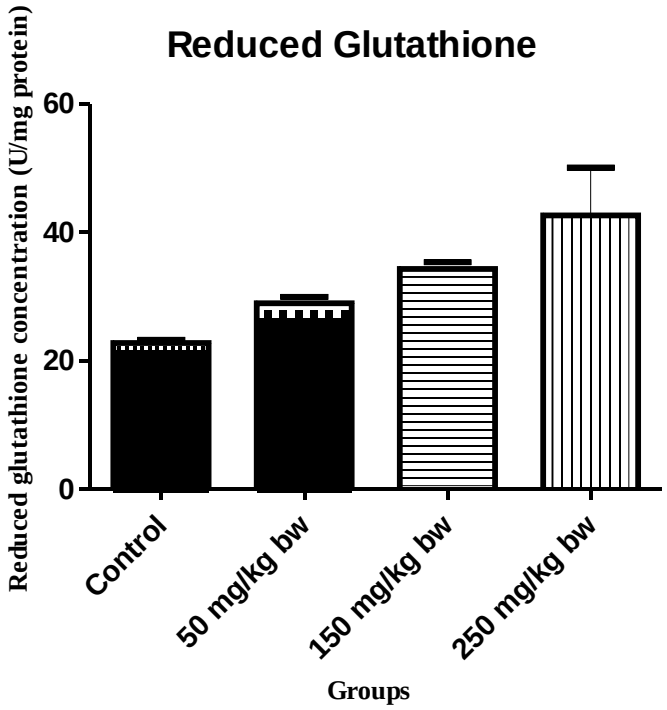

Figure 7: Concentration of reduced glutathione rats orally exposed to AgNPs for 12 weeks.

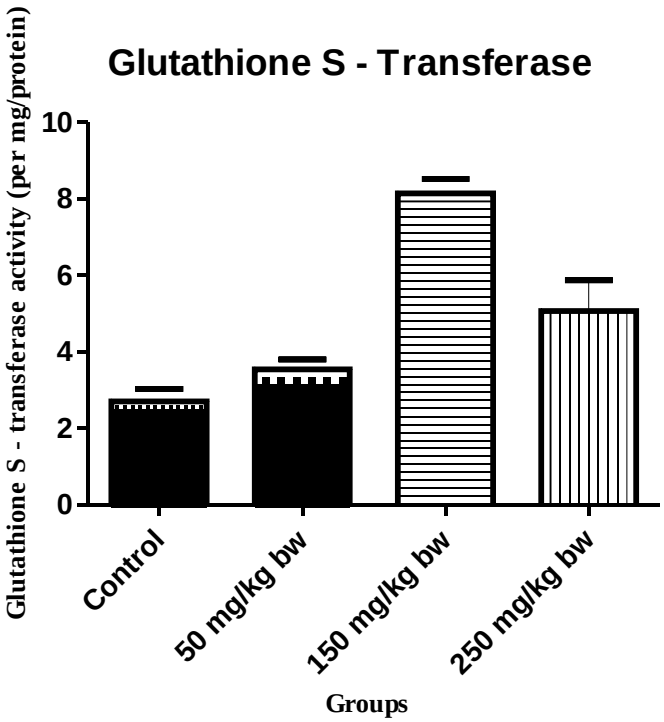

Figure 8: Glutathione S-transferase activities in rats orally exposed to AgNPs for 12 weeks.

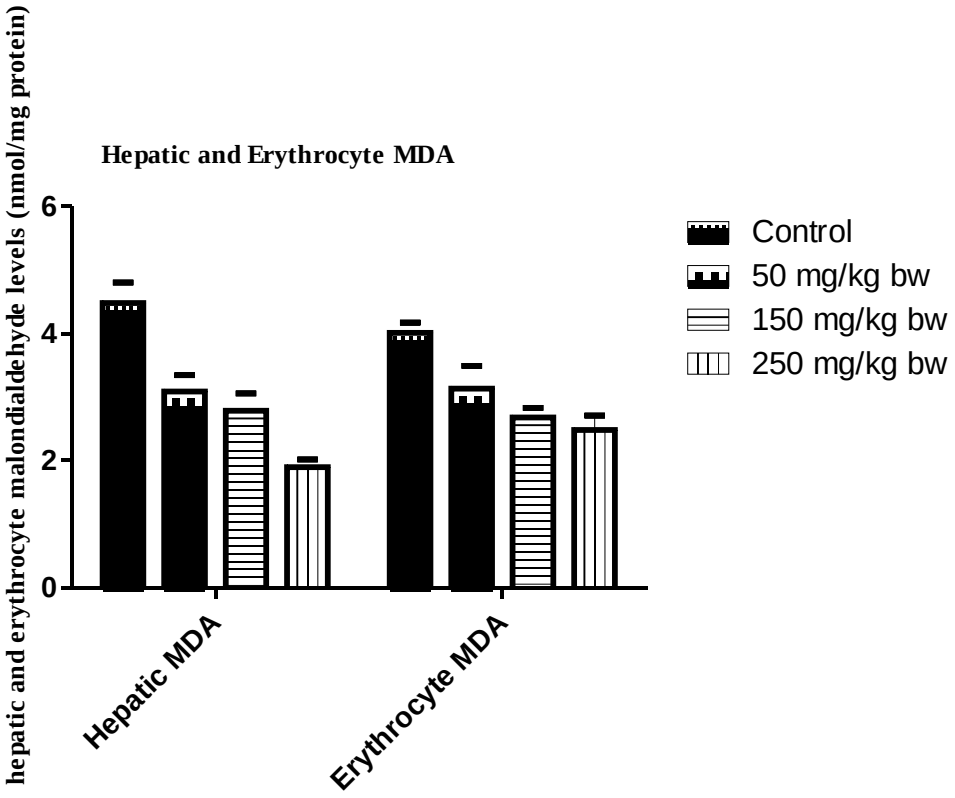

Figure 9: Hepatic and erythrocyte concentration of malonaldehyde (MDA) in rats orally exposed to AgNPs for 12 weeks.

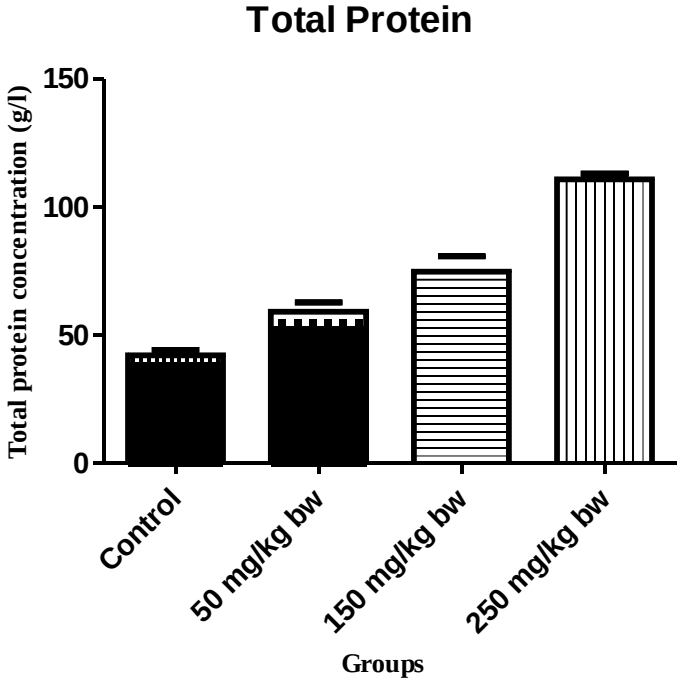

Figure 10: Total protein concentration in rats orally exposed to AgNPs for 12 weeks.