

## Organo Sliver Clay Particles Induced Oxidative Stress and Hepatotoxicity in Mice

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### Abstract

Toxicity of organo sliver clay particles (OSCPs) depends on chemical composition, particle size and atomic arrangement. In this study, the toxic effects of different size (OSCPs) 50, 100 and 200 nanometer with different concentration were evaluated on liver enzyme activities and oxidant/antioxidant status in mice treated daily those OSCP for 28 consecutive days. One hundred mice were randomly divided into ten groups: G1 (control without treatment), T1, T2, T3 treated with the same dose of 0.25 mg/kg body weight of OSCP at 50 nm, 100 nm and 200 nm size diameter, T4, T5 T6 treated with the same dose of 0.50 mg/kg.b.w at 50 nm, 100 nm and 200 nm size diameter and T7, T8 and T9 treated with the same dose of 1 mg/kg.b.w at 50 nm, 100 nm and 200 nm size diameter. Treatment groups were injected intraperitoneally with OSCP suspension, and at the end of study period, blood samples for sera preparations were collected and stored at -20°C until further analysis. Results obtained from this study showed that ALT, AST and ALP enzymes in all treated groups have increased in comparison with the control group and more significantly in high dose with low size diameter of OSCP treated. Adverse impacts on oxidant/antioxidant status were observed in a high dose-treated group (1.0 mg/kg.b.w) with less size diameter (50 nm), From result obtained, it was clear that lipid peroxidation marker; malondialdehyde (MDA) levels was higher whereas superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and glutathione peroxidase (GP<sub>x</sub>) levels decreased significantly ( $p < 0.001$ ) in all animals treated OSCP in comparison with the normal controls, but the most alteration was associated with higher dose at a low diameter size (1.0 mg/kg.b.w at 50 nm). Based on these results, it is suggested that the effect of OSCP on the tissues may cause organ toxicity in mice.

**Keyword:** *Organo Sliver Clay Particles; Liver Function*

### Introduction

Clay minerals are considered as natural material with particle size  $< 2 \mu\text{m}$ . Smectite, that classified as 2:1 phyllosilicate clays, have a crystal lattice unit formed by one alumina octahedral sheet sandwiched between two silica tetrahedral sheets. A negatively charged surface gives a rise when the ion substitution or the site vacancies at the tetrahedral and/or octahedral sheets happen. The exchangeable cation between the layers compensate the negative charge and may be easily exchanged by other metal cation, explaining the high ion exchange capacities of these minerals (70 - 120 meq/100g). Due to this crystalline arrangement, smectites are able to expand and contract the interlayer while maintaining the two dimensional crystallographic integrity. The interlayer between units contains positive cations and water molecules. Montmorillonite is some of the members of the smectite family [1].

Clays are used in human and veterinary health formulations as natural materials like peripients or active substances. The interaction between the drug and its excipients may delay the drug release and therefore, its absorption, by lowering the drug levels in the blood. This feature can be favorable when the slow, controlled desorption of the drug has a positive effect upon its therapeutic action [2]. Among these materials, montmorillonites have been the most extensively used minerals in this field of application. Montmorillonites exhibit large

specific surface and colloid properties and high cation exchange capacity, that give rise to optimum adsorbents of organic and inorganic substances. Metallic ion-exchanged montmorillonite dispersed in aqueous solution has shown to attract bacteria by electrostatic forces [3]. Furthermore, montmorillonites and fluoromicas intercalated with organic compounds with antibacterial effects such as cetylpyridinium, cetyltrimethylammonium, norfloxacin and tetracyclines have been also studied. The organo montmorillonites and fluoromicas showed excellent antibacterial activities [4,5].

Clay minerals are used in a wide variety of industrial applications [6] due to two important and contrasting properties: inertia and stability and reactivity and catalytic activity. The widespread use of clay minerals responds to their extensive distribution in the environment, the relatively simple extraction and economic feasibility they present. Large volumes of these materials are used in filtration processes of bleaching and clarification of various systems for animal feed pellets, in pesticides formulation and in water purification. They are also used as fats and oils adsorbents, and as catalyst or support catalysts in several organic syntheses [6,7]. The modification of the surface properties is of importance to extend the clay applications and has therefore received great interest [7]. The hydrophobic modification of the montmorillonite can enhance the thermal, rheological, and mechanical properties of the resultant materials. Although the modification of clay minerals can be performed by several methods, the ion exchange of the inorganic cations with organic cations, usually with quaternary ammonium compounds, allows changing the surface properties from hydrophilic to hydrophobic. Hence, these obtained organoclays thus obtained have been extensively investigated for hydrophobic contaminants immobilization. This study focuses on toxicological effect of several complexes formed between quaternary ammonium compounds and silver with kaolinite, in its natural and modified form.

The present study was carried out to investigate the particle-size effect of OSCPs on the hepatic enzyme activities and alteration in oxidant/antioxidant status in animals treated with those particles in an attempt to cover and understand the toxicity and potential threat of their therapeutic and diagnostic use in relation with the time of exposure.

### Materials and Methods

**Preparation of materials.** Silver modified clays (AgKaoF) was firstly prepared by the addition of 10g powder clays in 500 mL AgNO<sub>3</sub> (1000 ppm). The mixture was stirred for 16 hours at room temperature and then filtered to separate the solid and liquid fractions. The solid fraction was then dried in an oven at 90°C overnight and it was used for the preparation of CTAB modified AgKaoF (CTAB-AgKaoF). About 2g of dried samples was added with 200 mL of 2 mM CTAB solutions. This concentration was selected because it is slightly higher than CMC (Critical Micelle Concentration) value of CTAB (CMC CTAB: 1.0 mM). Following that, the mixture was stirred for 16 hours, filtered and finally the solid fraction was dried in an oven overnight.

### Characterization Techniques

Ag-clays have been characterized by X-Ray Diffraction (XRD), and XRD patterns were recorded. Fourier Transform-Infrared (FT-IR) spectrometer equipped with OMNIC software was used to detect the presence of CTAB on the prepared samples. The presence of silver in silver modified clays has been detected using Energy Dispersive X-Ray (EDX) analyzer equipped together with Field Emission-Scanning Electron Microscopy (FESEM). The size distribution of synthesized OSCPs at 50 nm, 100 nm and 200 nm were analyzed by microscope AFM.

### Laboratory animals

Male and female albino mice with the mean weight of  $28.3 \pm 1.2$  gram was used, these mice were prepared from AL Nahrain University; they were kept in animal house with standard conditions (12 hour darkness, 12 hour lightness, temperature of 25°C and suitable humidity). Animals were fed by standard food without limitation.

### Animals' treatment

The mice were divided to ten groups (10 mice in each group, five male and the other female randomly). And each group was kept in separate cage. G1 group as control group just received water and food. T1, T2 and T3 group received OSCPs with dose of 0.25 mg/kg intra-

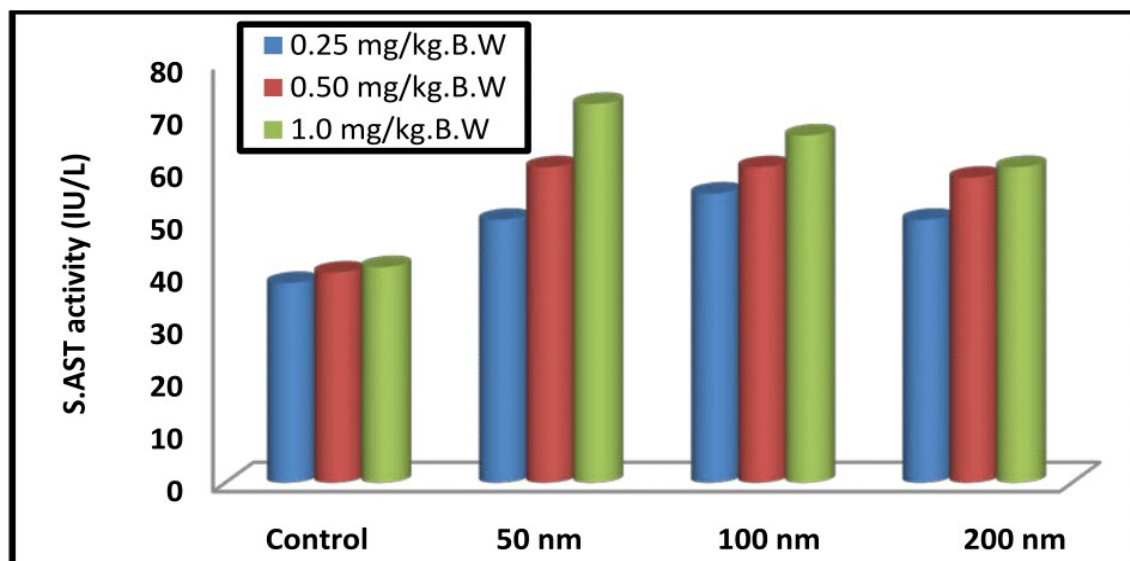
peritoneally in 28 successive days at 50, 100 and 200 nm. T4, T5 and T6 group received OSCPs with dose of 0.5 mg/kg at 50, 100 and 200 nm, and finally T7, T8 and T9 group received OSCPs with dose of 1.0 mg/kg at 50, 100 and 200 nm. At the end of experimental period all mice were anaesthetized by chloroform and blood sampling was done from heart of mice. Blood was collected in laboratory tubes carefully and kept for 15 minutes in the laboratory temperature. After coagulating the blood, serum was separated by centrifuge at 1500xg. For evaluating and comparing liver function in various groups, serum transaminases including AST, ALT, and ALP were measured colorimetrically using direct spinreact kits (Spain). Serum lipid peroxidation marker, malondialdehyde (MDA) was measured by precipitating lipoproteins with trichloroacetic acid and boiling with thiobarbituric acid to get pink colour as per the method [8]. Superoxide dismutase (SOD) was measured by using RANSOD kit and Glutathione peroxidase (GPx) was measured by using RANSEL kit (Randox Laboratories Ltd. Crumlin, United Kingdom) This method is based on Paglia and Valentine [9]. Catalase activity was determined by Goth method [10]. Reduced glutathione by method [11].

**Statistical Analysis**

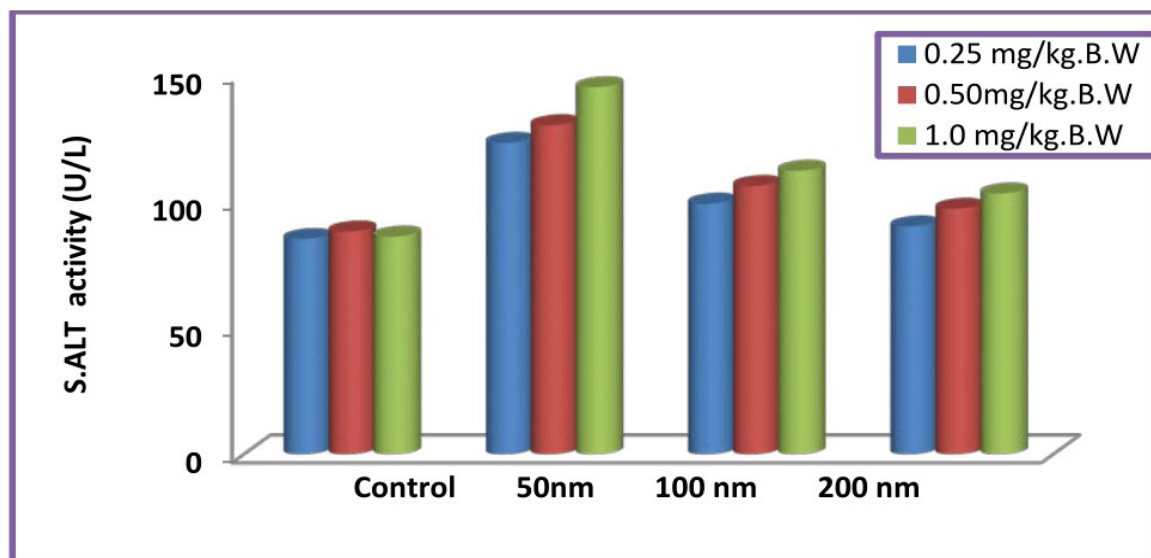
The results were expressed as Mean ± SD. Differences between means were analyzed using one-way ANOVA, and then the means were compared with Duncan. P values of 0.05 or less were taken as being statistically significant. Data were analyzed using version 16 of SPSS software (SPSS Inc., Chicago, IL, USA).

**Results**

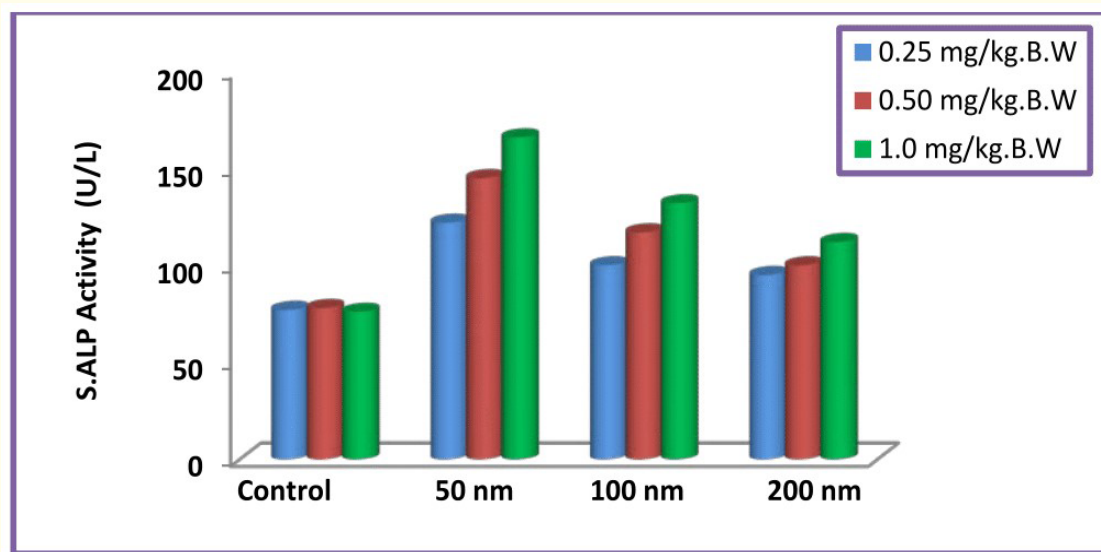
Serum AST levels in male mice showed statistically increase (P < 0.01) compared to control, and that increase depending on dose of OSCPs administrated intraperiotally. From figure 1 high level of serum AST 70 ± 11 IU/L was observed in group of animals treated with 1.0 mg/kg.b.w at 50 nm size diameter of particles compared with 62 ± 6 IU/L in animals treated with 0.5 mg/kg.b.w and 51 ± 5 IU/L in mice treated with 0.25 mg/kg.b.w of OSCPs, while control mice without treatment had levels of AST: 42 ± 4 IU/L. Different data were noticed in female mice treated with the same dose of sliver clay particles but it was not significant (P > 0.06) compared with male mice groups. On the other hand serum levels of ALT and ALP were increased as noticed in figure 2 and 3 but the levels of ALP elevated remarkably either in male or female groups compared with control mice. All treatment groups indicate statistically increased activities to their control (C1).



**Figure 1:** Serum aspartate transferase activity in male control, test group received three different doses (0.25 mg/kg, 0.50 mg/kg and 1.0 mg/kg) of organo sliver clay particle at three different size (50, 100 and 200 nm).



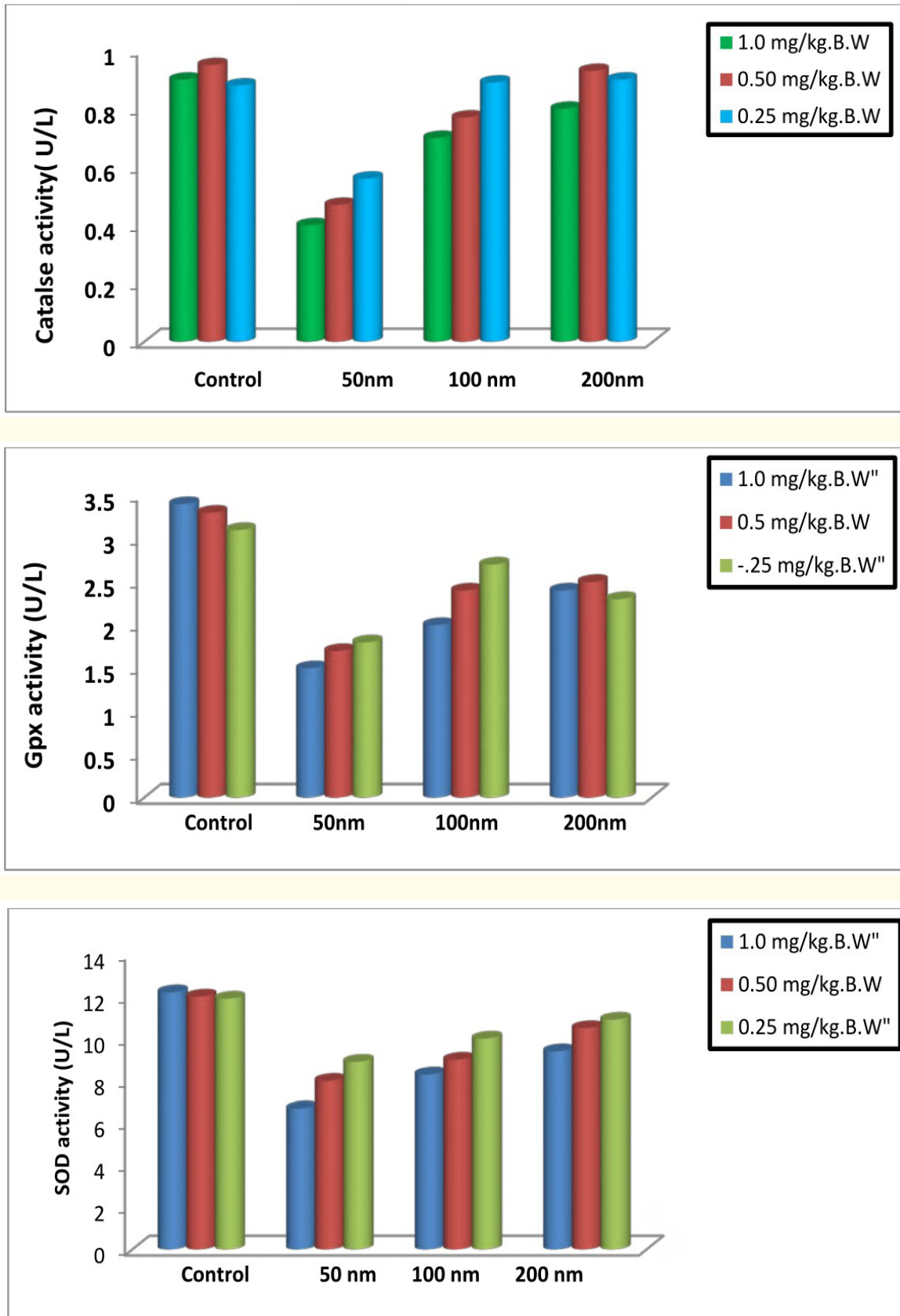
**Figure 2:** Serum alanine transferase activity in male control, test group received three different doses (0.25 mg/kg, 0.50 mg/kg and 1.0 mg/kg of organo sliver clay particle at three different size (50, 100 and 200 nm).



**Figure 3:** Serum Alkaline phosphatase activity in male control, and test groups received three different doses (0.25 mg/kg, 0.50 mg/kg and 1.0 mg/kg of organo sliver clay particle at three different size (50, 100 and 200 nm).

Results of oxidant/antioxidant status showed a significant alteration in all biochemical parameter measured. MDA was significantly increased when animals treated with a high dose (1.0 mg/kg.b.w) of OSCPs at 50 nm size (1.0 > 0.5 > 0.25 mg.kg.b.w). So we concluded

that the effect was depending on dose and size as shown in figure 4. On the other hand reduced glutathione was significantly decreased and this decrease noticed clearly at 1.0 mg/kg.b.w and at 50 nm size of OSCPs as shown in figure 5. Data from figures 6 observed a highly significant decrease in all enzymatic antioxidant GPx, sSOD and Cat when the animals treated highly dose of OSCPs, 1.0 mg/kg.b.w with small size (50 nm). So the alteration in the activities of those enzymatic antioxidants depend on dose and size.



**Figure 4:** Serum enzymatic antioxidant (Catalase, Glutathione peroxidase and superoxide dismutase activity in male control, and test groups received three different doses (0.25 mg/kg, 0.50 mg/kg and 1.0 mg/kg of organo sliver clay particle at three different size (50, 100 and 200 nm).

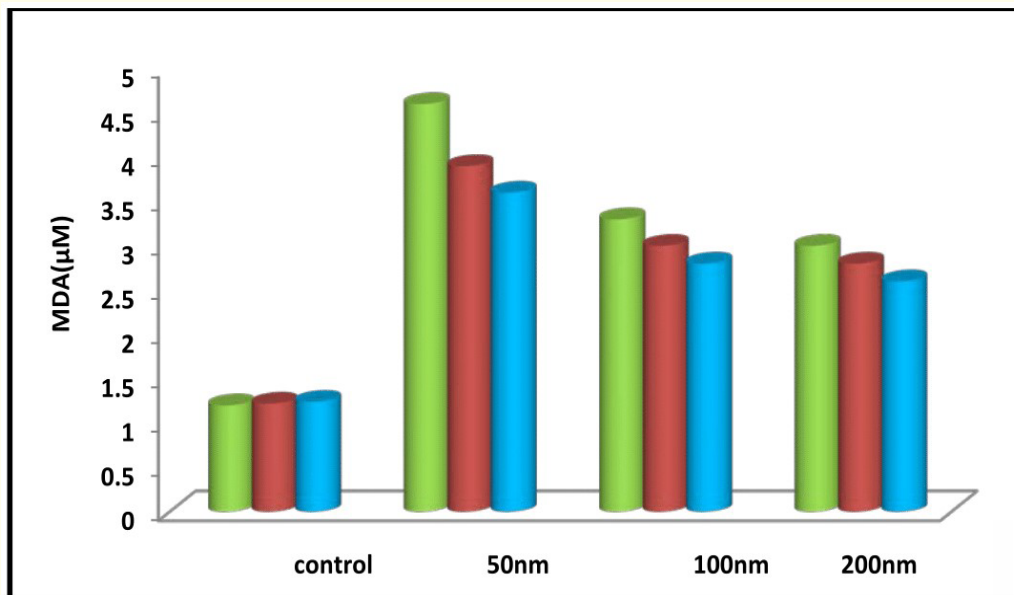


Figure 5: Serum lipid peroxidation marker (MDA) levels in male control, and test groups received three different doses (0.25 mg/kg, 0.50 mg/kg and 1.0 mg/kg) of organo sliver clay particle at three different size (50, 100 and 200 nm).

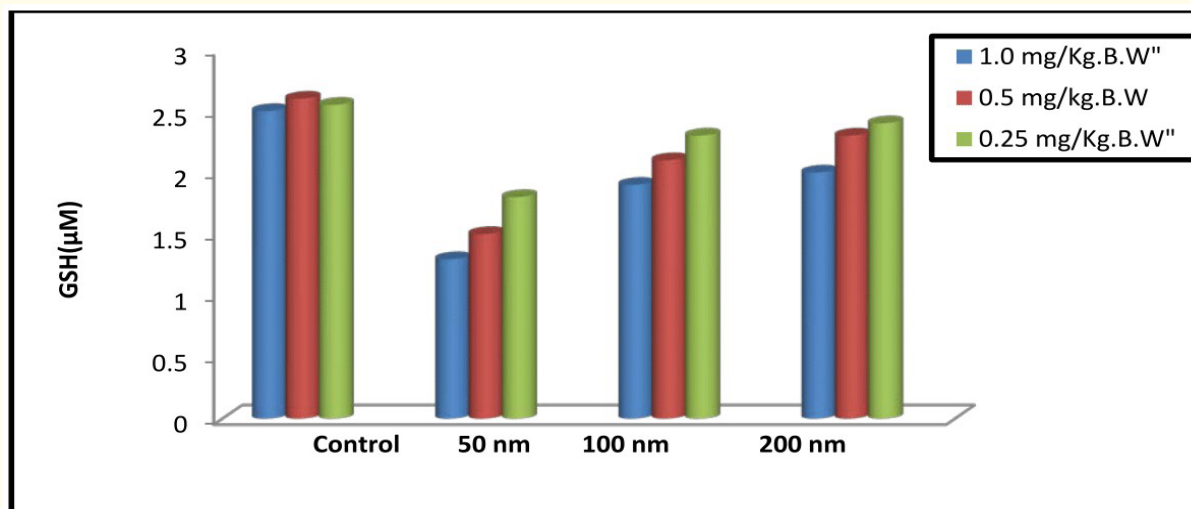


Figure 6: Serum reduced glutathione levels in male control, and test groups received three different doses (0.25 mg/kg, 0.50 mg/kg and 1.0 mg/kg) of organo sliver clay particle at three different size (50, 100 and 200 nm).

## Discussion

There is no consensus on the cytotoxicity of nanosilver, however, most publications do show reduced cell viability following exposure. Additional toxicological effects seen in the *in vitro* studies are glutathione depletion, mitochondrial deviations or destruction and damage to cell membranes. Nanosilver enters the body through the skin, respiratory system and gastrointestinal tract. The most important way to contact it [12], especially in the gastrointestinal tract is in colloidal form [12]. In this study more increase in AST levels in male mice, were observed in higher dose of 1.0 mg/kg with 50 nm size diameter group in comparison to other doses, 0.25 mg/kg and 0.50 mg/kg.b.w. Absorbed OSCPs bind to plasma proteins and can enter the cells, and they are distributed in organs such as liver, kidney, heart, lymph nodes, brain, lung, stomach and testicles depending on the route of administration [13]. Absorbed nanosilver from gastrointestinal tract enter liver through the portal vein and might have impact on the liver since the liver serves as the first checkpoint for everything absorbed before becoming systemic. Liver is able to actively remove compounds from the blood and transform them to chemical forms that can easily be excreted. It is a logical assumption that ingested OSCPs might have impact on the liver.

In the present work, physiological effects of OSCPs have been evaluated at different doses and different size on serum ALT, AST, ALP in male and female mice. Hepatic damage induced by intraperitoneal injection of OSCPs in mice, has possibly caused severe irritation of oxidant system in these cells. The smaller the diameter of the of OSCPs (50 nm) is, the more its influence to cells and its molecular effects on the intracellular mechanisms will increase. Previous studies, showed that reactive oxygen species (ROS) induced by nanoparticles can cause destruction of red blood cells [12-14] and the data showed different effects according to changes in the diameter of nanoparticles, their distribution in body tissues and the route of intake and period time of exposure. In fact ROS from the of OSCPs have attacked hepatocytes and released ALT stored in them and entering into the blood serum; whereas the immune response of mice to an external factor has been the increase of the number of white blood cells for phagocytosis of nanosilver particles [15]. Similar data in our research were observed in female mice for ALT, AST and ALP in all treatment groups elevated compared to control.

Considering the importance of role of hepatocytes in detoxification, any changes made in their structure and number can cause very large physiological changes for human body. On the other hand, wide use of different nanosilver OSCPs in the whole world requires more accurate and comprehensive studies on the effects of these particles on blood cells. The use of laboratory mice as animal models, and various treatment methods and OSCPs with different combinations and diameters presents new horizons for further research to investigate applications of nanotechnology in physiology [16]. Also we observed an increase in ALP levels in male mice G1 and G2 groups in comparison to control that can be justified with inflammatory process and destruction of hepatocytes [17]. In accordance with study by Chang who showed that repeated oral doses of nanosilver for 28 days did induce liver toxicity, as shown by increases in serum activity of ALP [18]. Malondialdehyde MDA levels were higher in the study group than control group (Figure 4). The increased MDA level, as measured by thiobarbituric acid reaction substance (TBARS) method, in animals treated with OSCPs is a good indicator of oxidative damage. In addition, MDA, a product of lipid peroxidation, is generated in excess amounts depending on oral dose and small size. As a result of continuous daily dose for 28 days, the animals might be subjected to peroxidative tissue injury by the OSCPs overload. These findings might support the idea that OSCPs intake in animals leads to an enhanced generation of reactive oxygen species and oxidative stress.

Data observed that all animals treated with OSCPs suffer from low levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP<sub>x</sub>) and glutathione reductase, when compared with control animals and this decrease was significantly clear ( $P < 0.001$ ). Superoxide dismutases are the proteins co-factor with copper, zinc, manganese, iron or nickel. In animals, it exists in three different forms including SOD1 found in cytoplasm, SOD2 present in cytoplasm, and SOD3 is extracellular. Superoxide is the main reactive oxygen species, which react with nitric oxide radical and forms peroxynitrite; thereby, causing oxidative stress and cellular damage. SOD is the essential antioxidant that decreases the formation of ROS and oxidative stress; thus, protecting the cells from damage. Erythrocyte SOD protects the erythrocyte from being damaged during oxidative stress [19]. SOD activity in mice treated with OSCPs is decreased, resulting in pronounced inhibition in the enzymatic blood antioxidant.

Catalase (CAT), widely distributed in all cells, is present in high amounts in erythrocytes. It is an intracellular enzyme made up of four polypeptide chains with four porphyrin heme groups. Catalase is responsible for detoxification of hydrogen peroxide in the cells [20]. In present study, the enzymatic antioxidants CAT were significantly lower in mice treated with highly dose (1.0 mg/kg.b.w) at small size diameter 50 nm. ( $44.88 \pm 19.87$ ) as compared with healthy untreated animals ( $120.29 \pm 25.2$ ). Decrease in the activity of CAT could be due to increase in the lipid peroxidation product malondialdehyde, which can form crosslink's, thereby inactivating several membrane bound enzymes [21].

Glutathione peroxidase ( $GP_x$ ) belongs to group of antioxidant selenoenzymes that protects the cell damage by catalyzing the reduction of lipid hydro peroxides. This action requires the presence of glutathione. Glutathione peroxidase levels in the body are in close relation with the glutathione, which is the most important antioxidant present in the cytoplasm of the cells [22]. The present study demonstrated significant reduction in  $GP_x$  in mice treated daily higher dose of OSCPs at lower size 50 nm, as compared with untreated animals. Decreased levels of  $GP_x$  is due to inactivation by the increased super oxide anion production leading to an increase in oxidative stress [23]. Low level of  $GP_x$  seems to result from the enzyme inhibition or reduced activity due to excessive production of hydrogen peroxide. This study showed significantly lower levels of all the antioxidants GSH, GPX and SOD in mice intake OSCPs compared with the matched untreated controls.

The present study reported a deficiency in reduced GSH levels. GSH, which considered as a major intracellular reducing agent, which is very sensitive to oxidative pressure and has several important function such as: protection against oxidative stress, regulation of gene expression, induction of a apoptosis activation and proliferation in T lymphocytes. In conclusion, the oxidative stress in animals treated with OSCPS is mainly caused by peroxidative injury. Production of free radicals by OSCPS intake, alteration in serum antioxidant enzymes status play an important role in the cytotoxicity of these OSCPs intake. Impairment of the antioxidant status is associated with elevated plasma levels of lipid peroxidation. These results suggest that the observed increase of spontaneous alternation in the liver function could result from OSCPs induced oxidative stress. Altogether, these results suggest that OSCPs in higher doses are capable of inducing oxidative stress, which is responsible for hepatotoxicity in experimental animals. In addition, spontaneous alternation in the oxidative stress biomarkers indicates toxicity of OSCPs accumulation. These data suggest that OSCPs can induce oxidative damage through a ROS-mediated process. However, it remains to be investigated whether OSCPs induce free radicals directly or indirectly through depletion of antioxidant defense mechanisms depending dose e.g. caused by interactions with antioxidant systems [24,25]. The Recent study have shown that small dose of OSCPs are more effective antioxidant than large OSCPs. In the present study OSCPs size is varied with different doses, high doses with lower size are more toxic. In conclusion, the present study clearly demonstrated that, intraperitoneally administration of OSCPs to male and female mice causes alterations in liver enzymes and alteration in oxidant/antioxidant status.

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