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Original Article

Study on Physiological and Piochemical Activity of Silver Nanoparticles in Male and Female mice

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ABSTRACT

This research was carried out to investigate the effects of Nano-silver (Ag-NPs) on some of blood Parameters in mice. Forty mice including twenty male and twenty female, were randomly divided into eight groups: C1 (male control), C2 (female control), T1, T2, T3 (male treatment) and T4, T5and T6 (female treatment). After the acclimatization period, treatment groups were gavaged with Silver Nanoparticle solution, T1 (3mg/kg), T2 (300mg/kg), T3 (1000mg/kg) and T4 (3mg/kg), T5 (300mg/kg) and T6 (1000mg/kg) for 15 days. At the end of study period, blood samples for sera preparations were collected from the tail vein and, centrifuged and removed serum stored at -20°C until analysis. Total cholesterol (TC), triglyceride (TG), Urea and creatinin were measured with Eliza kits (Pars Azmun). The results showed that nano-silver has no significant effect on serum creatinin. Serum levels of urea in male T2 and T3 groups was statistically decreased compared to their own control group (C1) (P<0.05), whereas in female groups groups T4 and T6 showed a considerably statistical elevation in contrast to control group(C2) (P<0.05). All serum levels of triglyceride in male treatment groups were statistically decreased by the administration Silver Nanoparticle solution in comparison to their control group (C1) (P<0.05)., also in females T4 and T5 indicate a considerable falling contrast to C2(P<0.05). Cholesterol levels in T1 was decreased statistically (P<0.05), conversely T2 showed an statistically elevation compared to C1(P<0.05).In female Cholesterol level in group T6 exhibited considerable increase compared to C2(P<0.05), and groupT4 displayed statistically drop to C2(P<0.05). No change in creatinin amount, positively decreasing concentration of triglyceride, erratic alteration in cholesterol levels and different effect of nanosilver particles on urea levels in female and male mice are our finding from this research.

Key words: Silver Nanoparticles, Blood parameters, Mice, Male, Female

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INTRODUCTION

In the recent years, nanotechnology had rapid progress in the most of different scientific branches and showed the effects on all parts of human, animal, environmental, and industrial life. One of the substances used in nano-formulation is silver nano-particle. It has been used since ancient times for jewelry, utensils, monetary currency, dental alloy, photography, explosives, etc [1]. While their small size makes AgNPs so useful in medicine and industries, it may potentially possess a hazard to human health and the environment. The smaller particles, which provide a much larger surface area to mass ratio, are more reactive and toxic than their bulk counterparts [2]. Previous studies have reported that AgNPs are able to interfere with cellular functions, cause toxic effects and, moreover, may interfere with specific biological systems *in vitro* [3, 4]. In addition, several studies in animals have indicated that AgNPs can be translocated in the blood circulation and distributed to several organs, including the liver, kidney and lung, after exposure via subcutaneous injection [5], inhalation [6]and oral administration [7]. In most cases, the liver is suggested to be the main target organ for AgNPs [6, 7]. In accord, a significant amount of silver was detected in the liver of rats after oral administration of AgNPs at doses of 30–500 mg/kg/day for 90 days [7].Furthermore; nanomaterials can be modified for better efficiency to facilitate their applications in different fields such as bioscience and medicine. Nanosilver (silver nanoparticle, Ag NP) materials have a wide range of applications including spectrally selective coating for solar energy absorption [8,9], catalysis in chemical reactions [10], surface-enhanced Raman scattering for imaging [11], and antimicrobial sterilization

[12.13,14]. Nanoparticles with at least one dimension of 100nm or less have unique physicochemical properties, such as high catalytic capabilities and ability to generate reactive oxygen species (ROS) [15], Serious concerns arise because nanoparticles may not only cause adverse effects in primary organs directly exposed but also in secondary organs, such as the cardiovascular or central nervous system (CNS) [16]. Also Silver Nanoparticles can evoke an increase intracellular calcium amount and Cause oxidative stress [17]. It has been shown that SNP can translocate through and accumulate in an in vitro BBB model composed of rat brain microvessel vascular endothelial cells[18]. In another study, SNP induced inflammation and affected the integrity of a BBB model
composed of primary rat brain microvessel endothelial cells [19]. Our aim in this study was evaluation the Effect of Silver Nanoparticles on some serum parameters in male and female mice.

METHOD AND MATERIAL

Experimental Animals

All procedures that involved animals were approved by the Veterinary Ethics Committee of the Faculty of para Veterinary Medicine of Ilam University. Forty mice including twenty male and twenty female, were randomly divided into eight groups: C1 (male control), C2 (female control), T1 (male treatment,3mg/kg),T2 (male treatment,300mg/kg), T3 (male treatment,1000mg/kg) and T4 (female treatment,3mg/kg),T5(female treatment,300mg/kg) and T6(female treatment,1000mg/kg).Each group comprise five mice.The animals of each group were housed in separate cages with sawdust bedding. Mice were kept in a 25 ^{o}C room with a 12h light: dark cycle, had free access to feed and clean water, and were stabilized for two weeks before the start of the experiment.

Synthesis of Silver Nanoparticles

The AgNPs were synthesized in a one-step reduction process in an aqueous solution. In a typical preparation, a 400-μL aliquot of a 0.1-M AgNO3 aqueous solution was added into 100 mL of an aqueous solution containing 0.10 wt. % of the soluble starch and vigorously stirred for 1 h. The pH of the resulting solution was adjusted to 8.0 by adding 0.1 M NaOH solution. Under this experimental condition, the initial reaction mixture was colorless, and the growth of the AgNPs was monitored at different intervals using UV–vis absorption spectroscopy. After about 1 h, the solution turned light yellow, which indicated the initial formation of the AgNPs. The mixture was maintained at 50°C for 24 h, and the color of the
reaction solution became yellow.

Treatment: After the acclimatization period, treatment groups were gavaged with Silver Nanoparticle solution, T1 (male treatment,3mg/kg),T2 (male treatment,300mg/kg), T3 (male treatment,1000mg/kg) and T4 (female treatment,3mg/kg),T5(female treatment,300mg/kg) and T6(female treatment,1000mg/kg).

Serum analyses: On the last day of the study the overnight fasted animals were anesthetized with formalin and blood samples for sera preparations were collected from the tail vein of each mouse into sterile plain tubes. Serum samples were separated from the clot by centrifugation at 3000 rpm for 15 min using a bench top centrifuge (MSE Minor, England). Serum samples were separated into sterile plain tubes and stored in the refrigerator for analyses. Determinations of parameters were performed using an automated biochemical analyzer (Chemistry analyzer photometer DANA-4500). Total cholesterol (TC), triglyceride (TG), Urea and creatinin were measured with Eliza kits (Pars Azmun).

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 levels of urea in male T2 and T3 groups was statistically decreased compared to their own control group (C1),(Fig1), whereas in female groups groups T4 and T6 showed a considerably statistical elevation in contrast to control group(C2),(Fig2). All serum levels of triglyceride in male treatment groups were statistically decreased by the administration Silver Nanoparticle solution in comparison to their control group (C1), (Fig3) also in females T4 and T5 indicate a considerable falling contrast to C2(Fig4).

Creatinin amount did not show any statistical change in male and female groups (Fig5, 6). Cholesterol levels in T1 was decreased statistically conversely T2 showed an statistically elevation compared to C1, (Fig7).In female Cholesterol level in group T6 exhibited considerable increase compared to C2 and groupT4 displayed statistically drop to C2 (fig8).

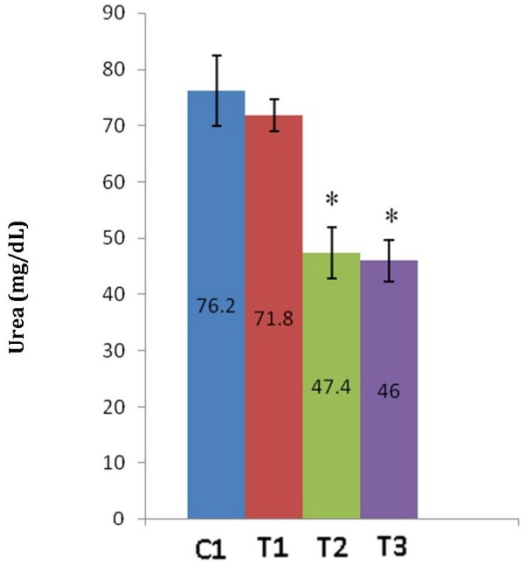

Fig. 1: The Urea levels in male control, test group received 3mg/kg (T1), test group received 300mg/kg(T2) and test group received 1000mg/kg (T3)Silver nano-particle. Each test group was compared with the control group. (* = P<0.05)

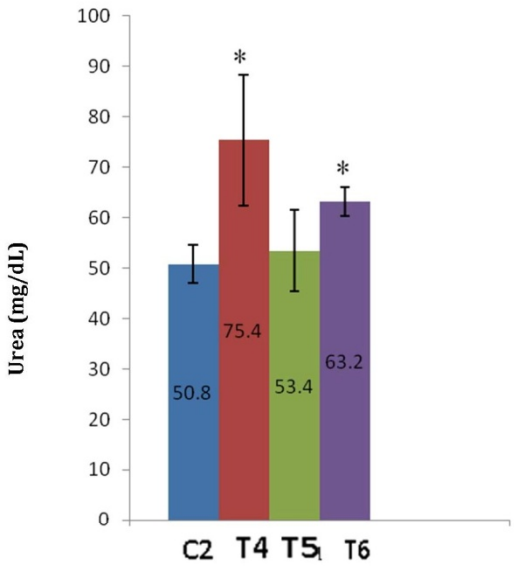

Fig. 2: The Urea levels in female control, test group received 3mg/kg (T4), test group received 300mg/kg(T5) and test group received 1000mg/kg (T6)Silver nano-particle. Each test group was compared with the control group. (* = P<0.05)

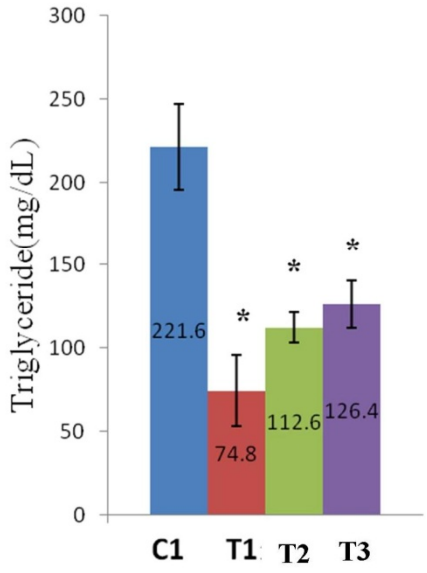

Fig. 3: The Triglyceride levels in male control, test group received 3mg/kg (T1), test group received 300mg/kg(T2) and test group received 1000mg/kg (T3)Silver nano-particle. Each test group was compared with the control group. (* = P<0.05)

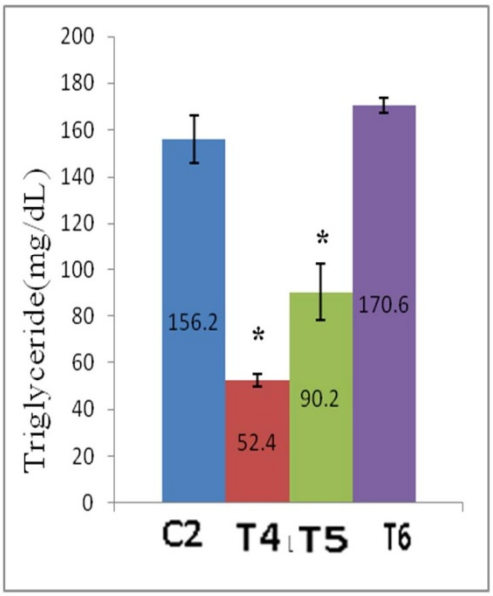

Fig. 4: The Triglyceride levels in female control, test group received 3mg/kg (T4), test group received 300mg/kg(T5) and test group received 1000mg/kg (T6)Silver nano-particle. Each test group was compared with the control group. (* = P<0.05)

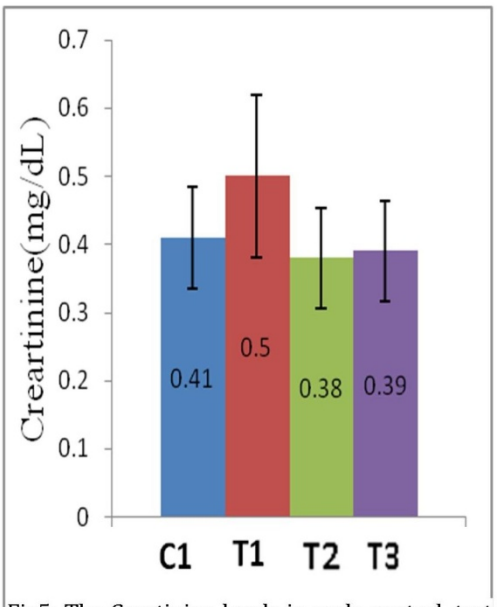

Fig5: The Creatinine levels in male control, test group received 3mg/kg (T_1), test group received 300mg/kg(T_2) and test group received 1000mg/kg (T_3)Silver nano-particle. Each test group was compared with the control group. (* = P<0.05)

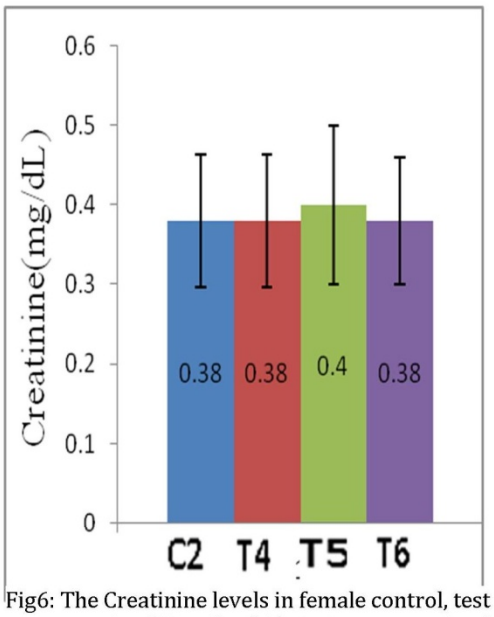

Fig6: The Creatinine levels in female control, test group received 3mg/kg (T_4), test group received 300mg/kg(T_5) and test group received 1000mg/kg (T_6)Silver nano-particle. Each test group was compared with the control group. (* = P<0.05)

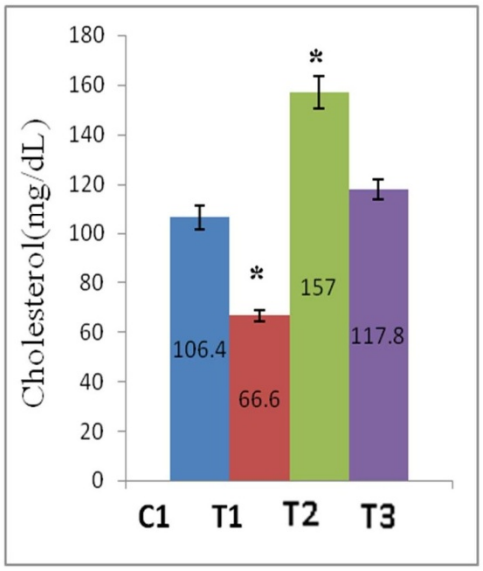

Fig 7: The Cholesterol levels in male control, test group received 3mg/kg (T_1), test group received 300mg/kg(T_2) and test group received 1000mg/kg (T_3)Silver nano-particle. Each test group was compared with the control group. (* = P<0.05)

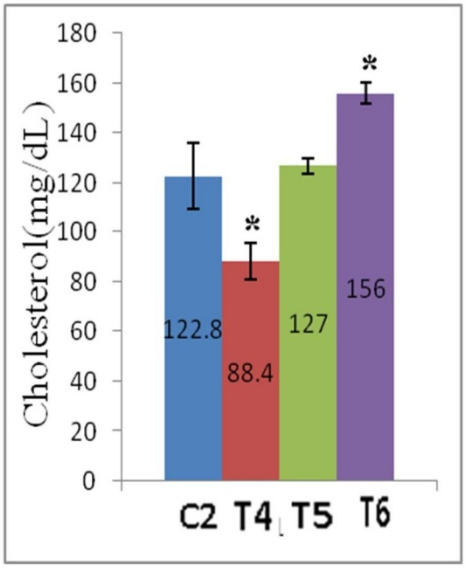

Fig. 8: The Cholesterol levels in female control, test group received 3mg/kg (T_4), test group received 300mg/kg(T_5) and test group received 1000mg/kg (T_6)Silver nano-particle. Each test group was compared with the control group. (* = P<0.05)

DISCUSSION

The increasing and widespread use of AgNPs is of increasing concern with regard to the safety of human health, especially in consumer products and medical applications.Our results indicated that administration of silver nano-particle was effective in decreasing triglyceride amount of blood in mice. Adversely Farhad Ahmadi reported that silver nano-particle was induce a statistically elevation in chicken serum Triglyceride. silver nano-particle s are able to produce oxidative stress that caused peroxidation of fat and release of free radicals in the body[20]. Research showed

function of mitochondria [21, 22] that exposure to silve nano-prarticles significantly decreased the mitochondria which seem to be sensitive targets to cytotoxicity of silver nano-particles. In the study with BRL 3A liver cell line, depletion of GSH level and increased ROS was found in association with mitochondrial perturbation, suggesting that oxidative stress might mediate the cytotoxicity of silver nano-particles. Recently, it has been found that Ag+ seems to perturb mitochondria through interactions with thiol groups of the mitochondrial inner membrane. As a result of the experimental studies by Gopinath *et al*. (2008) [23], it is known that nanomaterials can pass through cell membranes easily and cause severe toxic effects on human health. They have concluded that silver nanoparticles in higher concentrations (> 44.0 μg ml-1) are necrotic to cells, leading to rapid cell membrane rupture. Whereas in this study, it appears that triglyceride levels was not been affected from this hazardous process. Cholesterol levels in male mice group T2 was statistically increased compared to control group and also we observed a considerable elevation in T6 group that may be justified with mentioned hazardous effects of silver nanoparticles. However , Ewa Sawosz and his coworkers in a study found out that exposure chicken embryos to nano-silver particles has no effect on Triglyceride and cholesterol levels [24]. We are not observed any alteration in creatinin level in treatment groups. However the study of Rosenman revealed, creatinin clearance was depressed in humans orally administered silver nano-particles [25]. Sriram et al. in 2010 proved that nanosilver particles can activate Caspase mitochondrial enzymes, especially Caspase3 in lymphoid cancerous cells and cause planned death or apoptosis in them [26]. In the present study, considering the diameter and shape of used nanoparticles, anti-apoptotic pathways has been probably strengthened in white blood cells and apoptotic pathways in hepatocytes[27] and in other study it is clarified thet administration of Nanosilver caused depression and necrosis in liver cells[28]. In fact, free radicals from the nanosilver particles have attacked hepatocytes and therefore urea cycle in liver may be negatively affected; so probably the ammonia produced from proteins catabolism in body can not convert to urea. Hence decreased urea concentration in male animals in present study may be justified.

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