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Original Research

Ingestion of silver nanoparticles leads to changes in blood parameters

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Abstract

Objective(s): The silver nanoparticles, being very small size, can permeate the cellular membrane and interfere in the cell's natural process. In the present study, the effects of time, the dosage of these particles and their use on blood molecules and hormones, the volume of drinking water, and the urine parameters were analyzed.
Materials and Methods: Thirty six rats of the Wistar race, as subjects, were divided into six groups (one control group: C and five test groups: T1-T5). In the test groups, drinking water was replaced by the Nanosilver (NS) solution with concentrations of 5, 20, 35, 65, 95^{ppm}. After three and six months, three rats were chosen randomly from each group, and their blood was collected. Various blood parameters were measured instantly, and the results were processed by one-way analyses of variance and Tukey's test.
Results: The animal's uptake of water increased significantly in parallel with the increasing of the particles' concentration. Ketone bodies were noticed to be present in the urine of the female rats received high doses of the particles. The level of T4 decreased considerably ($p<0.05$) in parallel with the time and the concentration of the received particles. Depending on the dosage, and the time of use, blood testosterone increased, and the level of blood cortisol decreased. The observed effects were more evident in the proceedings with the concentration of 35ppm.
Conclusion: Ingestion of NS particles, especially by high doses and in long terms, can cause high blood pressure, tissue injury–particularly liver injury–and endocrine glands.

Keywords: Cortisol, Ketone bodies, Nanoparticles, T4, Testosterone, Silver

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Introduction

Nanoparticles are particles with a diameter of about 10-^{9} m. Owing to their minuscule dimensions, they have unique physical, chemical, electrical, and magnetic properties. For example, they can permeate cells and interfere with their natural process (1).

Nanoparticles of silver have turned into the most applied of these particles. The have great antimicrobial effects, and are used increasingly in various industries such as hygienic & cosmetic industries, catheters, antiseptic sprays, cleansers, and tooth-pastes. Their worldwide daily usage has made studies on the fitness of their use necessary. (2).

Bactericidal effects of silver are known for centuries. During recent decades, silver derivatives have been used as antiseptics in industry and medicine. Using silver sulfide as an antibiotic for babies' eye infection, and for the control of chlamydia or gonorrhea (3) are widespread.

More than 600 known microbes sensitive to Nanosilver (NS) are mentioned in different references such as HIV (4, 5).

Silver is naturally present in most tissues, but its biologic role remains indefinite (6). Some believe it to be for the proper function of the immune system (3). Silver colloidal (ionized) solutions have been produced and used. In recent years, and simultaneous with the beginning of nanotechnology, a new form of this metal, as the NS solution, has entered the market. The NS solution includes the suspension of deionized distilled water with silver which form has 80% metallic NS and 20% colloidal (ionized) silver.

These solutions are quite similar, but the dimensions of silver particles in the NS solutions are less than 5 nm, while in the colloids, they're about 10 nm.

The particles of NS, not only for their smaller dimensions but for their neutrality, are superior to silver colloidal ions. They have more surface area in the environment, which increases their effectiveness and improves their absorption and penetration into cells. In the NS solutions, a particular (metallic) form of silver, which is its active form, is more than its ionized form.

In the stomach and blood, ionized silver turns into insoluble silver with very low effectiveness (only 5-10% of them remain active), while metallic silver is resistant to stomach acid and conserves its activity.

The best colloids (20 ppm silver solution with 10% of 10 mm particles), in particle size, are at least five times bigger, and in density of metallic form are eight times weaker than the NS solution (1).

As a result, much stronger bactericidal effects, with less material, are expected through using NS, so that in the reports with lower amounts than 360 mg/day (an amount that EPA has affirmed as the recommended daily use of silver colloid), the desired results have been achieved (5, 7, 8).

It seems that if the possibility of toxicity and injury of these particles are evaluated, they can be a suitable substitute for the present antiseptic material in foodstuff industries such as nitrites. Silver isn't metabolized in the body (liver), and it is predicted to be collected in it with further use (3).

Also since the industrial manufacture of NS is less than one decade old, all of the carried studies concern the effects of the colloid silver, and the studies about NS are limited and few (2,8,9,10,11,12). Most of these studies focused on the effects of NS inhalation (9,12,13), skin absorption (14,15), the effects of cytotoxic (6,10,11,16,17), and their use in nutrition.

In this study, the changes in some of the biochemical, hematologic, humoral, and urinary parameters of rats of the Wistar race, through eating these particles, have been analyzed in order to evaluate the probable effects of these particles on the liver and bone marrow.

Materials and Methods

Animals

Thirty six eight-months-old rats of Wistar race, after a period of being kept undivided for two weeks in their room (25˚C and half-day lighting) were divided into six groups (one control group: C and five test groups: T1-T5).

The study began by replacing the water in the drinking cups of the cages with NS solution. The 4000 ppm NS solution was made by Nano Nasb pars Co. With respect to LC50 of about 10 ppm (18,19,20), the concentrations of 5 ppm, 20 ppm, 35 ppm, 65 ppm, 95 ppm were chosen until the used doses were close to the lethal dose, and the effect of high doses (95 ppm, 65 ppm), which is possible to be used by mistake, were assessed.

The aforementioned concentrations were stored in containers and used. After three and six months, three rats from each test group were chosen randomly and, after being anaesthetized by ether, their blood were collected directly from the heart. Different blood parameters, including sugar, triglyceride, cholesterol, HCT, HB, cortisol, testosterone, and T_4, were measured instantly by human kits (Pars Azmoon Co.). Counting the blood cells was done by an automatic H1 equipment. Ultimately, the results were processed by one-way analyses of variance and Tukey's test on a significance level $p<0.05$.

Results

Biochemical parameters

After three and six months of receiving different doses of NS, animals' blood cholesterol level decreased, but, compared to the control group, the fall was insignificant (Figure 1).

Also, after six months, progressive increase of the cholesterol level of subjects' blood was considerable. Subjects' blood sugar changes and its decrease, although insignificant, was dependent on the dose, particularly within 35 ppm.

The connection between duration of use and blood sugar level was evident both after three and six months. Figures 1 and 2 show the solution dose ($p<0.01$ after three months, and $p<0.05$ after six months) and the use duration ($p<0.01$) have both caused a great decrease in subjects' blood triglyceride.

The decrease in blood triglyceride was considerable in 35 ppm.

Hematologic parameters

Hematologic parameters, including the RBC, WBC counting, Hb, HCT, were assessed after six months (table 1). The WBC counting decreased ($p<0.01$) dose dependently.

The $p<0.001$ in 35 ppm, demonstrated special effects of this dose.

Humoral parameters

The cortisol decreased significantly ($p<0.001$ after three months, and $p<0.001$ after six months), and dose dependently. Also T_4 decreased ($p<0.001$) and the testosterone increased ($p<0.001$).

Water uptake & urine analysis

According to tables 2 & 3, by increasing the dose of the used solution, the water uptake index and animals urine volume increased significantly ($p<0.001$).

Among various urine parameters, only the presence of ketone bodies in the urine of female animals that used high doses was considerable, and the other parameters were normal.

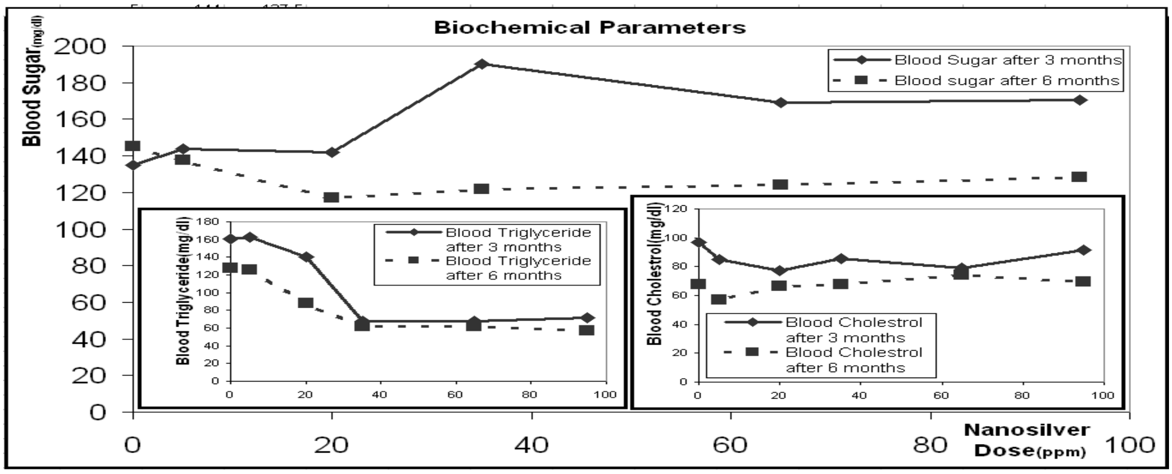

Figure 1. Biochemical parameters (sugar, triglyceride, cholesterol) changes in animal's blood after 3 and 6 months of NS consumption.

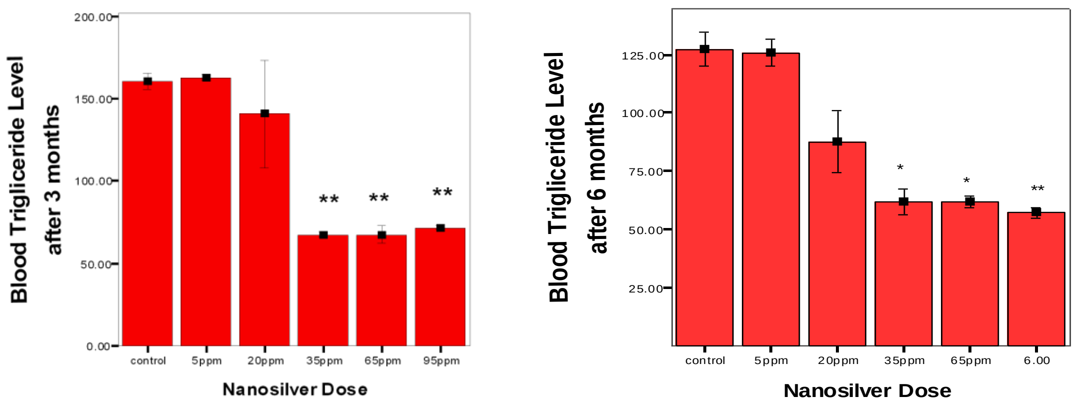

Figure 2. Animals blood triglyceride level after 3 and 6 months of NS consumption (*: p<0.05, **: p<0.01, ***: p<0.001).

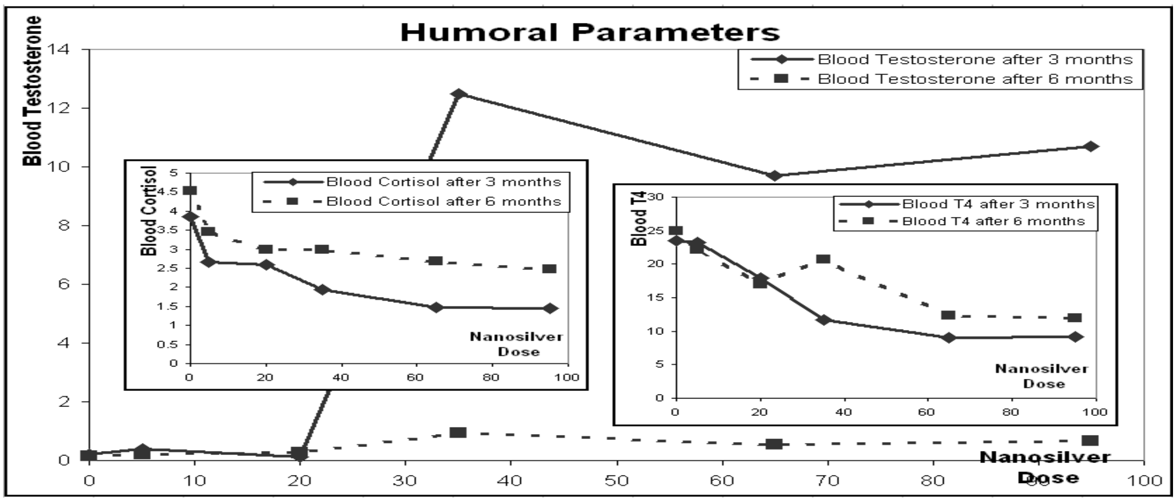

Figure 3. Animals blood Cortisol, Testosterone and T4 changes after 3 and 6 months of NS consumption.

Table1. Animals blood cells count, HB and HCT after 6 months of NS consumption.

NS Dose (ppm)	RBC (Count/µl)	Hemoglobin (g/dl)	WBC (Count/µl)	HCT (%)
0	5.53	12.9	11.4	30.4
5	5.46	14.3	10.85	28.2
20	6.1	13.55	8.62	32.3
35	5.77	13.7	5.4	30.57
65	5.7	13.9	9.2	29.93
95	6	13.86	8.4	31.13

Table 2. Total volume and dose of consumed solutions after 3 months of NS consumption**.**

Group	Total volume of consumed solution (Li/cage)	Total volume of consumed solution (Li/subjec	Number of subjects (/cage)	NS concentration of solutions (ppm)	Total consumed NS (ppm/cage)	Total consumed NS (ppm/subject)
C	20	3.33	6	0	0	0
T1	24	4	6	5	120	20
T2	26	4.33	6	20	520	86.66
T3	33	5.5	6	35	1155	192.5
T4	36	6	6	65	2340	390
T5	42	7	6	95	3990	665

Table 3. Total volume and dose of consumed solutions after 6 months of NS consumption.

Group	Total volume of consumed solution (Li/cage	Total volume of consumed solution (Li/Subject)	Number of Subjects (/cage)	NS concentratio n of solutions (ppm)	Total consumed NS (ppm/cage)	Total consumed NS (ppm/subject)
C	8	2.66	3	0	0	0
T1	11	3.66	3	5	5	18.33
T2	13	4.33	3	20	3	120
T3	16	5.33	3	35	5	186.66
T4	15	5	3	65	9	325
T5	25	8.33	3	95	2	791.66

Discussion

Biochemical analysis

No significant changes were observed in the blood cholesterol level, depending on dose or the time of NS used. But the increase in blood cholesterol was considerable depending on different doses. This increase shows the effects of these particles on the liver as the main place of the synthesis and regulation of the blood cholesterol in the long term.

The contradictory changes of animals' blood sugar, which were related to the solutions dose, shows the effect of the using period is obvious as the decrease of blood sugar, in comparison with the parallel amounts of any dose after six months, especially in the 35 ppm dose.

Table 4. Animal's urine (24h) parameters after 9 months of NS consumption**.**

Ascorbic Acid	Ketone	Protein	Blood	Nitrite	Glucose	PH	Urine Volume (ml)	Sex	NS concentration (ppm)	Group
+	-	30	-	+	-	7	1	M	0	Male
+	-	30	-	-	-	5	1	F	0	Female
+	-	30	+	-	+	6	13	M	35	T3
+	-	30	-	-	-	6	7.5	M	65	T4
+	-	30	-	-	-	5	3	M	95	T5
+	-	30	-	+	-	6	3	M	95	T5
+	+	30	-	-	-	5	1.5	F	95	T5
+	+	30	-	-	-	5	1.5	F	95	T5

On the other hand the expressive effect of time and the dose of used solution in the triglyceride level of subjects' blood make the probability of injury to the tissue of the liver by the silver nanoparticles more serious.

Hematologic analysis

The decrease in the counting of WBC after six months proves the occurrence of cell apoptosis through different ways, something that the results of the other sources have also pointed out. The observation of $p<0.001$, with respect to the dose of 35 ppm, shows the special effects of this limitation, which is similar to what is seen in figures 1, 2, and 3 on blood triglyceride.

The absence of measurable change in the counting of RBC and amount of HB and HCT shows the ineffectiveness of the studied material on the effectiveness of red bone bruin (red marrow) and the hematopoietic process.

Humoral analysis

The decrease in the animal blood cortisol and T4 levels, and the increase in their testosterone level, both significant and dependent on dose and duration of particles consumption, proved the effect of these factors on different glands.

Water uptake & urine analysis

Also in parallel to the used solution, the increase in water uptake index, blood osmotic pressure, and volume of animals urine increase are the effects of the use of these particles. The appearance of ketone bodies only in the urine of the female rats, after using high doses of the particles, is a sign of their interference in the function of kidneys, especially since it had happened dependent on the sex of the animals.

Based on results, ingestion of these particles, especially within high doses and in long term, causes high blood pressure, tissue injury–particularly liver injury–and endocrine glands.

Liver histopathology and the glands, by the measurement of the indicated parameters of the liver function, can be considered in the next studies.

The precise study of the causes of cell apoptosis, particularly in the immune system, and the decrease in the number of these cells in blood, is necessary. Also the study of the distribution of these materials in different tissues, and the method of their processing in the tissue of the liver, especially with bigger statistical population, have been emphasized.

Conclusion

Based on results, ingestion of these particles, especially within high doses and in long term, causes high blood pressure, tissue injury–particularly liver injury and endocrine glands. The pathological changes in treatment group 3 (35 ppm) was more intense compared to the other groups. Liver histopathology and the glands, by the measurement of the indicated parameters of the liver function, can be considered in the next studies.

On the other hand, worldwide use of different GNPs requires more accurate studies on the effects of these nanoparticles with different concentrations and shapes for further research on the usage of nano-technology.

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