Effect of silver nanoparticles on the Levels of Mineral Blood elements In Vivo

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Abstract

The present study, silver nanoparticles (AgNPs) were synthesized using an atmospheric pressure non-thermal plasma method at (6 min) exposure time. XRD and (FESEM) were used to investigate the structural and morphology properties of the silver nanoparticle. While optical properties were investigated using UV/VIS spectroscopy. This study was designed to investigate the dose and time dependent effect of AgNPs on the levels of Fe, Na, Ca, Cl in the blood of female rats. Rats were divided into (4) groups, each group contains (5) animals. The first group was a control group. The second group was dosed with (170 ppm) Ag nanoparticles ; the third group was dosed with (340 ppm) Ag nanoparticles, and the fourth group was dosed with (680 ppm) Ag nanoparticles . The time between one dose and another was one week. Every week, the blood levels were analyzed. The Fe , Ca concentrations in the blood of rats increased when the exposure period and the concentrations of silver nanoparticles were increased compared to the control group. There were significant differences in the effects of silver nanoparticles on (Na , Cl) concentrations in the blood serum with increasing doses and time of treatment.

Keywords: Silver Nanoparticles, Mineral Blood elements, IRON, Sodium, Calcium

Introduction

Nanoparticles are particles with lengths ranging from 1 to 100 nm , two or three dimensions, according to the ASTM standard definition[1] , Nanoparticles display new or fully optimized characteristics, such as molecule size, distribution, and morphology. The particles which have nano size are quite unique in nature due to the nano size increase surface area to the weight or volume ratio as well as their physical , biological and chemical properties is different to the relative of bulk material [2]. Among the diverse nanomaterials available, noble metallic nanoparticles have been used in a variety of application in the fields of electronic, magnetic, optoelectronics, and information storage [3]. Silver nanoparticles (Ag NPs) belong to the noble metal nanoparticles group. Due to their superior physical, chemical, and biological characteristics , silver nanoparticles (Ag NPs) have been extensively studied. Their superiority stems mostly from the shape, size, crystallinity, composition, and structure of Ag NPs as compared to their bulk forms. [4,5]. In addition the catalytic performance and anti-bacterialeffects of AgNPs have been implicated in fields of bio-sensing and antimicrobial applications [6]. Therefore, Ag NPs were used progressively in treatment of diseases with bacterial origin such as urinary tract infections [7]. Such wide application increases the incidence of exposure and accumulation of these particles within biological system to the level that may cause toxicity. Since, AgNPs can be hazardous to human health, because the small size of particles enhance their absorption through the skin and cell membranes including; blood-brain barriers and blood-tissue barrier [8], and distributed with circulation to different biological targets. At the cellular level, Ag-NPs are known to interfere with normal cellular functions that may led to toxic effect [9]. Animal research suggest that inhalation , ingestion or injection of nanoparticles can lead to the sedimentation of them in skin or lungs and their later movement from the primary sedimentation sites to secondary sites such as liver , spleen , kidneys , muscles , brain [10].

Experimental

1- Synthesis Ag Nanoparticles Colloidal

synthesize silver nanoparticles (AgNPs) by atmospheric pressure plasma jet. Aqueous solutions of silver nitrate ($AgNO_3$) were prepared in concentration (1 mM) with volume (100) ml of deionized water and equation (1) was used to calculate the required weight with a molecular weight of 169.88 gm /mol :

Concentration (mole) = (mass (g) / (Molecular weight (g / mol) × volume (liter)) … (1)

When the circuit of Cold plasma was switched on , plasma was formed between the stainless steel tube and the aqueous solution of $AgNO_3$ and the exposure time was 6 minutes. After few minute of plasma exposure to the aqueous solution $AgNO_3$, the color of the solution is changed to dark brown which is the property of Ag NPs formation show in UV–Vis absorbance.

2- Experimental animals

The study was conducted on 20 adult female rats . The rats were divided into four groups , and each group contained five rats. The first group was a control group, and three other groups received Ag nanoparticles that were administered orally. The second group was dosed with (170 ppm) Ag nanoparticles ; the third group was dosed with (340 ppm) Ag nanoparticles, and the fourth group was dosed with (680 ppm) Ag nanoparticles. The time between one dose and another was one week. Blood was drawn directly from all groups every week after each dose , to measure Fe, Ca, Na, Cl in blood. The doses were repeated regularly over four weeks.

Blood was drawn from rats over (7, 14, 21 and 28) days after dosing with different concentrations of silver nanoparticles. Serum was separated by centrifugation of blood sample at (3000) rpm for 15 min then kept frozen at (-20º C) until the time for using.

3- Statistical analysis

The obtained data of this study were expressed as mean ± SD. The data were statistically analyzed according to the design of the two-way factorial experiments by the method of analysis of variance according to the completely randomized design (CRD) using the statistical program (SPSS) and the use of the least significant difference (LSD). P-value less than 0.05 were considered to be significant for all measured parameters [11].

Results and Discussion

1- UV–visible (UV-Vis) absorbance

A cold plasma system was used to produce silver nanoparticles **.** The color change is an early indicator of the generation of Ag nanoparticles. The absorption spectrum of Ag nanoparticles is clarified in Figure (1) where the absorption peak appear at 400 nm for $AgNO_3$ concentration (1 mM) at (6) minute of plasma exposure , the color of the $AgNO_3$ solution changed from a transparent solution to a dark brown solution indicating the formation of Ag nanoparticles , because of the peak shifted to blue due to the surface plasmon resonance (SPR) of Ag nanoparticles [12, 13].

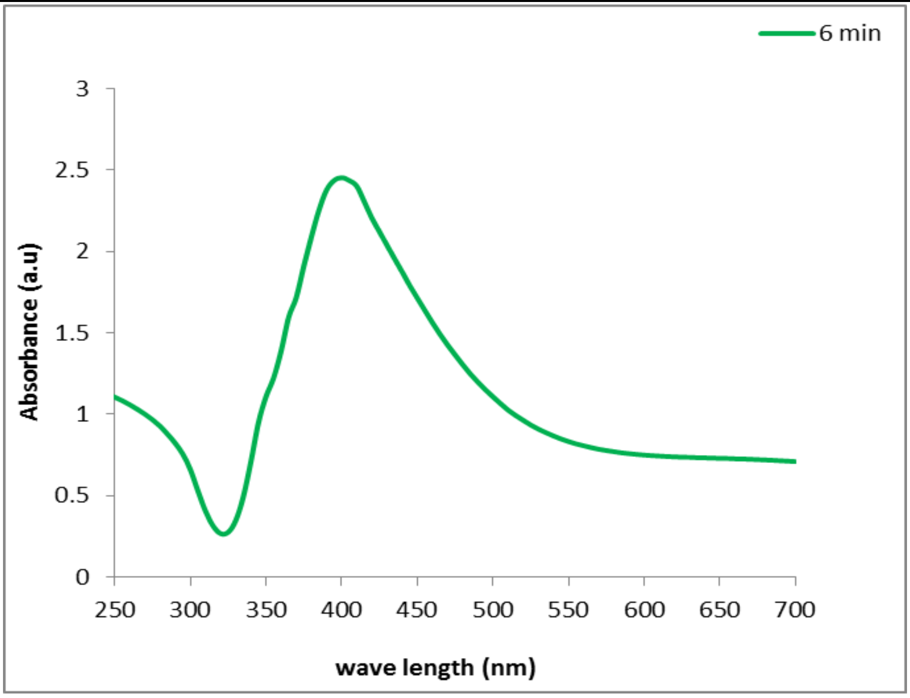

Figure 1: UV–VIS absorbance spectra of Ag NP_S at exposure time 6 min with concentration (1mM).

2- XRD analysis of Ag NP_S

Figure (2) shows the X-ray diffraction pattern of prepared silver nanoparticles at exposure time (6 min) at concentration (1 mM) of silver nitrate ($AgNO_3$), the peaks detected at (2Ө = 38.1°) and (2Ө = 44.27°) assigned to the formation of cubic silver nanoparticles (JCPDS reference code : 04-0783) , the results of XRD pattern showed adiffraction pattern from silver nanoparticles with a fcc crystalline structure [14, 15].

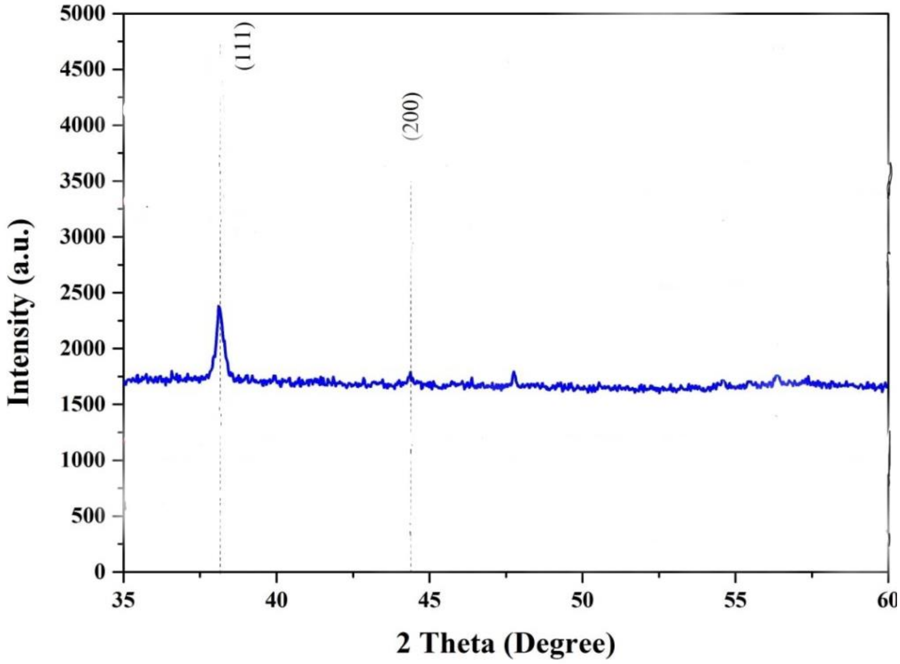

Figure 2: XRD pattern of Ag NPS at exposure time 6 min with concentration (1mM).

3 - FESEM Measurement of silver nanoparticles

The morphologies character of prepared silver nanoparticles were evaluated by FESEM measurement , Figure (3) shows the microscopic images of Ag nanoparticles of exposure time (6 min) of plasma at (1 mM) of $AgNO_3$ concentration, the obtained images reveals that the particle size of prepared silver nanoparticles is variable and is within the range of (28−58) nm. The average particle sizes are (47.16 nm) at the plasma exposure time (6 min). The Ag were almost spherical nanoparticles.

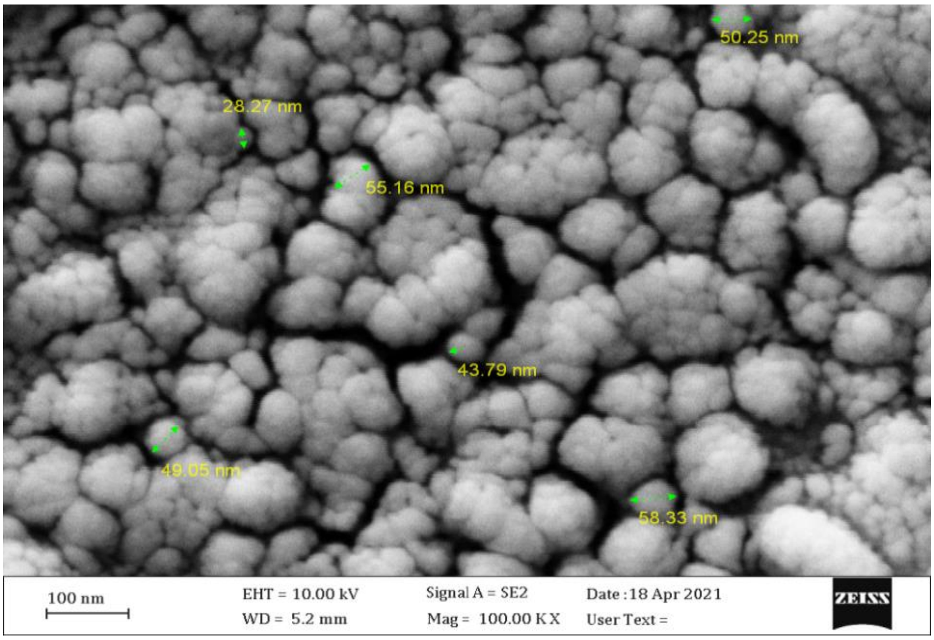

Figure 3: the microscopic images of Ag nanoparticles

4. Effect of silver nanoparticles on mineral blood elements

4.1 IRON Concentration (Fe)

The statistical analysis of iron concentration was demonstrated in figures (4 , 5) , which showed different doses (170, 340 and 680 ppm) of Ag NPs for different durations (7, 14, 21 and 28) days , the results showed a significant effect of the studied factors on the rate of iron concentration in the blood serum of rats , the highest rate of iron concentration in the 7-day exposure period (105.25 µg/dl) with a significant difference from the exposure period 21 and 28 , where the average iron concentration was (96.3 µg/dl) and (91.42 µg/dl), respectively, and the lowest rate of iron concentration in the control group was (81 µg/dl), significantly lower than the other of the treatments.

The results of treatment with different concentrations of silver nanoparticles showed that there were significant differences in the concentration of iron in the blood serum of rats, where the highest rate of iron concentration when treated with a concentration of (680 ppm) of silver nanoparticles was (116.3 µg/dl) and a significant difference from the other concentrations (170, 340) ppm and the iron concentration reached (92.6 , 107.3) µg/dl , respectively, and the lowest average iron concentration was in the control (81 µg/dl), with a significant decrease compared to the other of the treatments.

In Figure (4) data expressed as mean ± SD , ($P \geq 0.05$) among all groups. The figure shows an increase in the iron level in the blood of rats by increasing the dose of silver nanoparticles compared to the control group. By changing the periods of dosing, it was observed that the lowest iron rate at a time of 28 compared to the other of the dosing times.

in general, the level of iron in the blood of rats increased when the exposure period increased and the concentrations of silver nanoparticles increased compared to the control group [16].

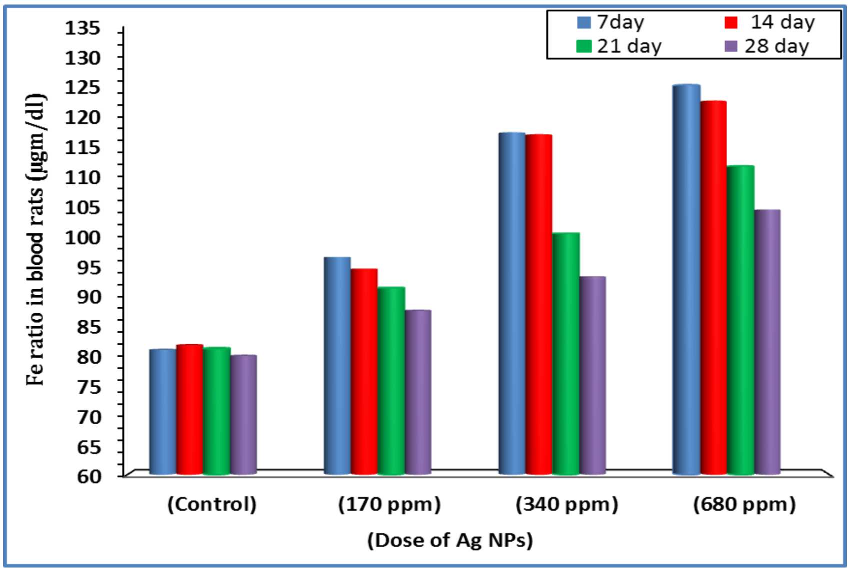

Figure 4: Iron (Fe) concentration in serum of rats during days of doses with Ag NP_S at different concentrations.

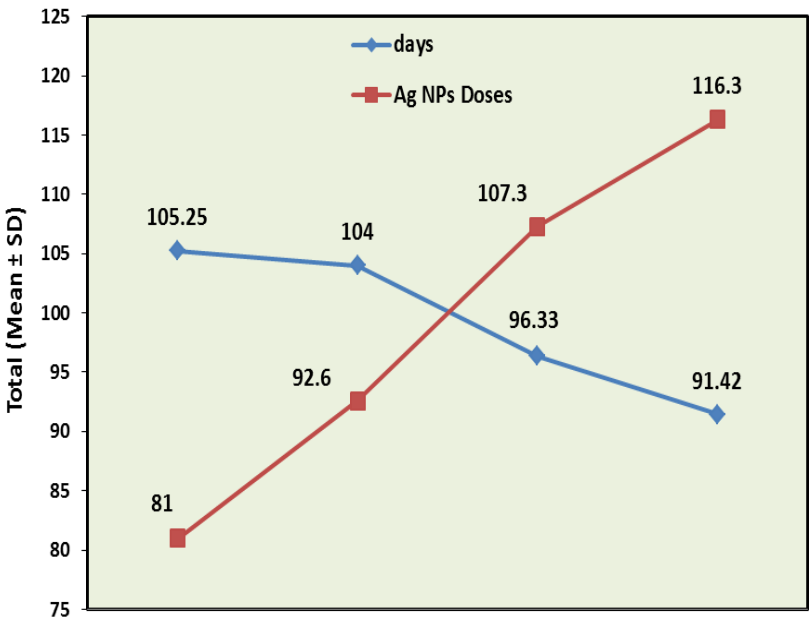

Figure 5: Total (Mean ± SD) of iron concentration in rats when the dose and time are variable.

4.2 Sodium Concentration (Na)

The results of the statistical analysis of the concentration of sodium in the blood serum of rats showed a significant effect for the exposure period and the concentrations of silver nanoparticles as shown in Figures (6 , 7) , as it reached the highest concentration of sodium (126.42 mmol/L) in a period of 28 days and for all concentrations of silver nanoparticles with a significant difference compared to the control group, where the concentration of sodium was (123 mmol/L).

The treatment of animals with different concentrations of silver nanoparticles showed significant differences, as it showed the superiority of the concentration of (680 ppm) Ag nanoparticles if the highest concentration of sodium reached Na (126.75 mmol/L), and a significant increase compared to the control group, where the concentration of sodium reached (123 mmol/L). There were no significant differences in the concentrations of (170 ppm) and (340 ppm) which the sodium concentration was reached (121 mmol/L) and (123 mmol/L).

In figure (6) the results of the statistical analysis of the interaction of the studied factors showed that there were no significant differences between all the studied interactions, and the highest rate of sodium was observed at the interaction of (680 ppm) Ag nanoparticles in a period of exposure of 28 days. It amounted to (135 mmol/L), while the lowest rate of sodium concentration was at a concentration of (170 ppm) of silver nanoparticles at a period of time exposure 7- days and amounted to (116.3 mmol/L).

the effect of cold atmospheric plasma on mineral blood is studied with different exposure durations (30,45,60) sec. As the plasma exposure duration increases, the sodium and chlorine elements decreased [16].

some researchers reported that silver and silver salts are distributed to the whole body. The researchers showed that, silver nanoparticles cause the permeability of cell membrane to potassium and sodium, and disrupt the activity of Na-Katpase and mitochondria [17].

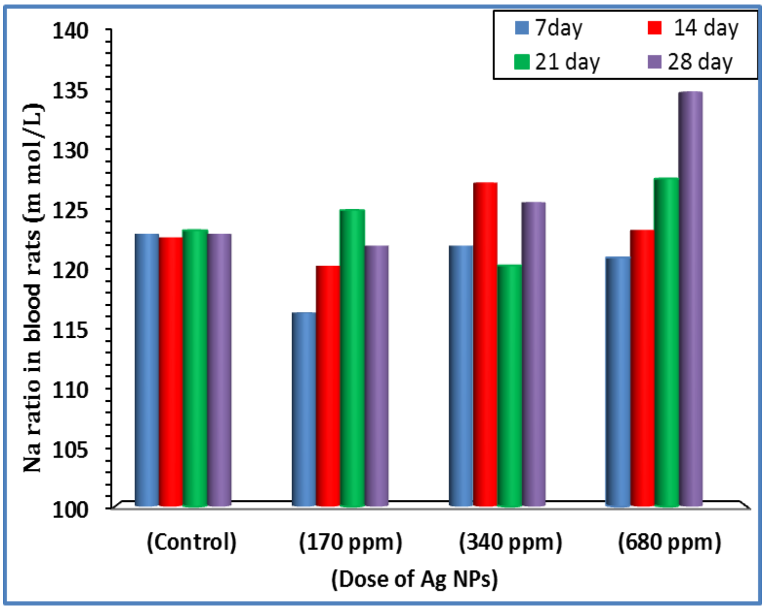

Figure 6: Na concentration in serum of rats during days of treatment with Ag NP_S at different concentration.

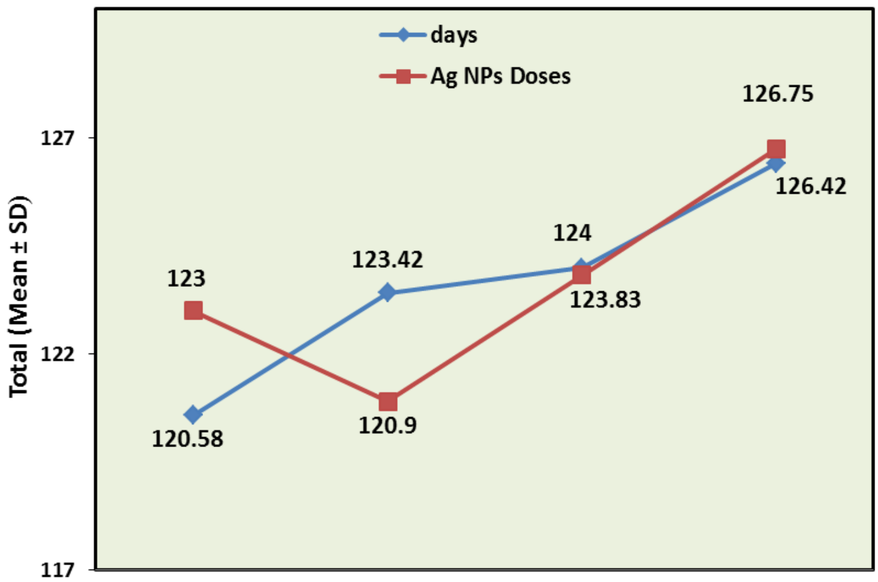

Figure 7: Total (Mean ± SD) of Na concentration in rats when the dose and time are variable.

4.3 Calcium Concentration (Ca)

Statistical analysis of data related to Ca Concentration was plotted in Figures (8,9).The results of the current study showed that there were no significant differences for the effect of silver nanoparticles on the calcium concentration in the blood serum of rats according to the exposure period. The highest rate of calcium concentration was recorded in the 28-day exposure period (10.03 mmol/L) without a significant difference from the other exposure periods (7, 14 , 21), as the calcium concentration reached (9.72, 9.84 and 9.77) mmol/L, respectively.

When studying the effect of concentrations of silver nanoparticles on the level of calcium in the blood serum of rats, significant differences were shown as fig (9). The highest concentration of calcium at the dose (680 ppm) was (10.25 mmol/L), with a significant difference from the other two concentrations (170 , 340) ppm . The calcium level were (9.84 mmol/L) and (9.76 mmol/L), respectively.

It was noted that the lowest concentration was in the control group, where the level of calcium reached (9.53 mmol/L) , and it was a significant decrease compared to the highest concentrations of calcium in the treatment (680, 170) ppm of silver nanoparticles.

When studying the effect of the interaction between the concentration of silver nanoparticles and the exposure period , no significant differences appeared , the highest rate of calcium concentration at the (7,14,21,28) day exposure period with a concentration of (680 ppm) silver nanoparticles was (9.96, 10.22, 10.15, 10.3 mmol/L) respectively , while the lowest level of calcium in the control experiment was (9.52 mmol/L).

The results of the effect of silver on calcium levels were comprable with those of the control group, indicating that the silver nanoparticles had no toxic effect on calcium levels because there was no significant decrease or very large increase in levels.

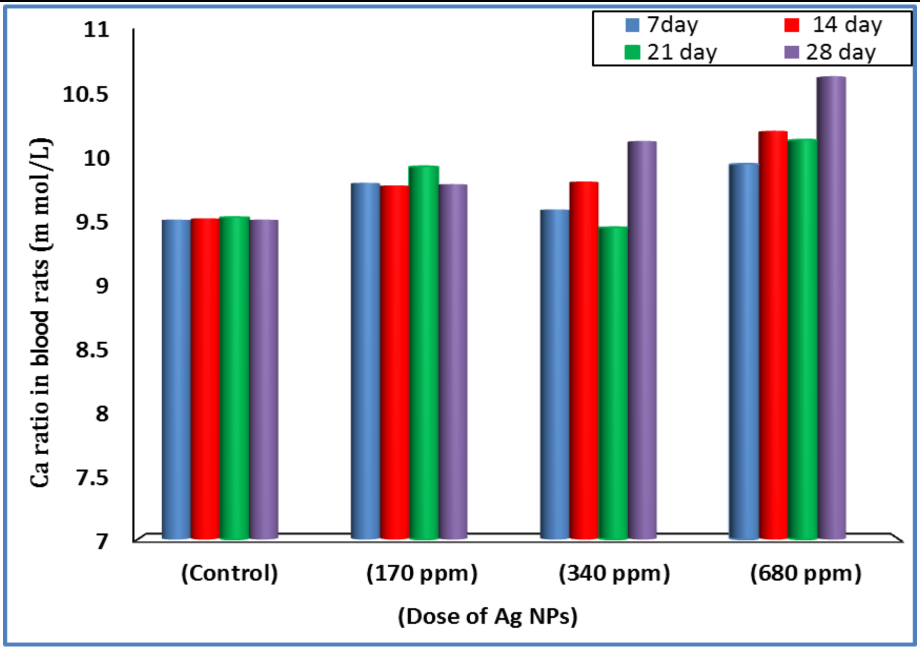

Figure 8: Ca concentration in serum of rats during days of treatment with Ag NP_S at different concentration

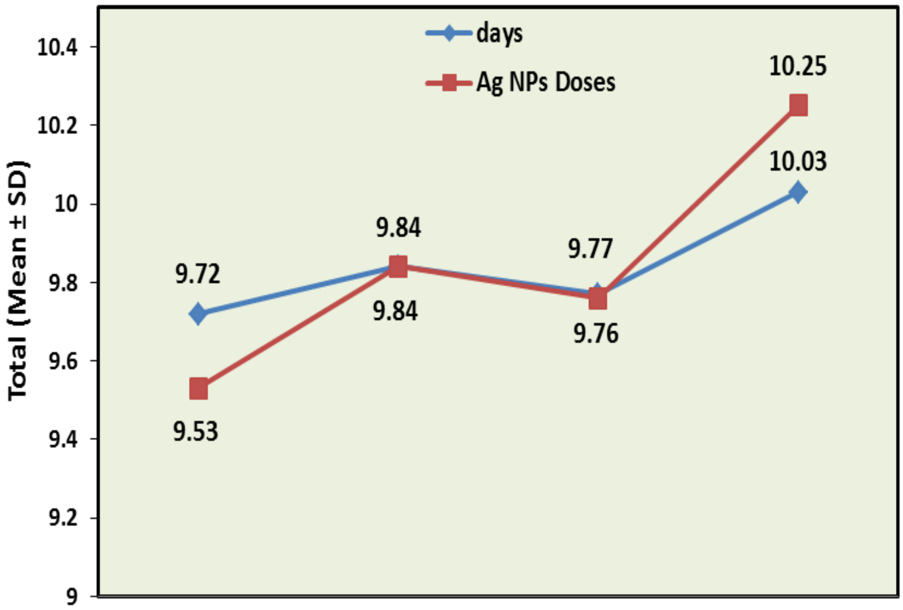

Figure 9: Total (Mean ± SD) of Ca concentration in rats when the dose and time are variable.

4.4 Chlorine Concentration (Cl)

The statistical analysis of Cl concentration was demonstrated in figures (10 , 11) , which showed different doses (170, 340 and 680 ppm) of Ag NPs for different durations (7, 14, 21 and 28) days. The results show that there are significant differences in the average concentration of Cl in the serum of rats treated with different concentrations of silver nanoparticles. The highest average Cl concentration reached (91.7 mmol/L) at the exposure period of 28 days, which is a significant increase compared to the exposure periods (7, 14, 21) days, when the average chlorine concentration reached (89.8, 90, 88.4) mmol/L, respectively, while the lowest average chlorine(Cl) concentration was recorded in the control experiment, which amounted to (87.6 mmol/L), with a significant decrease when compared to the different exposure periods.

The results of the current study showed that there were significant differences in the rate of chlorine concentration when treating rats with silver nanoparticles. The highest rate of chlorine concentration when treated with (680 ppm) silver nanoparticles was (93.75 mmol/L) , with a significant increase compared to all other treatments, while the lowest rate of chlorine concentration was in the control group. It was (87.6 mmol/L), significantly lower than the other of the treatments except for treatment (340 ppm) , where the chlorine concentration reached (88.3 mmol/L).

When studying the interaction between the studied factors, the results of the statistical analysis showed that there were differences between the interaction for the duration of exposure and the concentrations of silver nanoparticles, and there were significant differences between the studied interactions. The highest rate of Cl concentration at the 28 day exposure period with a concentration of (680 ppm) silver nanoparticles was (102.3 mmol/L). The lowest concentration of Cl at the treatment was (340 ppm) in a period of 21 days , which is (84.3 mmol/L).

The reduction of plasma chloride that was observed in higher concentrations of AgNPs, it is possible that due to the reaction of silver ions and chloride ions in the blood [18].

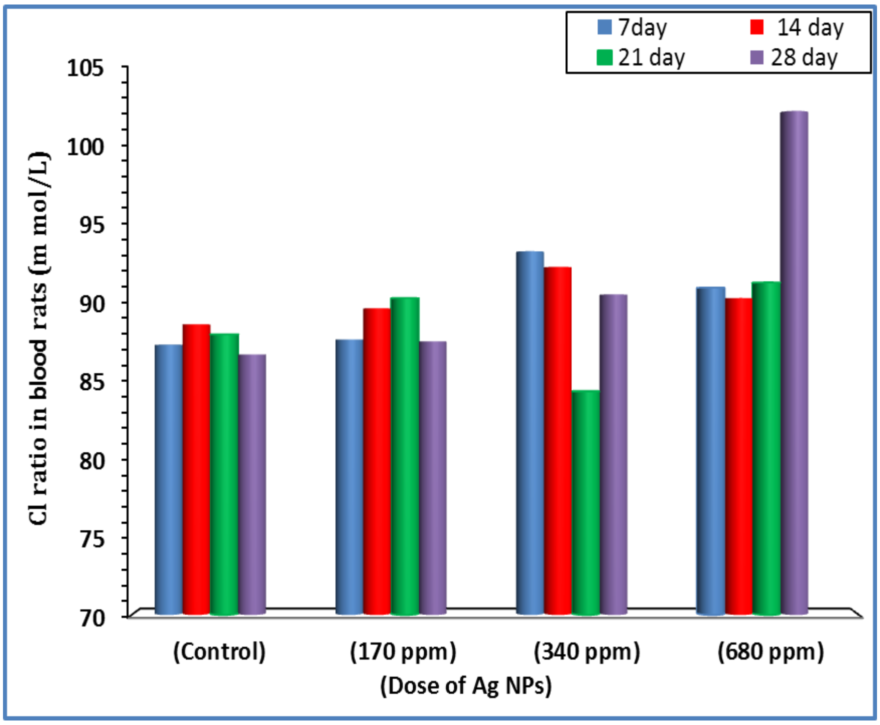

Figure 10: Cl concentration in serum of rats during days of treatment with Ag NP_S at different concentration

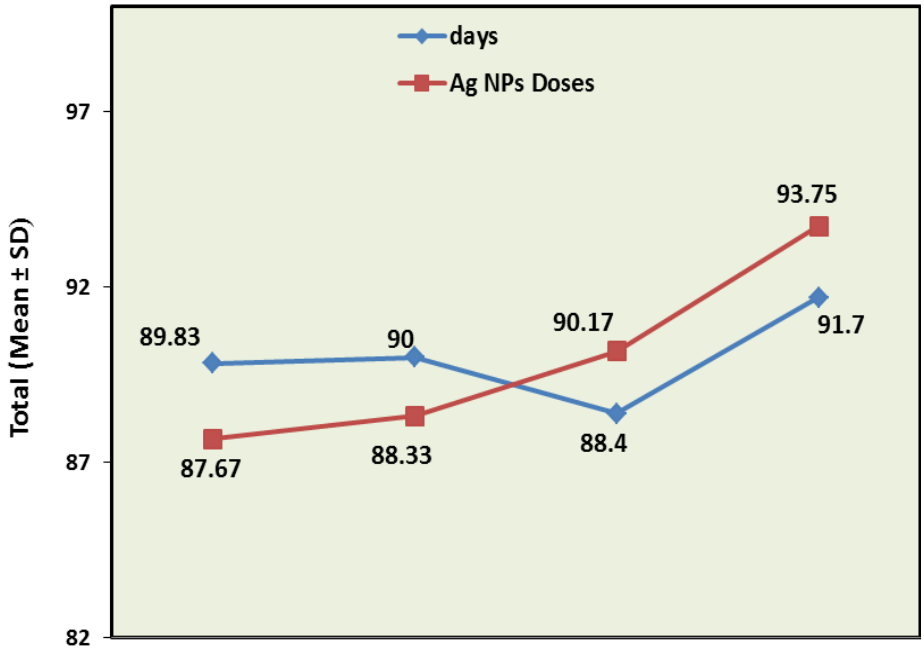

Figure 11: Total (Mean ± SD) of Cl concentration in rats when the dose and time are variable

Conclusions

Ag NPs have a strong positive effect on several mineral elements in the body of rats. In the case of iron, Ag NPs increase the proportion of the element in the blood, indicating that the use of nanoparticles to treat anemia. By increasing the level of calcium in the blood , this indicator is used in the treatment of joint problems and bone regrowth. Where the elements Na and Cl have a direct effect on blood pressure maintenance, these elements provide the nanoparticles a positive indication and a potential future in the treatment of blood pressure.

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