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Effect of caffeine-loaded silver nanoparticles on minerals concentration and antibacterial activity in rats

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ABSTRACT

Caffeine boosts metabolism and the neurological system. When extreme weakness or sleepiness occurs, it is used recreationally and medicinally to reduce physical and mental fatigue. Caffeine initially stimulates the central nervous system, increasing intellect, speed, accuracy, focus, and coordination. The aim of the study was to evaluate how caffeine nanoparticles affect potassium, calcium, zinc, and magnesium levels, in addition, the anti-bacterial activity of the samples has been measured. Eighteen male albino rats were divided into 3 separate groups. The first group (G1) was made up of 6 animals that served as a control group. The second group (G2) was made up of 6 animals that were given caffeine, and the third group (G3) was made up of 6 animals that were given silver nanoparticles from a caffeine solution. The particle size and structural morphology of caffeine and silver nanoparticles were analyzed using Brookhaven Instruments Corp., XRD and SEM, respectively. The structural results showed that after addition, caffeine was tube-shaped, and silver was spherical granular. Caffeine has more silver nanoparticles than caffeine solution. Caffeine solution affects potassium, calcium, zinc, and magnesium levels. Additionally, the solution has antibacterial activity against the following bacteria: *Staphylococcus aureus* (Gram +ve), *E. coli* (Gram –ve), and *Pseudomonas aeruginosa* (Gram-ve), but it has no effect against yeast (*Candida albicans*). It is concluded that caffeine-synthesized Ag NPs has significant biological effects on zinc, magnesium, calcium, and potassium levels in male albino mice and this also is antibacterial against *Staphylococcus*.

INTRODUCTION

Bacteria are among the oldest known forms of life on earth. There are thousands of bacterial species in every habitat and media in the globe; they inhabit the earth's crust, ocean, and soil. Several species of microorganisms that thrive in nuclear waste have also been identified by scientists. Many bacteria inhabit both human and animal bodies, including their skin, airways, mouths, digestive tracts, and reproductive systems.

There are several techniques for producing nanoparticles. The most prevalent approach for creating nanoparticles is chemical synthesis. Certain chemical processes, however, cannot avoid the usage of harmful substances in the synthesis procedure. Hence, there is an increasing need to create environmentally friendly nanoparticle production procedures that do not include the use of harmful chemicals by using biological approaches including microorganisms [1]. Enzymes and plant extracts have been proposed as possible ecologically acceptable alternatives to physical and chemical processes.

Caffeine, a plant alkaloid present in several plant species, serves as a natural pesticide that paralyzes and ultimately kills some insects that consume it [2]. Coffee, tea, and, to some degree, cocoa are the most prevalent caffeinated plants. The liver converts caffeine to three major metabolites: theobromine (12%), theophylline (4%) and paraxanthine (84%). After 45 min of consumption, the stomach and small intestine absorb all the caffeine. Upon consumption, it is transported throughout all bodily tissues and removed via first-order kinetics. Moreover, caffeine is utilized to prevent bronchopulmonary dysplasia in preterm newborns [3] and treatment [4]. It may increase weight growth during therapy [5], lessen cerebral palsy, and lessen linguistic and mental lag [6]. Also, caffeine is a central nervous system stimulant utilized to alleviate physical exhaustion and prevent or cure sleepiness. It results in greater alertness, a quicker and more exact mental process, higher concentration, and improved total body coordination.

Silver has long been known to suppress the growth of certain bacterial strains and germs prevalent in medical and industrial procedures [7]. Silver nanoparticles are most employed and well-known in the medical field. To prevent infection in burns and open wounds, they include topical ointments and lotions containing silver [8].

One of the key requirements for developing nanotechnology is the creation of green chemical processes that are clean, non-toxic, and environmentally beneficial since vital metallic silver nanoparticles (Ag NPs) are effective antibacterial and anti-inflammatory agents. Physical, chemical, and biological processes may all be used to create nanoparticles [9]. Depending on a special characteristics inclusive shape, size and distribution, the nanoparticles exhibit novel or enhanced characteristics. With an increase in the specific surface area of the nanoparticles, which may boost their biological efficiency in surface energy, the specific surface is suited for the catalytic reaction and other relevant qualities such as the antimicrobial activity of the silver nanoparticles [10-13]. Considering all above, the current study aimed to evaluate how caffeine nanoparticles affect potassium, calcium, zinc, and magnesium levels, and the anti-bacterial activity.

MATERIALS AND METHODS

Characterization techniques

Different devices were used to study the prepared samples, which are: Centrifuge type KM 3200, 6000 RPM - Made in Taiwan and was used to separate between liquid and solid, Atomic absorption spectrometry (Perkin-Elmer Model 3110) for the analysis and measurement of minerals (zinc, magnesium, calcium, and potassium), while X-ray diffraction scale (X'Pert Pro 40 kV, 30 mA, X-ray diffractometer) is used to know all the phases of the prepared materials, and to see the samples' form, a scanning electron microscope (SEM) is utilized.

Preparation of a standard caffeine solution

50 ml of distilled water is utilized to dissolve 500 mg of powdered caffeine at room temperature, and the concentration of this solution is 10 mg of caffeine/ml.

Chemical properties

The chemical structure of caffeine, a methylxanthine, is comparable to that of the nucleotide adenosine. Methylxanthines, sometimes referred to as xanthine, are

generated from amino acids, are necessary in nature, and typically form water-soluble salts. They contain a heterocyclic ring with nitrogen present in the ring structure. There are two types of A1 and A2 adenosine receptors: antagonists of the adenosine receptors A1 and A2. The maximal period of caffeine absorption is thought to be between (15-45) min after the first 15 min period of absorption. Caffeine's half-life for humans has been investigated to be between (5.2 and 6.7) hours, despite variations in numerous investigations. Yet, the metabolic rate of caffeine absorption varies per species [12] as shown in Figure 1.

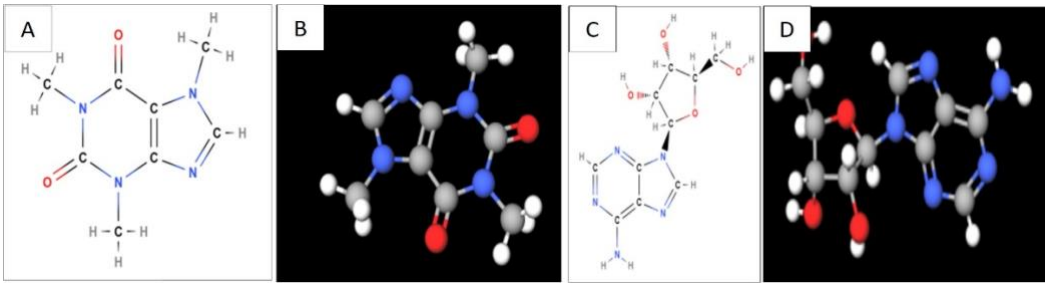

Figure 1. 2D to 3D molecular structure of caffeine (A, B) and adenosine (C, D), respectively.

Experimental animals

A total of 18 male adult rats (12-14) weeks old, weighing 30 g, were used in this study. Mice were housed in a university animal house facility with temperature controlled under a 12/12 h light/dark schedule at (22 ± 2) °C. The rats were then separated into three groups. In the first group (G1), six animals were used, and they were injected with distilled water intramuscularly for 30 days. Mice in each group were maintained in polypropylene cages and under hygienic conditions in a well-ventilated room in the animal house. The second group (G2) consisted of 6 animals injected intramuscularly with 0.5 ml/day of 10 mg/ml caffeine solution for 30 days. The third group (G3) included six animals treated by intramuscular injection with 0.5 ml/day of 10 mg/ml caffeine solution mixed with 90 ml of silver nitrate aqueous solution for 30 days for each animal. The samples studied are acquired according to ethical approval committee, approval number (152 at 13/10/2022).

Sample preparation for silver nanoparticle synthesis

After the silver nitrate was dissolved in distilled water, 10 ml of caffeine solution was added separately to reduce it to the Ag^{+1} ion. Then, they were brooded at ambient temperature (350 °C) for 48 h to obtain synthesis of 2 mmol/l of silver nanoparticles (Ag NPs). Group 2 animals were given 0.5 ml/day of 10 mg of caffeine solution, while group 3 was incubated at room temperature (350 °C) for 48 h. treat them with 0.5 ml/day of 10 mg caffeine solution with silver particles (SNP). Use the bio-reducing aqueous component (0.5 ml) for measurement and dilute to prevent mistakes owing to the high optical density for the solution, distilled water was utilized to tenfold dilute the particle suspension.

Blood samples collection

After 30 days of treating animals with caffeine and caffeine mixed with nanomaterials, animals were anesthetized with ether to collect blood samples by cardiac puncture in an EDTA solution (Ethylenediaminetetraacetic acid; $([CH_2N(CH_2CO_2H)_2]_2$, ≥99% Sigma-Aldrich) tube and then centrifuged for 15 min at 3000 rpm.

Measurement of XRD

The structural morphology of samples was supplied using Ital Structure XRD diffractometer model APD 2000, using CuKα (λ=1.542 A^{o}) at 40 KV, 20 mA anode current with 2θ= 10^{o} to 90^{o}. from (Nanotechnology and Advanced Research Center / University of Technology- Iraq, Baghdad, Iraq) operated at a voltage of 40 kV and a radiation.

Particle size analyzer

The grain size of the samples was supplied using Model: 90 Plus Signal Processing: Dynamic Light Scattering, DLS, from (Nanotechnology and Advanced Research Center / University of Technology- Iraq, Baghdad, Iraq).

Scanning electron microscopy (SEM) analysis

The sample images were supplied by SEM type (Tescan VEGA3 SB) from (Nanotechnology and Advanced Research Center / University of Technology- Iraq, Baghdad, Iraq). After fabricating the NPs, the nanoparticle suspension in water was used for SEM analysis by placing a drop of suspension on a clean electric and allowing the water to dissipate completely. Using a scanning electron microscope (SEM), the surface morphology of N.P.s was analyzed. The direct observation of the nanoparticles using electron microscopy identifies their size, shape, and surface morphology.

Measurement for the zone of inhibition

The antibacterial activity of the resulted samples was evaluated against *Candida albicans* (yeast), (*Pseudomonas aeruginosa* and *E. coli)* as negative and *S. aureus* as positive pathogens by well diffusion assay method. These samples were supplied by (Nanotechnology and Advanced Research Center / University of Technology- Iraq, Baghdad, Iraq). The samples were incubated for 18 h and spread on Mueller Hinton agar surface poured in petri plates. Holes of 6 mm were made, and each hole was packed with different test concentrations of samples as well as using distal water as a negative control. The plates were wrapped with parafilm tape and incubated at 37 ^{o}C overnight. Negative controls using only *Pseudomonas aeruginosa* and *E. coli* and *S. aureus* were used. The inhibition zones of bacterial growth were then measured in millimeters.

Analysis of antimicrobial activity

The antibacterial activity of caffeine using the agar diffusion method was well determined against three bacterial strains and one yeast, *Candida albicans* (yeast), *Pseudomonas aeruginosa* (Gram-ve), and *E. coli* (Gram-ve), *Staphylococcus aureus* (Gram+ve). All isolates have been sub-cultured and reactivated first to prepare Mueller-Hinton (MHA) agar plates, the culture of each strain was modified to McFarland tube No. (0.5) (CFU\ml), and MHA plates were spread with bacteria broth (~0.1 ml) and left to dry. Sterile tips were used to drill wells with a diameter of 6 mm in media plates and make different concentrations of caffeine stock solution (10 mg/ml) (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}) should be poured into the wells. The diameter of the inhibitory zones was then measured after an overnight incubation at 37 °C. The test was conducted in triplicate.

Measurement for biochemical parameters

Test kit is used to measure the concentration of zinc element in serum (Zn GIESSE DIAGNOSISICS, Zinco SL, Italy code; LOT: 21149, 00012 Guidonia Montecelio (Roma) Italy). A spectrophotometer that measures the absorbance at 546 nm may be used to estimate the quantity of Zn in a specimen.

Test kit is used to measure the concentration of magnesium element in serum (Magnessium ARSENAZO, Italy code; LOT: 21112, 00012 Guidonia Montecelio (Roma Italy). A spectrophotometer that measures the absorbance at 578 nm may be used to estimate the quantity of Mg in a specimen.

Test kit is used to measure the concentration of calcium element in serum (Ca Calcium ARSEMAZO, GIESSE DIAGNOSISICS, Italy code; LOT: 20109, 00012 Guidonia Montecelio (Roma Italy). A spectrophotometer that measures the absorbance at 630 nm may be used to estimate the quantity of Ca in a specimen.

Test kit is used to measure the concentration of potassium element in serum (K FUJI DRI-CHEM Na, K, Cl (24 tests), Italy code; LOT: 107207, 00012 Guidonia Montecelio (Roma Italy). A spectrophotometer that measures the absorbance at 406 nm may be used to estimate the quantity of K in a specimen.

Statistical analysis

The programs (SPSS) were used to do the analysis of Variance (ANOVA) test which is significance of the differences in the measured characteristics was determined using the analysis of variance method.

RESULTS

Effect of caffeine on distribution of grain size

Caffeine NPs' average particle diameter without and with loaded Ag using solution was shown in Figure 2. This Figure summarized the effectiveness of average particle diameter (Ag, Caffeine and Ag- Caffeine) NPs. The results in Figure 2, It can be seen from the figure that Ag–NPs with almost narrow particle size distribution at 25 ^{o}C with polydispersity index value with Std. error values around (0.293±0.012), GSD value (1.661), viscosity value (0.890 cP) and the effective particles size diameter of Ag NPs was (299.5±0.233) nm with PDI was 0.005. Also, a synthesis of Caffeine-NPs with narrow particle size from the figure with polydispersity index value with Std. error values around (0.287±0.144), GSD value (1.653), viscosity value (0.890 cP) and the effective particles size diameter of Caffeine-NPs was (520.4±0.6) nm with PDI was 0.004. in addition, a synthesis of mixed Caffeine-Ag–NPs with almost narrow particle size distribution with polydispersity index value with Std. error values around (0.305±0.016), GSD value (1.675), viscosity value (0.890 cP) and the effective particles size diameter of Ag NPs was (246.3±0.23) nm with PDI was 0.006.

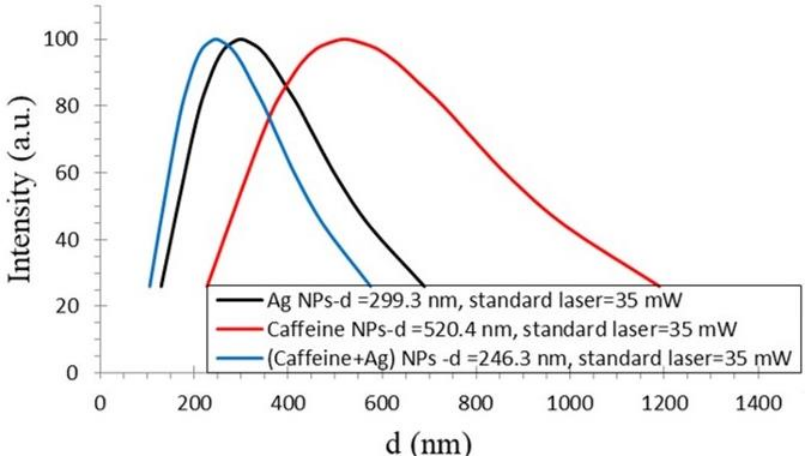

Figure 2. Distribution of sample particle sizes: silver NPs (black color), caffeine NPs (red color) and (silver+caffeine) NPs (blue color) using standard laser beam=35 mW red diode laser, nominal 640 nm wavelength.

Effect of caffeine using X-Ray diffraction analysis

The spectrum by X-ray spectroscopy enabled us to prove the existence of the constituent phases of the studied material. The structural characteristics of materials were determined using a D8 Advance Brucker diffractometer with X-ray tube CuKα 1,2 radiation (40 KV, 30 mA and λ= 0.15406 nm), a scan range (10-90)^{o} with scan speed 10 (deg/min), for X-ray measurements [14-24]. Figures 3 and 4 illustrate the peaks of caffeine and Ag NPs. The results in Figure 3, the caffeine is recognizable by its distinctive, prominent peaks at an angle 2θ of (12.2, 24.1, 26.6, 27.4, and 28.63) ° corresponding to (111), (200), (220) and (311) planes, respectively. While Figure 4, illustrate the initial detection of silver nanoparticles synthesized is recognizable by its distinctive, prominent peaks at an angle 2θ of (38.2, 44.4, 64.6, 77.5, and 81.6) ^{o} corresponding to (222), (444), (731), (822) and (751) planes, respectively.

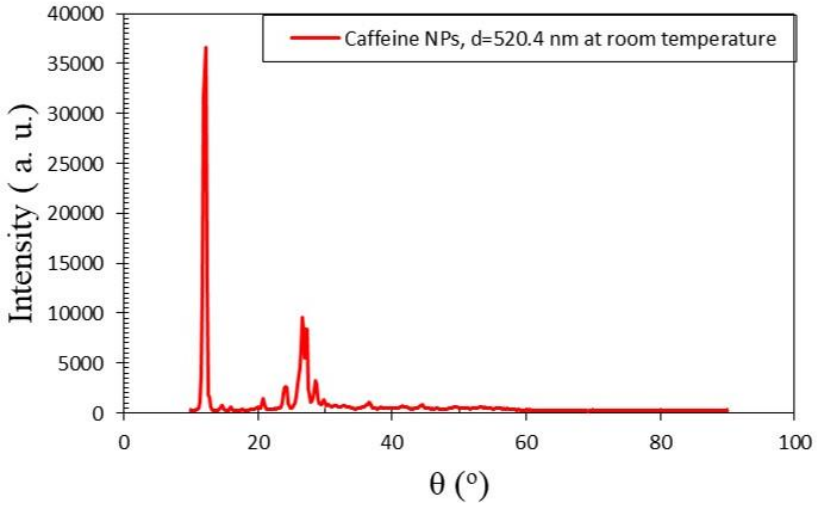

Figure 3. X-ray spectrum for caffeine NPs.

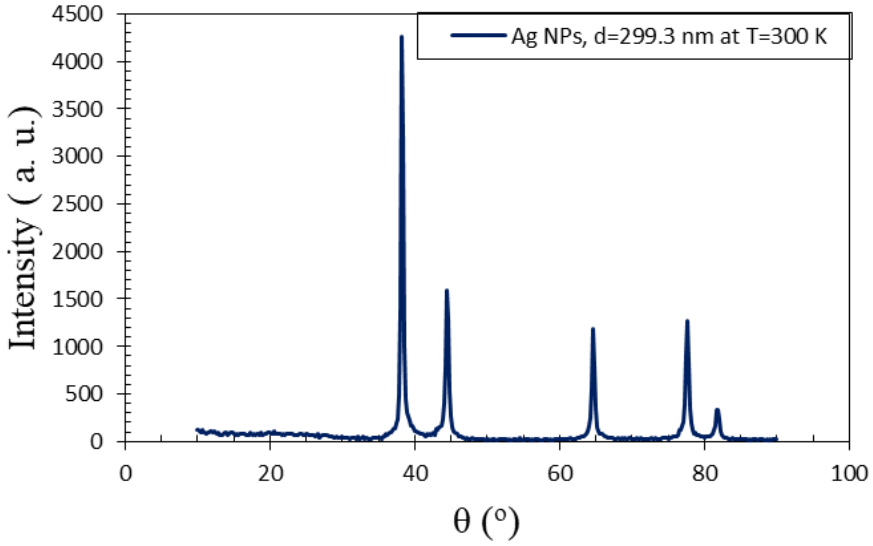

Figure 4. XRD analysis of silver NPs, α=β=90°, γ=120°.

Effect of caffeine using SEM Analysis

Utilizing a scanning electron microscope, the surface morphology of the caffeine sample was determined. Figure 4-5 (A), (B), and (C) illustrates the image that characterizes caffeine of different grades (5, 10, and 50) μm, where the surface area is covered with tight bundles in a cylindrical shape, and the one closest to the tubes is of the micron rank. After nanoparticle preparation, a suspension of nanoparticles in water was employed for SEM examination by producing a suspension droplet on a clean electrode and allowing the water to fully evaporate. The images in Figure 5 (A), (B), and (C), illustrate the caffeine nanoparticles as the particles are spherical. SEM magnification and HV for the image in Figure 5 (A), (B), and (C) are (SEM MG=1000 kx, HV=1000 kV, WD=26.77 mm), (MG=6.02 kx, HV=1000 KV, WD=26.57 mm) and (MG=990 kx, HV=4.46 KV, WD=20.45 mm) for (5, 10 and 50) μm, respectively. At the same time, the images in Figure 6 (A), (B), and (C) illustrate the silver NPs, are (SEM MG=1000 kx, HV=1000 KV, WD=27.95 mm), (MG=9.4 kx, HV=19.99 KV, WD=25.67 mm) and (500 kx, HV=1000 KV, WD=26.70 mm) for (6, 10 and 100) μm, respectively.

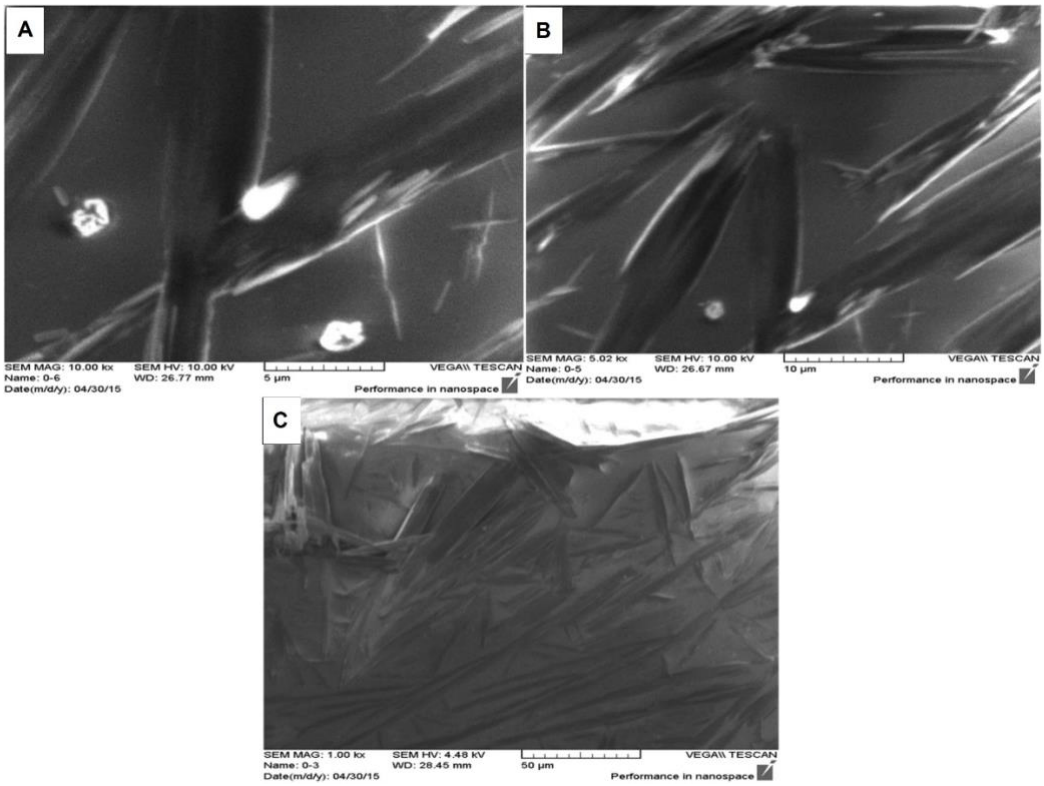

Figure 5. SEM images of caffeine: (A) 5, (B) 10, and (C) 50 µm.

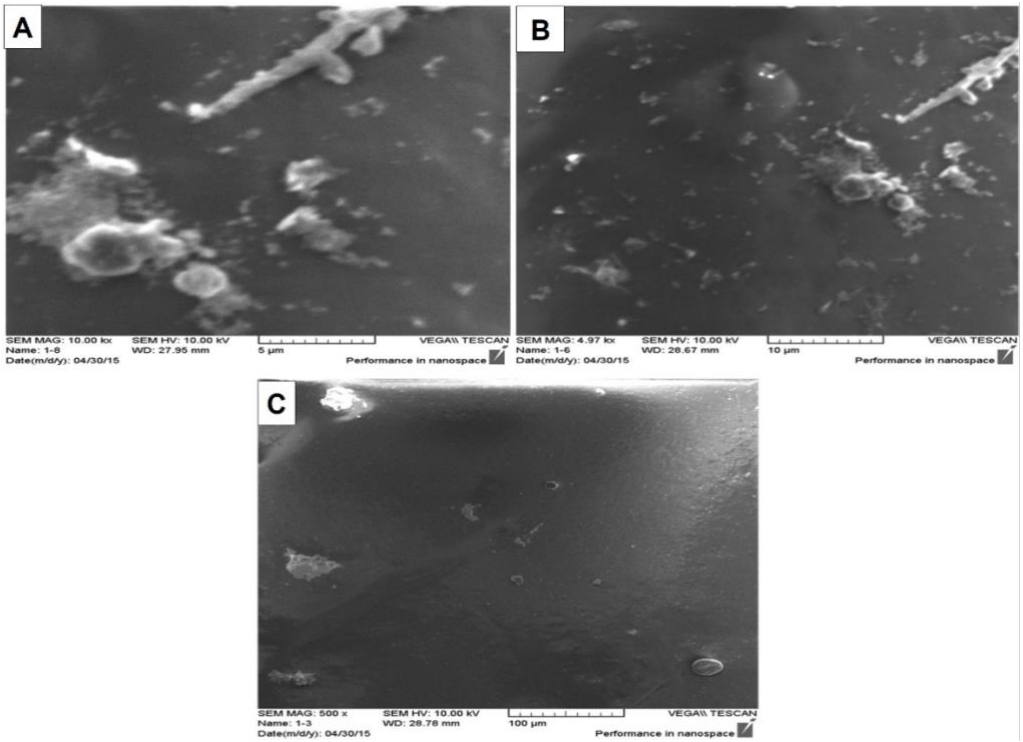

Figure 6. SEM images for silver NPs: (A) 5, (B) 10, (C) 100 µm.

Effect of caffeine on mineral concentration

Essential minerals are those that the body needs. Significant minerals (microminerals) and trace minerals are two types into which important minerals are sometimes classed

(microminerals). These two mineral kinds are equally important; however significant minerals are needed in greater amounts than trace minerals. The quantities needed by the body are not indicative of their significance.

The present result demonstrated the effect of intramuscular injection of 0.5 ml/day of caffeine solution 10 mg/kg for 30 days on the concentrations of minerals: zinc, magnesium, calcium, and potassium. There is a considerable change in the mean values of the concentration of zinc, magnesium, calcium, and potassium in the treated animals with caffeine and with silver nanoparticles produced with caffeine in comparison with treated animals as in Tables 1 and 2.

Table 1. Zinc, magnesium, calcium, and potassium serum levels are affected by caffeine.

K	Ca	Mg	Zn	Mineral
2.0±0.081 CV=0.0405%	1.35±0.005 CV=37%	0.405±0.0057 CV =1.2%	71.75±2.98 CV =4.15%	Control N=4
2.35±0.057 CV=0.024%	1.51±0.015 CV =0.009%	0.53±0.047 CV =0.088%	68.75±0.95 CV =0.0138%	Extract of caffeine

Table 2. Synthesize Ag NPs on serum levels of zinc, magnesium, calcium, and potassium.

K	Ca	Mg	Zn	Mineral
2.0±0.081 CV =0.0405%	1.35±0.005 CV =0.37%	0.405±0.0057 CV =1.2%	71.75±2.98 CV =4.15%	Control N=4
2.52±0.130 CV =0.051%	1.72±0.013 CV =0.007%	0.68±0.08 CV =1.279%	67±7.48 CV =0.111%	$AgNO_3$ & Extraction of caffeine

The results in Table 1 and 2 illustrate zinc has a good, helpful impact on the course of chemically induced preneoplasia in rats. In the susceptible population segment with a family history of carcinoma, it offers a viable dietary chemo preventive approach to illness. 0.5 ml/day of caffeine with silver nanoparticle reduced zinc concentrations in animals' serum levels in this study. Zinc has been implicated in the structural stability and activation of cytochrome p53, a key component of the apoptotic process and in activating a specific membrane of the caspase family. The results of the statistical analysis of Table 1 which is the zinc, magnesium, calcium, and potassium serum levels values are affected by caffeine showed that the highest value of CV (control) was examined for Ca (4.15%) while the lowest value of it was examined for K (0.0405%), and Mg (0.088%), Ca (0.009%) for the highest and lowest value of CV after using caffeine, Also; the other values was N=4, SS=7122.1962, df=3, MS=2374.0654, F=2072.5145, for total SS=7126.7781, df=7 then L.S.D.=1.77, in addition to the value of R=0.8885 then the P-value is 0.1112; thus, the results is not significant differences between the values the level of probability ($p < 0.05$). In the same time; the results of the statistical analysis of Table 2 which is the synthesize Ag NPs on serum levels of zinc, magnesium, calcium, and potassium showed that the CV value was examined for Mg (1.279%), Ca (0.007%) for the highest and lowest value of CV after adding Ag NPs with caffeine, Also; the other values was N=4, SS=6924.5312, df=3, MS=2308.1771, F=801.2556, for total SS=6936.0539, df=7 then L.S.D.=2.8, in addition to the value of R=0.8881 then the P-value is 0.1119; thus, the results is not significant differences between the values the level of probability ($p < 0.05$). The results of the statistical analysis showed that there were no significant differences between the group of laboratory animals treated with caffeine extract, Tables 1 and 2, indicates that the extract is safe and did not affect the vital tests of mice, which is an important step for evaluating its efficiency for use in preparing the nanocomposite in addition to confirming that it is a harmless substance

environmentally, which makes it a target for use in subsequent experiments. As the results of Tables 1 and 2 indicated there were no significant or statistically significant differences between the nanocomposite prepared from caffeine extract and silver and given to mice and the untreated control sample, as no biological changes appeared, as it is an important step in the fact that the nanocomposite has no side effects and can be used in biological activity applications.

Effect of caffeine on antimicrobial activity

In order to test and know the effectiveness of caffeine with Ag NPs in the biological field, four types of bacteria available to us were used that proliferate in the appropriate medium at 37 °C. The pellets' diameter and the inhibition area for each kind of bacteria were assessed after 24 hrs. The results are shown in Table 3. The inhibition zones created by caffeine against different microbial isolates are shown in Figure 7.

Table 3. (Caffeine + Ag) NPs exert antimicrobial action on test bacteria.

Microorganism (mg)	Inhibition Zones (mm)				
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
S. aureus (+ve)	24	23	20	16	8
E. coli (-ve)	18	15	-	-	-
P. aeruginosa (-ve)	24	21	14	-	-
Candid albicans	-	-	-	-	-

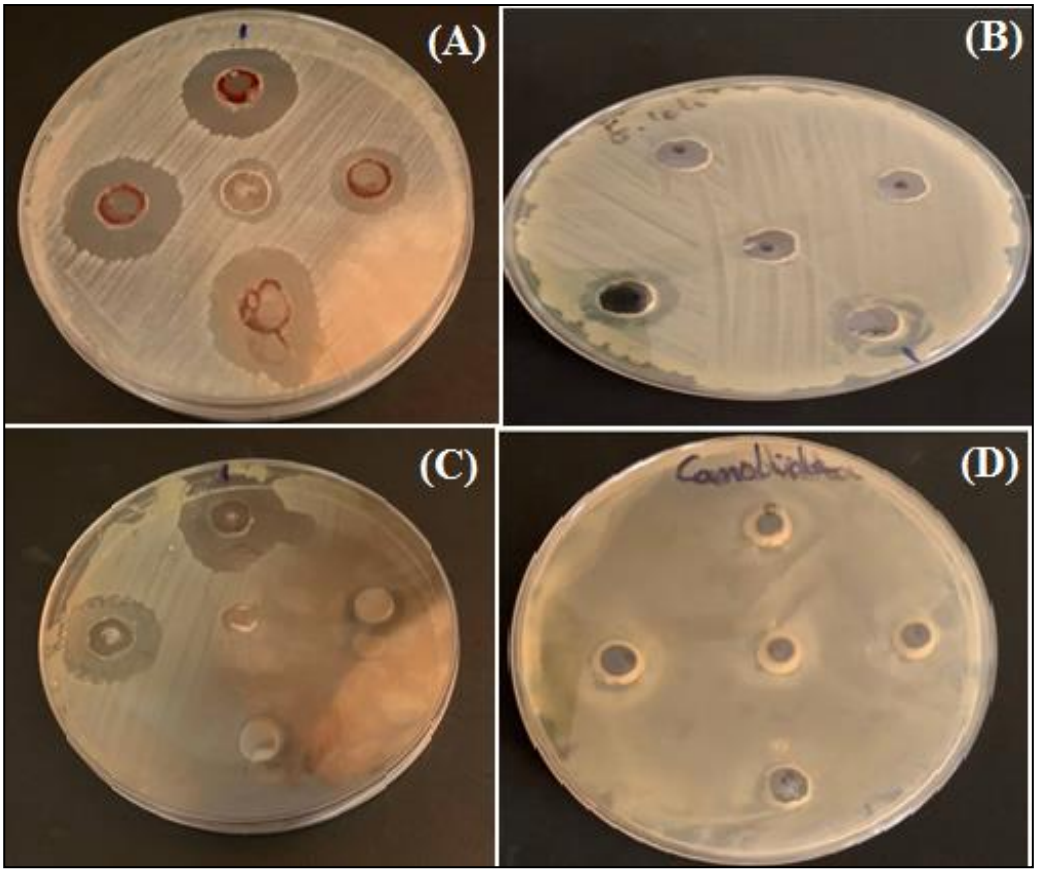

Figure 7. Antibacterial activity of (Caffeine with Ag) NPs show the diameter of the zone inhibition created by (caffeine +Ag NPs) with a higher dose 10^{-1} 10^{-5} mg at 37 °C and after 24 h with against different microbial isolates. (A) *S. aureus*, (B) *E. coli*, (C) *Pseudomonas aeruginosa*, (D) *Candida albicans*.

DISCUSSION

The scientists concentrate on a variation in the synthesis of nanoparticles for the development of antibiotics against microorganisms, as Ag NPs can enhance the bioactivity of natural compounds, but the activity is dependent on the nanoparticle size [36]. Biological or green synthesis of Ag NPs solution is considered safe and straightforward in comparison to conventional methods, which are linked to the release of toxic substances into the environment [37]. The antibacterial potential of synthesized (Ag with caffeine) NPs was evaluated in the current manuscript. The synthesized (caffeine with Ag) NPs extract was applied to four bacterial strains and demonstrated a growth inhibitory response against all strains, but particularly against three strains: *S. aureus*, a Gram +ve strain, *E. coli*, and *P. aeruginosa*, a Gram -ve strain. The findings revealed that, compared to gram-negative bacteria, gram-positive bacteria shown greater sensitivity to synthetic (caffeine with Ag) NPs. According to reports, the type of the particle and the test bacterium's surface makeup affect bactericidal action. Additionally, it was discovered that the electrostatic contact between (caffeine with Ag) NPs and the bacterium cell wall was what caused the bactericidal activity. Interaction between bacteria cells and NPs resulted in reduced size and count of the bacterial cell and physical rupture of a membrane which was observed. According to reports, the surface of bacterial cell walls has a strong -ve charge that allows Ag+ ions to engage with them and enter the cell with ease. After NPs interact and enter the bacteria cells, cell death is mediated by enzyme inhibition, proteins deactivation, induces oxidative stress, and imbalanced electrolytes and modifies the expression of a gene.

Primary zinc deficiency syndrome may be caused by zinc-poor diets [38] and occurs in an acquired form in exclusively breast-fed newborns owing to mutations in the zinc transport gene [39]. Diseases include malabsorption syndrome, complete parenteral nutrition, cirrhosis of the liver sickle cell disease, diabetes and chronic renal disease that may cause secondary zinc shortage.

In this study, the present result illustrated the increase in magnesium concentration in the serum of animals when treated with 0.5 ml/day of caffeine and caffeine with silver nanoparticles. Magnesium and calcium collaborate to control electrical impulses in the cell. There are important reasons why within healthy cells, magnesium concentrations are 10,000 times greater than calcium concentrations. Calcium enters the cell via cellular calcium channels only for as long as it is required to conduct an impulse; once its function is complete, magnesium ejects it from the cell. Constant vigilance is required to avert calcium buildup in the cell that may lead to harmful hyper-excitability, calcination, cell malfunction, and even cell death. When cells absorb too much calcium due to a magnesium deficiency, muscular contractions are prolonged for too long, causing, in moderate instances, twitches and tics. Chronic magnesium shortage causes heart disease symptoms such as arrhythmia, hypertension, and angina pectoris, as well as asthmatic spasms and contractions, migraine headaches, and severe menstrual cramps [25].

Supplemental iron usage reduces magnesium absorption. Magnesium is required more often when calcium supplements are used. If magnesium levels are insufficient, calcium will not be absorbed or processed properly, and it will end up dangerously accumulated in soft tissues. Magnesium is essential for vitamin D conversion to its active form, which enables calcium absorption, as well as regulating calcium's transit to its proper location in hard tissues. Together with excess potassium, phosphorus, and salt, lactose is also a substance inhibiting magnesium absorption (despite the fact that milk is not a very abundant source of the mineral) [25].

Physical stress and Mental, with it is accompanying constant a surge of adrenaline, quickly depletes magnesium, since adrenaline affects blood pressure, heart rate, muscular contraction, and vascular constriction, all of which need consistent supply of magnesium for normal operation. Magnesium is necessary for the nervous system's soothing effects, inclusive healthy sleep. Hibernating animals retain very high magnesium levels. Magnesium deficiency would speed a vicious cycle and exacerbate the effects of chronic stress, resulting in increased anxiety, irritability, weariness, and sleeplessness, many of the symptoms of adrenal depletion, in addition to hypertension and heart aches, signs of cardiovascular disease.

Stress and magnesium shortage are both associated with depression. Serotonin, the "feel-good" hormone, needs magnesium for it is careful release and uptake by brain cells. As suitable amounts exist, we can experience mental and emotional stability. The calcium concentration was higher in the treated animals than in the control group. Caffeine in high doses temporarily raises the calcium concentration in the urine. Nevertheless, compared to a placebo, 400 mg/day of caffeine did not substantially alter urine calcium excretion in premenopausal women over 24 h [26].

According to research, postmenopausal women who consume little calcium and drink two to three cups of coffee each day have increased bone loss [27]. In postmenopausal women, another research found no link between coffee use and bone loss [28]. The typical reduction in calcium retention from one 8-ounce cup of coffee is merely (2 to 3) mg [29].

A low calcium blood level (hypocalcemia) often indicates faulty parathyroid function, since the skeleton offers a considerable store of calcium for maintaining normal blood levels, particularly in the event of a low calcium intake from the food. Additional reasons of extremely low blood calcium concentrations include chronic renal failure, vitamin D insufficiency, and low blood magnesium levels, which are often detected in instances of severe alcoholism. Magnesium shortage may inhibit parathyroid hormone (PTH) release by the parathyroid glands and reduce osteoclast response to PTH. Thus, magnesium supplementation is necessary to treat hypocalcemia in individuals with low blood magnesium concentrations. Chronically low calcium consumption in growing people may impede adequate peak bone mass. Inadequate calcium consumption may lead to rapid bone loss and the development of osteoporosis after peak bone mass has been reached.

This study showed that the amount of potassium in the serum of animals treated with 0.5 ml/day of caffeine and caffeine with silver nanoparticles went up. Potassium intake is thought to be a significant cause of hypertension and CVD, with a 17 percent increase in risk of hypertension; systolic blood pressure [SBP]>140 mmHg attributable to low potassium intake [30]. By alone, increasing potassium consumption would result in a 17% reduction in the number of persons with recognized hypertension. When it comes to adult women, the risk of dying from a CVA decreases when the amount of food they eat goes up from about (60 to 80) mmol/day. Potassium's ability to prevent CVA is distinct from its ability to lower blood pressure.

Endothelium-dependent vasodilation depends on sodium and potassium balance. A potassium-rich meal elevates blood potassium (even within the physiologic range) due to endothelium-dependent dilatation by hyperpolarizing endothelial cells by stimulating the sodium pump and activating potassium channels [31]. As a consequence of endothelial hyperpolarization, cytosolic calcium levels fall, and blood vessels dilate. Experimental potassium deficiency reduces endothelial-dependent vasodilation [32-35].

The inhibition zones for each isolate were measured after incubation for 24h and illustrated in Figure 7. The results of the antimicrobial activity test showed that caffeine has antibacterial effects ranging from moderate (as in *E. coli*) and high effects (as in *S. aureus* and *Pseudomonas*). It affects Gram-positive and Gram-negative bacteria but has no effect on yeast (as in *Candida albicans*). The inhibition zones for each isolate were measured after incubation for 24 h are illustrated in this Table 3.

CONCLUSION

The anti-bacterial activity of caffeine with Ag (NPs) was assessed against four types of bacteria and the evaluation of caffeine nanoparticles affect potassium, calcium, zinc, and magnesium levels has been employed. The effective particles size diameter of Ag, caffeine, and mixed (Ag+caffeine) NPs was determined. X-ray analyses proved that it is a caffeine-dependent structure, without and with silver nanoparticles, through the two distinct peaks of caffeine and silver. In comparison, the analysis by scanning electron microscope showed that the compound has the image of tubes interspersed with spherical nano-granules, the first belonging to caffeine and the second to the luminous silver element. Group 2 animals were given 0.5 ml/day of 10 mg caffeine, and group 3 was treated with 0.5 ml/day of 10 mg caffeine with silver particles (SNPs). The effective particles size diameter of Ag, caffeine, and mixed (Ag+caffeine) NPs was (299.5, 520.4 and 246.3) nm respectively. The result of intramuscular injection of 0.5 ml/day of caffeine solution 10 mg/kg for 30 days showed an apparent effect on the mineral concentrations of magnesium, zinc, calcium, and potassium. There is a significant change in the mean values of zinc, magnesium, calcium, and potassium concentrations in animals treated with caffeine and silver nanoparticles produced in caffeine compared to animals treated with caffeine only. The application of caffeine solution applied to different types of bacteria also proved anti-bacterial activity for each type of bacteria.

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AUTHORS CONTRIBUTION

ThRM, MMNMS, MMT, IAH; methodology, ThRM, MR, MMNMS, RR, MNA; planned and conducted the tests, ThRM, MR, MMNMS, DB, MNA; data analysis and interpretation, and MR, DB, RR, MAS; prepared the manuscript. All authors have read and approved the final manuscript version.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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