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AMELIORATION OF SODIUM NITRATE-INDUCED KIDNEY AND LIPID DISORDERS THRU COMBINATION OF SILVER NANOPARTICLES WITH WHEAT GERM OIL, *INVITRO/INVIVO*

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ABSTRACT

Nanomaterial may aid in improving the human quality of life. This study supposed silver nanoparticles (Ag NPs) and" wheat germ oil (WGO)" eliminate the negative consequences of sodium nitrate ($NaNO_3$) on kidney and lipid disorders. Ag NPs were synthesized with two concentrations (10, 50 ppm). Spectroscopic techniques such as UV–Vis, X-ray diffraction (XRD), atomic force microscopy (AFM), and dynamic light scattering (DLS) were extensively used for the characterization of the Ag NPs. They had an average size of 16.8 nm, a poly disparity index (PDI) of 0.3, and a zeta potential (ZP) of -34.8. Bacillus subtilis (ATCC 6633) was utilised as the positive bacteria, and Salmonella typhimurium (ATCC 14028) was employed as the negative bacteria in this study. Combination of WGO, and Ag NPs showed an antibacterial effect reaching 91%, and 80% on Salmonella typhimurium and Bacillus subtilis respectively with a non-significant effect of both Ag NPs concentrations. Moreover, 70 male albino rats were separated into seven groups: G1 control, G2 positive control received NaNO3 orally15 mg/kg, G3 ate "WGO" only, and G4 fed Ag NPs 10ppm. Treatment groups (G5, G6, and G7) intake orally $NaNO_3$ 15 mg/kg with WGO, Ag NPs10ppm, and a combination of WGO and AgNPs10ppm respectively. In the positive control group were appeared; the body weight gain (BWG) and feed intake (FI) are decreased, increasing in renal function resulting from lipid profile syndrome, oxidative stress biomarker showed increasing in malondialdehyde (MDA), decreasing in glutathione (GSH), and catalase enzyme (CAT). However, all of these harmful impacts generated by $NaNO_3$ were dramatically reduced in the treated groups. The research managed to combine wheat germ oil and symbiosis Ag NPs diminished the preceding parameter, and that the histological structure of the kidney in the treatment groups was restored to normal group.

Keywords: Sodium Nitrate; Wheat Germ Oil; Silver Nanoparticles; Kidney Impairment

1. Introduction

Food can either be the safest and most effective kind of medicine or the slowest type of poison. The increased popularity of functional foods has necessitated the enhancement of traditional foods' health characteristics. Food additives present a long history of use. currently definite as "any substance not consumed as a food by itself, the addition of which to food for a technological purpose, processing, packing, or holding of such food results, or may be reasonably expected to result (directly or indirectly), in it or its by-products becoming a component of or otherwise affecting the physiognomies of such foods[1]. Nitrates and nitrites occur naturally in water and soil. They are also used as food preservatives in processed meats. They could play a role in the carcinogenicity of processed meat **[2].** Nitrites are frequently added to meat healing formulations to achieve the typical color of cured meat. These inorganic substances are man-made, although some can also be found in nature as minerals [3, 4]. To refine the association of dietary nitrate and nitrite intake with health outcomes, reliable measures of intake from dietary food records are required [5].While nitrates are stable, a non-

enzymatic mechanism transforms dietary nitrate to nitrite, which is then metabolized to nitric oxide (NO) by symbiotic bacteria in the mouth and stomach**[6].** The nitrate was absorbed and the plasma level peaked in 15-30 minutes with a half-life of roughly 5-8 hours after a high-nitrate diet. So because nitrate content was roughly ten times that of plasma [7]**.** The World Health Organization (WHO) advises the acceptable daily intake (ADI) 3.7 mg/kg of body weight per day (mg/kg bw/day) [8]. The intake or metabolism of nitrate, nitrite, and NO can affect vasodilatory and contractile functions, as well as the occurrence and development of inflammation, which is closely related to the pathogenesis of ischemic stroke and cerebral small vessel disease (CSVD) [9]. Increased nitrate levels have been associated to an increased risk of death from diseases like Vascular dementia, type 2 diabetes, hepatocellular carcinoma, and Vascular dementia, most likely due to nitrosamines' DNA-damaging effects. However, nothing is done to account for other factors that could influence epidemiological data[7]. Wheat germ oil (WGO) is indeed polyunsaturated oil manufactured from wheat germ, which is a by-product of flour manufacturing. It contains tocopherol, which has antioxidant and vitamin E capabilities, as well as property refers, particularly octacosanol, which have been shown to boost users' physical performance [10]. Nanotechnology is a rising industry that can be accustomed create Nano-scale constructions. Nano products are concerned with the approach and synthesis of particles with a diameter ranging from 1 to 100 nm [11]. Ag NPs have been used for a variety of medicinal reasons, due to their small size; they are known to interact easily with biological systems[12]. Ag NPs have newly received excessive devotion from the scientific world owing to their attractive features of excellent catalytic and antimicrobial actions, biocompatibility, high photo-electrochemical activity, chemical inertness, and simple synthesis [13] ,[14] . As a result, the primary goal of this research is to synthesize Ag NPs that are both stable and of a regulated size utilizing tri-sodium citrate as a reducing and stabilizing agent. In addition, explore the ability of WGO as a natural source of antioxidants and combination between them to minimize the deleterious side effects of sodium nitrate on male albino rats as a *in Vivo* experiential and impact their as antibacterial *In Vitro*.

2. Methodology

2.1. Chemicals

All of the chemicals were laboratory grade and of the greatest purity obtainable (A.R.), deionized water was used to prepare all the solutions. All glassware was thoroughly cleaned and rinsed with deionized water prior to use. Silver nitrate ($AgNO_3$) and oleic acid were purchased from Sigma-Aldrich, and tri-sodium citrate (TSC) $C_6H_5O_7Na_3$ was purchased from El Nasr Company for chemicals and wheat germ oil (WGO) was purchased from Agriculture Research Centre (ARC), Cairo, Egypt.

2.2. Ag NPs preparation

Silver nanoparticles are manufactured (Ag NPs) as stated by Al-Sherbini et al., [15] employed silver nitrate ($AgNO_3$) as a starting material and ($C_6H_5O_7Na_3$) as a reducing agent to covert Ag (I) ions to Ag^0.For this investigation, the experimental technique was used. To prepare (10 and 50 ppm) Ag NPs, heat 100mL of oleic acid in a 500mL beaker on a hot plate with a magnetic stirrer until the temperature reaches 120°C to 150°. Once boiling, add respectively

(1 ml and 5 ml) of ($AgNO_3$) stock solution 1000 ppm, drop wise, and 1 ml of a $C_6H_5O_7Na_3$ (1%)

Solution approximately 1 drop per second with strong stirring, continue stirring until the solution becomes yellow-to-yellow-brown in color. After that, the mixture was swirled for 20 minutes. The heating was then turned off, and the solution was allowed to cool at room temperature while being constantly stirred.

$$4Ag^+ + C_6H_5O_7Na_3 + 2H_2O \longrightarrow 4Ag^0 + C_6H_5O_7H_3 + 3Na^+ + H^+ + O_2$$

2.3. Ag NPs Physico - chemical Identification

The characteristic SPR of Ag NPs was measured in UV-Vis spectra with a resolution of 1ml in the range 200-800nm (Unicom UV 500UV/VIS Spectrophotometer). As blanks, the yellow-brown color solution was tested against pure water. Because of the Surface Plasmon Resonance (SPR) of Ag0, Ag NPs have a distinctive band at 400 nm. Transmission electron microscopy (TEM) (JEOL-JEM 1200) was used to measure the Ag NPs pictures and determine their size and morphology. The TEM was working at a voltage equal to 90 kV. For the TEM estimations, a drop of the sample containing Ag NPs was put on a copper grating enveloped with indefinite car-bon. After enabling the film to stand for two minutes, the extra solution was expelled using a drying paper, and the grid was permitted to dry before the examination. The average particle size distribution of Ag NPs obtained from TEM descriptions was calculated using known software nominated Image J4s. The dynamic Light Scattering (DLS) procedure was utilized to guesstimate the middling particle size circulation that was dignified by a zeta sizer (Malvern, ZS Nano, UK). On the other hand, the zeta potential value of Ag NPs was evaluated to give information about

the stability of nanoparticles. The description of Ag NPs structure was examined using an X-ray diffractometer using Cu Ka (0.15406 nm) radiation operating at 40 kV, 50 m A with a scanning rate of 0.02 s. 3D micrographs were obtained for each sample and the resolution of the micrographs was fixed at 1450–900 pixels [16]. To avoid undesirable borders, the graphed edges were removed. In nm, the roughness parameters were calculated with the same program. For each sample, the identical procedure was done in triplicate and averages were obtained. The 2D and 3D topography pictures of the formed Carbopol /Zn O hybrid nanoparticles gel were obtained using an Atomic Force Microscope (AFM, 5600LS, Agilent, California, CA, USA). Finally, a DLS and a zeta potential analyzer were used to determine the particle size and zeta potential of the synthesized Zn O NPs and Carbopol /Zn O hybrid nanoparticles gel samples.(Nano Sight NS500, Malvern Instruments Ltd., Kassel, Germany) [17, 18].

2.4. Antibacterial Activity Studies (In Vitro)

Six mixtures of WGO, Ag NPs with various concentration (10 and 50 ppm), and one‘s combination was evaluated as antibacterial effect of on *Bacillus subtilis* (ATCC 6633), a gram-positive bacteria, and *Salmonella typhimurium* ATCC14028, a negative gram bacteria, were evaluated using the micro-dilution technique in micro-titer plates (MTP), It was utilized to compute the inhibitory concentration minimal (MIC). The compounds were tested against pathogenic strains. Briefly, overnight cultures of the test strains were added to 200l of Muller Hinton broth media scattered in MTP with/without chemicals wells. Different volumes of mixtures (5, 10, 15, and 20 l) were added. After that, the plates were shaken for 24 hours at 37 °C (120 rpm). After the required incubation time, the cell growth was monitored using an ELIZA reader (Tecan Elx800, USA) at 620 nm. The MIC was measured in the last cell with no turbidity as the lowest concentration of chemicals that completely inhibited harmful bacteria[19]. The following formula was used to compute the antimicrobial activity Percentage (%) = (OD of Control - OD sample / OD of Control) x100. Where Sample OD is the absorbance of the test sample, and Control OD is the absorbance of the positive control (bacteria alone) [20].

2.5. In Vivo Study

Male albino was divided into seven groups of 70 rats weighed around 120 g± 20 g. At the Animal House of the Nutritional Chemistry and Metabolism Department, National Nutrition Institute (NNI) - Cairo, Egypt, all animals were housed separately in cages in a well-ventilated room. The animals were housed in conditions that were below the minimum requirements (12:12h light: dark cycle and 22 2°C temperature). They were given a conventional diet as well as fresh water. The experimental animals were kept and cared for in accordance with the International Guiding Principles for Animal Research. The experimental protocol was approved by the national hepatology & tropical medicine research institute (NHTMRI), The General Organization for Teaching Hospitals and Institutes, Cairo, Egypt. (Approval No. A1-2022).

2.5.1. Experimental Design

After a week of adaption on the regular diet, the rats were separated into seven groups (n=10 rats per group). For six weeks, the first group (G1) (control group) was fed merely a regular diet in relation to Reeves et al., [20] for six weeks. The second group (G2)(positive control) assimilated into a standard diet + sodium nitrate ($NaNO_3$) with a dose of 15 mg/kg BW by using a gavage needle [21]. The third group (G3) was fed on a standard diet; with slight modification, (corn oil was replaced by WGO). The fourth group (G4) was nurtured only on basal diet with slight alteration (corn oil was replaced by AgNPs10ppm). (G5, G6, and G7) consider the treatment groups, the fifth group (G5) received a standard diet, with meager adjustment (corn oil was replaced by WGO) + $NaNO_3$; 15mg/kg by using a gavage needle. The seventh group (G7) be given a standard diet, with small amendment (corn oil was replaced by Ag NPs 10 ppm) + $NaNO_3$; 15 mg/kg, the seventh group (G7) received a standard diet; with feeble modulation corn oil was replaced by a mixture of (WGO+ Ag NPs 10 ppm) + $NaNO_3$ with the same dose.

2.5.2. Biological Evaluation

For the biological evaluation of given diets, calculated feed intake (FI) and body weight gains (BWG) were utilized. Daily feed intake (FI; in gram) was estimated by subtracting the amount of food remaining in the cage from the amount of food served to each animal daily [22]Throughout the experiment, changes in (BWG) of rats in all groups were recorded weekly, and weight gain was determined for each group at the end of the feeding period (6 weeks), BWG = Final weight (g) – initial weight (g).

2.5.3. Biochemical Assays

2.5.3.1. Blood

Vacationer tubes were used in blood samples collection. All samples were centrifuged for 10 min at 4000 rpm at 37 °C, and the serum was collected and kept at 20 °C until analysis.

Serum total cholesterol and high density Lipoproteins (HDL) were determined as stated by

Burtis et al., [23] , serum tri glycerides(TG) was assessed as mentioned by Young, R [24] , the serum concentration very low density (VLDL) was calculated according to the following formula: (VLDL = Serum TG / 5) and the serum low density Lipoproteins (LDL) concentration was calculated according to the following formula: LDL = TC—(HDL + VLDL) [25] . cardio risk ratio (CRR) calculated by the formula of Wilson et al., [26] CRR= TC / HDL. Kinetic method of creatinine using spinreact kits as supposed by Young and Friedman [27]. Quantitative Enzymatic colorimetric determination of Blood Urea in serum using Stanbio laboratory kits as assumed by Tabacco et al., [28].

2.5.3.2. Tissue

Assessment of oxidative stress The tissue concentration of malondialdehyde (MDA), an indicator of oxidative stress-mediated tissue lipid peroxidation, was evaluated by Ohkawa et al.,[29]. The values of MDA in tissue homogenate were expressed as n mol/g tissue.

Reduced glutathione (GSH) is vital in maintaining cells from reactive oxygen species (ROS) (ROS). According to the manufacturer's instructions, tissue GSH (mg/g tissue) was measured in the homogenate [30]. Catalase (CAT) level determination using the approach to estimate the level of catalase enzyme. The fundamental principle is this: $2H_2O_2 + 2H_2O + O_2 = 2H_2O_2 + 2H_2O_2 + 2H_2O_2 + 2H_2$. Ammonium molybdate can immediately inhibit the decomposition of H2O2 by catalase (CAT). A yellowish compound is formed when leftover H2O2 interacts with ammonium molybdate. The formation of the yellowish complex [31].

2.5.4. Determination of Relative Weight of kidney (RW)

At the end of experiment, the animals sacrificed. The kidney examined carefully and measured in gram (absolute weight). According to the equation, the relative weight (RW) is determined as follows: Relative Organ Weight = **[Absolute organ weight (g) / Final body weight of rat (g)]** × 100 [32].

2.5.5. Histological examination

Kidney was organized for histological examination by dipping it in a saline isotonic solution (0.9 % NaCl) to remove excess blood, cleaning it, fixating it in 10% formalin for 1 day, dehydrating it, clearing it, embedding it in paraffin wax, serially sectioning it into four micron thick sections, and dyeing it with hematoxylin and eosin stain(H& E) [33].

2.6. Analytical Statistics

Microsoft EXCEL 2016 programmer was used to calculate the percent of inhibition for the In-Vitro tests, and Graph Pad Prism version 8.0software was used for data analysis. According to Duncan's multiple range tests, the letters reflect the significance of the differences. The data were analyzed in the in-vivo study using one-way analysis of variance (ANOVA), Duncan's multiple range tests, and Statistical Package for Social Science version 26. (SPSS, Chicago, IL, USA). P 0.05 was used to determine whether differences were significant. All of the data was provided as mean± SD. To identify significance between groups, the Kruskal-Wallis test and Mann-Whitney test were used to examine lesion scores for histopathological analysis. For the UV–Vis spectra, Origin 2019b was utilized.

3. Results and Discussion

3.1.-Ag NPs Characterization

3.1.1. UV-Vis Spectra of Ag NPs

UV–vis spectra are a useful technique used to determine the specific wavelength of a nanoparticle under study. The yellow brown solution produced due to the formation of Ag NPs, as illustrated in Figure 1, according to published studies. Surface Plasmon Resonance (SPR) absorption in Ag NPs has been found in the 380–420 nm range[34]. Because small metal nanoparticles absorb visible electromagnetic waves due to the collective oscillation of conduction electrons at the surface, the SPR may be used to detect the presence of metal nanoparticles. The previous findings were compatible with investigations that showed a single SPR band in the reaction mixture, revealing the spherical shape of the Ag NPs. At 410 nm, a distinctive signal for silver nanoparticles has been identified[35] ,[36].

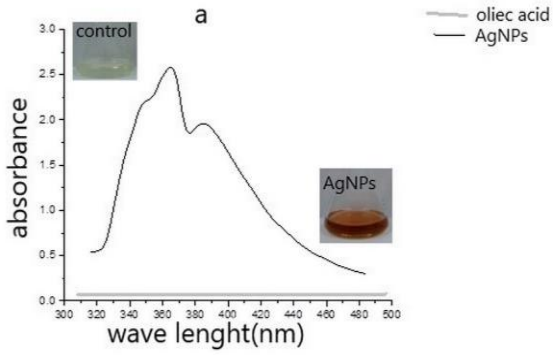

Figure 1. Uv–Vis of Ag NPs prepared in oleic acid

3.1.2. Transmission Electron Microscopy (TEM) of (Ag NPs)

The morphology of Ag NPs was investigated utilizing (TEM). Figures 2 and 3 showed TEM images of Ag NPs (10 and 50), respectively, while a

and b illustrate particle size, distribution, and homogeneity at two different magnifications. Ag NPs are generated with a small spherical size and well distributed in a Nano form, according to TEM images. Figures 2c and 3c show high-resolution TEM (HRTEM) images of Ag NPs (10 ppm and 50 ppm) that reveal the creation of distinct lattice fringes on the particle surface.

3.1.3. Ag NPs Zeta Potential Measurement

Figure 4b showed the zeta potential of Ag NPs. The zeta potential data of Ag NPs shows that the studied sample has a value of roughly – 34.6 mv. The goal of this type of analysis is to determine the nanoparticles' resistance to aggregation during preparation and storage. The negative value indicates that the OH groups are generating negative energy. Figure 4a shows the hydrodynamic size of Ag NPs prepared in oleic acid. The resulting graph shows that the average size of Ag NPs prepared in oleic acid was 16.8nm with a poly dispersity index (PDI).

3.1.4. Ag NP X-ray diffraction (XRD) Patterns

To evaluate the purity of the Ag NPs formed. The phase identification and characterization of the crystal structure of nanoparticles is done using XRD. As shown in Figure 4c, X-rays penetrate the nanomaterial, and the resulting diffraction pattern is compared to standards to obtain structural information. The graph shows four distinct strong peaks at 38, 44, 65, and 78, indicating that the nanomaterial is crystalline. These peaks correspond to the (111), (200), (220), and (311) set of planes for Ag NPs. The typical face centred cubic (FCC) silver lines correspond to all diffraction peaks. The peak intensity, location, and width of XRD patterns were determined. As a result, the XRD pattern clearly shows that Ag NPs are created from oleic acid [12] [37].

3.1.5. Atomic Force Microscope (AFM)

The non-contact style structural Atomic Force Microscope (AFM) 2D images and matching 3D images for the synthesized Ag NPS produced in oleic acid were shown in Figure 5 (a–d). The photos revealed a spherical particle form for the Ag NPs. In addition, an enormous number of random Nano pits are evenly distributed and monodisperselely. Furthermore, the automated batch-mode particle height functional analysis generated a matching particle size distribution histogram of the synthesized Ag NPs across the scanned region, indicating that Ag NPs had a homogeneous particle size distribution with a normal distribution. Furthermore, as shown in Figure 5 e and f, the highest peak height was found to be 14.7 nm, which represents the average particle size of manufactured. The related particle volume distribution was shown was shown in figure 5 g, and h [18].

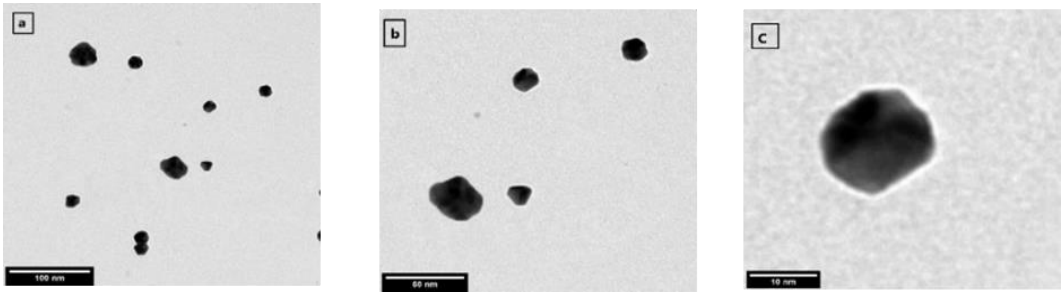

Figure 2. Characterization of Ag NPs (10ppm) by using TEM a) magnification 100 nm, b) magnification 50 nm, and c) HRTEM

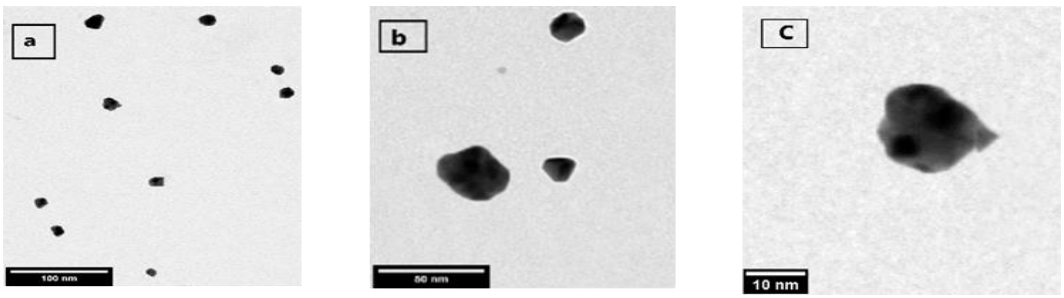

Figure 3. Characterization of Ag NPs (50ppm) by using TEM a) magnification 100 nm, b) magnification 50nm, and c) HRTEM

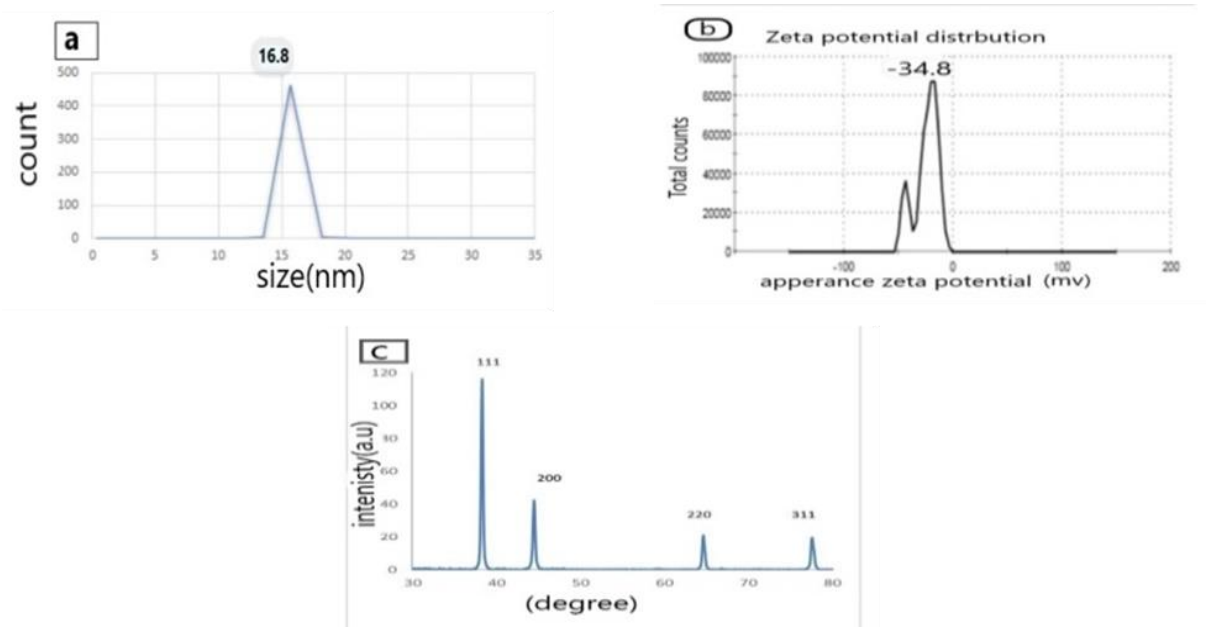

Figure 4. a) Average particle size, b) zeta potential, c) XRD of Ag NPs

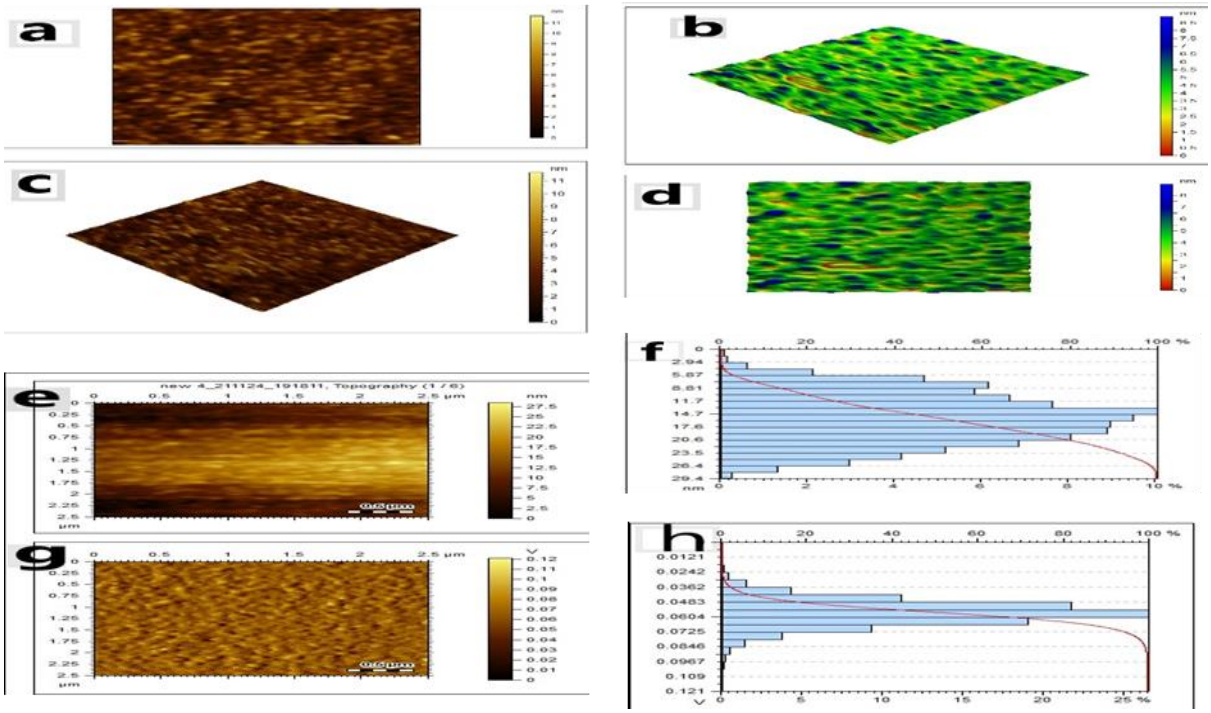

Figure 5. (a, b) Topographical 2D AFM images; (c, d) identical 3D AFM images; (e, f) typical histogram of particle size distribution; (g, h) typical histogram of particles volume distribution of the synthesized Ag NPs

3.2. Antimicrobial assay

The antibacterial properties of wheat germ oil (WGO) and prepared Ag NPs with different concentrations and their combinations were investigated as antibacterial agents against *Bacillus subtilis* (ATCC 6633) as positive gram and *Salmonella typhimurium* (ATCC 14028) as gram negative strain. *In vitro* tests with varied volumes (5, 10, 15, and 20µl) as shown in Fig.6 (a, and b) represented that all extracts have antibacterial actions and inhibited bacterial growth. WGO (Mixtures 2) revealed a low inhibitory percent; In Figure 6a it is obvious that, when the mixture volume was increased the bacterial inhibition (in case of *Bacillus subtilis* **ATCC 6633**) increased as well. On the other hand, no significant change was observed when the mixture was tested on the Gram-negative strain (*Salmonella typhimurium* ATCC14028) where the percentage of inhibition remained un-

changed Figure 6b. Furthermore, Ag NPs concentrations of 10 and 50 ppm, showed approximately the same effect on both strains.Combination of Ag NPs and WGO showed the best antibacterial effect, (80.5% and 91% for *Bacillus subtilis* ATCC 6633 and *Salmonella typhimurium* ATCC14028 respectively. Similar results were reported in previous studies. WGO was investigated for its antibacterial properties against bacteria that might cause infection in humans. Hospital-acquired infections are mostly caused by Staphylococcus aureus and *Escherichia coli*. These creatures live in and on the human body in their natural state [37] [38].

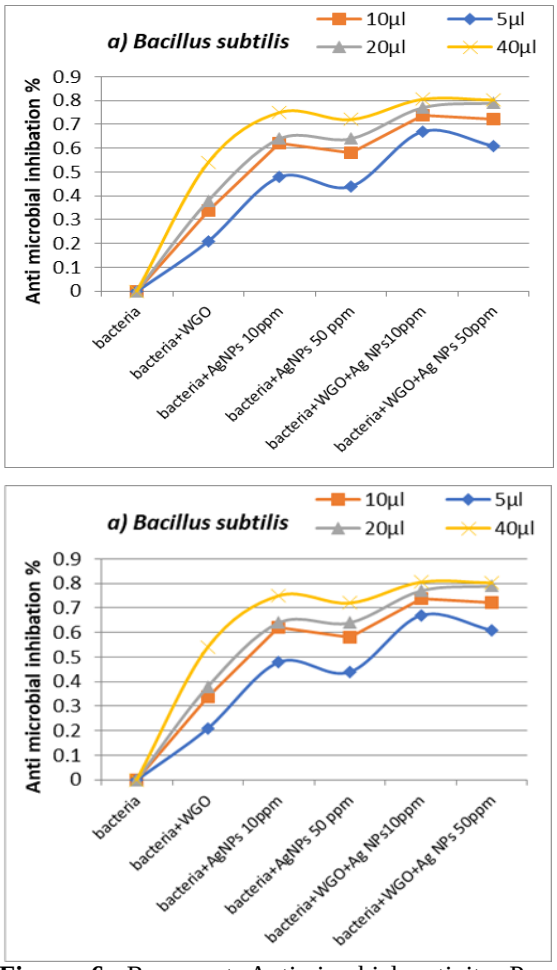

Figure 6. Represent Antimicrobial activity Percentage (%) of Ag NPs, WGO, and its combination where, Positive Control (bacteria only), bacteria + WGO, bacteria + Ag NPs 10ppm, bacteria + Ag NPs 50ppm, bacteria + WGO + Ag NPs 10 ppm, bacteria + WGO +Ag NPs 500ppm on a) Bacillus subtilis (ATCC 6633), b) Salmonella typhimurium ATCC14028.

This study's prior result corresponded to Robatjazi et al., [39], evaluated two standard strains of *Staphylococcus aureus* and *Pseudomonas eruginosa* as representative of gram+ve and gram-negative bacteria, respectively. *Staphylococcus aureus* had a -24mm inhibition zone, while *Pseudomonas eruginosa* showed 16mm inhibition zone[38]. Based on the findings of this study WGO suggested to be used as a food supplement or in the pharmaceutical applications as an easily accessible source of natural antioxidants and antibacterial. The previous suggestion was in agreement with Erjaee et al., [39] Ag NPs also demonstrated remarkable antibacterial efficacy against the pathogens examined, according to the researchers. Synthesized silver nanoparticles have an antibacterial action. Antimicrobial activity of Ag NPs against biologically tested bacteria was outstanding. Ag NPs were found to have a good inhibitory zone against all strains tested. *Salmonella typhimurium* is more susceptible to Ag NP inhibition than *Bacillus subtilis.* When compared to Gram-positive bacteria *S. aureus and B. subtilis*, Gram-negative bacteria *E. coli* and *Salmonella typhimurium* demonstrated a sensitive activity[14], [39].

This observation is supported by previous result of Abbaszadegan ,[40] who confirmed that Gram-positive bacteria are more resistant to silver's antibacterial action he structural differences in the cell membrane of Gram-positive and Gram-negative bacteria are responsible for these disparities. The cellular wall of Gram-positive strains is broader than that of Gram-negative strains. [41] . peptidoglycan, Gram-positive bacteria have a thicker cell wall than Gram-negative bacteria. On the cell wall of Gram-positive bacteria, several layers of peptidoglycans, as well as molecules of teichoic acids or lipoteichoic acids, generate a high negative charge, which may aid in the sequestration of free silver ions. Gram-positive bacteria's outer membranes may enable less silver to penetrate the cytoplasmic membrane in this way than Gram-negative bacteria's [42]. Consequently, Gram negative bacteria are more susceptible.

3.3. Effect of Administration of Wheat Germ Oil and Ag NPs

3.3.1. Feed Intake (FI), and Body Weight Gain (BWG)

Values of (FI), and (BWG) as screening for bioactivity of the tested groups were shown in Figure 7 (a, b). After 6 weeks, animals in the positive control group (G2) ($NaNO_3$ group) showed a decrease in (FI) and (BWG) equated to the control group. When nitrate is present in a rat's diet, food intake, body weight gain, and feed efficiency ratio all fall significantly, this decrease is due to the negative effects of nitrate and nitrite on nitrosamine production as well as the inhibitory action of nitrite on digestive enzymes. Helal et al., finding were in line with the current findings[43] who reported that increasing nitrate in the diet reduces food intake, body weight gain, and beta carotene consumption, resulting in a decrease in vitamin A levels in the

liver (60 and 80 %). By reducing food intake, nitrate causes weight reduction in the body. Influencing the neurological regulation of feeding habits, causes a decrease in growth hormone receptors in the liver. As a result, there is a deficiency of plasma somatomedins, which affects body growth.

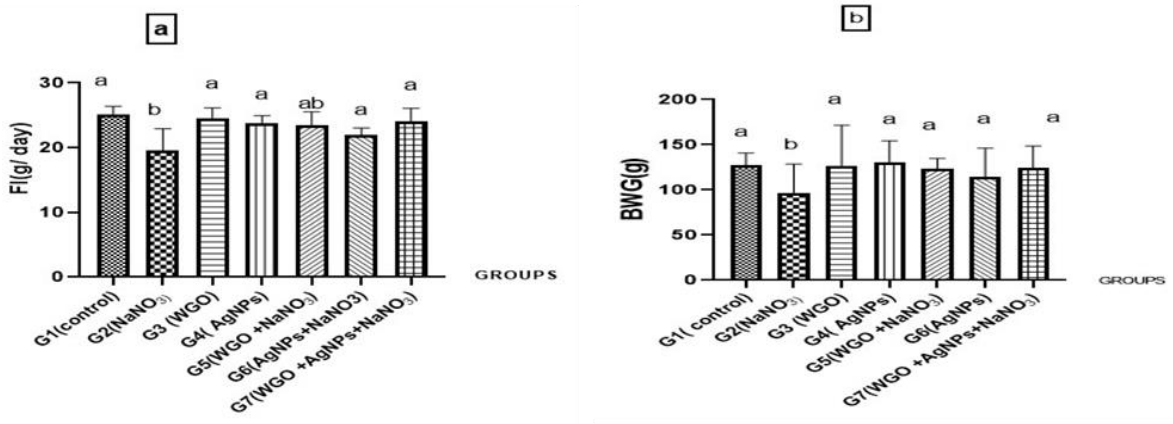

Figure 7. Effect of Ag NPs 10ppm and (WGO) and its combinations on: (a) Feed intake (FI) , (b) Body Weight Gain (BWG) in g, Where, G1 represented negative control group, G2: positive control ($NaNO_3$), G3: (WGO)control, G4: Ag NPs (10PPm) control, G5:(WGO+$NaNO_3$), G6:(Ag NPs+ $NaNO_3$), G7: WGO+ Ag NPs 10 PPm)+ $NaNO_3$. Represents the mean value ± S.D. (n=10 rats / group), significantly different using One-way ANOVA ($P < 0.05$), Values with the same letters indicate insignificant difference and vice versa.

3.3.2. Lipid Profile

Lipids are complicated and critical biological molecules that play a role in cellular membrane function. Bile acids, steroid hormones, and vitamin D are all produced. Circulating lipids are carried by lipoproteins, which are water soluble particles made up of a lipid nonpolar core of triglycerides and esterified cholesterol wrapped with lipoproteins, phospholipids, and other polar lipids. Lipoproteins and chylomicron-proteins are characterized as high density (HDL), low density (LDL), intermediate density (IDL), and very low density (VLDL).

HDL helps scavenge extra-hepatic tissue cholesterol-decrease the concentration of HDL has led to increased concentrations of cholesterol. There is evidence that serum cholesterol and LDL levels have already been linked to a higher risk of coronary heart disease (CHD) [46]. Cardiac risk ratio (CRR) is a relatively new metric that is frequently employed as a leading indication of dyslipidemia and related conditions such as cardiovascular disease [44].

As indicated in Figure 8, the mean value of total cholesterol, triglycerides, and LDL was considerably higher in the nitrate group (G2) than in the control group (a, b, and d). HDL, on the other hand, was considerably lower than control, as seen in Figure 8c very low-density lipoprotein (VLDL) in the positive control group (G2) were higher than in the control group. Where these parameters were attenuated in all treated groups (G5, G6, and G7). The previous findings matched those of Helal et al., [43] who studied the effect of nitrate on thyroids levels in rats. Increased T4, T3 levels and lower pituitary TSH production due to a negative feedback process. Akka, et al., [45] characterized decreasing FT4 and an increased TSH, and also linked to elevated total cholesterol, LDL-C, and serum triglyceride concentrations due to a reduced removal rate from plasma in such cases. The delivery of WGO, Ag NPs, and its combination to the nitrate treatment groups resulted in a significant decrease ($P< 0.001$) in total cholesterol, triglycerides (TG), LDL-c, and VLDL-c values In addition, as seen in Figure 8, there was a considerable rise in HDL-cholesterol (HDL-c) when compared to the equal positive treatment group (a-f). The previous findings matched those of Nagib, [8], who discovered a highly significant increase in TG, TC, LDL-c, and VLDL-c ratios in positive group rats. As well as a highly significant reduction in HDL-c. As WGO was given to rats, all of the lipid metrics were significantly improved when compared to the positive group of rats. When compared to a positive control, these findings support those of Saleh, H., [46]who reported that utilizing WGO resulted in a significant reduction in total cholesterol, TG, LDL-c, and VDL-c levels, as well as a significant increase in HDL-c. Wheat germ oil has a combination of Vitamin E, octacosanol, linoleic and linolenic acids, which are all beneficial. (WGO) has a number of additional nutritional and physiological benefits, including a high vitamin E and phytosterol

content, which may explain why it decreases triglyceride [46]. The decline in triglyceride and phospholipid levels could be due to those components. WGO also boosted high-density lipoprotein-cholesterol (HDL-c), a kind of cholesterol that absorbs and transports cholesterol from the bloodstream back to the liver. As a result, it is excreted from the body, reducing the negative effects of LDL cholesterol and the risk of atherosclerosis and cardiovascular disease [47] [22]. WGO has also been proven to help lower blood cholesterol, manage chronic inflammatory reactions, and treat neurological illnesses [48]. Previous studies demonstrated that Ag NPS reduces hyperlipidemia in G6 in agreement with Hussein et al., [34], who reported that both cholesterol and triacylglycerol levels dropped and returned to control values. However, the treated groups that drank a combination of (WGO and Ag NPs) showed no significant difference from the control group, indicating that the best effectiveness may be firm by comparing the different treated groups and determining the best outcome of this formula.

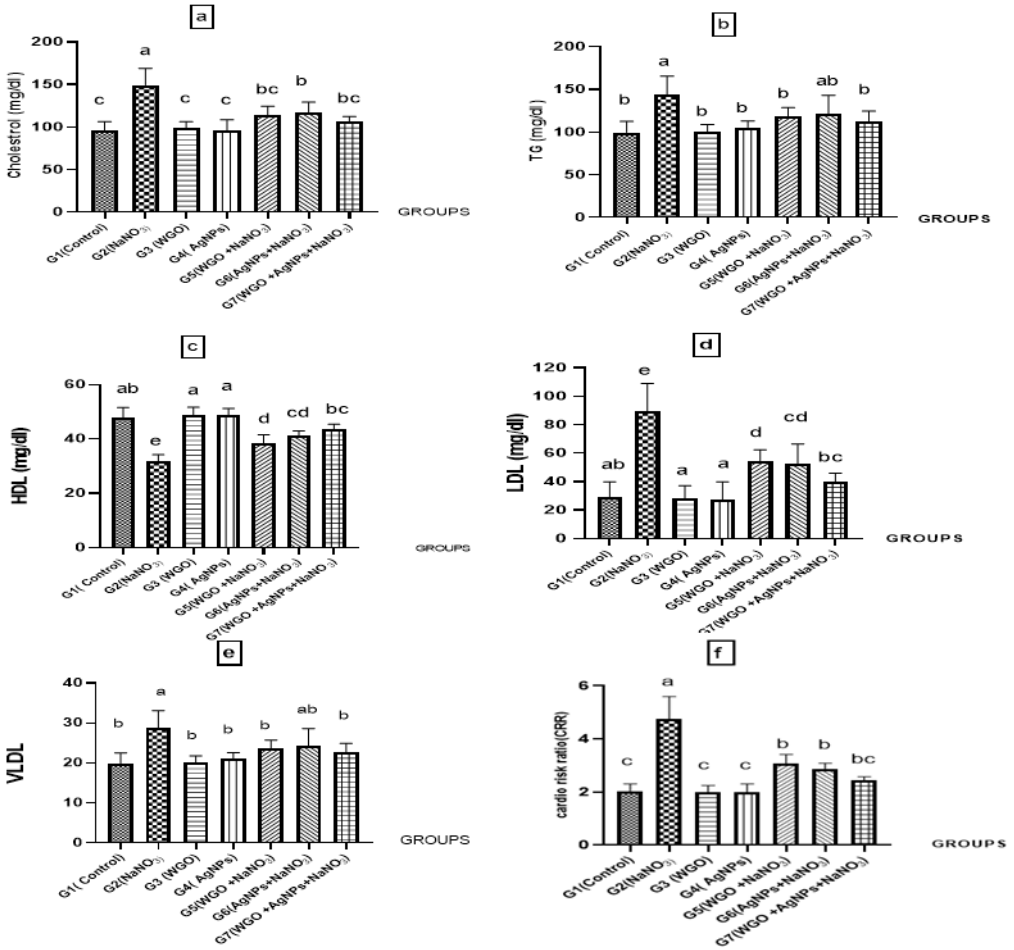

Figure 8. Effect of AgNPs 10ppm and (WGO) and its combinations on: (a) Cholesterol (mg/dl), (b) Triglyceride (TG) (mg/dl), (C) HDL (mg/dl), (d) LDL (mg/dl), (e) VLDL, (f) Antherogenic Index (AI), Where, G1 represented negative control group, G2: positive control ($NaNO_3$), G3: (WGO)control, G4: AgNPs(10PPm) control, G5:(WGO+$NaNO_3$), G6:(AgNPs+ $NaNO_3$), G7: WGO+ AgNPs (10 PPm)+ $NaNO_3$. Represents the mean value ± S.D. (n=10 rats / group), significantly different using One-way ANOVA ($P < 0.05$), Values with the same letters indicate insignificant difference and vice versa.

3.3.3. Kidney Function Parameters

Urea is a waste product that results from the breakdown of proteins. A high urea level ('uremia') suggests that the kidneys are not working properly. The term "creatinine" refers to a waste product produced by the muscles. Creatinine is a substance that enters the bloodstream and is excreted in the urine. $NaNO_3$ group there was a highly significant increase in blood urea nitrogen (BUN) and creatinine as shown in Figure9 (a, b). An increase in creatinine and urea-plasma concentrations suggests an

impairment of kidney function. These results were in agreement with an increase in protein catabolism in mammals. The previous results correlated with the findings with of Bouaziz et al., who realized on nitrate sodium was administered to adult rats. Nitrate induced considerable harm to the cortical and medullar portions of the kidney structure in the $NaNO_3$-treated group. Most glomeruli had congestion, lobulation, shrinkage, a wide glomerular gap, and peri-glomerular infiltration of mononuclear cells, all of which indicated inflammatory activity [49]. Some previous studies also indicated the same result with Piacenza et al., [50] who discovered that in an "acute kidney injury (AKI)" animal model. An augmented renal function could be the result of an increase in total cholesterol percentage, triglyceride,and LDL-c, these findings are inextricably linked to Visconti et al.,[51] who established that both total cholesterol and LDL-c increased glomerular basement permeability without chronic kidney disease (CKD), As a result of the lack of lipoprotein lipase activators, hyperlipidemia develops.

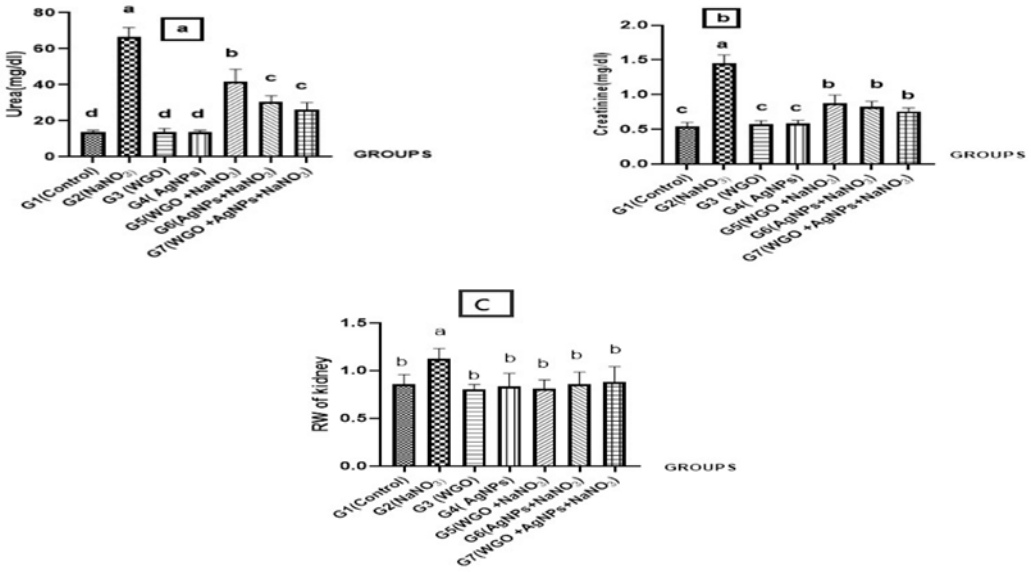

Figure 9. Effect of AgNPs 10ppm and (WGO) and its combinations on: (a) Urea (mg/dl), (b) Creatinine (mg/dl), Where, G1 represented negative control group, G2: positive control (NaNO3), G3: (WGO)control, G4: AgNPs(10PPm) control, G5:(WGO+NaNO3), G6:(AgNPs+ NaNO3), G7: WGO+ AgNPs (10 PPm)+ NaNO3. Represents the mean value ± S.D. (n=10 rats / group), significantly different using One-way ANOVA ($P < 0.05$), Values with the same letters indicate insignificant difference and vice versa.

This dyslipidemia stands linked to a higher risk of atherosclerosis and cardiovascular disease. Lowering the numeral and changing the type of cholesterol-resonant atherogenic lipoprotein particles is a key to lowering cardiovascular risk in these patients. In non-hemodialysis CKD patients, Statins are critical in the primary prevention of cardiovascular events and death. In terms of renal failure progression, the advantages are variable. CKD clinical therapy should involve patient education on dietary regimens [51]. In renal cells, scavenger receptors (SRs) such as SR-B (CD36), SR-AE, and SR-EA take up lipoproteins (LOX-1). In response to pathogenic activation, scavenger receptors are unregulated, and their level is connected to the severity of renal injury [20]. Furthermore, intracellular cholesterol does no effect on the expression of scavenger receptors (SRs). As a result, compartments expressing SRs can internalize a large number of cholesterol esters, resulting in the production of foam cells. All of these methods cause increased lipid absorption by renal cells [52]. While Ag NPs (G6) fell significantly in the WGO treated groups (G5) compared to the positive control group (G2), renal function was recovered to almost that of the treatment combination group (G7). Consumption of nitrate by adult rats affected also the soft organ weights kidneys. The relative weight was increased indicating a nephromegaly and its dysfunction. An increase in a relative kidney weight observed in Figure 9c in the nitrate group was confirmed by a previous study of by Bouaziz, et al., [49] found the same result in the rats treated with nitrate. Biochemical parameters are confirmed by histological examination. This was most likely caused by a blockade of salt and water resorption in the proximal renal tubules ascending limb of Henle's, increased blood flow to the renal medulla and diminished antidiuretic hormone response of the collecting duct.

3.3.4. Oxidative stress biomarker

Glutathione (GSH) is a non-protein thiol abundant in body cells. GSH is a potent antioxidant found in all living things. It acts as a cellular redox state regulator, protecting cells from lipid peroxides, reactive nitrogen, and oxygen species. GSH plays a protective role in the detoxification of carcinogens in healthy cells. In terms of other kidney dysfunction-related metrics, it is found that GSH, MDA, and CAT in the kidney homogenates tissue, which represents in Figure10 (a, b, and c). The positive control group ($NaNO_3$) had a considerable a significant drop GSH and effectiveness of (CAT) activity versus with negative control group. The data also revealed that GSH and CAT enzyme activity in the group fed with WGO improved slightly (G5) but not significantly when compared to the positive control. G6 and G7 showed a considerable increase in (GSH) and (CAT) when given Ag NPs, when WGO and Ag NPs were combined (G7) showed a good enhancement. These results may be due to antioxidants such as vitamin E, total phenols and flavonoid , which protect unsaturated fat in the body from oxidation [53]These records are in analogous with those attained by Nagib, [54]exhibited that oral supplementation of WGO showed increased glutathione, As a result, the oil has a significant amount of α-tocopherol. [55].

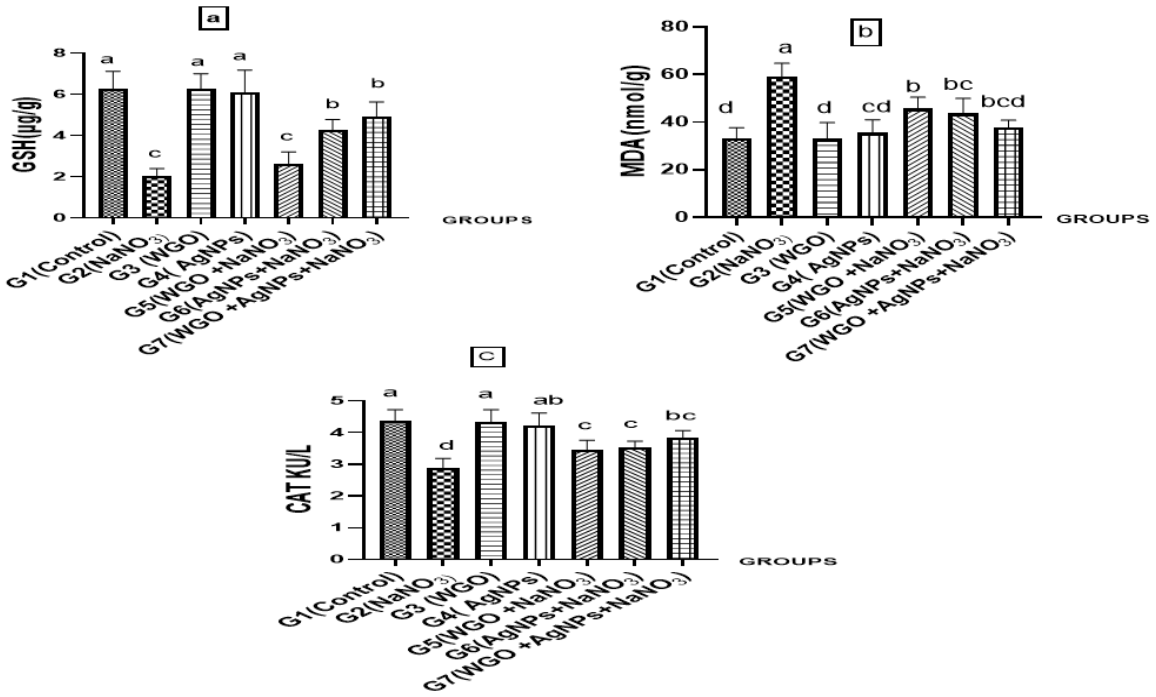

Figure 10. Effect of Ag NPs 10ppm and (WGO) and its combinations on: (a) glutathione (GSH)(µg/g), (b) malondialdehyde MDA (nmol/g), (C) catalase enzyme CAT KU/L, Where, G1 represented negative control group, G2: positive control (NaNO3), G3: (WGO)control, G4: Ag NPS(10PPm) control, G5:(WGO+NaNO3), G6:(Ag NPs+ NaNO3), G7: WGO+ Ag NPS(10 PPm)+ NaNO3. Represents the mean value ± S.D. (n=10 rats / group), significantly different using One-way ANOVA ($P < 0.05$), Values with the same letters indicate insignificant difference and vice versa.

As shown in Figure 10 b the level of MDA showed increasing significantly in the $NaNO_3$ group. The result is reliable with Abdulshahed et al., [56]. The creation of free radicals as a result of the $NaNO_3$ transformation after intake for NO_2 ione is the source of the elevated MDA concentration. There is oxidative stress owing to commensal bacteria on the tongue's surface that continue to metabolise NO, resulting in the generation of free radicals, particularly ONOO-, which causes damage to key biological molecules including as proteins, lipids, and nucleic acids. The findings also indicated statistically significant differences in WGO and Ag NPs therapy in groups G5 and G6. When compared to the positive control group, the WGO group improved first, followed by the Ag NPs group, and the largest improvement in the mixed group (G7) was a decrease in MDA, which was recovered to near-normal levels in the negative control. WGO treatment provides therapeutic effects, according to El-Shorbagy, by decreasing the generation of free radicals and boosting the levels of intestinal endogenous antioxidants. WGO's anti-inflammatory properties are due to its high vitamin E content, which can inactivate reactive free radicals and prevent the radical chain reaction from propagati [57]. Because it contains a considerable amount of unsaturated (81%) and several saturated (64%) fatty

acids, WGO has anti-inflammatory properties and can lower oxygen free radicals and nicotinamide adenine dinucleotide dinucleotide phosphate (NADPH) oxidase activity [46]. Additionally, the phenolic components in WGO have an antioxidant impact, which may aid to reduce pro-oxidative states and give significant antioxidant protection to the body's numerous organs[58].

Renal failure can also be caused by oxidative stress in the kidneys. The formation of reactive oxygen and nitrogen species, which oxidize proteins, lipids, and DNA, was a potential source of tissue harm. Furthermore, nitrate is now recognized as an oxidant product and a readily available source of nitric oxide. Chow and Hong [59] found that the latter reacted swiftly with superoxide anion to produce peroxynitrite. These radicals damage the cell membrane, causing it to disintegrate and destabilize, as well as lipid peroxidation. These findings corroborated prior findings mentioned above. Similarly, the enhanced lipid peroxidation seen following nitrate therapy suggested that oxidative stress had a role in the $NaNO_3$-induced kidney injury. Previous research findings in animal models of CKD has found that the disease promotes lipid accumulation in the artery wall and kidney, leading to atherosclerosis, glomerulosclerosis, and tubule interstitial damage. Increased lipid cellular influx, increased cellular synthesis and reduced cellular catabolism of fatty acids, and impaired antioxidant, anti-inflammatory, and reverse lipid transport characteristics of HDL appear to mediate these effects. This syndrome is characterized by oxidative stress and dyslipidemia, both of which are prevalent symptoms. Impaired clearance and increased oxidation of apo-lipoprotein B containing lipoproteins and their atherogenic remnants, as well as a reduction in plasma concentration, antioxidant, and anti-inflammatory properties of high density lipoprotein, are markers of dyslipidemia in patients with advanced CKD (HDL) [60].

3.5. Histopathology of the Kidney

The present research explored the effects of high lipid on kidney impairment which caused by nitrate. Microphotograph of rat kidney in (G2) represents positive control ($NaNO_3$) presented focal degeneration and complete necrosis of renal tubular epithelium with sloughed cells and renal cast inside their lumen. Some glomeruli show vascular congestion. Congestion of renal blood vessels associated with perivascular edema (HE, x200) represented in Figure11b); These data were in coherence with Abdulshahed et al., [56] substantial tissue alterations were recorded A rat kidney slice treated with sodium nitrate shows glomerular atrophy, glomerular tuft congestion, increased Bowman Space, renal tubular degeneration, and cortical dissolution. These histological alterations were most likely caused by the generation of free radicals and the promotion of lipid peroxidation. Observation groups (G1, G3, and G4) represented in Figure 11(a, c, and d) respectively showed normal structure of renal tubule and glomeruli (HE, x100).

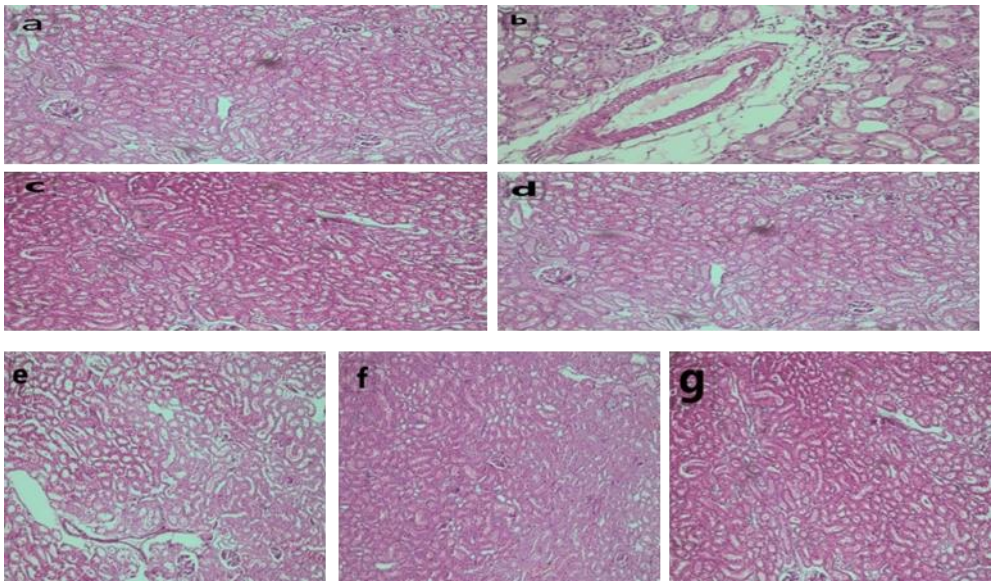

Figure 11. Microphotograph of rat's kidney for: (a, c, and d) normal control rats showing normal structure (HE, x200), (b) positive control rats' showing focal degeneration and complete necrosis of renal tubular (HE, x200), (e) Rats fed (WGO)showing slight degeneration of the epithelial lining of some renal tubules (HE, x100), (f) Rats fed AgNPs showing normal structure mild degenerative changes of some renal tubules and renal cast (HE, x100), (g) Rats fed combination between (WGO)and Ag NPs showing normal structure of renal tubule and glomeruli (HE, x100

Microphotograph of rat kidney in Treatment group (G5) which fed on (WGO) showed slight degeneration of the epithelial lining of some renal tubules (HE, x100). (G6) showed mild degenerative changes ~~of~~ in some renal tubules and renal cast (HE, x100). G7 fed on combinations of (WGO and Ag NPs) returned to show the normal structure of renal tubule and glomeruli (HE, x100). These findings were dependable with those of Soliman et al., [61] who reported that WGO had a favorable effect on rats exposed to radiation, resulting in inflammation; glomeruli cell infiltration, interstitial fibrosis, cloudy appearance, and loss of cellular architecture. The treatment of irritated rats with (WGO) improves the inflammatory process dramatically. The lipid peroxidation was reduced by WGO. The mechanism of WGO is related to linolenic and linoleic acids, which are sources of omega 3 and omega 6. These two fatty acids are involved in human metabolism and are precursors to prostaglandins, which aid in the healing inflammatory processes[62]. Rats were given Ag NPs as a treatment that significantly reduced pro- inflammatory cytokines to the normal levels, which could be due to Ag NPs' anti-inflammatory and antioxidant characteristics since excessive free radical accumulation causes inflammation and programmed cell death. Furthermore, Ag NPs are important in reducing inflammatory indicators and inhibiting inflammatory events as well as lowering the number of cytokines. The pro-inflammatory cytokines were dramatically decreased to normal levels, perhaps saving lives Because of the overabundance of free radicals, AgNPs have anti-inflammatory and antioxidant capabilities[63].

4. Conclusion

In this study, sodium citrate was utilized as a reducing and stabilizing agent for all silver ions, and silver nanoparticles (Ag NPs) were successfully formed in oleic acid. This study has an important meaning for the treatment of infections by examining the antibacterial properties of WGO and Ag NPs. generated Ag NPs with and without WGO was investigated in vitro versus Bacillus subtilis (ATCC 6633) as the positive bacteria, and Salmonella typhimurium (ATCC 14028) was employed as the negative bacteria in this study. Combination of WGO, and Ag NPs showed an antibacterial effect reaching 91%, and 80% on *Salmonella typhimurium* and *Bacillus subtilis* respectively *In Vitro*, . The research managed to combine wheat germ oil and symbiosis Ag NPs diminished the preceding parameter, intake orally $NaNO_3$ 15 mg/kg with WGO, Ag NPs10ppm, and a combination of WGO and AgNPs10ppm respectively. In the positive control group were appeared; the body weight gain (BWG) and feed intake (FI) are decreased, increasing in renal function resulting from lipid profile syndrome, oxidative stress biomarker showed increasing in malondialdehyde (MDA), decreasing in glutathione (GSH), and catalase enzyme (CAT). combination between WGO, AND Ag NPs minimize the deleterious side effects of sodium nitrate on male albino rats as an *in vivo.*

Abbreviations

Silver nanoparticles	AgNPs
Tri-sodium citrate	TSC
Wheat Germ Oil	WGO
Polyunsaturated fatty acids	PUFAS
Relative weight	RW
Transmission electron microscopy	TEM
Acute kidney injury	AKI
Renal inducible nitric oxide synthase	(iNOS)
Antherogenic index	AI
Cardiovascular diseases	CVD
X-ray diffraction	XRD
Atomic force microscopy	AFM
Poly dispersity index	PDI
Dynamic light scattering	DLS
Chronic kidney diseases	CKD

Conflict of interest

The author asserts that they have no competing interests in this paper's publication.

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تحسين اضطرابات الكلى والدهون التي يسببها نترات الصوديوم من خلال الجمع بين جسيمات الفضة النانومتريه مع زيت جنين القمح في المختبر/ الجسم الحي

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الملخص:

قد تساعد المواد النانومتريه في تحسين نوعية حياة الإنسان, لذا افترضت الدراسة أن جسيمات الفضة النانومتريه وزيت جنين القمح لها تاثير مضاد للبكتريا في المختبرويخففان اضطرابات الكلى والدهون في الجرذان الناتجة من استهلاك مادة نترات الصوديوم التي تدخل في غذاء الانسان كمادة حافظة في بعض الاغذيه. تم تحضير جسيمات الفضة النانومتريه بتركيزين (10 ، 50 جزء في المليون). وتم توصيف تلك الجزيئات المصنعة عن طريق التحليل الطيفي للأشعة فوق البنفسجية، وانحراف الأشعة السينية، والفحص المجهري للقوة الذرية، وتشتت الضوء الديناميكي؛ كان متوسط حجم تلك الجزيئات 16.8 نانومتر.تم تقييم تأثيرالمواد سالفة الذكر كمضادت للبكتيريا على باسيلس سيبتليس (*Bacillus subtilis ATCC 6633*) كبكتيريا موجبة والسالمونيلا (*Salmonella typhimurium(ATCC1402*) كبكتيريا سالبة حيث أظهر خليط زيت جنين القمح مع جسيمات الفضة النانومتريه أقصى فعالية للجراثيم على *Salmonella typhimurium* التي وصلت إلى 91٪ مع تأثير غير معنوي للتركيزات المختلفة لهذة الجزيئات. علاوة على ذلك، تمت التجربة علي الجرذان البيضاء حيث تم توزيع 70 من ذكورالجرذان إلى سبع مجموعات وتحتوي كل مجموعة علي عشرة من تلك الجرذان: المجموعة الاولي تمثل المجموعة الضابطة، المجموعة الثانية كانت المجموعة الإيجابية حيث تناولت نترات الصوديوم عن طريق الانبوب الفموي بتركيز 15 مجم / كجم من وزن الجرذ، المجموعة الثالثة تغذت علي زيت جنين القمح، المجموعة الرابعة اعطي لها جسيمات الفضة المحضرة بتركيز 10 جزء في المليون. تناولت المجموعات (الخامسة و السادسة و السابعة) عن طريق الانبوب الفموي نترات الصوديوم بنفس التركيز مع زيت جنين القمح، جسيمات الفضة النانومتريه ومزيج منهما على التوالي. وأظهرت النتائج في المجموعة الضابطة الإيجابية المتناولة لنترات الصوديوم انخفاض في وزن الجسم، وزيادة في وظائف الكلى الناتجة عن متلازمة ارتفاع الدهون الكلية، وأظهرت المؤشرات الحيوية للاجهاد التأكسدي زيادة في الدهون المؤكسدة ، وانحفاض مستوى الجلوتاثيون، وإنزيم الكاتلاز. بينما أظهرت المجموعات المعالجة تحسن ملحوظ في الوظائف الحيوية، وايضا تحسن في الانسجة الحيويه للكلي . يمكن أن يستنتج البحث أن الجمع بين زيت جنين القمح جزيئات الفضة النانومتريه فعال في الحد من المضاعفات المرتبطة بـتناول نترات الصوديوم كمادة حافظة.