Synthesis and Characterization of Silver Nanoparticles Using Nigella sativa Seeds and Study their Effects on the Serum Lipid Profile and DNA Damage in Rats' Blood Treated with Hydrogen Peroxide

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ABSTRACT

This study aimed to produce silver nanoparticles using aqueous extract of *Nigella sativa*, also to investigate the effects of green synthesized *Nigella sativa* seeds silver nanoparticles on dyslipidemia and DNA fragmentation in hydrogen peroxide-exposed rats. The produced *Nigella sativa* seeds silver nanoparticles were characterized through Ultraviolet-visible spectroscopy, Fourier-transform infrared spectroscopy, X-ray powder diffraction (XRD) style, and Scanning Electron microscope, and the morphology and size of these synthesized nanoparticles were investigated. Forty adults male rats were randomly and equally divided into five groups, which had been inspected daily for two months as followings: G1 group (Control), G2 group received tap water containing 1% H_2O_2, animals in G3 and G4 groups were injected IP with *the* nanoparticles in a dose of 25 and 50 mg/kg BW, respectively, and also received ordinary tap water containing 1% H_2O_2 , and in G5 group, the animals were injected IP with *Nigella sativa* seeds extract in 50 mg/kg BW and received ordinary tap water containing 1% H_2O_2. Blood samples were collected after one and two months of the experiment from each animal for DNA fragmentation measurements and serum lipid estimation. The results reported a case of dyslipidemia, as well significant elevation in DNA damage in G4 and G2 groups. The results also confirmed the hypolipidemic and cytoprotective effect of *Nigella sativa* seeds extract (G5 group) and silver nanoparticles, and group G3 clarified the correction between dyslipidemia, and the significant alleviation in DNA damage. In conclusion, the current study shows the effects of high doses of *Nigella sativa* seeds silver nanoparticles, and documents the ameliorative effect of *these* seeds extract and *their* silver nanoparticles on lipid profile and DNA damage.

Keywords: *Nigella sativa*, Nanoparticles, Lipids profile, DNA damage, Hydrogen peroxide

Introduction

Worldwide interest has been emerged on different aspects of nanotechnology research and more varied applications and developments for different kinds of nanoparticles have been identified involving energy, electronics, industries of space and medicine (1, 2). Metal nanoparticles are in electrical analysis and bio-electrochemical usage owing to their unique electro catalytic activity (3). Silver nanoparticles (AgNPs) based nanostructures were evaluated as appropriate carriers of diverse therapeutic molecules, involving, antimicrobial (4, 5), anti-inflammatory (6, 7), antioxidant (8, 9) and anticancer (10, 11).
AgNPs were found in medical devices like pacemakers, vascular prostheses, and wound dressings (12,13) and cardiovascular catheters (14). cardiovascular catheters (14).
Also used in cosmetics (15, 16), food stockpiling (17, 18), textile coatings (19, 20) and several environmental applications (21, 22). AgNPs attack various biological processes including cell membrane structure and its functions, degradation

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of enzymes, inactivation of cellular proteins, and breakage of DNA thereby results in cell lysis (23, 24). Previous studies have shown that AgNPs caused genotoxic effects in mice and rats after oral ingestion (25), systemic uses (26) and *in vitro* (27). AgNPs toxicity was correlated with releasing of silver ion and oxidative stress (28, 29) and always size and dose dependent (30-32).

Hydrogen peroxide, an antioxidant and non-radical, is produced by different physiological processes (33) and environmental pollutant, and could be a cause of lipid peroxidation and oxidative stress that could lead to oxidative DNA damage (34). Reactive oxygen species such as H_2O_2 was thought to promote tumorigenesis through oxidative DNA damage, inflammation, and genomic instability (35). Whatever, the mechanism that originates the ROS (H_2O_2), hydroxyl radical produced from H_2O_2 adducts of DNA, lipid peroxides (36), caused protein oxidation, lipid oxidation, DNA oxidation and DNA damage (37, 38). This study aimed to produce silver nanoparticles using aqueous extract of *Nigella sativa,* also to investigate the effects of green synthesized *Nigella sativa* Seeds silver nanoparticles on dyslipidemia and DNA fragmentation in hydrogen peroxide exposed rats.

Materials and Methods

Experimental animals. The current study was executed in the animal house of the college of Veterinary Medicine, AL-Qadisiya University through the period expanded from 1 November, 2017 to 31 October, 2018. Mature male Wistar rats aged 90 days and weighted 182 ± 5.6 g have been utilized. Green synthesis and characterization of silver nanoparticles by using *Nigella sativa* (black cumin) seed aqueous extract was performed by the following steps:

1. Collection of *Nigella sativa* seed: Aqueous extract of *Nigella sativa* seeds was prepared as described by (39, 40) with slight amendments.
2. Synthesis and characterization of *Nigella sativa* seeds silver nanoparticles (NSSSNPs): Green synthesis of AgNPs using *Nigella sativa* seed extract was prepared as described by (41-43). Characterization of *NSSSNPs* was performed by Ultraviolet–visible spectroscopy (Perkin Elmer Lambda 35 USA) as described by (43, 44), Fourier-transform infrared spectro-scopy (FTIR) (Shimadzu-8400S Japan) as described by (45, 46), X-ray diffraction (XRD) (Shemadzu-6000 Japan) as describe by (47, 48), Scanning Electron Microscope (SEM) (SEM-Tescan Vega III, Czech) as describe by (49).

Forty adult male rats were divided equally and randomly into 5 experimental groups, reared under normal conditions and treated daily for 8 weeks as follows: Control (G1) received tap water; G2: received tap water containing 1% of H_2O_2; G3: were injected IP with NSSSNPs (25 mg/kg BW) and received ordinary tap water containing 1% H_2O_2; G4: were injected IP with NSSSNPs (50 mg/kg BW) and received ordinary tap water containing 1% H_2O_2 and G5: were injected IP with *Nigella sativa* seed extract (50 mg/kg BW) and received ordinary tap water containing H_2O_2 1%. After one and two months, the blood samples were collected by orbital sinus technique from rats anesthetized by intramuscular injection with xylazine (40 mg/kg BW) and ketamine (90 mg/kg B.W), then serum was obtained for measuring the following: Lipid profile, including serum concentration of total cholesterol using TC kit (spinreact, ITALY) according to (50); triglyceride utilizing triglyceride kit (spinreact, ITALY), according to (51), low density lipoprotein-cholesterol and very-low density lipoprotein-cholesterol depending on Friedewald formula (52) and high density lipoprotein-cholesterol by utilizing HDL-c kit (spinreact, ITALY), according to (53). Besides, blood sample were also obtained for measuring DNA fragmentation percentage using comet assay kits (Trevigen, USA) as described by (54).

Statistical Analysis

Data were subjected to two-way ANOVA and least significant differences (LSD) to compare between means (55). The level of $P<0.05$ was considered significant.

Results and Discussion

Green synthesis and characterization of *Nigella sativa* seeds of silver nanoparticles: In the current study, the synthesized *NSSSNPs* were characterized by color alteration. The change in color of the mixture to dark brown occurred immediately after 24 hours of incubation in dark room as show in images (1-3). In the current study, better result for nanoparticles formed after reaction of $AgNO_3$ with *Nigella sativa* extract in percentage of 3:2 v: v ratio and pH 5.5. This reaction generated particles in crystallized form and sediment on bottom of beaker. The optical absorbance of the synthesized *NSSSNPs* was measured using UV-Vis spectroscopy between the lengths of 200 to 1100 nm, at resolution of 1 nm. An absorption peak between (430-460 nm) confirms the presence of AgNPs.

Using fourier-transform infrared spectroscopy (F-TIR), the peaks which refer to different functional groups t present in the compound prepared from reaction of $AgNO_3$ with *Nigella sativa* extract in percentage 3:2 v: v ratio in pH 5.5 was showed in (Figure 1). The most interesting peak bands in the FTIR spectrum of *NSSSNPs* were observed at 3286, 2962, 2931,1666, 1521,1446, 1390,846 and 526 cm^{-1} (Figure 2). Due to presence of N-H Stretching Vibrations groups in amide and O-H Stretching Vibrations groups in phenol, alkanes, C=O stretching vibration groups in amide C=C in aromatic, C-N stretching vibration groups in amide, CH aromatic Bending respectively. These results together showed that the functional groups of these bioactive compounds proved to have potential to act as reducing and stabilizing agents during the synthesis of silver nanoparticles. Image 4 showes the spherical shape of SNPs using SEM.

The pattern of X-ray diffraction peaks at theta angle-2 value of 15.3141, 37.8332, 44.2773, 64.0779, 77.0272 corresponding to hkl value from (110), (102), (400), (521) and (541) crystal planes was observed (Figure 3). According to the result of XRD analysis, the physical characteristics of particles in the prepared compound are tetragonal crystal, Centro symmetric, and the size of crystals was in range of 10.66-16.66 nm.

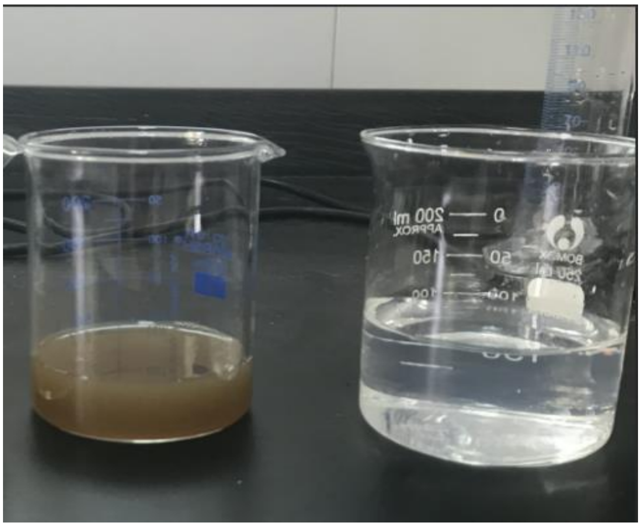

Image 1. Notice the $AgNO_3$ and *Nigella sativa* extract before mixing

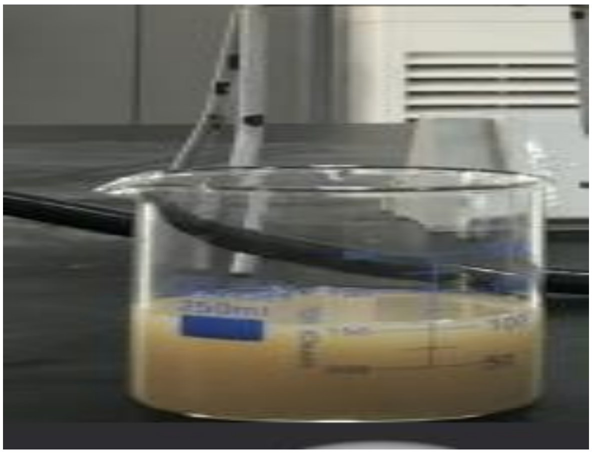

Image 2. Notice $AgNO_3$ with *Nigella sativa* extract after mixing and alteration of color to pale yellow

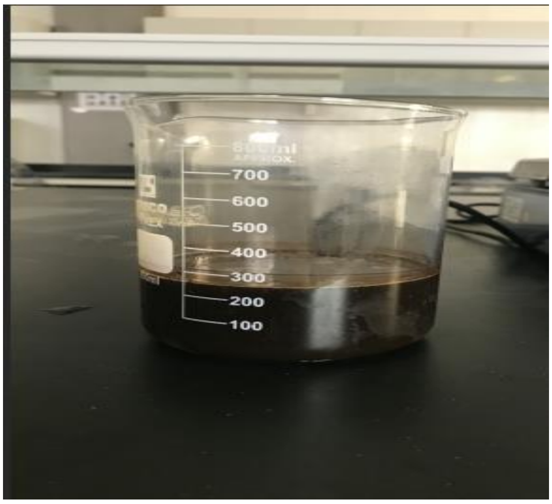

Image 3. Notice the $AgNO_3$ with *Nigella sativa* extract blend overnight after mixing whereas color become tan-brownish with the highest intensity

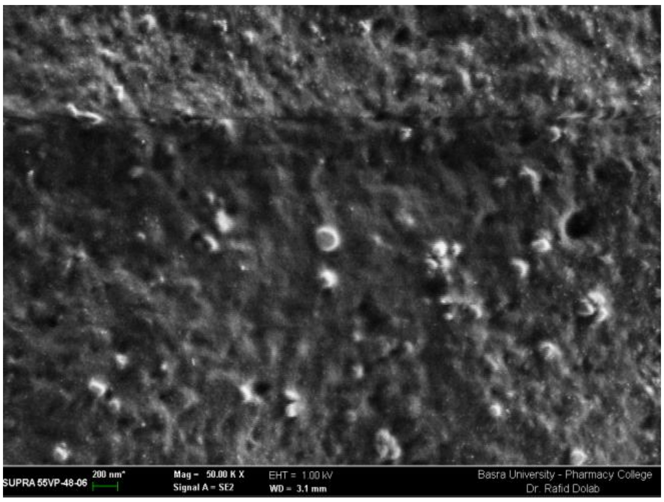

Image 4. SEM test image of the *Nigella sativa* seeds silver nanoparticles made from $AgNO_3$ with *Nigella sativa* extract in pH 5.5 (200 nm)

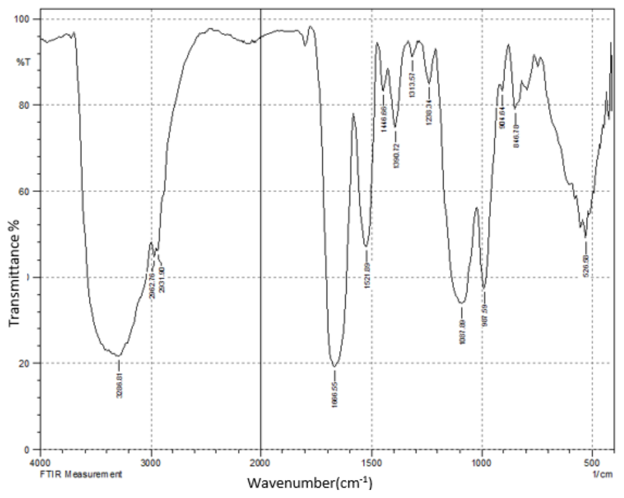

Figure 1. UV-Vis spectroscopy absorbance of *Nigella sativa* seeds silver nanoparticles made from $AgNO_3$ with *Nigella sativa* extract in pH 5.5

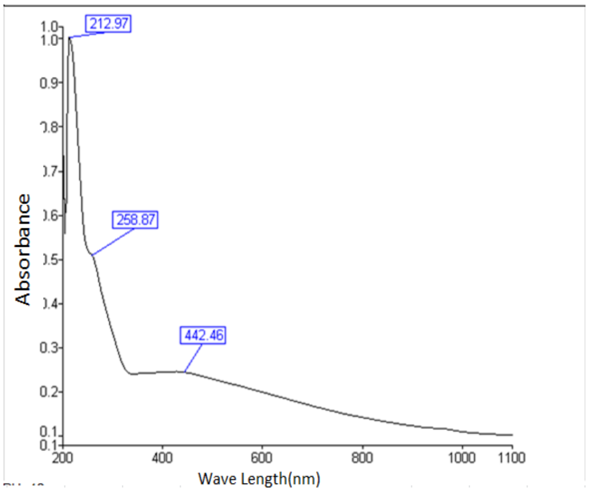

Figure 2. F-TIR spectroscopy for *Nigella sativa* seeds silver nanoparticles made from $AgNO_3$ with *Nigella sativa* extract in pH 5.5

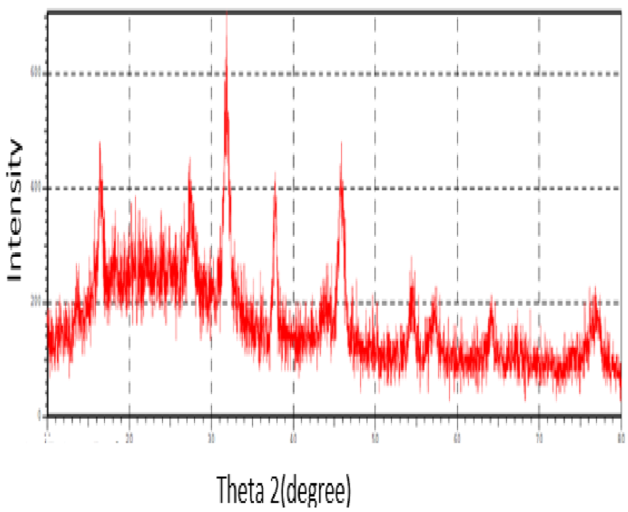

Figure 3. X-ray diffraction pattern for *Nigella sativa* seeds silver nanoparticles made from $AgNO_3$ with *Nigella sativa* extract in pH 5.5

At the end of the experiment, compared to the control and G5, there was significant elevation at $p<0.05$ in serum TC concentration observed in the treated groups that received 25 and 50 mg/kg B.W of *NSSSNPs* (G3+G4) and H_2O_2 groups (G2).

The result also showed that intra-peritoneal (IP) injection of NSSNP (group G3 and G4) caused significant reduction at $P<0.05$ in T-C concentration compared to the value in H_2O_2 (G2) group. Significant differences between groups G3 and G4 were also recorded (Figure 4).

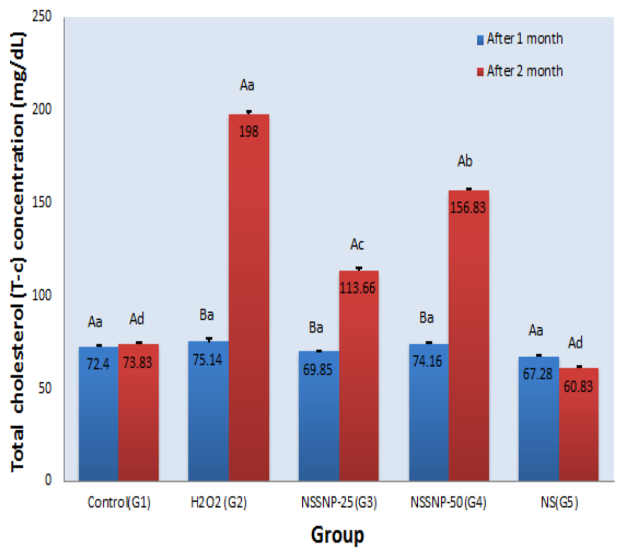

Figure 4. Effect of two concentrations of *Nigella sativa* seeds silver nanoparticles (*NSSSNPs*) and *Nigella sativa* extract on serum total cholesterol (TC) concentration (mg/dl) in H_2O_2 exposed rats. Values are expressed as mean±SE (n= 8). Various capital letters denote significant differences at $P<0.05$ between periods. Various small letters denote significant differences at $P<0.05$ between groups. Control (G1): Intact rats received drinking water daily for two months. H_2O_2 (G2): animals in this group received tap water containing 1% of H_2O_2. NSSNP-25 (G3): animals in this group were injected IP with *NSSSNPs* (25 mg/kg BW) and received ordinary tap water containing H_2O_2 1%. NSSNP-50 (G4): animals in this group were injected IP with *Nigella sativa* seeds silver nanoparticles (50 mg/kg BW) and received ordinary tap water containing 1% H_2O_2. (G5): animals in this group were injected IP *Nigella sativa* seed extract (50 mg/kg BW) and received ordinary tap water containing 1% H_2O_2

There was a significant ($P<0.05$) elevation in serum TAG concentration observed in groups G2, G3, and G4 after IP injection of *NSSSNPs* (25 and

50 mg/kg BW) or exposure to 1% H_2O_2 for one month compared to the value in groups G1 and G5. After two months of the experiment, IP injection of *NSSSNPs* (25-50) mg/kg BW or *Nigella sativa* (G5 group) caused significant decrease (P<0.05) in serum TAG concentration compared to the value in H_2O_2 treated group (Figure 5).

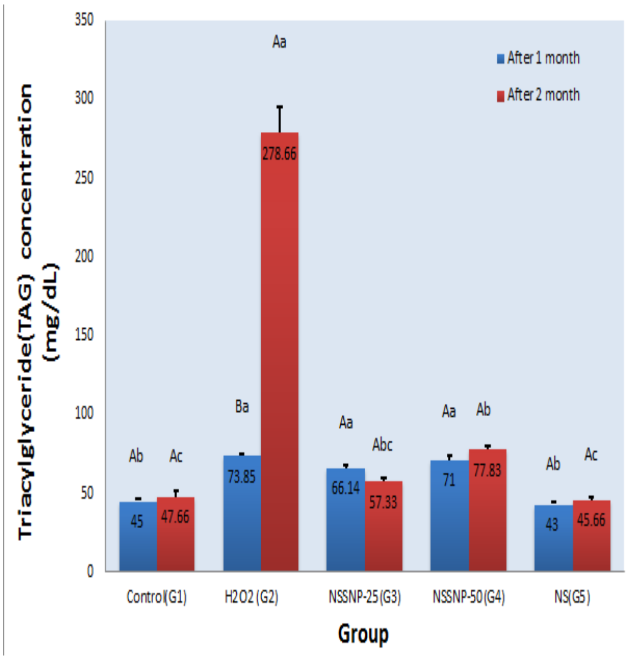

Figure 5. Effect of two concentrations of *Nigella sativa* seeds silver Nanoparticles *NSSSNPs* and *Nigella sativa* extract on serum triacylglyceride (TAG) concentration mg/dl in H_2O_2 exposed rats. Values are expressed as mean±SE (n= 8). Various capital letters denote significant differences at P<0.05 between periods. Various small letters denote significant differences at P<0.05 between groups. Control (G1): Intact rats received drinking water daily for two months. H_2O_2 (G2): animals in this group received tap water containing 1% of H_2O_2. NSSNP-25 (G3): animals in this group were injected IP *NSSSNPs* 25 mg/kg B.W and received ordinary tap water containing H_2O_2 1%. NSSNP-50 (G4): animals in this group were injected IP *Nigella sativa* seeds silver nanoparticles 50 mg/kg BW and received ordinary tap water containing1% H_2O_2. NS (G5): animals in this group were injected IP with *Nigella sativa* seed extract (50 mg/kg BW) and received ordinary tap water containing 1%H_2O_2

Significant elevation at P<0.05 in serum LDL-c concentration was observed after two months in H_2O_2 treated group, G4 group compared to the value of the treated groups G1and G3. The result also showed that IP injection of NSSNP 25 mg/K. BW or G5 group caused significant decrease at P<0.05 in in this parameter compared to the value in G4 and G2 groups (Figure 6).

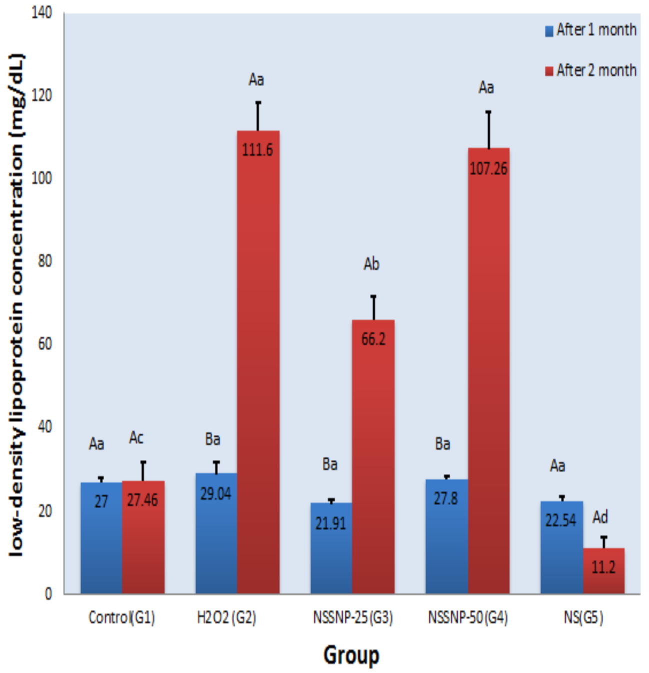

Figure 6. Effect of two concentrations of *Nigella sativa* seeds silver Nanoparticles *NSSSNPs* and *Nigella sativa* extract on serum low-density lipoproteins concentration (mg/dl) in H_2O_2 exposed rats. Values are expressed as mean±SE (n= 8). Various capital letters denote significant differences at P<0.05 between periods. Various small letters denote significant differences at P<0.05 between groups. Control (G1): Intact rats received drinking water daily for two months. H_2O_2 (G2): animals in this group received tap water containing 1% of H_2O_2. NSSNP-25 (G3): animals in this group were injected IP with *NSSSNPs* 25 mg/kg BW and received ordinary tap water containing 1%H_2O_2. NSSNP-50 (G4): animals in this group were injected IP with *Nigella sativa* seeds silver nanoparticles (50 mg/kg BW) and received ordinary tap water containing 1% H_2O_2. NS (G5): animals in this group were injected IP with *Nigella sativa* seed extract (50 mg/kg BW) and received ordinary tap water containing H_2O_2 1%

Significant elevation at P<0.05 in serum V-LDL-c concentration was observed after two months in H_2O_2 treated group compared to values of other treated groups. In the same period, IP injection of NSSSNP (25 or 50 mg/kg BW) or *Nigella sativa* (G5) caused significant decrease at P<0.05 in serum VLDL-c concentration compared to the value in H_2O_2 treated group (Figure 7).

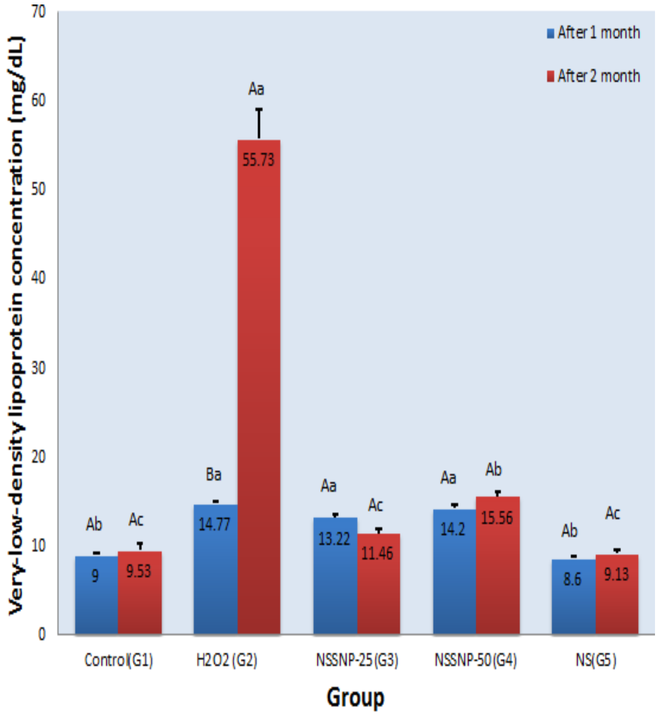

Figure 7. Effect of two concentrations of *Nigella sativa* seeds silver Nanoparticles *NSSSNPs* and *Nigella sativa* extract on serum very-low-density lipoproteins concentration (mg/dl) in H_2O_2 exposed rats. Values are expressed as mean±SE (n= 8). Various capital letters denote significant differences P<0.05 between periods. Various small letters denote significant differences P<0.05 between groups. Control (G1): Intact rats received drinking water daily for two months. H_2O_2 (G2): animals in this group received tap water containing 1% of H_2O_2. NSSNP-25 (G3): animals in this group were injected IP with *NSSSNPs* 25 mg/kg BWand received ordinary tap water containing 1%H_2O_2. NSSNP-50 (G4): animals in this group were injected IP with *Nigella sativa* seeds silver nanoparticles 50 mg/kg BW and received ordinary tap water containing 1% H_2O_2. NS (G5): animals in this group were injected IP with *Nigella sativa* seed extract (50 mg/kg BW) and received ordinary tap water containing 1% H_2O_2

In the current study, there was a significant (P<0.05) elevation in mean values of serum HDL-c concentration observed in G5 after one month of experiment, compared to the values in groups G2, G3, G4 as shown in (Figure 8). At the end of the experiment, significant (P<0.05) elevation in serum HDL-c concentration was observed after IP injection of *Nigella sativa* or NSSSNP in G3and G4 groups compared to the HDL-c value in H_2O_2 treated group.

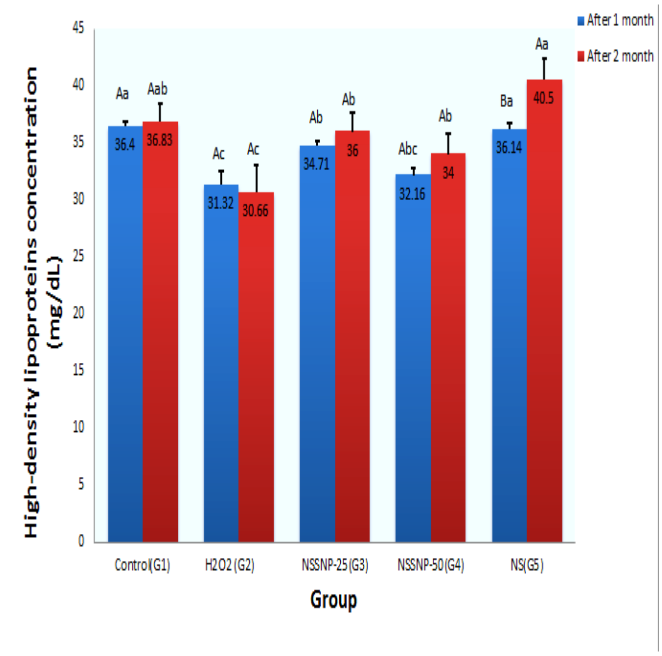

Figure 8. Effect of two concentrations of *Nigella sativa* seeds silver Nanoparticles *NSSSNPs* and *Nigella sativa* extract on serum high-density lipoproteins concentration (mg/dl) in H_2O_2 exposed rats. Values are expressed as mean±SE (n= 8). Various capital letters denote significant differences P<0.05 between periods. Various small letters denote significant differences P<0.05 between groups. Control (G1): Intact rats received drinking water daily for two months. H_2O_2 (G2): animals in this group received tap water containing 1% of H_2O_2. NSSNP-25 (G3): animals in this group were injected IP with *NSSSNPs* (25 mg/kg BW) and received ordinary tap water containing H_2O_2 1%. NSSNP-50 (G4): animals in this group were injected IP with *Nigella sativa* seeds silver nanoparticles (50 mg/kg BW) and received ordinary tap water containing H_2O_2 1%. NS (G5): animals in this group were injected IP with *Nigella sativa* seed extract (50 mg/kg BW) and received ordinary tap water containing 1%H_2O_2

The grade of DNA damage percentage was recorded as low, medium and high percentage. The result showed that G2 had higher percentage of high and medium DNA damage compared to that of G1 and G5, which showed higher percentage of low DNA damage.

The result also showed that IP injection of *NSSSNPs* at two concentrations (25 and 50 mg/kg BW) in H_2O_2 exposed rats failed to decrease percentage of high and medium DNA damage compared to G1 and G5 groups (Figure 9).

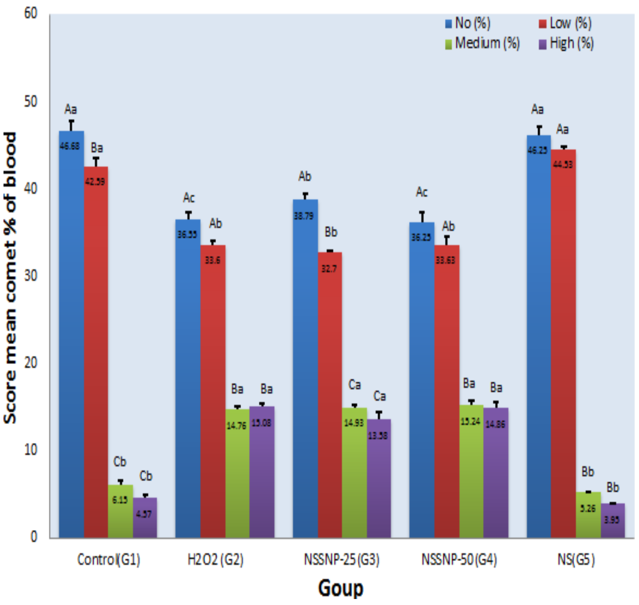

Figure 9. Effect of *Nigella sativa* seeds silver Nanoparticles by two doses and *Nigella sativa* extract on score mean comet % of blood in H_2O_2 exposed rats.Values are expressed as mean±SE (n= 8). Various capital letters denote significant differences P<0.05 between periods. Various small letters denote significant differences P<0.05 between groups. Control (G1): Intact rats received drinking water daily for two months. H_2O_2 (G2): animals in this group received tap water containing 1% of H_2O_2. NSSNP-25 (G3): animals in this group were injected IP with *NSSSNPs* (25 mg/kg BW) and received ordinary tap water containing H_2O_2. 1%. NSSNP-50 (G4): animals in this group were injected IP with *Nigella sativa* seeds silver nanoparticles (50 mg/kg BW) and received ordinary tap water containing 1% H_2O_2. NS (G5): animals in this group were injected IP with *Nigella sativa* seed extract (50 mg/kg BW) and received ordinary tap water containing 1% H_2O_2

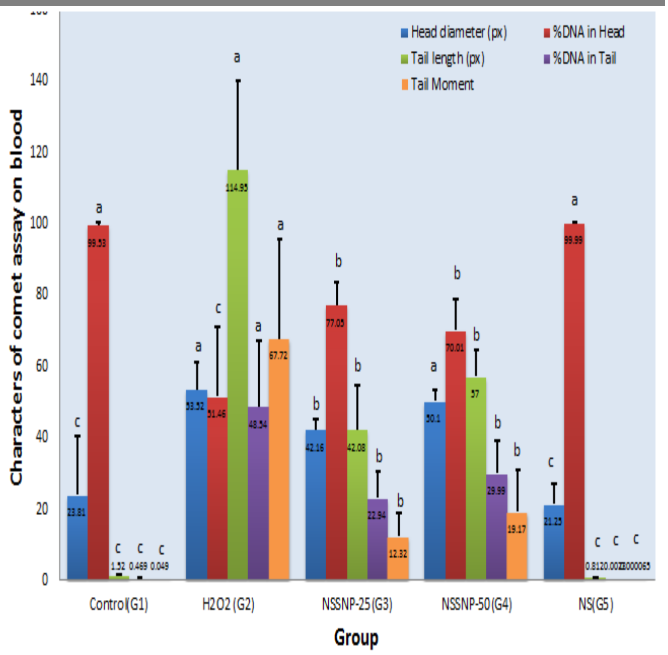

Figure 10. Effect of *Nigella sativa* seeds silver Nanoparticles by two dose and *Nigella sativa* extract characters of comet assay on blood in H_2O_2 exposed rats. Values are expressed as mean±SE (n= 8). Various small letters denote significant differences P<0.05 between groups. Control (G1): Intact rats received drinking water daily for two months. H_2O_2 (G2): animals in this group received tap water containing 1% of H_2O_2. NSSNP-25 (G3): animals in this group were injected IP with *NSSSNPs* (25 mg/kg BW) and received ordinary tap water containing 1% H_2O_2. NSSNP-50 (G4): animals in this group were injected IP with *Nigella sativa* seeds silver nanoparticles (50 mg/kg BW) and received ordinary tap water containing 1% H_2O_2. NS (G5): animals in this group were injected IP with *Nigella sativa* seed extract (50 mg/kg BW) and received ordinary tap water containing 1% H_2O_2

Figure 10 and light microscopic images 5 - 9 show characters of comet assay, it indicated a significant increase at P<0.05 in head diameter, tail length, DNA % in tail and tail moment with a significant decrease in DNA% in the head of H_2O_2 treated (G2) compared to the value in other treated groups except the head diameter in G4. The result showed that all mentioned criteria were opposed in G1 and G5. Intra-peritoneal injection of NSSSNP in two concentrations (25, 50 mg/kg B.W) with H_2O_2 caused significant (P<0.05) elevation in percentage of DNA in head with significant decrease (P<0.05) in the tail length, % DNA in tail and tail moment compared to value in G2.

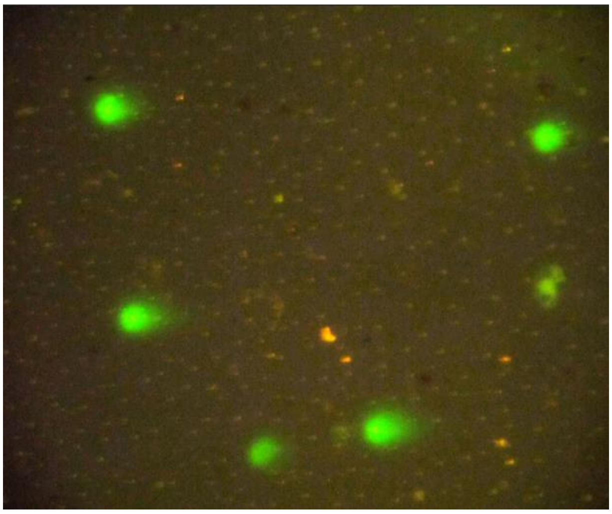

Image 5. Version sort of DNA damage (comet) in control group

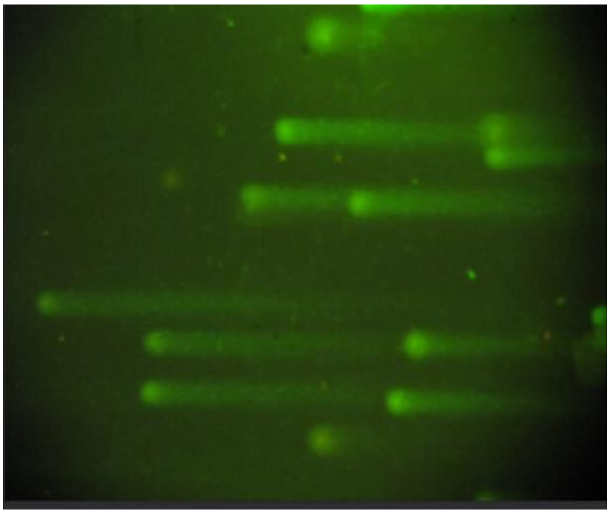

Image 6. Version sort of DNA damage (comet) in H_2O_2 group

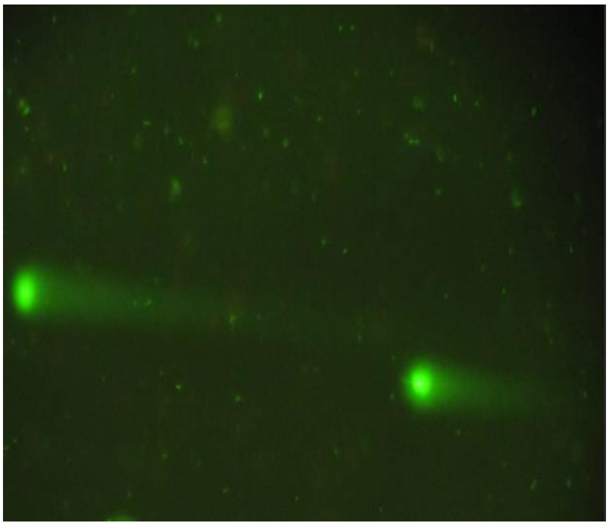

Image 7. Version sort of DNA damage (comet) in *NSSSNPs*-25 group

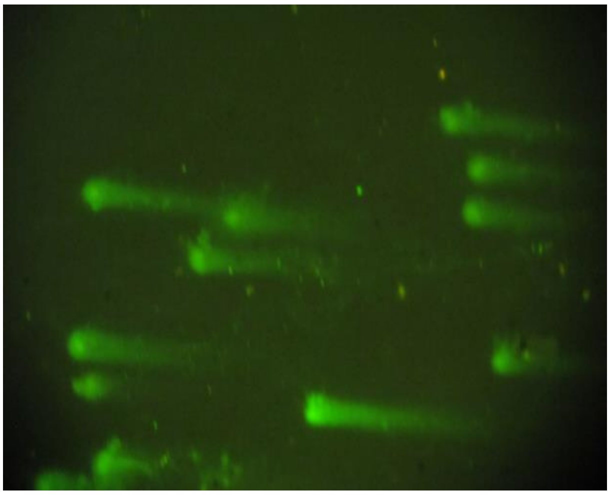

Image 8. Version sort of DNA damage (comet) in *NSSSNPs*-50 group

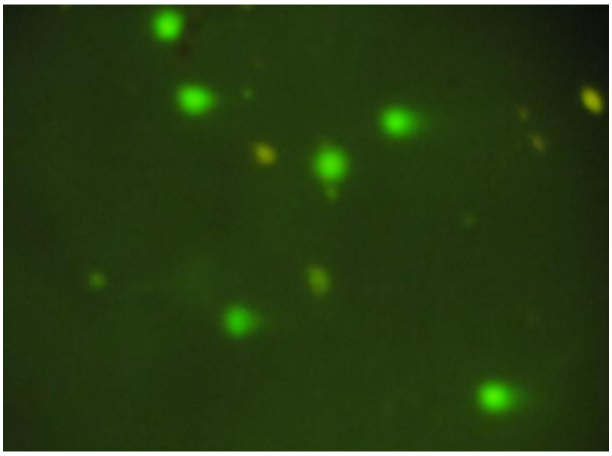

Image 9. Version sort of DNA damage (comet) in NS group

It is renowned that AgNPs exhibit deep yellowish brown color due to reduction of silver ion indicating formation of *NSSSNPs*, due to the excitation of surface Plasmon resonance (SPR) of the AgNPs (42, 56, 57). Most biological activities of *N. sativa* are back to thymoquinone, it is a major constituent of essential oils (58) and are responsible for efficient stable nanoparticles and reduction of metal ions (59).

The result of UV-visible absorption spectra of the aqueous solution of *NSSSNPs* agreed with several investigators (56 and 60). The SEM images showed spherical shape of the AgNPs, which is in accordance with (61).

The FT-IR was performed to identify the possible biomolecules present in the *Nigella sativa* seeds extract that are involved in the capping and reduction of AgNPs. It should be noted that using *Nigella sativa* as a reducing agent for synthetic AgNPs was first recorded by (41).

The observed functional groups of *NSSSNPs*, including amid, phenol, alkanes and halide, are going in line with Sangeetha and his colleagues (42). The carbonyl groups and aromatic rings are found to be involved in the nanoparticle formation (62). The XRD result in the current study confirms the crystalline nature of the silver nanoparticles and XRD peak widening was consistent with the small particles sizes of the nanoparticles (63).

The hypolipidemic effects of *Nigella sativa* in the current study could be attributed to upregulation of LDL-c molecules through receptor mediated endocytosis (64), decreased dietary cholesterol absorption and reduction of hepatocyte cholesterol synthesis (65, 66) as well as elevation in HDL-c level (67, 68).

Stimulation of primary bile acid synthesis and its fecal losses probably contributed to *Nigella sativa* dietary soluble fibers and sterols (69) leading to hypercholesterolemia. It was concluded that activation of peroxisome proliferator-activated receptor is responsible for cholesterol reducing mechanism of *Nigella sativa* seeds (64) in studies performed in rats and rabbits.

The data obtained from this study regarding the effect of *NSSSNPs* (25 mg/kg BW) showed significant elevation in serum HDL-c concentration with significant decrease in serum TC and LDL-c concentration indicating cardio protective effect of *NSSSNPs* in low concentration, this can be attributed to its antioxidant effect (57).

Low concentration of nanoparticles was effective in causing hyperlipidemia by changing the LDL-c, VLDL-c, HDL-c and in high fat diet rats (70).

On the contrary, a case of dyslipidemia after exposure to H_2O_2 or IP injection of 50 mg/kg BW concurrently with H_2O_2 has been reported, which indicated cardio toxic effect of AgNPs in high concentration (71). The results in the current study concerning the effect of H_2O_2 on lipid profile are consistent with the result of (72, 73). The postulated elevation in ROS after H_2O_2 exposure that influenced different tissues leading to lipid peroxidation (LPO) may result in alteration in sterol synthesis leading to elevation in cholesterol concentration and phospholipids degradation (74).

A decrease in DNA fragmentation after *Nigella sativa* seeds extract treatment observed in group G5 could be due to antioxidant effect of *Nigella sativa* which caused significant decrease in ROS and H_2O_2 production. Both glutathionedi-hydrothymoquinone and thymohydroquinone (the metabolites of Thymoquinone) have a powerful antioxidant activity. They have functional groups such as thiol (SH) and hydroxyl (OH) groups, which have strong antioxidant properties (75, 76).

It should be mentioned that there is no or scarce scientific research concerning correlation between DNA fragmentation and *Nigella Sativa*. Accordingly, it can be concluded that *Nigella sativa*as antioxidant may activate the antiapoptotic factors and down regulate apoptotic factors (77) leading to alteration in DNA fragmentation. Thymoquinone treatment significantly reduced DNA fragmentation through increase in the nuclear factor erythroid related factor (Nrf2), regulatory factor plays a role in production of several antioxidant gene including SOD, catalase (78) was postulated as mechanism for *Nigella sativa* cytogenetic effect. An elevation in DNA fragmentation and percentage of DNA damage in head and tail was recorded after H_2O_2 exposure and *NSSSNPs* in dose 50mg/kg BW compared to less DNA damage in *NSSSNPs* (25mg/kg) group.

Lack of induction of DNA damage by *NSSSNPs*-25 is possible due to the coating which may protect the cells from direct interaction with AgNPs either by reducing ion leaching from particles or by causing extensive agglomeration of NPs with possible reduction of cellular uptake (79).

Cytotoxicity and genotoxicity of AgNPs as well as Ag ions (27, 80) were due to oxidative stress through elevation in the gene expression of reactive oxygen species *in vitro* (81, 82).

Depending upon their size, concentration (83) and surface chemistry, internalization (84, 85).

AgNPs may then get translocated to target organelles, such as the mitochondria and nucleus, where they interact with membrane proteins and elicit in the host biological effects, including altered cell morphology, oxidative stress, DNA damage, inflammation (28, 86), mitochondrial dysfunction, and consequent cell death by apoptosis or necrosis (87, 88). Likely, small Ag NPs form ROS, such as hydroxyl radicals (92, 89), where the Ag ions/complexes react with thiol groups of protein, leading to depletion of glutathione (90, 91), disrupting their physiological activity leading to cell death (92). Besides, H_2O_2 as ROS may cause depression in Nrf2 and thus decrease in expression of this cytoprotective factor (93) leading to oxidative stress and DNA damage (94). Whatever is the mechanism that originates, the ROS, hydroxyl radical produced from H_2O_2 adducts of DNA, lipid peroxides (95), caused protein oxidation, lipid oxidation, DNA oxidation, and DNA damage (96).

Conflict of Interest

The authors declare that there is no conflict of interest.

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تصنيع وتوصيف جسيمات الفضة النانوية المصنعة باستعمال حبة السوداء ودراسة تأثيرها على مستوى الدهون في المصل الدم وتلف الحمض النووي في دم الجرذان المعالجة ببيروكسيدالهيدروجين (الجزءالأول)

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الخلاصة

صممت هذه الدراسة لتصنيع جسيمات الفضة النانوية باستخدام مستخلص مائي حبة السوداء كعامل اختزال وتغطية، وكذلك لاستقصاء تأثير جسيمات القضة النانوية – حبة سوداء على مستوى الدهون في مصل الدم وتلف الحمض النووي في دم الجرذان المعرضة لبيروكسيد الهيدروجين. تم تمييز جسيمات الفضية النانوية المصنعة من خلال التحليل الطيفي للأشعة فوق البنفسجية المرئية عند الحزمة 442 نانومتر؛ أكد التحليل الطيفي للأشعة تحت الحمراء أن الجسيمات النانوية الفضية مغلفة بمركبات نباتية ؛كشف تحليل حيود الأشعة السينية بشكل واضح عن توليد جسيمات الفضة النانوية بلورية الشكل ذات حجم يتراوح بين (10.66 - 16.66) nm، وتم استخدام المسح الإلكتروني المجهر للتحقيق في شكل وحجم جسيمات الفضة النانوية في المركب، إذ تم تقسيم أربعين (40) من الجرذان الذكور البالغين بشكل متساوٍ وعشوائي إلى خمس مجموعات وكانوا يعالجون يومياً لمدة شهرين على النحو التالي: المجموعة الأولى (السيطرة)، والمجموعة الثانية: تلقت الفئران في هذه المجموعة مياه الصنبور التي تحتوي على 1 ٪ بيروكسيد الهيدروجين، بينما الجرذان في المجموعتين الثالثة والرابعة فقد حُقِنتْ بجسيمات الفضة النانوية – حبة السوداء (25 و 50) ملغم / كغم من وزن الجسم على التوالي بالإضافة الى انها حصلت على مياه الصنبور العادية التي تحتوي على 1 ٪ بيروكسيد الهيدروجين $_2$،المجموعة الخامسة: تم حقن الحيوانات في هذه المجموعة بالمستخلص المائي لبذورالحبة السوداء 50 ملغم / كغم من وزن الجسم وبالإضافة الى تلقيها مياه الصنبور العادية التي تحتوي على 1 ٪ بيروكسيدالهيدروجين . تم جمع عينات من الدم بعد شهرواحد وشهرين من التجربة، تم استخدام جزء منها لقياسات تجزئة الحمض النووي واستخدمت عينات المصل لتقديرمستوى الدهون، ونتيجة لذلك في الدراسة، كشفت عن حالة خلل الدهون في الدم عن طريق الارتفاع الكبير في تركيز الدهون الثلاثية, البروتين الدهني منخفض الكثافة، بروتينات الشحمية الوضيعة الكثافة والكوليسترول ،مع انخفاضٍ كبيرٍ في بروتين شحمي مرتفع الكثافة، بالإضافة إلى الارتفاع الكبير في تلف الحمض النووي في المجموعات الرابعة والثانية. أكدت النتائج أيضاً بأن المستخلص المائي للبذور حبة السوداء (مجموعة G5) وتركيز المنخفض لجسيمات القضة النانوية – الحبة السوداء بجرعة 25 ملغم / كغم من وزن الجسم لها تأثير خافض لمستوى الدهون بالإضافة الى انها عامل حماية خلوية ،تم توضيحها من خلال تصحيح عسرشحميات الدم، جنباً إلى جنب مع تخفيف كبيرفي تلف الحمض النووي. نستنتج من هذه الدراسة ان مستخلص بذور نبات الحبة السوداء وجسيمات الفضة النانوية المصنعة باستعمال حبة السوداء لها دور في تحسين مستوى الدهون في مصل الجرذان وكذلك تحطم الحامض النووي منقوص الاوكسجين.

الكلمات المفتاحية: الحبةالسوداء، جسيمات الفضةالنانوية، مستوى الدهون، تلفDNA, بيروكسيدالهيدروجين

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