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Study The Role of Nigella Sativa Silver Antiparticles on Some Liver Biomarkers and Hepatocyte Comet Assay in Stressed Rats

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Abstract:

Nigella sativa silver nanoparticles (NS-Ag-NPS) were prepared and characterized for investigation the liver dysfunction induced by H2O2 in rats. forty healthy adult male rats selected and divided randomly into five groups (eight rats per group). The control group (G1) was administrated with tap water for 2 months. The second group (G2) was administrated tap water with H2O2 1% for 2 months. Third group (G3) was IP injected with Nigella sativa seed aqueous extract (60 mg/ Kg/ body weight) and receive tap water with 1% H2O2 for 2 months. The fourth group (G4) was IP injected with NS-Ag NPs (30mg / Kg/ body weight) and receive tap water with 1% H2O2 for 2 months. Finally, the fifth group (G5) was IP injected with NS-Ag NPs (60 mg / Kg/ body weight) and receive tap water with 1% H2O2 for 2 months. At the end of the experiment, rats were anesthetized and blood samples were collected through cardiac puncture procedure, for measurement of serum AST, ALT, and ALP, and liver tissue homogenized for measurement of (GPx) and (MDA) level. The histological changes examination of liver tissue and comet assay technique for DNA fragmentation of hepatocyte was studied. The results showed significant ($P<0.05$) elevation of AST, ALT, ALP, and MDA in G2, G4, and G5 group as a compared with G1 and G3 group, A significant ($P< 0.05$) decrease of GPx level in G2, G4, and G5 groups as a compared with G1 and G3 group. A significant ($P <0.05$) elevation of medium % and high % of comet appearance in G2, G4, and G5 groups as a compared with G1 and G3 groups. On the other hand, the percentage of no comet appears significantly ($P < 0.05$) in G1 and G3 groups. All comet parameters showed a significant ($P <0.05$) elevation in G4 and G5 groups as compared with the control group except in %DNA in Head parameter which showed a significant ($P< 0.05$) decrease when compared with the G1 group. On the other hand, the G3 group showed no significant differences from the control group. Histological section of the liver was illustrating damage of hepatocyte, mononuclear cell, infiltration of inflammatory cell, vacuolated hepatocytes and focal necrosis area was observed in liver tissue after administration with H2O2, NS-Ag NPs (30mg / Kg/ body weight), and NS-Ag NPs (60mg / Kg/ body weight). Finally, the current study's findings explained the cytotoxic activity of NS-Ag- NPs, and H2O2 at high concentrations, as well as the ameliorative impact of NS and NS-Ag- NPs at low concentrations on certain liver biomarkers.

Key words: Nigella Sativa Silver Nanoparticles, liver enzyme, liver comet assay, oxidative stress of liver, GPx, MDA.

Introduction

Nanoscience is quickly developed, and the nanoparticles(NPs) applications have been found in many arenas **(Han et al., , 2016; Canaparo et al., , 2021).** As compared with micron sized particles and as a result to physicochemical properties, NPs are absorbed through the lung, lymph node, kidney, spleen, and liver **(Fubini, et al., , 2010; Shabbir et al., , 2021),** also accumulated in brain **(Attia et al., , 2018; Ju et al., 2021).**

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Silver NPs (AgNPs) are repeatedly used in the manufacture of a variety of commercial product including cosmetics, ointments and creams, also paint **(Park and Yeo 2016; Skalska et al., , 2016).** The antimicrobial activity of AgNPs has been exploited in variety industries in fungicidal and bactericidal, water purification, medical devices, finally in air quality management **(Arora et al., 2009; Pelletier et al., 2010).** All of these applications of AgNPs have increased the ecological exposure of AgNPs, which has elevated concern regarding possible toxicity dangers to human health **(Massarsky et al., ; 2014; Park and Yeo 2016)**. AgNPs that are absorbed by many vital organs **(Skalska et al., 2016)**. Liver has been suggested as a major target organs for the assessment toxicity of silver nanoparticles in rodents **(Kim et al., 2010).**

Nigella sativa (NS) also have a many names (black caraway, black seed, and back cumin), implant in Northern Africa, Middle East, Eastern Europe, and in South Asia **(Golkar and Nourbakhsh 2019)**. NS seeds are traditionally used as diet **(Mazaheri et al., , 2019)**, flavor additive (Dubey,Singh, Mishra, Kant, & Solanki, 2016), and natural medication for the numerous health profits for the treatment of diarrhea, asthma, infections and hypoglycemia, and hypertension **(Hallajzadeh et al., 2020)** hypolipidimic, hepatoprotective role, antioxidant activity and pain-relieving **(Hallajzadeh et al., 2020)**

The reactive oxygen species (ROS) are products formed under normal physiological circumstances due to the partial reduction of oxygen moleculese. Hydrogen peroxide (OH-) is a molecule has low reactivity, however it can readily enter cell membrane and produce the most reactive types of oxygen (hydroxyl radical) by Fenton's reaction.**(Nita and Grzybowski 2016)** The generation of (OH-) has been dramatically elevated via oxidative burst throughout inflammatory circumstances **(Marinho et al., 2014).**

In our study, we investigated the effects of AgNPs synthesized from *Nigella sativa* aqueous extract on Wistar rats. The acute and sub-chronic toxicity was observed for different concentrations. The effect was observed for liver enzymes which give a direct correlation between hepatic injury and toxicity. The enzymes such as ALP, AST, ALT and histological changes were observed and evaluation of DNA damage of hepatocyte by comet assay technique.

Material and method:

Preparation and characterization of Nigella sativa-silver nanoparticles (NS-Ag NPs):

Nigella sativa (NS) seed aqueous extract (10%) was ready according to **(Awan et al., 2018)** thereafter mixed with solution of (2 mill mole) of silver nitrate (AgNO3) in request to green synthesis of (NS-Ag NPs) according to the technique used by **(ali and Khudair 2019)**. Physical characterization of (NS-Ag NPs) via Scanning electron microscopy (SEM) **(Khoshnamvand et al., , 2019)**, X-ray diffraction (XRD) **(ali and Khudair 2019)** and Fourier transform infrared (FT-IR) spectroscopic **(Mittal et al., 2015; Rolim et al., 2019)** all of these physical tests was demonstrating the purity, crystals shape and size range from 15 to 18 nm.

Experimental Animals Design

The forty adult male rats in age of (3 month) and body weight range (200 -230 g). Experimental rats were gained from the animal house of Veterinary Medicine College for Baghdad University. They were housed eight per each cage, and were placed in room for 2 weeks for adaptation. Room temperature was kept at (21 - 25˚c), with changed of air continuously and with light /dark cycle of 12:12 hour/day.

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The experimental rats were divided randomly to five groups (eight rats per group). Control group (G1) were administrated with tap water for 2 months. Second group (G2) were administrated tap water with H_2O_2 1% for 2 months. There'd group (G3) were IP injected with Nigella sativa seed aqueous extract (60 mg/ Kg/ body weight) and receive tap water with 1% H_2O_2 for 2 months. Forth group (G4) were IP injected with NS-Ag NPs (30mg / Kg/ body weight) and receive tap water with 1% H_2O_2 for 2 months. Finally, fifth group (G5) were IP injected with NS-Ag NPs (60 mg / Kg/ body weight) and receive tap water with 1% H_2O_2 for 2 months. **(Ali.Z.S and Khudair.K.K 2019**).

Specimen Preparation

Rats were anesthetized by intramuscular injection of (40 mg/kg) Xylazine and (90mg/kg) of Ketamine. Through cardiac puncture procedure, blood samples were collected in gel tube for serum preparation , thereafter kept it by freezing at (-20˚ C) till it used **(David, 2005)** for measurement of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and liver tissue homogenized for measurement of glutathione peroxidase (GPx) and Malondialdehyde (MDA) level by using ELISA kit and according to Kit manufacturer's instructions. The histological changes examination of liver tissue and comet assay technique for DNA fragmentation of hepatocyte **(Husain et al., , 2019)** was studied.

Statistical Analysis

The **(SAS 2012)** program was used to statistical analysis of study parameters. Least significant difference –LSD test was used to significant compare between means in this study.

Results:

Serum enzyme (AST, ALT and ALP) in control group and all treated group are showed in figure (1,2 and 3) respectively. The result register that significant ($P<0.05$) elevation of AST, ALT and ALP in G2, G4 and G5 group as a compared with control group and animals receive Nigella sativa aqueous extract (60 mg/ Kg/ body weight). There are significant ($P< 0.05$) decrease of GPx level in G2, G4 and G5 groups as a compared with G1 and G3 treated group, whereas the significant ($P<0.05$) elevation of GPx in G3 group as a compared with control group, this result demonstrated in figure (4). Figure (5) illustrate MDA level in control and other four treated groups, this figure showed significant ($P<0.05$) elevation in MDA in G2, G4 and G5 when compared with control group.

The data of comet incidence clarified in figure (6) these result showed significant ($P <0.05$) elevation of medium % and high % of comet appearance in G2, G4 and G5 groups as a compared with G3 and control group. On the other hand, the percentage of no comet appear significantly ($P < 0.05$) in control and G3 groups. Comet parameters are shown in figure (7) all comet parameters showed a significant ($P <0.05$) elevation in G4 and G5 group as a compared with control group except in %DNA in Head parameter which showed significant ($P< 0.05$) decrease when compared with control group. On the other hand, G3 group showed no significant differences with control group.

Histological section of liver was demonstrated in figure (8) illustrate the normal structure of hepatocyte in (a) control group and (b) in G3 group. Figure (9 and 10 a and b) illustrate damage of hepatocyte, mononuclear cell, infiltration of inflammatory cell, vacuolated hepatocytes and focal necrosis area was observed in liver tissue after administration with H2O2**,** NS-Ag NPs (30mg / Kg/ body weight) and NS-Ag NPs (60mg / Kg/ body weight).

Figure (1): The effect of silver nanoparticles was synthesized by Nigella sativa aqueous extract on serum aspartate aminotransferase (AST) concentration (U/L) in adult male rats along 2 months of administrated.

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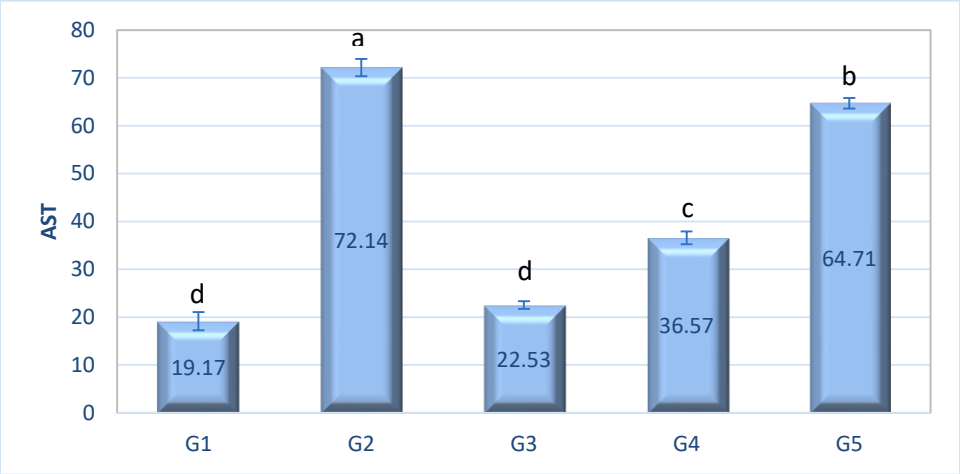
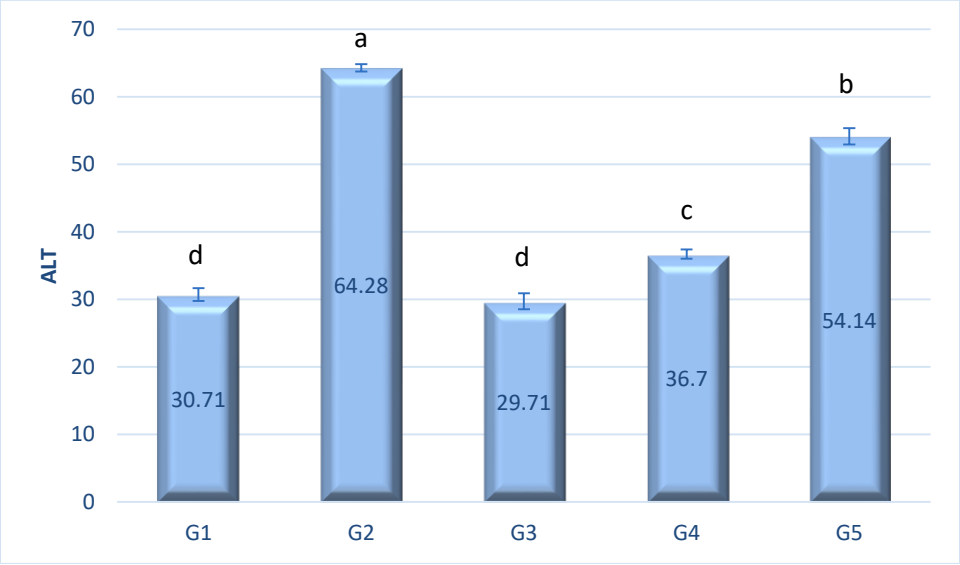


The values represented as M ± SE, n =6 rats.
G1: administrated with tap water for 2 months. G2: administrated tap water with H_2O_2 1% for 2 months. G3: IP injected with Nigella sativa seed aqueous extract (60 mg/ Kg/ body weight) and receive tap water with 1% H_2O_2 for 2 months. G4: IP injected with NS-Ag NPs (30mg / Kg/ body weight) and receive tap water with 1% H_2O_2 for 2 months. G5: IP injected with NS-Ag NPs (60 mg / Kg/ body weight) and receive tap water with 1% H_2O_2 for 2 months.

Figure (2) The effect of silver nanoparticles was synthesized by Nigella sativa aqueous extract on serum alanine aminotransferase (ALT) concentration (U/L) in adult male rats along 2 months of administrated.



The values represented as M ± SE, n =6 rats.
G1: administrated with tap water for 2 months. G2: administrated tap water with H_2O_2 1% for 2 months. G3: IP injected with Nigella sativa seed aqueous extract (60 mg/ Kg/ body weight) and receive tap water with 1% H_2O_2 for 2 months. G4: IP injected with NS-Ag NPs (30mg / Kg/ body weight) and receive tap water with 1% H_2O_2 for 2 months. G5: IP injected with NS-Ag NPs (60 mg / Kg/ body weight) and receive tap water with 1% H_2O_2 for 2 months.

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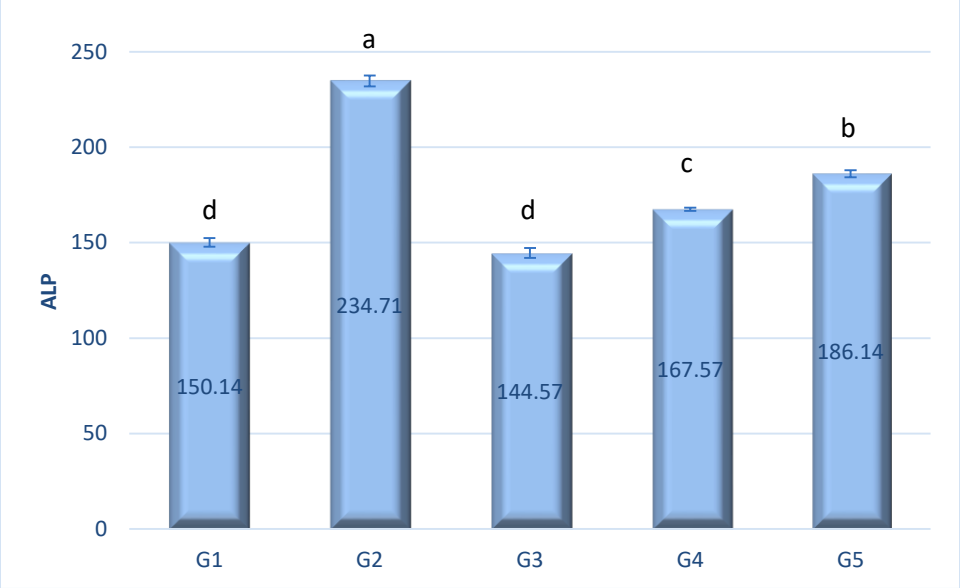
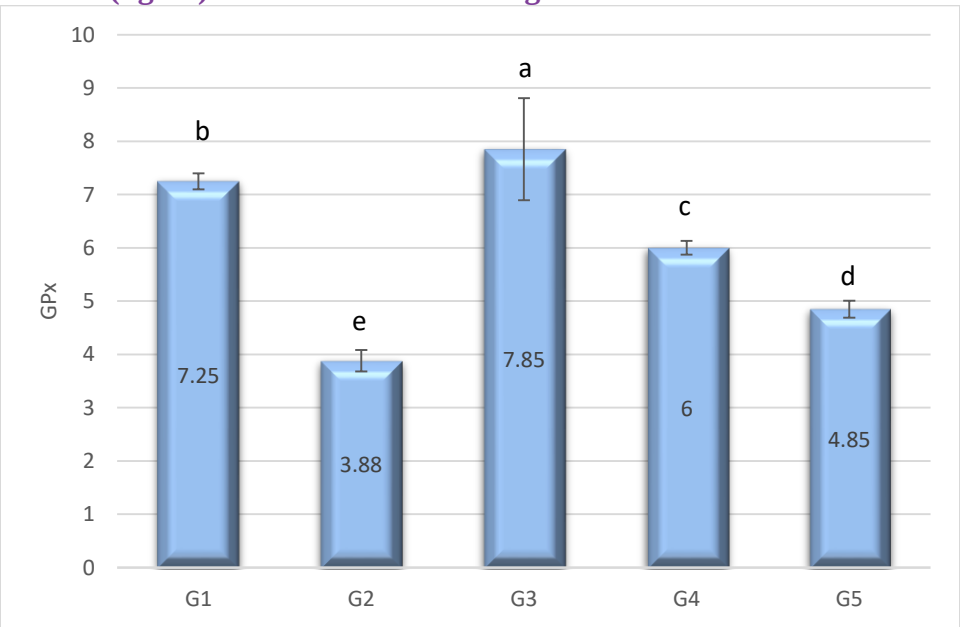
Figure (3) The effect of silver nanoparticles was synthesized by Nigella sativa aqueous extract on serum alkaline phosphatase (ALP) concentration (IU/L) in adult male rats along 2 months of administrated.



The values represented as M ± SE, n =6 rats.
G1: administrated with tap water for 2 months. G2: administrated tap water with H_2O_2 1% for 2 months. G3: IP injected with Nigella sativa seed aqueous extract (60 mg/ Kg/ body weight) and receive tap water with 1% H_2O_2 for 2 months. G4: IP injected with NS-Ag NPs (30mg / Kg/ body weight) and receive tap water with 1% H_2O_2 for 2 months. G5: IP injected with NS-Ag NPs (60 mg / Kg/ body weight) and receive tap water with 1% H_2O_2 for 2 months.

Figure (4) The effect of silver nanoparticles was synthesized by Nigella sativa aqueous extract on liver tissue GPx concentration (ng\ml) in adult male rats along 2 months of administrated.



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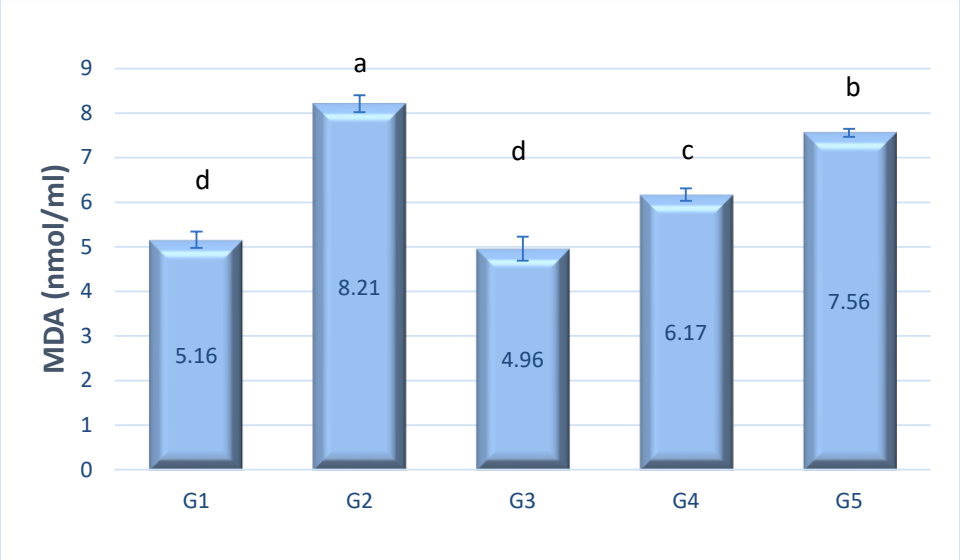
The values represented as M ± SE, n =6 rats.
G1: administrated with tap water for 2 months. G2: administrated tap water with H_2O_2 1% for 2 months. G3: IP injected with Nigella sativa seed aqueous extract (60 mg/ Kg/ body weight) and receive tap water with 1% H_2O_2 for 2 months. G4: IP injected with NS-Ag NPs (30mg / Kg/ body weight) and receive tap water with 1% H_2O_2 for 2 months. G5: IP injected with NS-Ag NPs (60 mg / Kg/ body weight) and receive tap water with 1% H_2O_2 for 2 months.

Figure (5) The effect of silver nanoparticles was synthesized by Nigella sativa aqueous extract on liver tissue MDA concentration (ng\ml) in adult male rats along 2 months of administrated.



The values represented as M ± SE, n =6 rats.
G1: administrated with tap water for 2 months. G2: administrated tap water with H_2O_2 1% for 2 months. G3: IP injected with Nigella sativa seed aqueous extract (60 mg/ Kg/ body weight) and receive tap water with 1% H_2O_2 for 2 months. G4: IP injected with NS-Ag NPs (30mg / Kg/ body weight) and receive tap water with 1% H_2O_2 for 2 months. G5: IP injected with NS-Ag NPs (60 mg / Kg/ body weight) and receive tap water with 1% H_2O_2 for 2 months.

Figure (6) The effect of silver nanoparticles was synthesized by Nigella sativa aqueous extract on liver tissue comet incidence in adult male rats along 2 months of administrated.

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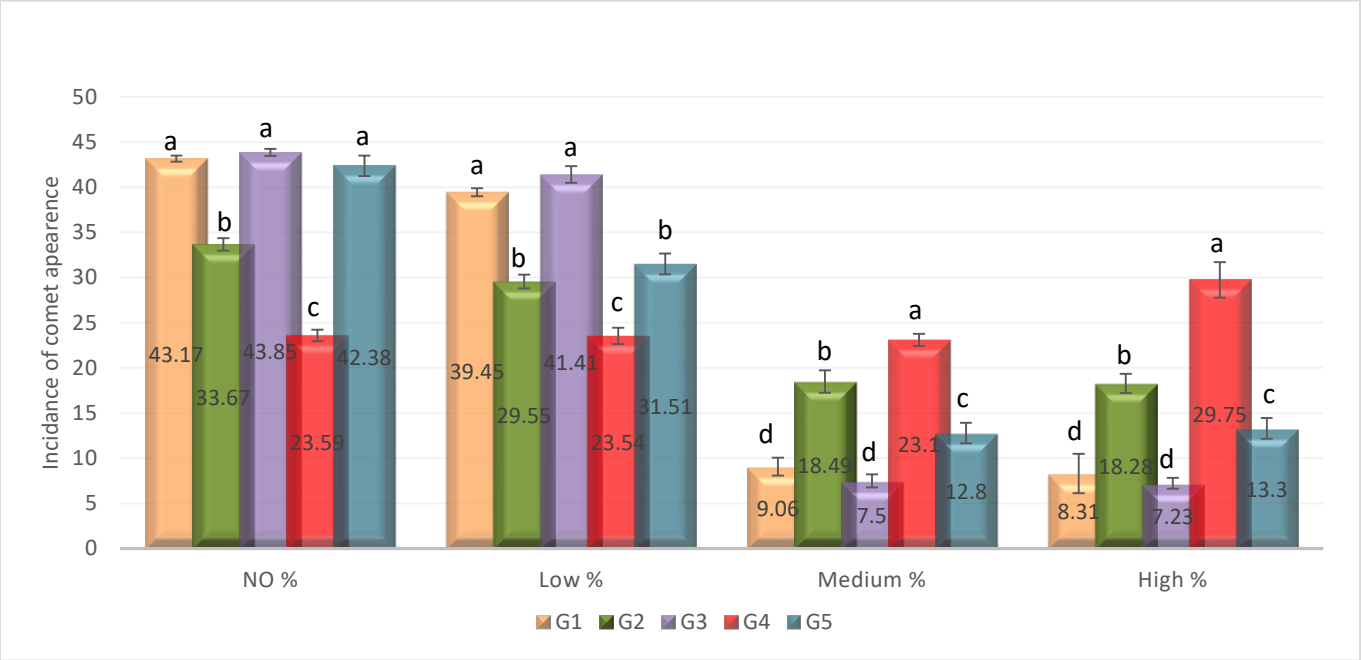
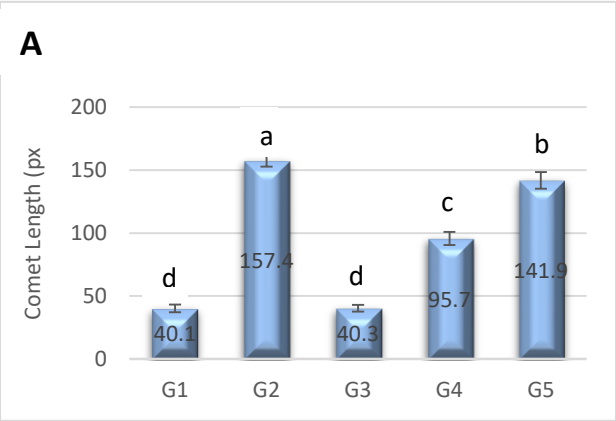
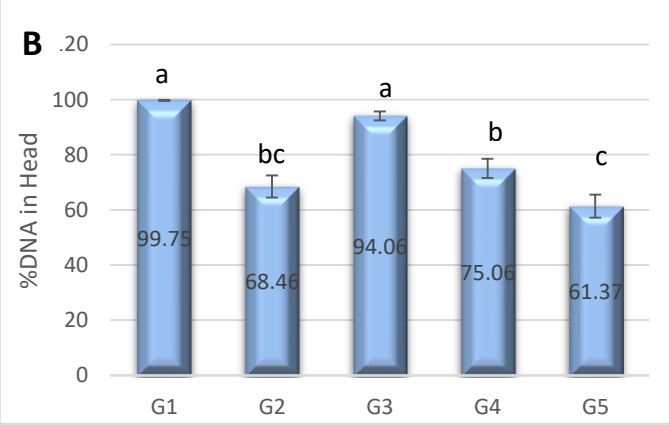


The values represented as M ± SE, n =6 rats.
G1: administrated with tap water for 2 months. G2: administrated tap water with H_2O_2 1% for 2 months. G3: IP injected with Nigella sativa seed aqueous extract (60 mg/ Kg/ body weight) and receive tap water with 1% H_2O_2 for 2 months. G4: IP injected with NS-Ag NPs (30mg / Kg/ body weight) and receive tap water with 1% H_2O_2 for 2 months. G5: IP injected with NS-Ag NPs (60 mg / Kg/ body weight) and receive tap water with 1% H_2O_2 for 2 months.

Figure (7) Represent the effect of silver nanoparticles was synthesized by Nigella sativa aqueous extract on liver tissue comet parameters in adult male rats along 2 months of administrated.





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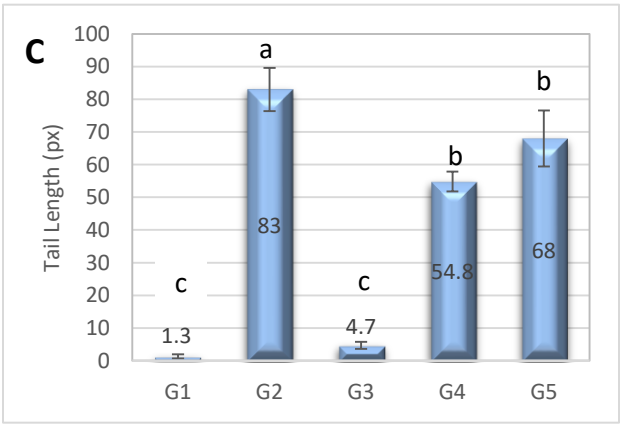
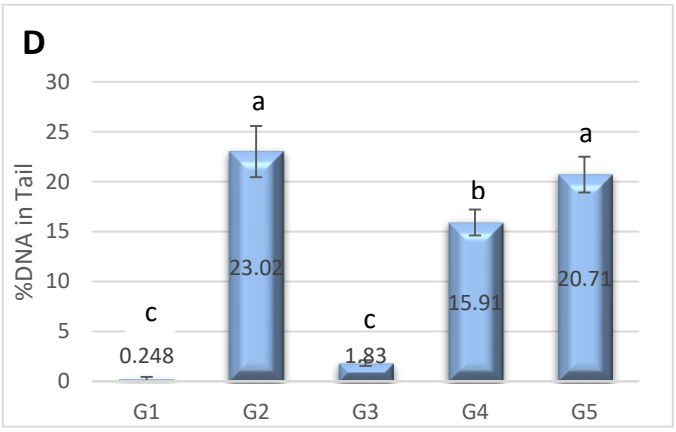
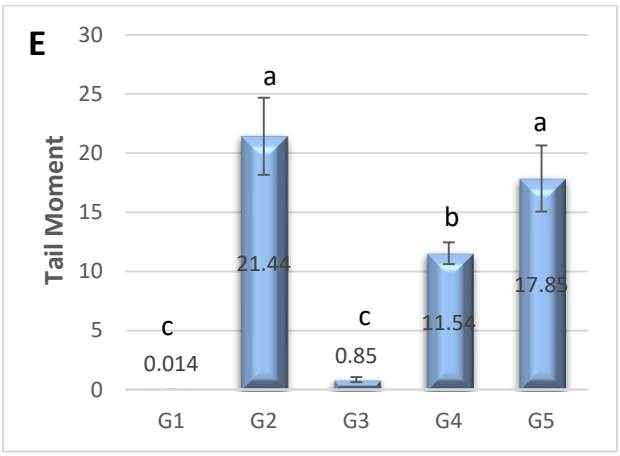
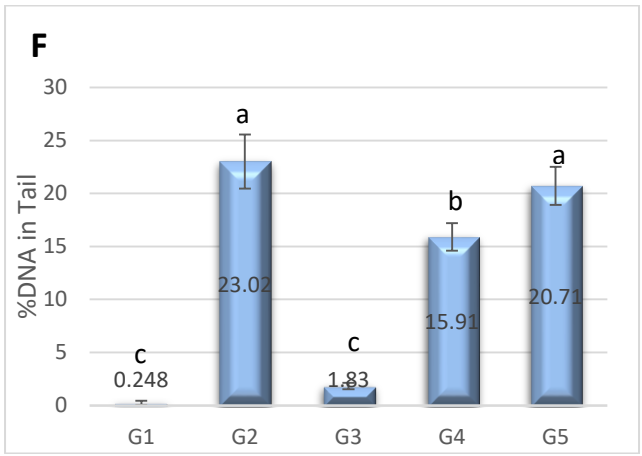
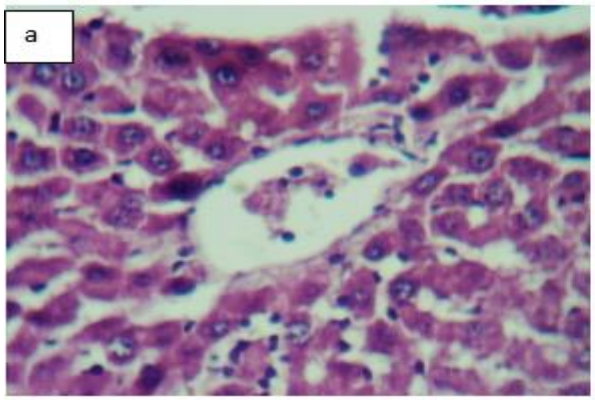








The values represented as M ± SE, n =6 rats.
G1: administrated with tap water for 2 months. G2: administrated tap water with H_2O_2 1% for 2 months. G3: IP injected with Nigella sativa seed aqueous extract (60 mg/ Kg/ body weight) and receive tap water with 1% H_2O_2 for 2 months. G4: IP injected with NS-Ag NPs (30mg / Kg/ body weight) and receive tap water with 1% H_2O_2 for 2 months. G5: IP injected with NS-Ag NPs (60 mg / Kg/ body weight) and receive tap water with 1% H_2O_2 for 2 months.



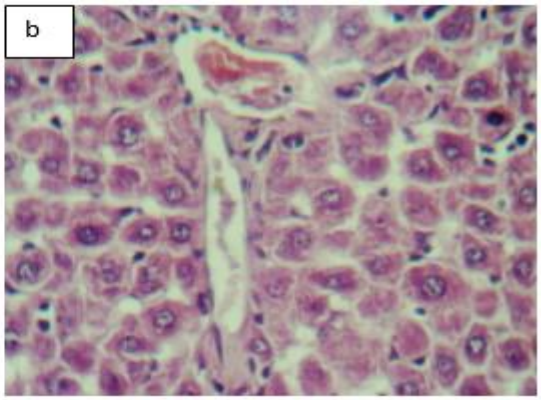


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Figure (8) illustrate the normal hepatocyte in (a) control group and (b) after group was IP injected with NSs aqueous extract (60 mg/ Kg/ body weight) and receive tap water with 1% H_2O_2 for 2 months).

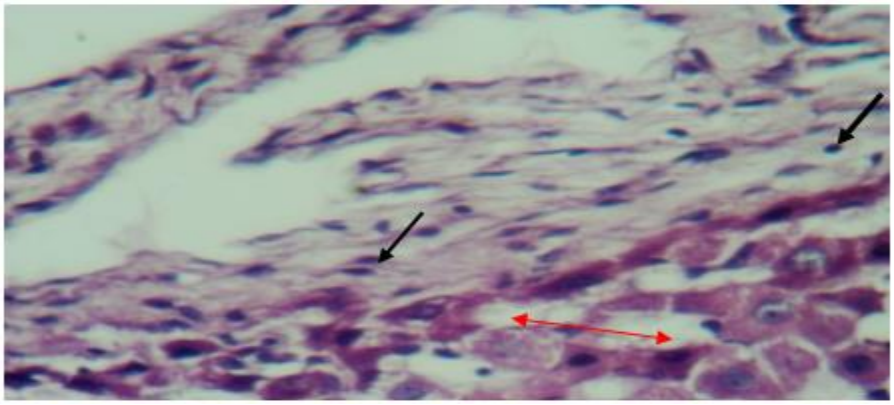

Figure (9) illustrate the histological changes on the hepatocyte after rats administrated tap water with H_2O_2 1% for 2 months (G3). Showed the infiltration of inflammatory cell (⟶) and focal necrosis area (⟷).

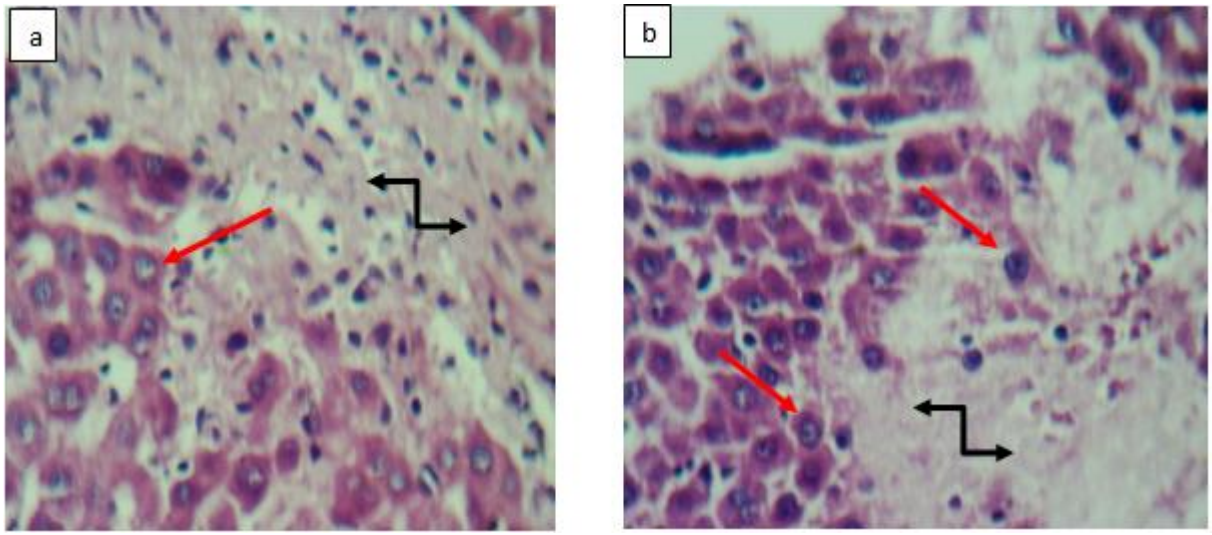

Figure (10) illustrate the histological changes on the hepatocyte after rats administrated (a) NS-Ag NPs (30mg / Kg/ body weight) and (b) NS-Ag NPs (60mg / Kg/ body weight).Damage of hepatocyte, mononuclear cell (⟶) , necrosis area (⮌) was observed in liver tissue.

Discussion:

To assess physiological effect of NS, NS-Ag NPs and H_2O_2, liver function was studied through classical biomarkers of serum transaminases. Raised activities of AST and ALT consider specific hepatocyte damage, while ALP is a non-specific pointer to the liver function. The current results show level of these enzymes in the serum of treated rats in groups (2, 4 and 5) increased significantly. The increment of these liver biomarkers within the serum is typically indicating injury in both liver morphology and function **(Badraoui et al., , 2020;**

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Saoudi et al., , 2021). Hepatic injury after NS-Ag-NPs and H_2O_2 IP injection have possibly produced due to sever irritation of oxidant- antioxidant system in liver cells **(Rana et al., , , 2021).** The free radicals generated by NPs and H_2O_2 have attached hepatocyte and lead to release enzyme that stored in hepatocyte to blood stream. However the immune response to exterior factors lead to increase number of inflammatory cell and mononuclear cell as shown in histological changes of our study (image 9 &10) because to phagocytic of AgNps **(Gulboy et al., , , 2015).**

The present results show that administration of NS-Ag NPs and H_2O_2 to rats induced oxidative stress in the hepatocyte as pointed by elevated values of MDA compared to control and NS treated rats, Malondialdehyde is a gauge of lipid peroxidation **(Das et al., , 2021)** , so, it's a most essential markers of oxidative stress that influence on different organs **(Cherian et al., , 2019)**. In our result demonstrate the raise of GPx and diminution of MDA level in animals group treated with NS and NS-Ag NPs 30 mg /kg Body weight as a compared with NS-Ag NPs 60 mg /kg body weight and H_2O_2 treated groups, these results pointed on the antioxidant activity of NS and NS-Ag NPs 30 mg /kg body weight as a minor dose. The component of NS such as thymohydroquinone, a thymoquinone phenolic derivatives **(Venkatachallam et al., , 2010; Silva et al., 2020)** have a strong antioxidant capacity which attributed with their hydroxyl (OH) group and thiol (SH) **(Flesar et al., , 2010)**. However, the decrease in Gpx and raise of MDA level after animal IP injected with NS-Ag NPs 60 mg /kg body weight concurrently with H_2O_2 and H_2O_2 IP injection groups indicated on the generation of oxidative stress condition. The several mechanism may be related to the toxicity prompted by AgNPs , one of them associated with ROS generation (Y. Li et al., 2017) , as well as; large cellular uptake and particular interaction with macromolecule of cells which owing to chemical structures and surface coating or release of silver ions from silver nanoparticles **(Gliga et al., , 2014)**.

Ag ion complexes react with thiol groups, causing glutathione depletion **(Martirosyan et al., 2016)** and the cascade of events that results in oxidative stress and damage cells **(Teodoro et al., 2011)**. As a result, superoxide dismutase and glutathione antioxidant systems are depleted, resulting in oxidative stress, mitochondrial membrane permeabilization; cell cycle detention, and eventually apoptosis **(Liu et al., 2010)**.

It can be suggested that a higher concentration of NS-Ag-NPs (60 mg/kg B.W.) could increase the amount of Ag produced, leading to oxidative stress and an increase in MDA levels. AgNPs' cytotoxicity and genotoxicity, as well as the activation of oxidative stress, is caused by an increase in ROS gene expression in vitro (Y. Li et al., 2017). Several studies have shown a close link between mitochondrial damage and the formation of reactive oxygen species (ROS) in cells **(Christiansen et al., 2015)**. In biological environments, redox-active metals are also effective catalysts for lipid oxidation. **(Cadet et al., 2017)**. Thus, increased H2O2 levels were associated with increased MDA levels and severe TAC depletion in the H2O2 treated population, indicating that thiol proteins are H2O2 targets **(Sies 2017)**. It is a well-known phenomenon that Ag toxicity manifests itself in the form of free radicals. Increased production of reactive oxygen species (ROS) has also been linked to NP toxicity. Molecules have a lot of surface area and are very reactive, so they have a lot of oxidizing power **(Rana et al., 2021)**.

The results of comet assay showed decrease of percentage of comet incidence in control and G3 group, whereas elevated in of percentage in G2, G34 and G5. Since NS contains polyphenol groups, mostly glutathione-dihydrothymoquinon and thymohydroquinon, which have potent antioxidant function, these findings pointed to the antioxidant impact of NS **(Darakhshan et al., 2015)**. Thymoquinone therapy reduced DNA fragmentation by increasing the nuclear factor erythroid related factor (Nrf2), a regulatory factor that plays a role in the development of many antioxidant genes **(Gore et al., 2016),** which was proposed as a mechanism for the NS cytoprotective impact. Elevation in DNA fragmentation and percentage of DNA damage

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in head and tail was recorded after H_2O_2 exposure and NS-Ag-NPs in 60 mg/kg B.W., comparing to less DNA damage in NS-Ag-NPs in 30 mg/kg B.W. NS-Ag-NPs -30 does not cause DNA damage because of its covering, which may preserve cells from direct contact with AgNPs by reducing ion leaching from particles or causing extensive agglomeration of NPs, thereby reducing cellular uptake. **(Nymark et al., 2013)**. The genotoxicity of AgNPs, as well as Ag ions, has been linked to various fundamental pathways **(Guo et al., 2016; M. Li et al., 2017)**. Internalized AgNPs can then be translocated to target organelles like the mitochondria and nucleus, where they interact with membrane proteins and cause a variety of biological effects like altered cell morphology, oxidative stress, DNA destruction, inflammation **(Sudha et al., 2017**), mitochondrial dysfunction, and cell death via apoptosis and necrosis **(AshaRani et al., 2012**). AgNPs' cytotoxicity and genotoxicity is caused by oxidative stress, which was manifested in vitro by an increase in the gene expression of reactive oxygen species **(Y. Li et al., 2017)**. After AgNP therapy, oxidative stress causes a number of DNA base lesions, including Micronuclei, which indicate chromosomal damage, and oxidative DNA damage (oxidative base damage and single strand breaks), which were widely found in vitro in cultured cells and in vivo in rodents **(Li et al., 2014)**

Increased DNA damage was also found in the H_2O_2 (G2) treated rats, indicating oxidative stress and genotoxicty. Via oxidative DNA destruction, inflammation, and genomic instability, reactive oxygen species such as H_2O_2 were thought to promote tumorigenesis **(Sies et al., 2017; Tudek et al., 2017)**. Whatever mechanism produces ROS (H_2O_2), lipid peroxides induced protein oxidation, lipid oxidation, DNA oxidation, and DNA damage by producing hydroxyl radical from H_2O_2 conjugates of DNA **(Huang et al., 2016)**. As a ROS, hydrogen peroxide can depress Nrf2, resulting in a decrease in the expression of this cytoprotective factor, resulting in oxidative stress and DNA damage **(Bakunina et al., 2015)**

The current research discovered variable histological variations in rat hepatocyte sections in groups NS-Ag-NPs -60 and H_2O_2, indicating hepatotoxicity caused by cytotoxicity and oxidative stress caused by NS-Ag-NPs at elevated concentrations or H_2O_2. The association of nanoparticles with serum proteins determines their bioaccumulation. Since interacting with serum proteins, nanoparticles undergo endocytosis. Furthermore, their intracellular degradability can play a role in their cytotoxicity **(Rana et al., 2021)**.

Conclusion:

Finally, the current study's findings explained the cytotoxic activity of NS-Ag- NPs and H2O2 at high concentrations, as well as the ameliorative impact of NS and NS-Ag- NPs at low concentrations on certain liver biomarkers.

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Conflict of Interest

No conflict of interest.

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