Ginkgo Biloba Alleviates Silver Nanoparticles Induced Cardiotoxicity in Adult Male Albino Rats; Role of 8-Ohdg and Microrna-21 Expression

Doaa Elsayed Abdelrazzak[1], Mona El-demerdash Ibrahim[1], Manar Hamed Arafa[1], Samah Mohamed Ahmed[2]
1 Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Zagazig University, Zagazig, Egypt
2 Department of Medical Histology and Cell Biology, Faculty of Medicine, Zagazig University, Zagazig, Egypt
Corresponding author: Doaa Elsayed Abdelrazzak Metwaly
E-mail: doaaabdelrazzak2@gmail.com, doaametwaly@medicine.zu.edu.eg
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Abstract
Background: Silver nanoparticles (AgNPs) are the most commercialized nanoparticles worldwide. However, they have negative effects on the majority of organs including the heart. Ginkgo biloba extract (GBE) is one of the most widely sold phytomedicine.
Objectives: to evaluate the potential protective effect of ginkgo biloba on AgNPs induced cardiotoxicity in adult male albino rats.
Methods: Sixty adult male albino rats were divided equally into 6 groups; two control groups and four experimental groups (GBE treated group, AgNPs treated group, GBE & AgNPs treated group and follow up group). At the end of the study, rats were subjected to biochemical analysis; plasma nitrite, serum cardiac troponin I (cTnI), plasma microRNA-21, heart 8-OHdG and TNF-α as well as histopathological examination by H&E stain.
Results: Our results clarified that AgNPs induced significant increases in plasma nitrite, serum cTnI, plasma microRNA-21 expression as well as heart levels of 8-OHdG and TNF-α as compared to control group. AgNPs induced pale eosinophilic cytoplasm with small dark nuclei and marked cardiac muscle fibers affection with wide intercellular spaces and disorganized cardiomyocytes. GBE ameliorated all these hazardous effects. The follow up group showed some toxic effects.
Conclusion: GBE has a protective effect on AgNPs induced cardiotoxicity by antioxidative, anti-inflammatory and antiapoptotic mechanisms with improvement of molecular mechanisms through affection of microRNA-21expression. Histological improvement of cardiac tissue also strengthened the defensive effects of GBE.
Keywords: Silver nanoparticles, Heart, Oxidative stress, MiRNA-21, Ginkgo biloba extract.

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Introduction:

Nanotechnology is a highly promising and rapidly developing field of science **(1)**. Silver is the most commercialized nano-compound according to the Woodrow–Wilson database on nano products due to their unique antimicrobial properties. The wide range of industrial and medical applications of AgNPs increases their short and long term induced toxicities **(2,3)**.

The cardiac toxicity induced by AgNPs involves several different mechanisms including oxidative stress, mitochondrial disruption, nitric oxide (NO) affection, inflammation, apoptosis and affection of coagulability and cardiac muscle contractility **(4)**.

Silver nanoparticles induce oxidative stress resulting in DNA damage with increased level of 8-hydroxy,2-deoxyguanosine (8-OHdG), altered cell cycle and proliferation capacity in addition to apoptosis and necrosis. They can also physically interact with cell membranes and proteins **(5)**.

MicroRNAs (miRNAs) are small, single-stranded, natural, endogenous, non-coding ribonucleic acid (RNA) with a length of around 18-25 nucleotides. These molecules serve as regulators of cellular growth, differentiation, proliferation, apoptosis and metabolism by affecting gene expression at the post transcriptional level. The expression patterns of miRNAs change in various human diseases suggesting their promising potential as biomarkers for diagnostic, prognostic and treatment responses **(6,7)**.

Recently, microRNA-21 (miR-21) has received increased attention due to its involvement in many biological processes, mainly in cardiovascular diseases. Deregulated expression of miR-21 was also observed in the heart and vasculature under certain conditions **(8)**.

Ginkgo biloba (GB) is an important plant of the Traditional Chinese Medicine (TCM). It is the only surviving member of Ginkgo, which is the oldest living tree species on the earth **(9)**. Ginkgo biloba extract (GBE) is one of the most widely sold phytomedicine. Its cardioprotective effects are mediated through antioxidant, anti-inflammatory and antiplatelet activities together with increased blood flow by release of NO and prostaglandins (PGs) **(10, 11)**.

Flavonoids in GBE are major antioxidants among various polyphenols and act as heavy metal chelators due to their phenolic structures. They have been clinically explored in cardiovascular disorders **(12)**.

Materials and Methods:

Chemicals

Silver Nanoparticles (AgNPs): was purchased from Sigma-Aldrich chemical company, USA in the form of nano-powder <100 nm particle size. Its CAS number 576832.

Ginkgo Biloba (GB): was purchased from EMA Pharm Pharmaceuticals, Egypt as ginkgo biloba leaf powder extract capsules 260 mg.

Distilled water: obtained from El- Nasr Company, Egypt and used as a solvent for both; AgNPs and GBE.

Experimental design

Ethical approval

The experiment design was permitted by the Institutional Animal Care and Use Committee (IACUC) of Zagazig University in Egypt with approval number (ZU-IACUC/3/F/131/2020).

The study was carried out on 60 adult male albino rats, each rat weighing 150–200 gm with an average age of 50–60 days. They were obtained from the animal house, Faculty of Medicine, Zagazig University. The rats received balanced food rich in all stuffs necessary to maintain their health before and during drug administration. All animals received human care in compliance with the Animal Care Guidelines and Ethical Regulations in accordance with 'The Guide for the Care and Use of Laboratory Animals' **(13)** and IACUC of Zagazig University (IACUC-ZU).

Grouping of animals: The rats were classified into 6 groups, 10 rats in each. All rats received treatment by oral gavage daily for 8 weeks except group VI (follow up) was extended for 16 weeks as follow: Group I: (Negative control group): each rat received only regular diet and tap water to measure the basic parameters. Group II: (Positive control group): each rat received 1ml distilled water, the solvent of AgNPs **(14)** and GBE **(15)**. Group III: (GBE treated group): each rat received GBE at dose of (100 mg/kg/day) **(16)**. Group IV: (AgNPs treated group): each rat received AgNPs at dose of (100 mg/kg/day) which represents 1/20 of oral rat LD50 {LD50 of AgNPs is 2000 mg/kg according to Kim et al. **(17)**}. Group V: (GBE & AgNPs): each rat received GBE and AgNPs with the previously mentioned doses. Group VI: (Follow up group): each rat received the daily oral dose of AgNPs for 8 weeks then followed up for another 8 weeks without intervention.

Methods:

I- **Characterization of AgNPs:** To study the particles size and morphology, the aqueous dispersion of the nanoparticles was drop-cast onto a carbon- coated copper grid. The grid was air dried at room temperature and visualized using Transmission Electron Microscope (JEOL JEM-2100, Jeol Ltd, Tokyo, Japan) at Electron Microscope Research Unit, Faculty of Agriculture Mansoura University, Egypt **(Figure. 1) (18)**.

II- **Biochemical study**

Blood samples (about 2 ml) were collected in clean test tubes either with anticoagulant (EDTA) for plasma or without anticoagulant for serum (that were left for 30-60 minutes for spontaneous clotting). Then the sera and plasma were separated by centrifugation of blood at 3000 round per minute (r.p.m) for 10 minutes. The supernatant sera and plasma were pipette off by using fine tipped automatic pipettes and stored in deep frozen at -80 °C for estimation of plasma nitrite, serum cTnI and plasma miR-21 levels. After blood samples collection, the anaesthetized rats were sacrificed, hearts were dissected, and each heart was equally divided into two halves, one half was homogenized for estimation of 8-OHdG together with inflammatory biomarker, TNF-α.

a- **Assessment of plasma nitrite level.** The Nitrite Assay Kit utilizes the Griess Reagent, a classic protocol for the estimation of nitrite according to Green et al. **(19)** and Kleinbongard et al. **(20)**.

b- **Assessment of serum cardiac troponin I (cTnI):** This assay employs the quantitative sandwish enzyme immunoassay technique according to the manufacturer's instructions (CUSABIO chemical company, Japan) as described by Wang and Chatham. **(21)**.

c- **RT-PCR for assessment of plasma microRNA-21 expression level.** Plasma miR-21 expression level was measured by real time PCR (RT-qPCR). It was extracted from plasma by miR extraction kit then it was reverse transcribed into cDNA by reverse transcription kit. The RT-product was amplified by real time PCR **(22)** (QIAGEN Kit and a miScript SYBR Green PCR Kit were purchased from Sigma-Aldrich chemical company).

d- Assessment of oxidative stress biomarker, 8-hydroxy,2-deoxyguanosine (8-OHdG). 8-OHdG was estimated with ELISA Kits, according to the manufacturer's instructions (CUSABIO chemical company, Japan) as described by Zhang et al. **(23)**.

e- Assessment of inflammatory biomarker, tumor necrosis factor alpha (TNF-α). The level of TNF-α in heart tissue was determined with ELISA kits, according to the manufacturer's instructions (CUSABIO chemical company, Japan) as described by Irwin et al. **(24)**.

III- Histopathological study.

The other part of the heart from each rat was immediately immersed in 10% formol saline for 48 hours to be processed and stained by Hematoxylin and Eosin stain for light microscope study **(25)**.

IV- Statistical analysis

Analysis of data was done using SPSS program version 25.0. For normally distributed data, comparison between the six studied groups was analyzed using one way analysis of variance (ANOVA) and least significant Difference (LSD) test. All data were expressed as (mean+SD). P values less than 0.05 were considered statistically significant while values less than 0.001, were considered extremely significant **(26)**.

Results

As regard the control groups and GBE treated group: There was no statistically significant difference among GBE group (group III), negative control group (group I) and positive control group (group II) as regard all studied parameters. So, we considered the negative control group as the control one for comparison with other groups **(Table 1)**.

As regard the treated groups (AgNPs treated group, AgNPs & GBE treated group and follow up group):

I- Biochemical study:

a- **Plasma nitrite:** There were highly statistically significant increases in AgNPs treated group, GBE & AgNPs group and follow up group as compared to negative control group ($P< 0.001$). A highly statistically significant decrease in combined GBE & AgNPs group as compared to AgNPs treated group ($P<0.001$) was observed. As regard follow up group, there was high statistically significant decrease ($P<0.001$) as compared to AgNPs treated group and high statistically significant increase ($P<0.001$) as compared to combined GBE & AgNPs group **(Table 2)**.

b- **Serum cardiac troponin I (cTnI):** There were highly statistically significant increases in AgNPs treated group, GBE & AgNPs group and follow up group as compared to negative control group ($P< 0.001$). A highly statistically significant decrease in combined GBE & AgNPs group as

compared to AgNPs treated group (P<0.001) was observed. As regard follow up group, there was high statistically significant decrease (P<0.001) as compared to AgNPs treated group and high statistically significant increase (P<0.001) as compared to combined GBE & AgNPs group **(Table 2).**

c- **RT-PCR for estimation of plasma microRNA-21 (miRNA-21):** There were highly statistically significant increases in AgNPs treated group, GBE & AgNPs group and follow up group as compared to negative control group (P< 0.001). A highly statistically significant decrease in combined GBE & AgNPs group as compared to AgNPs treated group (P<0.001) was observed. As regard follow up group, there was high statistically significant decrease (P<0.001) as compared to AgNPs treated group and high statistically significant increase (P<0.001) as compared to combined GBE & AgNPs group **(Table 2).**

d- **Oxidative stress biomarker: 8-OHdG in heart tissue:** There were highly statistically significant increases in AgNPs treated group, GBE & AgNPs group and follow up group as compared to negative control group (P< 0.001). High statistically significant decreases in combined GBE & AgNPs group as compared to AgNPs treated group (P<0.001) were observed. As regard follow up group, there were high statistically significant decreases (P<0.001) as compared to AgNPs treated group and high statistically significant increases (P<0.001) as compared to combined GBE & AgNPs group **(Table 2).**

e- **Inflammatory biomarker: TNF-α in heart tissue:** There were highly statistically significant increases in AgNPs treated group, GBE & AgNPs group and follow up group as compared to negative control group (P< 0.001). High statistically significant decreases in combined GBE & AgNPs group as compared to AgNPs treated group (P<0.001) were observed. As regard follow up group, there were high statistically significant decreases (P<0.001) as compared to AgNPs treated group and high statistically significant increases (P<0.001) as compared to combined GBE & AgNPs group **(Table 2).**

II- **Histopathological study:**

Histological examination of heart tissues of the negative and positive control groups showed similar histological results, so negative control group was considered as the control group. Sections of the control left ventricle showed long parallel myocytes. They had acidophilic cytoplasm and central oval nuclei. The intercellular spaces were narrow and had fibroblasts and blood capillaries. Intercalated disc (specialized cell junction) between the cells and intracellular striations were also seen **(Figure 2.a).** Examination of H&E stained heart sections (the left ventricle) of GBE group (III) showed the same normal histological structure as control group **(Figure 2.b).**

Examination of H&E stained sections of AgNPs group showed marked morphological changes in the form of pale eosinophilic cytoplasm with small dark nuclei. Marked cardiac muscle fibers affection; some fibers were pale with empty cytoplasm while others with small apoptotic nuclei. Fibers showed wide intercellular spaces and small fragmented fibers were also detected. Disorganization of cardiomyocytes was observed. Vacuolated cytoplasm and extravasated blood

between the cells together with congested blood vessels with thickened walls were noticed **(Figure 2.c & 2.d & 2.e).**

Also, examination of sections in GBE + AgNPs group showed improvement of the left ventricular structure in the form of some fibers with eosinophilic striated cytoplasm and central vesicular nuclei. Intercellular spaces were narrow except few spaces were detected **(Figure 2.f).**

The follow up group showed fibers with wide spaces and some apoptotic nuclei while others are oval vesicular. Fragmented fibers and capillaries were detected in the intercellular spaces **(Figure 2.g).**

Table (1): Statistical comparison among (-ve) control, (+ve) control and GBE treated groups as regard mean values of plasma nitrite, serum cardiac troponin I, plasma microRNA-21 ratio and heart levels of 8-OHdG and TNF-α using ANOVA test:

Group / Parameter	Group (I) -ve Control	Group (II) +ve Control	Group (III) GBE	F	p
	Mean ± SD				
Plasma nitrite (nmol/ml)	5.38±0.53	5.25±0.57	4.80±0.81	2.188	0.132 (NS)
Serum cardiac troponin I (pg/ml)	76.99±6.41	77.02±5.45	82.33±7.51	2.233	0.127 (NS)
Plasma microRNA-21 ratio	1.02±0.07	1.01±0.04	1.07±0.09	2.197	0.131 (NS)
Heart 8-OHdG (ng/g tissue)	0.63±0.055	0.64±0.058	0.68±0.092	1.622	0.216 (NS)
Heart TNF-α (pg/g tissue)	87.05±4.92	88.03±4.94	83.74±3.64	2.452	0.105 (NS)

Number of rats in each group=10 rats **NS:** non-significant (P>0.05) **SD:** standard deviation

F: ANOVA test **P:** level of significance **GBE:** Ginkgo biloba extract

8-OHdG: 8-hydroxy-2′-deoxyguanosine **TNF-α:** Tumor necrosis factor alpha

Table (2): Statistical comparison among (-ve) control, AgNPs treated, GBE & AgNPs treated and follow up groups as regard mean values of plasma nitrite, serum cardiac troponin I (cTnI), plasma microRNA-21 ratio, heart 8-OHdG and TNF-α using ANOVA test:

Group / Parameter	Group (I) -ve Control	Group (IV) AgNPs	Group (V) GBE+AgNPs	Group (VI) Follow up	F	p
	Mean ± SD					
Plasma nitrite (nmol/ml)	5.38±0.53	13.50±2.10	7.32±0.42	9.21±0.89	84.903	<0.001**
Cardiac troponin I (pg/ml)	76.99±6.41	1109.75±121.53	455.66±59.46	805.79±70.21	341.348	<0.001**
Plasma miRNA-21 ratio	1.02±0.07	3.74±0.58	1.93±0.14	2.78±0.23	128.034	<0.001**
Heart 8-OHdG (ng/g tissue)	0.63±0.055	12.27±0.82	6.79±1.14	9.16±0.56	422.674	<0.001**
Heart TNF-α (pg/g tissue)	87.05±4.92	893.91±13.21	420.36±50.56	730.72±40.28	1160.012	<0.001**

Number of rats in each group=10 rats ****:** high significant (P<0.001) **SD:** standard deviation
F: ANOVA test **P:** level of significance **GBE:** Ginkgo biloba extract
AgNPs: silver nanoparticles **8-OHdG:** 8-hydroxy-2′-deoxyguanosine
TNF-α: Tumor necrosis factor alpha

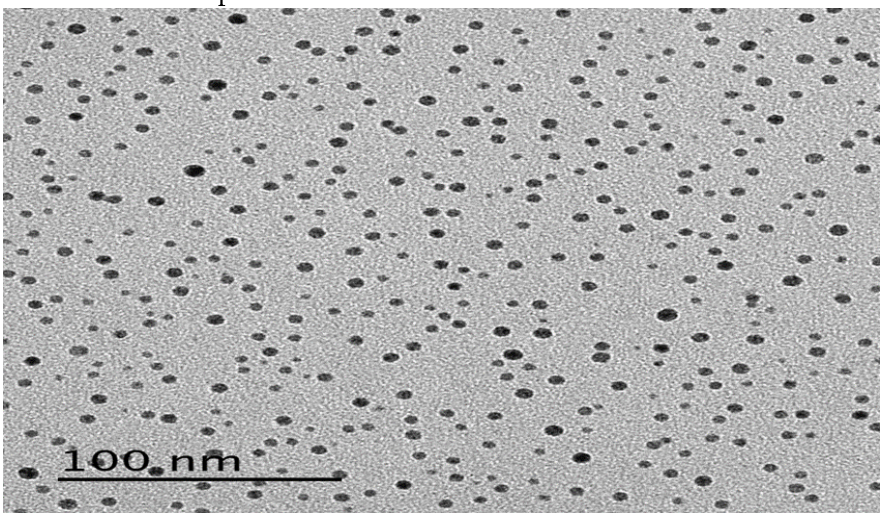

Figure 1: Scanning electron microscope photogragh showing silver nanoparticles less than 100 nm (scale bar 100 nm).

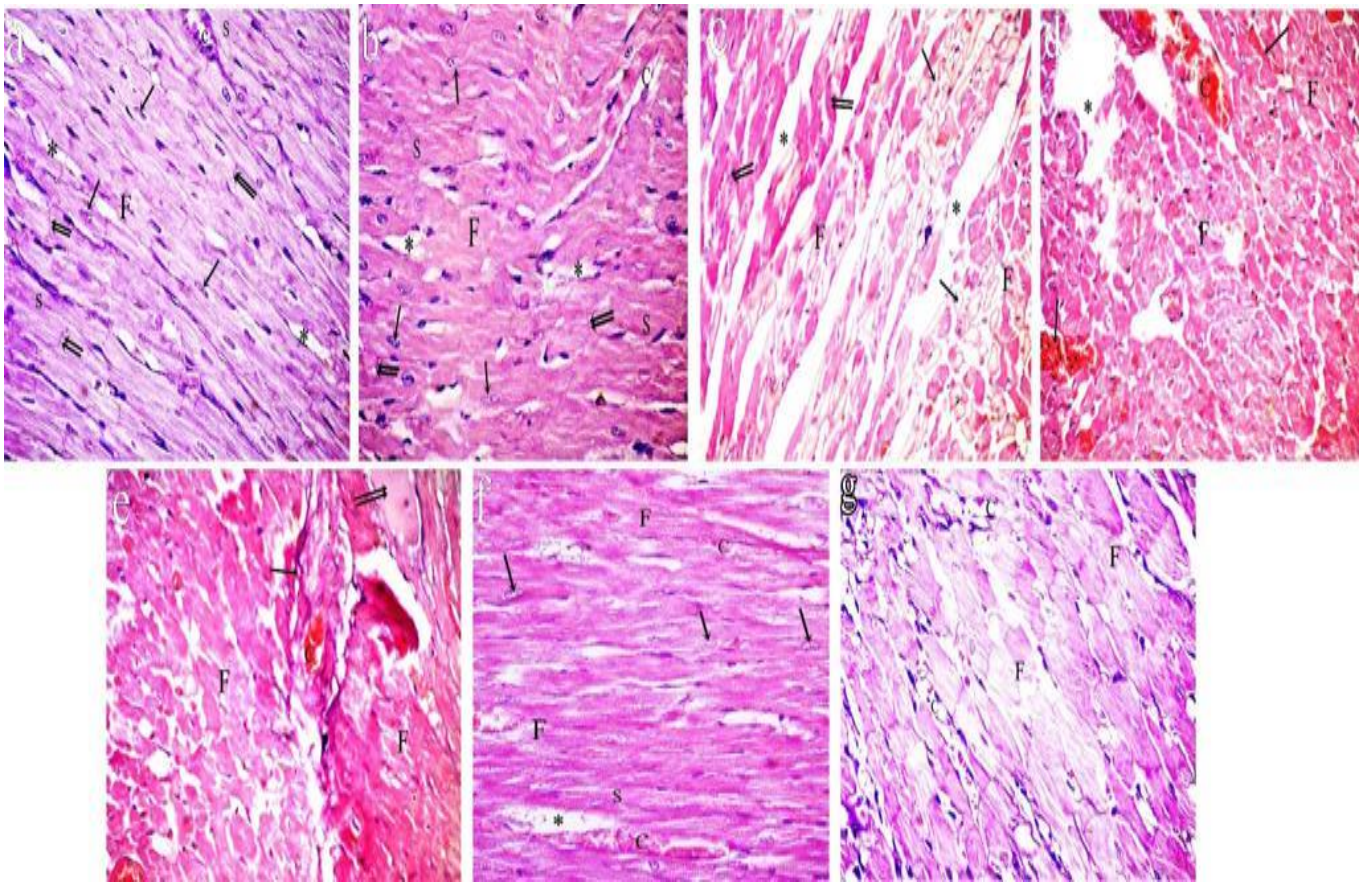

Figure 2: H&E-stained sections of **a:** negative control group showing cardiac muscle fibers (F) with acidophilic striated cytoplasm (s) and oval vesicular nuclei (arrow). Narrow spaces (*) between cardiac muscle fibers containing blood capillaries (c) are noticed. Intercalated discs (specialized cell junction) are seen between muscle fibers (double arrow). **b:** GBE group showing cardiac muscle fibers (F) with acidophilic striated cytoplasm (s) and oval vesicular nuclei (arrow). Narrow spaces (*) between cardiac muscle fibers containing blood capillaries (c) are noticed. Intercalated discs are seen between muscle fibers (double arrow). **c:** AgNPs group showing marked cardiac muscle fibers (F) affection; fibers are pale with empty cytoplasm (arrow), others with small apoptotic nuclei (double arrow) and wide spaces between muscle fibers (*). **d:** AgNPs group showing disorganized cardiac muscle fibers (F) with extravasated blood (arrow) and wide spaces between muscle fibers (*). Congested blood vessels are also detected (C). **e:** AgNPs group showing disorganized cardiac muscle fibers (F). Congested blood vessels (C) with thickened wall (arrow) are seen. Muscle fibers are detected with pale acidophilic non striated cytoplasm (double arrow). **f:** GBE+AgNPs group showing cardiac muscle fibers (F) with acidophilic striated cytoplasm (s) and oval vesicular nuclei (arrow). Narrow spaces (*) between cardiac muscle fibers containing blood capillaries (c) are noticed. **g:** follow up group showing cardiac muscle fibers with pale acidophilic non striated cytoplasm (F). Dilated blood vessels (C) are seen in the spaces between muscle fibers. All groups: H&Ex400.

Discussion

Silver nanoparticles are used in extensive applications due to their antimicrobial properties. Ginkgo biloba extract has a long history of being used as a phytotherapeutic product for medicinal

purposes, in the early 1970s **(27, 28)**. In the present study, AgNPs treated rats showed significant increase in plasma nitrite as compared to control group. Plasma nitrite is more than just a byproduct of NO, it also can be converted back into NO by different biochemical pathways. Nitric oxide is a free radical signaling molecule synthesized by conversion of arginine to citrulline by the family of nitric oxide synthase (NOS) **(29)**.

The ability of AgNPs to induce NO production and subsequently increase plasma nitrite coincides with Zielinska et al. **(30)** who found that AgNPs increased NO production and inducible NOS (iNOS) expression. Nitric oxide directly or via conversion to reactive nitrogen species could induce apoptosis. Additionally, Rosas-Hernández et al. **(31)** found that AgNPs can induce NO production by activating endothelial NOS through its phosphorylation on endothelial cells derived from coronary vessels. It can mediate cardiovascular injury through the formation of secondary oxidants or by inhibition of mitochondrial respiration thus it increases the oxidizing stress and cellular damage brought about by oxidants **(32)**.

The results of the current investigations revealed a significant increase in heart levels of 8-OHdG in AgNPs treated group as compared to control group which coincides with Yousef et al. **(33)**. Additionally, Nayek et al. **(34)** observed that AgNPs induced ROS which subsequently led to production of 8-dihydrodeoxyguanosine triphosphate (8-oxo-dGTP), which in turn could produce 8-OHdG.

Silver nanoparticles can enter the mitochondria and nucleus and induce DNA oxidation with subsequent impairment of cellular functions due to liberation of Ag^{+} from AgNPs that can induce cell specific, localized cellular toxicity. The ability of AgNPs to form ROS is considered the main pathway for their toxic effects. Increased ROS is related to permanent membrane damage, DNA, lipid and protein damage with apoptosis or necrosis **(35)**. 8-OHdG is a good marker of oxidative DNA damage and considered as a factor of the initiation and promotion of carcinogenesis **(36)**.

A study by Estévez and Jordán **(37)** suggested that NO can be coupled with O2 to produce peroxynitrite, that has been linked to several interactions causing lipid peroxidation and nitrosylation of some molecules. While Janzadeh et al. **(38)** stated that oxidative stress caused by AgNPs can trigger inflammatory responses, including the activation of innate immunity and increased permeability of endothelial cells. Inflammation and increased oxidative stress followed by apoptosis are likely to be the main mechanisms of AgNPs induced cardiotoxicity.

Upon administration of GBE to AgNPs treated rats, there were significant decreases in plasma nitrite and heart levels of 8-OHdG which pass parallel with Erdogan et al. **(39)** who stated that GBE prevented high NO production in rats. It could also ameliorate depletion of SOD and GSH in plasma of rats that coincides with Yalçın et al. **(40)** who stated that GBE could reduce free radical formation, prevent the 8-OHdG formation and oxidative DNA damage and induce DNA repair after oxidative stress.

Chen et al. **(41)** stated that GBE had inhibitory effects on oxidative stress via phosphorylation of protein kinase B (Akt) pathway enhancing the translocation of nuclear factor erythroid 2 (Nrf2) into the nucleus. Nrf2, a transcription factor, has a role in the activation of antioxidant cascade through binding to the antioxidant response element.

The results of the present study showed a highly statistically significant increase in serum cTnI in AgNPs treated group as compared to control group. Cardiac troponin I is a sensitive tool for documenting myocardial damage. It has been used as a reliable marker for cardiac risk assessment. It is more sensitive and significantly more specific for the diagnosis of cardiovascular diseases, especially myocardial injury **(42)**.

Damage to myocardial cell integrity is followed by release of cTnI into the blood. Silver nanoparticles could damage myocardial tissue and suppress the activities of myocardial transmembrane potential (TMP), Na and K channels **(43)**.

In the present study, there was a significant decrease in serum cTnI in combined (GBE & AgNPs) and follow up groups when compared to AgNPs treated group. This coincides with Jasim et al. **(44)** who stated that the cardioprotective effect of GBE is attributed to augmentation of endogenous antioxidants and inhibition of lipid peroxidation.

In a study by Guo et al. **(45)**, GBE administration downregulated the high levels of cTnI and improved the damaged myocardial cells in rats. They clarified that GBE treatment could suppress endoplasmic reticulum stress induced ROS and apoptosis.

MicroRNA-21 (miR-21) is one of the earliest identified and most investigated miRNAs. It is tumor promoting miRNA that stimulates cancer occurrence and development through regulation of multiple tumor suppressor genes. MicroRNA-21 is expressed abnormally in multiple cancer cell types and increased in most malignant tumors **(46)**. It could serve as a novel biomarker of inflammation and apoptosis. It is up regulated in inflammatory disorders and may be a promising therapeutic target **(47)**.

The results of the present work showed a highly significant increase in plasma miR-21 expression as compared with the control group which pass parallel with Abdel Hamid et al. **(48)**. They showed that miR-21 is related to apoptosis of cardiomyocytes and reported that increased miR-21 was associated with increased 8-OHdG denoting that oxidative stress induces upregulation of miR-21 running in parallel to the study by Tu et al. **(49)**.

Wei et al. **(50)** found that programmed cell death gene 4 (PDCD4) is targeted by miR-21 in cardiomyocytes under oxidative stress. Also, the H2O2 stimulated miR-21 is regulated by nuclear factor kappa B (NF-κB). These results indicate a novel mechanism in oxidative stress induced cell death program in cardiomyocytes which suggested that miR-21 contributed an important role in ROS mediated cardiomyocytes injury.

According to the present findings, GBE administration induced high statistically significant decrease in plasma miR-21 expression in AgNPs treated rats which coincides with marked downregulation in miR-21 expression level in the liver after GBE administration in the study by Sherif and Al-Shaalan **(51)**. They attributed the hepatoprotective mechanism of GBE against methotrexate (MTX) induced hepatotoxicity to the downregulation of miR-21 hepatic expression and the modulation of the IL-6/STAT3 signaling pathway.

At normal conditions, the transcription factor signal transducer and activator of transcription 3 (STAT3) is a vital transcription factor that organizes several biological processes such as apoptosis, proliferation, cell differentiation and inflammation **(52)**. It is located in the cytoplasm and when activated by proinflammatory cytokines such as IL-6, the phosphorylation of

STAT3 on tyrosine 705 occurred and formed dimers with the STATs family members. The activated STAT3 then translocated to the nucleus from the cytoplasm and initiated gene transcription concerning apoptosis and inflammation **(53)**. The processing and expression of miR-21 are controlled by the janus kinases (Jaks), STAT3 and bone morphogenetic proteins **(54)**.

In the present study, rats treated with AgNPs showed high statistically significant increases in heart levels of TNF-α as compared to control group which coincides with Yousef et al. **(33)** and Elblehi et al. **(55)**. This indicates the ability of AgNPs to induce inflammation as Ansar et al. **(56)** reported that TNF-α is an effective cytokine of inflammation and endothelial functions which plays an important role in inflammatory response.

Silver nanoparticles can generate intracellular ROS with subsequent cell damage and initiation of an inflammatory response **(57)**. Tumor necrosis factor alpha is cardiotoxic as it induces depression of cardiac functions and myocardial contractility and causes auto-destructive inflammation and apoptosis of cardiomyocytes **(58)**.

The toxicity of AgNPs is time and dose dependent; this phenomenon has also been identified in a previous study by Williams et al. **(59)**. Ginkgo biloba extract administration showed significant decreases in heart levels of TNF-α in AgNPs treated rats indicating the anti-inflammatory ability of GBE which coincide with Dong et al. **(60)** who stated that GBE is a known bioactive extract widely explored for its remarkable anti-inflammatory activity, especially in cardiovascular and neurological disorders.

Chen et al. **(41)**; Tao et al. **(61)** reported that administration of GBE caused significant decrease in serum TNF-α in rats. The components of GBE, mainly ginkgolide A, can suppress cyclooxygenase-2 (COX-2) and 5-lipo-oxygenase, which are limiting enzymes for the conversion of arachidonic acid to prostaglandin and leukotrienes. It also can reduce endoplasmic reticulum stress, which would be responsible for boosting inflammation.

The histological examination of H&E-stained sections of AgNPs treated rats revealed marked morphological changes in the heart tissue in the form of pale eosinophilic cytoplasm with small dark nuclei. Marked cardiac muscle fibers affection with wide intercellular spaces and small fragmented fibers were also detected. Disorganization of cardiomyocytes was observed. Vacuolated cytoplasm and extravasated blood between the cells together with congested blood vessels with thickened walls were noticed. This is in accordance with Yousef et al. **(33)**.

Also, Rathore et al. **(62)** concluded that the heart muscle fibers treated with AgNPs demonstrated mild edema and separation of myofibrils while Adeyemi and Faniyan **(63)** documented that AgNPs induced inflammation, loss of cross striations with myocardial degeneration and cellular alteration in the cardiac tissue of male wistar rats.

Co-administration of GBE with AgNPs, showed improvement of the left ventricular structure in the form of some fibers with eosinophilic striated cytoplasm and central vesicular nuclei. Intercellular spaces were narrow except few spaces were detected which is consistent with El-Boghdady. **(58)** who reported that GBE showed a remarkable improvement in histopathological damage induced by Adriamycin in heart tissue of rats as reflected by decreasing vacuolization of myocardial cells.

Moreover, Zhang et al. **(64)** showed that GBE decreased lymphocyte infiltration and inflammatory cells in myocardial muscle tissue induced by high fat diet. Furthermore Kadhim et al. **(65)** reported that GBE treatment decreased the pathological changes induced in heart tissue by I/R injury in the form of focal necrosis and leukocytes infiltration.

Matching with these findings, Badore et al. **(66)** revealed that treatment with GBE showed maximum protective effect in the form of mild myocardial edema with no infarction or inflammatory cells. They attributed the cardioprotective effect of GBE to the increase in the basal myocardial antioxidant enzyme activities.

Conclusions

The biochemical and histopathological results of the present work showed that increased NO production, DNA oxidation, inflammation and apoptosis could be the contributing factors in AgNPs induced cardiotoxicity. Over expression of plasma microRNA-21 could also be a novel biomarker of cardiotoxicity. Administration of GBE could offer protective effects against these harmful consequences. It has antioxidant, anti-inflammatory and molecular protective mechanisms together with restoration of histological structure so, it can be used as a herbal supplement to alleviate the harmful effects of AgNPs.

Disclosure statement

The author (s) did not disclose any potential conflicts of interest.

Data availability

The data arrangements used and evaluated in this present study were available from the corresponding author upon reasonable request.

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