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The efficiency of blackberry loaded AgNPs, AuNPs and Ag@AuNPs mediated pectin in the treatment of cisplatin-induced cardiotoxicity in experimental rats

Jihan Hussein [a], Mehrez E. El-Naggar [b,*], Moustafa M.G. Fouda [b,*], Osama M. Morsy [c], Jamaan S. Ajarem [d], Ahmed M. Almalki [d], Ahmed A. Allam [e], Enas Mahmoud Mekawi [f]

[a] Medical Biochemistry Department, National Research Centre, Dokki, Giza, Egypt
[b] Pre-Treatment and Finishing of Cellulosic based Fibers Department, Textile Industries Research Division, National Research Center, 33 El- El Buhouth St, Dokki, Giza 12622, Egypt
[c] Arab Academy for Science, Technology & Maritime Transport, Arab League, Cairo 2033, Egypt
[d] Zoology Department, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
[e] Department of Zoology, Faculty of Science, Beni-suef University, Beni-suef 65211, Egypt
[f] Agricultural Biochemistry Department, Faculty of Agriculture, Benha University, Egypt

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ABSTRACT

Cisplatin (cis-Diaminedichloroplatinum) is one of the most effective chemotherapeutic because of its anti-neoplastic properties against various types of tumor. However, it has a wide variety of side effects such as hepato, gastrointestinal, neuro, nephro, and cardiotoxicity (acute and/or chronic) that highly restricted its usage. Thus, research work was planned to detect the role of gold (AuNPs), silver nanoparticles (AgNPs) and their corepshell (Ag@AuNPs) as a carrier for blackberry extract and to enhance its benifit in treatment of cisplatin-induced cardiotoxicity. In our work, solid-state process was used in order to prepare these nanoparticles using pectin as an ecologically friendly-polymer acting as reductant for ions and at the same time as stabilizing agent for the produced nanoparticles. This nominated method for large-scale preparation of nanoparticles is simple, efficient, and convenient. The presence of individual metallic Ag, Au and both has been proven by UV-vis spectroscopy. Transmission electron microscopy (TEM) and particle size analyzer confirmed the preparation of spherical small size with a main diameter <40 nm. The data obtained from zeta potential evaluation displayed the well stabilization for the produced nanoparticles. Transmission electron microscopy (TEM), scanning electron microscopy (SEM) and particle size analyzer have verified that the spherical small size is <40 nm in diameter. Data from zeta potential assessment revealed the good stability of the produced nanoparticles. To this end, fifty sex rats were used in this study and divided into control, cisplatin (cispt), and five treated groups. After the experimental period, lipid profile was estimated and atherogenic coefficient (AC), atherogenic index (AI), and cardiac risk ratio (CRR) were calculated. Oxidant and antioxidant parameters were also estimated. Cardiovascular disease markers were estimated by ELISA. The mean levels of cholesterol, triglycerides, malondialdehyde (MDA), advanced oxidative protein products (AOPP), and cardiovascular markers were significantly increased in cispt group compared to control; whereas these parameters were attenuated in all treated groups in particular that received blackberry (bb) loaded Ag@AuNPs. Based on these results, it can be concluded that bb has antioxidant and antilipidemic effect that help in protecting against cardiovascular disease specially when loaded with Ag@AuNPs.

1. Introduction

Cisplatin is considered one of the most powerful used chemotherapeutic agents due to its anti-neoplastic properties against different tumor types. It is used in managing solid tumors like bladder, head & neck, and ovarian tumors; in addition to hematological malignancies, and lymphoma [1]. However, it has a number of side effects including neuro, nephro, hepato, and gastrointestinal toxicity [2]. Nevertheless, cardiotoxicity (acute and/or chronic) is one of the important factors restrict the use of cisplatin [3].

These complications in cisplatin-induced cardiotoxicity (CD) are due to ROS release; reactive oxidative species, thereby decreasing glutathione (GSH), producing oxidative stress, triggering cell membrane lipid peroxidation, as well as protein degradation and DNA [4,5]. Therefore, an operational method to improve the CD and myocardial injury may be an effective antioxidative stress involvement [6].

* Corresponding authors.
E-mail addresses: mehrez_chem@yahoo.com (M.E. El-Naggar), drmmfouda@gmail.com (M.M.G. Fouda).

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Blackberries are described as types of fruits with high contents of antioxidant compounds like phenolic compounds, in particular ellagitannins and anthocyanins [7]. These compounds have several health benefits in cancer, diabetes, inflammation, neurodegenerative disorders and cardiovascular diseases [8]. The mechanism by which these compounds can attain the health benefits comprising limitation of oxidative stress. Additionally, unbalanced oxidative status is considered one of the important and critical causes of several diseases or a secondary impairment associated with the illness [9]. However, using plant extract alone in the treatment of different diseases is still ineffective or very slow and need more and more enhancement to appear their benefit. Recently, there are great advances of nanotechnology and its applications in science and technology fields.

Metallic nanoparticles such as gold (AuNPs), silver (AgNPs), and zinc oxide (ZnONPs) have shown huge challenges in the field of medicine and health [10,11]. Previously, several studies developed the consumption of different plant extract and natural products in order to bind them with silver or gold as nanocarriers to be served as anti-tumor agent [12], anti-diabetic [13], anti-neurotoxicity [14] and anti-renal failure agents [15].

Metal nanoparticles have been widely prepared using several different methods such as chemical reduction, electrochemical, microbiological reduction, ultrasonication method, microwave radiation and so on [10,16–20]. Most of the methods require aqueous or organic media for preparation. The reaction relies on utilizing the chemical compounds or radiations or waves for ions reduction and, other compounds can be used to stabilize the formed nanoparticles.

In this current work, an efficient technique nominated as solid-state synthesis has been used for the clean synthesis and high-throughput production of metal and core shell metal nanoparticles [11,17,21]. Pectin is well known for its outstanding biodegradable and biocompatible properties as a natural polymer. Commercially, pectin is extracted in mildly acidic conditions out of various citrus products. In various potential biomedical applications, pectin has been widely investigated [22].

Polysaccharides have been reported to undergo various transformations within the alkaline medium, including depolymerization and destruction by oxidation [22,23]. It has been reported that in alkaline medium, polysaccharides undergo chemical interaction with metal ions to produce two types of compounds; non-reducing compound such as galacturonic acid and reducing monosaccharide compounds such as glucose, galactosis, rhamnesis, arabinose. Such compounds work to very efficiently reduce the metal ions to nanometric particles and in the same stabilize such these formed nanomaterials and prevent them from aggregation by the formation of coordination bonds with huge hydroxyl groups of these saccharides.

Solid state technique has been used for nanoparticles preparation. The advantage for using such technique is to save time, chemicals and solvents that can be used largely in the traditional synthesis in addition to ease of transportation from the lab to different industrial fields.

Ultimately, the aim of the current research was planned to develop a highly efficient, and reliable method of reducing the use of hazardous chemical compounds, save a time and energy using a simple and environmentally friendly method (solid-state synthesis). Hereby, pectin as both reducing and stabilizing agent has been used for the preparation of silver nanoparticles (AgNPs), gold nanoparticles (AuNPs) and core shell of both (Ag@AuNPs) with no need to use an extra compound and organic or aqueous solvents via apply the solid-state method. As a result, we sought to investigate the function of AgNPs, AuNPs and Ag@AuNPs in improving the benefit of blackberry in the treatment of cisplatin-induced cardiotoxicity passing on its characteristics in attenuating oxidative stress and cardiac injury.

2. Materials and methods

2.1. Materials

Cisplatin (1 mg/mL) was purchased from "EIMC United Pharmaceuticals, Egypt". Full-ripe blackberries were purchased from "local market in Egypt". Silver nitrate and gold (II) chloride hydrate were purchased from "Fischer Co. Germany". Pectin and sodium hydroxide pellet (NaOH) were purchased from WIN LAB, India. All other chemical were used as received. Deionized water has been used for preparation, characterization and *in-vivo* studies.

2.2. Preparation of blackberry extract

Leaves of blackberry were collected, cleaned, washed with deionized water and left for drying for 4 days followed by well grinding of these cleaned leaves. Then, the final powder (10 g) was mixed well with absolute ethanol (99%; 50 mL). The supernatants were collected and subjected for rotary evaporation at 40 °C. The dried extract was resuspended in deionized water and filtered through a 25 μm filter paper and lyophilized to obtain dried active ingredient then re-dissolved in deionized water (400 mg/25 mL) and stored at −20 °C for further characterization. The resultant solution of the blackberry extract was labeled as bb [24,25].

2.3. Solid state synthesis of silver, gold and core-shell of silver-gold nanoparticles

Nanoparticles of silver (AgNPs), gold (AuNPs) and their core shell (Ag@AuNPs) were synthesized using solid state technique. In this technique, pectin was used as both stabilizing and reducing agent. In this technique, pectin (0.2 g) was grinded with 0.02 g of NaOH (pellet). After complete grinding, 0.1 g of silver nitrate ($AgNO_3$) was added and the grinding process was continued for another 3 min till the color was changed from colorless to yellowish color affirming the formation of AgNPs. For the preparation of AuNPs, gold (III) chloride hydrate ($HAuCl_4.3H_2O$) was used as a selective precursor (0.1 g) for such preparation. The color of solid mixture was changed to reddish violet after the addition of chloroauric acid owing to the formation of AuNPs. On the third side, to prepare core shell, 0.05 g of silver nitrate was added firstly to Pectin/NaOH mixture followed by the addition of 0.05 g of gold (II) chloride hydrate. All the solid mixture was kept under continuous grinding for 10 min till the color was changed to deep reddish brown. The final products of AgNPs, AuNPs and Ag@AuNPs were kept at room temperature for further characterization and application.

2.4. Preparation of solution mixture of extracted blackberry /nanoparticles

First of all, 0.1 g of the formed AgNPs, AuNPs and Ag@AuNPs were dissolved in 80 mL of deionized water and kept under magnetic stirring for 20 min and followed by sonication for another 20 min to ensure the complete dissolution. Afterward, bb (20 mL; 10 mg/mL) was added dropwise to the previous solutions and kept for another 30 min of homogenization. In this step, the metal nanoparticles are considered as a carrier for bb (model drug). The produced mixture solutions were kept for analysis prior to administration to rats.

2.5. Characterization of the prepared metal nanoparticles

The wavelength and absorbance of the prepared metal nanoparticles were examined using UV-vis spectra "Shimadzu UV-2450 spectrophotometer, Japan" at a wavelength ranged from 250 to 700 nm. Prior evaluation thru UV-vis, a definite weight (0.001 g) of the analyzed samples were dissolved in 20 mL of deionized water and submitted for ultrasonication for 15 min. The particles shape and the core shell of the nanoparticles were further investigated by the means of

"Transmission electron microscopy"; "TEM; JEOL JEM-2100" operating at 200 kV. Each sample of AgNPs, AuNPs and Ag@AuNPs were examined at three different magnification. Selected area diffraction pattern (SAED) was obtained for each sample to outline the crystallinity of the formed nanoparticles.

The surface structure of the fabricated nanoparticles of AgNPs, AuNPs and Ag@AuNPs were studied by examine the topographic images obtained from "field emission scanning electron microscopy": "FESEM; JSM 7600 F FESEM with EXD, JEOL, Ltd., Tokyo, Japan". Each figure has been taken at different magnifications. In addition, the elemental analysis for the scanned samples were carried out using "energy-dispersive X-ray spectroscopy"; "EDX". The average particle size diameter and zeta potential of AgNPs, AuNPs and Ag@AuNPs was confirmed by a "ZETASIZER Nano series; Nano ZS Malvern Instrument Ltd., Malvern, UK". A known weight (0.001 g) of the formed powder nanoparticles was suspended in 20 mL of deionized water and sonicated for 15 min prior to examinations using DLS and zeta sizer.

2.6. Entrapment efficiency

One ml of the prepared solution of bb loaded AgNPs, AuNPs and Ag@AuNPs was distributed in deionized water (10 mL) and the absorbance was measured using UV spectrophotometer "Shimadzu1601; Shimadzu, Kyoto, Japan" at 515 nm using water as blank. The amount of the untrapped bb extract in the supernatant was estimated and the amount of the entrapped bb extract was determined by subtracting amount of free bb extract in the supernatant from the original amount of bb extract taken. For each sample, the experiment was carried out in triplicate and the average was calculated. The efficiency of nanoparticles for bb extract trapping (EE %) was calculated from Eq. (1) as follows:

$$EE(\%) = \frac{(bb\,extract\,loaded - bb\,extract\,loss)}{bb\,extract\,loaded} \times 100 \quad (1)$$

2.7. Experimental animals

Fifty sex male albino rats, weighing 160–180 g were obtained from the animal house of the National Research Centre (NRC), Giza, Egypt. Animals were kept in polypropylene cages and maintained in a controlled room temperature ranged from 20 to 25 °C with light/dark cycle, fed on a standard commercial diet and water freely available along the experimental time. The experiment was achieved in accordance with the "guidelines of the Institutional Animal Ethics Committee of National Research Centre (NRC), Giza, Egypt".

2.8. Induction of cardiotoxicity

Cispt was intraperitoneal injected in animals in a single dose of 20 mg/kg body weight once according to the method described by El-Sayed et al., [15].

2.9. Experimental design

Fifty sex rats were used in this study and divided into seven groups as follow: control group: rats received deionized water, cispt group: animals received a dose of cispt and a daily dose of DW, treated group I: this group received a single dose of cispt followed by blackberry (bb) extract (10 mL plant extract /kg b.w./day) orally for 14 days, treated group II: received a single dose of cispt followed by bb/AgNPs (1 mg/kg b.w./day) orally for 14 days, treated group III: rats received a single dose of cispt followed by bb/AuNPs (1 mg/kg b.w./day) orally for 14 days, treated group IV: rats received a single dose of cispt followed by Ag@AuNPs (1 mg/kg b.w. /day) orally for 14 days, treated group V: rats received a single dose of cispt followed by bb extract loaded Ag@AuNPs (1 mg/kg b.w./day) orally for 14 days [15]. After the experimental period finalization, all groups were kept fasting for12 h before blood sampling. To this end, blood was collected in dry clean test tubes and centrifuged at 3000 rpm using cooling centrifuge "Laborzentrifuge, 2K15, Sigma, Germany" for 15 min; samples were divided into aliquots and stored at −20 °C for estimation of different biochemical parameters. Serum was separated and then stored at −20 °C. Heart was removed quickly from each rat, washed with ice-cold saline and kept at −80 °C until used for determination of other biochemical parameters.

2.10. Preparation of tissue homogenate

Heart tissues were cut into small pieces and homogenized in 5 ml phosphate buffer [0.5 g of Na_2HPO_4 and 0.7 g of NaH_2PO_4 per 500 ml of deionized water (pH 7.4) per gram tissue] followed by centrifugation at 4000 rpm for 10 min at 4 °C. Supernatant was separated to evaluate oxidant and antioxidant markers.

2.11. Biochemical estimations

2.11.1. Lipid profile

Serum total cholesterol (TC) and triglycerides (TGs) were determined by enzymatic colorimetric methods according to Jacobs and Vandemark [26] and Allain et al., [27] respectively. Serum high-density lipoprotein cholesterol (HDL—C) was estimated by precipitated method that described previously by Fruchart et al., [28]. Low-density lipoprotein cholesterol (LDL-C) was calculated from the value of cholesterol and triglycerides using Friedewald equation [29]. Other parameters were calculated using lipid profile data:

- Atherogenic index (AI): was calculated from 10 logarithms of the TG/HDL, according to Dobiásová and Frohlich [30] as follow:

$$Atherogenic\ index = \log\left(\frac{TG}{HGL} - C\right)$$

- Cardiac risk ratio (CRR): was calculated from the ratio between total cholesterol and HDL-C [26].
- Atherogenic coefficient (AC) was calculated from the ratio of LDL-C to HDL-C according to Kinosian et al., [31].

2.11.2. Oxidant and antioxidant profile

Total antioxidant capacity (TAC) in serum was carried out on the base of free radical-scavenging activity according to Janaszewska and Bartosz [32]; briefly, samples were acidified with perchloric acid for protein precipitation and centrifuged at 4000 rpm for 15 min at 4 °C; the supernatant was then separated and diluted with phosphate-buffered saline (PBS) and incubated for 20 min. After that, the absorbance was measured at 517 nm; the results expressed as percent of scavenging activity compared to the control without sample.

Serum advanced oxidation protein products (AOPPs) level was determined according to Witko-Sarsat et al., [33] using ELISA kit from Glory Science Co. Ltd. (Del Rio, Texas, USA). Whereas, cardiac malondialdehyde (MDA) was determined according to Ohkawa et al., [34].

2.11.3. Biochemical markers of cardiotoxicity

Serum lactate dehydrogenase (LDH) and troponin-I levels were determined by an enzyme-linked immunosorbent assay (ELISA) Kit accorded to the manufacturer instructions.

3. Results and discussion

The present art work is pertaining to improve the availability of very interesting natural food resource through using nanotechnology in term

of drug; blackberry (bb) loaded nanoparticles; AgNPs, AuNPs and Ag@AuNPs were successfully prepared using a naturally occurring biomaterials; firstly, pectin was activated by sodium hydroxide which, in turn, produced sodium pectinate. Such this produced active polymer has the ability to reduce and stabilize the metal nanoparticles with no need to use an extra materials or solvent. The utilized technique; solid state facilitates the preparation of nanoparticles in a large scale with well distribution capability [11,17]. To this end, fixed amount of previously prepared blackberry extract solution was loaded to all the prepared nanoparticles. It is expected that the utilization of nanoparticles as a carrier for the selected natural drug (bb) enhances its effect as antioxidant and antilipidemic to act as protecting agent for cardiovascular disease especially when loaded nanoparticles.

Before starting the *in-vivo* study in rats, it is necessary to evaluate the prepared nanoparticles in term of particle shape, particle size, stability and morphological features as well. Below are the characteristic tools as well as the obtained results.

3.1. Physicochemical characterization of the as-synthesized AgNPs, AuNPs and Ag@AuNPs

The first observation for the formation of nanoparticles is the color change. It is well known that pectin colorless. Via utilizing this polymer as reductant for the silver ions, the color started to change from colorless to yellow color ended by brown by the time. The same trend for AuNPs formation, the color was changed from colorless to reddish violet color due to the absorbance of surface plasmon resonance of AgNPs and AuNPs. The color change could be attributed to the potential power of pectin that reduce and stabilize the formed nanoparticles thru its primary alcoholic reducing groups and the abundant hydroxyl groups respectively. Firstly, UV-vis absorbance is very important tool to clarify the definite wavelength for each nanoparticle under investigation (Fig. 1). It is observed that AgNPs has specific wave length at 410 nm affirming the successful preparation of AgNPs using pectin with the aid of solid- state synthesis. Accordingly, AuNPs has an absorbance band at wavelength equal to 524 nm as shown in Fig. 1. It is obviously stated that, the wavelength of AgNPs has been shifted to greater wavelength; 500 nm (Fig. 1) while formed directly with AuNPs affirming that the successful formation of core shell rather than alloy. In case of alloy formation, there are two peaks will be formed, one for AgNPs and the other for AuNPs. Thus, in our synthesis, Ag can be acted as a core and AuNPs as a shell which illustrate by the presence of one peak [20,35].

Moving to the particle shape, it is depicted by TEM evaluation shown in Fig. 2 (A, B, C) that AgNPs appears as small spherical shape with well distribution. The well distribution owing to the potential power of pectin to reduce Ag ions into very small clusters of AgNPs. Fig. 2 (D, E, F) displays TEM images of the produced AuNPs. It is clearly seen that the particles are also formed with spherical shape but with less stability. The particles are formed with AuNPs have large diameter than that of AgNPs which could be attributed to the easily reduction of Ag ions than Au ions. Meanwhile, Fig. 2 (G, H, I) illustrates the particle shape of Ag@AuNPs. That depicts that the particles are clearly formed with two phases which confirm that one of the two metal nanoparticles are covered or coated with the other. As mentioned in the experimental part, AgNPs was prepared firstly followed by the preparation of AuNPs. Therefore, it is expected the inner black particles is AgNPs and coated with the white shaded of AuNPs.

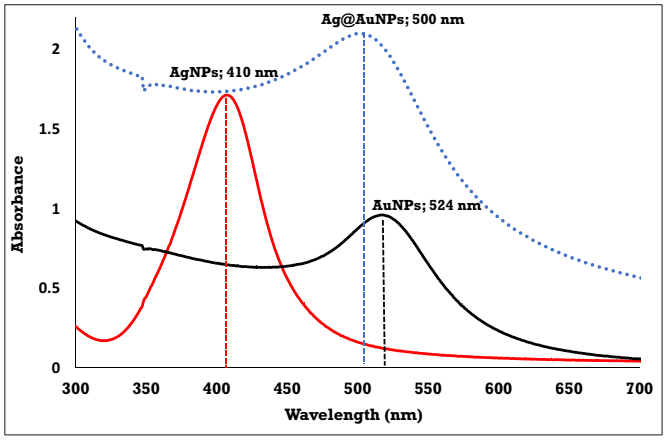

Fig. 1. UV-vis spectra of AgNPs, AuNPs and Ag@AuNPs.

The inset images in Fig. 2 (C, F, I) represent the selected area diffraction patterns (SAED) of AgNPs, AuNPs and Ag@AuNPs respectively. It is well known that SAED patterns reveal the crystalline nature of the synthesized nanoparticles. All the SAED patterns exhibit clear and bright spots in a circular ring, which originate from the various crystallites from the diffraction planes of the fcc crystal. The SAED patterns of AgNPs show the diffraction ring from inner to outer which can be indexed 111, 200, 220, 311 where most of the spherical crystalline Ag NPs are present. SAED confirmed the spherical crystalline nature of AgNPs. The SAED patterns of AuNPs shows the diffraction ring from inner to outer which can be indexed as (111), (200), (220), (311) and (222) reflections respectively of fcc gold. Regarding to SAED pattern of Ag@AuNPs, SAED displays distinct ring patterns demonstrating their crystallinity. The diffraction pattern agrees well with the standard d -spacing values of both Au^o and Ag^o.

The data obtained from TEM was confirmed with that of particle size analyzer (Fig. 3 A) determined by dynamic light scattering (DLS). the data obtained from Fig. 3 A is the average hydrodynamic size for the three obtained nanoparticles; AgNPs, AuNPs and Ag@AuNPs. It is depicted that the average particle size of AgNPs, AuNPs and Ag@AuNPs is 11 nm, 21 nm and 37 nm respectively. It is shown that the size of AgNPs is smaller than that of AuNPs which is in accordance with the data obtained from TEM images. Meanwhile, the particle size analyzer of Ag@AuNPs gives a size larger than that of AgNPs and AuNPs respectively confirming that the successful preparation of two nanoparticles in one form with two phases.

For further confirmation, zeta potential tool was carried out to outline the stability of the formed nanoparticles. All the prepared samples have zeta potential value more than −30 mv which, in turn, reflect the well stabilized nanoparticles. They exhibit zeta potential value equal to −70 mv, −58 mv and − 41 mv for AgNPs, AuNPs and Ag@AuNPs respectively. As expected, AgNPs has excellent zeta potential than AuNPs which could be attributed to the same reason that mentioned before. The low zeta potential value is for Ag@AuNPs (−41 mv). However, the formed Ag@AuNPs is assigned to be a good stabilized sample and protected from agglomeration even stored for a long time [36–38]. The negative signal could be attributed to the stabilizing compound (sodium pectinate).

Fig. 4 shows the morphological structures of blackberry (bb), bb loaded AgNPs, bb loaded AuNPs and bb loaded Ag@AuNPs. As descripted in our experiment that the formed nanoparticles are considered as a carrier for bb (natural model drug). The designed nanoparticles have very small particles (<50 nm). Thus, they have high surface area that has the ability to deliver the drug to specific organ without loss in its efficiency. Fig. 4 (A, B, C) illustrated the surface structure and elemental analysis of AgNPs, AuNPs and Ag@AuNPs respectively. As observed from these images that the scanned samples exhibit bright appearance due to the deposition of nanoparticles on the surface of sodium pectinate. it is clearly seen that the formed particles exhibit very small size affirming the high surface area. The elemental analysis of these nanoparticulate systems operated via EDX tool are outlined in Fig. 4 (D, E, F). From the obtained data, there specific elements; C, O, Na, Ag and Au for the characterized samples. It is observed that C and O are attributed to pectin compound. While Na is due to the reacted

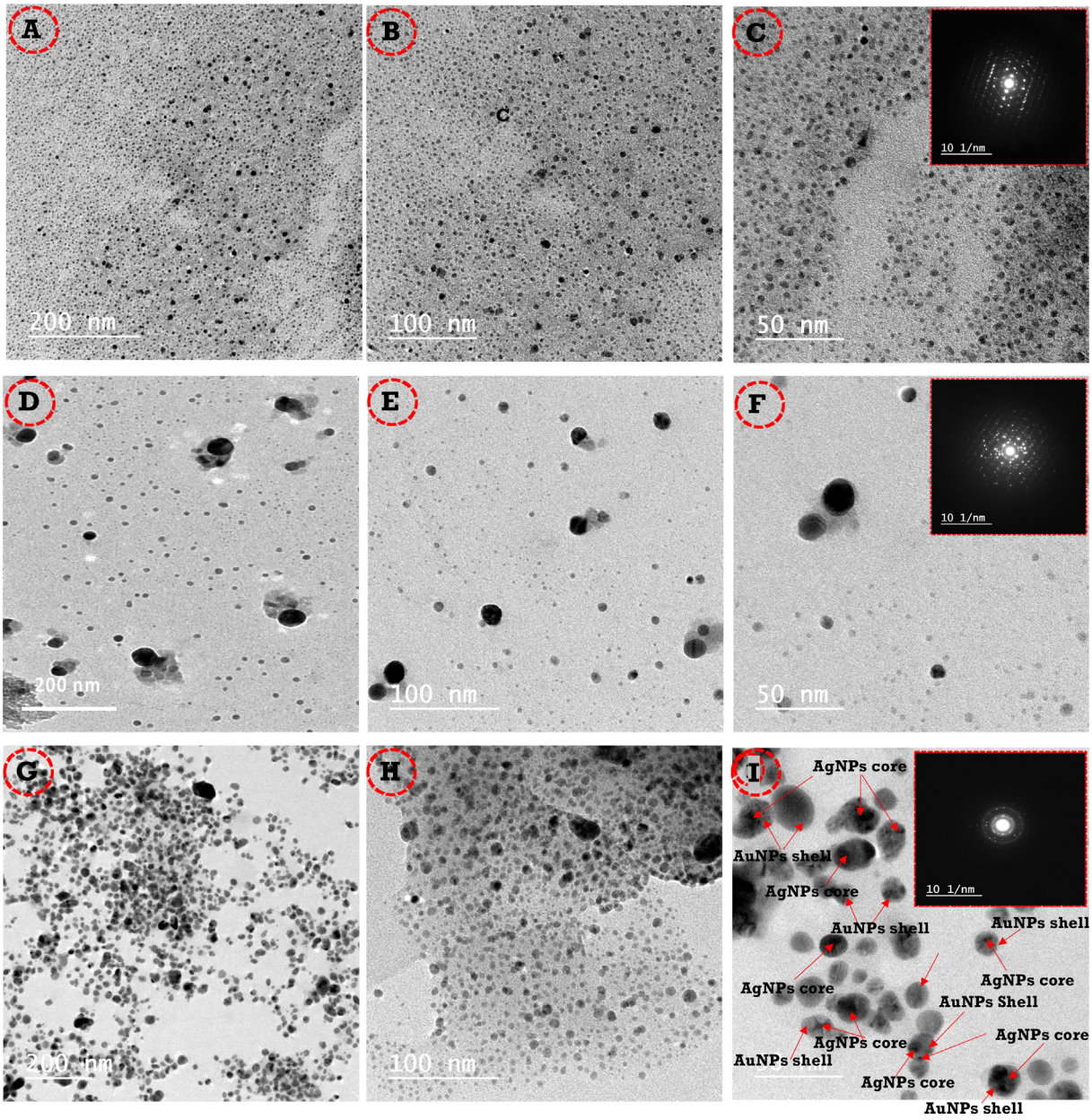

Fig. 2. 2: TEM at different magnifications for (A, B, C) AgNPs, (D, E, F) AuNPs and (G, H, I) Ag@AuNPs. The inset images in fig. 2 (C, F, I) is SAED of AgNPs, AuNPs and Ag@AuNPs respectively.

sodium hydroxide with pectin molecules prior to the nanoparticles formation. From the three EDX images, it is observed that the scanned samples are pure and impurities free.

The entrapment efficiency of bb extract loaded in the prepared formulations of AgNPs, AuNPs and Ag@AuNPs was calculated. It is seen that the entrapment efficiency of bb extract loaded AgNPs, AuNPs, Ag@AuNPs is 88.64%, 86.05% and 97.47% respectively. Moreover, the highest entrapment of bb extract is obtained with formula based on Ag@AuNPs which could be attributed to the high affinity and the high surface area of the formula that combined two active nanoparticles. While the value of entrapment efficiency of bb extract loaded AgNPs decreases to 88.64%. Whereas, the lowest entrapment efficiency is observed with AuNPs (86.05%). The low entrapment efficiency values indicate relatively low affinity of bb extract for AuNPs. The low affinity when compared with AgNPs and Ag@AuNPs due to the large particle size and thus, producing low surface area which hinder the good loading of AuNPs to bb extract.

3.2. In-vivo study in rats using AgNPs, AuNPs and Ag@AuNPs as a carrier for bb extract

Cisplatin (Cis) is a potent and extremely efficient therapy in cancer diseases such as breast cancer, solid tumor, leukemia, and small cell carcinoma. In this work, we aimed to clarify the role of blackberry extract, as a rich source of polyphenols and have antioxidant properties, in attenuating the side effects produced by injection of cispt particularly cardiotoxicity; in addition to clarify the role of nanoparticles (AgNPs, AuNPs and Ag@AuNPs) in enhancing the beneficial effect of the using extract.

In this study, the mean value of total cholesterol, triglycerides and LDL aesignificantly increases in cispt group compared to control, whereas HDL is significantly decreased compared to control (Table 1). Additionally, the calculated atherogenic indices are also increased in cispt compared to control. Whereas, these parameters are attenuated in all treated groups (Table 2). The significant elevations in AI, CRR,

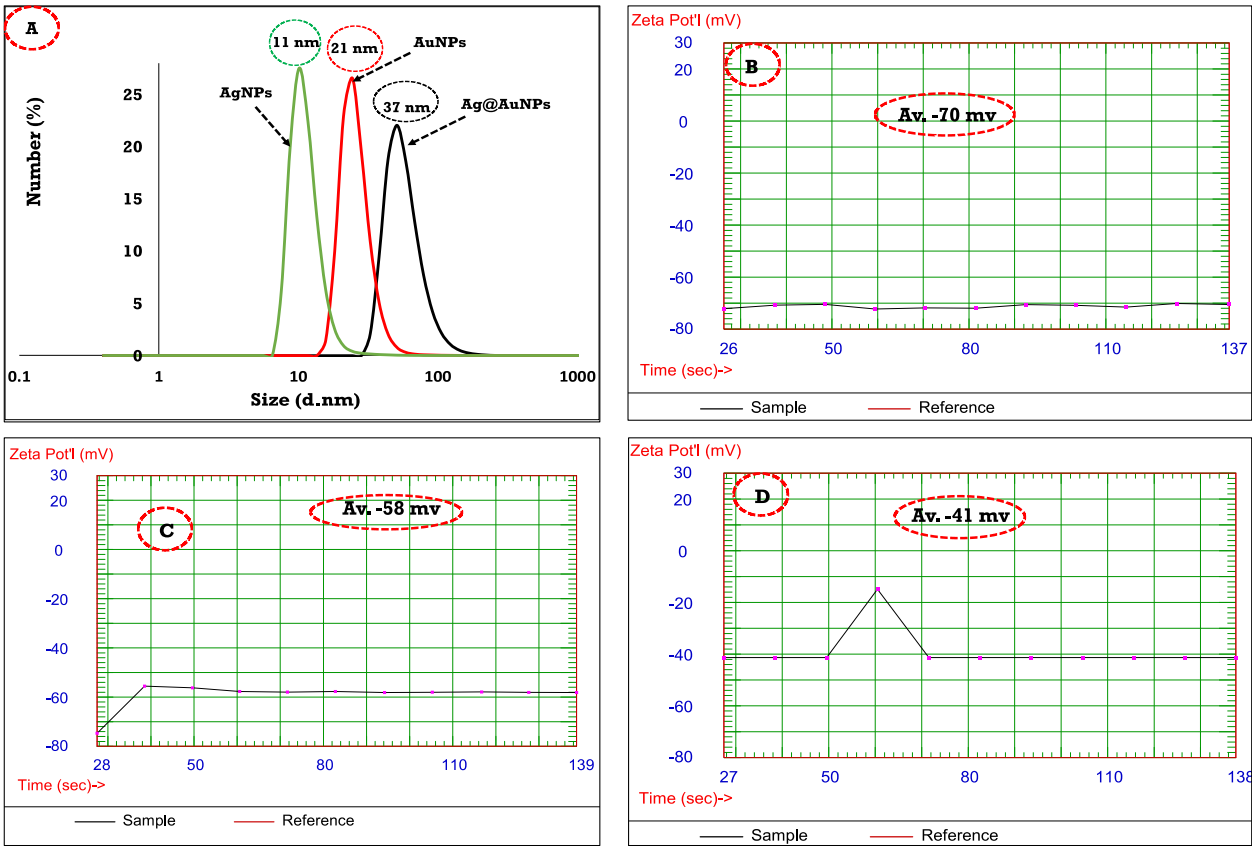

Fig. 3. (A) particles size analyzer of AgNPs, AuNPs and Ag@AuNPs, (B) zeta potential of AgNPs, (C) zeta potential of AuNPs and (D) zeta potential of Ag@AuNPs.

and AC clarify the harmful effect of cispt on the heart. This clarification is in agreement with the reported via Sharma et al., [39].

The elevation of lipid profile in Cis group may be attributed to the interference of cispt with metabolism or biosynthesis of lipids. Also, cispt may lower the cytochrome P 450 which in turn depresses the activity of cholesterol 7-hydroxylase which is responsible for the synthesis of bile acids from cholesterol [26].

Chemotherapy including cispt therapy might induce cardiotoxicity through the suppression of fatty acids oxidation that associated with elevation of total cholesterol, triglycerides, and LDL-C. The inhibition of fatty acids oxidation, results in cardiomyopathy due to the shortage of energy supply [40]. Elevation of lipid profile in cispt -treated group may be due to the inhibition of carnitine palmitoyl-transferase system (CPT I), a primary step in fatty acid oxidation [41] in addition to the decline of cholesteryl esters hydrolase and lipoprotein lipase (LPL) activities and also the increasing of the activity of cholesteryl esters synthetase [42]. Dependently, this hyperlipidemia induced by cispt, is due to the reduction of fatty acids' β-oxidation.

bb consumption attenuates hyperlipidemia in all treated groups. In agreement, Azofeifa et al., [43] indicated that consumption of bb beverage (25%) decreased both cholesterol and triacylglycerol and returned back to the control levels in experimental diabetes. However the results of the treated groups explored that, the treated group that consumed bb loaded Ag@AuNPs is the only group has no significant difference with the control group to clarify the best effectiveness through the different treated groups and appears as the best result of this formula.

In this study the mean value of oxidative stress markers (MDA and AOPP) are significantly increased in cispt group compared to control while TAC is significantly decreased. However, these parameters were attenuated in all treated groups (Table 3).

In agreement, Yilmaz et al., [44] and Mansour et al., [45] reported that, cisplatin increases malonaldehyde levels and reduces the antioxidant enzymes. Yilmaz et al., [46] suggested that cispt' treatment provokes oxidative stress attributable to the reduction of reduced glutathione (GSH).

Cispt and other chemo anti-tumors have a tetracyclic quinone-hydroquinone moiety, an aminosugar (daunosamine) and also a short side chain with a carbonyl group. The quinone moiety is converted into a semiquinone radical by a reduction of single electron. The parent quinone is then regenerated by a decline of molecular oxygen to superoxide anion and produce H_2O_2 by dismutation. Thus, the redox cycling of the quinone moiety exposes free radicals can get electrons from the cell membranes lipids resulting in lipid peroxidation that shares in cell death. MDA, the major and final end product of peroxidation, is considered an important marker of lipid peroxidation [47]. Indeed, the produced reactive oxygen species (ROS) can damage mitochondria (functional and structural), which may result in cardiomyocytes apoptosis or death [48].

Accordingly, oxidative stress is playing an important role in cardiovascular and atherosclerosis [49]. In Experimental studies, pharmacological strategies have been extensively applied to elevate the antioxidant levels of the tissues and reduce releasing of free radicals associated with different diseases [50]. Functional foods, such as garlic, onion, pomegranate berries, were studied previously as a source of exogenous antioxidants derived from diet that play important roles in managing diseases [51].

In the current study, the reduction of MDA and AOPP levels and the elevation of TAC in the blackberry treated group confirmed that the consumption of bb extract alone or loaded nanoparticles increases the antioxidant status in tissues because of their antioxidant capacities [52] and due to the high contents of phenolic compounds like ellagitannins and anthocyanins [7].

Additionally, there is a significant elevation in troponine 1 and LDH level in cispt group. As known, LDH is considered as an important

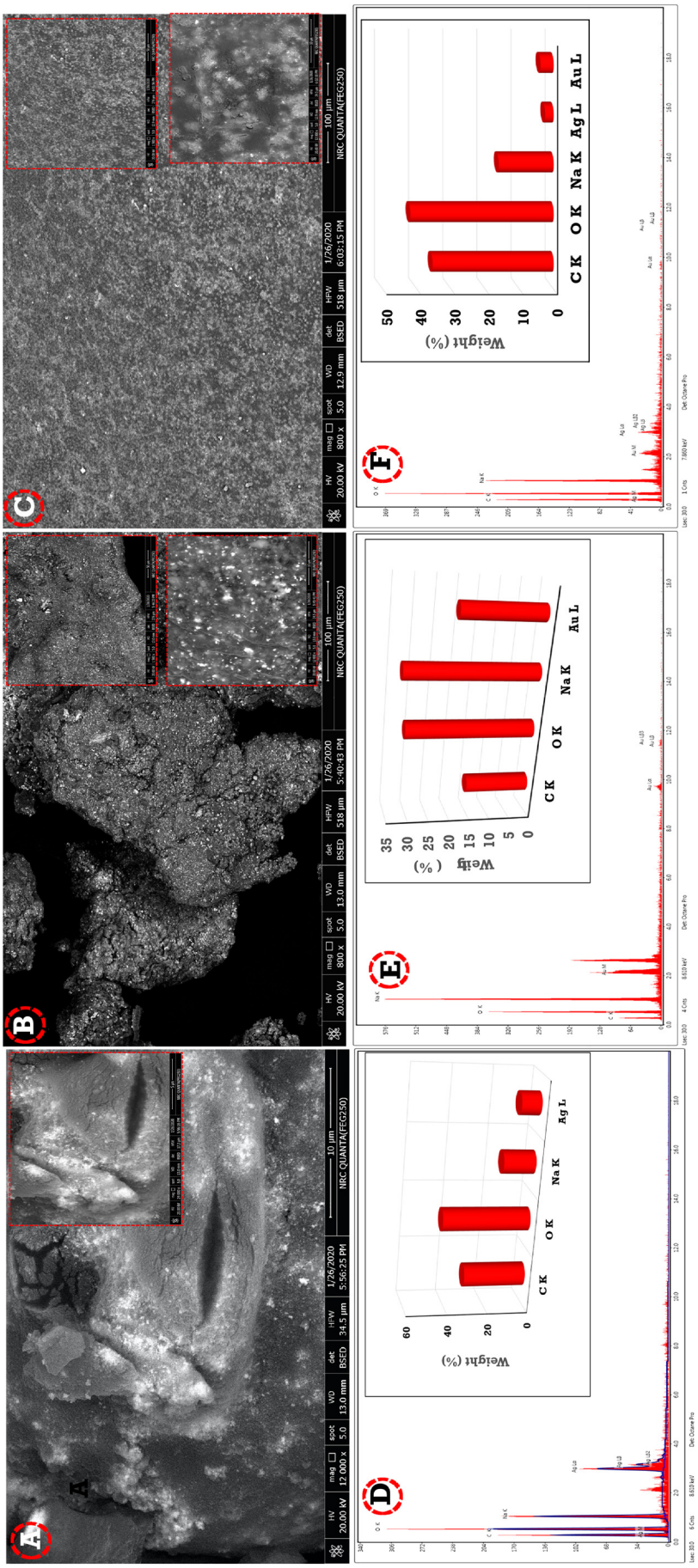

Fig. 4. SEM of (A) AgNPs, (B) AuNPs, (C) Ag@AuNPs, EDX of (D) AgNPs, (E) AuNPs and (F) Ag@AuNPs.

Table 1
Lipid profile in different studied groups.

Groups	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Control	82.02 ± 1.24	72.22 ± 1.42	36.30 ± 0.81	31.60 ± 0.71
Cispt	113.00 ± 1.33 [a]	98.17 ± 1.84 [a]	22.32 ± 0.45 [a]	71.04 ± 0.24 [a]
Cispt + bb	104.92 ± 2.03 [a,b]	93.45 ± 1.22 [a,b]	30.20 ± 0.72 [a]	56.03 ± 0.21 [a,b]
Cispt + bb /AgNPs	99.27 ± 1.53 [a,b]	90.11 ± 2.13 [a,b]	29.76 ± 0.32 [a,b]	51.48 ± 0.41 [a,b]
Cispt + bb/AuNPs	95.10 ± 1.11 [a,b,c]	83.76 ± 1.90 [a,b,c]	31.20 ± 0.12 [a,b]	47.14 ± 0.32 [a,b,c]
Cispt + bb/ Ag@AuNPs	84.85 ± 2.10 [b,c]	73.43 ± 1.53 [b,c]	35.12 ± 0.47 [b,c]	35.04 ± 0.67 [b,c]
Cispt + Ag@AuNPs	91.7 ± 1.78 [a,b,c]	79.70 ± 1.65 [a,b,c]	32.44 ± 0.29 [b]	43.32 ± 0.47 [a,b,c]

[a] Significant difference at P ≤ 0.05 as compared to control group.
[b] Significant difference at P ≤ 0.05 as compared to cispt group.
[c] Significant difference at P ≤ 0.05 as compared to bb treated group.

Table 2
Atherogenic indices in different studied groups.

Groups	Atherogenic index (AI)	Cardiac risk ratio (CRR)	Atherogenic coefficient (AC)
Control	0.298 ± 0.65	2.259 ± 0.96	0.870 ± 1.01
Cispt	0.643 ± 0.43[a]	4.61 ± 0.84 [a]	3.182 ± 0.92 [a]
Cispt + bb	0.490 ± 0.86[a,b]	3.47 ± 0.78 [a,b]	1.855 ± 0.89 [a,b]
Cispt + bb/AgNPs	0.481 ± 0.42[a,b]	3.33 ± 0.89 [a,b]	1.729 ± 0.78 [a,b]
Cispt + bb/AuNPs	0.428 ± 0.57[a,b,c]	3.04 ± 0.68 [b]	1.510 ± 1.02 [a,b]
Cispt + bb /Ag@AuNPs	0.320 ± 0.61[b,c]	2.41 ± 0.74 [b,c]	0.997 ± 0.75[b,c]
Cispt + Ag@AuNPs	0.449 ± 0.85[abc]	3.12 ± 0.77 [bc]	1.435 ± 0.85 [a,b]

[a] Significant difference at P ≤ 0.05 as compared to control group.
[b] Significant difference at P ≤ 0.05 as compared to cispt group.
[c] Significant difference at P ≤ 0.05 as compared to bb treated group.

marker of myocardial injuries [53]. In agreement, El-Awady et al., [54] indicated that cispt stimulates myocardial injury and increases LDH. Thus, the elevation of lipid peroxidation level induced by injection with cispt leads to the hydroperoxides' formation in the cardiomyocyte cell membrane which in turn leads to an injury of the membrane structure, resulting in an impairment of its functions including permeability and releasing different proteins including troponine 1 and LDH [55]. In treatment groups (bb loaded Ag@AuNPs), the mean value levels of LDH and troponine 1 are returned back to become more or less near the control group (Table 4).

From all current results we can notice that bb extract has a beneficial effect as antioxidant and antilipidemic beside its role in protecting against cardiovascular diseases. However, the role of nanoparticles in this study as an enhancer for the bb' properties is very clear due to the improvement of its absorbability as well as bioavailability toward the targeted organ in the body.

Thus, the results displays a significant change between different treated groups and bb treated groups in particular, the treated group that consumed bb loaded AuNPs. Moreover, the treated group that received bb/Ag@AuNPs give the best results between all treated groups that indicated the effectiveness of the using nanoparticles as a carrier for bb extract. Whereas the treated group with Ag/Au- NPs give an important result to clarify the role of nanoparticles alone in this study.

4. Conclusion

On the basis of the results achieved, it is possible to infer that as an excellent method for the preparation of various nanoparticles such as silver nanoparticles (AgNPs), gold nanoparticles (AuNPs) and core shell of silver -gold nanoparticles (Ag@AuNPs) is the solid state method. For such preparation, pectin was successfully used to reduce and stabilize the above-mentioned nanoparticles. The data obtained revealed the effective preparation of nanoparticles based on AgNPs, AuNPs and Ag@AuNPs with spherical size and small size of 11 nm, 21 nm and 37 nm respectively. Additionally, AgNPs and AuNPs have been produced in one phase while Ag@AuNPs have two phases confirming the development of core shell. Moreover, for AgNPs, AuNPs and Ag@AuNPs, the stability of these nanoparticles is well and equivalent to −70 mv, −58 mv and − 41 mv respectively. In doing so, the obtained nanoparticles were effectively used as a carrier for blackberry extract (bb) in order to enhance its benefits in the treatment of cisplatin-induced cardiotoxicity. The obtained in vivo results, revealed the positive role that bb have on the experimental cardiovascular disease. This can help to reduce the oxidative stress and elevation of TAC associated with an improvement in lipid homeostasis, due to the anti-hyperlipidaemic features that suggests a potential adjuvant for the treatment of cardiovascular disease through the combining of anthocyanins and ellagitannins. Furthermore, it can be inferred that the beset results for all the treated group of the experimental rats were obtained via utilizing bb loaded Ag@AuNPs followed by bb/AuNPs. Ultimately, it is possible to conclude that, using nanotechnology in terms of nanoparticles, several drug molecules/natural expedient extract can be loaded to these nanoparticles, which, in turn, contribute to improve their absorbability as well as bioavailability toward the targeted organ in the body.

Table 3
Oxidants and antioxidants parameters in different studied groups.

Groups	Serum AOPP (ng/ml)	Cardiac MDA (nmol/g tissue)	Serum TAC (Mm/L)
Control	10.22 ± 1.11	28.23 ± 2.30	2.12 ± 0.17
Cispt	18.1 ± 1.02[a]	41.35 ± 2.81 [a]	0.92 ± 0.12 [a]
Cispt + bb	17.21 ± 2.1[a]	37.23 ± 1.67 [a]	1.20 ± 0.38 [a]
Cispt + bb/AgNPs	15.31 ± 0.95 [a,b]	35.21 ± 2.14[a,b]	1.32 ± 0.24 [a]
Cispt + bb/AuNPs	14.56 ± 0.86 [a,b]	34.23 ± 1.76 [a,b]	1.45 ± 0.41[a,b]
Cispt + bb/Ag@AuNPs	11.53 ± 1.43[b,c]	30.21 ± 2.25[b,c]	1.97 ± 0.34 [b,c]
Cispt + Ag@AuNPs	13.37 ± 1.62 [a,b]	34.24 ± 2.41 [a,b]	1.76 ± 0.52[a,b]

[a] Significant difference at P ≤ 0.05 as compared to control group.
[b] Significant difference at P ≤ 0.05 as compared to cispt group.
[c] Significant difference at P ≤ 0.05 as compared to bb treated group.

Table 4
LDH and troponin-I levels in different studied groups.

Groups	Serum LDH (U/l)	Serum troponin-I (pg/ml)
Control	997 ± 11.2	28.2 ± 0.92
Cispt	1442 ± 10.3. [a]	132.0 ± 0.83[a]
Cispt + bb	1210 ± 10.3 [a,b]	117.7 ± 0.7[a,b]
Cispt + bb/AgNPs	1138 ± 9.65 [a,b]	88.2 ± 0.62[a,b,c]
Cispt + bb/AuNPs	1094 ± 9.71 [a,b]	67.4 ± 1.01[a,b,c]
Cispt + bb/Ag@AuNPs	1004 ± 11.21[b,c]	33.6 ± 0.94[b,c]
Cispt + Ag@AuNPs	1099 ± 10.34 [a,b,c]	64.8 ± 1.13[a,b,c]

[a] Significant difference at P ≤ 0.05 as compared to control group.
[b] Significant difference at P ≤ 0.05 as compared to cispt group.
[c] Significant difference at P ≤ 0.05 as compared to bb treated group.

Author statement

The corresponding author is responsible for ensuring that the descriptions are accurate and agreed by all authors.

√ Jihan Hussein, Mehrez E. El-Naggar, Moustafa M. G. Fouda: Conceptualization, Methodology.
√ Osama M. Morsy, Ahmed A Allam and Enas Mahmoud Mekawi: Investigation; Validation; Visualization
√ Jamaan S. Ajarem, Ahmed M. Almalki: Funding acquisition; formal analysis
√ Jihan Hussein, Mehrez E. El-Naggar: Supervision
Jihan Hussein, Mehrez E. El-Naggar, Moustafa M. G. Fouda, Enas Mahmoud Mekawi and Jamaan S. Ajarem: Writing- Reviewing and Editing.

Compliance with ethical standards

All procedures by this study were in accordance with international ethical standards. The research involved no human participants.

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