Potassium bromate-induced nephrotoxicity and potential curative role of metformin loaded on gold nanoparticles

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Abstract

Potassium bromate (KBrO₃) is classified by the International Agency for Research on Cancer as a carcinogenic compound, where it causes renal tumors. The present study investigated the potential curative effect of metformin loaded on gold nanoparticles (MET AuNPs) in attenuating KBrO₃-induced nephrotoxicity. Rats were divided into eight groups (control, MET, AuNPs, MET AuNPs, KBrO₃, KBrO₃/MET, KBrO₃/AuNPs, and KBrO₃/MET AuNPs). KBrO₃ administration resulted in a significant elevation in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), albumin (Alb), total bilirubin (TB), direct bilirubin (DB), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), creatinine, urea, uric acid. Also, KBrO₃ significantly increased renal malondialdehyde (MDA), protein carbonyl (PC), and nitric oxide (NO) levels and reduced the activities of antioxidant molecules superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), and Reduced glutathione (GSH). It also caused damaged DNA spots in comet assay and increased inflammatory IL-6 and apoptotic markers (caspase 3, Bax) while antiapoptotic Bcl-2 was significantly reduced. MET, AuNPs, MET AuNPs reduced the extent of renal damage induced by KBrO₃ as indicated by decreased (AST, ALT, ALP, Alb, TP, TB, DB, creatinine, urea, uric, Lipid profile). MET, AuNPs, MET AuNPs showed a good curative effect against KBrO₃-induced nephrotoxicity and MET AuNPS group showed better results compared with monotherapy.

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Metformin (MET), metformin loaded on gold nanoparticles (MET AuNPs), potassium bromate (KBrO₃), nephrotoxicity

Introduction

KBrO₃ is used as food additive in cheese production, beer making, and is commonly added to fish paste products. It is also important in pharmaceutical and cosmetic industries and is a constituent of cold wave hair solutions. In addition, KBrO₃ is found as a byproduct of ozone disinfection in drinking water samples.¹

KBrO₃ has been classified by the International Agency for Research on Cancer as a carcinogenic compound, where it causes renal tumors, mesotheliomas of the peritoneum, and follicular cell tumors of the thyroid in rats. Administration of KBrO₃ to rats was found to induce oxidative stress and reduced the antioxidant capacity of rat’s deference mechanism system.² KBrO₃ is highly injurious to tissues especially the central nervous system tissue. The nephrotoxicity caused by KBrO₃ has been attributed to its ability to trigger the production of reactive oxygen species (ROS), lipid peroxidation (LPO), also, it caused primary DNA oxidative damage and increased 8-hydroxydeoxyguanosine (8-OHDG) DNA adduct levels, a representative marker of oxidative DNA modification, in vivo and in vitro. The oxidative stress induced by KBrO₃ far exceeds the cellular antioxidative defense capacity leading to marked nephrotoxicity in humans and animals as well as the carcinogenicity in experimental animals.³

When ingested, KBrO₃ is rapidly absorbed, reduced to bromide in the tissues and then partly excreted in the urine as a bromide ion. Since the kidney is the primary target organ of KBrO₃, the toxic effects of this compound in humans arise from acute poisoning causing renal failure. Yet, the exact mechanism(s), particularly the interplay among several endogenous concurrent contributing factors behind KBrO₃ acute renal toxicity and the ways to overcome its toxic effects by natural products remain hardly known.⁴

Metformin (MET) (N,N-dimethyl biguanide) is one of the oral drugs used for more than 40 years to treat patients with type 2 diabetes mellitus (T2DM) without causing over hypoglycemia. It has recently been recommended that metformin therapy be initiated at the time of diagnosis of T2DM, in conjunction with lifestyle modification.⁵

Various studies had emphasized the anti-inflammatory and antioxidant role of MET through multiple mechanisms. So, MET can be an appropriate treatment option for many diseases, when inflammatory processes and oxidative stress play a role in their pathogenesis.⁶

Metformin was found to protect against tubular injury by restoring the biochemical alterations. Moreover, MET protected podocytes in diabetic nephropathy in rats.⁷

Recent studies have suggested that MET may have therapeutic or renoprotective effects against nephrotoxic agents. It has also been shown to have a good
efficacy in diabetic nephropathy. Furthermore, it significantly decreased albuminuria in patients with DM.8

Gold nanoparticles (AuNPs) have become an important biomedical tool for scientists in research because of the several advantages of AuNPs like high loading efficiency for the target substance, enhanced ability to cross various physiological barriers, and low systemic side effects. Hyaluronic acid (HA) was used as both a reducing and a stabilizing agent in the formation of AuNPs in sizes ranging from 5 to 30 nm by thermal treatment. HA have been widely used as novel drug carriers for target-specificity and long-acting delivery, they have been employed both as delivery vehicles and angiostatic agents in cancer therapy.9 AuNPs obtained from extracellular synthesis from C. sorokiniana extract are 5–15 nm in size and spherical shape. TEM images of extracellular synthesis show noticeable cell wall and membrane damages, cytoplasm dissolutions, and irregularities. AuNPs obtained by intracellular synthesis are in 20–40 nm size and localized in the cell wall and cytoplasm.10

Drug nanoencapsulation is emerging as an effective alternative to reduce the side effects of drug administration while accomplishing controlled release and site-specific delivery.11

Based on the previous data, the present study aimed to evaluate the possible therapeutic effect of metformin loaded on gold nanoparticles against KBrO3-induced nephrotoxicity in rats.

Materials and methods

Experimental animals

Forty-eight healthy male Sprague Dawley (SD) rats 6–7 weeks old, with an average weight 95 g were used for the experiment. Rats were obtained from the Egyptian Institute for Serological and Vaccine production, Helwan, Egypt and were housed in the animal house of the Department of Zoology, Faculty of Science, Kafrelsheikh University. Rats were placed in stainless steel cages containing wood-chip bedding, renewed every day. They were kept in a temperature-controlled environment with a 12 h light/dark cycle. All rats were acclimatized for 1 week before the commencement of the experiments. All rats were fed a normal diet and water was allowed ad libitum during the study. The experimental protocol was carried out in accordance with the guide of the National Research Council for the Care and Use of Laboratory Animals and was approved by the ethics committee of the Department of Zoology, Faculty of Science, Kafrelsheikh University.

Animal grouping

After 1 week of acclimatization period, animals were divided randomly into eight groups, each consisting of six animals as follows:

**Control group:** Rats of this group did not receive any treatment.

**Metformin (MET) treated group:** Rats of this group were administered MET 200 mg/kg bw by intragastroluminal gavage (i.g.) twice a week for 4 weeks.12
Gold nanoparticles (AuNPs) treated group: Rats of this group were administered AuNPs 5 μg Au/Animal by i.g. twice a week for 4 weeks.\(^{13}\)

Metformin loaded on gold nanoparticles (MET AuNPs) treated group: Rats of this group were administered MET AuNPs 200mg/kg bw by i.g. twice a week for 4 weeks.\(^{14}\)

Potassium bromate (KBrO\(_3\)) treated group: Rats of this group were administered KBrO\(_3\) 100 mg/kg bw by i.g. twice a week for 4 weeks.\(^{15}\)

Potassium bromate and Metformin (KBrO\(_3\) + MET) treated group: Rats were administered KBrO\(_3\) 100 mg/kg bw by i.g. twice weekly for 4 weeks alone, then rats were administered MET 200 mg/kg bw twice a week for another 4 weeks after termination of KBrO\(_3\) administration.

Potassium bromate and Gold nanoparticles (KBrO\(_3\) + AuNPs) treated group: Rats were administered KBrO\(_3\) 100 mg/kg bw by i.g. twice a week for 4 weeks alone, then rats were administered AuNPs 5 μg Au/Animal twice a week for another 4 weeks after termination of KBrO\(_3\) administration.

Potassium bromate and Metformin loaded on gold nanoparticles (KBrO\(_3\) + MET AuNPs) treated group: Rats were administered KBrO\(_3\) 100 mg/kg bw by i.g. twice a week for 4 weeks, then rats were administered MET AuNPs 200 mg/kg bw twice a week for another 4 weeks after termination of KBrO\(_3\) administration.

**Chemicals**

Potassium bromate (KBrO\(_3\)) is an odorless white crystalline powder. It was obtained in powder form from El-Gomhouria Chemicals Company (Cairo, Egypt). KBrO\(_3\) was dissolved in distilled water.

Metformin (MET) was obtained from Chemical Industries Development (CID) (Giza, Egypt). MET was dissolved in distilled water.

Gold nanoparticles (AuNPs) are suspension form with spheroidal to rod shape. It was obtained from Nano Gate Company (25 Ibrahim Abo Elnaga-street, Abbas ElAkkad, Nasr City, Cairo, Egypt).

Citrate-capped gold nanoparticles (GNPc) were synthesized by slightly modifying the method used in previous experiments.\(^{16,17}\)

Citrate-capped gold nanoparticles (GNPc) were synthesized following the Turkevich–Frens method and by slightly modifying the method used in our previous experiments. Briefly, 100 ml of hydrogen tetrachloroaurate (III) trihydrate HAuCl\(_4\)-3H\(_2\)O (10\(^{-3}\) M) was heated to a boiling temperature and then mixed to trisodium citrate (10 ml, 38.8 × 10\(^{-3}\) M) (Merck) under vigorous stirring. After the formation of a deep-red burgundy colloid, the stirring and boiling processes were continued for 10–15 min. Subsequently, the heating was stopped and the stirring process was continued for another 15 min. Then, 3 ml of 10\(^{-3}\) M HAuCl\(_4\) were mixed with 18 ml of 2 mg/ml chitosan solution (medium molecular weight), and then heated to 50°C. The colloidal solutions were centrifuged and suspended in ultrapure water. Loading of Met (Sigma Aldrich) onto the surface of GNPc was
performed by incubating the colloidal GNPc with Met at pH 3.5. The pH of the mixture was then adjusted to 8 with 1 M NaOH. The obtained Met gold Nano conjugates 232. Met delivery using chitosan-capped gold nanoparticles were subsequently washed to remove the free drug.

**Structural characterization of NPs, gold concentration, and size**

The absorption maxima were scanned at the wavelength of 200:800 nm. On the other hand, the TEM has shown a majority of nearly spherical AgNPs with diameters ranging from 15.8 to 19.6 nm. It was done according to Aldea et al.17

**Methods**

**Blood sampling and kidney tissue preparation**

At the end of the experimental period (8 weeks) rats were fasted overnight, sacrificed 24 h after the last treatment and blood samples were collected in clean centrifuge glass tubes, left to clot in a water bath at 35°C for 30 min then centrifuged at 3000 rpm for 15 min. The clear non-hemolyzed supernatant was quickly collected. In labeled Eppendorf’s tubes, the sera were divided into aliquots and frozen at −20°C for different biochemical analyses. Kidney samples were homogenized (10% w/v) in ice-cold saline. The homogenate was kept at −20°C in labeled Eppendorf’s tubes till used for biochemical estimations. Other samples of kidney tissue were stored in neutral buffered formalin (10%) for histopathological studies.

**Biochemical assays**

Total protein (TP) was estimated using the Biuret method of Doumas et al.18 Albumin (Alb) was evaluated according to the method of Doumas et al.19 Total bilirubin (TB) and direct bilirubin (DB) was evaluated according to Abd Elhalem et al.20 The levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were accomplished using the method of Schumann and Klauke.21 Alkaline phosphatase (ALP) level was measured according to the method of Belfield and Goldberg.22 Serum urea (Ur) level was determined by Mohamed and Ashour23 and creatinine (Cr) concentration was calculated according to Solt.24 Moreover, serum total cholesterol (TC) was determined as described by Iwata et al.25 Triglycerides (TG) were determined by the method of Rojkin et al.26

**Estimation of antioxidant enzymes**

Superoxide dismutase (SOD) activity was determined by measuring the inhibition of autoxidation of epinephrine at pH 10.2 and 30°C according to Misra and Fridovich.27 Catalase (CAT) activity was measured using hydrogen peroxide as the substrate according to the method previously described by Manubolu et al.28 Reduced glutathione (GSH) was determined according to the method of Jollow
et al.\textsuperscript{29} The determination of glutathione-S-transferase (GST) activity was assayed according to the method of Habig et al.\textsuperscript{30}

\textit{Estimation of oxidative stress markers}

MDA level was assayed according to the method of Doğru-Abbasoğlu et al.\textsuperscript{31} The levels of nitric oxide (NO) production were measured according to the method of Yousef and Hussien.\textsuperscript{32} The levels of protein carbonyl according to the method of Levine.\textsuperscript{32}

\textit{Estimation of apoptotic markers}

Kidney homogenates were made in lysis buffer and analyzed using a colorimetric caspase-3 assay kit (Product number: CASP3C; Sigma-Aldrich Co. USA) according to the manufacturer’s instructions. The concentrations of caspase-3 in renal lysates were calculated with the help of the calibration curve generated using known amounts of standards. Bcl-2 (Cat. No. LS-F10920) and Bax (Cat. No. LS-F5064) levels were measured in the renal tissue lysates by ELISA kits (LifeSpan BioSciences, Inc., Seattle, WA, USA). The procedure was performed according to the instructions of the manufacturer. Levels were expressed as ng/mg tissue protein according to Hamed et al.\textsuperscript{33}

\textit{Estimation of inflammatory markers}

Frozen organs were sectioned at 10-\textmu m intervals by microtome and total protein was extracted as recommended by R&D Systems (Minneapolis, MN). Total protein in each extract was measured by Bradford assay (Sigma-Aldrich, St. Louis, MO). ELISA for IL-6 was performed using 50 \textmu g of total protein as described by the manufacturer (R&D Systems) and the amount of IL-6 in each extract is expressed as nanograms of IL-6 per milligram of protein. It was assayed according to the method of Ono et al.\textsuperscript{34}

\textit{DNA fragmentation by comet assay}

The alkaline comet assay was performed as described by Singh et al.\textsuperscript{35} For each sample, 100 isolated comets (single-strand breaks of DNA migrate from nucleus to anode) were randomly selected and measured for comet tail length, % DNA in tail, and tail moment according to the definition.

\begin{equation*}
\text{Tail moment} = \text{Tail length} \times \%\text{DNA in tail} / 100
\end{equation*}

\textit{Histopathological examination}

Kidney specimens were dehydrated in ascending grades of ethyl alcohol (70\%, 90\%, and 100\%), cleared in xylene and impregnated and embedded in paraffin
wax. Serial sections of 4–5 \( \mu \text{m} \) thick were obtained using a rotary microtome and stained with Harris’s\(^{36} \) Hematoxylin and Eosin stain for general histological examination.

**Ultra-structural preparations**

Specimens from the kidney of all animal groups were cut into small pieces, each piece measured about 1 mm\(^3 \), then fixed immediately at 4°C for 18–24 h in freshly prepared 3\% glutaraldehyde-formaldehyde. The specimens were washed in phosphate buffer (pH 7.4), and postfixed for 1 h at 4°C in isotonic 1\% osmium tetroxide.\(^{37} \) Prepared sections for electron microscopic examination followed the method of Bozzola and Russell.\(^{38} \) Semithin sections were stained with toluidine blue for detection of the area of interest, then ultrathin sections were prepared by using the ultramicrotome glass knives. Sections were stained by uranyl acetate and lead citrate, then examined by a JEOL CX 100 transmission electron microscope.

**Statistical analysis**

Results were expressed as mean ± standard error of the mean (SEM) \((n = 6)\). Statistical significance was calculated using one-way analysis of variance (ANOVA) followed by Waller and Duncan’s\(^{39} \) multiple range test. All the statistical analyses were carried out with the use of Graphpad prism 5 software (Graphpad prism software Inc., San Diego, California, USA). Differences were considered significant at \( p \leq 0.05 \).

**Results**

**Effect of different treatments on lipid profile levels**

As shown in Figure 1, administration of KBrO\(_3\) resulted in significant increases in serum levels of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) as compared to normal control group. On the other hand, KBrO\(_3\) administration resulted in a significant reduction in serum level of high-density lipoprotein cholesterol (HDL-C) as compared to the normal control group. Treatment with either MET, AuNPs or MET/AuNPs significantly reduced serum levels of TC, TG, and LDL-C and caused a significant increase in HDL-C as compared to KBrO\(_3\) group.

**Effect of different treatments on liver function tests**

As shown in Figure 2, administration of KBrO\(_3\) resulted in significant increases in serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TB), and direct bilirubin (DB) as compared to the normal control group. On the other hand, KBrO\(_3\) administration resulted in a significant reduction in serum levels of total protein (TP) and albumin (Alb) as compared to the normal control group. Treatment with either MET,
AuNPs or MET/AuNPs significantly reduced serum levels of ALT, AST, ALP, TB, DB, and caused a significant increase in serum levels of TP, Alb as compared to KBrO₃ group.

**Effect of different treatments on kidney function tests**

As shown in Table 1, administration of KBrO₃ resulted in significant increases in serum levels of creatinine (Cr), urea (Ur), uric acid (UA) as compared to the normal control group. Treatment with either MET, AuNPs or MET/AuNPs significantly reduced serum levels of Cr, Ur, and UA as compared to KBrO₃ group.
Table 1. Kidney function tests of control and different treated rat groups.

| Parameter | C  | MET | AuNPs | MET AuNPs | KBrO₃ | KBrO₃ + MET | KBrO₃ + AuNPs | KBrO₃ + MET/AuNPs |
|-----------|----|-----|-------|-----------|-------|------------|--------------|------------------|
| Cr (mg/dl)| 0.71 ± 0.06 | 0.66 ± 0.03 | 0.67 ± 0.04 | 0.56 ± 0.03 | 1.46 ± 0.07<sup>a</sup> | 0.78 ± 0.01<sup>b</sup> | 0.95 ± 0.03<sup>a,b</sup> | 0.72 ± 0.04<sup>b</sup> |
| Ur (mg/dl)| 21.1 ± 2.63 | 19 ± 1.95 | 21 ± 1.73 | 17 ± 2.08 | 44 ± 1.54<sup>a</sup> | 35 ± 1.73<sup>a</sup> | 36 ± 3.46<sup>a</sup> | 27.3 ± 1.45<sup>b</sup> |
| UA (mg/dl)| 3.2 ± 0.28 | 3.2 ± 0.28 | 3.3 ± 0.33 | 2.9 ± 0.06 | 8.8 ± 0.26<sup>a</sup> | 7.8 ± 0.23<sup>a</sup> | 7.8 ± 0.11<sup>a</sup> | 5.4 ± 0.48<sup>a,b</sup> |

AuNPs: gold nanoparticles; C: control; KBrO₃: potassium bromate; MET: metformin; MET AuNPs: metformin loaded on gold nanoparticles.

Results are presented as means ± SE for six rats in each group.

<sup>a,b</sup>Significant as compared to control and KBrO₃ groups, respectively at p ≤ 0.05.
Effect of different treatments on oxidative stress markers in renal tissues

As shown in Table 2, administration of KBrO3 resulted in significant increases in renal tissue contents of malondialdehyde (MDA), protein carbonyl (PC), nitric oxide (NO) as compared to the normal control group. On the other hand, KBrO3 administration resulted in a significant reduction in superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), and reduced glutathione (GSH) as compared to the normal control group. Treatment with either MET, AuNPs or MET/AuNPs significantly reduced tissue contents of MDA, PC, NO, and caused a significant increase in tissue contents of SOD, CAT, GST, and GSH as compared to KBrO3 group.

Effect of different treatments on apoptotic markers in renal tissues

As shown in Figure 3, administration of KBrO3 resulted in significant increases in renal tissue contents of cysteine-aspartic acid protease (Caspase 3) and B-cell lymphoma-2 associated X (Bax) as compared to the normal control group. On the other hand, KBrO3 administration resulted in a significant reduction in B-cell lymphoma-2 (Bcl-2) as compared to the normal control group. Treatment with either MET, AuNPs or MET/AuNPs significantly reduced tissue contents of Caspase 3, Bax, and caused a significant increase in tissue contents of Bcl-2 as compared to KBrO3 group.

Effect of different treatments on inflammatory marker in renal tissues

As shown in Table 3, administration of KBrO3 resulted in significant increases in renal tissue contents of interleukin-6 (IL-6) as compared to the normal control group. Treatment with either MET, AuNPs or MET/AuNPs significantly reduced tissue contents of IL-6 as compared to KBrO3 group.
Table 2. Oxidative stress markers in renal tissue of control and different treated rat groups.

| Animal groups | Parameter          | C    | MET   | AuNPs | MET AuNPs | KBrO3  | KBrO3 + MET | KBrO3 + AuNPs | KBrO3 + MET/AuNPs |
|---------------|--------------------|------|-------|-------|-----------|--------|-------------|---------------|-------------------|
|               | MDA (nmol/g)       | 325.7 ± 4.86 | 310.5 ± 5.14 | 314.9 ± 3.98 | 277.4 ± 12.96 | 1878 ± 57.5<sup>a</sup> | 637 ± 37.54<sup>a,b</sup> | 962.6 ± 53.29<sup>a,b</sup> | 467.7 ± 61.45<sup>a,b</sup> |
|               | PC (μmol DNPH/mg)  | 0.8 ± 0.05 | 0.8 ± 0.04 | 0.8 ± 0.06 | 0.7 ± 0.05 | 2.8 ± 0.16<sup>a</sup> | 1.9 ± 0.06<sup>a,b</sup> | 1.9 ± 0.19<sup>a,b</sup> | 1.01 ± 0.12<sup>a,b</sup> |
|               | NO (mg/g)          | 77.1 ± 2.65 | 74.2 ± 4.63 | 76.6 ± 4.87 | 65.6 ± 3.00 | 373.4 ± 16.84<sup>a</sup> | 245.3 ± 12.41<sup>a,b</sup> | 307.9 ± 13.92<sup>a,b</sup> | 100.9 ± 8.09<sup>a,b</sup> |
|               | SOD (U/g)          | 284.3 ± 4.1 | 300.1 ± 6.72 | 293.7 ± 4.49 | 341 ± 10.02 | 64.67 ± 3.53<sup>a</sup> | 156.3 ± 17.46<sup>a,b</sup> | 90.73 ± 5.052<sup>a</sup> | 232.1 ± 15.09<sup>a,b</sup> |
|               | CAT (U/g)          | 193.8 ± 6.15 | 205 ± 7.83 | 200.4 ± 6.05 | 246 ± 12.17 | 148.8 ± 6.28<sup>a</sup> | 181.2 ± 5.23 | 168.1 ± 8.60 | 196.3 ± 3.18<sup>b</sup> |
|               | GST (U/g)          | 4.8 ± 0.05 | 4.9 ± 0.11 | 4.927 ± 0.09 | 5.2 ± 0.30 | 2.8 ± 0.11<sup>a</sup> | 3.9 ± 0.07<sup>a</sup> | 3 ± 0.09<sup>a,b</sup> | 4.5 ± 0.28<sup>b</sup> |
|               | GSH (mg/g)         | 241.3 ± 10.56 | 267 ± 7.572 | 245 ± 14 | 308.7 ± 9.80 | 73.9 ± 2.81<sup>a</sup> | 118 ± 8.19<sup>a</sup> | 93.5 ± 3.43<sup>a</sup> | 159.7 ± 15.98<sup>a,b</sup> |

AuNPs: gold nanoparticles; C: control; KBrO3: potassium bromate; MET: metformin; MET AuNPs: metformin loaded on gold nanoparticles.

Results are presented as means ± SE for six rats in each group.

<sup>a,b</sup>Significant as compared to control and KBrO3 groups, respectively at p ≤ 0.05.

Table 3. Inflammatory marker in renal tissue of control and different treated rat groups.

| Animal groups | Parameter | C    | MET   | AuNPs | MET AuNPs | KBrO3  | KBrO3 + MET | KBrO3 + AuNPs | KBrO3 + MET/AuNPs |
|---------------|-----------|------|-------|-------|-----------|--------|-------------|---------------|-------------------|
|               | IL-6 (pg/mg) | 0.4 ± 0.04 | 0.4 ± 0.03 | 0.4 ± 0.05 | 0.3 ± 0.03 | 1.3 ± 0.06<sup>a</sup> | 0.8 ± 0.05<sup>a,b</sup> | 0.9 ± 0.04<sup>a,b</sup> | 0.6 ± 0.03<sup>a</sup> |

AuNPs: gold nanoparticles; C: control; KBrO3: potassium bromate; MET: metformin; MET AuNPs: metformin loaded on gold nanoparticles.

Results are presented as means ± SE for six rats in each group.

<sup>a,b</sup>Significant as compared to control and KBrO3 groups, respectively at p ≤ 0.05.
Effect of different treatments on body weight

No mortality or obvious clinical signs were observed in any group of animals throughout the experimental period. The body weights were significantly increased at the end of the experiment without intergroup differences. No significant differences were detected in final body weights between treated and non-treated rats are shown in Table 4.

Effect of different treatments on DNA damage by comet assay

As shown in Table 5 and Figure 4 administration of KBrO₃ resulted in significant increases in renal tissue contents of tail length (TL), tail DNA (% DNA), and tail moment (TM). Treatment with either MET, AuNPs or MET/AuNPs significantly reduced tissue contents of TL, % DNA and TM as compared to KBrO₃ group.

Histological examinations

Results are shown in Figures 5 to 12. Figures 13 to 16 showing portions of kidney in normal control group. Figures 17 to 19 showing portions of kidney in recovery rats administered metformin loaded on AuNps. Figures 20 to 22 showing portions of kidney of rats administered KBrO₃.

Characterization of AuNPs

Nanoparticles characteristics were monitored by UV-VIS spectrophotometer and by Transmission Electron Microscope (TEM). The identification results are presented in Figures 23 and 24. For the UV-VIS data, the absorbance was detected between λ 200 and 800 nm (Figure 23) which ensures the presence of the AuNPs in the suspension. On the other hand, the TEM images (Figure 24) have shown a majority of nearly spherical AuNPs with diameters ranging from 15.8 to 19.6 nm. The physical appearance of the AuNPs suspended in trisodium citrate was dark red (Figure 25). Presently, there have been stunning efforts to improve the synthesis of nanoparticles with anticipated sizes and characteristics to grow their biomedical applications, TEM is one of the reliable methods to detect AuNPs sizes.

Discussion

The present study was designed to investigate curative effect of METAuNPs on KBrO₃-induced nephrotoxicity. Administration of KBrO₃ caused a marked increase in TC, TG, and LDL-C levels but decreased HDL-C level, similar findings were reported.⁴⁰⁻⁴² The studied groups (MET, AuNPs, METAuNPs) decreased TC, TG, and LDL-C and increased HDL-C. This finding correlates with the report of Owolabi and Omogbai⁴³ which showed beneficial effects of MET in diabetic rats. Also, Singh
Table 4. Body weight changes of control and different treated rat groups.

| Animal groups | Parameter      | C      | MET    | AuNPs  | MET    | KBrO3  | KBrO3 + MET | KBrO3 + MET/AuNPs | KBrO3 + MET/AuNPs |
|---------------|----------------|--------|--------|--------|--------|--------|-------------|-------------------|-------------------|
|               | Initial weight | 95 ± 4.56 | 95 ± 2.89 | 95 ± 3.79 | 95 ± 4.09 | 95 ± 3.57 | 95 ± 2.82 | 95 ± 3.16 | 95 ± 4.69 |
|               | 1st week (g)   | 107 ± 3.58 | 110 ± 5.87 | 108 ± 7.46 | 110 ± 4.34 | 116 ± 2.83 | 110 ± 6.32 | 110 ± 4.89 | 115 ± 5.06 |
|               | 2nd week (g)   | 125 ± 3.03 | 122 ± 4.29 | 123 ± 13.02 | 122 ± 3.58 | 128 ± 5.79 | 123 ± 6.35 | 132 ± 9.05 | 127 ± 4.69 |
|               | 3rd week (g)   | 138 ± 6.26 | 134 ± 2.89 | 140 ± 11.84 | 132 ± 2.61 | 140 ± 8.32 | 140 ± 9.06 | 147 ± 11.58 | 145 ± 11.76 |
|               | 4th week (g)   | 152 ± 6.23 | 145 ± 2.37 | 159 ± 12.95 | 144 ± 2.61 | 154 ± 11.88 | 150 ± 10.71 | 157 ± 13.89 | 159 ± 19.05 |
|               | 5th week (g)   | 161 ± 6.63 | 158 ± 2.28 | 180 ± 7.24 | 156 ± 3.16 | 170 ± 16.33 | 161 ± 11.42 | 171 ± 14.21 | 172 ± 20.21 |
|               | 6th week (g)   | 174 ± 6.03 | 171 ± 2.61 | 191 ± 6.90 | 168 ± 3.74 | 179 ± 15.71 | 175 ± 12.33 | 183 ± 15.39 | 181 ± 21.68 |
|               | 7th week (g)   | 188 ± 6.07 | 185 ± 2.61 | 202 ± 8.94 | 183 ± 4.10 | 189 ± 15.43 | 184 ± 12.74 | 192 ± 12.96 | 193 ± 22.29 |
|               | 8th week (g)   | 201 ± 4.05 | 198 ± 3.85 | 214 ± 11.35 | 197 ± 5.51 | 203 ± 13.93 | 194 ± 13.34 | 202 ± 13.71 | 204 ± 24.05 |
|               | 9th week (g)   | 217 ± 6.78 | 214 ± 5.47 | 225 ± 12.99 | 212 ± 6.36 | 217 ± 13.70 | 210 ± 12.43 | 218 ± 16.84 | 219 ± 22.62 |

AuNPs: gold nanoparticles; C: control; KBrO3: potassium bromate; MET: metformin; MET/AuNPs: metformin loaded on gold nanoparticles.
Results are presented as means ± SE for six rats in each group.

Table 5. DNA damage in renal tissue of control and different treated rat groups.

| Animal groups | Parameter                  | C      | MET    | AuNPs  | MET    | KBrO3  | KBrO3 + MET | KBrO3 + MET/AuNPs | KBrO3 + MET/AuNPs |
|---------------|----------------------------|--------|--------|--------|--------|--------|-------------|-------------------|-------------------|
|               | Tail length (μm)           | 1.5 ± 0.05 | 1.9 ± 0.12 | 1.4 ± 0.12 | 1.6 ± 0.06 | 5.5 ± 0.23 | 3.1 ± 0.06 | 3.8 ± 0.11 | 3.2 ± 0.09 |
|               | Tail DNA (%)               | 1.4 ± 0.06 | 1.6 ± 0.06 | 1.6 ± 0.05 | 1.5 ± 0.06 | 5.2 ± 0.12 | 3.1 ± 0.12 | 3.4 ± 0.18 | 3.1 ± 0.12 |
|               | Tail moment (μm)           | 2.143 ± 0.09 | 3.107 ± 0.1 | 2.333 ± 0.15 | 2.497 ± 0.15 | 27.97 ± 0.58 | 9.493 ± 0.29 | 13.21 ± 1.19 | 10.09 ± 0.64 |

AuNPs: gold nanoparticles; C: control; KBrO3: potassium bromate; MET: metformin; MET/AuNPs: metformin loaded on gold nanoparticles.
Results are presented as means ± SE for six rats in each group.

*Significant as compared to control and KBrO3 groups, respectively at p ≤ 0.05.
et al.\textsuperscript{14} demonstrated that MET improved lipid profile that was deteriorated due to streptozotocin, similar findings were also reported.\textsuperscript{44} Moreover, Dhas et al.\textsuperscript{45} demonstrated that AuNPs improved alterations in lipid profile induced by alloxan. While MET loaded pectin-chitosan biohybrids ameliorated streptozotocin induced alterations in lipid profile.\textsuperscript{46}

\textbf{Figure 4.} Detection of DNA damage by comet assay in kidney cells: (a) control group rats with normal spots and round shape (untailed) (arrows) (comet assay; Scale bar = 100 \textmu m), (b–d) MET group, AuNPs group, and MET/AuNPs group, respectively showing normal spots and round shape (comet assay; Scale bar = 100 \textmu m), (e) KBrO$_3$ group showing increase the number of damaged spots in which the length of the comet was greater than the diameter of the basal nuclear DNA (tailed) (arrowheads) (comet assay; Scale bar = 100 \textmu m), and (f–h) KBrO$_3$ group treated with MET, KBrO$_3$ group treated with AuNPs, KBrO$_3$ group treated with MET/AuNPs, respectively showing decreased the number of damaged spots (comet assay; Scale bar = 100 \textmu m).

\textbf{Figure 5.} Photomicrograph of kidney from group normal control showing; normal renal corpuscles (white arrow), normal PCT and DCT with intact acidophilic brush border (T). H&E.

et al.\textsuperscript{14} demonstrated that MET improved lipid profile that was deteriorated due to streptozotocin, similar findings were also reported.\textsuperscript{44} Moreover, Dhas et al.\textsuperscript{45} demonstrated that AuNPs improved alterations in lipid profile induced by alloxan. While MET loaded pectin-chitosan biohybrids ameliorated streptozotocin induced alterations in lipid profile.\textsuperscript{46}
Figure 6. Photomicrograph of kidney from group MET showing slightly normal kidney tissue architecture with renal corpuscle (black arrow), with well identified PCT and DCT (T). H&E.

Figure 7. Photomicrograph of kidney from group AuNPs (a and b) showing; intact renal corpuscle with intact glomeruli (arrow) and intact renal tubules (T). H&E.

Figure 8. Photomicrograph of kidney from group MET + AuNPs showing: (a) renal cortex appeared with normal structure of renal corpuscle (arrow) and different types of renal tubules PCT and DCT (T) and (b) higher magnification of the previous showed normal renal corpuscle (arrow) and intact renal tubules (T). H&E.
Administration of KBrO₃ caused significant increases in ALT, AST, ALP, TB, and DB but decreased Alb and TP; these results were in accordance with those of other studies.⁴⁷–⁴⁹ MET, AuNPs, and METAuNPs groups significantly decreased ALT, AST, ALP, and bilirubin and increased Alb and TP. Balamash et al.⁴⁴ reported a similar effect for MET against streptozotocin. Also, MET suppressed the effect of thioacetamide on hepatic markers⁵⁰ and Dhas et al.⁴⁵ demonstrated that AuNPs ameliorated the alterations in hepatic markers induced by alloxan. Similarly, MET loaded pectin-chitosan biohybrids decreased ALT, AST, ALP, and bilirubin and increased Alb and TP that were altered by streptozotocin.⁴⁶

Figure 9. Photomicrograph of kidney from group KBrO₃ showing: (a) shrinkage glomeruli of renal corpuscle with widen capsular space (white arrow). Detached brush border with degeneration of some renal tubules (black arrow) and (b) the renal medulla showed severe degeneration of the epithelium lining of collecting tubules (arrow head). H&E.

Figure 10. Photomicrograph of kidney from group KBrO₃ + MET showing: (a) histological structure of kidney with renal corpuscles (black arrow), kidney tubules (T) and (b) higher magnification to the previous figure showing mild degeneration in the glomerular tissue (black arrow) and mild degeneration in some tubular epithelium. H&E.
Administration of KBrO₃ caused significant increases in Ur, UA, and Cr serum levels. These results indicated that KBrO₃ induced acute kidney injury, similar findings were reported.²,⁴⁷,⁴⁹,⁵¹

In the present study, MET, AuNPs, and METAuNPs groups decreased Ur, UA, and Cr serum levels. This finding correlates with Owolabi and Omogbai⁴³ which showed beneficial effects of MET in diabetic rats. MET inhibits gentamicin induced renal injury.⁵² Balamash et al.⁴⁴ confirmed the renoprotective effect of MET against streptozotocin. Also, Dhas et al.⁴⁵ demonstrated that AuNPs

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**Figure 11.** Photomicrograph of kidney from group KBrO₃ + AuNPs showing: (a) renal cortex contains intact renal corpuscles (arrow) and renal tubules (T) and (b) higher magnification of renal cortex showing renal corpuscles (arrow) and mild pathological lesions in renal tubules (arrowhead). H&E.

**Figure 12.** Photomicrograph of kidney from group KBrO₃ + MET AuNPs showing: (a) renal cortex with intact renal corpuscles (arrow) and different tubules PCT and DCT (T) and (b) higher magnification of the previous figure showing intact renal corpuscles (arrow) and tubular epithelium (T). H&E.
Figure 13. An electron micrograph of normal kidney showing proximal convoluted tubule with normal brush borders (white arrows) and normal mitochondria (black arrows).

Figure 14. An electron micrograph of normal kidney showing distilled convoluted tubule, blood vessel BV, and microvilli (white arrows).

Figure 15. An electron micrograph of normal kidney showing glomerulus tubule with normal basement membrane (white arrows).
Figure 16. An electron micrograph with high magnification of normal kidney showing portion contain filtration sites (black arrow) and basement membrane (white arrow).

Figure 17. An electron micrograph of kidney showing Proximal convoluted tubule with recovery brush borders (white arrows) and slightly vacuoles (black arrows) and lysosomes (LY).

Figure 18. An electron micrographs of recovery kidney showing distilled convoluted tubule with recover brush borders (white arrows).
ameliorated the alterations in renal markers induced by alloxan and Chinnaiyan et al.\textsuperscript{46} reported that MET loaded pectin-chitosan biohybrids ameliorated nephrotoxic effects of streptozotocin.

Administration of KBrO\(_3\) significantly elevated oxidative stress markers such as MDA, NO, and PC. Similar results were previously reported\textsuperscript{2,49,53,54} Meanwhile, MET, AuNPs, and METAuNPs groups decreased MDA, NO, and PC. It has been reported that MET reduced oxidative stress induced by streptozotocin.\textsuperscript{14} Lipopolysaccharide induced oxidative damage was reduced by AuNPs\textsuperscript{55} and Arun et al.\textsuperscript{56} demonstrated a synergistic antioxidant effect of novel chitosan combined

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure19}
\caption{An electron micrograph of recovery kidney showing glomerulus tubule with recovery basement membrane which is slightly irregular and thick (white arrows).}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure20}
\caption{An electron micrograph of kidney showing proximal convoluted tubule which lost brush borders (white arrows) with much vacuoles (black arrows) and many lysosomes (LY).}
\end{figure}
metformin drug on streptozotocin-induced diabetes mellitus. Administration of KBrO₃ decreased the activity of renal antioxidant enzymes such as SOD, CAT, GST, and GSH levels as previously reported.²,⁴⁹,⁵³ MET, AuNPs, and METAuNPs groups increased SOD, CAT, GST, and GSH. Similarly, MET improved the antioxidant enzymes that were reduced by streptozotocin.¹² A similar effect was caused by AuNPs⁵⁷ and chitosan with MET.⁵⁶

**Figure 21.** An electron micrograph of kidney showing plasma cell (inflammatory cell) (white arrows).

**Figure 22.** An electron micrograph of kidney showing glomerulus tubule with irregular basement membrane with a large thickening and fusion in pedicel (white arrows).
Administration of KBrO₃ significantly elevated renal apoptotic markers such as caspase3, Bax but decreased Bcl-2 that results were in agreement with others.² MET, AuNPs, and METAuNPs groups decreased caspase 3 and Bax and increased Bcl-2. Guigas et al.⁵⁸ demonstrated that MET downregulated caspase 3 and Bax.

Figure 23. Gold nanoparticles (AuNPs) were examined by UV/VIS spectrophotometer particles’ absorbance was maintained at a range between 200 and 800 nm.

Figure 24. Electron micrograph of AuNPs suspension on transmission electron microscopy (TEM) showing a majority of almost spheroidal to rod shaped gold nanoparticles with diameters ranging between 15.8 and 19.6 nm.
and upregulated Bcl-2. Similarly, MET inhibited apoptosis induced by gentamicin\textsuperscript{59} or cisplatin\textsuperscript{60} or streptozotocin.\textsuperscript{12} Moreover, Zheng et al.\textsuperscript{61} reported that AuNPs reduced caspase 3 and Bax and increased Bcl-2. Hyaluronic acid co-functionalized gold nanoparticle complex for the targeted delivery of MET downregulated caspase 3 and Bax and upregulated Bcl-2.\textsuperscript{9}

Administration of KBrO\textsubscript{3} significantly elevated IL-6, this is consistent with other previous studies.\textsuperscript{62–64} MET, AuNPs, and METAuNPs groups decreased IL-6. These findings correlate with the report of Al-Hashem et al.\textsuperscript{50} which showed that metformin suppresses thioacetamide inflammatory response. Qu and Qu\textsuperscript{65} confirmed the anti-inflammatory effect of MET against lipopolysaccharide. Dhas et al.\textsuperscript{45} demonstrated the anti-inflammatory effect of AuNPs against alloxan. Also, MET loaded polydopamine reduced the inflammatory cytokines in Parkinson’s disease.\textsuperscript{66}

Administration of KBrO\textsubscript{3} induced DNA fragmentation, as monitored by the release of nucleotides from damaged DNA, both in the renal cortex and medulla when compared to control. Previous studies showed an increased level of DNA single strand breaks and alkali labile sites in the kidney by the Comet assay.\textsuperscript{67,68} MET, AuNPs, and METAuNPs groups reduced the genetic alterations, micronucleus formation, and chromosomal aberrations. Diniz Vilela et al.\textsuperscript{69} confirmed the protective effect of MET against streptozotocin. Similarly, Abd El-Rahim et al.\textsuperscript{70} confirmed the antigenotoxic effect of MET against streptozotocin. While Fraga et al.\textsuperscript{71} demonstrated the protective effect of AuNPs against DNA damage. Also, the protective effect of hyaluronic acid co-functionalized gold nanoparticle complex for the targeted delivery of MET was established against DNA damage.\textsuperscript{9}

Administration of KBrO\textsubscript{3} didn’t have an effect on the bodyweight of rats, which is consistent with previous studies.\textsuperscript{72,73}

Examination of hematoxylin and eosin stained sections of rats treated with KBrO\textsubscript{3} revealed shrunken glomeruli with widening capsular space and tubular damage, similar results were reported.\textsuperscript{2,4,67} MET, AuNPs, and METAuNPs groups improved the histopathological changes caused by KBrO\textsubscript{3}, this was in agreement with Amini et al.\textsuperscript{74}
Transmission electron microscope showed proximal convoluted tubule with lost brush borders and many vacuoles, glomerulus tubule with irregular basement membrane with large thickening and fusion in pedicel in the KBrO3 group. These observations were consistent with others.75 Meanwhile, MET, AuNPs, and METAuNPs groups ameliorated effects on these ultrastructural changes in the kidneys, the thickness was significantly decreased. These results agree with other studies.45,75

**Conclusion**

The obtained data showed that MET, AuNPs, METAuNPs, particularly METAuNPs, had a potent therapeutic effect against KBrO3-induced nephrotoxicity. This can be explained by their antioxidant, anti-inflammatory, and anti-apoptotic properties. The present work provides insight into the roles of the METAuNPs, which may be used as a therapy for KBrO3-induced nephrotoxicity.

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