Egyptian Journal of Zoology (EJZ), Vol. 79: 29-47 (June, 2023) p-ISSN: 1110-6344
Publisher: The Zoological Society of A. R. Egypt e-ISSN: 2682-3160
DOI: 10.21608/ejz.2022.135884.1081

RESEARCH ARTICLE

PROTECTIVE EFFECT OF AQUEOUS EXTRACT OF *MORINGA OLEIFERA* LEAVES AGAINST POTASSIUM BROMATE-INDUCED RENAL TOXICITY IN RATS

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Article History:
Received: 25 April 2022
Accepted: 27 June 2022

Published Online:
12 July 2022

Keywords:
Inflammation
$KBrO_3$
Moringa oleifera
Nephrotoxicity
Oxidative stress

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ABSTRACT

$KBrO_3$ is a vital component used in food, beer, pharmaceutical, and cosmetic productions; it produces moderate-to-dangerous toxic insults to a variety of organs. This study aimed to investigate if *Moringa oleifera* leaves aqueous extract (MOE) can protect rats from $KBrO_3$-induced renal toxicity. Four experimental groups of male albino rats (Sprague Dawley) were used here (n=8): control, MOE (400 mg/kg body weight), $KBrO_3$ (100 mg/kg body weight), and $KBrO_3$ in combination with MOE groups. Daily for six weeks, each group received orally its unique treatment. After the experimental period kidneys and serum were collected for biochemical, molecular, and histological investigations. The $KBrO_3$ treatment was associated with a significant rise in serum levels of urea, creatinine, sodium, and potassium. $KBrO_3$ also caused a significant increase in the renal tissue levels of malon-dialdehyde and nitric oxide, while reducing the activities of antioxidant enzymes in the renal tissues. Moreover, $KBrO_3$ led to kidney inflammation and fibrosis by increasing the tumor necrosis factor-α, interleukin-6, and tumor growth factor-β1, which was followed by upregulation in the renal expression of *miRNA* 21 (*miR-21*), *miR-29*, and *miR-192*. In comparison to the control group, histopathological evaluation of the $KBrO_3$ group revealed degenerative alterations and damage in the kidney tissues. Conversely, co-treatment with MOE revealed a noticeable alleviation of the harmful effects of $KBrO_3$ in almost all examined parameters. In conclusion, MOE could be utilized as an alternative therapy to alleviate the detrimental effects of $KBrO_3$ on kidneys due to its antioxidant, anti-inflammatory, and anti-fibrotic activities.

INTRODUCTION

The crystalline chemical potassium bromate ($KBrO_3$) is colorless, hydrophilic, and odor-less[1]. It is a vital component used in food, pharmaceutical, and cosmetic productions, where it's used for making beer, drugs, and hair care products[2]. Also, $KBrO_3$ has been recognized being as an oxidizing agent, and its aggregation in the body has been shown to resolve various organ

toxicity[3]. Furthermore, after *in vivo* metabolism, $KBrO_3$ produces more aggressive metabolites (bromate and bromide radicals). They extend invade cellular components through reactive oxygen species (ROS) and nitric oxide (NO), which damage the cellular structures, containing membranes, nucleic acids, and the essential proteins[4]. As a result, *in vivo*, $KBrO_3$ produces moderate-to-dangerous toxic insults to a variety of organs, including the kidney, liver, and brain. Because of its oxidizing property and mutagenicity *in vivo*, $KBrO_3$ is categorized as a class 2B carcinogen[4].

The kidney is responsible for a variety of functions, including detoxification and fluid balance. The kidney might be regarded as one of the principal target organs of exogenous toxicants due to its physiological importance[5]. In this regard, $KBrO_3$ has been confirmed to labialize cell membranes of the kidney of rats, which can also result in renal failure[6]. The capacity of $KBrO_3$ to stimulate the generation of ROS, lipid peroxidation, and 8-hydroxyguanosine alteration in renal DNA has been linked to its nephro-toxicity[7].

Consequently, therapeutic mediation including the usage of natural products to alleviate and/or pharmacologically reduce the $KBrO_3$-caused organ toxicity may be a good therapy methodology[8]. *Moringa oleifera* (MO) is a highly prized medicinal plant[9]. It is employed for the treatment of numerous diseases[10]. It has several biological effects, all of which have been linked to its high concentration of bioactive substances such as flavonoids, alkaloids, phytosterols, and glucosinolate[11]. Specifically, the leaves and some other parts of the *Moringa* plant have been found to contain high concentrations of flavonoids such as quercetin, kaempferol, and apigenin, and these are believed to be responsible for the potent antioxidant activity of the plant[12]. Therefore, the present investigation was designed to consider the role of the MO leaves aqueous extract (MOE) for its nephroprotective effects versus $KBrO_3$ toxicity in rats.

MATERIAL AND METHODS

Preparation of MOE

The MO leaves were authenticated and acquired from the Egyptian Scientific Society for *Moringa* at National Research Center, Dokki, Giza, Egypt. To eliminate contaminants, the MO leaves were soaked in water for 15 minutes. The leaves were dehydrated at 55ºC in an air dryer. Then leaves were ground into a powder using a domestic grinder (BRAUN), sieved through a 60 mesh sieve, and kept at 7ºC. The MOE was made by mixing 40 g of dry powder with 100 mL of hot water and leaving it at room temperature for 24 hours, stirring constantly with a glass rod. The extract was achieved by filtration using Whatman No. 1 (Maidstone, UK). The filtrate was condensed utilizing Rotary Evaporator (Model RE52A; Wincom Company Ltd., Changsha, Hunan, China) to 8% of its original amounts at 55ºC. The concentrated filtrate was dry in the oven at 60ºC for 48 hours[13]. The high-performance liquid chromatography (HPLC) analysis of the phenolic composites was performed using a Waters 2695 Alliance HPLC system (Waters Inc., Milford, CT, USA), supplied with a UV-Vis DAD according to Mizzi *et al*.[14].

Experimental animals

Thirty-two adult male albino, Sprague Dawley, rats (*Rattus norvegicus*) weighing 200±10g were used throughout the study. The rats were purchased from the breeding unit of Medical Research of National Research Center, Giza, Egypt. Animals were bred and kept in an air-conditioned animal house in conjunction with 12 hours light-dark cycle and unlimited access to food and water. The animals were acclimated for one week before the start of the experiments.

Experimental design

Rats were randomly allotted into four experimental groups (eight rats/per group).

The experimental groups were illustrated as follows: Group (A) acted as a control group and received only distilled water, group (B) received 400 mg MOE/kg body weight[15], group (C) received 100 mg $KBrO_3$/kg body weight[16], and group (D) received MOE combined with $KBrO_3$. All experimental regimens were administrated orally/daily for six weeks. The $KBrO_3$ white crystals (molecular weight: 167, code: L26221) were purchased from El-Gomhoria Co., Cairo, Egypt.

Sample collection.

After the completion of the experiment, animals were sacrificed under light diethyl ether anesthesia. Blood samples were collected in clean, dry centrifuge tubes and were left for clotting and then centrifuged for 10 minutes at 1800 ×*g* and 4ºC to separate the serum. Sera were stored at –20ºC in polypropylene vials until analysis. The kidneys were also removed for biochemical, molecular, and histological analysis.

Serum biochemical assays

Urea and creatinine concentrations were determined by the endpoint colorimetric methods (CHEMELEX, S.A, Barcelona, Spain). Concentrations of electrolytes (Na^+ and K^+) were estimated by direction-selective electrode systems (ADVIA 1800 Chemistry System; Siemens Healthineers Headquarters, Erlangen, Germany).

Renal biochemical assays

In an ice-cold medium containing 10 mmol/L phosphate-buffered saline, pH 7.4, kidney tissue was homogenized to yield 10% (weight/volume) homogenate for investigations of nitric oxide (NO) and malondialdehyde (MDA) levels, as well as antioxidant enzymatic activities (SOD, CAT, and GPx). In a cooling centrifuge at 4ºC, the homogenate was spun at 1800 ×*g* for 10 minutes. The nitric oxide assay was performed using the OxiSelect™ NO assy kit (catalog number: STA-800, Cell Biolabs, San Diego, CA, USA). MDA concentration was measured using a colorimetric assay by OxiSelect™ TBARS assay kit (catalog number: STA-330, Cell Biolab). However, the superoxide dismutase (SOD) and catalase (CAT) activities were measured by OxiSelect™ Assay Kit (Catalog numbers: SAT-340 and SAT-341, respectively, Cell Biolab). The glutathione peroxidase (GPx) activity was measured colorimetrically by GPx assay kit (catalog number: EGPX-100, BioAssay System, Hayward, CA, USA).

Western blotting assay

The expressions of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and tumor growth factor-β1 (TGF-β1) were performed by Western blotting assay. The kidneys were pounded using a homogenizer (PRO Scientific Inc, Oxford, CT, USA) in a lysis solution containing (50 mmol Tris, 150 mmol sodium chloride, 1% triton, 0.1 sodium dodecyl sulfate "SDS", and 1.0 mmol phenylmethyl-sulfonylfluoride). The homogenates were sonicated for 10 seconds per kidney after centrifugation at 12000 ×*g* and 4ºC for 20 minutes. The supernatants containing the kidney lysates were collected then equal volumes of protein (20 µg) were separated by 10% SDS polyacrylamide gel electrophoresis (SDS-PAGE). Fractioned proteins were transferred to polyvinylidene (PVDF) membranes, which were blocked for one hour with 5% non-fat, dry milk in tris buffer saline (1×) containing 0.01 tween 20 (TBST). After blocking, the membranes were washed three times for 5 minutes each. This was followed by the incubation of membranes overnight at 4ºC on a sharker with primary antibodies: antibody TNF-α antibody (PA5-19810), antibody IL-6 antibody (PA1-26811), and antibody TGF-β1 antibody (PA1-29032). In addition, β-actin was used as an internal control. The concentration of all antibodies was 1:10000. After incubation, the primary antibody was discarded and the membranes were washed with TBST three times for 5 minutes each, at 37ºC for one hour. Next, the membranes were incubated with the secondary antibody: Horseradish peroxidase-

linked goat antirabbit (HRP). Then, the secondary antibody was discarded and the membranes were washed 3 times by TBST. All primary and secondary antibodies were purchased from (Thermo Fisher Scientific, Waltham, MA, USA). For detection, the membranes were incubated overnight in 10 mL of the blocking buffer mixed with 5 µL of the substrate "Qdot R 625 streptavidin conjugate; catalog number: W10142, Thermo Fisher Scientific, Waltham, MA, USA" on a rocking platform. Then, the substrate was discarded and the membranes were washed 3 times with 1× washing buffer. Calculation of protein concentration relative to the internal control protein (β-actin) was performed using the Image J software.

***miRNA* (*miR*) expression in renal tissue**

Total RNA from kidney tissue was isolated using a *miR*Neasy Mini Kit, catalog number: 217004 (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Then the total RNA was eluted from the column in RNase-free water and stored at –80ºC. The quality and concentration of the isolated RNA were evaluated by using NanoDrop (R) 1000 spectrophotometer (Thermo Fisher Scientific); all samples showed an A260/280 > 1.6. Total RNA was reverse transcribed using miScript II RT Kit, catalog number: 218160 (Qiagen). Target-specific polymerase chain reaction (PCR) primers (*miR-21*, *miR-29*, and *miR-192*) were obtained from Applied Biosystems (Waltham, MA, USA). Real-time PCR (qPCR) amplifications were performed using the SYBR-Green fluorescent-based primer assay. The qPCR was performed in the 5-plex Rotor-Gene PCR System (Qiagen, Hilden, Germany). Approximately 20 µL reaction mixture consists of 2× QuantiTect syber green PCR master mix, 10× miscript universal primers, 2 µL primer assay, and 50 pg-3ng cDNA was used for qPCR. All objectives were amplified in duplicates for each sample. The thermal protocol consists of 15 minutes for HotStarTaq DNA polymerase activation at 95ºC, followed by 40 cycles of denaturation at 95ºC for 15 minutes of primer annealing for 30 seconds at 55 ºC and extension at 70ºC for 30 seconds. The $2^{\Delta\Delta}$Ct method was conducted for the analysis of the tested *miRs* expression amounts, using RUN6 as an endogenous reference control for standardization determinations.

Histological investigation.

For histological investigation, the kidney was dissected and immediately fixed in 10% neutral buffered formalin. Tissues were dehydrated in a series of ethanol solutions, then cleaned in xylene, embedded in paraffin, and processed for histological investigation[17]. Using a rotary microtome, sections of 4-5 µm thickness were cut and stained with hematoxylin and eosin for general histological examination and Masson Trichrome for fibrosis.

Statistical analysis.

Data were reported as mean ± standard error. Statistical analyses were performed by using the statistical package for social sciences (SPSS) version 26 (IBM corp., Armonk, NY, USA). One-way analysis of variance was used for comparison of means followed by an LSD post-hoc test. Differences between means were considered to be significant when $P<0.05$.

RESULTS

Analysis and detection of MOE ingredients

Table "1" revealed the existence of the six most important polyphenolic compounds in MOE, which are coumarin (4.25 ppm), ferulic acid (4.65 ppm), resorcinol (0.31 ppm), quercetin (4.19 ppm), kaempferol (3.98 ppm) and, phenanthrene (117.71 ppm).

Effects of MOE on kidney functions and serum levels of electrolytes in potassium bromate-treated rats

Serum urea and creatinine concentrations exhibited a significant ($P<0.05$) increase in the group treated with $KBrO_3$ alone when compared with the control group.

Table 1: Phenolic compounds in aqueous extract of *Moringa oleifera* leaves.

Peak name	Retention time (min)	Area (mAU∗min)	Height (mAU)	Relative area (%)	Relative height (%)	Amount (ppm)
Coumarin	1.323	0.304	1.083	8.28	8.26	4.25
Ferulic acid	1.983	0.309	1.095	8.40	8.35	4.65
Resorcinol	2.907	0.309	1.086	8.40	8.28	0.31
Quercetin	3.583	0.331	1.169	9.01	8.92	4.19
Kaempferol	4.350	0.320	1.168	8.71	8.91	3.98
Naphthaline	nd	nd	nd	nd	nd	nd
Phenanthrene	5.263	0.298	1.046	8.10	7.98	117.71

nd: Not detectable

However, the impairment in kidney functions in $KBrO_3$-treated group were modulated significantly (P<0.05) by the co-administration of MOE (Table 2). Similarly, serum levels of Na^+ and K^+ increased significantly (P<0.05) in the $KBrO_3$ group compared with the control group. Conversely, co-administration of MOE with $KBrO_3$ lowered significantly (P<0.05) the Na^+ and K^+ levels compared with the $KBrO_3$ alone treated group (Table 2).

Table 2: Effects of aqueous extract of *Moringa oleifera* leaves (MOE) and potassium bromate on kidney functions and serum levels of electrolytes in male rats.

Experimental groups	Urea (mg/dL)	Creatinine (mg/dL)	Na^+ (mEq/L)	K^+ (mEq/L)
Group A	0.66±0.02	23.40±1.43	133.53±0.82	4.60±0.04
Group B	0.71±0.02	23.60±0.69	134.48±0.87	4.73±0.11
Group C	1.63±0.13[a]	45.57±1.48[a]	163.07±0.79[a]	6.52±0.13[a]
Group D	0.91±0.04[b]	31.55±1.15[b]	144.30±0.45[b]	4.72±0.11[b]

Values are expressed as mean ± standard errors, n = 8. Group A: control group; group B: MOE group; group C: $KBrO_3$ group; group D: $KBrO_3$+MOE group; [a]: significantly different from the control group at P<0.05; [b]: significantly different from the $KBrO_3$ group at P<0.05.

Effects of MOE on renal NO and MDA levels, as well as enzymic antioxidants in potassium bromate-treated rats

In comparison to the control group, $KBrO_3$ induced a significant rise (P<0.05) in NO and MDA contents in kidney tissue (Figure 1). On the other hand, there was a substantial drop (P<0.05) in the activities of enzymic antioxidants (SOD, CAT, and GPx) in $KBrO_3$-tretad groups. When compared with the KBO_3 group, the group that received both KBO_3 and MOE demonstrated a large reduction (P<0.05) in the NO and MDA contents as well as a significant increase (P<0.05) in the activities of enzymic antioxidants (SOD, CAT, and GPx).

Effects of MOE on renal TNF-α, IL-6, and TGF-β1 content in potassium bromate-treated rats

$KBrO_3$ was found to trigger renal inflammation as manifested by the upregulation (P<0.05) of pro-inflammatory cytokines (TNF-α and IL-6) as shown in (Figure 2). Similarly, renal expression of

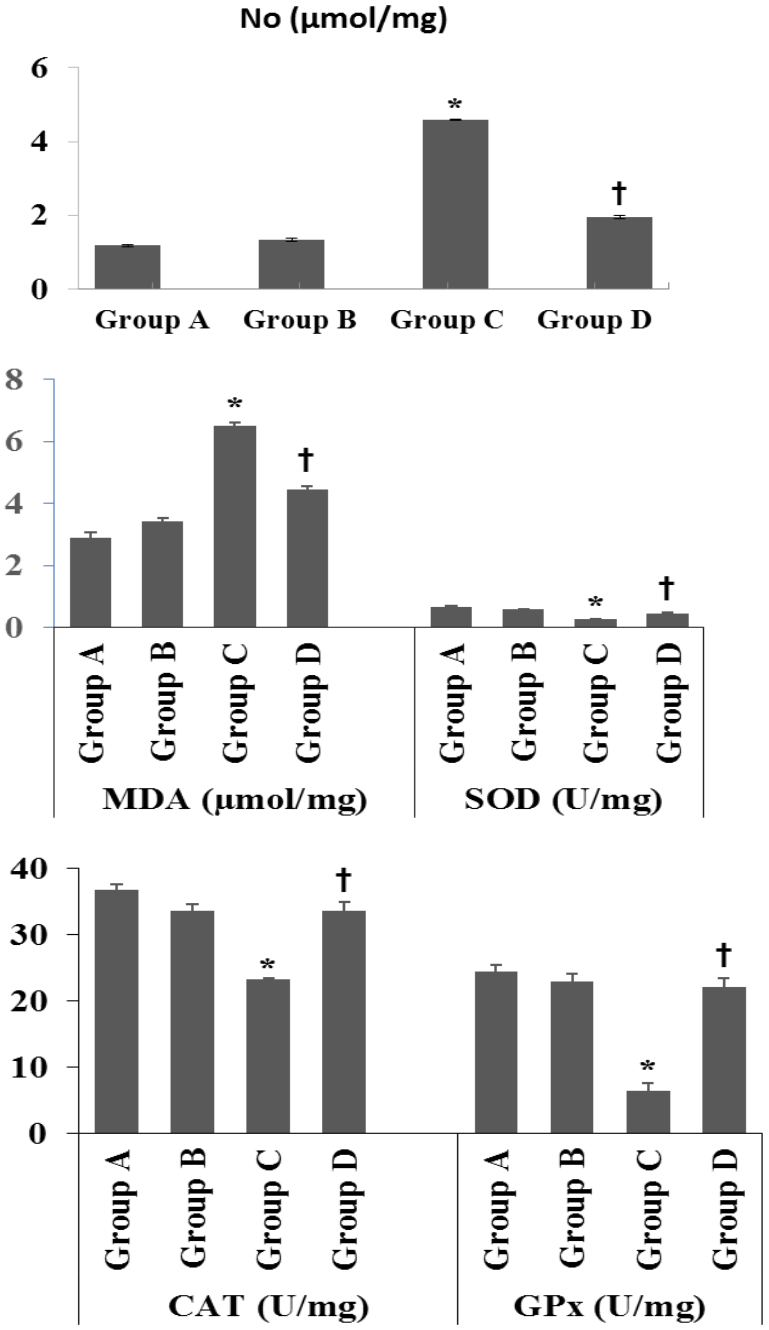


Figure 1: Effects of aqueous extract of *Moringa oleifera* leaves (MOE) and potassium bromate on renal nitric oxide (NO) and malondialdehyde (MDA) levels, as well as enzymic antioxidants in male rats. Group A: control group; group B: MOE group; group C: $KBrO_3$ group; group D: $KBrO_3$+MOE group; SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidease; *****: significantly different from the control group at $P<0.05$; **†**: significantly different from the $KBrO_3$ group at $P<0.05$.

TGF-β1 was increased significantly ($P<0.05$) in $KBrO_3$-treated rats. In contrast, the cotreated group with KBO_3+MOE showed downregulation in the expression of pro-inflammatory cytokines (TNF-α and IL-6) and fibrotic agent TGF-β1.

Effects of MOE on renal *miR-21*, *miR-29*, and *miR-192* expression in potassium bromate-treated rats

The data showed a significant increase ($P<0.05$) in the expressions of *miR-21*, *miR-29*, and *miR-192* in the $KBrO_3$-treated group when compared with the control group. In contrast, the combination between $KBrO_3$ and MOE caused a significant reduction ($P<0.05$) in the expressions of *miR-21*, *miR-29*, and *miR-192* as compared with the $KBrO_3$ alone treated group (Figure 3).

Effects of MOE on renal histology of potassium bromate-treated rats

In the control group, bowman's capsule and convoluted tubules displayed a normal histological appearance (Figure 4). Like

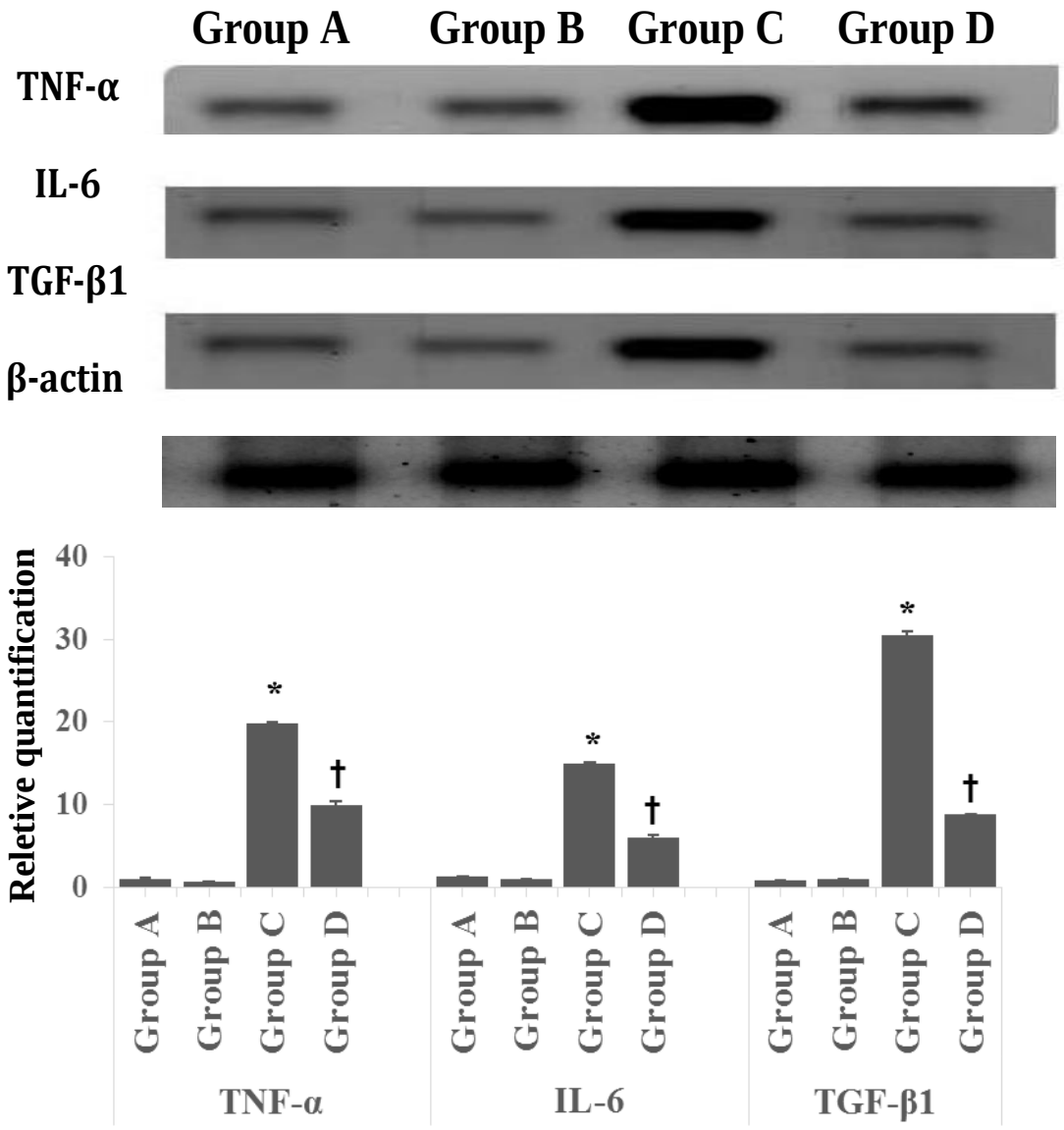
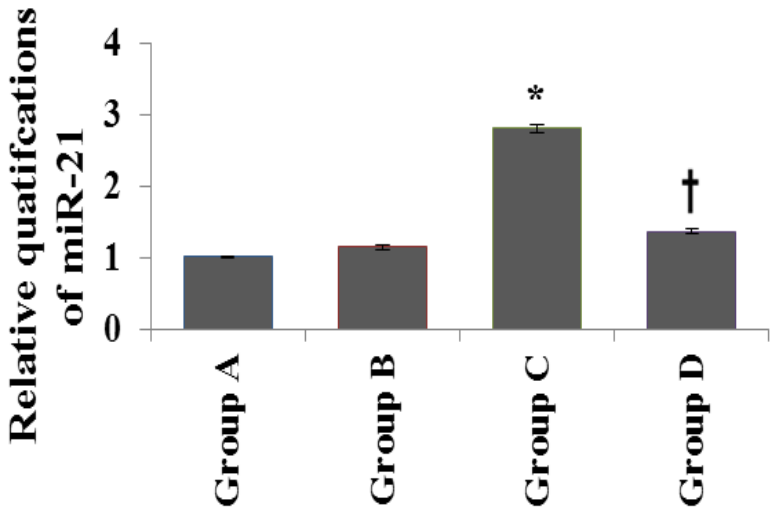
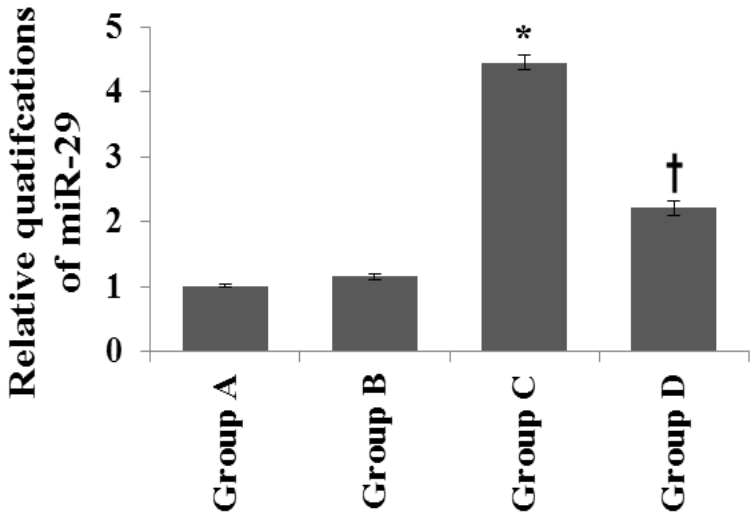


Figure 2: Western blot analysis showing the effects of aqueous extract of *Moringa oleifera* leaves (MOE) and potassium bromate on renal tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and tumor growth factor-β1 (TGF-β1) content in male rats. Group A: control group; group B: MOE group; group C: $KBrO_3$ group; group D: $KBrO_3$+MOE group; *****: significantly different from the control group at $P<0.05$; **†**: significantly different from the $KBrO_3$ group at $P<0.05$.

results were achieved in the kidney of rats treated with MOE (Figure 4). The current investigation showed that $KBrO_3$ at a dose of "100 mg/kg" for 6 weeks induced marked histopathological alterations in the renal tissue of rats (Figure 5). The kidney sections of $KBrO_3$-treated rats revealed separation in intratubular connective tissue and atrophy of glomerular tufts, tubules were vacuolated, and hemorrhage was also noticed. In addition, the tubules were dilated with desquamation and loss of cellular boundary, hyaline degeneration, and lymphocytic infiltration (Figure 5). However, rats given MOE (400 mg/kg) demonstrated significant improvements in the histological features and protection against renal tissue degeneration, vestiges of these alterations were even visible in the rats given $KBrO_3$+MOE, but some sections demonstrated significant improvements in tubule architecture (Figure 6).





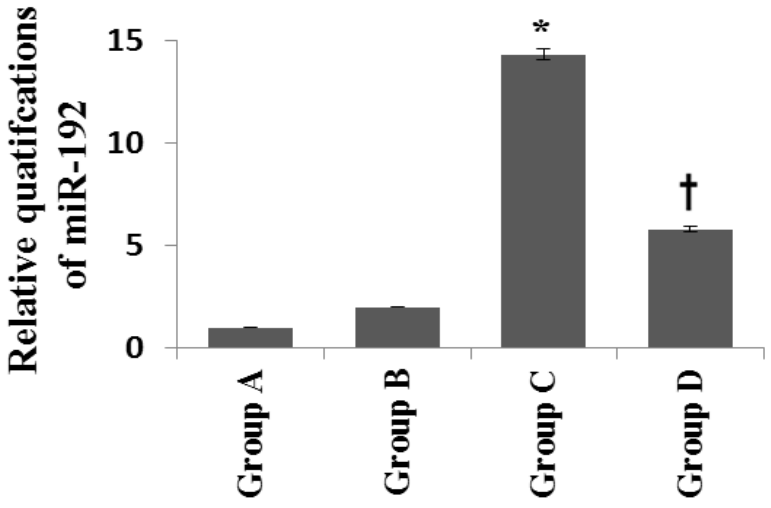


Figure 3: Effects of aqueous extract of *Moringa oleifera* leaves (MOE) and potassium bromate on the expression of renal *miR21*, *miR29*, and *miR192* in male rats. Group A: control group; group B: MOE group; group C: $KBrO_3$ group; group D: $KBrO_3$+ MOE group; *****: significantly different from the control group at $P<0.05$; **†**: significantly different from the $KBrO_3$ group at $P<0.05$.

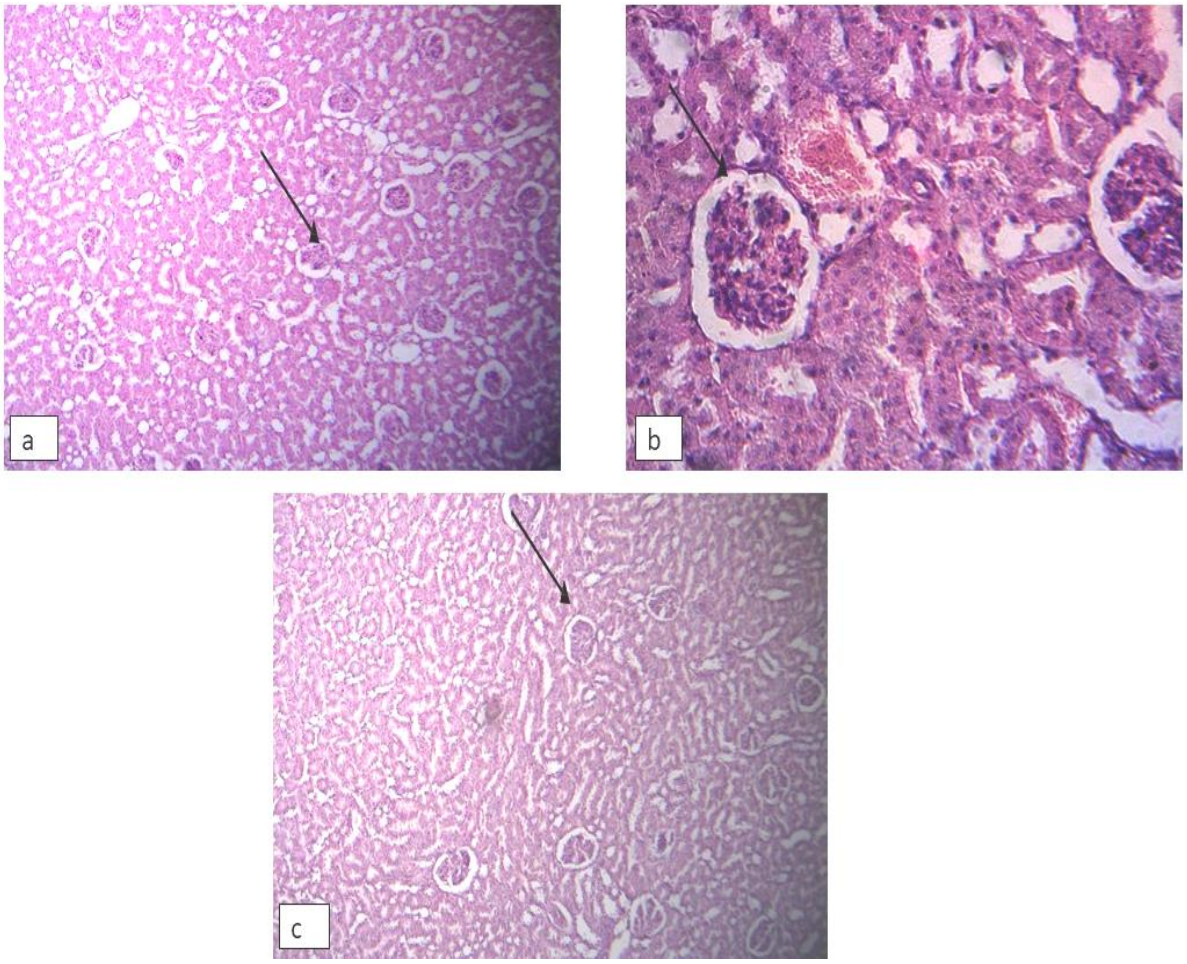


Figure (4): Light micrograph of kidney sections of the control group (**a** and **b**) and the aqueous extract of *Moringa oleifera* leaves (MOE)-treated group **(c)** (hematoxylin and eosin stain). **(a** and **b)** Kidney sections of control rats showing normal renal glomeruli (black arrows) and tubules. The renal glomeruli are normal in number and morphology (magnification: 200× and 400×, respectively), and **(c)** Kidney section of MOE-treated rats showing normal renal glomeruli and tubules (magnification: 200×).

Effects of MOE on renal fibrosis of potassium bromate-treated rats

The tubulointerstitial fibrosis score was evaluated in renal tissues by measuring the fibrotic region in the Masson trichrome-stained section. No evidence of fibrosis was detected in the renal tissues of the control group (Figure 7a). Similar morphological findings were observed in the MOE-treated group (Figure 7b). The $KBrO_3$-treated rats revealed marked tubulointerstitial fibrosis, which was indicated by a dense deep red staining, the fibrosis with tubules scored 92% (Figure 7c, d). On the other hand, a marked improvement was detected in the MOE-treated group, which was proved by the reduction in fibrotic score to 62% (Figure 7e).

DISCUSSION

Given the present results, $KBrO_3$ administration resulted in a significant increase in serum concentration of creatinine and urea compared with the control group. This result is in harmony with reports of Akomolafe *et al*.[8] and Abd Elmaksoud *et al*.[18]. These results may be expected to the kidney's incapability to carry out its functions of purification and removal of metabolites as a result of the structural alterations in the kidney tissues after administration of $KBrO_3$ as repotred

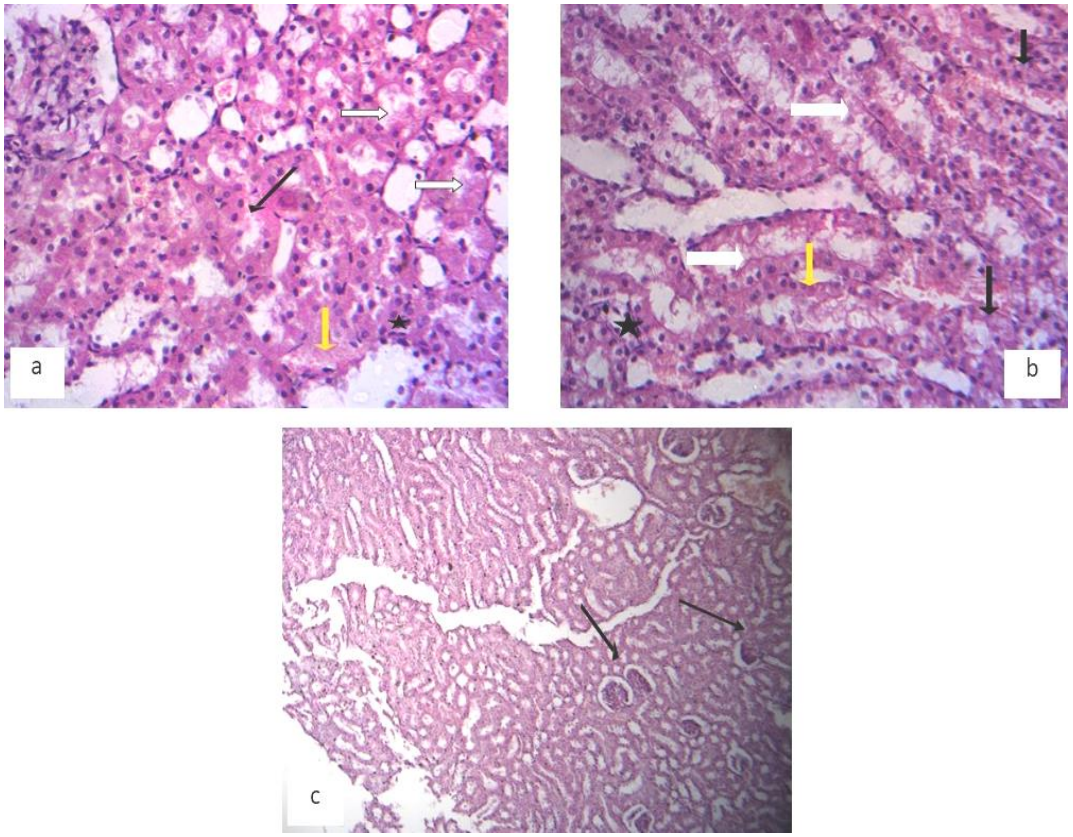


Figure (5): Light micrograph of kidney sections of the $KBrO_3$-treated group (hematoxylin and eosin stain) showing (a) the renal cortex is infiltrated with inflammatory cells (black star) associated with extravasation hemorrhage (black arrow), dilated tubules with loss of cellular boundary (white arrows), and hyaline degeneration (orange arrow) (magnification: 400×), **(b)** renal tubules infiltration with inflammatory cells (black star) associated with extravasation hemorrhage (black arrow), dilated tubules with loss of cellular boundary (white arrows), and hyaline degeneration (orange arrow) (magnification: 400×), and **(c)** a marked reduction in glomeruli number and size, glomerular atrophy (black arrows), associated with marked interstitial spaces widening of renal tubules (magnification: 200×).

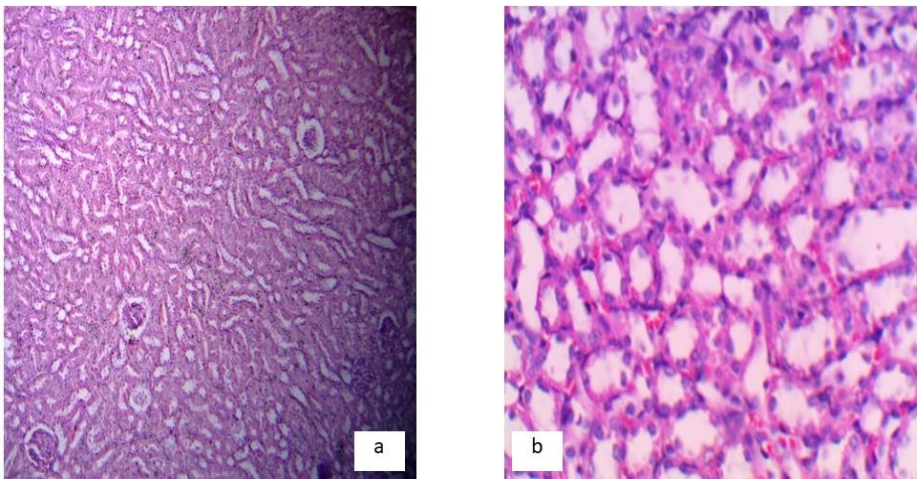


Figure (6): Light micrograph of kidney sections of $KBrO_3$+MOE-treated group (hematoxylin and eosin stain) showing **(a)** moderated reduction in glomeruli number and size, associated with mild retraction of the interstitial spaces with a widening of renal tubules (magnification: 200×) and **(b)** marked improvement of architecture of tubules (magnification: 400×).

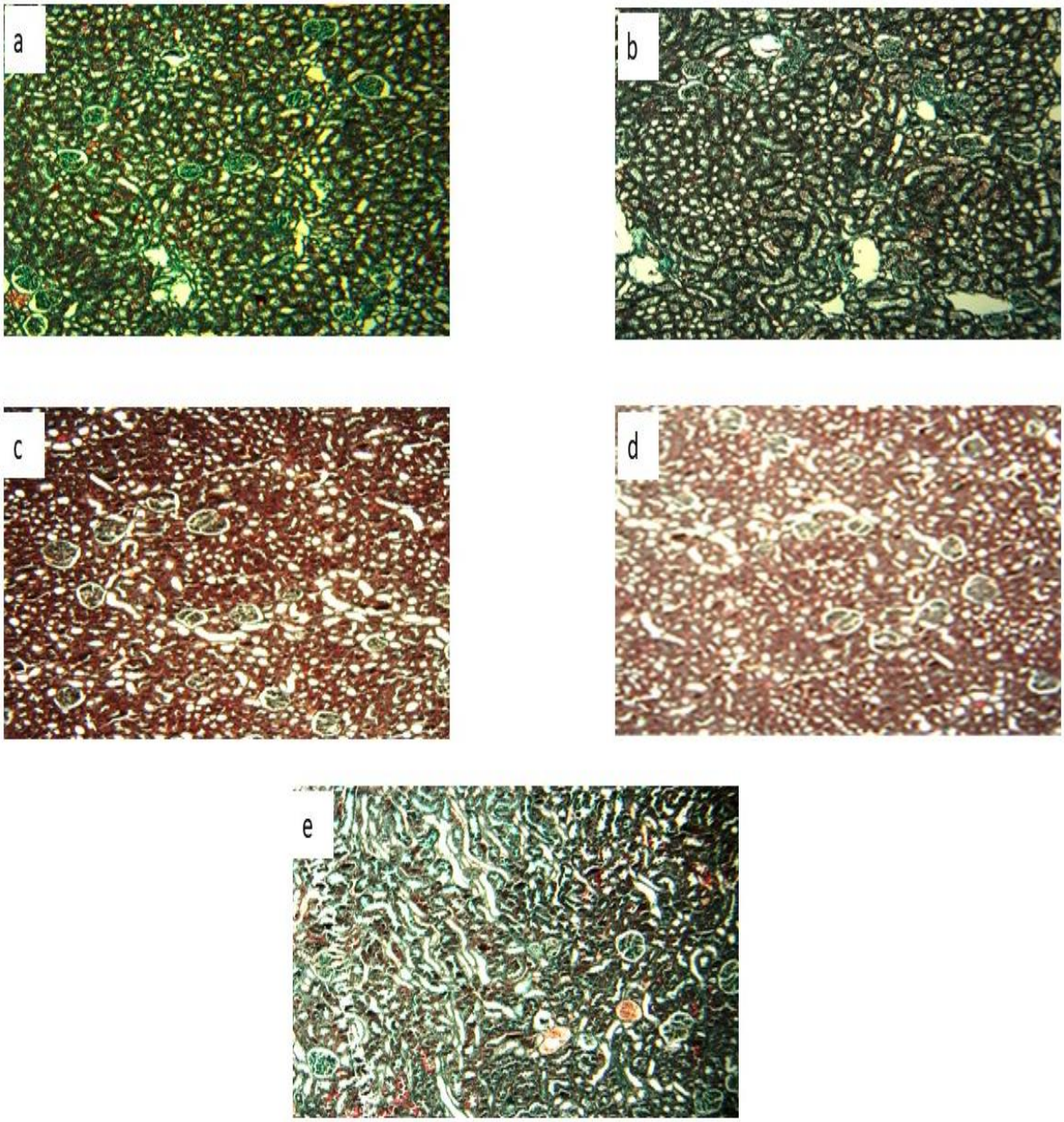


Figure 7: Photomicrographs of renal sections stained with Masson trichrome showing: **(a** and **b)** no evidence of fibrosis (negative control and MOE groups, respectively); **(c** and **d)** evidence of tubulointerstitial fibrosis indicated by dense deep red staining, and fibrosis with tubules score = 92% ($KBrO_3$ group); (e) a marked improvement proved by a reduction in the fibrotic score to 62% ($KBrO_3$+MOE group). MOE: aqueous extract of *Moringa oleifera* leaves.

previously[19]. The kidney is responsible for the management of different electrolytes and the care of homeostasis[20]. Sodium (Na^+) and potassium (K^+) are chief components of extracellular and intracellular fluids, respectively, therefore the raised levels of these electrolytes could signify renal dysfunction, mainly at glomerular and tubular levels[19]. The current result showed that $KBrO_3$ was correlated with a significant increase in serum levels of Na^+ and K^+ ions; this agrees with Adewale *et al*.[6] who mentioned that oral ingestion of $KBrO_3$ alone increased significantly the serum electrolytes "Na^+, Cl^-, HCO_3^- and K^+". Meanwhile, the animal group received

MOE with $KBrO_3$ showed a significant decrease in serum urea, creatinine, Na^+, and K^+ levels as compared with the $KBrO_3$-treated group. In addition, Adedapo *et al*.[21] found a significant improvement in urea/creatinine ratio after co-exposure to MO stem methanolic extract and glycerol that could be linked with restored tubular architecture compared with the toxicant group. Also, Omodanisi *et al*.[22] found that MO administration reduced the creatinine level, suggesting MO's ability to restore/enhance the kidney functional status in diabetic-nephrotoxic rats.

In comparison to the control group, the group received $KBrO_3$ showed a significant rise in NO and MDA levels in kidney tissue, as well as a significant decline in the enzymic activities of renal antioxidants "SOD, CAT, and GPx". These findings might be attributed to the nephrotoxic effect of $KBrO_3$, which can mediate renal oxidative stress[23]. The kidney is sensitive to oxidative stress because of its minimal amounts of antioxidant protection systems including antioxidant enzymes[8]. The elevated levels of NO in rats of the $KBrO_3$ group correlated with the report of Adewale *et al*.[6], which demonstrated that $KBrO_3$ treatment signified tissue damage in rats by increasing NO. Likewise, Watanabe *et al*.[24] mentioned that $KBrO_3$ is established to reduce the activity of glutathione peroxidase and increase the formation of free radicals as superoxide anion radical, NO, and peroxynitrite anion ($ONOO^-$), and increase lipid peroxidation in the rat's kidney. Akomolafe *et al*.[8] showed a substantial drop in the SOD, CAT, and GPX activities and GSH level in the $KBrO_3$-treated group compared with the control group, which is consistent with our findings. Furthermore, when compared with the control, $KBrO_3$ caused a substantial rise in MDA levels in the kidneys of rats. In the present study, the inadequate antioxidant enzyme activity and the elevated MDA concentrations suggested oxidative injury to the kidney tissues of rats. After $KBrO_3$ treatment, Abd Elmaksoud *et al*.[18] discovered a large increase in renal tissue MDA, as well as a significant decrease in renal tissue antioxidants (SOD and GPx). Khan *et al*.[2] stated also that the reduction of antioxidant responses has been a concern in the kidney toxicity with $KBrO_3$. The current results stated that oral administration of MOE with $KBrO_3$ during the experiment period declined significantly the levels of renal NO and MDA, as well as increased significantly the activity of renal SOD, CAT, and GPx enzymes when compared with the $KBrO_3$-treated group. Ijaz *et al*.[25] reported also that MO protected from paracetamol-induced nephrotoxic in rabbits.

TNF-α and IL-6 are pro-inflammatory cytokines that are thought to play a role in the pathophysiology of chronic kidney disease[26]. ROS can cause inflammation by activating transcription factors, which cause pro-inflammatory cytokines like IL-6 and TNF-α to be secreted[27]. The current results showed that $KBrO_3$ enhanced the inflammatory response in the kidneys, as seen by increasing the expression level of renal TNF-α and IL-6. These findings are consistent with those of Elsayed and Barakat[28] who found a high level of renal IL-6 in $KBrO_3$-intoxicated rats. Also, Okoko[29] revealed that $KBrO_3$ caused a significant release of TNF-α and IL6, which indicates that the molecule activates macrophages. However, the animal group received both MOE and $KBrO_3$ in the present study revealed a suppression in the overproduction of TNF-α and IL-6 in renal tissue that provided a protective effect against the kidney pathological changes. The anti-inflammatory influence of MOE has been earlier reported using different experimental designs, Edeogu *et al*.[30] confirmed that MOE prevented the progress of renal inflammation in response to gentamicin administration by reducing the concentrations and expressions of IL-1β, IL-6, TNF-α, NO, nuclear factor(NF)-κB, and inducible nitric oxide synthase. Abdel-Daim *et al*.[15] revealed that pre-treating the rats with MOE suppressed significantly the progression of kidney inflammation following lead acetate exposure. The major

mechanism of action MO as an anti-inflammatory was indicated to be through the suppression of the NF-κB pathway[15].

Statistical analysis revealed a rise in the expression of TGF-β1 protein in the renal tissues of the $KBrO_3$-treated group when compared with the control group. This result was consistent with Bayomy *et al*.[31] who reported that $KBrO_3$ treatment is associated with inflammatory cell infiltration and deposition of a massive amount of collagen fibers in the tissues; they reasoned that ROS and oxidative stress stimulated the expression of proinflammatory and pro-fibrotic molecules. Our results also agree with Ali *et al*.[23] who declared that $KBrO_3$ daily administration for 28 days resulted in inflammatory cell infiltration and fibrosis in rat kidneys, which increased gradually with increasing the $KBrO_3$ dose. In the current experiment, the administration of MOE reduced the expression of TGF-β1; this reduction was attributed to MOE's role in attenuating the $KBrO_3$-induced fibrosis process. In addition, Susanto *et al*.[32] showed the significant activity of MO in decreasing TGF-β1 expression in the mice model that developed hepatocellular carcinoma.

Prolonged inflammation causes failure of regeneration and extracellular matrix accumulation; thus, one of the mechanisms for inhibiting fibrosis is inflammatory inhibition. This study proved that MOE has an anti-inflammatory effect that inhibited the fibrosis process and associated with a decrease in the proinflammatory cytokine "TNF-α and IL-6: production. Farid and Hegazy[33] stated that MOE utilized the excretion of cytokines to realize the equilibrium between the pro-and counter inflammatory signaling paths. The active substances in MO such as quercetin could inhibit the NF-κB pathway by inhibiting the translocation of NF-κB factor p65 to the nucleus, so that it inhibits the inflammatory effect[34].

The *miRs* are epigenetic controllers of gene regulation that can influence a variety of cellular processes, including development and illness[35]. The *miRs* like *miR-192*, *miR-194*, *miR-21*, *miR-200a*, and *miR-204* are concentrated in the kidney as well as other organs, according to evaluations of m*iRNA* expression patterns[36]. The *miRs* are also important in standardizing renal physiology functions, from blood pressure management to fluid and electrolyte equilibrium throughout the body[37]. TGF-β1 stimulates the transcriptional and post-transcriptional invention of the profibrotic *miR-21* in cultured proximal tubular epithelial cells through mothers against decapentaplegic homolog 3 (Smad3) signaling pathways[38]. In the present experiment, animals that received $KBrO_3$ showed an elevation of the expression of *miR-21*, which was associated with upregulation in the renal TGF-β1. Also, the expression of *miR-29* increased significantly in the current study in $KBrO_3$-treated group. Long *et al*.[39]. noticed also that an increase in quantities of *miR-29c* promoted cell apoptosis and fibronectin synthesis, which is associated with TGF-β1 signaling.

A functional relationship between *miR-192* and TGF-β1-motivated renal fibrosis has also been recognized, even though the effect of TGF-β1 on *miR-192* expression is not constant between different studies. Likewise, *miR-192* was noticed to be overexpressed in fibrotic kidneys of rodents following unilateral ureteral obstruction or renal mass excision[40], but upregulated or downregulated on models of experimental diabetic nephropathy[41]. The present study recorded a significant rise in renal expression of both TGF-β1 and *miR-192* in the $KBrO_3$-treated group. Several investigations in experimental animal models have concentrated on the beneficial possibility of *miRs* in chronic kidney disorders and hopeful results in stopping renal fibrosis have been acquired by knocking down *miR-21*[42], *miR-29c*[39], and *miR-192*[43]. In the current study, animals that received both $KBrO_3$ and MOE showed a reduction in renal expression levels of *miR-21*, *miR-29*, and *miR-192*.

The molecular parameters reported in the current investigation were validated by histopathological analyses. The renal histopathology abnormalities identified in the $KBrO_3$ confirmed the renal damage that might be caused by oxidative destruction. Earlier, research has suggested that $KBrO_3$ can cause glomerular injury, tubular necrosis, and other alterations[8]. This is consistent with the findings of the current investigation, which show degeneration of corpuscular tissues after $KBrO_3$ injection as compared with the control group. The current study found that $KBrO_3$ induced obvious histopathological changes including disruption in the architecture of kidney tissue; similar results have been reported by Eldurssi *et al*.[44]. Opara *et al*.[16] indicated that administration of $KBrO_3$ might produce labialization of the cell plasma membrane due to the occurrence of elevated oxygen content in each molecule of $KBrO_3$. Such disturbance of the regular lipid bilayer of the plasma membrane has been followed by a leak of the enzymes to the extracellular fluid[45]. It is established that the main mechanism of $KBrO_3$-produced nephrotoxicity is the construction of ROS, which begins lipid peroxidation and reduces both enzymatic and non-enzymatic antioxidants[7]. Yet, the animal group that received both $KBrO_3$ and MOE showed improvement in kidney tissue architecture. This finding was compatible with Abdel-Daim *et al*.[15] who emphasized the reno-protective impact of MOE against histopathological alteration in kidney tissue induced by lead acetate.

Renal sections of $KBrO_3$ model rats stained with Masson trichrome revealed marked tubulointerstitial fibrosis, which was indicated by a dense deep red staining and retraction of tubules with fibrosis. The fibrotic activity of $KBrO_3$ was reduced by the MOE, as indicated in the current study. Furthermore, Abd-Elhakim *et al*.[46] discovered that the ethanol extract of MO leaves inhibited fibrogenesis by reducing a tissue inhibitor of metalloproteinases "TIMP1" expression; the anti-fibrosis mechanism is thought to be derived from MOE's antioxidant and anti-inflammatory properties. The MO contains compounds with hydroxyl complexes, such as quercetin and kaempferol. The hydroxyl complex easily donates electrons to other unstable atoms such as free radicals; thereby, stabilizing, and neutralizing potentially pathological effects. The dried leaves of MO are a rich source of polyphenol components such as phenolic acids and flavonoids. As shown in the current study, MOE has high levels of coumarin, ferulic acid, resorcinol, quercetin, kaempferol, and phenanthrene. Flavonoids like quercetin, for example, are potent antioxidants, hypo-lipidemic, antidiabetic, and hypotensive, and they decrease oxidative stress and apoptosis[47]. Ferulic acid and resorcinol, which are phenolic chemicals found in MOE, have anti-inflammatory, antioxidant, and antiapoptotic properties[48]. As a result, the phenolic compounds and flavonoid concentration of MOE can be contributed to its antioxidative and anti-inflammatory characteristics. In conclusion, the current study showed that exposure to $KBrO_3$ was associated with a considerable rise in renal dysfunction markers, oxidative stress, pro-inflammatory, and profibrotic cytokines, in addition to histological alterations in kidney tissue, indicating a nephrotoxic impact. On the other hand, MOE administration inhibited $KBrO_3$ nephrotoxicity, probably *via* mitigating renal functions, ROS-mediated oxidative injury, inflammation, and fibrosis, as well as improving kidney tissue architecture.

COMPLIANCE WITH ETHICAL STANDARDS

Animal care and experimental procedures were carried out following the guidelines of the Committee of Care and Use of Experimental Animal Resources, Medical Research Center. Faculty of Medicine, Ain Shams University.

FUNDING SOURCE DISCLOSURE

This research received no funds**.**

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

AHK, MAF, and HAM designed the research. AHK and HAM performed the biochemical investigations, molecular assays, and statistical analysis. MAF presented the histological examinations. All authors carried out the experiments, drafted the manuscript, as well as revised and approved the manuscript.

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How to cite this article:
Kamel, A. H.; Fouad, M. A. and Mohamed, H. A. (2023). Protective effect of aqueous extract of *Moringa oleifera* leaves against potassium bromate-induced renal toxicity in rats. Egyptian Journal of Zoology, 79: 29-47 (DOI: 10.21608/ejz.2022.135884.1081).

التاثير الوقائي للمستخلص المائي لأوراق نبات "*Moringa oleifera*" ضد التسمم الكُلوي الناتج عن برومات البوتاسيوم في ذكور الجِرذان

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برومات البوتاسيوم هو عنصر حيوي يستخدم في المنتجات الغذائية والبيرة والأدوية ومستحضرات التجميل، وينتج عنه تأثير سام متوسط إلى خطير لمجموعة متنوعة من الأعضاء. هدفت هذه الدراسة إلى التحقق مما إذا كان المستخلص المائي لأوراق نبات المورينجا يمكن أن يحمي الجِرذان من السُمية الكُلوية التي يسببها برومات البوتاسيوم. تم استخدام أربع مجموعات تجريبية من ذكور الجِرذان المهقاء (ن=8) في هذه الدراسة: المجموعة الضابطة، مجموعة مستخلص أوراق المورينجا (400 مجم/كجم من وزن الجسم)، مجموعة برومات البوتاسيوم (100 مجم/كجم من وزن الجسم)، مجموعة مستخلص أوراق المورينجا + برومات البوتاسيوم، وذلك عن طريق الفم يوميا لمده ستة أسابيع. بعد انتهاء فترة التجربة تم فصل الكُلى ومصل الدم لإجراء التحاليل البيوكيميائية، والجزيئية، والنسيجية. وقد ارتبطت المعاملة ببرومات البوتاسيوم بارتفاع ذو دلالة إحصائية في مستويات اليوريا والكرياتينين والصوديوم والبوتاسيوم في مصل الدم، وفي مستويات المالونديألديهايد وأكسيد النيتريك في الأنسجة الكلوية، مع تقليل أنشطة الإنزيمات المضادة للأكسدة الخلوية. علاوة على ذلك، فقد أدت المعاملة ببرومات البوتاسيوم إلى إلتهاب وتليف الكُلى عن طريق زيادة عامل نخر الورم-ألفا والإنترلوكين-6، وعامل نمو الورم بيتا-1، متبوعا بزياده في التعبير الجيني الكُلوي لكل من "*miRNA21* و *miRNA29* و *miRNA192* ". وبالمقارنة مع المجموعة الضابطة، أظهر الفحص النسيجي للمجموعة المعاملة ببرومات البوتاسيوم تغيرات تنكسية وتلف في أنسجة الكُلى. على العكس من ذلك، أظهرت المعاملة المشتركة بمستخلص أوراق المورينجا + برومات البوتاسيوم عن انخفاضًا ملحوظًا في التأثيرات الضارة لبرومات البوتاسيوم في معظم المَعلمات التي تم قياسها. والخلاصة، يمكن استخدام المستخلص المائي لأوراق المورينجا كعلاج بديل للتخفيف من التأثرات الضارة لبرومات البوتاسيوم على الكُلى بسبب أنشطته المضادة للأكسدة وللإِلتهاب وللتليف.