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Effect of rosmarinic acid on potassium bromate induced renal cortical oxidative stress and apoptosis in adult male albino rat

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SUMMARY

Potassium bromate (KBrO3) is widely used as a food additive and is a major water disinfection by-product, in spite of its well-known oxidative cell and tissue damage. Therefore, the therapeutic efficacy of rosmarinic acid is examined to alleviate KBrO3 mediated renal oxidative damage. For this purpose, 24 adult male albino rats were categorized into four groups; group 1 (control); group 2: received 50 mg/Kg/day rosmarinic acid orally for 4 weeks; group 3: received 20 mg/Kg/dose KBrO3 orally twice weekly for 4 weeks, and group 4: received both KBrO3 and rosmarinic acid. After 4 weeks, serum was collected for analysis of kidney functions and kidneys were sampled for histopathological and biochemical analysis. The results indicated that treatment with rosmarinic acid significantly abated most of the indices and biomarkers of the renal toxicity caused by KBrO3. It significantly ameliorated histopathological changes and the changes in the immunoexpression of proapoptotic protein (Bax), antiapoptotic protein (Bcl2) and inducible nitric oxide synthase (iNOS) induced by KBrO3. Taken together, it could be concluded that the rosmarinic acid has a beneficial effect against KBrO3-induced nephrotoxicity by its antioxidant and antiapoptotic effects.

Key words: Kidney – KBrO3 – Rosmarinic acid – Bax – BCL2 – iNOS

INTRODUCTION

Potassium bromate (KBrO3) is an oxidizing agent and a well-known flour enhancer which exists as a white crystals or powder. It is primarily used as a flour maturing agent and as a dough conditioner. It has been in use for the past 90 years as a food additive that gives strength and sponge like characters to the dough. Potassium bromate was decomposed into a stable compound potassium bromide (KBr) during the bread - baking process. However, this reduction is sometimes incomplete and residual KBrO3 remains in the bread, making a source of potential oxidative damage (Oloyede and Sunmonu, 2009). It is commonly used in cosmetic products (such as permanent hair weaving solutions and textile dying). In addition, KBrO3 may appear as a by-product in bromide containing water ozonization. It is also used in the beer processing of barley and is commonly added in Japanese fish paste products. It is still used (legally and illegally) as an improver for bread and cake in several countries, including the United States, although it has been associated with the development of several organ damage (Kakehashi et al., 2013). The International Cancer Research Agency (IARC) has classified KBrO3 as a potential human

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carcinogen (Group 2B) and has restricted its use in food processing (Kujawska et al., 2013). Several research on the safety assessment of potassium bromate has been conducted. Biotransformation of KBrO3 may result in oxidative damage to essential cellular macromolecules resulting in hepatotoxicity, neurotoxicity, genotoxicity and cancer in experimental animals (Ajarem et al., 2016; Bayomy et al., 2016). KBrO3's nephrotoxicity is attributed to its ability to trigger reactive oxygen species (ROS), lipid peroxidation, and 8-hydroxyguanosine modification in renal DNA (Spassova et al., 2015).

Cellular defense against oxidative stress is provided by several mechanisms. Reinforcing the endogenous antioxidant defenses and counterbalancing the reactive oxygen species by using natural antioxidants safeguards against drug-promoted toxicity (Brewer, 2011). Rosemary (Rosmarinus Officinalis) is generally used in food processing as a flavoring factor and spice. It consists of dried leaves and flowers and is a good source of biologically active phytochemicals because it consists of a variety of phenolic components, including rosmarinic acid, rosmanol, carnosol, carnosic acid, rosmadial 7-methyl-epirosemanol, isorosmanol and caffeic acid (Genena et al., 2008). It has many biological activities, including antioxidant (Bakirel et al., 2008; Lee et al., 2008), anti-inflammatory, antiapoptotic, and antitumor (Lee et al., 2008; Venkatachalam et al., 2013).

Therefore, this work was conducted to estimate the potential effects of rosmarinic acid as a preventive agent in rats against potassium bromate-evoked cortical oxidative stress and apoptosis by using histopathological, immunohistochemical and biochemical methods.

MATERIALS AND METHODS

Study design

The present study was carried out on 24 pathogen-free adult male albino Wistar rats weighing 200-250g. They were housed under similar conditions in clean, well-ventilated polypropylene cages and had a good supply of food and water throughout the experiment. The animal procedures were approved by the local Institutional Animal Ethical Committee of Faculty of Medicine, Tanta University, Egypt. All experimental procedures were performed from 9 to 11 a.m. Animals were acclimatized to their environment for at least 2 weeks before starting the experiment.

After the acclimatization period, the rats were randomly divided equally into four experimental groups in this study:

Group I: Served as a control group and received 1.0 ml of distilled water orally.

Group II (RA group): Received rosmarinic acid at a dose of 50 mg/kg/day dissolved in distilled water by oral gavage for 4 weeks (Makino et al., 2012).

Group III: Rats received potassium bromate (KBrO3) orally.

Group IV: Rats received rosmarinic acid orally, two hours before KBrO3 administration.

KBrO3 and rosmarinic acid used in the current study was purchased from Sigma Chemicals (Sigma, St Louis, MO, USA).

The concentration of KBrO3 used was 25 g per liter of distilled water. The corresponding groups were treated with KBrO3 (20 mg/kg; aqueous) intragastric twice a week for four weeks (Khan et al., 2012). The rosmarinic acid was given orally using a gastric tube in a dose of 50 mg/Kg/ day; for 4 weeks alone in group II and two hours before KBrO3 in group IV.

Processing of specimens

At the appropriate time, the rats were anesthetized by 50 mg/kg ketamine and 5 mg/kg xylazine intraperitoneally (Struck et al., 2011). The heart was exposed after incision of the chest wall, then 5 ml of intracardiac blood was drawn and serum was separated to estimate blood urea nitrogen (BUN) and serum creatinine. Then the rats were sacrificed, and kidneys were removed and perfused with a fixative solution (2% paraformaldehyde and 2% glutaraldehyde solution in 0.1 M phosphate buffer pH 7.2 and then weighed and sampled for histopathological studies and biochemical studies.

Assessment of nephrotoxicity

Level of blood urea nitrogen (BUN) and serum creatinine concentrations were measured using standard laboratory techniques to assess the extent of nephrotoxicity and the results were expressed as mg/dl (Altoom et al., 2018).

Assessment of renal oxidative stress

Left kidney tissues from all experimental rats were minced and homogenized (10%, w/v) separately in ice-cold saline. Homogenates were centrifuged at 18,000×g (+4°C) for 15 min and used for estimating renal malondialdehyde (MDA), reduced Glutathione (GSH), Glutathione peroxidase (GSH-Px) and Superoxide Dismutase (SOD) activities.

The thiobarbituric acid substrate assay was used to measure malondialdehyde (MDA; nmol/g wet tissue) as an indicator of lipid peroxidation, with a spectrophotometer (at 535 and 520 nm). Antioxidant activity was checked by estimating reduced GSH, GSH-Px and SOD. Reduced GSH (Glutathione Assay Kit, Item No. 703002, Cayman Chemical Company, Ann Arbor, USA) as a biomarker of protective oxidative injury was measured according to Ellman's method with a spectrophotometer and the results were expressed as µmol/g tissue protein (Altoom et al., 2018). The GSH-Px enzyme activity analysis was measured at 340 nm by spectrophotometer and expressed as U/g-tissue protein (Buyuklu et al., 2014). To estimate superoxide dismutase (SOD, Superoxide Dismutase Assay Kit, Item No. 706002, Cayman

Chemical Company, Ann Arbor, USA) activity (an antioxidant) (Units/mg protein) xanthine/xanthine oxidase (XO) assay was used by measuring the amount of reduced nitroblue tetrazolium (NBT), with one unit of SOD defined as the amount of protein that inhibits the rate of NBT reduction by 50% (Buyuklu et al., 2014).

Histological and immunohistochemical examination

The right kidney from each animal was cut longitudinally into two halves. The specimens were fixed in 10% neutral-buffered formalin for 24 hours, washed, dehydrated, cleared and paraffin-immersed. Then, 4 µm sections were stained with Mallory's trichrome and hematoxylin/eosin (H&E), and were examined under a light microscope, to detect the histological changes (Gamble, 2008). The histopathological findings in the sections were graded as grade 0: no change; grade1: mild, usually single-cell necrosis in scattered tubules; grade2: moderate with more than one cell in scattered tubules; and grade 3: marked which exhibiting total necrosis in almost every power field (Farombi and Ekor, 2006).

For immunohistochemical study four µm sections were stained to estimate immunoexpression of Bax (proapoptotic protein), Bcl2 (antiapoptotic protein), and inducible nitric oxide synthase (iNOS). According to the manufacturer's protocol, sections were incubated with a monoclonal antibody against Bax and Bcl2 (Dako, Carpinteria, California, USA); in a dilution of 1:200, and a polyclonal anti-iNOS antibody (Thermo Fisher Scientific, Massachusetts, USA) in a dilution of 1:100 using the streptavidin-peroxidase method. They were then washed in PBS and incubated at room temperature for 30 min with specific secondary antibody. The sections were then washed in PBS, revealed by treating with liquid diaminobenzidine, and then counterstained with hematoxylin. Cells were considered positive for Bax, Bcl2 and iNOS expressions if they showed brown precipitation. The same protocol was applied to the negative controls, but without applying the primary antibody.

Quantitative morphometric measurement

Leica Qwin 500 C Image analyzer computer system (Leica Imaging System LTD., Cambridge, England) in (Central Research Lab, Tanta Faculty of Medicine, Egypt) was used to obtain the morphometric data in the current work. Ten non-overlapping fields in slides of each animal in each group were examined to estimate:

The percentage area of Bcl2 and Bax immunoreactions at a magnification of 400X: The image analyzer was used to measure the area of Bcl2 and Bax and was expressed in relation to the area of the measuring frame of a known area (estimate area% /20µm2 frame).

The mean of optical density of iNOS reactions at a magnification of 400X: It was measured using the color detect menu and in relation to a standard measuring frame.

Statistical Analysis

The data were presented as the mean ± SD (standard deviation). Mann-Whitney U- test was used for the statistical analysis of the histological scores. Comparisons between two groups in all other data were analyzed by unpaired Student "t" test. However, the difference among the groups was assessed by One-way analysis of variance (ANOVA) followed by Post Hoc Tukey's test. The probability of chance (P value) < 0.05 was considered statistically significant. Analyses of all data were performed using the software Statistical Package for Social Sciences version 17 (SPSS Inc,

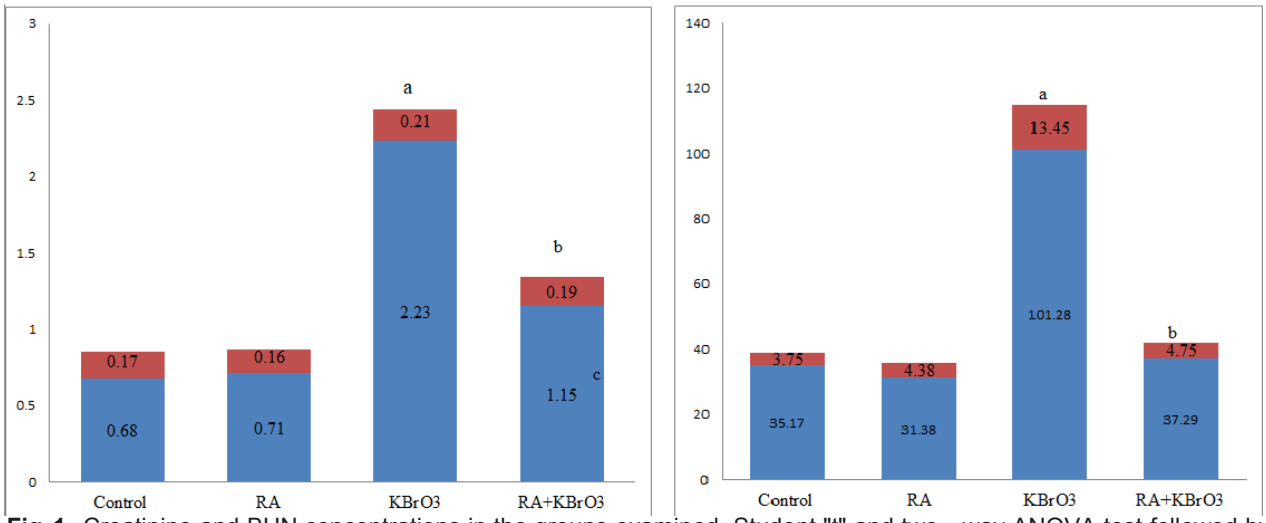


Fig 1. Creatinine and BUN concentrations in the groups examined. Student "t" and two - way ANOVA test followed by post - hoc test of Tukey were used. **a** *P*< 0.05 *vs* other groups; **b** *P*< 0.05 *vs* KBrO3 group. Data is expressed as mean ± SD. RA: rosmarinic acid.

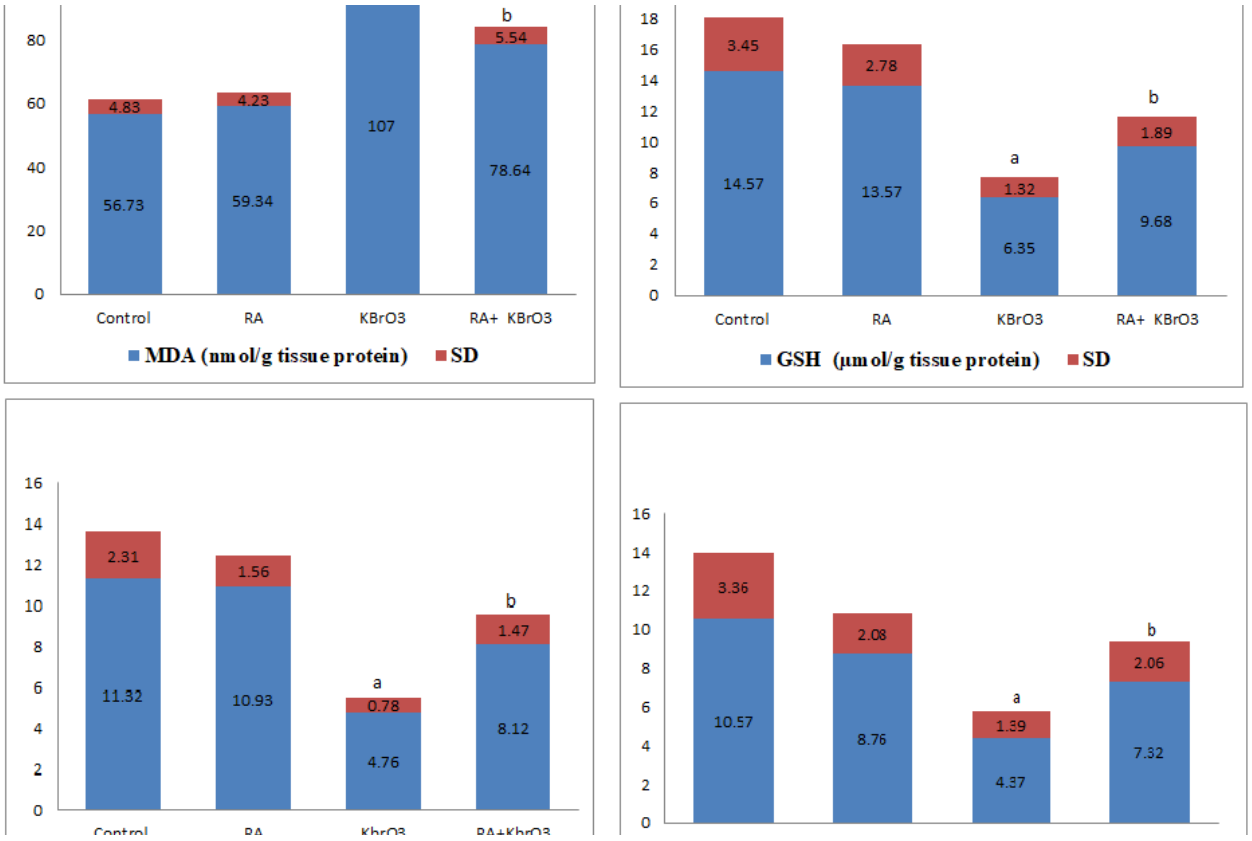


Fig 2. Measures of MDA, reduced values of GSH, GSH-Px and SOD in the groups examined. The student "t" and two - way ANOVA test followed by the post – hoc test of Tukey were used. a P< 0.05 vs other groups; b P< 0.05 vs KBrO3 group. Data is expressed as mean ± SD. RA: rosmarinic acid.

Chicago, IL, USA).

RESULTS

Biochemical results

Rosmarinic acid effect on serum creatinine and BUN

Figure 1 showed the effect of rosmarinic acid on serum creatinine and BUN. KBrO3-treated group showed significant increases in serum creatinine and BUN when compared to other groups. Treatment with rosmarinic acid in the KBrO3 group significantly ($P < 0.05$) decreases the rise in serum creatinine and BUN levels caused by KBrO3.

Rosmarinic acid effect on oxidative stress parameters

The group administered by KBrO3 showed a significant increase in the level of MDA. The same group also showed a significant reduction in GSH, GSH-Px and SOD levels compared to other groups. Treatment with rosmarinic acid in the KBrO3 group ($P < 0.05$) significantly improved the increase in MDA and the decrease in the levels of GSH, GSH-Px and SOD (Fig. 2).

Histological results

H&E-stained sections of control and rosmarinic acid groups were examined and showed normal renal cortex histological architecture. Multiple glomeruli were seen surrounded by the capsule of Bowman. The proximal convoluted tubules (PCT) were lined with pyramidal cells with rounded nuclei and eosinophilic cytoplasm. However, the distal convoluted tubules were lined by cuboid cells with less eosinophilic cytoplasm (Fig. 3).

Rats treated with KBrO3 showed shrunken glomeruli and capsular space dilation in Malpighian

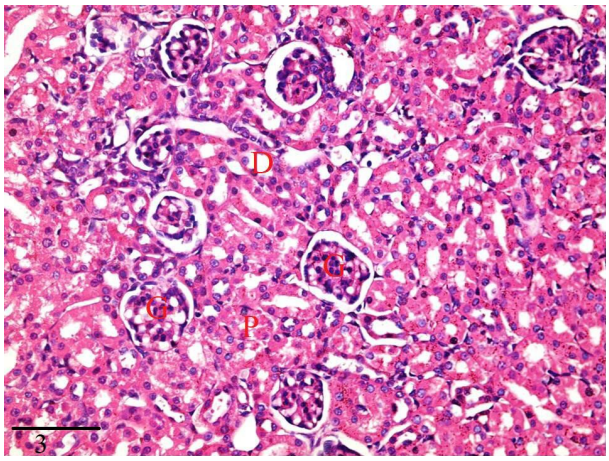


Fig 3. A control rat renal cortex showing the Malpighian corpuscles' normal architecture surrounded by a double walled capsule of Bowman and containing capillary tufts (**G**). PCTs with narrow lumen are seen around the corpuscles of Malpighi, lined by pyramidal cells (**P**). Wide lumen, DCTs are lined by cubical cells (**D**) (Hematoxylin & eosin stain, Bar: 20 µm).

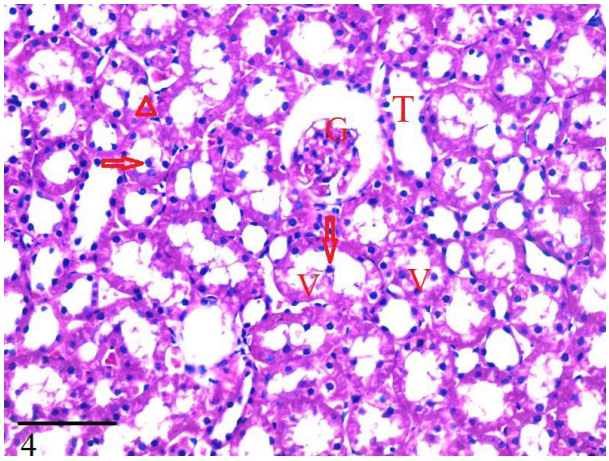


Fig 4. A KBrO3 treated rat renal cortex showing corpuscles of Malpighi with damaged shrunken glomeruli and widening of the capsular space (**G**) with dilatation of the surrounding tubules (**T**), vacuolar degeneration (**V**) and tubular wall disruption (**Δ**). Note: The presence of pyknotic nuclei desquamation of tubular lining cells (arrows). (Hematoxylin & eosin stain, Bar: 20 µm).

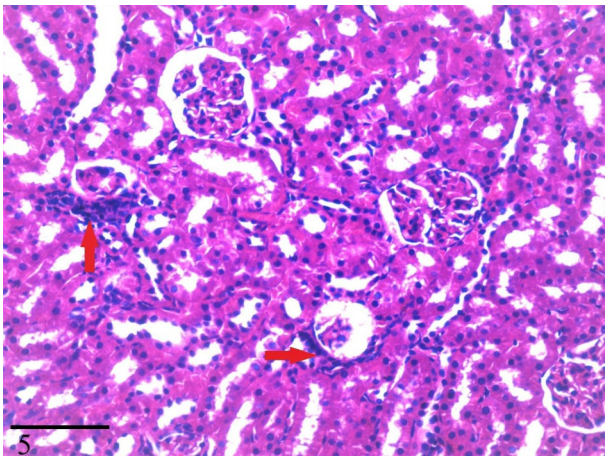


Fig 5. Showing interstitial cellular infiltration (arrows). (Hematoxylin & eosin stain, Bar: 20 µm).

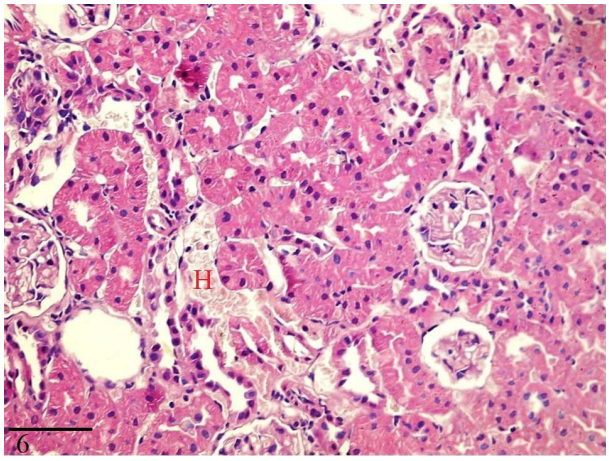


Fig 6. Showing interstitial hemorrhage (H). (Hematoxylin & eosin stain, Bar: 20 µm).

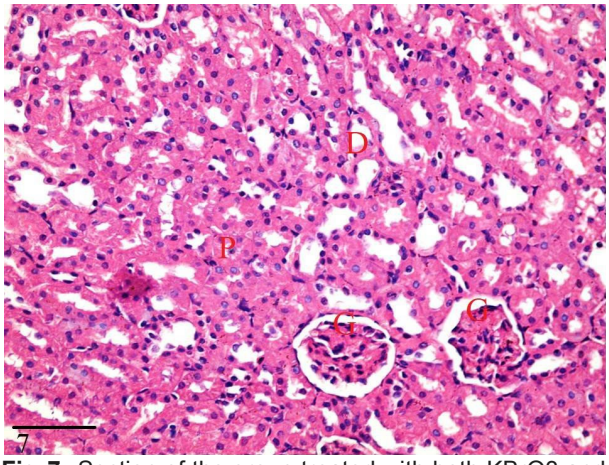


Fig 7. Section of the group treated with both KBrO3 and rosmarinic acid showing Malpighian corpuscles (**G**), PCTs (**P**) and DCTs (**D**) are more or less similar to control (Hematoxylin & eosin stain, Bar: 20 µm).

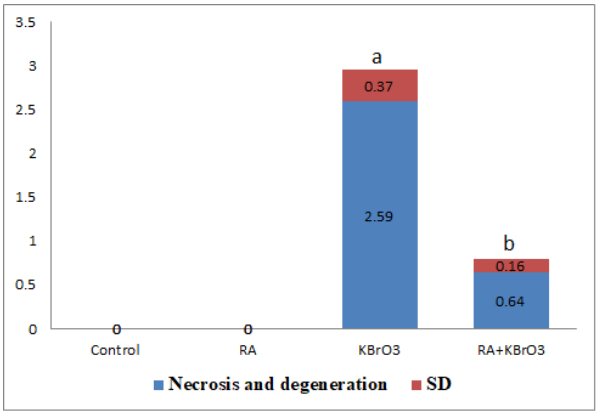
corpuscles. Degenerative changes have been observed in surrounding tubules as vacuolation with a loss of the lining epithelium. Additionally, a tubular wall interruption was occasionally observed. Several tubules also recorded outbreak of necrosis with pyknosis of the nuclei (Figs. 4-6). Interpretation of the histological score between the rats treated with KBrO3 and the normal rats showed a very significant variation (2.87 ± 0.34, P<0.001). Furthermore, interstitial hemorrhage (2.79 ± 0.26, P < 0,001) appeared significantly with mononuclear cell infiltration (Figs. 4-6).

Rosmarinic acid administration with KBrO3 improved the histological changes that induced by KBrO3. The renal cortex appeared with histological architecture nearly like control group (Fig. 7). The histopathologic score recorded showed significant (p<0.05) decrease (Fig. 8).

Table 1. The mean area % of Bax and Bcl2 and the mean optical density of iNOS in the different groups studied.

Parameters	**Group I** (control group)	**Group II** (rosmarinic acid)	**Group III** ($KBrO_3$)	**Group IV** (rosmarinic acid with $KBrO_3$)
Bax (area %)	0.065 ± 0.03	0.074 ± 0.02	18.453 ± 3.18 ^{a}P	2.37 ± 0.46 ^{b}P
Bcl2 (area %)	38.82 ± 3.17	36.89 ± 3.17	14.65 ± 1.58 ^{a}P	35.47 ± 2.21 ^{b}P
***i*NOS (Optical density)**	0.263 ± 0.056	0.276 ± 0.082	1.873± 0.168 ^{a}P	0.467 ± 0.214 ^{b}P

Data is expressed as mean ± standard deviation.
P. value = probability of chance, $P < 0.05$ is significant tested by Student "t" test
^{a}***P*** ($P < 0.05$) *vs* the control group (Group I).
^{b}***P*** ($P < 0.05$) *vs* $KBrO_3$ group (group III).



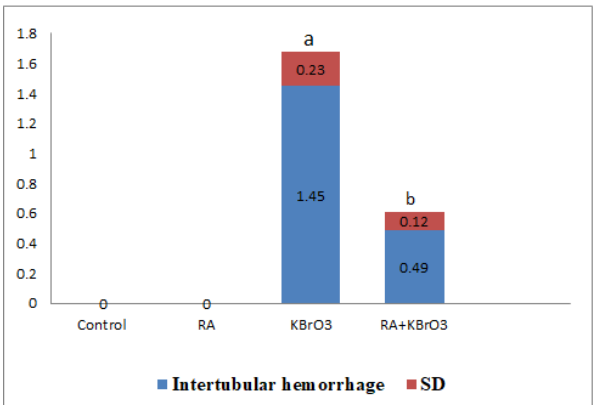


Fig 8. Mann-Whitney U-test was used to test the histopathological score in the examined groups. **a** P< 0.05 vs other groups; **b** P< 0.05 vs KBrO3 group. (group III). RA: rosmarinic acid.

Results of Immunohistochemistry

Immunohistochemical reaction of Bax antigen

In the control and rosmarinic acid groups, the kidney cortex showed no immunostaining reaction for Bax, while scarce dispersed cells showed weak reaction (Fig. 9A). Most of renal cortical cells in the KBrO3 group showed strong positive reaction (Fig. 9B); KBrO3 rats that received rosmarinic acid showed weak reaction (Fig. 9C). In comparison with the group of control animals, statistical analysis of morphometric data showed a significant increase in the area percentage of Bax positive cells in the KBrO3 group; while rats that were administered both KBrO3 and rosmarinic acid showed a significant decrease (P < 0.05) in the Bax area percentage compared to KBrO3 rats (Table 1).

Immunohistochemical reaction of Bcl2

Examination of cortical sections of control rats showed moderate to marked reactivity of Bcl2 (Fig. 10A). In the KBrO3 group, cortical tissue appeared with a weak reaction compared to rat control (Fig. 10B). Rats received both rosmarinic acid and KBrO3 showed a strong reaction of Bcl2 when compared to rats that received KBrO3 (Fig. 10C). Statistical analysis of morphometric data showed a significant reduction in the area percentage of positive Bcl2 cells in the KBrO3 group compared to the control group, while KBrO3 rats receiving rosmarinic acid showed a significant increase (P < 0.05) in the Bcl2 area percentage compared to rats administered KBrO3 (Table 1).

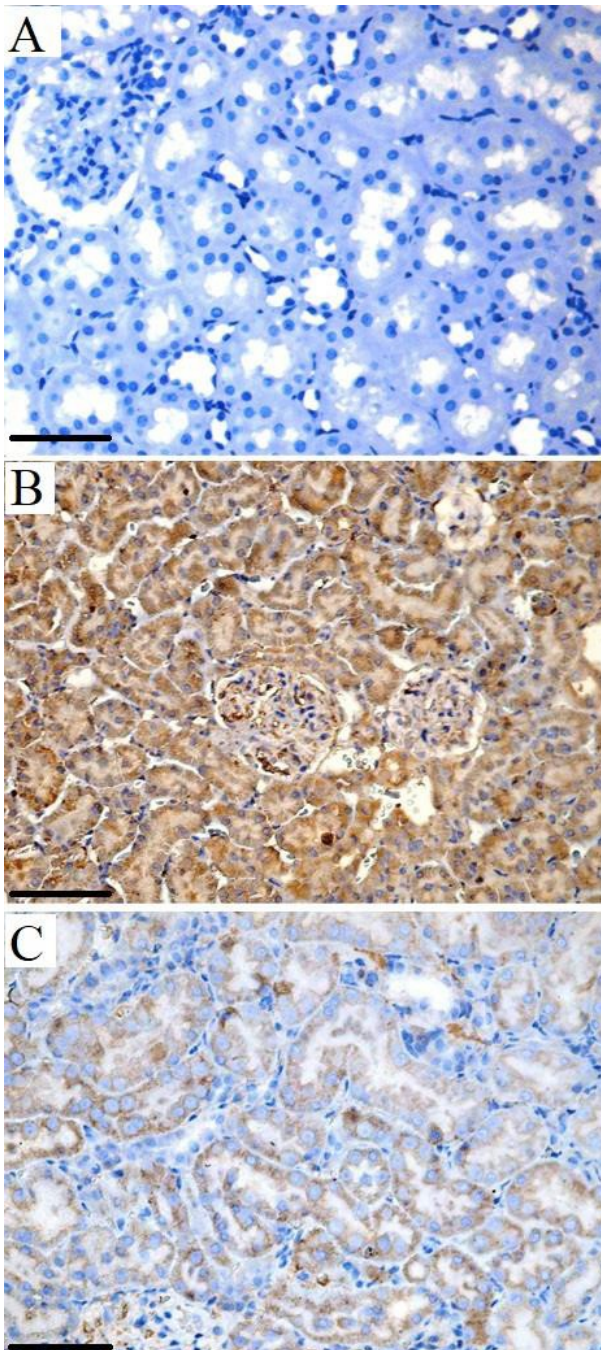


Fig 9. (**A**) Cortical tissue from control showing no Bax activity. (**B**) Marked expression of Bax in cortical tissue of KBrO3 group. (**C**) Weak expression of Bax activity in KBrO3 + Rosmarinic acid administrated group (Bax, immunostaining, Bar: 20 µm).

Immunohistochemical reaction of iNOS

The kidney cortex showed weak expression of iNOS reaction in the control and rosmarinic acid groups (Fig. 11A). Strong immunoreaction for iNOS was mostly shown in cortical tubules in rats

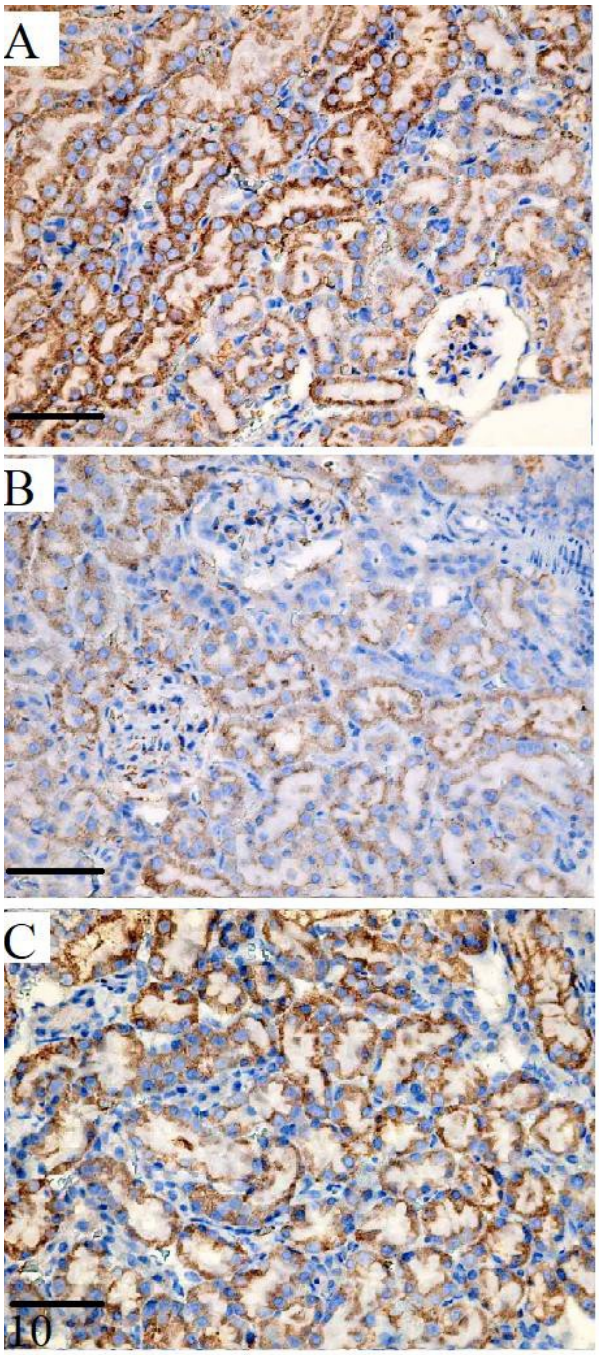


Fig 10. (**A**) Cortical tissue from control showing strong immunostaining reaction of Bcl2. (**B**) Weak positive immunostaining reaction at most in KBrO3-treated renal cortex tubules. (**C**) Strong positive reaction of Bcl2 in the renal cortex of the rosmarinic acid and KBrO3 treated group (Bcl2 immunostaining, Bar: 20 µm).

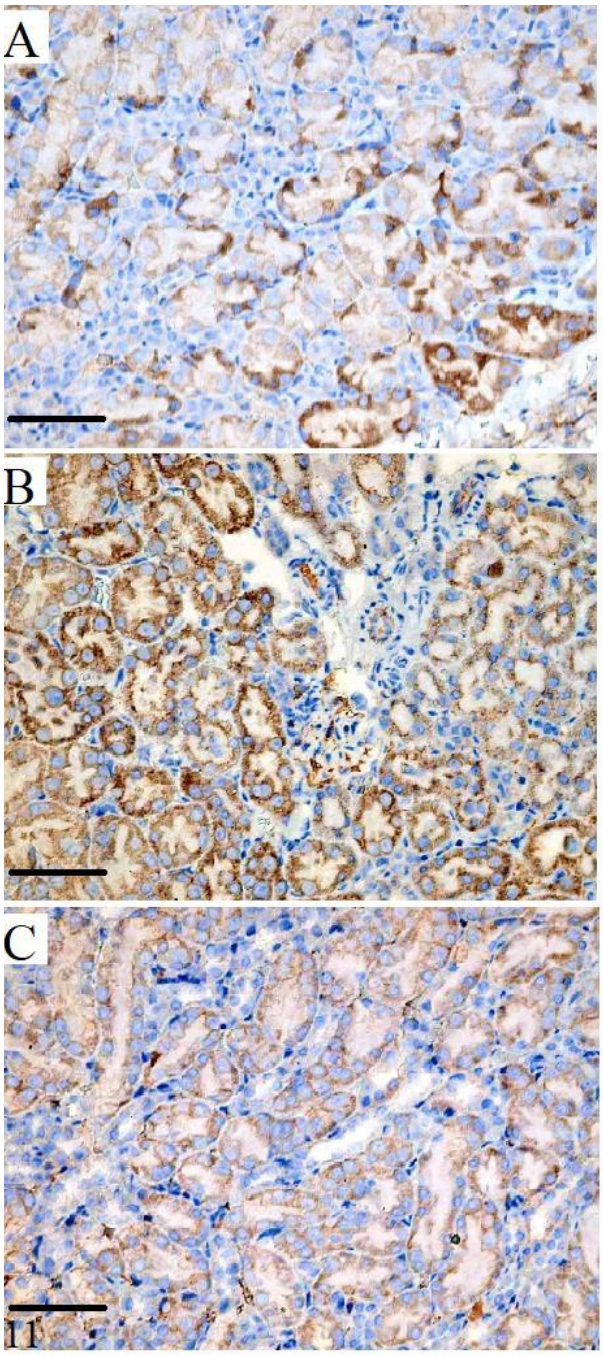


Fig 11. (**A**) Showing control rat cortical tissue with weak activity of iNOS immune expression. (**B**) KBrO3 treated rat cortical tissue with marked activity of iNOS immune expression. (**C**) Weak activity of iNOS immune expression in group treated with rosmarinic acid and KBrO3 (iNOS, immunostaining, Bar: 20 µm).

administered by KBrO3 and was scarcely noticed in glomeruli (Fig. 11B). Rats that were administered both rosmarinic acid KBrO3 showed low expression of iNOS activity (Fig. 11C). Statistically, the KBrO3 group showed a significant increase in iNOS optical density compared to rat control, while rats receiving rosmarinic acid and KBrO3 showed significant ($P < 0.05$) decrease in iNOS optical density compared to KBrO3 group (Table 1).

DISCUSSION

Several researchers reported that KBrO3 caused renal impairment in various species and strains of animals and at different doses (Ben Saad et al., 2016). However, in Fischer 334 rats, other authors found little or no evidence of renal impairment (Dodd et al., 2013). Our objective here was to study this agent's nephrotoxicity in male Wistar rats at an oral dose of 20 mg/kg/ twice per week

(Khan et al., 2013). We also tested whether co-treatment with a rosmarinic acid previously reported in rodents to abate the severity of cisplatin-induced kidney injury and diabetic nephropathy can also mitigate KBrO3 nephrotoxicity (Domitrovic et al., 2014; Tavafi et al., 2011).

The present study revealed that, compared to the control group, the administration of KBrO3 caused a marked increase in urea and creatinine serum levels. These results indicate that the KBrO3 induced acute kidney injury similarly to those previously noted. Hence, bromate is rapidly absorbed from the gastrointestinal tract, at least in part unchanged, so that its toxicity could appear in a few hours after ingestion (Watanabe et al., 2004; Bao et al., 2008; Khan et al., 2011).

In rats pretreated with rosmarinic acid, serum creatinine and BUN levels were significantly reduced compared to the KBrO3 group, suggesting their ability to protect the kidneys from destruction. These data are in agreement with previous results concluding amelioration of serum urea, creatinine, BUN and creatinine clearance in cisplatin-induced nephrotoxicity treated with rosmarinic acid (Domitrovic et al., 2014) and with other antioxidants (Ali et al., 2018).

Examination of H&E-stained sections of rats treated with KBrO3 revealed shrunken glomeruli with widening capsular space and tubular damage. There was a significant difference between the KBrO3 group and the control group in histological score. Previous reports supported the histological results recorded in the present study (Dodd et al., 2013; Ali et al., 2018). In the kidney tissues of rats pretreated with rosmarinic acid compared to the KBrO3 group, marked histological amelioration was observed as most of the Malpighian corpuscles and surrounding tubules (PCT and DCT) appeared normal, having the same characteristics as those of the control group. Domitrović et al. (2014) reported that rosmarinic acid improved histopathological changes evoked by cisplatin. These results are in agreement with the results of previous studies in which other antioxidants against KBrO3 nephrotoxicity have been used (Ali et al., 2018).

The pathophysiology of KBrO3-mediated kidney damage is related to the generated ROS as emphasized by the elevated renal MDA level and decreased renal levels of reduced GSH, GSH-Px and SOD in the present work. Malondialdehyde was measured to assess thiobarbituric acid reactive substances to indicate lipid peroxidation, and is used not only as an available parameter of oxidative stress but also to translate ROS into active chemicals and to magnify the function of ROS through the chain reaction, including cellular metabolism and functional impairment (Cheeseman, 1993; Halliwell and Gutteridge, 2007). Reduced glutathione is an important antioxidant molecule that can be used to withstand the induced oxidative stress by many organs, including the kidney and liver. Previous studies have shown that KBrO3 can reduce this molecule's tissue content (Altoom et al., 2018; Ali et al., 2018; Parsons and Chipman, 2000). In accordance with these previous results, the present study showed that after treatment with KBrO3, renal levels of GSH were reduced. These changes were well correlated with the renal histological findings, suggesting that oxidative stress plays a major role in the nephrotoxicity induced by KBrO3, and explaining the extensive and marked tubular necrosis that appeared throughout the cortex. Rosmarinic acid pretreatment significantly increases SOD, GSH, GSH-Px renal levels and significantly decreases MDA compared to rats treated with KBrO3. Its protective effect may be due to its high capacity for ROS scavenging (Zhang et al., 2015). It is shown that rosmarinic acid is an external source of vital antioxidant enzyme, superoxide dismutase, and β-carotene.

These results come in line with previous studies that indicated that rosmarinic acid, by activating antioxidant proteins, provides a protective effect against oxidative stress (Kafeshani, 2016).

Accumulating evidence suggests that apoptosis plays a critical role in different kidney injury experimental models (Servais et al., 2008; Bae et al., 2009). Bcl-2 family proteins, either pro- or anti-apoptotic, function as mitochondrial pathway molecular integrators. The pro-apoptotic proteins (Bax and Bak) undergo structural modifications after exposure to death signals and alter the integrity of the mitochondrial membrane, releasing cytochrome c and other pro-apoptotic molecules (Lalier et al., 2007). In the present work, the quantitative morphometric analysis of the immunohistochemical study showed a significant increase in the percentage area of positive Bax cells and a decrease in the mean optical density of Bcl2 immunoreactivity in the KBrO3 group compared to the control group. Ali et al. (2018) reported KBrO3-induced apoptosis. It is suggested that reactive oxygen species (ROS) are an important mediator of apoptosis induced by KBrO3, and are often responsible for the apoptosis signaling pathway mediated by mitochondria. Apoptotic stimuli cause cytochrome c release from mitochondria, resulting in a series of reactions that cause caspase activation (Servais et al., 2008).

Apoptosis inhibition seems to be one of the most interesting therapeutic approaches since renal injury has been reported to be suppressed after apoptosis inhibition (Homsi et al., 2006). Compared to rats treated with KBrO3, pretreated rats with rosmarinic acid showed a significant decrease in Bax activity in cortical tissues and a significant increase in Bcl2 expression, and thereby suppressing apoptosis. This is consistent with the result of Lee et al. (2008) reporting protective effects of rosmarinic acid against hydrogen peroxide-induced apoptosis as effectively suppressing Bax up-regulation and Bcl-2 down-regulation. Production

of nitric oxide (NO) by induction of inducible NO synthase (iNOS) is important for non-specific host defense, helping to kill tumors and intracellular pathogens. Enhanced iNOS formation of NO can contribute to the process of inflammation. Inducible expression of NOS could induce a substantial and sustained release of NO that could react further with superoxide form peroxynitrite and hydroxyl radicals, resulting in cellular injury (Trachtman et al., 2002). iNOS engages in deteriorated cellular media and oxidative environments (Forstermann and Sessa, 2012). iNOS may be absent or present in very small amounts in normal kidney tissue, but it increases in nephropathy (Manikandan et al., 2011). This data is consistent with the outcome of the current work as statistical analysis showed a substantial increase in iNOS immune expression in KBrO3 group compared to control rats. However, rosmarinic acid administration has significantly reduced the protein expression of iNOS in kidney tissues in the current research. This is consistent with previous researchers who have shown that rosmarinic acid inhibits the expression of NOS activity in rats treated with gentamicin in the kidney tissues (Bayomi et al., 2017). Thus, reducing the expression of iNOS and/or rosmarinic acid's antioxidant property may contribute to attenuating NO formation in the KBrO3-treated kidney tissues.

Conclusion

It could be concluded that, as indicated by biochemical, histological and immunohistochemical changes, KBrO3 has deleterious effects on the kidney. By reducing renal oxidative stress and apoptosis, rosmarinic acid has protective effects against KBrO3-induced nephrotoxicity. Accordingly, prohibiting the use of KBrO3 and using rosmarinic acid as nephroprotective agents are highly recommended.

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