Histopathological Study

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A B S T R A C T

Ruta chalepensis L. (Rutaceae) is a perennial herb, widely distributed in the Mediterranean area, with glabrous stem, alternate bipinnatisect leaves. It is an ancient medicinal plant still being used in the traditional medicine of many countries. Potassium bromate (KBrO₃) is a strong oxidizing agent that has been used in flour milling, as a cheese making, in beer malting, as a component of cold hair-wave liquids. This study studies the protective and curative effects of R. chalepensis oil extract against KBrO₃ toxicity on kidney of male rats. Accordingly, a total number of fifty male albino rats were divided into five groups. The first group served as a control animals. The second group was administered Rue at an oral daily dose of 0.5 g/Animal for four weeks. The third group received KBrO₃ 100 mg/kg/b. w. for four weeks. The fourth group (protective group) was initially administered with Rue alone for 2 weeks and followed by KBrO₃ in association with Rue for 2 weeks. The fifth group (therapeutic group) was first given KBrO₃ alone for 2 weeks and was secondly administered Rue in association with KBrO₃ for 2 weeks. At the end of 2nd and 4th weeks of treatment, the kidney tissues were collected for histopathological studies. Histopathological studies revealed that the renal reactions of rats from the Rue group showed normal architecture pattern same that observations as in the control rats. The rats treated with KBrO₃ showed vacuolation and contraction of glomerular tuft, separation in intertubular connective tissue, necrotic changes in some areas and hyaline changes in tubules. Furthermore, lymphoid infiltration and intratubular hemorrhage, dilated in tubules with loss of cellular boundary. In the protective group minimal changes in the structure of the kidney was revealed compared to control. Both glomeruli and convoluted tubules gained the normal features. In therapeutic group, renal sections revealed improvement in the histological structure though there was a mild necrosis and slight in inflammatory leukocytic infiltration. The results of this study suggest the nephroprotective effect of KBrO₃ and the ameliorative effect of Rue when administrated for protective and therapeutic purposes.

Key words: Rats, Kidney, Potassium Bromate, Ruta chalepensis, Histopathology.

INTRODUCTION

Potassium bromate KBrO₃ is colorless and odorless crystals, which can be a granular or powder form and have negligible vapor pressure, soluble and can dissociate in water to the metal and bromate ions¹,² stated that the biopsy of the kidney exposed to KBrO₃ showed atrophy, necrosis, degeneration and regeneration of the proximal tubular epithelium and in the later stages, sclerosis of the glomeruli and interstitial fibrosis have been reported. Cardio-toxicity and hepatotoxicity have also been observed. KBrO₃ caused degeneration and necrotic change in the kidney³ Small fatty vacuoles in the epithelial cells of the renal proximal convoluted tubules, hemorrhage and congestion of the blood vessels of the heart were reported by⁴. Kidney showed dilated urinary
spaces with many glomeruli showing lobulated tufts, glomeruli and desquamation of tubular epithelia after treated with KBrO₃ in goats³. KBrO₃ in mice caused degenerative changes of tubular cells, cytoplasm vacuolation, cellular infiltration, tubular dilation with eosinophilic debris and clear cell cytoplasm⁶/⁷ showed unsteadiness in movement, difficulty in breathing and two rats died after the 18 days when administrated 150 mg/kg b. w. of KBrO₃ and showed degenerative changes and haemorrhage in the cerebellum after administrations of 100 and 150 mg/kg b. w. of KBrO₃.

R. chalepensis L. is a native herb of the Mediterranean region⁸. This plant is widely used in folk medicine as an antihemorrhagic, antispasmodic and as a treatment for snake bites, headaches and wounds⁹. Additionally, from Ruta use as an abortifacient, rue was also used in ancient Greece and Egypt to reinforce vision.¹⁰ Ruta species are sources of different classes of natural products with biological activities, including antifungal, antioxidant, phytoxic, abortive depressant, antidotal and anti-inflamatory activities.¹¹ Ethanol extract of R. chalepensis from 200 to 5000 mg/kg, p. o. dose did not produce mortality or weight loss during the observation period of 14 days. Rue is used in Mediterranean folk medicine to treat pulmonary conditions, for example tuberculosis and to decrease swelling of the spleen, as well as outwardly to treat wounds.¹² Herbal medicines derived from plant extracts are being progressively used to treat an extensive diversity of diseases. Nevertheless, relatively little knowledge about their mode of action is available.¹³ Therefore, this study investigates the protective and therapeutic effects of Ruta chalepensis against KBrO₃ toxicity on kidney of male rats.

MATERIALS AND METHODS

Animals:

Fifty male albino rats (Rattus norvegicus), weighing between 275-300 g were used throughout the present study. They were obtained from the animal house of Zoology Department, Faculty Science, Omar Al-Mukhtar University. The animals were housed in groups of five in standardized cages and were located in the same room with constant environmental conditions such as temperature (22 ± 3°C) and humidity (50 - 60 %). They were supplied with enough rat feed and drinking water ad-libitum. All animals were allowed to acclimatize in the environment for two weeks before the commencement of the study which lasted for four weeks.

Chemicals:

Potassium bromate (KBrO₃): Potassium bromate with the empirical formula (KBrO₃) obtained from (BDH) company (England). KBrO₃ was dissolved in distilled water freshly prepared.⁷

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Rue (Ruta chalepensis L.): Leaves of R. chalepensis were collected from Al-Jabal Al-Akhdar region on the east coast of Libya during the period of March 2016. The extraction process for the rue essential oil followed the methodology described by.¹⁴ The collected flowers and leaves were weighed and washed with water dried and then placed in acetone inside sealed jars for 48 hrs. Solvent was removed from samples by rotary evaporator and then oils were collected. R. chalepensis was orally administrated at a dose of 0.5 g/Animal¹⁵. Both doses were orally given through a special stomach tube with a smooth tip to protect the interior lining of the oral and buccal cavity from injury.

Experimental animals grouping:

The animals were divided into 5 equal groups, each contains 10 male rats: 1) Control Group (G1): Animals of this group received distilled water daily by oral gavage for four weeks. 2) The Rue Treated Group (G2): Rats received Rue orally in a daily dose of (0.5 g/Animal), for four weeks. 3) The (KBrO₃)-Treated Group (G3): This group included rats that were administrated (KBrO₃) in a daily dose of (100 mg/kg b. w.) for four weeks. 4) The Protected Group (G4): Animals of this group were first administrated Rue orally in a dose of (0.5 g/Animal) daily for two weeks and secondly administrated daily oral doses of Rue (0.5 g/Animal) in association with (KBrO₃) (100 mg/kg b. w.) for an additional two weeks. 5) The Therapeutic Group (G5): Animals of this group were first provided with oral dose of (KBrO₃) (100 mg/kg b. w.) daily for two weeks, then were treated orally with (KBrO₃) (100 mg/kg b. w.) in association with Rue (0.5 g/Animal) for an additional two weeks.

Preparation of tissue samples:

At the end of experiment, animals from control and treated groups were sacrificed 24 h after the last dose. The Kidneys were dissected out, washed in saline and dried on filter paper. Kidney samples were kept in 10 % neutral buffered formalin solution for histopathological examination.

Histopathology:

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 micrometers thick were obtained using a rotary microtome and stained with Harris's Haematoxylin and Eosin stain for general histological examination.

RESULTS

The kidneys of group 2 showed normal architecture of tissue, as well as normal appearance similar to that in the control group (Figures 1 and 2). In group 3 examination
of the kidney sections of KBrO$_3$-treated rats, after 2 weeks revealed separation in intertubular connective tissue and the glomerular tufts were contracted and vacuolated. Focal areas of necrosis and hyaline cast in tubules, haemorrhage and chronic inflammatory cells were also noticed. In addition, Tubules were dilated with loss of cellular boundary, atrophy of glomerular tufts, cloudy swelling, hyaline degeneration (Figures 3 and 4). After 4 weeks the kidney sections showed congestion of intertubular blood vessels, lymphocytic infiltration in tubules and separation in intertubular connective tissue. In addition, necrotic areas were invaded by fibroid tissue bands and degenerations with desquamation of tubular epithelial cells, some vacuolization, congestion, necrosis, and lymphocytic infiltration in tubules (Figures 5 and 6). Sections from group 4 showed a normal histological structure of kidney tissue. No pathological changes were observed. Kidney sections from the protective group that was treated with Rue for 2 weeks followed by double treatment with Rue and KBrO$_3$ for another 2 weeks and sacrificed after 4 weeks, revealed an almost complete prevention of histopathological alterations. There were minimal changes in renal structure, both glomeruli and convoluted tubules gained partial near to normal features compared with KBrO$_3$-treated group (Figure 7). In group 5, kidney sections of rats administered of KBrO$_3$ for 2 weeks revealed different alterations in comparison with control.

The kidney sections revealed lymphatic infiltration, homogenous eosinophilic masses. The convoluted tubules were distorted and some glomeruli were shrunken with intratubular hemorrhage, tubular necrosis and cortical interstitial edema, also, loss of the brush border of renal tubules were showed. These alterations are similar to those of group 3 after 2 weeks (Figures 3 and 4). On the other hand, at the end of experimental duration (4 weeks), the kidneys markedly recovered the toxic changes to near the control kidneys. During 4 weeks, there was an improvement in the histological structure of the kidney though there was a mild necrosis and slight of inflammatory leucocytic infiltration (Figure 8).

**DISCUSSION**

Potassium bromate has been considered as a food additive for the past 90 years. It is also used in pharmaceutical and cosmetic industries.\(^\text{18}\) *Ruta chalepensis* (Rue) is an aromatic evergreen shrub belongs to the family Rutaceae. It is native to the Mediterranean and is currently distributed worldwide.\(^\text{19}\) Rats orally receiving a daily dose of Rue for two and four weeks showed near to normal architecture pattern.

The glomerulus and proximal and distal convoluted tubules showed normal appearance. It is accepted that ethanolic and aqueous extracts of *R. graveolens* seeds at dose 50 mg/kg/day via the oral route caused glomerular alteration, fatty change and dilatation of the renal tubules. The present study indicated that KBrO$_3$ induced marked histopathological alterations in the kidney tissue of rats after 2 weeks. These alterations include disturbance in architecture of kidney tissue, vacuolation and contraction of glomerular tuft, separation in intertubular connective tissue, necrotic changes in some areas and hyaline cast in tubules. Furthermore, degenerative lesions, lymphoid infiltration, intratubular and intertubular connective tissue hemorrhage, dilated tubules with loss of cellular boundary, glomerular atrophy, and cloudy swelling hyaline degeneration were also observed. The same injuries occurred after four weeks, in addition to severe disorganization of kidney, fibrosis tissue bands, desquamated epithelial cells and loss of brush border. Similar results have been reported by\(^\text{21}\) where changes in the epithelium of Bowman’s capsule and vacuolization of glomerular cells were revealed. Proximal convoluted tubule and distal tubules of nephron showed marked changes, such as, ragged tubular cells and necrosis of individual tubular epithelial cells.

Dilatation of tubules with loss of brush border was reported by\(^\text{22}\) Degenerative changes in the proximal convoluted tubules reinforce the views of\(^\text{21,23}\) where it was found that many chemicals have a direct nephrotoxic action and exerted their effects principally on the proximal convoluted tubule. Hemorrhage and congestion of the blood vessels of the kidneys were seen in the rats fed KBrO$_3$. There was congestion of the blood vessels of the kidneys, focal necrosis of renal proximal convoluted tubules with packing of the glomeruli and with lymphocytic infiltration\(^\text{16, 21}\) and\(^\text{24}\). The inflammatory infiltration revealed in the kidney may be due to deposition of immune complexes probably generated by the chemical compound.

The results of this study, thus, indicate that administration of KBrO$_3$ might lead to labialization of the cell plasma membrane due to the presence of high oxygen content per molecule of KBrO$_3$. Such disruption of the ordered lipid bilayer of the plasma membrane has resulted in leakage of the enzymes to the extracellular fluid.\(^\text{25}\) The presence of necrosis may be related to the depletion of ATP, which finally leads to the death of the cells.\(^\text{26}\) Renal medullary necrosis occurs as a primary manifestation of renal disease. The mechanism of which is poorly understood, but it seems to involve a vascular change. Prostaglandin synthetase is Also found in the kidney, primarily in the medulla, and inhibition of this enzyme resulted in decreased production of prostaglandin E2 (PGE2) and loss of its vasodilatory effect on juxtedamillary arterioles.\(^\text{27}\) In the protective group, after 2 weeks the histological patterns of the kidney showed somewhat healthy appearance as the kidney.
Figure (1): Photomicrograph of a section of kidney of control rat showing, the glomerulus (G), surrounded by Bowman’s capsule (arrow), outer and inner visceral layers and a capsular space ( ), the proximal (P) and distal (d) convoluted tubules (H & E, X 400).

Figure (2): Photomicrograph of a section in kidney of rat treated daily with Rue (0.5 g/Animal) for 4 weeks, showing normal architecture of renal tissue almost similar to that demonstrated by the control group (H & E X 400).

Figure (3): Photomicrograph of a section in kidney of rat treated daily with KBrO₃ (100 mg/kg) after 2 weeks showing, contraction of the glomerular tuft (G), degenerative lesions in some of the renal tubules (arrows), lymphoid infiltration (Head arrows) and intratubular hemorrhage (H) (H & E, X 400).

Figure (4): Photomicrograph of a section in kidney of rat treated daily with KBrO₃ (100 mg/kg) after 2 weeks showing, tubular degeneration (D) and hemorrhagic changes in intertubular connective tissue infiltrated by chronic inflammatory cells (arrow). Tubules are dilated with loss of cellular boundary (Head arrows) (H & E, X 400).

Figure (5): Photomicrograph of a section in kidney of rat treated daily with KBrO₃ (100 mg/kg) after 4 weeks showing, marked injury with desquamated epithelial cells (Head arrow), loss of brush border (B), dilation of tubules and degenerated epithelial cells are visible in the lumens of necrotic tubules (arrow) (H & E, X 400).

Figure (6): Photomicrograph of a section in kidney of rat treated daily with KBrO₃ (100 mg/kg) after 4 weeks showing, sloughing of tubular epithelial cells (S), some vacuolization, congestion, necrosis. Endothelial rupture in capsule (ERC), damaged glomeruli and tubules are dilated with loss of cellular boundary (H & E, X 400).

Figure (7): Photomicrograph of a section in kidney of rat from protective group after 4 weeks revealed approximately normal appearance of glomeruli and renal tubules, evidenced by preservation of tubular histology compared with KBrO₃-treated group (H & E, X 400).

Figure (8): Photomicrograph of a section in kidney of rat from therapeutic group after 4 weeks showing, improvement the histological structure of the kidney though there was a mild necrosis and slight of inflammatory leucocytic infiltration (H & E, X 400).
tissue displayed a near to normal architecture and kidney restored their morphological feature. This study may be the first attempt on the combined analysis of protective effects of Rue against KBrO₃ toxicity in kidneys. These results are similar to the findings of 28. In the therapeutic group, there was mild improvement as a result of Rue administration where moderate destruction of renal structure. Histological features of kidneys were variable in different parts. These alterations were manifested in disorganization of the renal structure which was observed during the first 2 weeks of KBrO₃ administration. Evidently, histopathological examinations of kidney also did not support Rue therapy as it did not help in improving cellular architecture. This appearance indicates poor treatment of the kidney against the toxic agent. Nevertheless, this study proves that Rue has some protective effect against the toxic agent KBrO₃; yet the obtained results could not confirm their effectiveness as therapeutic agents. No significant results

REFERENCES