Original Article

Evaluation Cyto-Genotoxicity and Nephrotoxicity Caused by Potassium Bromate and the Protective Impact of Sesame Oil in Male Rats

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ABSTRACT

Introduction: Potassium bromate (KBrO3) is commonly used in baking to add flavour and improve flour quality, it can also hurt many organs, especially the kidneys, by changing signs of kidney function.

Aim of the Work: The objective of this study is identifying the protective impact of sesame oil against the toxic influence of potassium bromate in bone marrow cells and kidney cortex tissue of male albino rats by usig cytogenetic tests and histopathological examination of the kidney.

Materials and Methods: Thirty-two male rats (Rattus norvegicus) were used, and the animals were divided into four groups; each group consisted of eight animals. The first group treated distilled water as a control, the second group treated sesame oil (4 ml /kg body weight), the third group received about (12 mg of potassium bromate per one kg body weight) and the fourth group treated with potassium bromate and sesame oil for 21 days.

Results: The results revealed a significant decrease of ($p \leq 0.05$) in the mitotic index in the KBrO3 group and the KBrO3 plus sesame oil group compared to the control group. The study recorded a significant increase ($p \leq 0.05$) in the chromosomal aberration rate in groups treated with potassium bromate doses of 12 mg /kg body weight compared to control groups administrated with distilled water and sesame oil after 21 days. Moreover, the current research identified histological changes in kidney cortex tissue in the group that administrated potassium bromate compared to the control. In addition, the results showed the positive role of sesame oil in significantly reducing potassium bromate toxicity in bone marrow cells and renal damage in the group animals treated by potassium bromate and sesame oil compared with the animal group treated by potassium bromate alone.

Conclusion: sesame oil had protective role against the toxicity and adverse side effect of potassium bromate in bone marrow cells and kidney cortex tissue.

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Key Words: Cyto-genotoxicity; nephrotoxicity; potassium bromate; sesame oil.

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INTRODUCTION

Potassium bromate ($KBrO_3$) is related to causing many diseases and is classified as a carcinogen[1]. Despite being a genotoxic chemical with numerous adverse effects on various human organs, bakers use significant amounts of potassium bromate[2]. The salt of bromate ion is a nephrotoxic and carcinogenic agent in humans and animal rats following exposure to drinking water or their feeding[3]. Although many studies elucidated the adverse negative effect of potassium bromate as an oxidizing agent and mentioned its potential human carcinogenicity, it is used widely to improve the baking industry by enhancing bread properties[4]. Potassium bromate exerts a various degree of toxicity. It is classified as a class 2 carcinogen depending on its dose and duration of exposure consumed with food and water in living organisms[5].

The sesame scientific name (Sesamum indicum L.), the earliest oil seed crops farmed and used by humans, belongs to the Pedaliaceae family[6]. The seeds of the sesame crop are cultivated in Ghana's northern regions for use as food and medicine derivatives and have the advantage of being organic antioxidants[7]. Sesame seeds can make sesame oil a valuable product containing various naturally occurring phytochemicals with anti-inflammatory and anticancer effects, such as sesamin, sesamol, and sesamolin[8]. Bioactive substances and nutrients in sesame oil include lignans, tocopherols, and phytosterols. Its qualities that promote neurological health, lower cholesterol, prevent degeneration, and are anti-lipogenic all help to improve human health[9].

The main objective of this work was to identify the protective and curative role of sesame oil in preventing cytotoxicity genotoxicity in bone marrow cells and avoiding renal damage stimulated by potassium bromate treatment through the calculating chromosomal aberration rate, mitotic index and histological changes assessment of kidney cortex in male rats.

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MATERIALS AND METHODS

The treatments and animals grouping

This study was conducted in the animal house and Genetics Laboratory of the Department of Biology - Faculty of Science - Kufa University. The period of study was from 18 February to 8 April 2023. Thirty-two animals of male rats (*Rattus norvegicus*), They weighed about (130 – 292) gm and were housed in the animal home with food and water supplements, regular light and dark cycles, and for a minimum of two weeks before the experiment's commencement.

Ethics statement

The guidelines for laboratory animals of ethics used to care were followed during every animal experiment., after receiving approval from the Central Committee for Bioethics of Kufa university. The research was approved by the University of Mosul's Ethical Approval No. um.VET.2021.5.

Experimental Design

Four groups were used, each group contained eight animals. The group one animal stayed without treatment, was administered with distilled water. In contrast, the second group received sesame oil (4 ml /kg body weight). The third group received about (12 mg of potassium bromate per one kg body weight) and the fourth group was treated with potassium bromate plus sesame oil for 21 days and

Chemicals

1. Potassium bromate (KBrO3) was used as a powdered white material.
2. Sesame oil.
3. Colchicine Solution was prepared according to[10].

Cytogenetic study

Mitotic index and chromosomal preparations of bone marrow cells of animals were calculated according to[10,11].

Histopathological study

Kidney tissues were fixed in 10% formalin for at least 24 h, and all sections of tissue were prepared to assess histopathological changes in the kidney cortex of animals using a rotary microtome according to[12]. Hematoxylin and eosin dyes were used to stain the sections, and the kidney cortex's histopathological alterations were examined under a light microscope.

Analytical Statistics

The SPSS (Statistical Package for Social Sciences) was used to evaluate the data statistically. ANOVA (analysis of variance), independent sample t-test. The results are presented as mean ± standard deviation. Differences between treatments at $p \leq 0.05$ were considered statistically significant.

RESULTS

The results of the research indicated a significant $P \geq 0.05$ decrement in the mitotic index in the group that was administrated $KBrO_3$ and the group was administrated $KBrO_3$ plus sesame oil in comparison with the controls were administrated distilled water and sesame oil. The study observed a significant increment $P \geq 0.05$ in the rate of division in the animal group that was administrated $KBrO_3$ plus sesame oil compared with a group that was administrated $KBrO_3$ only (Table 1).

The findings showed from (Table 2) significant increment ($P \geq 0.05$) in total average aberration per 100 cells in the chromosome in male rats that were administrated $KBrO_3$, and the rats group was administrated $KBrO_3$+ sesame oil for 21 days in comparison with the negative control and positive control. Moreover, the study elucidated that a significant $P \geq 0.05$ decrease in chromosomal aberration rate in the group was administrated $KBrO_3$+ sesame oil for 21 days compared with the group was administrated $KBrO_3$ alone, (Table 2).

The current research indicated some types of structural chromosomal aberrations in the group were administrated by potassium bromate and $KBrO_3$+ sesame oil, including sticky and fragmentation chromosomes (Figures 1,2).

The present work revealed the presence of histopathological alterations stimulated by potassium bromate administration in kidney cortex tissue of male albino rats when they were potassium bromate treatment at a dosage of about 12 mg/kg body weight for 21 days. Additionally, the results indicated the protective role of sesame oil at a dose of 4ml/kg and its ability to reduce histological changes in the renal tissue, as mentioned in (Figures 3,4).

Table 1: Effect of potassium bromate and sesame oil on the mitotic index in male albino rats after 21 days

Treatment	MI (%)
	Mean±SD
Control (distilled water)	20.67±1.97[a]
Control sesame oil	24.00±1.55[b]
Potassium bromate	14.67±1.37[abc]
Potassium bromate + sesame oil	21.17±1.33[abc]

Notes: Mean ± standard deviation, the small letters refer to significant differences at ($P \leq 0.05$) a: significant difference with negative control .b: significant difference between treatments and positive control, c: significant difference between treatments.

Table 2: Effect of potassium bromate and sesame oil on the chromosomal aberration rate in male albino rats after 21 days

Control Distilled water	Type of aberration	Control Sesame oil	Potassium bromate	Potassium bromate + Sesame oil
Sticky Chromosome	0.02±0.00[a]	0.02±0.00[b]	20.71±3.57[abc]	5.98±1.16[bc]
Fragmentation chromosome	2.96±0.04[a]	0.12±0.11[b]	23.00±2.37[abc]	14.55±2.24[abc]
Total %	2.98±0.04	0.14±0.11	43.71±5.94	20.53±3.4

Notes: Mean ± standard deviation (Mean ± S.D), the small letters refer to significant differences at ($P \leq 0.05$) a: significant difference with negative control, b: significant difference between treatments and positive control ve+, c: significant difference between treatments

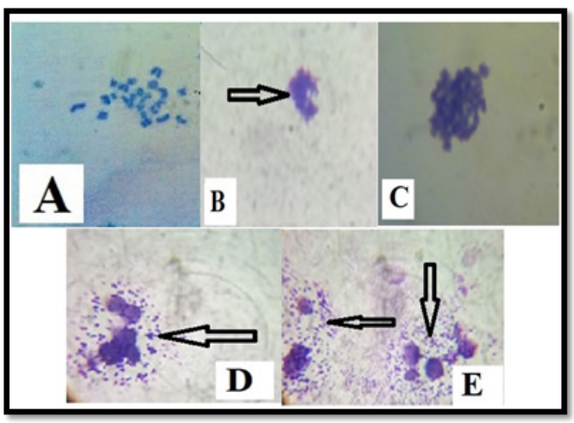

Fig. 1: Potassium bromate Impact on the percentage of chromosomal aberration in the group treated by potassium bromate.
All pictures were photographed at 1600X magnification.

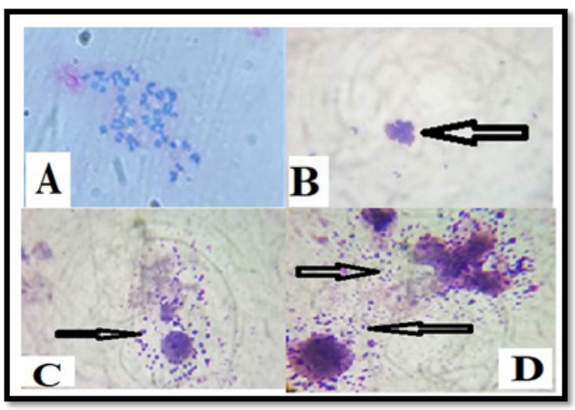

Fig. 2: Potassium bromate and sesame oil influence the percentage of chromosomal aberration in the group treated by potassium bromate+ sesame oil of All pictures photographed at 1600X magnification.

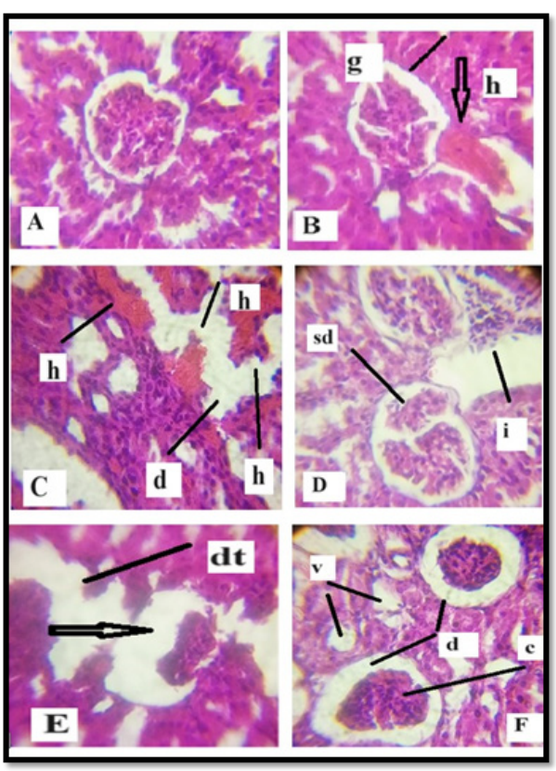

Fig. 3: Cross section of kidney cortex tissue of male animal rats, A: showing standard architecture without changes from the control group, B: showing dilation in glomerulus capsule space (g) and bleeding in renal tubules, C: showing hemorrhage in renal tubules (h) and dilation in renal tubules (d), D: showing infiltration of leukocytes (i) and minor degradation in glomeruli (sd), E: showing degradation of glomeruli (pointed arrow) and dilation in renal tubules, F: showing dilation in capsule space(d), congestion in glomeruli (c) and vacoulation in cytoplasmic tubular cells, B-F pictures from KBrO3 treated groups. All animal tissue specimens were stained by H&E and photographed at 400 X magnification.

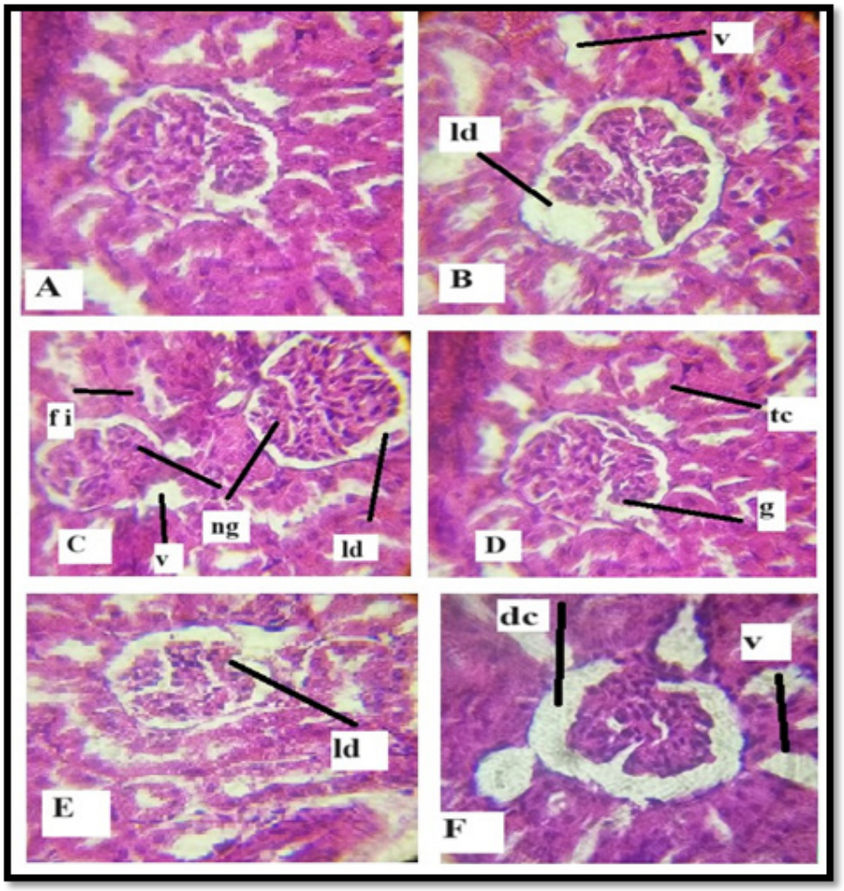

Fig. 4: Cross section of kidney cortex tissue in male animal rats, A: showing standard architecture without changes in a control group, B: showing little degradation in glomerulus capsule space (ld) and vacuolated in the cytoplasm- hemorrhage in renal tubules (v), C: showing glomerulus near to the standard (ng), and slight dilation in renal tubules (ld), vacuolated in the cytoplasm of tubular cells (v) and few infiltrated leukocytes (fi). D shows semi-standard glomerulus (g) and tubular cell (tc) architecture. E: showing little degradation of glomeruli (ld). F: showing dilation in capsule space(dc) and vacation in the cytoplasm of tubular cells (v). B-F pictures from $KBrO_3$ + sesame oil treated groups. All animal tissue sections were stained by H&E and photographed at 400 X magnification.

DISCUSSION

According to table 1, the results indicated significant $P \geq 0.05$ decrement in the mitotic index in the group that was administrated $KBrO_3$ and the group was administrated $KBrO_3$+ sesame oil in comparison with the controls. These findings agreed with[13] who recorded that $KBrO_3$ induced cellular damage and apoptosis in the jejunum mucosa of adult rats. In addition, the results incorporated with[14] who mentioned that $KBrO_3$ caused severe toxicity for antioxidant activities and exhibited apoptosis in bone marrow mesenchymal stem cells in female rats. These results were supported by[15], who recorded that $KBrO_3$ decreased the number of cells and produced apoptosis at high doses was identified in a cultured cell line and caused decreasing in mitotic index.

According to Table 1, the study observed significant increment $P \geq 0.05$ in the rate of division in the animal group that was administrated $KBrO_3$+ sesame oil compared with a group that was administrated $KBrO_3$ alone. These results in agreement with[16], who recorded that sesame oil functions as a natural antioxidant and contains bioactive components that protect body cells against various disorders by scavenging free radicals produced by harmful substances. The outcomes were supported by[17], who recorded that sesame oil has many kinds of natural phytochemicals that pose many benefits and healthy effects such as anti-tumour, anticancer, and anti-inflammatory activities. Similar to our results[18] noticed that sesame oil contains vitamins and lignans with several health-promoting benefits against genotoxicity in Wistar rats.

The findings from Table 2 and figure 1,2 showed significant increment ($p \geq 0.05$) in total average aberration per 100 cells in the chromosome in male rats that were administrated $KBrO_3$. These results were similar to[19], who recorded that potassium bromate is a generator of reactive oxygen species that is clastogenic and carcinogenic; it also promotes DNA oxidative damage, mutations, structural chromosomal abnormality, DNA-strand breakage, and micronuclei in many in *vivo* cells, these effects are toxic and genotoxic. In addition, the outcomes incorporated with[20], who reported that the creation of micronuclei, fragmented

DNA percentage in animal tissues, and the chromosomal aberrations frequency were all increased by $KBrO_3$, which also had a hazardous effect. The results agreed with[21], who recorded that $KBrO_3$ the most prevalent forms of structural chromosomal abnormalities were higher frequencies of fragment and sticky chromosomes.

Furthermore, the findings supported by[22], who mentioned that $KBrO_3$ is a DNA oxidizing and genotoxic agent mainly related to DNA damage that induces chromosomal aberrations micronuclei. Moreover, the study elucidated that a significant $P \geq 0.05$ decrease in chromosomal aberration rate in the group was administrated $KBrO_3$+ sesame oil compared with the group was administrated $KBrO_3$ alone Table 2 and figure 2. These findings were agreed with[23] who demonstrated that sesame oil at different doses significantly declined the aberrant metaphases and chromosomal aberration frequencies. Furthermore, the results supported by[24] who reported sesame oil possesses a promising protective activity against the aberrant chromosome and DNA damage. As well as ,the findings in agreement with[25] who recorded bioactive components of sesame oil play potent anti-cancer role against oxidative stress through scavenging free radicals in *vitro* and in *vivo* by regulate various stages of the cell cycle and apoptosis.

The present work revealed the presence of histopathological alterations stimulated by potassium bromate administration in kidney cortex tissue of male albino rats when they were treated by potassium bromate. Additionally, the results indicated the protective role of sesame oil and its ability to reduce histological changes in the renal tissue, as mentioned in Figures 3 and 4. These results were supported by[26], who reported that potassium bromate is well-known for promoting free radicals in the renal cortex and tissue damage. The findings also were agreed with[27], who indicated that potassium bromate is considered a human carcinogen and a potent nephrotoxic agent; it is found in the environment, and its harmful effects are demonstrated by plasma biomarkers (levels of urea, uric acid, and creatinine) and kidney tissue, as well as oxidative stress-related parameters included superoxide dismutase, glutathione, catalase, malondialdehyde and peroxidase. Furthermore, the result was similar to[28], who reported in his study that KBrO3 decreased kidney organ-body weight and its adverse effect represented by causing histological changes and oxidative damage in the kidney and liver, which may result in many health problems, cell necrosis, degeneration, dislocation, and death. In addition, the outcomes were supported by[29], who recorded that regular sesame oil was protective against KBrO3 nephrotoxicity and acts as a potential antioxidant by improving kidney function parameters and antioxidant enzymes in rats. Furthermore, the present results were supported by[30], who mentioned that sesame oil supplement efficiently ameliorated adverse effects represented by oxidative stress-induced kidney injury by reducing lipid peroxidation and oxygen free radicals in chemical reagents as carbon tetrachloride exposed rats and increased the capacity of antioxidant. Supported by[31], who demonstrated that sesame oil was a beneficial supplementation and had improved the rabbit's body health, this positive role of sesame oil belongs to solid antioxidant activity because it possesses phenolic contents, flavonoids, and pigments.

CONCLUSION

The study can be concluded that sesame oil can be used as an antioxidant because it has abundant beneficial properties for applications in food; the data showed it had a potent protective effect against potassium bromate-induced cytotoxicity, genotoxicity in bone marrow cells and histopathological alterations in male albino rat's kidney cortex tissue.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تقييم السمية الخلوية الجينية والكلوية المتسببة عن برومات البوتاسيوم والأثر الوقائي لزيت السمسم في ذكور الجرذان

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مقدمة: يُستخدم برومات البوتاسيوم ($KBrO_3$)بشكل شائع في الخبز لإضافة نكهة مميزة وتحسين جودة الدقيق، إلا أنه قد يُلحق الضرر بالعديد من الأعضاء، وخاصة الكلى، من خلال تغيير مؤشرات وظائفها.

هدف الدراسة: تهدف هذه الدراسة إلى تحديد التأثير الوقائي لزيت السمسم ضد التأثير السام لبرومات البوتاسيوم في خلايا نخاع العظم وأنسجة قشرة الكلى لدى ذكور الجرذان البيضاء، وذلك باستخدام الاختبارات الوراثية الخلوية والفحص النسيجي للكلى.

المواد والطرق: استُخدم اثنان وثلاثون جرذًا من الذكور من نوع Rattus norvegicus، وقُسِّمت الحيوانات إلى أربع مجموعات؛ كل مجموعة تتكون من ثمانية حيوانات. عوملت المجموعة الأولى بالماء المقطر كمجموعة سيطرة، وعوملت المجموعة الثانية بزيت السمسم (٤ مل/كجم من وزن الجسم)، وتلقت المجموعة الثالثة حوالي (١٢ ملغ من برومات البوتاسيوم لكل كيلوغرام من وزن الجسم)، وعوملت المجموعة الرابعة ببرومات البوتاسيوم وزيت السمسم لمدة ٢١ يومًا.

النتائج: أظهرت النتائج انخفاضًا معنويًا $p\leq$٠,٠٥ في مؤشر الانقسام الخيطي في مجموعة $KBrO_3$ ومجموعة $KBrO_3$ +زيت السمسم مقارنةً بمجموعة السيطرة. وسجلت الدراسة زيادة معنوية $p\leq$٠,٠٥ في معدل التشوه الكروموسومي في المجموعات المعالجة بجرعات برومات البوتاسيوم ١٢ ملغم/كغم من وزن الجسم مقارنةً بمجموعات السيطرة المعاملة بالماء المقطر وزيت السمسم بعد ٢١ يومًا. علاوة على ذلك، حدد البحث الحالي تغيرات نسيجية في أنسجة قشرة الكلى في المجموعة التي عوملت ببرومات البوتاسيوم مقارنةً بمجموعة السيطرة. بالإضافة إلى ذلك، أظهرت النتائج الدور الإيجابي لزيت السمسم في تقليل سمية برومات البوتاسيوم في خلايا نخاع العظم وتلف الكلى بشكل ملحوظ في حيوانات المجموعة المعاملة ببرومات البوتاسيوم وزيت السمسم مقارنةً بمجموعة الحيوانات المعاملة ببرومات البوتاسيوم وحده.

الخلاصة: كان لزيت السمسم دور وقائي ضد السمية والآثار الجانبية الضارة لبرومات البوتاسيوم في خلايا نخاع العظم وأنسجة قشرة الكلى.