Volume 6 Issue 2 Page 66-73, 2025

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https://doi.org/10.59298/NIJBAS/2025/6.2.667300

Antioxidant and therapeutic potentials of *Abelmoschus esculentus* water extract against potassium bromate intoxication

Eze-Steven Peter E^{1*}, Okpara Lilian O¹ and Aloh Godwin S².

- ¹Leiomyoma and Nutrigenomics Research Unit, Applied Biochemistry Department, Enugu State University of Science and Technology, Enugu State, Nigeria.
- Biochemistry Department, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.
- *Corresponding author email address: peter.ezesteven@esut.edu.ng

ABSTRACT

Potassium Bromate (KBrO₃) is a known environmental toxin that poses significant health risks, including oxidative stress. Abelmoschus Esculentus (Okra) is a commonly used fruit in sub-Sahara Africa. This study investigated the protective and therapeutic effects of A. esculentus leaf extract on bromate-induced toxicity in albino rats. Thirty (30) adult Wistar male albino rats were divided into six (6) groups of five rats each. Group 1 served as the normal control, while Group 2 was the negative control, receiving 50 mg/kg of KBrO₃. Group 3 was treated with 100 mg/kg of Vitamin C plus 50 mg/kg of KBrO₃. Groups 4, 5, and 6 received Okra leaf extract at doses of 200 mg/kg, 400 mg/kg, and 800 mg/kg, respectively, along with 50 mg/kg of KBrOs. All treatments were administered orally over a 28-day period. Body weights were recorded at the beginning and end of the study. Post-treatment, rats were euthanized using standard method with blood samples collected for biochemical analyses. Results indicate that the scavenging effects of the leaf extract on DPPH radicals are dose-dependent. Similarly, ascorbic acid exhibited a dose-dependent inhibition of DPPH radical. However, the effect of ascorbic acid was significantly higher compared to the leaf extract of Abelmoschus esculentus. The ferric-reducing antioxidant power of ascorbic acid was significantly lower when compared to leaf extract of Abelmoschus esculentus suggesting that the leaf extract of Abelmoschus esculentus displayed a concentration-dependent ferric-reducing ability that was significant when compared to the standard ascorbic acid used. Results further indicate that treatment with Abelmoschus esculentus significantly increased the levels of GSH $(5.49\pm0.14,\ 5.91\pm0.07,\ 6.24\pm0.28 \text{mg/mg}\ \text{protein})$, GPx $(21.56\pm1.46,\ 23.47\pm0.61,\ 6.24\pm0.28 \text{mg/mg}\ \text{protein})$ 24.07±0.75u/mg protein), and SOD (31.43±1.54, 33.93±1.40, 36.30±0.46u/mg protein) in a dose-dependent manner compared with the Potassium Bromate-induced toxicity only group. Therefore, A. esculentus leaf extract significantly mitigates the adverse effects of bromate-induced toxicity.

Keywords: Antioxidants, Oxidative stress, Abelmoschus esculentus, Potassium bromate, Wistar rats.

INTRODUCTION Background of Study

Toxicity refers to a substance's capability to harm a living organism and hinges on the specific dose required to induce damage [1]. This damage may manifest as observable alterations in anatomy or biological processes, which are often driven by previously undetected alterations in specific biochemical processes within the organism [2]. Every substance possesses a degree of toxicity, implying that everything can be considered a toxin, with the critical factor being the dosage administered. Moreover, various species react differently to toxins, reflecting the complex interplay between toxicity and an organism's response to varying chemical dosages [3]. Toxicity assessment relies on an organism's reactions to different chemical dosages. Toxicity can encompass the impact on an entire organism, such as an animal, bacteria, or plant, as well as the impacts on particular organismal components, including cells This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited

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(cytotoxicity) or organs like the liver (hepatotoxicity) and kidneys (nephrotoxicity) [4]. Moreover, some toxins exhibit extreme potency by inflicting substantial damage rapidly, while others, which infiltrate the body more gradually, can cause equally severe or even more extensive harm. Certain chemicals or compounds can show toxicity even with modest or one-time exposure, a phenomenon known as acute toxicity [5]. All substances possess acute toxicity potential, and some can be highly toxic acutely, even in a single exposure [6]. One chemical of interest is potassium bromate, which is generated by passing bromine through a heated potassium hydroxide solution [6]. It is commonly used in the food sector, notably in bakeries, to modify dough elasticity. However, potassium bromate Page | 67 causes oxidative stress in living organisms, resulting in a variety of harmful consequences. Abelmoschus esculentus, commonly known as okra, lady's finger, or gumbo, belongs to the family Malvaceae. This is an easily accessible and cost-effective vegetable crop containing significant quantities of nutritional and bioactive components associated with therapeutic effects against chronic diseases like type-2 diabetes, cardiovascular ailments, and digestive disorders

Aim of the Study

This study aims to evaluate the therapeutic and antioxidant potentials of ethanol extract of Abelmoschus esculentus leaf against potassium bromate intoxication in albino Wistar rats.

Justification for the Study

Bromate is a well-known toxic compound that poses a significant health risk to both humans and animals. Its harmful effects on various physiological systems, make it a public health concern. Oxidative stress plays a crucial role in the pathophysiology of many diseases. Antioxidants are essential for protecting cells and tissues from oxidative damage induced by free radicals. Given the possible health advantages associated with antioxidants, it is of major scientific and practical interest to examine the antioxidant capacity of okra leaf extract in bromate intoxication. Although every part of the Abelmoschus esculentus plant, including the fresh leaves, buds, flowers, and pods, are recognized for its nutritional value and potential health benefits, okra is farmed primarily for its immature pods, which are often eaten as a green vegetable. In Nigeria, the green leaves of okra are rarely eaten. Okra leaves have been linked to a variety of ethnomedicinal claims, although scientific research on their antioxidant properties is limited. Hence, this research work aims to address this knowledge gap and explore the potential health-promoting properties of okra leaves, especially in KBrO₃-intoxicated albino rats.

Oxidative Property of KBrO₃

It is an ionic molecule composed of potassium ions (K+) and bromate ions (BrO₃-). It is used as a dough enhancer in bakeries because it is a strong oxidizing power.

Antioxidants

Oxidation processes are essential for life because the body continually produces free radicals as a result of its frequent consumption of oxygen. These free radicals are extremely reactive entities with one or more unpaired electrons in the outermost shell. When they are produced, the chain reaction begins, causing damage to critical biological molecules and contributes to a variety of health issues, including heart disease, diabetes, macular degeneration, and cancer. Antioxidants are substances that, when present at low quantities relative to an oxidizable molecule, considerably delay or prevent its oxidation, hence lowering oxidative stress on the physiological system. Antioxidants react with these free radicals to stop the chain reaction by eliminating free radical intermediates and preventing additional oxidation processes, and they are oxidized in the process. Antioxidants are effective because they are prepared to sacrifice their own electrons to free radicals. When a free radical receives an electron from an antioxidant, it no longer needs to assault the cell, and the chain reaction of oxidation is broken. After donating an electron, an antioxidant is defined as a free radical. Antioxidants in this form are harmless because they can tolerate electron changes without becoming reactive [8]. Antioxidants can function via two pathways: chain-breaking and preventive [8]. Plants and animals contain a complex system of many forms of antioxidants, such as vitamin C and vitamin E, as well as enzymes like catalase (CAT), superoxide dismutase (SOD), and different peroxidase.

Uses of Antioxidant

In the chemical or food industry, antioxidants act as primary antioxidants by breaking the radical chain reactions between substrates (plastic, rubber, or food products) and ROS by interfering at various phases of radical formation (propagation or initiation). Primary antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG), are utilized in the food sector to neutralize free radicals and prevent off-flavor formation in food items and prevent food spoilage [9]. Foods high in antioxidants, such as fruits and vegetables, are advised because their antioxidant content will help prevent cardiovascular disease and protect against cancer (eg, Lycopene prevents cancer of the mouth, pharynx, esophagus, stomach, colon and rectum [10].

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ONLINE ISSN: 2992-5797 PRINT ISSN: 2992-6122

MATERIALS AND METHODS

Materials

Collection of samples

Fresh leaves of *Abelmoschus esculentus* (Okra) were collected from Umudike in Ikwuano Local Government Area of Abia State, and authenticated at the Department of Forestry, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike. A voucher number MOUAU/ZEB/HERB/21/008 was assigned to a specimen sample which was deposited at a herbarium in the Department of Plant Science and Environmental Biology. The leaves were chopped into smaller particles and airdried when kept on the a laboratory bench for 30 days before being pulverized into powder using a locally made motor blender. Crude extract was prepared from the powdered material.

Animals

A total of 30 adult male Wistar rats were used for the study. Experimental animals were obtained from the Animal House of the Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike, housed in aluminum cages and allowed to acclimatize within two weeks before commencement of the studies. Experimental rats were allowed free access to Chikkun finisher's mash (Chikkun Feed, Nigeria) and clean water, ad litibum, but starved for 12 hours prior to the commencement of any of the experiments to help standardize conditions, reduce variability, establish baseline parameters, minimize interference from food, and enhance the safety of the experimental procedures. All animal experiments were conducted in compliance with international guidelines for care and use of laboratory animals [11] at the leiomyoma and nutrigenomics laboratory in the Department of Applied Biochemistry, Faculty of Biological Sciences, Enugu State University of Science and Technology, Enugu State, Nigeria.

METHODS

Preparation of extract

Cold maceration technique was adopted in the preparation of plant extract as described by [12].

Design of Animal Studies

Thirty (30) adult male albino rats assigned to 6 groups of 5 rats each were treated according to the order below:

Group 1: Normal control

Group 2: Negative control (50 mg/kg of KBrO₃ only)

Group 3: Vitamin C (100 mg/kg) + 50 mg/kg of KBrO₃

Group 4: Okra leaves extract (200 mg/kg) + 50 mg/kg of KBrO₃

Group 5: Okra leaves extract (400 mg/kg) + 50 mg/kg of KBrO₃

Group 6: Okra leaves extract (800 mg/kg) + 50 mg/kg of KBrO₃

All treatments were via the oral route and lasted for 28 days. Body weights were taken at the beginning and at the end of the treatment using an electronic balance (DJA1000, China). At the end of the period, animals were sacrificed and blood samples collected for various biochemical analyses.

Statistical analysis

Statistical Package for Scientific Solution (SPSS, Version 20.0, IBM SPSS Inc, Chicago, IL) was used for data analysis. Level of significance was calculated by One-Way Analysis of Variance (ANOVA). Data were analyzed using Duncan Multiple Range Test and complemented with Student's t test for post-hoc test for comparisons of the means of the various doses and fractions. P- values less than 0.05 were considered statistically significantly different between the test and control groups as well as among test groups for measured value.

RESULTS

In-vitro Antioxidant capacity of leaf extract of Abelmoschus esculentus (Okra)

DPPH radical scavenging capacity

The DPPH scavenging activity of the leaf extract of *Abelmoschus esculentus* (Okra) is shown in Figure 1. Results indicate that the scavenging effects of the leaf extract on DPPH radicals are dose-dependent. Similarly, ascorbic acid, which was the standard drug used, exhibited a dose-dependent inhibition of DPPH radical. However, the effect of ascorbic acid was significantly higher compared to the leaf extract of *Abelmoschus esculentus*.

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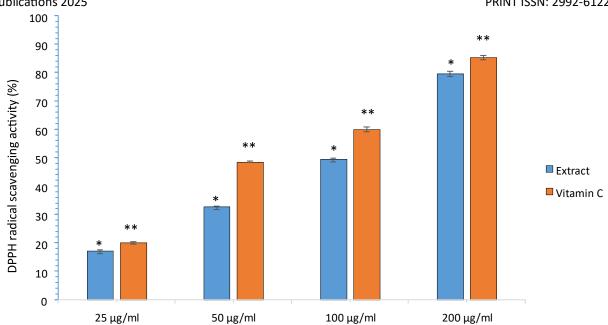
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OPEN ACCESS ONLINE ISSN: 2992-5797 **Publications 2025** PRINT ISSN: 2992-6122

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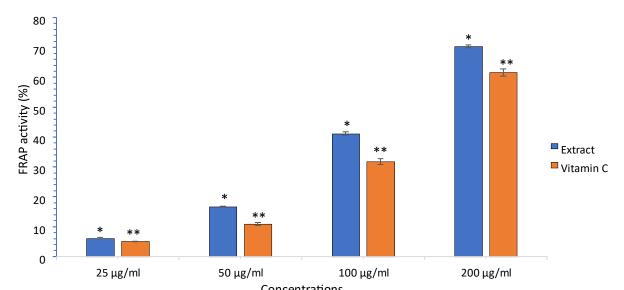
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Conc of extract & Vit C Fig. 1: DPPH radical scavenging activity of leaf extract of Abelmoschus esculentus Bars are presented as mean \pm standard deviation (n = 3), bars with double asterisk (**) are significantly (P < 0.05) different from paired bars with a single asterisk (*).

Ferric reducing antioxidant power (FRAP)

In Figure 2, the ferric reducing antioxidant power (FRAP) of the leaf extract of Abelmoschus esculentus (Okra) is depicted. The findings revealed that the ferric-reducing antioxidant power of ascorbic acid was notably lower in comparison to that of the leaf extract of Abelmoschus esculentus. This outcome suggests that the leaf extract of Abelmoschus esculentus (Okra) displayed a concentration-dependent ferric-reducing ability that was significant when compared to the standard (ascorbic acid).



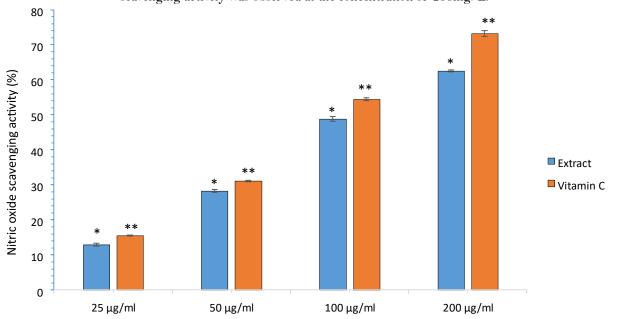
Concentrations Fig.2: Ferric reducing antioxidant power (FRAP) of Leaf extract of Abelmoschus esculentus (Okra). Bars are presented as mean \pm standard deviation (n = 3), bars with double asterisk (**) are significantly (P<0.05) different from paired bars with single asterisk (*).

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Nitric oxide scavenging antioxidant power

As depicted in Figure 3, the nitric oxide scavenging activities of Abelmoschus esculentus (Okra) leaf extract increased with the concentration, ranging from 25 mg/L to 200 mg/L. However, the nitric oxide scavenging activities of ascorbic acid were significantly higher as compared to Abelmoschus esculentus leaf extract. The highest nitric oxide scavenging activity was observed at the concentration of 200mg/L.

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Concentrations Fig. 3: Nitric oxide scavenging antioxidant power of Leaf extract of *Abelmoschus esculentus* (Okra) Bars are presented as mean \pm standard deviation (n = 3), bars with double asterisk (**) are significantly (P < 0.05) different from paired bars with single asterisk (*).

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Antioxidant activities of Leaf extract of Abelmoschus esculentus (Okra) in Potassium Bromate induced oxidative stress in rats

Table 1 displays the antioxidant activities of the leaf extract of Abelmoschus esculentus (Okra) in Potassium Bromateinduced toxicity in rats. The induction of Bromate toxicity led to a reduction in GSH (4.15±0.57mg/mg protein). GPx (18.57±1.53u/mg protein), SOD (28.43±1.63u/mg protein), and CAT (9.50±0.30u/mg protein) levels when compared with the control group (6.41±0.21mg/mg protein, 25.03±0.61 u/mg protein, 35.63±1.30u/mg protein, Page | 71 12.17±0.75u/mg protein), respectively. Additionally, the MDA levels in the Bromate-induced toxicity group (0.77±0.03 mmol/mg protein) significantly increased in comparison to the control group (0.24±0.04 mmol/mg protein). However, treatment with Abelmoschus esculentus significantly increased the levels of GSH (5.49±0.14, 5.91 ± 0.07 , 6.24 ± 0.28 mg/mg protein), GPx (21.56 ± 1.46 , 23.47 ± 0.61 , 24.07 ± 0.75 u/mg protein), and SOD (31.43±1.54, 33.93±1.40, 36.30±0.46u/mg protein) in a dose-dependent manner compared with the Potassium Bromate-induced toxicity only group. Regarding CAT levels, there was a nonsignificant increase in a dose-dependent manner when compared with the Bromate-induced only group (negative control group). Furthermore, the MDA levels after treatment with 200 mg/kg (0.40 ± 0.04 mmol/mg protein), 400 mg/kg (0.34 ± 0.03 mmol/mg protein), and 600mg/kg (0.30±0.05 mmol/mg protein) resulted in a dose-dependent decrease when compared with the negative control group (0.77±0.03mmol/mg protein).

Table 1: Antioxidant activities of Leaf extract of Abelmoschus esculentus (Okra) in Potassium Bromate induced oxidative stress in rats

Treatments	GSH (mg/mg protein)	GPx (u/ mg protein)	SOD (u/ mg protein)	CAT (u/ mg protein)	MDA (mmol/ mg protein)
Control	6.41 ± 0.21^{d}	25.03±0.61°	$35.63\pm1.30^{\circ}$	12.17±0.75°	0.24±0.04ª
$\mathrm{KBrO_3}$ (50 mg/kg) only	4.15 ± 0.57^{a}	$18.57 \pm 1.53^{\mathrm{a}}$	28.43 ± 1.63^{a}	$9.50 \pm 0.30^{\rm b}$	0.77 ± 0.03^{d}
Vit. C (100 mg/kg) + KBrO $_3$ (50 mg/kg)	5.24 ± 0.26^{b}	$21.76 \pm 1.25^{\mathrm{b}}$	31.67 ± 1.54^{b}	11.10±0.36 ^b	0.39±0.05°
Okra leaf extract (200 mg/kg) + KBrO ₃ (50 mg/kg)	$5.49\pm0.14^{b,c}$	21.56 ± 1.46^{b}	31.43 ± 1.54^{b}	11.03±0.71 ^b	0.40 ± 0.04^{c}
Okra leaf extract (400 mg/kg) +	$5.91 \pm 0.07^{c,d}$	$23.47\pm0.61^{\text{b,c}}$	33.93±1.40 ^{b,c}	$10.93 \pm 0.42^{\rm b}$	0.34±0.03 ^{b,c}
KBrO ₃ (50 mg/kg) Okra leaf extract (800 mg/kg) + KBrO ₃ (50 mg/kg)	6.24±0.28 ^d	24.07±0.75°	36.30±0.46°	12.13±0.31°	0.30±0.05a,b

Values are presented as mean \pm standard deviation; means with different letter superscripts are significantly (P<0.05) different from any paired mean with the column.

DISCUSSION

Abelmoschus esculentus, commonly known as Okra, stands out for its rich reservoir of bioactive agents, endowing it with significant ethnobotanical, pharmaceutical, and economic importance, as noted by [13]. This plant has been the subject of extensive research, unveiling a vast array of bioactive substances. These encompass antidiabetic, hypolipidemic, antioxidant, antimicrobial, and anticancer properties, as well as its effects on wound healing, hepatoprotection, immunomodulation, neurological health, gastroprotection, weight management, and cardioprotection [147]. Conversely, potassium bromate (KBrO₃) has gained notoriety as a potent oxidizing agent utilized to investigate the protective potential of various natural products, both in vitro and in vivo, as reported by [15], and [16]. Oxidative stress can inflict damage on cells, proteins, and DNA, thereby contributing to the aging process. Furthermore, it may play a pivotal role in the development of an array of health conditions, including diabetes, cancer, and neurodegenerative disorders such as Alzheimer's [17]. The primary objective of our study was to examine the potential protective and therapeutic attributes of ethanol extract Abelmoschus esculentus leaves in alleviating KBrO₃-induced toxicity in albino rats. Additionally, we analyzed the *in vitro* antioxidant properties of the extract. Results of the in vitro antioxidant properties of the ethanol leaf extract of Abelmoschus esculentus showed that the ethanol leaf extract of Abelmoschus esculentus displayed a concentration-dependent ferric reducing ability This is an Open Access article distributed under the terms of the Creative Commons Attribution (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited

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that was significant when compared to the standard (ascorbic acid). This result was in line with previous studies conducted by [18] and [19], which reported the antioxidant properties of Abelmoschus esculentus. The observed concentration-dependent ferric-reducing ability of the Abelmoschus esculentus ethanol leaf extract underscores its significant antioxidant potential. This property is essential for combating oxidative stress, which is implicated in the development various health conditions, including aging and chronic diseases The study revealed that rats with Bromate-induced toxicity experienced significant weight loss. It is in line with the assertions of WHO which states that at higher doses of KBrO₃, rat growth becomes retarded and the weight Page | 72 significantly reduced. This was probably due to the binding of KBrO₃ to the iodine receptors, minimizing iodine uptake by the thyroid gland and causing iodine insufficiency leading to growth retardation [21]. However, treatment with Abelmoschus esculentus leaf extract resulted in a dose-dependent increase in body weight and percentage weight gain, indicating the potential for Okra to counteract the weight loss associated with toxic insults. The study revealed that Abelmoschus esculentus leaf extract possesses strong antioxidant properties. These findings suggest that Okra leaf extract can effectively counteract the oxidative damage caused by Bromate-induced toxicity. Antioxidants are crucial for preventing and managing various diseases, including those associated with oxidative stress.

CONCLUSION

This study underscores the remarkable potential of ethanol leaf extract of Abelmoschus esculentus as a natural remedy for alleviating the adverse effects of potassium bromate-induced toxicity. The extract had a strong antioxidant property by protecting against oxidative stress. These findings suggest that A. esculentus holds promise as a valuable source of bioactive compounds with potential therapeutic applications in countering toxic assault and promoting overall well-being. Further research and exploration of its diverse health benefits are warranted, with the aim of harnessing its full potential in the realm of natural medicine and health promotion.

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CITE AS: Eze-Steven Peter E, Okpara Lilian O and Aloh Godwin S. (2025). Antioxidant and therapeutic potentials of *Abelmoschus esculentus* water extract against potassium bromate intoxication. NEWPORT INTERNATIONAL JOURNAL OF BIOLOGICAL AND APPLIED SCIENCES 6(2): 66-73.

https://doi.org/10.59298/NIJBAS/2025/6.2.667300