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In vivo attenuation of experimentally-induced oxidative stress by common African vegetable (*Corchorus olitorius*)

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Abstract

Despite decades of study, the significance of free radicals' function in organ damage remains questionable and is still a significant problem in the diagnosis of many diseases. Therefore, the goal of the current investigation was to determine whether *Corchorus olitorius* leaves could reduce oxidative stress caused by potassium bromate ($KBrO_3$). Twenty-four rats were put into groups A, B, C, and D. Group A received distilled water as the control. Animals in groups C and D were additionally given 100 and 200 mg/kg body weight of *C. olitorius*, respectively, in addition to the 100 mg/kg body weight of $KBrO_3$ given to groups B, C, and D on a daily basis for 28 days. The animals were sacrificed while being gently sedated with diethyl ether and the blood, heart, liver, and kidneys were collected. The results showed that $KBrO_3$ decreased plasma, hepatic, renal, and cardiac CAT, SOD, and GPx activities as well as GSH concentrations, but raised MDA levels, as compared to the control group. Rats were given extract from *C. olitorius* leaves at doses of 100 and 200 mg/kg body weight, with the results of the 200 mg/kg dose being comparable to those observed in the control group. These treatments resulted in significant increases in antioxidant levels (except GPx) and decrease in MDA levels in their plasma and tissues. The study showed that potassium bromate increased the levels of oxidative enzymes leading to oxidative stress. This effect was attenuated by *C. Olitorius* demonstrating its protective effect on the $KBrO_3$-induced oxidative stress.

Keywords: Attenuation effect; *Corchorus olitorius* (jute); Oxidative stress; Potassium bromate

1. Introduction

Oxidative stress results when oxidation outpaces the body's antioxidant defense systems. It is brought about by an imbalance between the body's antioxidant defenses against reactive oxygen species (ROS) and their production [1]. Oxidative stress is caused by metabolic processes that use oxygen and upset the balance of pro- and anti-oxidant reactions in living things [2]. Under these circumstances, reactive oxygen species (ROS) can damage proteins, lipids, and

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nucleic acids, leading to cell death or transformation [3]. Therefore, ROS can be hazardous to biological systems and cause diseases if they are not effectively scavenged by cellular components [4]. Cardiovascular diseases are significantly impacted by oxidative stress [5]. The importance of oxidative stress in cardiovascular disease processes such ischemia-reperfusion damage, atherogenesis, and cardiac remodeling has been understood for a few decades [6].

When manufacturing bread, potassium bromate ($KBrO_3$) is frequently used as a food addition (like a flour enhancer, strengthening the dough and enabling higher rise) [7]. Additionally, fish paste and fermented drinks contain $KBrO_3$. Studies on the risk of potassium bromate have revealed that it is extremely harmful because it results in DNA oxidative damage and lipid peroxidation [8]. Cardiotoxicity is brought on by the $KBrO_3$ and results in alterations in lipid profile of the heart and blood [9].

Antioxidants are substances that prevent or slow down the development of oxidative chain reactions as well as lipid oxidation [10]. Antioxidants combat oxidative stress by neutralizing excess free radicals and preventing them from starting chain reactions that result in various illnesses and premature aging [11].

The vast amount of research studies regarding the pharmacological actions of the bioactive components of plant materials and their potential to treat various ailments have raised concerns about natural medicines that are spreading around the world [12]. *Corchorus olitorius* is one of such plants. The plant *Corchorus olitorius* (Malvaceae) is indigenous to both tropical and subtropical areas of the world, and its mallow leaves are frequently eaten as a leafy vegetable. According to reports, the leaves have been used in traditional medicine to cure tumors, gonorrhea, chest pains, diarrhea, malaria, enteritis, fever, and other conditions [13]. The Yoruba people of southwestern Nigeria usually refer to *C. olitorius* as "Ewedu", while the plant is also referred to as jute mallow or bush okra in English, it is known as "Ahihara" among the Igbo people of southeastern Nigeria. The *C. olitorius* plant can also be found in Egypt, Sudan, Malaysia, South America, and the Caribbean in addition to Nigeria [14]. The plant contains a number of nutrients, such as calcium, potassium, phosphate, iron, ascorbic acid, carotene, and a significant amount of mucilaginous polysaccharides [15]. *C. olitorius* is employed as a demulcent, diuretic, purgative, bitter tonic, laxative, refrigerant, carminative, and lactagogue in medicine [16]. The care of chronic cystitis and dysuria has shown promising outcomes when using the leaf extract. Its significant antibacterial activity, which has been recorded, supports the idea that it has historically been used to treat gonorrhea, fever, and dysentery [15,16]. Additionally, its hypolipidemic and hypoglycemic effects have been documented [17]. The goal of this study is to determine whether it can reduce the oxidative stress that potassium bromate causes in the body's essential organs.

2. Material and Methods

2.1. Extraction of Plant Materials

Fresh *Corchorus olitorius* (jute) plants were harvested from the Institute of Agricultural Research and Training, Moor Plantation, Ibadan, Nigeria. Carefully separating the leaves from the stem, the damaged ones were discarded. To get rid of contaminants, they were thoroughly washed under running water. In an open laboratory setting, they were allowed to air dry for 14 days at room temperature before being ground into powder with an electric blender (Moulinex). Using a soxhlet apparatus and ethanol as the solvent, the extraction was completed in accordance with the steps described by Airaodion et al. [18,19]. About 25 g of the sample powder and a round bottom flask with a capacity of 250 mL of ethanol were added to the soxhlet extractor and condenser on a heating mantle. The solvent was heated by the heating mantle and began to evaporate as it passed through the apparatus to the condenser. The condensate dropped into a reservoir that housed the sample-containing thimble. When the solvent level reached the siphon and was poured back into the flask with a flat bottom, the cycle was resumed. The operation was given a total of 18 hours. Once the process was done, the ethanol was evaporated in a rotary evaporator at 35 ^{o}C with a yield of 2.28 g which equals a percentage yield of 9.12%. Until it was needed, the extract was kept in the refrigerator.

2.2. Experimental Design

Twenty-four (24) mature male Wistar rats (*Rattus norvegicus*) weighing between 140 and 160 g were used in the experiment. They were acclimated in a laboratory setting for seven (7) days prior to the experiment. The rats were housed in wire-mesh cages with free access to commercial rat food and water. The animals were kept in standard temperature and humidity conditions with 12-hour cycles of light and dark. This inquiry was carried out in accordance with the Declaration of Helsinki and the guidelines established by the Committee for the Purpose of Control and Supervision of Experiments on Animals. Additionally, NIH policy was followed when doing animal experiments [20]. At random, they were put into groups A, B, C, and D. Group A received oral distilled water as the usual control. Animals in groups C and D were additionally given 100 and 200 mg/kg body weight of *C. olitorius*, respectively, in addition to the

100 mg/kg body weight of potassium bromate given to groups B, C, and D. Rats were given *C. olitorius* extract and freshly prepared potassium bromate on a daily basis for 28 days using oral gavage. The animals were slaughtered while being gently sedated with diethyl ether twenty-four hours following the last treatment. Through a heart puncture, blood was taken. The animals' hearts, livers, and kidneys were also taken.

2.3. Preparation of Tissue Homogenates

The techniques described by Ugwu et al. [21] for generating tissue homogenates were applied. One gram of liver, kidney, and heart tissues were homogenized in 100 ml of ice-cooled 1.15% potassium chloride solution and 50 mM potassium phosphate buffer solution (pH 7.4), producing 10 percent homogenate (W/V). A 0.9 percent NaCl solution was used to wash the tissues, and they were subsequently homogenized. For homogenization, Sonicator's 4710 Ultrasonic Homogenizer was employed (Cole-Parmer Instrument Co., USA). The homogenate was centrifuged at 4000 rpm for 15 minutes at 4°C before the supernatant was collected for additional analysis.

2.4. Determination of Oxidative Stress Parameters

Oxidative stress parameters were assessed in plasma and tissue homogenates using the methods described by Airaodion et al. [22]. In order to quantify malondialdehyde (MDA), a consequence of lipid peroxidation (LPO), reactive thiobarbituric acid (TBARS) molecules were used. Measurements were made of both enzymatic (superoxide dismutase, catalase, and glutathione peroxidase) and non-enzymatic (GSH) antioxidants.

2.5. Statistical Analysis

The outcomes are shown as the mean $\pm$ standard deviation. The level of group homogeneity was assessed using Tukey's test and one-way Analysis of Variance (ANOVA). All analyses were carried out using the Graph Pad Prism software, and P values less than or equal to 0.05 were considered statistically significant.

3. Results

The results of plasma, hepatic, renal, and cardiac oxidative stress indicators in rats at the end of 28 days after ingesting potassium bromate and *C. olitorius* extract are presented in Tables 1, 2, 3, and 4, respectively. The results showed that potassium bromate decreased plasma, hepatic, renal, and cardiac CAT, SOD, and GPx activity as well as GSH concentrations, but raised MDA levels, as compared to the control group. Rats were given extract from *C. olitorius* leaves at doses of 100 and 200 mg/kg body weight, with the results of the 200 mg/kg dose being comparable to those observed in the control group. These treatments resulted in significant increases in antioxidant levels (except GPx) and decreases in MDA levels in their plasma and tissues.

Table 1 Effect of *C. olitorius* on Plasma Oxidative Stress Biomarkers of Potassium Bromate-induced Rats

Oxidative Stress Biomarkers	Control	$KBrO_3$ Only	$KBrO_3$ + 100 mg/kg *C. olitorius*	$KBrO_3$ + 200 mg/kg *C. olitorius*	p-value
CAT (µmol/mg protein)	78.55±4.18	49.93±2.76	55.16±7.15	71.77±5.38	0.02
SOD (µmol/mg protein)	98.12±3.83	62.18±2.04	74.88±6.33	89.34±4.27	0.01
GSH (µg/mg protein)	40.46±1.38	25.18±1.11	33.18±3.02	38.00±2.52	0.01
GPx (µmol/mg protein)	42.85±2.05	27.88±1.62	26.92±2.03	29.76±1.81	0.04
MDA (nmol/mg protein)	99.79±5.21	150.02±5.57	141.28±8.74	117.64±5.67	0.02

Values are presented as Mean±SD, where n = 6.; **Legend**: CAT = Catalase, SOD = Superoxide Dismutase, GSH = Glutathione, GPx = Glutathione Peroxidase, MDA = Malondialdehyde

Table 2 Effect of *C. olitorius* on Hepatic Oxidative Stress Biomarkers of Potassium Bromate-induced Rats

Oxidative Stress Biomarkers	Control	$KBrO_3$ Only	$KBrO_3$ + 100 mg/kg *C. olitorius*	$KBrO_3$ + 200 mg/kg *C. olitorius*	p-value
CAT (µmol/mg protein)	94.18±4.49	58.29±2.11	69.74±3.96	83.16±3.94	0.00
SOD (µmol/mg protein)	108.72±4.29	68.63±1.83	74.85±4.27	96.96±3.56	0.02
GSH (µg/mg protein)	61.92±2.09	33.28±2.05	48.23±2.19	56.28±3.11	0.03
GPx (µmol/mg protein)	55.94±1.75	30.30±1.93	35.64±2.85	34.26±2.18	0.04
MDA (nmol/mg protein)	107.52±3.38	148.87±3.88	135.94±3.27	119.71±4.84	0.00

Values are presented as Mean±SD, where n = 6.; **Legend**: CAT = Catalase, SOD = Superoxide Dismutase, GSH = Glutathione, GPx = Glutathione Peroxidase, MDA = Malondialdehyde

Table 3 Effect of *C. olitorius* on Renal Oxidative Stress Biomarkers of Potassium Bromate-induced Rats

Oxidative Stress Biomakers	Control	$KBrO_3$ Only	$KBrO_3$ + 100 mg/kg *C. olitorius*	$KBrO_3$ + 200 mg/kg *C. olitorius*	p-value
CAT (µmol/mg protein)	82.82±3.47	56.46±3.29	63.62±4.41	77.28±3.71	0.03
SOD (µmol/mg protein)	119.56±4.17	81.35±2.25	93.19±3.82	110.65±3.90	0.02
GSH (µg/mg protein)	36.46±1.38	20.92±1.01	25.62±2.78	34.37±3.23	0.01
GPx (µmol/mg protein)	42.43±3.11	29.37±2.17	30.44±1.52	33.18±1.98	0.04
MDA (nmol/mg protein)	102.61±4.73	144.99±3.28	130.12±3.84	116.81±3.82	0.00

Values are presented as Mean±SD, where n = 6.; **Legend**: CAT = Catalase, SOD = Superoxide Dismutase, GSH = Glutathione, GPx = Glutathione Peroxidase, MDA = Malondialdehyde

Table 4 Effect of *C. olitorius* on Cardiac Oxidative Stress Biomarkers of Potassium Bromate-induced Rats

Oxidative Stress Biomarkers	Control	$KBrO_3$ Only	$KBrO_3$ + 100 mg/kg *C. olitorius*	$KBrO_3$ + 200 mg/kg *C. olitorius*	p-value
CAT (µmol/mg protein)	76.76±2.45	50.64±2.21	59.36±2.71	68.83±3.12	0.02
SOD (µmol/mg protein)	100.74±4.25	60.00±2.69	74.68±4.40	89.92±3.56	0.02
GSH (µg/mg protein)	39.27±1.91	25.75±2.03	30.38±2.34	36.32±1.73	0.02
GPx (µmol/mg protein)	47.23±1.78	28.14±3.72	28.42±1.44	32.82±1.78	0.04
MDA (nmol/mg protein)	100.18±3.05	141.84±3.18	134.23±8.18	112.30±5.53	0.00

Values are presented as Mean±SD, where n = 6.; **Legend**: CAT = Catalase, SOD = Superoxide Dismutase, GSH = Glutathione, GPx = Glutathione Peroxidase, MDA = Malondialdehyde

4. Discussion

Malondialdehyde (MDA) and reduced glutathione (GSH) have generally been found to respectively increase and decrease in numerous tissues under oxidative stress conditions [23]. When combined with additional markers, such as cellular quantities of different antioxidants, MDA and GSH are effective markers for the detection of cellular damage caused by reactive oxygen species (ROS) [24,25].

Malondialdehyde (MDA) levels in the plasma, liver, kidney, and heart of the rats in this investigation were considerably higher after exposure to $KBrO_3$ than they were in the control group. This is an indication of the collapse of the antioxidant defense mechanisms and increased peroxidation. Malanoldehyde and 4-hydroxynonenal are examples of lipid hydroperoxide decomposition products that can result in chaotic cross-linkage with proteins and nucleic acids, which is a crucial step in the development of cancer [26]. In this study, $KBrO_3$ toxicity is shown to significantly increase the plasma, hepatic, renal, and cardiac lipid peroxidation (LPO) levels. Furthermore, when a free radical-mediated LPO causes significant tissue damage, the membrane is damaged, which reduces the amount of fluid in the membrane. Concurrent administration of $KBrO_3$ and *C. olitorius* completely reversed these changes, resulting in a considerable drop in MDA levels, indicating its antioxidant defense against $KBrO_3$-induced oxidative damage. This is in line with a research by Airaodion et al. [27], which found that methanolic extract of *C. olitorius* leaves reduced the oxidative stress that ethanol caused in rats.

Antioxidant enzymes, like superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), are found in many tissues and physiologically decrease oxidative stress by facilitating the elimination of reactive oxygen species (ROS)[28]. In our study, $KBrO_3$-treated rats had significantly lower SOD, catalase, and GPx activities than the rats in the control group. Therefore, the oxidative stress condition caused by $KBrO_3$ was probably caused by a decrease in the activity of cytoplasmic antioxidant enzymes [29]. That is, the increase in cellular hydrogen peroxide, which is primarily eliminated by these enzymes, was brought about by the reductions in cytoplasmic SOD, catalase, and GPx activity. When given alongside $KBrO_3$, *C. olitorius* considerably lowered the amount of SOD and catalase in the plasma, liver, kidney, and heart, demonstrating its protective impact. This is consistent with the results of Adedosu et al. [30], who examined the effects of *Corchorus olitorius* leaf extract on specific antioxidants and biochemical indicators in sodium arsenite-exposed rats.

Furthermore, *C. olitorius* leaf extract effectively reduced the decline in plasma, hepatic, renal, and cardiac GSH content caused by $KBrO_3$ in this present study. These findings imply that the *C. olitorius* leaf has a suppressive impact on the level of oxidative stress caused by $KBrO_3$. Since the leaf of *C. olitorius* does not scavenge free radicals, its suppressive impact on oxidative stress is most likely due to a decrease in the production of nitric oxide (NO). It is well-known that NO reacts quickly with O_2 to produce $ONOO^-$, a potent oxidant with cytotoxic properties [31]. O_2^- generation from $KBrO_3$ in the presence of GSH, albeit being an *in vivo* experimental result, suggests that one of the mechanisms of $KBrO_3$-induced oxidative stress is the rise of cellular $ONOO^-$ generated from O_2^- and NO.

In rats treated with $KBrO_3$, the treatment of *C. olitorius* leaf extract considerably reduced catalase activity but not GPx. In the cytoplasmic component of the cell, catalase is known to have substantially lower hydrogen peroxide-removing capacity than GPx [32]. Therefore, it is probable that *C. olitorius* leaf extract's inability to restore the GPx activity was what caused it to only partially reduce $KBrO_3$-induced oxidative stress. According to reports, the element selenium inactivates the enzyme GPx by non-enzymatically reacting with $ONOO^-$ in the active site [32]. It is likely that O_2^- produced by $KBrO_3$, rather than NO, is directly involved in the $KBrO_3$-induced reduction of GPx activity because *C. olitorius* leaf extract had no effect on the decrease in GPx activity caused by $KBrO_3$ treatment in all the tissues investigated.

Rahman et al. [18] made the suggestion that NO functions as an antioxidant in $KBrO_3$-induced oxidative stress in a recent study. However, the current data strongly hints that oxidative stress and tissue damage caused by $KBrO_3$ are accelerated by NO and $ONOO^-$.

5. Conclusion

The study demonstrated that $KBrO_3$ caused significant increase in the plasma, hepatic and cardiac lipid peroxidase level resulting in oxidative stress. This effect was attenuated by *C. Olitorius* through the restoration of antioxidant enzymes demonstrating its protective impact.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no competing interests.

Statement of ethical approval

This study was approved by relevant ethical committee.

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