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Original Research Article

Ameliorative Effect of *Parkia biglobosa* (African Locust Bean) Seed against Potassium Bromate-induced Oxidative Stress

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Abstract

This study sought to investigate the ameliorative effect of *Parkia biglobosa* against potassium bromate-induced oxidative stress in Wistar rats. *P. biglobosa* was extracted with soxhlet extractor with ethanol as the solvent. Twenty-four Wistar rats were grouped into A, B, C and D after seven days acclimatization. Group A was given distilled water orally. Animals in groups B, C and D were administered 100 mg/kg body weight of potassium bromate, but groups C and D were also treated with 100 and 200 mg/kg body weight of *P. biglobosa* respectively. At the end of 28 days after receiving potassium bromate and locust bean extract, the results of plasma, hepatic, renal, and cardiac oxidative stress indicators in rats are shown in Tables 1, 2, 3, and 4 respectively. The findings demonstrated that, as compared to the control group, potassium bromate caused a significant decrease ($p \leq 0.05$) in the plasma, hepatic, renal, and cardiac catalase, superoxide dismutase (SOD), and glutathione peroxidase activity (GPx), reduced glutathione (GSH) concentrations, and increased Malondialdehyde (MDA) levels. Rats treated with extract from locust bean seeds at doses of 100 and 200 mg/kg body weight experienced significant increases in antioxidant levels and decreases in Malondialdehyde levels in their plasma and tissues, with the findings of the 200 mg/kg dose being comparable to those seen in the control group. *P. biglobosa* was found to cause significant increase in antioxidant levels among the rats thereby ameliorating the oxidative damage caused by potassium bromate.

Keywords: Ameliorative effect, Oxidative Stress Biomarkers, *Parkia biglobosa,* Potassium Bromate

INTRODUCTION

One of the main risk factors for developing many chronic diseases is oxidative stress. Reactive oxygen species (ROS) are produced in excess compared to the body's antioxidant defenses, which causes tissue damage like hepatonephrotoxicity and cardiotoxicity. The body's antioxidant defenses give the system the ability to get rid of ROS, get back to a reducing environment, and fix tissue damage (Hala and Thanaa, 2013). Nitric oxide (NO) and superoxide ions are examples of free radicals that are created as second messengers, especially by

immune cells. Nitric oxide synthase quickly converts superoxide to peroxynitrite while hydrogen peroxide (H_2O_2) decomposes slowly into the extremely reactive hydroxyl radicals. Peroxynitrite and hydroxyl radicals are both extremely reactive oxidizing agents that can oxidize DNA, lipids, and proteins (Reuter *et al*., 2010). Numerous chronic diseases, including hepatotoxicity, nephrotoxicity, atherosclerosis, hypertension, diabetes mellitus, and malignancies, are largely caused by oxidative stress (Krajcovicova *et al*., 2012).

As a common food additive in the production of bread, potassium bromate ($KBrO_3$) is also discovered in drinking water samples as a result of ozone disinfection. It was discovered that administering $KBrO_3$ to rats caused oxidative stress and passively reduced the blood's antioxidant capacity (Ahmed and Mahmood, 2012). Antioxidants in the diet can stop or slow down the oxidation of cellular substrates that are vulnerable, preventing oxidative stress. Due to their high antioxidative activity, phenolic compounds like flavonoids, phenolic acids, diterpenes, saponins, and tannins have drawn a lot of attention (Rubiolo *et al*., 2008). As a result, it's critical to add antioxidants to our diets to safeguard against a variety of chronic conditions linked to oxidative damage. Additionally, antioxidants are crucial for maintaining food quality because they can stop lipids from degrading owing to oxidation (Erukainure *et al*., 2012).

The common name for *P. biglobosa* is "African locust bean." It is a perennial tree in the Leguminosae genus of legumes (Olabinri *et al*., 2013). The plant's seeds are contained in an edible pulp that is yellowish, mealy, and sweet tasting (Aliero*et al*., 2001). It is a plant whose high concentration of phenolic chemicals is well known (Miollogo-Kone *et al*., 2008). Epigallocatechin, epicatechin 3-O-gallate, and epigallocatechin 3-O-gallate were all present in the plant's bark (Alabi *et al*., 2005). Heart and saponin glycosides can be found in leaf extract (Miollogo-Kone *et al*., 2009). Protein and lactose are abundant in the fruit's pulp and seeds (Alabi *et al*., 2005). Oxalate, hydrogen cyanide, tannin, and phytate are examples of antinutritional components found in the seeds (Olabinri *et al*., 2013). The *P. biglobosa* extract is antibacterial (Miollogo-Kone *et al*., 2008), antidiabetic (Odetola *et al*., 2019), antifungal (Kouadio *et al*., 2000), anti-inflammatory (Fawole and Abioye, 2002), anti-diarrheal (Agunu *et al*., 2005), anti-hypertensive (Airaodion and Ogbuagu, 2020), as well as hypoglycemia and hypolipidemic (Airaodion *et al*., 2019a) in addition to having other beneficial effects. In a recent study, Ezirim *et al*. (2022) reported that *P. biglobosa* seed possessed therapeutic potential against potassium bromate-induced testicular toxicity. The purpose of this investigation was to ascertain whether it could protect Wistar rats from oxidative damage caused by potassium bromate.

MATERIALS AND METHODS

Procurement of Chemical and Kits

Potassium bromate ($KBrO_3$) and the biochemical kits for the determination of oxidative stress parameters were purchased from Cephas Global Resources Limited (A division of Deliving Stone Int'l), E Line 444 (along Fin Bank/Eco Bank), Head Bridge Market, Onitsha, Anambra State, Nigeria.

Collection and Extraction of *Parkia biglobosa*

P. biglobosa (African locust bean) seed was purchased from a local market at Orita-Challenge area of Ibadan, Nigeria and were identified by a botanist. They were sun dried, then a mechanical blender (Moulinex) was used to grind them into powder. According to the procedures outlined by Airaodion *et al*. (2019b; 2020a), the extraction was carried out using a soxhlet device with ethanol as the solvent. A round bottom flask with a capacity of 250 mL of ethanol was connected to the soxhlet extractor and condenser on a heating mantle along with approximately 25 g of the sample powder. After being heated by the heating mantle, the solvent started to evaporate as it moved through the device to the condenser. The sample-containing thimble was housed in a reservoir that the condensate dropped into. The cycle restarted when the solvent's level reached the siphon and it was poured back into the flask with a flat bottom. A total of 18 hours were given to the procedure. Once the process was completed, the ethanol was evaporated in a rotary evaporator at 35 ^oC with a yield of 2.55 g which represents a percentage yield of 10.20%. The extract was kept in the fridge until it was required.

Animal Treatment

The experiment involved twenty-four (24) mature male Wistar rats (*Rattus norvegicus*) weighing between 140 and 160 g. Before the experiment began, they were acclimated for seven (7) days in a laboratory setting. The rats were kept in wire-mesh cages with unlimited access to water and commercial rat food. The animals were housed in conventional temperature and humidity settings with 12-hour light/dark cycles. The Declaration of Helsinki and the regulations set forth by the Committee for the Purpose of Control and Supervision of Experiments on Animals were followed in the conduct of this investigation. Additionally, animal experiments were conducted in compliance with NIH protocol (National Research Council, 2011). They were assigned to groups A, B, C, and D at random. Oral distilled water was administered to group A as the normal control. Animals in

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

Oxidative Stress Biomarkers	Control	$KBrO_3$ Only	$KBrO_3$ + 100 mg/kg *P. biglobosa*	$KBrO_3$ + 200 mg/kg *P. biglobosa*	p-value
CAT (µmol/mg protein)	78.55±4.18	49.93±2.76	62.33±3.02	76.27±4.01	0.03
SOD (µmol/mg protein)	98.12±3.83	62.18±2.04	80.00±2.81	92.92±2.19	0.03
GSH (µg/mg protein)	40.46±1.38	25.18±1.11	31.66±1.83	37.14±2.00	0.01
GPx (µmol/mg protein)	42.85±2.05	27.88±1.62	36.58±1.11	38.25±1.77	0.02
MDA (nmol/mg protein)	99.79±5.21	150.02±5.57	135.35±4.11	107.82±3.29	0.03

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 *P. biglobosa* on Hepatic Oxidative Stress Biomarkers of Potassium Bromate-induced Rats

Oxidative Stress Biomarkers	Control	$KBrO_3$ Only	$KBrO_3$ + 100 mg/kg *P. biglobosa*	$KBrO_3$ + 200 mg/kg *P. biglobosa*	p-value
CAT (µmol/mg protein)	94.18±4.49	58.29±2.11	73.19±3.53	87.87±3.27	0.04
SOD (µmol/mg protein)	108.72±4.29	68.63±1.83	82.47±2.34	99.27±2.94	0.03
GSH (µg/mg protein)	61.92±2.09	33.28±2.05	44.05±2.72	58.51±1.56	0.04
GPx (µmol/mg protein)	55.94±1.75	30.30±1.93	51.29±1.29	56.11±1.43	0.02
MDA (nmol/mg protein)	107.52±3.38	148.87±3.88	139.63±3.28	124.26±3.45	0.01

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

groups B, C and D were administered 100 mg/kg body weight of potassium bromate, but groups C and D were also treated with 100 and 200 mg/kg body weight of *P. biglobosa* respectively. Rats received daily doses of freshly produced potassium bromate and *P. biglobosa* by oral gavage. Twenty-four hours after the last treatment, the animals were sacrificed while being lightly sedated with diethyl ether. Blood sample was collected through cardiac puncture. Liver, kidney and heart of the animals were also harvested.

Preparation of Tissue Homogenates

The Hala and Thanaa (2013) described methods for creating tissue homogenates was used. Briefly, 100 ml of ice-cooled 1.15% potassium chloride solution and 50 mM potassium phosphate buffer solution (pH 7.4) were used to homogenize one gram of liver, kidney, and heart tissues, yielding 10 percent homogenate (W/V). The tissues were collected separately, washed in a 0.9 percent NaCl solution on ice, and then homogenized. Sonicator's 4710 Ultrasonic Homogenizer was used for homogenization (Cole-Parmer Instrument Co., USA). The supernatant was collected for further examination after the homogenate had been centrifuged at 4000 rpm for 15 minutes at 4°C.

Determination of Oxidative Stress Parameters

Using the techniques outlined by Airaodion *et al*. (2019c), oxidative stress parameters were measured in plasma and tissue homogenates. Malondialdehyde (MDA), a byproduct of lipid peroxidation (LPO), was measured in terms of reactive thiobarbituric acid compounds (TBARS). Enzymatic (superoxide dismutase, catalase, and glutathione peroxidase) and non-enzymatic (GSH) anti-oxidants were also measured.

Statistical Analysis

The results are presented as the mean ± standard deviation. One-way Analysis of Variance (ANOVA) and Tukey's test were used to determine the degree of homogeneity among the groups. P values under 0.05 were regarded as statistically significant for all analyses, which were conducted using Graph Pad Prism Software (Version 8).

RESULTS

At the end of 28 days after receiving potassium bromate and locust bean extract, the results of plasma, hepatic, renal, and cardiac oxidative stress indicators in rats are shown in Tables 1, 2, 3, and 4 respectively. The findings demonstrated that, as compared to the control group, potassium bromate caused a significant decrease ($p \leq 0.05$) in the plasma, hepatic, renal, and cardiac CAT, SOD, and GPx activity, GSH concentrations, and increased MDA levels. Rats treated with extract from locust bean seeds at doses of 100 and 200 mg/kg body

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

Oxidative Stress Biomakers	Control	$KBrO_3$ Only	$KBrO_3$ + 100 mg/kg *P. biglobosa*	$KBrO_3$ + 200 mg/kg *P. biglobosa*	p-value
CAT (µmol/mg protein)	82.82±3.47	56.46±3.29	68.88±3.89	79.44±3.12	0.05
SOD (µmol/mg protein)	119.56±4.17	81.35±2.25	99.93±5.14	108.23±3.45	0.04
GSH (µg/mg protein)	36.46±1.38	20.92±1.01	27.38±2.22	34.00±2.18	0.00
GPx (µmol/mg protein)	42.43±3.11	29.37±2.17	36.36±1.87	39.45±3.13	0.02
MDA (nmol/mg protein)	102.61±4.73	144.99±3.28	123.78±4.73	111.35±5.54	0.01

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 *P. biglobosa* on Cardiac Oxidative Stress Biomarkers of Potassium Bromate-induced Rats

Oxidative Stress Biomarkers	Control	$KBrO_3$ Only	$KBrO_3$ + 100 mg/kg *P. biglobosa*	$KBrO_3$ + 200 mg/kg *P. biglobosa*	p-value
CAT (µmol/mg protein)	76.76±2.45	50.64±2.21	61.18±2.11	75.00±2.62	0.02
SOD (µmol/mg protein)	100.74±4.25	60.00±2.69	87.15±3.72	96.13±3.83	0.03
GSH (µg/mg protein)	39.27±1.91	25.75±2.03	32.21±1.25	38.78±3.18	0.00
GPx (µmol/mg protein)	47.23±1.78	28.14±3.72	35.23±1.27	43.49±2.00	0.02
MDA (nmol/mg protein)	100.18±3.05	141.84±3.18	131.86±3.75	103.26±4.01	0.00

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

weight experienced significant increases in antioxidant levels and decreases in MDA levels in their plasma and tissues, with the findings of the 200 mg/kg dose being comparable to those seen in the control group.

DISCUSSION

The increased creation of reactive oxygen species (ROS) that causes an oxidative stress in cells is a common feature of the toxicity of compounds, including $KBrO_3$ (Khan *et al*., 2001). Superoxide anions and their derivatives, particularly the extremely reactive and harmful hydroxyl radical that causes the peroxidation of cell membrane lipids, are the most common ROS produced in living cells (Airaodion *et al*., 2020b). One of the metabolic byproducts of lipid peroxides produced by the lipid oxidation reaction brought on by oxygen free radicals in tissues is MDA. Alterations in membrane structure and enzyme inactivation have been associated with lipid peroxidation, a kind of oxidative degradation of polyunsaturated fatty acids (Airaodion *et al*., 2019d). The potassium bromate's oxidant effects on rat blood, liver, kidney, and heart are indicated by the considerable decrease in endogenous antioxidant and the elevation of MDA. The body's arsenal of antioxidants, including CAT, SOD, GSH, and GPx, are created by the body to'mop up' or neutralize free radicals that can damage cells and so protect it from oxidative stress. Genetic makeup and environmental exposure, including diet and chemical exposure, have an impact on the body's capacity to produce these antioxidants (Oyewole, 2011). The complex process of lipid peroxidation impairs the structure and operation of cells. Cell lyses are brought on by the peroxidation of the lipids in the cell membrane, which starts the process of the membrane's integrity being lost. However, lipid peroxides or protein carbonyls are likely to damage tissue as a result of the decreased activity of tissue antioxidant enzymes (Airaodion *et al*., 2019c). Numerous studies show that the use of $KBrO_3$ resulted in significant oxidative stress and elevated lipid peroxidation (Khan *et al*., 2003; Abd El-Ghany *et al*., 2011; Ahmed *et al*., 2012). It is possible to hypothesize that enhanced lipid peroxidation is caused by antioxidant enzymes working less efficiently (Airaodion *et al*., 2019e). One of the key substances for the control of numerous cellular processes is glutathione. By interacting with superoxide, peroxyl, and singlet oxygen to produce oxidized glutathione and other disulfides, it performs a direct antioxidant role. A GSH-dependent antioxidant enzyme is called GPx (Airaodion *et al*., 2020c). As opposed to this, SOD catalyzes the dismutation of the superoxide anion (O_2) into H_2O_2, which catalase then detoxifies to H_2O (Airaodion *et al*., 2020b). Catalase is a typical enzyme that is present in almost all living things. Catalyzing the breakdown of hydrogen peroxide into water and oxygen is one of its roles (Ogbuagu *et al*., 2019; Airaodion *et al*., 2019f). The current findings are consistent with the theory that the antioxidant defense system depletion is a contributing factor in $KBrO_3$ toxicity

(Ahmed *et al*., 2012). The routines of frequently used chemotherapy frequently result in a considerable decrease in antioxidant enzyme activities and an increase in free radicals in experimental models as well as in people, and this is particularly relevant to $KBrO_3$ treatment (Ballmaier and Epe, 2015).

In this current investigation, it was found that rats treated with $KBrO_3$ had lower levels of GSH than those in the control group, indicating that low levels of GSH increased lipid peroxidation, which in turn increased GSH consumption. When compared to the control group, the activity of the GSH-dependent antioxidant enzyme GPx in the $KBrO_3$treated animals was considerably lower. The decreasing availability of reduced glutathione, the GPx enzyme's substrate, may be the cause of the observed drop in GPx activity (Airaodion *et al*., 2019d).

In the $KBrO_3$-treated rats, the decline in SOD activity may have contributed to the development and spread of lipid peroxidation (Ajith*et al*., 2007). The superoxide anion formed during the regular metabolic process cannot be removed by the lowered SOD activity (Badary *et al*., 2004). The increased production of ROS like superoxide and hydrogen peroxide, which in turn causes the inhibition of these enzymes' activities, may be the cause of the decreased SOD and catalase activities (Airaodion *et al*., 2020c). The continual production of ROS *in vivo* for physiological functions is widely recognized (Ogbuagu*et al*., 2019). However, ROS generation that exceeds the capacity of an antioxidant system can lead to oxidative stress and oxidative damage to proteins, nucleic acids, and lipids (Airaodion *et al*., 2019c). H_2O_2 produced by free radicals or by SOD during the elimination of superoxide anions is scavenged by catalase.

The decline in SOD, CAT, and GPx activity in rats treated with *P. biglobosa* at doses of 100 and 200 mg/kg body weight in combination with $KBrO_3$ treatment was prevented. The presence of polyphenols in *P. biglobosa* may contribute to its protective properties. In actuality, polyphenols influence the expression of a crucial enzyme in cellular antioxidant defenses as well as the detoxification of xenobiotics, according to Moskaug *et al*. (2005). It is possible that *P. biglobosa* is an effective chemopreventive agent against oxidative stress and can reduce potassium bromate-mediated hepatic, renal, and cardiac oxidative damage in rats because of the considerable recovery of plasma, hepatic, renal, and cardiac antioxidant contents (Agarwal, 2005). This outcome is consistent with the research of Ogunyinka *et al*. who found that *P. biglobosa* reduced oxidative stress in the liver (2017) and heart (2019) of streptozotocin-induced diabetic rats.

The beneficial effects of *P. biglobosa* were comparable to those described by Airaodion *et al*. (2019c), who found that *P. biglobosa* ameliorated the oxidative stress caused by ethanol in Wistar rats. *P. biglobosa*'s high level of omega-3 polyunsaturated fatty acid (PUFA) can be ascribed to its antioxidant activity; thus, vegetarians who cannot ingest fish oil can obtain the same health advantages from locust beans, which contain omega-3 PUFA (Alabi *et al*., 2005). The extract's potent antioxidant activity may have reduced the formation of oxygen radicals by white blood cells and improved cardiovascular health. In fact, *P. biglobosa* contains a high concentration of phenolic compounds and strong antioxidant activity, according to Tamfu *et al*. (2021).

CONCLUSION

The findings from this study showed that potassium bromate induced oxidative stress could cause damage to the kidneys, liver and the heart. *P. biglobosa* was found to be effective chemopreventive agent against the oxidative stress induced by potassium bromate and can reduce the oxidative damage induced by it. It is recommended that this study could be replicated in clinical trials among human volunteers.

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COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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