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Therapeutic Potential of *Parkia biglobosa* Seed against Potassium Bromate-induced Testicular Toxicity

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was aimed at assessing the therapeutic potential of *Parkia biglobosa* (*P. biglobosa*) seed against potassium bromate-induced testicular toxicity.
Methodology: *P. biglobosa* was extracted with soxhlet extractor with ethanol as the solvent. Twenty-four adult male Wistar rats were acclimatized under laboratory conditions and were randomly grouped into A, B, C and D. 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. Both potassium

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bromate and *P. biglobosa* were freshly prepared on daily basis and administered to rats by oral gavage. After 28 days of treatment, the animals were sacrificed under mild diethyl ether anaesthetization 24 hours after cessation of last treatment. The testes were removed homogenized in the ice cold 0.25 M sucrose solution. The homogenates were centrifuged at 5000 ×g for 10 minutes in a refrigerated centrifuge. The supernatant was collected and stored frozen for further analysis. The parameters were measured using standard methods.

Results: When compared to animals in the control group, animals intoxicated with $KBrO_3$ had lower testicular concentrations of total cholesterol, total protein, glycogen, sialic acid, MDA, and GSH, as well as higher levels of ALP, SOD, and CAT activity. Additionally, it was shown that as compared to the animals in the control group, $KBrO_3$ boosted the testicular ACP's activity. However, *P. biglobosa* treatment of intoxicated rats reduced these alterations in a dose-dependent manner.

Conclusion: The results of this investigation demonstrated that potassium bromate caused testicular toxicity, and that *P. biglobosa* treatment counteracted this effect. Thus, it is recommended that these results be investigated in clinical trials in human volunteers.

Keywords: Parkia biglobosa; potassium bromate; testicular toxicity.

1. INTRODUCTION

The human reproductive system must operate at its peak for there to be life. About 15% of couples experience infertility as a health issue worldwide [1]. It is now clear that poor semen quality causes the issue in at least 50% of all cases [2,3]. Low sperm count is one of the recognized causes of infertility and may be brought on by hormonal imbalance caused by oxidative stress. Recent research has suggested that the fundamental cause of infertility may be changes to the sperm's molecular components (paternal genome, mitochondrial DNA, and transcripts) [4,5].

It has been demonstrated that potassium bromate ($KBrO_3$) causes oxidative stress in the kidney and liver of rats as well as thyroid follicular cell tumors [6]. Swiss albino rats' physiological and metabolic processes are also negatively impacted [7]. Additionally, $KBrO_3$ has been delisted from the list of permitted additives for treating flour due to evidence that it is carcinogenic [8]. However, under carefully controlled baking circumstances, potassium bromate is transformed into potassium bromide, which is thought to be safe for consumption [8]. Although this salt is currently prohibited in some nations [9], it is still used in the USA and Japan. Because it is inexpensive, accessible, and possibly the best and most effective oxidizing agent, acting slowly throughout the fermentation period and altering the structure and properties of the dough, there is still evidence of some illegal use of $KBrO_3$ in countries such as Nigeria where its use is prohibited [10,11].

A perennial tropical plant known as *Parkia biglobosa* is primarily found in West Africa's savannah [12]. *P. biglobosa* is also known as the African locust bean, and in Nigeria it is referred to 'Ogiri' by the Igbos, 'Dorowa' by the Hausas, and 'Iru' by the Yorubas [13]. The consumption and financial worth of the bean seeds from the African locust bean have contributed to its popularity. The beans are typically fermented in West Africa, particularly Nigeria, to produce a food item known as "Dawadawa." Dawadawa is a protein-rich, dark-colored flavor that is frequently added to regional soups and used as a source of protein [14–16]. Additionally, other plant components including the fruit and stem have also been used. Fresh fruit pulp can be used as a supplement for minerals, and the stem bark has been said to have anti-snake venom properties [17]. The African locust bean has been studied for its potential as a chemotherapeutic in addition to its culinary uses [18-20]. A decoction of the stem bark has been employed in traditional medical procedures as a hot mouthwash to treat toothaches [21]. It has been claimed that a blend of the root and leaves is an effective cure for tooth decay and painful eyes [22]. According to Airaodion et al. [23], *P. biglobosa* seed has hepatoprotective properties. *P. biglobosa* seed was found by Airaodion and Ogbuagu [24] to reduce hypertension in a different investigation. This study is therefore aimed at assessing the therapeutic potential of *Parkia biglobosa* seed against potassium bromate-induced testicular toxicity.

2. MATERIALS AND METHODS

2.1 Procurement of Chemical and Kits

Potassium bromate ($KBrO_3$) and the biochemical kits for the determination of testicular 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.

2.2 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. [25,26], 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 ^{o}C 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.

2.3 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 [27]. 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 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. After receiving sequential treatments for 28 days, the animals were slaughtered and their testicles were harvested. Twenty-four hours after the last treatment, the animals were sacrificed while being lightly sedated with diethyl ether. The testes were taken out and put in an isotonic 0.25 M sucrose solution (1:5 w/v) solution. The 0.25 M sucrose solution was extremely cold when the testes were weighed and homogenized in it. In a chilled centrifuge, the homogenates were spun at 5000 g for 10 minutes (TDL-5000B Shanghai Anke company, Ltd., China). For further study, the supernatant was collected and frozen-stored.

2.4 Estimation of Biochemical Indices

Using a UV/VIS spectrophotometer, the biochemical parameters in the testicular homogenate of rats were examined. Total protein was quantified using the method described by Gornall et al. [28]. The procedure outlined by Kemp et al. [29] was used to determine the amount of glycogen in the testes. According to Fredrickson et al. [30]'s procedure, the amount of total cholesterol in the testes was examined using a reagent assay kit. The calorimetric approach as published by Yao et al., [31], was used to determine a total sialic acid. The technique outlined by Wright et al. [32] was used to measure the activity of acid phosphatase (ACP) and alkaline phosphatase (ALP). The procedure outlined by Airaodion et al. [23] was used to measure the amount of reduced glutathione (GSH) in the samples. Using the technique outlined by Airaodion et al. [23], thiobarbituric acid reactive substances (TBARS) were quantified as an estimation of malondialdhyde (MDA), a byproduct of lipid peroxidation. Using the technique outlined by Airaodion et al. [23], the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were measured.

2.5 Data Analysis

One-way ANOVA was used to analyze the data, and the Tukey post hoc mean comparison test was employed to see whether there were any statistically significant differences between the variables. The analyzed data were expressed as the mean and standard error (SEM) of the mean for six replicates. Statistical significance was defined as a P-value of 0.05 or below ($P \leq 0.05$).

Graph Pad Prism was used for all statistical analyses (version 8.0).

3. RESULTS

When compared to animals in the control group, animals intoxicated with $KBrO_3$ had lower testicular concentrations of total cholesterol, total protein, glycogen, sialic acid, MDA, and GSH, as well as higher levels of ALP, SOD, and CAT activity. Additionally, it was shown that as compared to the animals in the control group, $KBrO_3$ boosted the testicular ACP's activity. However, *P. biglobosa* treatment of intoxicated rats reduced these alterations in a dose-dependent manner.

4. DISCUSSION

Testicular secretory components include total protein, glycogen, sialic acid, and cholesterol are useful for assessing the functional capacity of the testicles [33]. Comparing rats exposed to $KBrO_3$ alone to those in the control group, a significant rise in cholesterol levels was seen in the testes (table 1), which may indicate damage to Sertoli cells, which can result in phagocytosis and the deposition of cell membrane lipid [34]. Testicular cells require cholesterol for membrane biogenesis, cell communication, and as a precursor for androgen synthesis in order to function normally [35]. Low testicular cholesterol has been linked to Leydig cell-inhibited androgen production. When induced by pituitary luteinizing hormone (LH), leydig cells secrete a group of hormones known as androgens, including testosterone, androstenedione, and dehydroepiandrosterone (DHEA). LH stimulates the Leydig cells' production and secretion of testosterone by increasing the activity of the enzyme cholesterol desmolase [36]. However, when compared to those exposed to $KBrO_3$ without treatment, treatment with *P. biglobosa* extract at doses of 100 and 200 mg/kg significantly raised the concentration of total cholesterol. This might be attributed to *P. biglobosa*'s ability to protect the testis by synthesizing cholesterol, a precursor in the steroidogenesis of the male sex hormone. This study clearly shows that $KBrO_3$ is the cause of the decreased cholesterol levels observed in the animals exposed to $KBrO_3$ alone, but the increased testicular cholesterol levels in the rats treated with *P. biglobosa* extract were indicative of the extract's protective effect on the testicular cells.

The growth of the testicles and spermatogenesis depend heavily on protein production. The spermatogenic process, which produces the proteins needed for germ cell development, is regulated by sertoli cells [37,38,39]. When compared to animals in the control group, animals exposed to $KBrO_3$ alone showed a decrease in testicular protein (table 1). This drop may be attributable to enzymatic inhibition of protein synthesis caused by $KBrO_3$'s effect on genetic information. The results of the current investigation showing that $KBrO_3$ intoxication caused a considerable decrease in protein content in the testis were corroborated by Ahmad and Mahmood [40] and Mohamed and Saddek [41]'s findings.

Glycogen controls germ cell survival and is an essential component of healthy testicular growth and function [42]. The primary energy source in the animal reproductive system's testes is testicular glycogen [43]. It is essential for gonadal maturation and healthy operation [44]. It provides seminiferous tubular cells with glucose stores. Steroid hormones and glycogen levels are inversely correlated [45]. Only animals exposed to $KBrO_3$ showed a significant ($P<0.05$) drop in testicular glycogen when compared to animals in the control group. The drop might be the result of an increase in testicular activity brought about by $KBrO_3$, which causes the testes to consume a lot of glucose. It's also possible that the potassium bromate-intoxicated group's decreased testicular glycogen was caused by an inhibition of the enzymes involved in glycogen synthesis. Following $KBrO_3$ injection, a decrease in glycogen levels prevented glycogen synthesis, which might ultimately reduce spermatogenesis [46]. The difference in glycogen concentration between the groups treated with *P. biglobosa* extract and the group exposed to $KBrO_3$ only may be an indication of the extract's facilitative effects.

Sialic acid (N-acetylneuraminic acid) is a product of pyruvic acid and N-acetylmannose [47]. It is a crucial component of glycoproteins and glycolipids. The findings of this investigation showed that, in comparison to the healthy control group, $KBrO_3$ exposure caused a considerable drop in testicular sialic acid. This decrease in spermatogenesis rate may be the cause of this drop in testicular sialic acid levels. The current study's findings, which are corroborated by earlier findings by Nwonuma et al. [48], showed that rats given potassium bromate intoxication had significantly less testicular sialic acid. Sialic acid levels recovered in the *P. biglobosa* treatment groups, suggesting that the extract

Table 1. Effect of *P. biglobosa* seed on the biochemical parameters of testes-homogenate of potassium bromate induced testicular toxicity of Wistar rats

Treatment Group	Total Cholesterol (mg/g)	Total Protein (mg/g)	Glycogen (mg/g)	Total Sialic Acid (mg/g)	ACP (IU/L)	ALP (IU/L)
Control	8.61 ± 0.84^{a}	4.73 ± 0.26^{a}	3.39 ± 0.21^{a}	5.13 ± 1.01^{a}	148.63 ± 9.36^{b}	183.33 ± 11.20^{a}
100 mg/kg $KBrO_3$ only	5.38 ± 1.11^{c}	2.26 ± 0.03^{c}	1.96 ± 0.03^{b}	2.85 ± 0.17^{c}	204.35 ± 12.93^{a}	132.39 ± 12.83^{c}
100 mg/kg $KBrO_3$ + 100 mg/kg *P. biglobosa*	6.52 ± 0.59^{bc}	3.38 ± 0.27^{b}	2.47 ± 0.37^{ab}	3.93 ± 0.83^{b}	157.64 ± 13.62^{b}	154.83 ± 8.27^{b}
100 mg/kg $KBrO_3$ + 200 mg/kg *P. biglobosa*	7.93 ± 0.92^{ab}	4.46 ± 0.62^{a}	3.25 ± 0.32^{a}	4.76 ± 0.75^{ab}	151.38 ± 8.94^{b}	177.64 ± 10.33^{ab}

Results are presented as mean±SEM with n = 6. Values with different superscripts along the same column are significantly different at P<0.05

LEGEND: ACP = Acid Phosphatase, ALP = Alkaline Phosphatase

Table 2. Effect of *P. biglobosa* seed on the oxidative stress parameters of testes-homogenate of potassium bromate induced testicular toxicity of wistar rats

Treatment Group	MDA (U/mg)	GSH (U/mg)	SOD (U/mg)	CAT (U/mg)
Control	0.54 ± 0.00 [a]	52.33 ± 3.23 [a]	62.86 ± 5.02 [a]	0.85±0.00 [a]
100 mg/kg $KBrO_3$ only	0.34 ± 0.00 [c]	38.82 ± 2.46 [c]	47.28 ± 4.45 [c]	0.52 ± 0.01 [c]
100 mg/kg $KBrO_3$ + 100 mg/kg *P. biglobosa*	0.40 ± 0.00 [b]	45.45± 2.64 [b]	55.30 ± 4.72 [b]	0.65 ± 0.01 [b]
100 mg/kg $KBrO_3$ + 200 mg/kg *P. biglobosa*	0.52±0.00 [a]	53.17±4.39 [a]	63.19±3.85 [a]	0.81±0.00 [a]

Results are presented as mean±SEM with n = 6. Values with different superscripts along the same column are significantly different at P<0.05.

LEGEND: MDA = Malondialdehyde, GSH = Reduced Glutathione, SOD = Superoxide Dismutase, CAT = Catalase

may be able to improve the production of components of the testicular secretory system. The results of Mohamed and Saddek [41], who observed that potassium bromate decreased the content of sialic acid but that this effect was reversed in rats treated with taurine and/or vanillin, are similarly compatible with this outcome.

With a pH-optimum in the acid zone, acid phosphatase (ACP) is a hydrolase enzyme that catalyzes the hydrolysis of different phosphate esters [26]. ACP's prostatic isoenzyme is a crucial testis diagnostic sign. ACP is located in cellular lysosomes, thus lipid peroxidation that compromises the integrity of the membrane may cause an increase in the enzyme in the testicles. Animals exposed to $KBrO_3$ had higher levels of ACP activity than those in the control group. The lysosomal membrane rupturing and enzyme liberation by $KBrO_3$ that results in the enhanced testicular activity of ACP may be the cause [49,50]. The production of additional lysosomes as a result of lipid peroxidation may also be the cause of the enzyme's increased activity [51]. This outcome is consistent with the research of Nwonuma et al. [48], who found that exposure to $KBrO_3$ significantly increased the activity of testicular ACP. The outcome, however, conflicts with those of Nwonuma et al. [52], who found no difference in ACP activity between rats treated with lower dose of $KBrO_3$ and control animals. Rats given 100 and 200 mg/kg of *P. biglobosa* demonstrated improved ACP activity, which may have been caused by the extract's effects.

Alkaline phosphatase (ALP) is involved in mobilizing carbohydrates and lipid metabolites for use by the spermatozoa in the seminal fluid or inside the cells of the accessory sex structures [53]. For the germ cells of many mammalian species, including rats, ALP is a superb histochemical and biochemical marker [54]. ALP is most suited for oligo- and azoospermia distinction since it derives largely from testicles and the epididymis [55,56]. Any decrease in sperm cells will result in a drop in the degree of ALP activity since the acrosomic system of the sperm head is made up of ALP [56]. Animals exposed to $KBrO_3$ had lower ALP activity than animals in the control group, which could be a sign of impaired spermatogenesis. This outcome is consistent with Nwonuma et al. [48]'s findings that testicular ALP activity significantly decreased after exposure to $KBrO_3$. The outcome, however, conflicts with those of Nwonuma et al. [52], who found no difference in ALP activity between animals treated with lower dose of $KBrO_3$ and control animals. Rahman et al. [57] hypothesized that the decreased ALP activity in various tissues may be caused by the treated animals' enhanced plasma membrane permeability or cellular necrosis. Rats given 100 and 200 mg/kg of *P. biglobosa* demonstrated improved ACP activity, which may have been caused by the extract's effects.

A team of defense against reactive oxygen species (ROS) that includes antioxidant enzymes plays a crucial function in the detoxification of oxidative damages [58]. The results of the current study showed that the intoxication of rats with $KBrO_3$ significantly altered the activities of antioxidant enzymes, which may be related to the presence of catechin, kaempferol, rutin, and quercetin, which spread free radicals like peroxyl radicals and transformed reactive free radicals into inactive products. Other investigations [59,60] that characterized Digera muricata and Launaea procumbens in rats reported similar outcomes.

Malondialdehyde (MDA) is a byproduct of the cell membrane's lipid peroxidation [41]. It measures cellular structure oxidative damage [61]. MDA levels in testicular tissue significantly decreased

as a result of potassium bromate toxicity when compared to the control group. However, *P. biglobosa* treatments of 100 and 200 mg/kg body weight returned MDA levels to those of the rats in the control group. The worsening of the sperm's biochemical characteristics may be due to increased lipid peroxidation. The findings of the current study that reactive oxygen species (ROS) creation by $KBrO_3$ toxicity and subsequent oxidative damage may enhance sperm deformation were corroborated by the findings of Acharya et al. [62]'s study. One of the mechanisms that help to cause the death of germ cells is oxidative stress [63].

An effective glutathione peroxidase/glutathione reductase system keeps the tripeptide glutathione (G-S-S-G) in a reduced state. A powerful endogenous antioxidant called glutathione shields cells from a variety of harmful stimuli, such as free radicals produced by oxygen [64]. A measurement of the cellular redox status is the level of reduced glutathione [65]. Therefore, a change in glutathione levels may have an impact on the cell's total redox status. Extreme oxidative stress may cause glutathione and glutathione s-transferase levels to drop [66]. Animals exposed to $KBrO_3$ alone had significantly lower glutathione concentrations than those in the control group. A 20–30% reduction in glutathione levels might compromise a cell's ability to defend itself against xenobiotic toxicity and perhaps cause cell damage or death [67,68]. The metabolism and excretion of bromate in rats are significantly influenced by glutathione and other sulfhydryls [69]. The well-characterized *in vitro* thiol-mediated oxidation of DNA by bromine radicals and bromine oxides is expected to contribute to DNA damage *In vivo* [70]. While intracellular glutathione may promote the development of DNA reactive compounds, extracellular glutathione pools may be crucial in shielding target organs from bromate absorption and oxidative DNA damage [71-74]. This decline in antioxidant status allows the target tissue to develop the mutagenic lesion 8-oxodeoxyguanosine (8-oxodG) and to experience an increase in cellular proliferation. Compared to rats merely exposed to $KBrO_3$, *P. biglobosa* treatment increased the decreased glutathione levels. The significant increase seen may be attributed to the *P. biglobosa* extract's superior ability to shield the testes from free radicals through lipid peroxidation protection caused by $KBrO_3$. Additionally, this study revealed a negative correlation between glutathione level and peroxidation which is consistent with other studies [75–78]. Lack of glutathione can cause sperm motility defects by causing instability in the middle component of the sperm [47]. It suppresses the production of O_2 and shields the plasma membrane from lipid peroxidation [79].

When compared to the activities of superoxide dismutase (SOD) and catalase (CAT) in the control rats, only the animals treated with $KBrO_3$ showed a significant decrease. The current study's findings corroborate earlier findings that the primary line of defense against oxidative stress caused by free radicals was provided by antioxidant enzymes, namely SOD and CAT [80]. Superoxide radicals, which are extremely reactive and may be hazardous, were catalytically dismutated to hydrogen peroxide by SOD. In our investigation, we found that the activities of SOD and CAT significantly decreased when rats were given $KBrO_3$ alone. The action of $KBrO_3$ on metabolic blockage or displacement of Zn and Cu ions, the cofactor of the enzyme, may be concurrent with the decrease in SOD activity in rats exposed to $KBrO_3$ only [81]. Our findings, which demonstrated that $KBrO_3$ directly promoted the depletion of superoxide dismutase and catalase, supported the role of enzymes as antioxidants by converting superoxide radicals to hydrogen peroxide, which was then broken down into molecular oxygen and water. The decrease in catalase activity is a sign that the testes are unable to remove the hydrogen peroxide produced by $KBrO_3$ and its metabolites or that there is an excess of ROS in the testes, as has already been proven [82].

By increasing the production of ROS, such as singlet oxygen, hydrogen peroxide, and hydroperoxides, as well as by directly depleting antioxidant reserves, potassium bromate is known to harm tissues by free radicals [83-85]. Rats were given *P. biglobosa* and $KBrO_3$ in the current study, and when compared to rats given $KBrO_3$ alone, the level of SOD was higher. In general, the effects of *P. biglobosa* on the reproductive system have not yet been fully determined. This investigation focused on *P. biglobosa* extract's impact on various reproductive outcomes following exposure to $KBrO_3$ and its potential to mitigate $KBrO_3$'s detrimental effects on rat testicular integrity. The rise in SOD and CAT activities in the rats treated with *P. biglobosa* extracts may be the result of the extract inducing the activity of the enzyme. The results of this investigation demonstrated

that *P. biglobosa* extract is efficient in reducing testicular toxicity.

5. CONCLUSION

The results of this investigation demonstrated that potassium bromate caused testicular toxicity, and that *P. biglobosa* treatment counteracted this effect. Thus, it is recommended that these results be investigated in clinical trials in human volunteers.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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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