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Research Article

Mitigation of Experimentally-Induced Testicular Toxicity by *Corchorus olitorius* Leaves

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Abstract

Background: In order to learn more about the extent to which *Corchorus olitorius* (*C. olitorius*) leaves may affect experimentally-induced testicular toxicity in light of the fact that the literature in this area is quite scanty, this study investigated the mitigative effects of *C. olitorius* leaf extract against infertility induced by potassium bromate on male Wistar rats.

Methodology: *C. olitorius* 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 *C. olitorius* respectively. Both potassium bromate and *C. olitorius* 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 0.25M 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 levels of total cholesterol, total protein, glycogen, sialic acid, GSH, as well as ALP, SOD, and CAT activity. Additionally, it was shown that as compared to the animals in the control group, $KBrO_3$ boosted the testicular MDA and ACP's activity. However, *C. olitorius* treatment of intoxicated rats reduced these alterations in a dose-dependent manner.

Conclusion: Potassium bromate induced testicular toxicity by unhinging the biochemical indices and increasing lipid peroxidation in the testes of exposed animals. This effect was mitigated by coadministration with *C. olitorius* leaf extract. Therefore, it is advised that clinical trials involving human volunteers be conducted to further explore these findings.

Keywords: *Corchorus olitorius; Oxidative Stress; Potassium Bromate; Testicular Toxicity*

Introduction

Male infertility, which has about 30% global incidence rate and is thought to account for 50% of the world's cases directly, is recently gaining international attention [1]. It has been difficult to comprehend the prevalence and nature of male infertility throughout Africa due

to a paucity of statistics and the fact that African males rarely submit to fertility testing and typically hold women responsible for the bulk of infertility cases in the family [2]. Male infertility is reported to account for 20-50% of all infertility cases among Nigerians in different parts of the country [3]. Male infertility can occur for a variety of reasons, including poor semen quality, hypothalamus-pituitary disease, testicular disease, anomalies in sperm transport, and idiopathic male diseases [4]. Experiencing extremely high temperatures, erectile dysfunction, a history of developmental disorders including cryptorchidism, diabetes, and respiratory infections, as well as prior cancers and surgical procedures, are further acknowledged causes. A person's lifestyle choices, such as exposure to environmental pollutants like cadmium, mercury, potassium bromate, arsenic compounds, hydrocarbons, alcohol, smoking, and pesticides, are also potential causes [5,6]. A rise in occurrences of male infertility has been related globally to deteriorating semen quality [7]. Sperm parameters are at or below optimal values in only 2% of men. Infertility may be caused by low sperm counts, poor sperm motility, abnormal morphological characteristics, or a combination of these things [8]. Another significant factor that is diminishing or inadequate is male sex hormone secretion. Even though it is reported that endocrine illnesses only account for fewer than 3% of cases of male infertility, male hormone assays are performed to both identify the sources of these endocrine abnormalities and to obtain predictive information. Agents that inhibit spermatogenesis in the testicles or affect the body's hormonal balance may both interfere with spermatogenesis and prevent conception [9].

Oxidative stress leads to the damaging effects on the testicles. It is well recognised that an unbalanced pro-oxidant and antioxidant value amount in tissue, and particularly macromolecules, can seriously harm cell membranes, including proteins, carbohydrates, and DNA, and ultimately harm tissues and all systems [10,11]. Exogenous antioxidant supplements would therefore play a crucial role in activating the antioxidant defences of the cell to counteract any intoxication. According to oxidative free radicals, lipid peroxidation is recognised as the oxidative degradation of lipids [12,13]. Superoxide dismutase (SOD), a type of cellular antioxidant enzyme, metabolises the alteration of O_2 to H_2O_2 and other less reactive species [14]. In order to remove H_2O_2, SOD collaborates with other cellular enzymes like glutathione transferase (GST), glutathione peroxidase (GPx), and catalase (CAT) [15]. CAT play a crucial role in catalysing the breakdown of H_2O_2 into H_2O and O_2 [16]. Glutathione (GSH) is a multifunctional non-enzymatic antioxidant that is primarily found inside cells. In the cell, it serves as a thiol disulfide buffer. One of the enzymes that defend cells from reactive oxygen species is GSH, which becomes GSSG when it is oxidised [17]. Accordingly, the concentration of these enzymes reveals the cellular toxicity.

Numerous herbs have been known to affect male fertility for a long time [18]. Plants may have spermicidal, estrogenic, or ecotoxic effects or their extracts may have negative effects on the reproductive system, such as reducing fertility [19]. However, *Corchorus olitorius* is one of the plants whose fertility potential has recently been evaluated [20]. The plant belongs to the *Corchorus* genus of the Tiliaceae family, which contains over 60 distinct species spread across the globe, with 30 of those species being found in Africa. In addition to being known as jute mallow in English, the leafy vegetable *C. olitorius* is also referred to as "Ewedu" and "Ahihara" in the southwest and southeast of Nigeria, respectively. *C. olitorius*, also known as "Jute," is a vegetable that is widely grown throughout the world, particularly in tropical African countries, Malaysia, South America, and the Caribbean [21]. The leaves of *C. olitorius* are used as food and herbal medicine in many countries throughout the world, particularly in India and the Philippines [22]. Among the ethnomedical applications of the plant's leaves are the treatment of tumour growths, chronic cystitis, fever, pain, and fever. While the seeds are said to have estrogenic action, the plant's polyphenols can be extracted and are known to have anti-obesity properties [23].

Effects of various extracts of *C. olitorius* leaves have been reported [24,25]. In order to learn more about the extent to which *C. olitorius* leaves may affect experimentally-induced testicular toxicity in light of the fact that the literature in this area is quite scanty, this study investigated the mitigative effects of *C. olitorius* leaf extract against infertility induced by potassium bromate on male Wistar rats.

Figure 1: *Corchorus olitorius plant.*

Materials and Methods

Extraction of plant extract

Fresh *Corchorus olitorius* (jute) plants (Figure 1) 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. Under flowing water, they were well cleaned to remove contaminants. In an open laboratory setting, they were allowed to air dry for 14 days at room temperature before being ground into powder using an electric blender. Using a soxhlet device and 95% ethanol as the solvent, the extraction was performed in accordance with the instructions provided by Airaodion., *et al*. [26,27]. A rotary evaporator was used to recover the ethanol at 35^{o}C, yielding 2.28 g or a 9.12% yield. Until it was needed, the extract was kept at 4^{o}C in the refrigerator.

Experimental design

The experiment involved twenty-four (24) mature male Wistar rats (*Rattus norvegicus*) weighing between 140 and 160g. They had seven (7) days to get used to the lab environment before the experiment. The rats were kept in cages made of wire mesh, and they had complete access to rat food and water. The animals were housed in conditions with constant temperatures, humidity, and 12-hour light/dark cycles. The Declaration of Helsinki and the rules set by the Committee for the Control and Supervision of Experiments on Animals were both followed during the conduct of this investigation. Additionally, animal experiments were carried out in compliance with NRC policy [28]. They were randomly placed into groups A, B, C, and D. Oral distilled water was administered to Group A, which served as the control group. In addition to the 100 mg/kg body weight of potassium bromate given to groups B, C, and D, animals in groups C and D also received *C. olitorius* extract at doses of 100 and 200 mg/kg body weight, respectively. Rats were orally administered *C. olitorius* extract and freshly made potassium bromate solution every day for 28 days. After twenty-four hours from the last treatment, the animals were softly sedated with diethyl ether before being killed. The testes were removed and placed in a 0.25M sucrose solution (1:5 w/v) that was isotonic. The testes were weighed and homogenised in the 0.25 M sucrose solution. The homogenates were spun at 5000g for 10 minutes in a refrigerated centrifuge (TDL-5000B). China's Shanghai Anke business Ltd. The supernatant was gathered and frozen-stored for further research.

Estimation of biochemical indices

The biochemical parameters in the testicular homogenate of rats were investigated using a UV/VIS spectrophotometer. The Gornall., *et al*. [29] technique was used to measure total protein. The amount of glycogen in the testes was measured using the method described by Kemp., *et al*. [30]. The approach described by Fredrickson., *et al*. [31] involved utilising a reagent assay kit to measure the total cholesterol levels in the testes. The total sialic acid was calculated using the calorimetric method described by Yao., *et al*. in their publication [32]. To assess the activity of acid phosphatase (ACP) and alkaline phosphatase, Wright., *et al*.'s method [33] was used. The samples' reduced glutathione (GSH) levels were determined using the method described by Airaodion., *et al*. [34]. Thiobarbituric acid reactive substances (TBARS) were measured as an estimation of malondialdehyde (MDA), a byproduct of lipid peroxidation, using the method described by Airaodion., *et al*. [34]. The activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were assessed using the method described by Airaodion., *et al*. [34].

Data analysis

The Tukey post hoc mean comparison test was used to determine whether there were any statistically significant differences between the variables after one-way ANOVA was used to analyse the data. The mean and standard deviation for six replicates of the studied data were expressed. A P-value of 0.05 or less (P0.05) was used to determine statistical significance. For all statistical evaluations, Graph Pad Prism was utilised (version 8.0).

Results

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

Discussion

To evaluate the functional capability of the testicles, testicular secretory components such as total protein, glycogen, sialic acid, and cholesterol are helpful [35]. Rats exposed to $KBrO_3$ alone experienced significantly higher cholesterol levels in the testes than rats in the

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	8.610.84^{a}	4.730.26^{a}	3.390.21^{a}	5.131.01^{a}	148.639.36^{b}	183.3311.20^{a}
100 mg/kg $KBrO_3$ only	5.381.11^{c}	2.260.03^{c}	1.960.03^{b}	2.850.17^{c}	204.3512.93^{a}	132.3912.83^{c}
100 mg/kg $KBrO_3$ + 100 mg/kg *C. olitorius*	6.470.16^{bc}	3.110.09^{b}	2.080.08^{ab}	3.270.11^{b}	189.229.74^{b}	156.276.66^{b}
100 mg/kg $KBrO_3$ + 200 mg/kg *C. olitorius*	7.610.37^{ab}	4.030.13^{a}	3.080.11^{a}	4.680.81^{ab}	166.058.22^{b}	170.038.16^{ab}
p-value	0.02	0.02	0.03	0.00	0.01	0.03

Table 1: *Effect of C. olitorius Seed on the biochemical parameters of testes-homogenate of potassium bromate induced testicular toxicity of wistar rats.*

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

Treatment Group	MDA (U/mg)	GSH (U/mg)	SOD (U/mg)	CAT (U/mg)
Control	0.54 0.00^{c}	52.33 3.23^{a}	62.86 5.02^{a}	0.850.00^{a}
100 mg/kg $KBrO_3$ only	0.83 0.01^{a}	38.82 2.46^{c}	47.28 4.45^{c}	0.52 0.01^{c}
100 mg/kg $KBrO_3$ + 100 mg/kg *C. olitorius*	0.68 0.00^{b}	46.004.12^{b}	53.87 3.35^{bc}	0.61 0.00^{b}
100 mg/kg $KBrO_3$ + 200 mg/kg *C. olitorius*	0.580.00^{bc}	50.552.74^{a}	59.353.03^{a}	0.780.01^{a}
p-value	0.01	0.03	0.03	0.02

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

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

control group (Table 1), which may be a symptom of harm to the sertoli cells, which can cause phagocytosis and the deposition of cell membrane lipid [36]. To function normally, testicular cells need cholesterol for membrane biogenesis, cell communication, and as a precursor for androgen production [37]. Leydig cell-inhibited androgen synthesis has been linked to low testicular cholesterol. Leydig cells secrete an assortment of hormones known as androgens, such as testosterone, androstenedione, and dehydroepiandrosterone (DHEA), when pituitary luteinizing hormone (LH) stimulates them. By raising the activity of the enzyme cholesterol desmolase, LH enhances the Leydig cells' ability to produce and secrete testosterone [38]. However, treatment with *C. olitorius* extract at doses of 100 and 200 mg/kg significantly increased the levels of total cholesterol when compared to those exposed to $KBrO_3$ without treatment. This may be explained by *C. olitorius*' capacity to produce cholesterol, a precursor in the steroidogenesis of the male sex hormone, as a form of self-defense. The results of this study clearly demonstrate that $KBrO_3$ is the cause of the lower cholesterol levels seen in the animals exposed to $KBrO_3$ alone, but the higher cholesterol levels found in the testicles of the rats given *C. olitorius* extract were a sign of the extract's protective effect on the testicular cells.

Protein synthesis is crucial for spermatogenesis and testicular development. Sertoli cells control the spermatogenic process, which generates the proteins required for germ cell growth [39,40]. Animals treated to $KBrO_3$ alone showed a decrease in testicular protein compared to those in the control group (Table 1). This decrease could be a result of enzymatic protein synthesis suppression brought on by $KBrO_3$'s impact on genetic information. Ahmad and Mahmood [41] and Mohamed and Saddek [42] also found results that supported the current study's conclusions that $KBrO_3$ poisoning significantly reduced the amount of protein in the testes.

Glycogen regulates germ cell survival and is crucial for normal testicular development and function [43]. Testicular glycogen serves as the main energy source in the testes of animals with reproductive systems [44]. It is necessary for proper gonadal development and operation [45]. It supplies glucose stores to seminiferous tubular cells. Glycogen levels and steroid hormones are inversely related [46]. The only animals that showed a significant ($P \leq 0.05$) decrease in testicular glycogen compared to those in the control group were those that were exposed to $KBrO_3$. The decrease may be due to $KBrO_3$-induced increased testicular activity, which results in the testes consuming a lot of glucose. It's also plausible that an inhibition of the enzymes responsible for glycogen production contributed to the decreased testicular glycogen in the potassium bromate-intoxicated group. Glycogen synthesis was prevented after $KBrO_3$ injection because of a drop in glycogen levels, which may have a negative impact on spermatogenesis [47]. A sign of the extract's facilitative effects may be seen in the difference in glycogen levels between the groups treated with *C. olitorius* extract and the group exposed to $KBrO_3$ alone.

Pyruvic acid and N-acetylmannose combine to form sialic acid (N-acetylneuraminic acid) [48]. It is an essential part of glycolipids and glycoproteins. The results of this study demonstrated that testicular sialic acid levels significantly decreased as a result of $KBrO_3$ expo-

sure as compared to the healthy control group. Testicular sialic acid levels may have fallen due to this decline in spermatogenesis rate. The present investigation shown that rats administered potassium bromate poisoning had much reduced testicular sialic acid, which is supported by earlier findings by Nwonuma., *et al*. [49]. In the *C. olitorius* treatment groups, sialic acid levels increased, indicating that the extract may be able to enhance the production of elements of the testicular secretory system. This conclusion is also consistent with the findings of Mohamed and Saddek [42], who found that potassium bromate lowered the level of sialic acid but that this effect was reversed in rats given taurine and/or vanillin.

Acid phosphatase (ACP) is a hydrolase enzyme that catalyses the hydrolysis of various phosphate esters and has a pH-optimum in the acid zone [50]. ACP is found in cellular lysosomes; hence, lipid peroxidation that undermines the integrity of the membrane may produce an increase in the enzyme in the testicles. The prostatic isoenzyme of ACP is a critical testis diagnostic indicator. ACP activity was more prevalent in animals exposed to $KBrO_3$ than it was in animals in the control group. The enhanced testicular activity of ACP may be caused by the lysosomal membrane being ruptured and enzyme liberated by KBrO3 [51,52]. The lipid peroxidation process may also result in the creation of extra lysosomes, which would explain the enzyme's increased activity [53]. This result is consistent with the findings of Nwonuma., *et al*. [49]'s study, which showed that testicular ACP activity was markedly increased by exposure to $KBrO_3$. The results, however, disagree with those of Nwonuma., *et al*. [54], who discovered no distinction between rats treated with $KBrO_3$ and control animals in terms of ACP activity. The effects of the extract may have contributed to the increased ACP activity seen in rats given doses of 100 and 200 mg/kg of *C. olitorius*.

Alkaline phosphatase (ALP) is involved in releasing lipid and carbohydrate metabolites into the seminal fluid or within the cells of the accessory sex structures for usage by spermatozoa [55]. ALP is an excellent histochemical and biochemical marker for the germ cells of several mammalian species, including rats [56]. Since ALP is mostly derived from testicles and the epididymis, it is best suited for oligo- and azoospermia differentiation [57,58]. The acrosomic system of the sperm head is made up of ALP, so any decrease in sperm cells will result in a decrease in the level of ALP activity [58]. Lower ALP activity in $KBrO_3$-exposed mice compared to animals in the control group may indicate decreased spermatogenesis. This result supports Nwonuma., *et al*. [49]'s observation that testicular ALP activity considerably decreased following exposure to $KBrO_3$. However, the results disagree with those of Nwonuma., *et al*. [54], who discovered no difference in ALP activity between animals treated with $KBrO_3$ and controls. Rahman., *et al*.'s [59] theory proposed that the increased plasma membrane permeability or cellular necrosis in the treated animals may have contributed to the lower ALP activity in diverse tissues. The effects of the extract may have contributed to the increased ACP activity seen in rats given doses of 100 and 200 mg/kg of *C. olitorius*.

Public health has always been concerned about the relationship between chemical pollution and reproductive health. These days, $KBrO_3$-related reproductive disorders with regard to male infertility are more prevalent. Animal and human food have both had KBrO3 found in them [60,61]. According to Iwuoha., *et al*. [62], it decreases the production of oestrogen, which is a major factor in male infertility and oxidative stress. It is well known that certain endocrine disruptors, such as $KBrO_3$, cause oxidative stress [63,64]. In testicular tissues of rats given $KBrO_3$, this study demonstrates that there was a significant decrease in the activities of both SOD and catalase as well as the concentration of GSH, but a significant increase in MDA levels (Table 2). Chemical induction in rats significantly decreased the GSH contents and increased lipid peroxidation [65]. Reduced testosterone secretion and poor sperm quality may be caused by abnormalities in the lipid peroxidation and anti-oxidant status of testicular tissues. Recent research has demonstrated that $KBrO_3$ treatment reduced the sperm quality in treated rats [66,67]. Similar reports indicate that $KBrO_3$ has a negative impact on male rat sex hormone secretion [62,68]. Therefore, $KBrO_3$ is likely to cause testicular toxicity. Rats' blood capillaries were damaged and constricted as a result of $KBrO_3$ induction, which in turn caused the concentration of nitrite in tissue supernatant to increase [69].

The antioxidant system in the testicular tissues was strengthened by concurrent administration of $KBrO_3$ and *C. olitorius* extract, which increased SOD and catalase activities as well as GSH levels significantly. According to this study, *C. olitorius* extract prevented testicular

toxicity caused by $KBrO_3$ by preventing lipid peroxidation. The findings of this study demonstrate a significant decrease in lipid peroxidation (MDA levels) in the testes of rats treated with *C. olitorius* extract. The inhibitory effects of catechin, quercetin, and other flavonoids on *in vitro* lipid peroxidation have been reported in a number of studies [70,71]. Typically, this effect is measured colorimetrically by measuring the formation of thiobarbituric acid-reactive substance. This study concurs with studies on various flavonoids, which are a constituent of *C. olitorius*, and which reported the inhibitory effects of *C. olitorius* extract on lipid peroxidation [72]. The results of this study demonstrated that *C. olitorius* extract improves the oxidative status of the testicles. The research also demonstrates that administration of *C. olitorius* extract prevents $KBrO_3$-induced testicular toxicity by raising tissue enzymatic antioxidant activities (SOD and catalase) and lowering lipid peroxidation.

Conclusion

Potassium bromate induced testicular toxicity by unhinging the biochemical indices and increasing lipid peroxidation in the testes of exposed animals. This effect was mitigated by coadministration with *C. olitorius* leaf extract. Therefore, it is advised that clinical trials involving human volunteers be conducted to further explore these findings.

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Consent for Publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

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