

Common African Vegetable (*Corchorus olitorius*) Alleviated Potassium Bromate-Induced Sperm Abnormalities

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Abstract

Background: Effects of various extracts of *Corchorus olitorius* leaves have been reported. In order to learn more about the extent to which *C. olitorius* leaves may affect experimentally-induced infertility in light of the fact that the literature in this area is quite sparse, we evaluated the ameliorative effects of ethanolic extract of *C. olitorius* leaves against infertility induced by potassium bromate on male Wistar rats.

Methodology: Twenty-four male Wistar rats were split into groups A, B, C and D. Group A served as the control group and received oral distilled water. Animals in groups C and D received *C. olitorius* at doses of 100 and 200 mg/kg body weight, respectively, in addition to the 100 mg/kg body weight of potassium bromate given to groups B, C and D. Every day for 28 days, *C. olitorius* extract and freshly prepared potassium bromate were given orally to rats.

Results: When compared to animals in the control group, exposure to 100 mg/kg body weight of KBrO₃ significantly decreased sperm count, sperm motility, sperm viability and seminal pH but elevated sperm morphology. In a dose-dependent manner, these perturbations were reduced by *C. olitorius* leaf extract at concentrations of 100 and 200 mg/kg body weight.

Conclusion: If the findings from this study, which was conducted on rats, can be extrapolated to men, we come to the conclusion that KBrO₃ consumption may impair male reproductive processes due to lowered sperm quality and increased sperm abnormalities. The results also demonstrated that the *Corchorus olitorius* leaf extract has pharmacological potentials to enhance sperm count, viability, and motility as well as afterwards reduce sperm abnormalities in male rats exposed to KBrO₃, a function essential for a healthy fertility status. There is a need for further studies on *C. olitorius* to identify the active ingredients that improve sperm quality which in turn could be used to produce new drugs/supplements.

Thus, this pharmacological investigation could be used to produce new drugs from organic plant materials.

Keywords: *Corchorus olitorius* Leaves; Fertility; Potassium Bromate; Sperm Qualities

Introduction

With a global incidence rate of about 30%, infertility among males is fast becoming a matter of global concern with males reported to be directly involved in about 50% of global infertility prevalence [1]. Due to a lack of data and the fact that African men seldom consent to fertility testing and typically prefer to hold women accountable for the majority of infertility cases in the family, it has been challenging to understand the frequency and nature of male infertility across Africa [2]. According to reports, male infertility accounts for 20–50% of all infertility cases among Nigerians in various regions of the nation [3]. Poor semen quality, hypothalamus pituitary disease, testicular disease, abnormalities of sperm transport, and idiopathic male disease are some of the reasons that lead to male infertility [4]. Other recognized reasons include exposure to very high temperatures, erectile dysfunction, a history of developmental conditions such as cryptorchidism, diabetes, and respiratory infections, as well as previous malignancies and surgical procedures. Other causes include aspects of one's lifestyle, such as contact with chemicals found in the environment, such as cadmium, mercury, potassium bromate, arsenic compounds, hydrocarbons, alcohol, smoking, and pesticides [5,6].

Semen quality deterioration has been linked to an increase in male infertility cases worldwide [7]. Only 2% of males have sperm parameters that are at or below optimum levels. Low sperm counts, poor sperm motility, aberrant morphological features, or a combination of these factors may all contribute to infertility [8]. Male sex hormone secretion is also another important contributing component that is declining or secreting them improperly. Male hormone assays are carried out to both determine the causes of these endocrine abnormalities and to get prognostic information, even though it is reported that male infertility caused by endocrine diseases only accounts for less than 3% of male infertility cases. Agents that prevent conception may do so *via* altering the body's hormonal composition, interfering with spermatogenesis at the testicular level, or both [9].

It has long been known that a variety of herbs might impair male fertility [10]. Plants can have spermicidal, estrogenic, and ecobolic effects, or their extracts can be harmful to the reproductive system and cause anti-fertility effects [11]. One of the plants that have recently had its fertility abilities assessed is *Corchorus olitorius* [12]. The plant is a member of the Tiliaceae family's *Corchorus* genus, which has roughly 60 different species distributed throughout the world, with 30 of those species being found in Africa. The leafy vegetable *C. olitorius* is also known as jute mallow in English, "Ewedu" and "Ahihara" in the southwest and southeast of Nigeria, respectively. The vegetable *C. olitorius*, also referred to as "Jute," is widely cultivated around the world, especially in tropical African nations, Malaysia, South America, and the Caribbean [13]. Many nations around the world, notably India and the Philippines, employ the leaves of *C. olitorius* as food and herbal medicine [14]. The treatment of pain, fever, chronic cystitis and tumor growths are a few of the ethnomedical uses of the plant's leaves. The plant's polyphenols can be extracted and are known to have anti-obesity properties, while the seeds are claimed to have estrogenic action [15].

Effects of various extracts of *C. olitorius* leaves have been reported [16,17]. In order to learn more about the extent to which *C. olitorius* leaves may affect experimentally-induced infertility in light of the fact that the literature in this area is quite sparse, we evaluated the ameliorative effects of ethanolic extract of *C. olitorius* leaves against infertility induced by potassium bromate on male Wistar rats.

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. The leaves were carefully separated from the stem, and the spoilt ones were thrown away. They were properly cleansed under running water to remove impurities. They were allowed to air dry for 14 days at room temperature in an open laboratory setting before being processed with an electric blender to make powder. Following the steps given by Airaodion., *et al.* [18,19], the extrac-

tion was carried out utilizing a Soxhlet device with 95% ethanol as the solvent. The ethanol was recovered at 35°C using a rotary evaporator, producing 2.28g or a yield of 9.12%. The extract was kept in the fridge at 4°C until it was required.



Figure 1: *Corchorus olitorius* plant.

Experimental design

Twenty-four (24) mature male Wistar rats (*Rattus norvegicus*) weighing between 140 and 160g were used in the experiment. Before the experiment, they were given seven (7) days to get acclimated to a laboratory setting. The rats were housed in wire-mesh cages and had full access to rat food and water. The animals were kept in environments with consistent humidity levels, 12-hour light/dark cycles, and temperatures. This inquiry was carried out in accordance with the Declaration of Helsinki and the guidelines established by the Committee for the Control and Supervision of Experiments on Animals. In addition, animal experimentation was conducted in accordance with National Research Council policy [20]. At random, they were split up into groups A, B, C and D. Group A, which acted as the control group, was given oral distilled water. Animals in groups C and D also received *C. olitorius* at doses of 100 and 200 mg/kg body weight, respectively, in addition to the 100 mg/kg body weight of potassium bromate given to groups B, C and D. Every day for 28 days, *C. olitorius* extract and freshly produced potassium bromate solution were given orally to rats. The animals were gently sedated with diethyl ether after twenty-four hours of the last treatment before being put to death. The cauda epididymis were separated from both testes and tinged with 2 mL of normal saline then teased the cauda epididymis of each rat. The suspension was mixed through a metallic net to avoid any other tissue contamination. This suspension was used for the determination of the sperm parameters.

Analyses of epididymal sperm characteristics

Sperm motility and count

Small cuts were made in the cauda epididymis in accordance with Ogbuagu, *et al.* [21]'s procedures to remove the spermatozoa, which were then put in 0.05M phosphate buffered saline (pH 7.4). Sperm suspension was tested for sperm concentration and motility. The spermatozoa's slow and fast motions (also known as progressive movements), as well as any kind of flagellar or head movement, were all taken into account when calculating the percentage of mobility. The sperm count was determined using a Neubauer hemocytometer.

Sperm viability

Sperm viability was assessed using the Eosin/Nigrosin staining method. One drop of fresh semen to two drops of staining agent was used to analyze the staining on a microscope slide. A smear was applied and allowed to dry on a different slide. At a magnification of

3100, spermatozoa with damaged membranes and unstained spermatozoa were counted. Eliasson's research [22] found a correlation between sperm viability and the percentage of intact cells.

Sperm morphology

To ascertain the morphology of the sperm, Airaodion, *et al.* [23]'s described approach was applied. Sperm suspension was stained with eosin, and smears were created on slides, dried by air and made permanent to identify the aberrations in spermatozoa. The slides were viewed under a microscope at x100 magnification with an oil immersion objective. The number of morphologically abnormal spermatozoa was recorded, and the fraction of these was computed.

Seminal pH

Using pH paper with a range of 6.0 to 14.0, the seminal solution's pH was determined in accordance with the process described by Airaodion, *et al* [24]. The mixture was thoroughly blended and evenly distributed in a drop on the pH paper. The impregnated zone's color stabilized after about 30 seconds and the matching value was recorded after the color was matched to the calibration strip to ascertain the pH.

Statistical investigation

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

Results

When compared to animals in the control group, exposure to 100 mg/kg body weight of KBrO_3 significantly decreased sperm count (Figure 2), sperm motility (Figure 3), sperm viability (Figure 4) and seminal pH (Figure 6) but elevated sperm morphology (Figure 5). In a dose-dependent manner, these perturbations were reduced by *C. olitorius* leaf extract at concentrations of 100 and 200 mg/kg body weight.

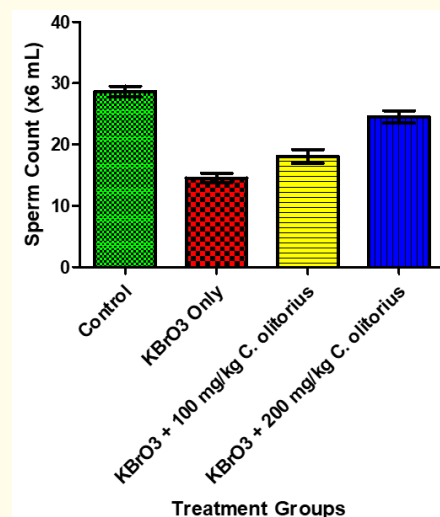


Figure 2: Effect of *C. olitorius* on the sperm count of potassium bromate-induced male rats.

Bars are presented as mean standard deviation with $n = 6$.

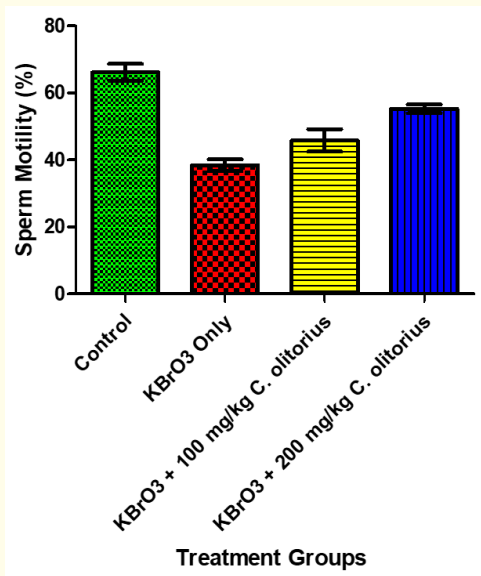


Figure 3: Effect of *C. olitorius* on the sperm motility of potassium bromate-induced male rats.

Bars are presented as mean standard deviation with n = 6.

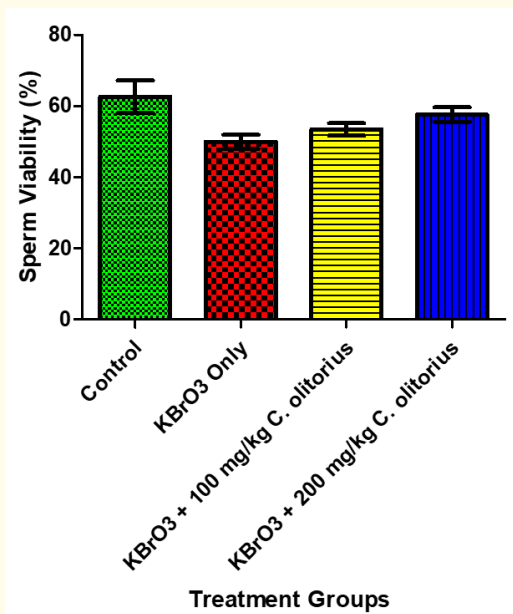


Figure 4: Effect of *C. olitorius* on the sperm viability of potassium bromate-induced male rats.

Bars are presented as mean standard deviation with n = 6.

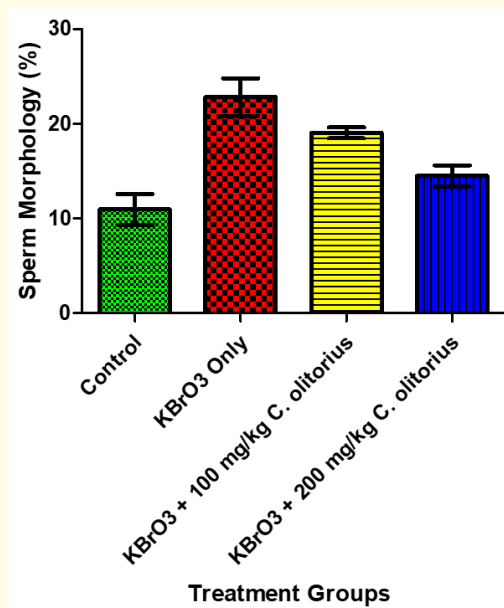


Figure 5: Effect of *C. olitorius* on the sperm morphology of potassium bromate-induced male rats. Bars are presented as mean standard deviation with n = 6.

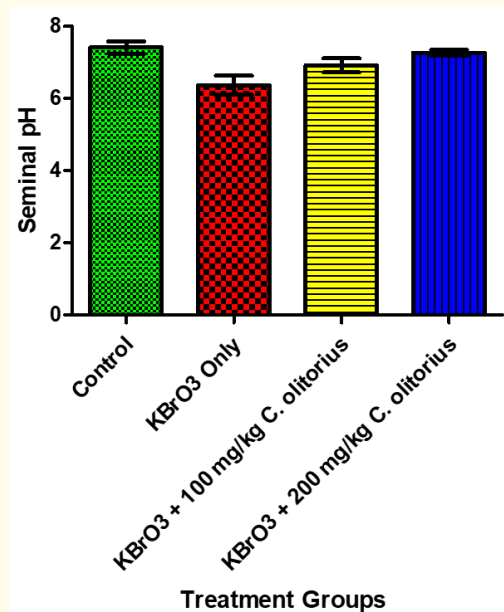


Figure 6: Effect of *C. olitorius* on the seminal pH of potassium bromate-induced male rats. Bars are presented as mean standard deviation with n = 6.

Discussion

The process of spermatogenesis involves intricate differentiation [25]. However, exposure to hazardous substances can cause spermatogonial degeneration [26]. The current study's findings verified that KBrO_3 intoxication led to severe tubular degeneration, meiotic stoppage, sperm concentration decline, and destruction of germinative epithelium.

One of the main problems the food industry is currently facing is the use of food additives [27]. All food additives should be investigated for their potential short- and long-term genetic and toxic effects because they have not yet been sufficiently controlled. Due to the daily ingestion of food additives through food, which can accumulate in the body over the course of a lifetime due to the excessively prolonged exposure, they appear to be of utmost relevance to human health [28]. Numerous studies have demonstrated that KBrO_3 (a food additive) causes genotoxicity and cancer in both experimental animals and human. The genotoxic harm caused by KBrO_3 is now being evaluated using a range of short-term genotoxicity testing techniques [29]. The therapeutic effect of various medicinal plants as natural antioxidants to treat chronic diseases [30] as DNA damage, mutagenesis, and carcinogenesis are studied through the use of therapeutic secondary metabolites and therapeutic essential oils, which are abundant in products from medicinal plants. Standard laboratory techniques are testicular biopsies and sperm analysis to assess male fertility. Semen vitality, morphology, and motility have all been found to contribute to male infertility [31], along with additional factors like starvation, genetic disorders, and severe illness [32]. Another study discovered that the majority of males turn to herbal or plant-based formulations for self-medication because they are either too embarrassed or timid to visit a clinic for formal medical consultations [33]. As a result, very few men seek appropriate medical care to finally address their infertility problems. A reliable method for determining the genotoxicity and germ cell toxicity of various substances is the spermatozoa morphology test.

The effect of *C. olitorius* leaves on KBrO_3 -induced sperm morphology in male rats was assessed in the current investigation. The results of the investigation are depicted in figure 2 through 6, where it can be seen that KBrO_3 significantly ($P \leq 0.05$) reduced sperm count, motility, and viability in comparison to the control group. The testicles and other body tissues of animals treated with potassium bromate exhibited noticeably elevated levels of free radical activity, according to reports [34,35]. Unchecked free radical production has the potential to cause cancer [36,37]. A few instances of the reproductive dysfunction brought about by the change in sperm characteristics include reduced sperm count, motility, and viability [38]. A related study found that growing rats' growth was impeded by drinking water containing KBrO_3 , which in turn resulted in lower testicular and epididymal weights [39].

Sperm cells are produced in the seminiferous tubules of the testes and atrophy there, leading to lower spermatids, decreased fertility, problematic spermatogenesis, and poor reproductive function [40]. The seminiferous tubule's atrophy may have reduced the amount of raw materials required for sperm formation as well as the available space, which ultimately affects the number of sperms formed and raises the likelihood of abnormalities in the head, mid-piece, and tail of the sperm cells that are produced [39]. Increases in sperm abnormalities can often affect fertility by reducing sperm count, motility and viability. It is well known that defective sperm and infertility are related [41].

The pH of the sperm sample is a significant factor in determining the quality of the sperm and is a well-known predictor of the survival rate of sperm cells. One of the causes of infertility is acidic media, which can be found in the female vagina or in the sperm itself and is known to increase sperm mortality, impair sperm performance, and prevent conception [42]. Since alkaline medium balances the female vagina's acidity and creates an environment that encourages optimal sperm motility and enhances the processes leading to ovum fertilization, it is the most ideal environment for sperm performance [41]. When compared to the control group, the KBrO_3 -treated rats' low seminal pH (Figure 6) may have an inhibitory effect on sperm survival and reproduction. It is also significant to note that this treatment group's sperm motility and viability were all lower than those of the control group, further supporting the idea that consumption of KBrO_3 by men has a negative impact on their ability to reproduce. This is in line with the findings of Ezirim, *et al.* [43] and Iwuoha, *et al.*

[44], who found that 100 mg/kg body weight of KBrO_3 adversely affected the male Wistar rats' reproductive hormone. Recent research and publications have connected high fertility index with sperms' progressive motility [45]. The fructose content of sperm samples has been shown to increase sperm motility, and the use of KBrO_3 in this study may have decreased the fructose content of the sperm samples, decreasing sperm motility and sperm cell survival.

However, the pH of the animals was restored by simultaneously administering KBrO_3 and various doses of *C. olitorius* leaf extract. The alkaline semen environment seen in the rats treated with 100 and 200 mg/kg of *C. olitorius* shows that the doses are favorable for rat reproduction. This outcome is consistent with that of Uloneme [46], who noted a significant increase in active motility in all groups treated with aqueous leaf extract of *Corchorus olitorius* at various doses compared to the rat control group. It is conceivable that the same factors that support spermatogenesis and increase sperm count after treatment with an ethanol extract of *C. olitorius* leaves may have also increased spermatozoa's rate of motility [47]. It was found that the increment pattern was dose-dependent, which is consistent with the conclusion made by Osuchukwu, *et al* [48].

On the other hand, rats treated with *Corchorus olitorius* leaves had a considerable reduction in the morphology of their sperm cells (Figure 5). This suggests that the Yoruba tribe of Nigeria's consumption of *Corchorus olitorius* leaves as food components could be used to address and treat sperm abnormalities issues. The product's method of action is unclear, although it may be related to how it affects testosterone synthesis and secretion, which promotes spermatogenesis and increases sperm quality, quantity, and motility [26]. It could possibly be due to better spermatogenesis and sperm motility caused by increased blood flow to the testis as a result of vasodilatation of its arteries.

According to a study by Orieko, *et al.* [12], experimental rats' sperm quality improved at lower doses of *C. olitorius* leaf extract (up to 500 mg/kg body weight), but it degraded at higher dose of 1000 mg/kg. Their research seems to support the findings of Oyedeji, *et al.* [16] that large doses of *C. olitorius* leaf extract had detrimental effects on the male rats' reproductive systems. Therefore, it is recommended to consume it in moderation.

Conclusion

If the findings from this study, which was conducted on rats, can be extrapolated to men, we come to the conclusion that KBrO_3 consumption may impair male reproductive processes due to lowered sperm quality and increased sperm abnormalities. The results also demonstrated that the *Corchorus olitorius* leaf extract has pharmacological potentials to enhance sperm count, viability, and motility as well as afterwards reduce sperm abnormalities in male rats exposed to KBrO_3 , a function essential for a healthy fertility status. There is a need for further studies on *C. olitorius* to identify the active ingredients that improve sperm quality which in turn could be used to produce new drugs/supplements.

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