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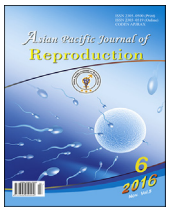

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Effect of *Thaumatococcus daniellii* leaf rat-feed on potassium bromate induced testicular toxicity

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ABSTRACT

Objective: To evaluated the antioxidant and protective effect of *Thaumatococcus daniellii* (*T. daniellii*) on rat feed on potassium bromate ($KBrO_3$) toxicity in male rat testes.
Methods: Thirty male albino rats of average weight 140 ± 5 g were randomly grouped into 5 with six rats per group. The rats in group A (positive control) and test groups (C, D and E) were orally given 0.5 mL of 10 mg/kg body weight of $KBrO_3$ daily. The animals in the negative control (group B) and positive control were fed with commercial rat feed while the animals in the tests groups were fed with 10%, 20% and 30% *T. daniellii* leaf rat feed respectively. The treatment was carried out for 14 days consecutively, and the animals were sacrificed 24 h after the last day of the treatment.
Results: Biochemical assays were carried out on the testicular homogenates. The results showed significant increase ($P < 0.05$) in malondialdhyde, total protein, and superoxide dismutase as well as testicular glycogen in the positive control compared to test groups. The histopathological result showed testicular cellular degeneration in the positive control compared to the test animals which showed normal cell due to protective effect of the leaf.
Conclusions: The biochemical and histopathological results in this present study showed testicular toxicity in the rats administered with $KBrO_3$ and *T. daniellii* leaf protective effect on the testicular function toxicity in rats fed with *T. daniellii* leaf rat feed.

1. Introduction

The optimal function of the reproductive system in human is vital for sustenance of life. Infertility is a health problem affecting approximately 15% couples world-wide. It is now evident that in 50% of all cases at least, reduced semen quality contributes to the problem [1]. Some of the reported infertility cases are attributed to low sperm count which could be a result of hormonal imbalance induced by oxidative stress. Recent studies have shown that alterations in the sperm molecular factors (paternal genome, mitochondrial DNA and transcripts) maybe is the underlying cause of infertility [2,3]. Potassium bromate ($KBrO_3$) has been shown to cause oxidative stress in the kidney and liver of rats as well as cell tumours and follicular cell tumours of the thyroid [4]. It also has an adverse effect on the physiological and biochemical functions of Swiss albino rats [5]. Scientific evidence has also implicated $KBrO_3$ to be carcinogenic, and has since been removed from the list of acceptable additives for flour treatment [6]. However, under controlled baking conditions, $KBrO_3$ is converted into potassium bromide, which is considered to be harmless to the consumer [6]. This salt is now banned in some countries [7], but is still being used in USA and Japan. However, some degree of illegal use of $KBrO_3$ in dough preparation is still recorded in countries with a ban on its use apparently, because it is cheap, easily accessible and perhaps the best and most effective oxidizing agent, acting sluggishly through the fermentation period and changing the structure and properties of the dough. Some plants' leaf due to their phytochemical content has the potential to abate or prevent oxidative stress. One of such is *Thaumatococcus daniellii* (*T. daniellii*) known as the sweet prayers plant, mainly because its seed is a good sweetener. The sweet prayers' plant, *T. daniellii* is a rhizomatous plant found in tropical rain forests and coastal areas of west Africa, particularly, Nigeria, Ghana and Cote d'Ivoire [8]. *T. daniellii*, whether cultivated or in the wild, contributes to the economy of the rural people in most parts of southern Nigeria through its stalks, leaves, fruits and rhizomes [9]. It is locally used in mat weaving (stalks), roof thatching (stalks and leaves), food

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wrapping (leaves), as potherbs (leaves and rhizomes) and for sweetening drinks and foods (fruits). The most exciting use of *T. daniellii*, for which it has earned global interest, is its use as a sweetener and taste modifier [10]. There are unscientific claims that food wrapped with the leaf usually have better taste and prevent food from related poison. The testes of humans and other mammals are highly susceptible to be damaged by genetic disorders, environment or occupational exposure to chemical or other means. Specific causes of testicular damage have been catalogued [11]. Reports on the abatement of $KBrO_3$ induced toxicity in rats through compounded feed is scanty. This study has therefore, evaluated the effect of $KBrO_3$ in some selected testicular function indices and abetment by *T. daniellii* rat feed on the adverse effect of the substance on rats' testicular function.

2. Materials and methods

2.1. Preparation of plant material and feed compounding

The leaves of *T. daniellii* were purchased at Oja-Tutu market at Ilorin Kwara State Nigeria and authenticated at the Department of Plant Biology University of Ilorin. The leaves were washed under running water, air-dried and pulverised. The pulverised leaves were filtered. Each of the rats in this experiment was fed 9 g daily ratio of feed. The preparation of the feed was as follow: 10%, 20% and 30% rats' daily ration were substituted with 10%, 20% and 30% pulverised *T. daniellii* leaves respectively at the ratio of 1:4 (w/w).

2.2. Preparation of the $KBrO_3$

0.5 mL of 10 mg/kg body weight of $KBrO_3$ was orally administered daily to the rats through gavage during the experiment. The selection of $KBrO_3$ dosage was premised upon our previous findings [12] which showed that 10 mg/kg body weight of $KBrO_3$ caused testicular damage.

2.3. Animal grouping and treatment

Thirty male Wistar albino rats of average weight of 140 ± 5 g were randomly assigned into 5 groups A, B, C, D and E. Group A and B were the positive and negative control respectively while C, D and E were the test groups. Rats in Groups A, C, D and E were all administered 0.5 mL of 10 mg/kg body weight of $KBrO_3$ daily dose. Subsequently, the rats in test groups C, D and E were fed 10%, 20% and 30% of *T. daniellii* rat feed respectively. Rats in groups A and B were fed commercial rat feed only. All the animals were allowed access to drinking water *ad-libitum*. The treatments were consistent each day for 14 d.

2.4. Preparation of testicular homogenate

The testes were harvested from the sacrificed rats and immediately homogenized in ice-cold 0.25 mol/L sucrose solution using a mortar and pestle placed on ice to reduce heat generated from the friction between the mortar and pestle. The homogenates were diluted in 1:5w/v ratio. The homogenates were then centrifuged at 5000 r/min for 15 min. The supernatants were collected into clean sample bottles and kept frozen until required for biochemical assays.

2.5. Biochemical assay

Digital UV/VIS spectrometer was used to investigate the biochemical parameters in the rats' testicular homogenate. Total protein concentration in the testicular homogenate was estimated according to the method described by Gornall *et al.* 1949 [13]. The enzyme activities of alkaline phosphatase (ALP), acyl carrier protein (ACP), and superoxide dismutase (SOD) were estimated by the methods described by Wright *et al.* 1972 [14], and Misra *et al.* 1972 [15] respectively. The level of sialic acid, reduced glutathione, cholesterol and glycogen in the testes were evaluated according to the methods described by Warren *et al.* 1959 [16], Jollow, *et al.* 1974 [17], Fredrickson *et al.* 1987 [18] and kemp *et al.* 1959 [19] respectively. Thiobarbituric acid reactive substances (TBARS) was measured as an estimate of malondialdhyde (MDA) which is a product of lipid peroxidation using the method described by Satoh [20].

2.6. Histology of testes

The preparation of tissues for histological examination was carried out as described by Adeyemi *et al.* [21] The representative portions of the testes removed from sacrificed rats were fixed in 10% buffered formalin (pH 7.4) for 12 h, and then embedded in paraffin. The paraffin embedded tissues were cut into 5 μm sections using a microtome and then stained with hematoxylin and eosin and mounted in Canada balsam [22]. The stained sections were viewed under light microscope and were captured using Sony DSC-W35.

2.7. Statistical analysis

The data were expressed as mean ± SEM. Two ways analysis of variance was used followed by Duncan post hoc mean comparison test to assess for significant differences among the variables at *P*-value less than 0.05. All the statistical evaluations were carried out using the statistical package for social science (SPSS version 19).

3. Results

The percentage organ body weight of testes of the rats in the positive control group showed a significant decrease (37.6%) when compared to the percentage organ body weight of the testes of the rats in the teste group fed with 10%, 20% and 30% of the *T. daniellii* leaf which showed an increase (2.4%, 28.8% and, 8.8% respectively) (Figure 1). ACP activity in the positive control animals was elevated (46%) when compared to the test groups fed with 20% and 30% of *T. daniellii* leaf rat feed which showed a decrease in ACP activity (27% and 32.5% respectively) (Figure 2A). On the contrary, the ALP activity in the positive control was decreased significantly (4.5%) when compared with the groups fed with *T. daniellii* leaf rat feed which showed an increase in ALP (109% and 40.9%) (Figure 2B). There was a significant decrease in the total protein concentration in the positive control animals (14.9%) compared to the group fed with 30% *T. daniellii* leaf rat feed which

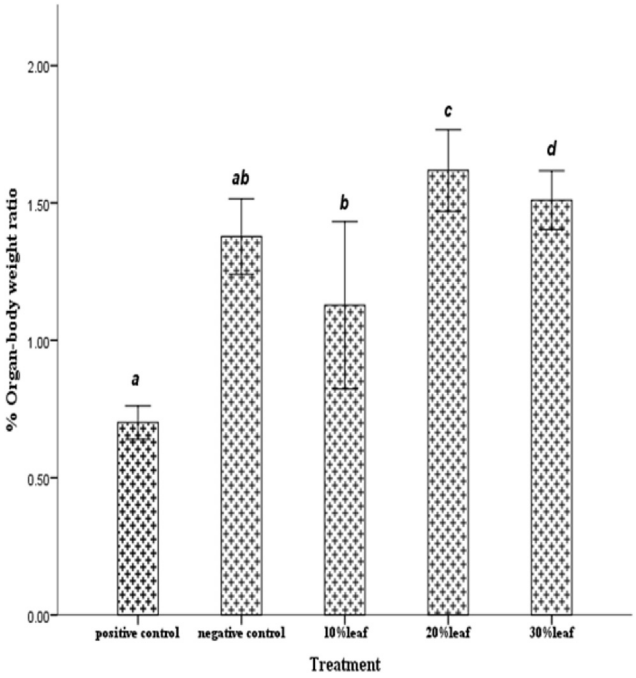

Figure 1. The organ-body weight ratio of rats administered with KBrO3 and fed with *T. daniellii* leaf. Each value is represented as mean of three replicates ± SEM. [abcd]Statistical difference relative to the positive control at $P \leq 0.05$.

showed an increase in total protein concentration (3.9%) (Figure 2C). The testicular cholesterol level in the positive control showed a decrease (26.7%) compared to the test groups animals which were fed *T. daniellii* leaf rat feed, which also showed a reduction (22.7%) (Figure 3A).Similarly, testicular glycogen in rats fed 20% and 30% *T. daniellii* leaf rat feed showed decrease (3.25% and 11.23%) in comparison with the result obtained for the positive control which also was reduced (59.4%) (Figure 3B). The level of sialic acid showed a significant increase in group fed 30% *T. daniellii leaf rat* compared to the positive control (Figure 3C). Rats in the control group showed a significant increase in the MDA level (21.82%) when compared to the test group which showed a decrease in MDA (7.41%) in the group fed 20% *T. daniellii leaf rat* (Figure 4A). GSH level increased significantly in the positive control group compared to the groups fed 10% and 20% *T. daniellii* leaf rat feed (5.12% and 5.98%), conversely the rats fed 30% *T. daniellii* leaf rat feed showed a significant increase in the level of glutathione reductase (52.14%) (Figure 4B). SOD enzyme activity decreased significantly (57.1%) in the positive control rats compared to the test animals fed 10% and 30% *T. daniellii* leaf rat feed which showed an increase in SOD activity (85% and 795%) respectively (Figure 4C). The histopathological result of the testes of the animals fed with 10% of the *T. daniellii* leaf showed well differentiated germ cells and increased proliferation of the sperm cells compared to the positives control animals

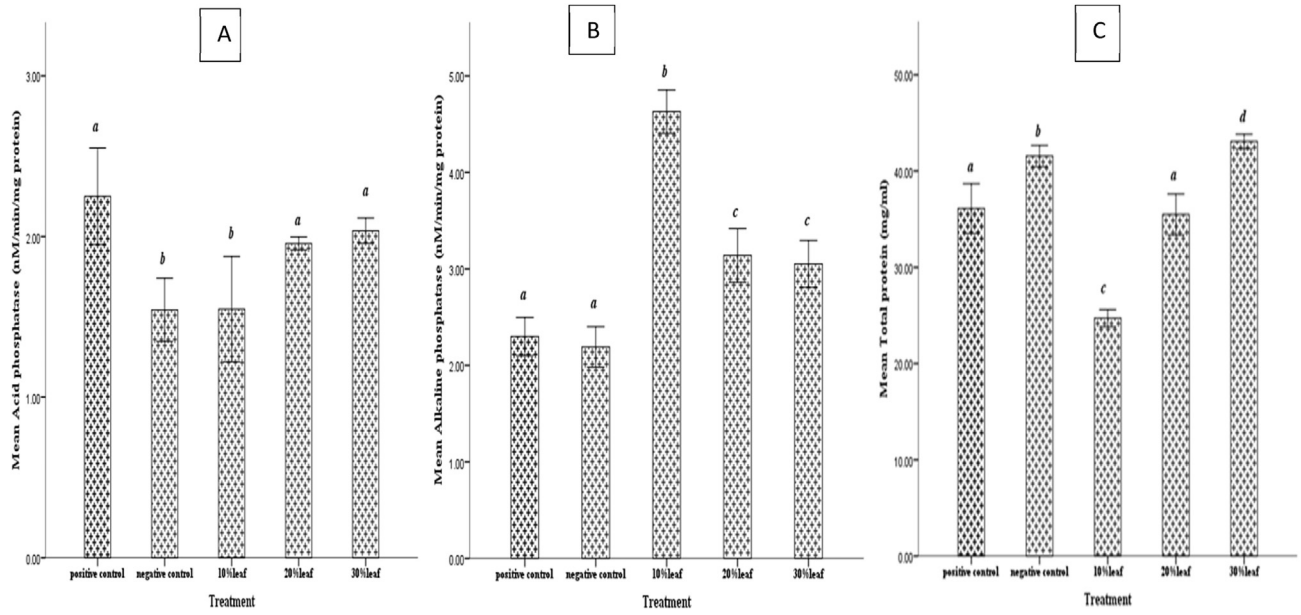

Figure 2. (A–C) The levels of Acid phosphatase (A), Alkaline phosphatase and Total protein (C) in the testes of rats administered with KBrO3 and fed with *T. daniellii* leaf rat-feed. Each value is represented as mean of three replicates ± SEM. [abcd]Statistical difference relative to positive control at $P \leq 0.05$.

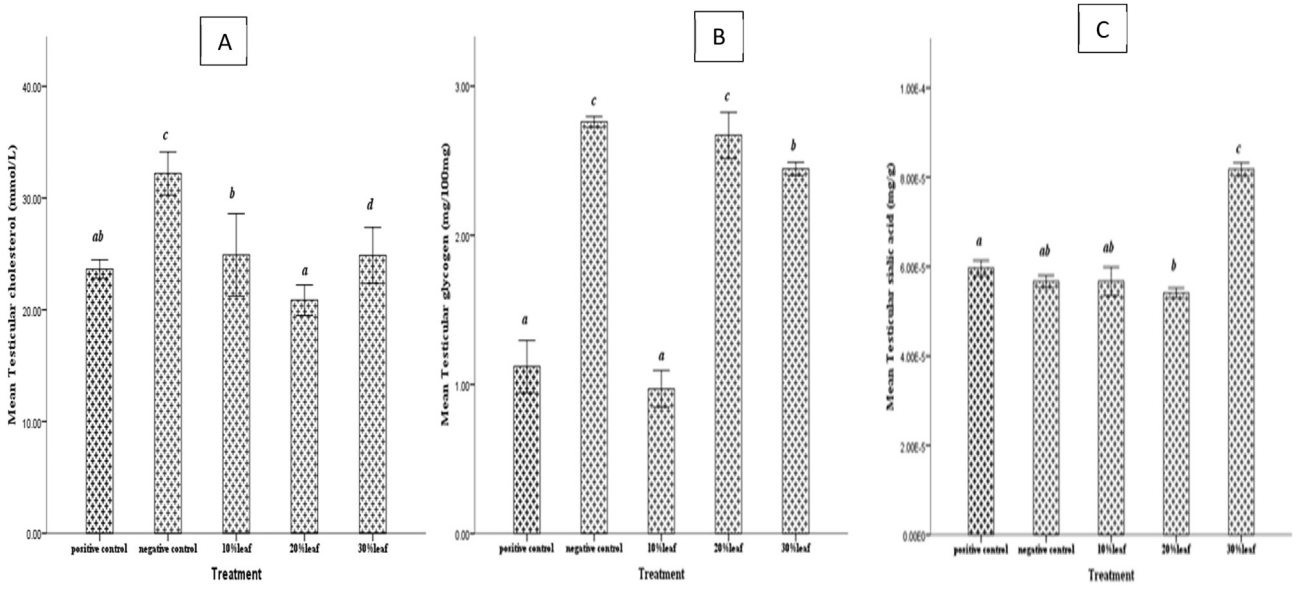

Figure 3. (A–C) The levels of Cholesterol (A), Glycogen (B) and sialic acid(C) in the testes of rats administered with KBrO3 and fed with *T. daniellii* leaf rat-feed. Each value is represented as mean of three replicates ± SEM. [abcd]Statistical difference relative to positive control at $P \leq 0.05$.

which showed reduced sperm cell and degeneration of the testicular tissue (Figure 5).

4. Discussion

The significant reduction in the organ body ratio of the testes of rats fed with $KBrO_3$ may be due to $KBrO_3$ induced toxicity. This reduction could be as a result of reduced tubule size, spermatogenic arrest and inhibition of steroid biosynthesis of leydig cells, a site of steroid biosynthesis [23,24]. In addition, this may also be an indicative of impairments at testicular, pituitary or hypothalamic level [25]. The increase in organ body weight in the test groups, therefore presupposes that *T. daniellii* leaf rat feed may have accorded the rat testes protection from the toxic effect of $KBrO_3$. Acid phosphatase is an enzyme (hydrolase), which catalyses the hydrolysis of various phosphate esters with pH-optimum in the acid zone [26]. The prostatic isoenzyme of ACP is a vital diagnostic marker of testes. ACP is localized in cellular lysosome consequently compromised to the membrane integrity due to lipid peroxidation may result in the testicular increase in the enzyme. The increased testicular activity may be due to lysosomal membrane breakdown and the liberation of the enzyme by $KBrO_3$ [27,28]. The increased activity in the enzyme could also be due to the synthesis of new lysosome as a consequence of lipid peroxidation [29]. The ACP activity in rats fed 10% *T. daniellii* leaf showed improvement which could be due to the effect of the leaf. ALP is an excellent histochemical as well as biochemical marker for the germ cells of several mammalian species including rats [30]. ALP is primarily of testicular and epididymal origin and, therefore, suitable for differentiation of oligo-and azoospermia [30,31].

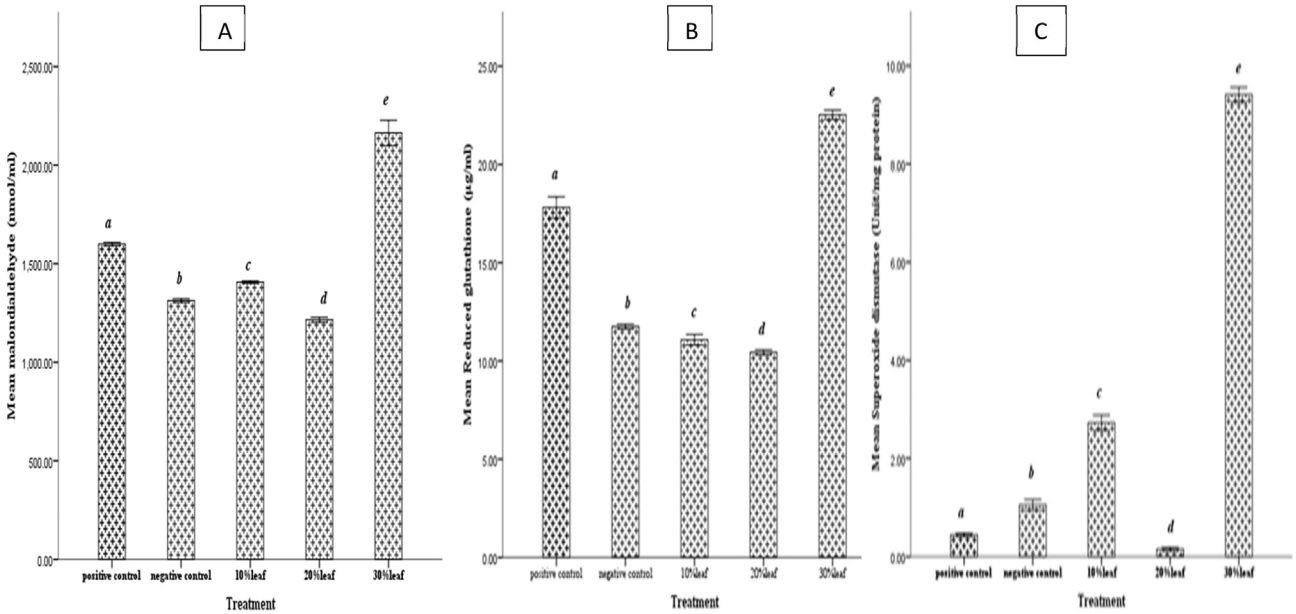

Figure 4. (A–C) The levels of malondialdhyde (A), Glutathione reductase (B) and superoxide dismutase(C) in the testes of rats administered with KBrO3 and fed with *T. daniellii* leaf rat-feed. Each value is represented as mean of three replicates ± SEM. [abcde]Statistical difference relative to positive control at $P \leq 0.05$.

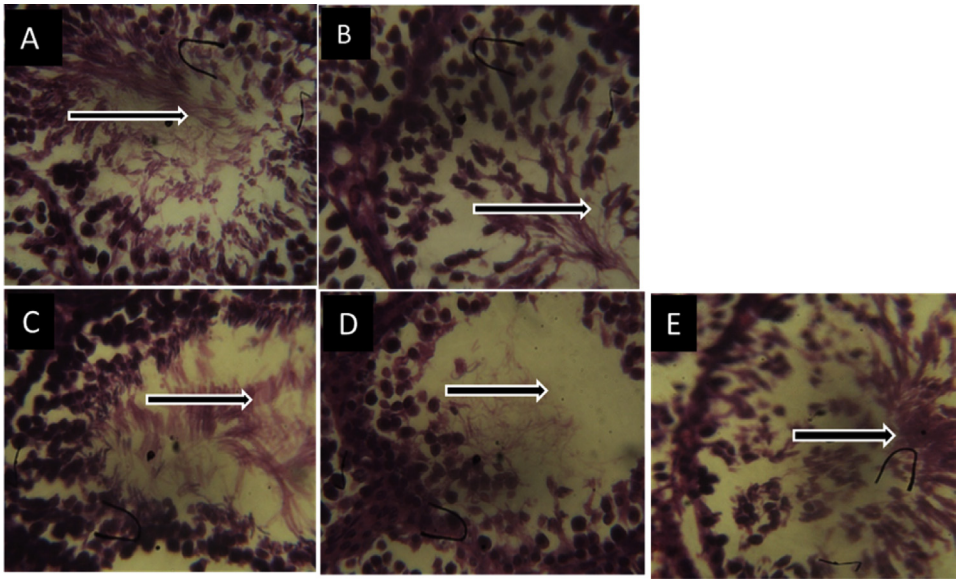

Figure 5. A-E photomicrograph of testis section (H&E ×400).
Group A (positive control) showed a well differentiated germ cell with proliferation of spermatogenic cells as a result of a mild infiltration. Group B, (negative control) showed well differentiated testicular tissue with highly proliferated spermatogenic cells and mild infiltration. Group **C**, 10% *T. daniellii* leaf rat-feed, showed a highly proliferated testicular cell with poor differentiation because of an acute infiltration. Group D, 20% *T. daniellii* leaf rat-feed, showed a highly proliferated testicular tissue. Group E, 30% *T. daniellii* leaf rat-feed, showed a poorly differentiated tissue with slight proliferation of spermatogenic cells as a result of infiltration.

The acrosomic system of sperm head is composed of ALP [32] hence any decrease in sperm cells will lead to decrease in the level of ALP activity. The decreased ALP activity seen in the positive control rats may be indicative of reduced spermatogenesis, resulting from a reduction in the number of sperm cells. The decreased ALP activity could be correlated to decreased acrosomic heads of the sperm cell. Rahman *et al.* [33] suggested that the decrease in the activities of ALP and ACP in different tissues might be due to the increased permeability of plasma membrane or cellular necrosis in treated animals. Protein biosynthesis is vital in the testicular development and spermatogenesis. Sertoli cells coordinate the spermatogenic process by synthesizing a variety of proteins required during the different phases of germ cell maturation [34]. The increased testicular protein in the animal fed 30% *T. daniellii* leaf rat feed could be associated to the protective effect of the leaf. The reduction in protein content observed in the positive control group could also be attributed to disturbances in protein synthesis or metabolism due to toxicity by $KBrO_3$ [35]. For the normal testicular activity, testicular cells require cholesterol for membrane biogenesis and cell signalling as well as precursor required for androgen synthesis [36]. It is apparent from this study that $KBrO_3$ is responsible the reduced cholesterol level seen in the animals in the positives control, however the increased testicular cholesterol level in the rats fed *T. daniellii* rat leaf was indicative of the protective property of the leaf on the testicular cells. The energy storage of testicular glycogen is a crucial requirement for gonadal maturation and proper functioning [37]. The decreased testicular glycogen may be due to loss of sertoli cell as a result of degeneration or inhibition of enzyme activity in the synthesis of glycogen. The increase in the glycogen concentration in the test compared to the positive control could be indicative of the abetment effect of the *T. daniellii* leaf in the compounded feed. Sialic acid (N-acetylneuraminic acid), a derivative of N-acetylmannose and pyruvic acid is an important constituent of glycoproteins and glycolipids. Mann [38] reported that declined sialic acid level may be due to decrease in the rate of spermatogenesis. This study showed increased level of sialic acid in the group fed 30% *T. daniellii* leaf rat fee which may implied that the leaf have the potential to enhance the testicular synthesis of sialic acid. MDA is an index of oxidative damage to cellular structures [39]. The protection of the leaf against lipid peroxidation was appreciable at the lower percentage of the leaf in the compounded feed, which showed lower MDA concentration compared to the positive control. The low MDA supported the speculation that the leaf has antioxidant property. Glutathione deficiency can lead to instability of the mid-piece of sperm, resulting in defective sperm motility. It protects plasma membrane from lipid peroxidation, scavenges superoxide and prevents O_2 formation [40]. The increase in the GSH may be because of enhanced synthesis due oxidative stress by $KBrO_3$ in the positive control and the group fed 30% leaf. Since GSH level decreased in rats fed lower concentration of the leaf but increased in those given 30% or higher concentration of the leaf, it means that the concentration above 20% may be deleterious rather than protective for the animal. However, the GSH level in the group fed 10% and 20% of the leaf respectively showed protective effect of the leaf against oxidative stress by the $KBrO_3$. The decrease in the activity of SOD in rats in the positive group may be in tandem with the effect of $KBrO_3$ in obstruction of metabolism or displacement of Zn and Cu ion which are the cofactor of the enzyme [41]. The increase in SOD activity in the rats fed 30% of *T. daniellii* -rat feed may be due to induction in the activity of the enzyme activity by the leaf. The histopathological changes in the testicular cells in this study supported the biochemical changes due to $KBrO_3$ induced toxicity on the testes function indices. Testicular cell degeneration was very obvious in the positive control which was due to toxicity of $KBrO_3$. The reduced

cellular degeneration in the groups fed with *T. daniellii* rat feed could be indicative of the protective property of *T. daniellii* leaf. The degeneration could be as a result of $KBrO_3$ induced lipid peroxidation on the testicular cell membrane. The biochemical and histopathological results in this study showed $KBrO_3$ induced testicular toxicity in the rats' testes and protective property of *T. daniellii leaf* rat feed on the testicular functions of rats administered with $KBrO_3$.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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