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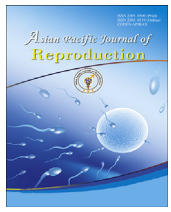

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Effect of *Thaumatococcus daniellii* leaf rat-feed on potassium bromate induced testicular toxicity

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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

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