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Ameliorative activity of α-tocopherol against potassium bromate-induced reproductive functional and morphological impairments in male rats

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Abstract

α-tocopherol (AT) acts as oxidative radical scavenger and protects against toxicants that impair reproductive functions and structures. Potassium bromate (PB) exhibits toxic effects on tissues via generation of reactive oxygen species (ROS) and impairment of antioxidant system. This study was aimed to evaluate the ameliorative effect of AT on PB-induced reproductive impairments in rats. Twenty male rats (150-170 g) were equally divided into four groups: group 1 (Control - Distilled water); group 2 (50 mg/kg AT only); group 3 (50 mg/kg AT + 25 mg/kg PB co-treated) and group 4 (25 mg/kg PB only). All treatments were done daily and orally for 28 days except PB (administered as single dose intraperitoneally at the start of study). Blood samples were collected for biochemical and hormonal assays. Testicular tissues were harvested for sperm analysis and histological studies. Data was presented as mean±standard deviation (SD) and significant difference level was p<0.05. Repeated oral administration of AT after exposure to PB caused significant increase in serum levels of catalase (CAT), superoxide dismutase (SOD), total antioxidant capacity (TAC), follicle stimulating hormone (FSH), leutenizing hormone (LH), testosterone, 17β-hydroxysteroid dehydrogenase (17HSD) and sperm parameters compared to PB only treated group. Further, significant reduction in serum levels of ROS, RNS, and abnormal sperm parameters were observed in the co-treated group compared to PB only treated group. Testicular histoarchitecture also revealed an improvement of the deleterious effect of PB due to the AT treatment. Hence, AT ameliorates PB-induced reproductive impairments in rats.

Keywords: α-tocopherol, Potassium bromate toxicity, Reproductive hormones, Testicular histomorphology

1. Introduction

Potassium bromate (PB) is a white crystalline salt which is readily soluble in water. It is a food additive that is used in making dough and fish paste, in the production of beer, cheese or fermented beverages, and a constituent of cold-wave hair solutions [1]. Emeje et al. [2] reported the persistent use of PB for baking in some parts of Nigeria and the amount found in the bread samples (1.16 - 10.44 µg/g) was significantly greater than the permissible safe level of 0.02 µg/g allowed for bread production by the Food and Drug regulating agency (FDA) in the United States. A report on the assessment of its toxicity showed that PB is a toxic chemical substance that could cause lipid peroxidation and oxidative tissue damage in humans and lower mammals [1]. Studies in experimental animals have shown that the reactive oxygen species (ROS) synthesis and impairment of antioxidant system are the major mechanisms of its tissue toxicity and damage [3,4]. The study by Elsheikh et al [5] reported that the exposure of pre-pubertal rats to PB caused growth retardation, testicular hypoplasia and impairment of spermatogenesis, thereby predisposing the animals to infertility or sterility.

In the biological system, the main mechanism for the removal of ROS is mediated by the antioxidant system which comprises the enzyme-based and non-enzymatic compounds which act to mitigate the potential damage caused by oxidative stress [4]. The non-enzymatic antioxidant compounds include the glutathione (GSH), ascorbic acid and α-tocopherol [6]. α-tocopherol (AT) commonly known as Vitamin E is an antioxidant which inhibits the free radicals

synthesis in tissues by reacting with them to produce tocopheryl radicals that will in turn be reduced by a hydrogen donor [7,8]. Its fat-solubility enables its affinity to the cell membranes and thereby protects the membranes from oxidative damage [7]. Other functions include enzymatic activities, gene expression and neurological functions [9]. Studies by Fang et al. [10] and Oyeyemi et al. [11] have shown that AT exhibits protective effect against chemical toxicants that impair the male reproductive functions.

The National Agency for Food and Drug Administration (NAFDAC), the Nigerian regulatory agency had banned the use of potassium bromate as a food additive due to its toxic effect [12]. However, its use has persisted in Nigeria especially in the bakery industry. This study was aimed to assess the ameliorative activity of AT against the potassium-bromate induced reproductive functional and morphological impairments in male Wistar rats.

2. Materials and methods

2.1 Experimental animals

Twenty male Wistar rats (150-170 g, 14 weeks old) were sourced from and housed in the Animal House of the University of Medical Sciences, Ondo City, Ondo State, Nigeria. The animals were kept under standard conditions of humidity, temperature, 12 h light/dark cycle. They were given free access to standard feed (pellet chow) and drinking water was provided *ad libitum.* The animals were handled with care and all the experimental procedures were carried out in compliance with the experimental animals' guidelines by the National Institutes of Health and National Research Council [13,14].

2.2 Design of the study

Experimental rats (20) were randomly divided into four equal groups consisting of five rats per group (n=5). The animals received the following treatments: group 1 (control) received distilled water (1 mL) once daily for 28 days orally; group 2 received AT only (50 mg/kg) once daily for 28 days orally; co-treated group 3 received AT (50 mg/kg) once daily for 28 days orally + PB (25 mg/kg) once at the beginning of the study intraperitoneally (IP) and group 4 received PB only (25 mg/kg) once at the beginning of the study IP. The doses of PB and AT employed were considered safe for the experimental study [5,11]. After the end of the study, the rats were anesthetized with an IP injection of Sodium thiopental (50 mg/kg). Blood sample was collected from each animal through cardiac puncture for subsequent biochemical and hormonal assays. Anti-coagulant containers with ethylenediaminetetraacetic acid (EDTA) were used to collect the blood samples and QBC Auto read Plus centrifugal system (Becton, Dickinson and Company, New Jersey, USA) was used to centrifuge the blood samples to obtain the serum. The testicular tissue of the animals was harvested for sperm analysis and histological study.

2.3 Evaluation of oxidative stress markers

The serum levels of the malondialdehyde (MDA), catalase (CAT) and superoxide dismutase (SOD) were estimated according to the method by Oyeyemi et al [15]. The total antioxidant capacity (TAC) assay was performed using the OxiSelect TAC kit (Cell Biolabs, Inc. San Diego, CA, USA) while the ROS and reactive nitrogen species (RNS) assays were performed using the OxiSelect ROS/RNS kit (Cell Biolabs, Inc. San Diego, CA, USA). The analytical setup for the ROS and RNS assays were provided by the manufacturer's manual. The reagents were freshly prepared, and the study and standard samples were assayed in duplicate. The DCFH solution was prepared by diluting dichlorodihydrofluorescin DiOxyQ (DCF-DiOxyQ) with the priming agent (in ratio 1:5), vortexed to homogenity and incubated for 30 min at room temperature. For each assay, 50 µL of sample was added to wells of a 96-well plate. 50 µL of catalyst was added, mixed and allowed to incubate for 5 min at room temperature. 100 µL of DCFH solution was added and the plate reaction wells were covered to protect them from light. The solution was incubated at room temperature for 30 min and the fluorescence was read with a fluorescence plate reader at 480 nm excitation.

2.4 Hormonal assay

The serum levels of the follicle stimulating hormone (FSH), leutenizing hormone (LH) and testosterone were evaluated by the enzyme-linked immunosorbent assay (ELISA) method and performed by using the ELISA kit (Calbiotech, Inc, CA, USA). The serum activities of 3β-hydroxysteroid dehydrogenase (3HSD) and 17β-hydroxysteroid dehydrogenase (17HSD) were determined according to the method by Oyeyemi et al. [16].

2.5 Evaluation of sperm parameters - motility, viability, count and morphology

The epididymis from the animals was removed and placed in a petri dish containing pre-warmed 2 mL phosphate buffer saline (PBS, pH 7.4). Sperm cells were obtained from the caudal epididymis of the animals and used to prepare

the sperm suspension used for the sperm analysis according to the guidelines by the World Health Organization (WHO) [17]. The sperm motility evaluation was performed by placing one drop of the sperm suspension on a warmed microscope slide, examined under the microscope and values obtained were expressed as percentage. For the determination of sperm viability, 10 µL of sperm suspension was placed on microscope slides and stained with eosin-nigrosin. The slides were examined under the microscope, the stained (non-viable) and non-stained (viable) sperms were counted, and the values obtained were expressed as percentage. For the evaluation of sperm count, the Sodium Bicarbonate-Formalin solution was used to dilute the sperm suspension (dilution ratio 1:20). One drop of the diluted sperm suspension was introduced into a modified Neubauer hemocytometer chamber, the count of sperm cells was taken in 2 mm^2 under the microscope and expressed in million/mL. For the determination of sperm morphology, some drops of sperm suspension were smeared on the microscope slides and stained with eosin-nigrosin. The stained slides were examined under the microscope (magnification X1,000). A total of 100 sperm cells were evaluated per sample and the values of sperm cells with abnormal morphology was expressed as percentage.

2.6 Assessment of the sperm DNA integrity

The DNA integrity of the sperm cells was assessed, and observable DNA damage was evaluated using the aniline blue (AB) staining technique. Fresh sperm smear was air dried and fixed in 3% buffered glutaraldehyde in 0.2 M phosphate buffer saline (PBS, pH = 7.2). The smear was treated with 5% aqueous AB stain in 4% acetic acid. In each slide, about 200 spermatozoa were counted via light microscopy. Pale blue stained or unstained cells and dark blue stained cells represented the normal and abnormal spermatozoa respectively. The percentage of abnormal spermatozoa was recorded [18].

2.7 Assessment of testicular histoarchitecture

The testicular tissue of the rats was fixed in Bouin's fluid for 24 h, processed into tissue block and sectioned at 5 µ thickness. The sections were mounted on microscope slides and stained with Mayer's hematoxylin-eosin (H & E) [19]. The photomicrograph of stained tissue sections was taken following examination under the microscope.

2.8 Statistical analysis

Data obtained was analyzed using Graphpad prism software (version 8). The results were expressed as mean ± standard deviation (SD). Comparison of data was carried out by one-way analysis of variance (ANOVA) and Tukey's honestly significant difference test with the level of significant difference set at $p<0.05$.

3. Results

3.1 Effect of AT on antioxidant and oxidative stress markers in rats exposed to PB toxicity

As seen in Table 1, the serum CAT and SOD levels and TAC were significantly reduced while the MDA, ROS and RNS levels were significantly increased in PB only treated group when compared with the control group. However, the serum CAT and SOD and TAC levels for the co-treated group were significantly increased while their MDA, ROS and RNS levels were significantly reduced when compared with the PB only treated group.

Table 1 Effect of AT on antioxidant and markers of oxidative stress in rats exposed to PB toxicity.

Serum parameters	Control group	AT only treated group	Co-treated group	PB only treated group
CAT (µ/M)	10.0 ± 0.68	10.6 ± 0.45	9.4 ± 0.65**	5.2 ± 0.28*
SOD (µ/M)	2.7 ± 0.22	2.3 ± 0.31	2.3 ± 0.30**	1.5 ± 0.17*
MDA (µ/M)	4.7 ± 0.13	5.0 ± 0.23	4.8 ± 0.25**	5.8 ± 0.53*
TAC (mM Trolox equivalent)	7.5 ± 0.66	9.6 ± 1.56	5.3 ± 0.90**	2.4 ± 0.24*
ROS (µM)	16.2 ± 1.28	5.1 ± 0.37*	14.1 ± 1.37**	29.1 ± 1.28*
RNS (µM)	2.3 ± 0.58	1.2 ± 0.18	4.8 ± 0.41*,**	6.8 ± 0.55*

*,** indicates significant difference (at $p<0.05$) compared to control group and PB-treated group only respectively.

3.2 Effect of AT on reproductive hormones in rats following exposure to PB toxicity

As seen in Table 2, the serum levels of FSH, LH, 3HSD and 17HSD in the co-treated group and PB only treated group were significantly reduced when compared with the control group. However, the values in co-treated group showed significant increase compared with the PB only treated group. The serum testosterone level showed significant reduction in the PB only treated group when compared with the control and co-treated groups.

Table 2 Effect of AT on reproductive hormones in rats exposed to PB toxicity.

Parameters	Control group	AT only treated group	Co-treated group	PB only treated group
Testosterone (ng/mL)	2.8 ± 0.08	3.1 ± 0.09	2.7 ± 0.1**	1.8 ± 0.11*
FSH (mIU/mL)	2.4 ± 0.15	2.4 ± 0.05	1.7 ± 0.04*,**	1.0 ± 0.02*
LH (mIU/mL)	7.6 ± 0.11	7.8 ± 0.20	6.2 ± 0.13*,**	4.4 ± 0.37*
3HSD (ng/mL)	13.1 ± 1.43	13.2 ± 1.31	10.1 ± 0.96*,**	7.2 ± 0.44*
17HSD (ng/mL)	28.2 ± 3.08	26.3 ± 3.61*	19.6 ± 1.22*,**	12.4 ± 0.47*

$^{*, **}$ indicates significant difference (at $p<0.05$) compared to control group and PB-treated group only respectively.

3.3 Effect of AT on the sperm motility, viability, count, morphology and DNA integrity in rats exposed to PB toxicity

As seen in Table 3, the sperm motility and count in the co-treated and PB only treated groups and sperm viability in the PB only treated group were significantly reduced compared with the control group. Conversely, these parameters showed significant increase in the co-treated group when compared with the PB only treated group. The results also showed that the abnormality in sperm morphology was significantly increased in the co-treated and PB only treated groups compared with the control group. However, a significant reduction can be observed in the co-treated group when compared with the PB only group. Furthermore, the normal sperm chromatin condensation was significantly increased in co-treated group compared with the PB only group and the abnormal sperm chromatin condensation was significantly elevated in PB only group in comparison to the control group. However, a significant decrease in the abnormal sperm chromatin condensation can be observed in the co-treated group when compared with the PB only treated group.

Table 3 Effect of AT on sperm parameters (motility, viability, count and morphology) and DNA integrity in rats exposed to PB toxicity.

Parameters	Control group	AT only treated group	Co-treated group	PB only treated group
Sperm motility (%)	61.3 ± 6.60	58.8 ± 7.00	42.2 ± 3.44*,**	25.6 ± 3.80*
Sperm viability (%)	88.3 ± 2.11	89.0 ± 2.92	84.0 ± 1.87	81.4 ± 4.19*
Sperm count (x10^6/mL)	31.4 ± 3.41	33.4 ± 4.20	20.3 ± 5.02*,**	11.5 ± 2.61*
Sperm morphological abnormality (%)	7.9 ± 1.49	7.0 ± 1.23	20.0 ± 2.89*,**	30.2 ± 3.61*
Normal sperm chromatin condensation (%)	98.5 ± 0.37	98.2 ± 0.29	98.1 ± 0.53**	96.0 ± 0.37
Abnormal sperm chromatin condensation (%)	1.5 ± 0.38	1.8 ± 0.20	1.9 ± 0.53**	4.0 ± 0.28*

$^{*, **}$ indicates significant difference (at $p<0.05$) compared to control group and PB-treated group only respectively.

3.4 Effect of AT on testicular histoarchitecture of rats exposed to PB toxicity

The results of this study (Figure 1) show the testicular histoarchitecture of the experimental animals. Testicular sections from the control and AT only treated group showed normal testicular histomorphological features which include normal orientation of seminiferous tubules and tubular lumen, normal distribution of sperm cells and interstitial spaces and cells. In comparison with control group, the co-treated group showed minor derangement of some germinal epithelium and mildly enlarged seminiferous tubular lumen. Conversely, PB only treated group showed major distortion of the germinal epithelium, prominent vascular congestion, and derangement of interstitial cells in comparison with control animals.

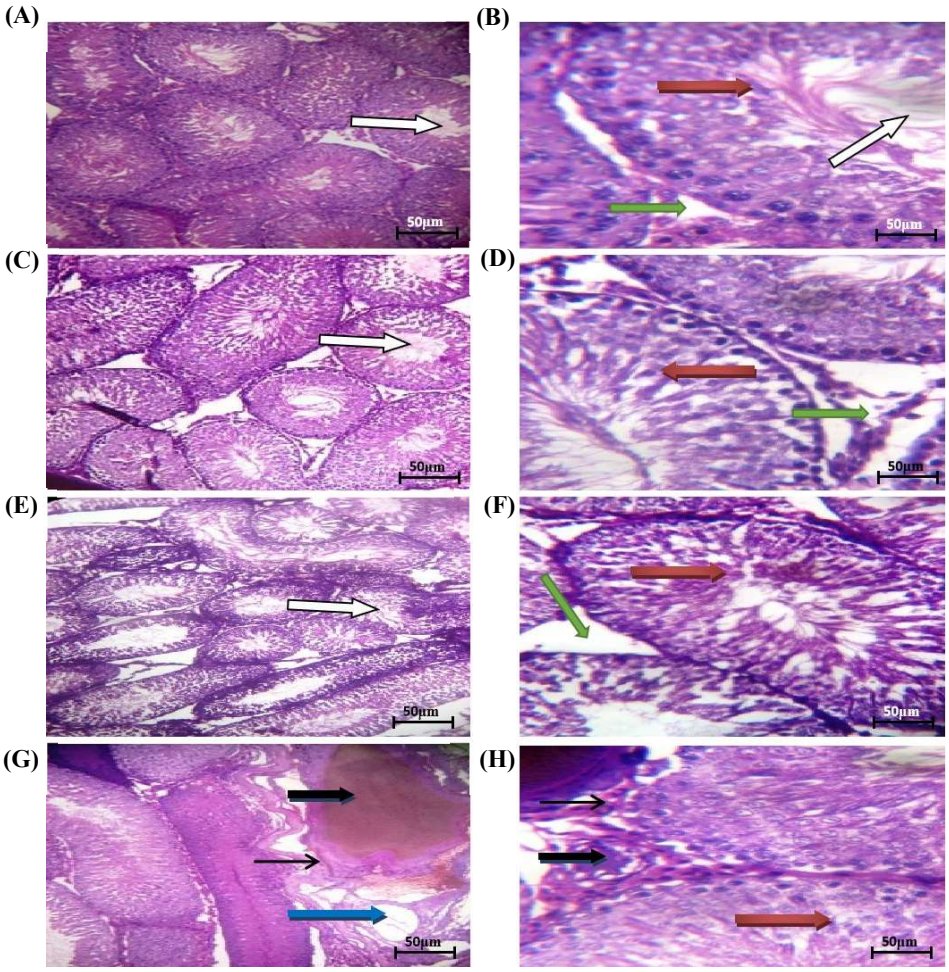

Figure 1 Testicular histoarchitecture of rats exposed to PB toxicity and treated with AT H&E the control X100 (A), Control X400 (B), only treated group X100 (C), only treated group X400 (D), co-treated group X100 (E), co-treated group X400 (F), PB only group X100 (G), and PB only group X400 (H). The white arrows indicate seminiferous tubular lumen, red arrows indicate the sperm cells, green arrows indicate the interstitial spaces, blue arrow indicates distorted interstitial space, thick black arrows indicate vascular congestion and slender black arrows indicate distorted interstitial cells.

4. Discussion

In mammalian cells, the CAT and SOD enzymes are among major components of the antioxidant defence system that function to defend the body against the deleterious effect of the ROS. The reduced levels of SOD, CAT and TAC observed in this study may result from the generation of free radicals in the experimental animals following exposure to PB toxicity. This was similar to the results reported by Ali et al. [20]. Essentially, oxidative stress usually occurs in tissues when ROS overwhelm the antioxidant defence system leading to their pathological effects which include reproductive dysfunction and infertility [21]. PB is a strong oxidant which elicit ROS and RNS production leading to tissue damage and cellular dysfunction [22]. Based on the results of this study, the ROS and RNS increased significantly in PB only treated animals. This may be directly related to the decrease in the serum SOD, CAT and TAC levels observed in this study. As a potent antioxidant, AT treatment was observed to mitigate the generation of oxidative stress markers occasioned by the exposure to PB toxicity in the study animals.

The successful development of male germ cells is based on the balanced endocrine interplay of the hypothalamus, the pituitary gland, and the testes as an entity. In this study, the exposure to PB toxicity caused a decrease in the serum levels of reproductive hormones which include the FSH, LH, and testosterone. This finding was similarly reported in a study by Khan et al. [23]. According to the study by Oyeyemi et al. [16], oxidative stress has been reported to cause degeneration of the Leydig cells leading to a significant decline in testosterone production. The reduction in testosterone synthesis would in turn lead to down-regulation of FSH and LH production by the pituitary gland and culminate into reproductive dysfunction and infertility.

In this study, the PB only treated group showed significant reduction in the serum 3HSD and 17HSD levels. This may be attributed to the decrease in serum LH level and elevation of serum ROS and RNS levels. The LH has been reported to play vital role in the commencement of steroidogenesis [23]. Moreover, the HSDs are the major enzymes that facilitate androgenesis due to their important role they play in the process of steroidogenesis [24]. Therefore, the reduction in serum 3HSD and 17HSD activities in animals exposed to PB toxicity could account for concurrent reduction in the level of serum testosterone as observed in this study. However, the results of this study showed that the AT treatment ameliorated the adverse effect of the exposure to PB toxicity on the reproductive hormones.

Furthermore, the findings of this study showed that the exposure to PB toxicity caused significant decrease in the sperm motility and count. According to studies by Pant et al. [25], the reduction in sperm motility can be due to the oxidative inhibition of some associated enzymes. This could be a primary effect of the exposure to PB toxicity observed in this study. The probable cause of significant reduction in the sperm count is the significant decrease in the FSH, LH and testosterone levels which would in turn impair the process of spermatogenesis. Essentially, oxidative stress that results from the exposure of toxicants to testicular tissues would cause deleterious effect on sperm cells leading to reduction of the sperm count [26]. According to Venkatesh et al. [27], the elevated levels of ROS and RNS following exposure to tissue toxicants would lead to a significant increase in the percentage of abnormal sperm morphology. The observed increase in the sperm morphological anomalies in the PB only treated group can be associated with the ROS and RNS generation. However, the results of this study also indicated that the AT treatment was potent in ameliorating the adverse effect of PB toxicity on the sperm parameters and morphology.

According to study by Barroso et al. [28], oxidative stress results into sperm DNA damage and the integrity of the sperm DNA essentially determines the male reproductive proficiency. According to the findings of this study, the exposure to PB toxicity caused significant elevation in the abnormal sperm chromatin. The oxidative alterations of the sperm DNA could be as a result of the modifications of the nucleotide bases, frameshifts or cross-linkages, chromosomal reshuffle, single-strand or double-strands disruptions and gene alterations [29]. Moreover, the testicular histoarchitecture of the experimental animals exposed to PB toxicity showed vascular congestion and disruption of the interstitial spaces and cells (of Leydig). Studies have reported that oxidative stress exerts deleterious effects on the body tissues including the testis [26,27]. This implies that the adverse effects of exposure to PB toxicity include the testicular histomorphological derangement. However, the AT counteracts against the deleterious effect of the exposure to PB toxicity and ameliorates the associated sperm DNA damage and testicular histomorphological derangement. Essentially, the AT is a potent antioxidant which can function to scavenge free radicals and ameliorate the damaging effect of oxidative stress induced in tissue by exposure to toxicants [30].

5. Conclusion

Based on the findings of this study, the AT exhibit potent ameliorative effect against PB-induced reproductive functional and morphological impairments in male rats. The ameliorative effects of the AT against the exposure to PB toxicity can be attributed to its antioxidant activity. However, the non-exposure to the PB and its associated toxicity is preferable to the therapeutic mitigation of its toxic effect after exposure. Hence, it is recommended that the ban on the consumption of potassium bromate especially in the bakery industry should be enforced in countries where such policy has been enacted and promptly enacted in countries that currently do not have such policy.

6. Ethical approval

This research was approved by the Research and Ethics Committee of the University of Medical Sciences, Ondo City, Ondo State, Nigeria (PHS/16/0460).

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