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Research article

Comparative effects of *Azanza garckeana* fruit pulp and melatonin on serum and testicular oxidative stress changes, live sperm cells and spermatozoa abnormalities evoked by chronic administration of bisphenol A in rabbit bucks

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Abstract

The study aimed to elucidate serum and testicular oxidative stress changes induced by bisphenol A (BPA) and their amelioration by Azanza garckeana (AG) pulp extract and melatonin in rabbit bucks. Forty-two adult New Zealand White rabbit bucks (n = 6), aged 10 ± 0.05-months-old weighing 1.2 ± 0.03 kg were fed ad libitum on a commercial diet. They were randomly divided into seven groups of six (6) bucks each. Group A was administered distilled water (1.5 mL); group B, BPA (100 mg/kg); group C, AG (500 mg/kg); group D, melatonin (1.0 mg/kg); group E was pre-dosed for six weeks with BPA (100 mg/kg), then AG (500 mg/kg) for another six weeks; group F was pre-dosed for six weeks with BPA (100 mg/kg), then melatonin (1.0 mg/kg) for another six weeks; and group G was pre-dosed for six weeks with BPA (100 mg/kg), then AG (500 mg/kg) and melatonin(1.0 mg/kg) for another six weeks. There was a significant increase ($p < 0.05$) in the activities of both serum and testicular superoxide dismutase, catalase and glutathione peroxidase as well as a decrease in malondialdehyde concentration in treatment groups. The percentage dead sperm and spermatozoa abnormalities such as detached sperm heads, free, coiled and bent tails in the groups exposed to BPA increased significantly ($p < 0.05$) compared to control. It is concluded that BPA-induced oxidative stress. The administration of AG only ameliorated these negative effects better than melatonin. However, optimum results were seen when both substances were administered synergistically.

Keywords: Catalase, Glutathione peroxidase, Malondialdehyde, Spermatozoa abnormalities, Superoxide dismutase.

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INTRODUCTION

Bisphenol A (BPA) is a chemical with a high manufacturing rate, commonly used in dental sealants, medical devices, food packaging materials, and heat receipts (Rochester, 2013). BPA exposure can occur through eating, breathing, or skin contact. (Vandenberg et al., 2007; Meli et al., 2020). In mammals, BPA causes oxidative damage. It undergoes oxidation to produce a multitude of metabolites, several of which may have a greater physiological effect than the original chemical. The most frequent source of damage to the sperm is induced by reactive oxygen species (ROS). Natural antioxidants are crucial for combating ROS's harmful effects in humans and animals (Noori, 2012) by exerting protection against various medications or chemically-induced toxicity (Baiomy and Mansour, 2015).

Azanza garckeana (AG) was identified by Professor August Garcke (1819-1904), other names for this plant are African chewing gum, tree hibiscus, snot apple, quarters, wild hibiscus (Palmer and Pitman, 1972). The plant is widely grown in Tula (latitude 9° 48'51"N, longitude, 11° 18′ 32″E and altitude of 610 m), Nigeria. It is also found in Kankiya (latitude 12° 32′ 57″N and longitude 7° 49′ 31″E) and the Daggish Kali highlands (latitude 10° 37′ 38″N and longitude 13° 24′ 53″E), Nigeria (Edward et al., 2021).

The AG is very rich in antioxidants, scavenging ROS that harm DNA and cell membranes (Capasso, 2013). It also demonstrates antioxidant properties in the reproductive system of the males. *Azanza garckeana* contains nutrients, minerals and phytochemical compounds, such as flavonoids, phenols and tannins that are beneficial for human and animal health (Maroyi and Chiekh-Youssef, 2017). A powerful endogenous chemical generated from tryptophan with pleiotropic effects and broad-spectrum antioxidant activities is melatonin. It is a sleep promoter (Lerner and Case, 1959), in some vertebrates, this chemical signal light and darkness serving as a regulator of photo-dependent seasonal reproduction (Tan et al., 2015). BPA exposure results in increased ROS generation of ROS in the testes, which decreases the antioxidant level of the body, and impairs spermatogenesis (Omran et al., 2018). The stem bark of AG extract possesses potent scavenging properties, when compared to antioxidants like vitamins C and E (Mshelia et al., 2016). It has been suggested that AG may be used as an antioxidant, which may reduce the dependence on synthetic drugs as an ROS-scavenging agent (Dikko et al., 2016).

There is need to also compare the antioxidant properties of AG to a standard antioxidant like melatonin due to its availability and affordability. Currently, due to their alleged safety and predominance in wild edible fruits, natural phenolics are increasingly being used as antioxidants and functional additives on a global scale (Wu et al., 2017; Abubakar et al., 2021; Saad et al., 2021). It has been well established that oxidative stress is a significant factor in the pathophysiology of diseases in rabbit bucks (Bisht et al., 2017). Optimal strategies to treat both idiopathic and BPA-induced toxicities may be achieved by using combination antioxidant therapy such as *Azanza garckeana* and melatonin (Fadi et al., 2021).

MATERIALS AND METHODS

Study Area

The study was carried out at Theriogenology and Production Department, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

Plant material

Azanza garckeana (AG) ripe fruits were obtained from Tula, Nigeria between February and April 2021, and sent to the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria for identification. Furthermore, voucher number: **ABU07276** was issued. The plant was further verified by comparison with attributes from databases (http://www.theplantlist.org and http://www.ipni.org).

Methanol Extraction

Sigma Chemical Company, St. Louis, MO, USA, provided the analytical grade methanol that was utilized to extract the plant material. All additional compounds were made in distilled water and were also of analytical grade.

The seed of the AG nut was removed after being thoroughly cleaned under running water. Two weeks were spent air-drying the pulp. A grinding mill was used to ground the dried material into a coarse powder. At a temperature of 50 °C, methanol was used to extract precisely 5 kg of the plant material. The solvent was recovered, concentrated, and then dried on a rotary evaporator to a constant weight. After that, it was kept for later usage in an airtight container (Wu et al., 2013). Methanol used for extraction of the plant material was of analytical grade (Sigma Chemical Company, St. Louis, MO, USA). All other chemicals used were also of analytical grade and were prepared in distilled water.

Percentage Yield of Azanza garckeana from Methanol Extraction

Using the formula shown below, the weight of the extract was recorded, and the percentage extract yield was calculated (Table 1) (Tsado et al., 2015) :

$$\% \text{ Yield} = \frac{\text{Weight of Extract (g)}}{\text{Weight of Dried powedered sample (g)}} \text{ x100}$$

Table 1 Quantitative Phytochemical Composition of *Azanza garckeana* (AG) fruit pulp

Phytoconstituents	(w/w) (%)
Tannins	14.9 ± 0.4
Flavonoids	25.5 ± 2.4
Saponins	18.9 ± 0.2
Phenols	36.5 ± 3.7
Alkaloids	19.0 ± 0.1

Phytochemical analysis and acute toxicity test of AG pulp extract

Following the phytochemical screening, the found compounds were quantified using accepted techniques (Ogbu et al., 2020).

The acute toxicity profile of AG pulp extract was performed in two phases (Lorke, 1983). Briefly, out of the 30 rabbit bucks used in acute toxicity study, 15 rabbit bucks were divided into three groups of five rabbit bucks each for phase I screening. Rabbit bucks in groups 1, 2, and 3 received 10, 100 and 1000 mg/kg body weight of methanol AG fruit pulp extracts, respectively and was administered by oral gavage.

The bucks were monitored for 24 hours. Higher doses were selected for phase II based on the findings of phase I. In this phase, the remaining 15 rabbit bucks were divided into three groups of five rabbit bucks each and given extracts of AG fruit pulp in doses of 1600, 2900, and 5000 mg/kg body weight, respectively, for groups 4, 5, and 6. The rabbit bucks were then monitored for 24 hours for mortality, lethality, and any morphological or behavioral indicators of toxicity, such as dullness, changes in the look or color of the eyes and fur, hyperactivity, changes in feeding habits, sedation and mortality.

Table 2 Qualitative phytochemical screening of methanol extract of *Azanza garckeana* fruit pulp

S/no	Constituents	Test	Inference
1	Carbohydrates	Molisch	+
2	Anthraquinones	Bontragers	–
3	Alkaloids	Wagner's Reagent	+
4	Cardiac glycosides	Kelle-Killiani	+
5	Flavanoids	Sodium Hydroxide	+
6	Saponins	Frothing	+
7	Steriod	Iron chloride	+
8	Triterpenes	Libermann Buchard	+
9	Tannins	Iron chloride	+
10	Phenols	Libermann Buchard	+

+ Present - Absent

Animals

Forty-two (n = 6), healthy New Zealand White rabbit bucks (*Oryctolagus cuniculus*), aged 10 ± 0.05-months-old weighing 1.2 ± 0.03 kg were used for the study. The bucks were sourced from rabbit farms within Zaria and environs. They were screened and treated against endoparasites and bacterial infection before starting the experiment using standard methods. The bucks were housed in standard rabbit cages, one buck per cage (40 *x* 50 *x* 35 cm). They were all given access to water and standard rabbit feeds (Labar Feed Mills, Zaria, Nigeria) *ad libitum*. Before the study began, rabbit bucks were allowed to acclimatize for 14 days prior to experimental procedures.

Experimental Diet

The American Organization of Analytical Chemists' method was used to do the proximate analysis of the diets (AOAC, 2012). The diet consisted of values of isonitrogenous and isocaloric food (Table 3) (Pauzenga, 1985).

Table 3 Composition of Experimental Diet

Feedstuff	Composition (%)
Maize	30.2
Groundnut cake	28.1
Rice offals	35.3
Vitamin premix	0.5
Palm oil	1.0
Bone meal	4.0
Methionine	0.4
Salt	0.5
Total	100.0
Proximate Composition	
Dry Matter	89.5
Crude protein	16.8
Ether extract	1.3
Crude fibre	8.7
Nitrogen free extract	54.0
Ash	7.2
ME (kCal/kg)	2,640.4

Metabolisable energy was calculated according to formula of Pauzenga (26):
ME = 37 × % CP + 81 × % EE + 35.5 × % NFE

Experimental Design, Duration and Sample Collection

Forty-two (42) rabbit bucks were randomly divided into seven (7) groups of six (6) bucks each, designated as groups A, B, C, D, E, F and G.
Group A: Distilled water (1.5 mL) only for seven consecutive days per week for 12 weeks.

Group B: BPA (100 mg/kg) for five consecutive days per week for 12 weeks.

Group C: AG (500 mg/kg) for seven consecutive days per week for 12 weeks.

Group D: melatonin (1.0 mg/kg) seven consecutive days per week for 12 weeks.

Group E: six weeks pre-dosed with BPA (100 mg/kg) for five consecutive days per week, then AG (500 mg/kg) for six weeks.

Group F: six weeks pre-dosed with BPA (100 mg/kg) for five consecutive days per week, then melatonin (1.0 mg/kg) for six weeks.

Group G: six weeks pre-dosed with BPA (100 mg/kg) for five consecutive days per week, then AG (500mg/kg) + melatonin (1.0 mg/kg) for six weeks.

The length of the investigation was three months (90 days), during which serum samples were collected fortnightly (weeks 2, 4, 6, 8 10 and 12). For assessment of serum enzyme activities and LPO determination, a total of 252 serum samples were collected and the testes were harvested from five sacrificed bucks in each group at the end of the study. (Nair, 2015).

Exactly 3 mL of blood was taken from the marginal ear vein of each buck weekly using a 27- G needle and placed immediately on ice in heparinised tubes. Serum was collected from blood by centrifuging for 5 minutes at 3000 × *g* and kept at temperature of 25-26 ℃ (Attia and Kamel, 2012).

Evaluation of Oxidative Stress Biomarkers in the Testes

The epididymides and testes were carefully collected, separated, and weighed individually before analysis. Testicular homogenate was diluted in nine volumes of buffered saline and centrifuged at 3000 x g for 15 minutes. The oxidative stress indicator such as malondialdehyde (MDA) and antioxidant enzyme activities such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured in the supernatant after it had been removed. (Oda and Waheeb, 2017).

Evaluation of testicular and serum antioxidant enzyme activities

Superoxide dismutase

The method outlined by Fridovich (1997) was used to measure superoxide dismutase (SOD) activity. The assay's foundation is SOD's capacity to prevent the auto-oxidation of adrenaline at pH 10.2. Testicular tissue homogenate of 0.1 mL was diluted in 0.9 mL of distilled water to create a 1:10 dilution of micro some. 0.2 mL of the diluted micro-some and 2.5 mL of 0.05 M carbonate buffer were put together in an aliquot. The procedure was started by adding 0.3 mL of 0.3 mM adrenaline. The reference mixture consisted of 2.5 mL of 0.05 M carbonate buffer, 0.3 mL of 0.3 mM adrenaline, and 0.2 mL of distilled water. The absorbance was assessed over a period of 30 seconds and reached up to 150 seconds at 480 nm, using a spectrophotometer (BIOXYTECH SOD-525).

Increase in absorbance per minute = (A_2-A_1) /2.5

Percentage inhibition = 100 – [(increase in absorbance for sample/ increase in absorbance of blank) × 100].

The amount of SOD required to achieve 50 % suppression of the oxidation of adrenaline to adrenochrome in one minute was one unit of SOD activity. The SOD activity was expressed in IU/mg tissue protein concentration.

Catalase

Catalase (CAT) activity was determined by the method described by Karkhanei *et al.* (2021). The ability of one unit of CAT to decompose 1.0 µmole of H_2O_2 per minute at pH 7.0 at 25˚C, while the hydrogen peroxide (H_20_2) concentration falls from 10.3 mM to 9.2 mM. The rate of disappearance of H_20_2 is followed by observing the rate of decrease in the absorbance at 240 nm)

Glutathione peroxidase (GPx)

According to Rajagopalan et al. (2004) description, the concentration of glutathione peroxidase (GPx) was assayed in accordance with Ellman's (1959) methodology. It was based on the interaction between glutathione peroxidase and 5, 5-dithiobisnitro 179 benzoic acid (DNTB) (GPx). In a nutshell, 1.5 mL of 10% Trichloroacetic acid (TCA) was added to 150 L of testicular tissue homogenate (in phosphate-saline buffer, pH 7.4) and centrifuged at 1500 g for 5 minutes. The supernatant was treated with 1 mL of Ellman's reagent and 3 mL of phosphate buffer in exactly the same amounts (0.2 M, pH 8.0) at 412 nm, read. The graph of the GPx, standard curve was used to determine the amount of GPx. The tissue protein concentration was used to express the GPx activities as IU/mg.

Evaluation of serum and testicular lipid peroxidation

The reaction's fundamental premise was that lipid peroxidation (LPO) can produce peroxide intermediates, which, upon cleavage, release MDA. Malondialdehyde concentration functioned as an indicator of the degree to which LPO, a byproduct of the reaction between thiobarbituric acid (TBA) and MDA occurred. A complex color was produced by the reaction, which was detected by light that was absorbed at 535 nm and measured by spectrophotometer (BIOXYTECH -525).

150 mL of tissue homogenate were treated with 2 mL of 0.37 % TBA solution, 15 % trichloroacetic acid solution, and 0.25N HCl reagent (1:1:1) before being heated in a water bath for 60 minutes at 90°C. After cooling and centrifuging the mixture at 3000 *x g* for 5 minutes, the pink supernatant (TBA-MDA complex) was tested for absorbance at 535 nm. The molar extinction value of 1.56 10^{-5} cmM^{-1} was used to compute the concentration of MDA produced.

Malonaldehyde concentration (µM/mg protein) = Absorbance of sample/1.56 × 10^{-5} × protein concentration (mg).

Percentage of live and dead sperm

This was established in accordance with Esteso et al. (2006)'s description. On a clean, grease-free slide, a tiny smear of semen was applied, and it was stained with two drops of Eosin-Nigrosin stain penetrated and marked dead sperm cells pink whereas live spermatozoa remained colorless. When the slides were dried, stained and unstained sperm cells were counted using light microscopy at a magnification of × 40.

Sperm abnormalities

A small smear of the semen sample was made on a clean, grease-free glass slide and stained with two drops of Eosin-Nigrosin to identify any aberrant sperm). Sperm cells were counted per slide using hand counter under light microscopy at × 100 magnification using oil immersion. All abnormal cell types such as detached heads, coiled, bent and free tails were counted and recorded. The slide is examined under high power and at least 200 cells (both stained and unstained) are counted and a percentage of each estimated (Esteso et al., 2006).

Ethical Approval

Approval for the study was sought and obtained from the Ahmadu Bello University Committee for Animal Use and Care (ABUCAUC) with the approval number: **ABUCAUC/2021/062**.

Statistical analyses

The data was presented as mean ± SEM. A two-way analysis of variance (ANOVA) was used for the analyses, followed by Tukey's multiple comparison test, $p \leq 0.05$ were considered significant in 213 cases. GraphPad Prism version 5.0 for Windows 2003 from GraphPad Prism Software, San Diego, California, USA, was used to conduct the analyses (www.graphpad.com).

RESULTS

Tannins, flavonoids, saponins, phenols and alkaloids were found in the methanol extract of AG fruit pulp. Phenols were the most abundant component in the fruit (36.5 ± 3.7 %). Flavonoids and saponins were found to be present in the amounts of 25.5 ± 2.4 % and 18.9 ± 0.2 %, respectively. Alkaloids occurred in smaller quantity (19.0 ± 0.2 %), tannins had the least quantity (14.9 ± 0.4 %) (Table 1).

Acute toxicity profile of AG pulp

Up until the dose of AG pulp delivered, which was 5000 mg/kg body weight, there was no fatality noted. Additionally, no notable changes in body weight or behavior were noticed within 24 hours of the acute toxicity trial. The findings revealed that AG pulp was suitable for consumption.

Testicular Antioxidant Enzyme Activities

The mean ± SEM of testicular SOD, catalase, glutathione peroxidase activities and malondialdehyde concentrations of rabbit bucks in the treatment groups A, B, C, D, E, F and G sampled at week 12 of the study (Table 4).

Superoxide dismutase activity

Testicular SOD activity was significantly ($p < 0.05$) highest in group C (5.4 ± 0.2 U/mg protein), moderately compared to groups A (2.8 ± 0.6 U/mg protein), D (3.5 ± 0.5 U/mg protein), E (4.3 ± 0.7 U/mg protein), F (2.9 ± 0.7 U/mg protein), G (3.3 ± 0.8 U/mg protein) and B (1.5 ± 0.08 U/mg protein) (Table 4).

Catalase

Testicular catalase activity (U/mg protein) were significantly ($p < 0.05$) higher in group C (32.32 ± 4.9 U/mg protein), moderate in groups D (12.2 ± 0.5 U/mg protein), E (10.6 ± 1.5 U/mg protein) and G (11.3 ± 2.1 U/mg protein), but significantly lowest in group B (2.2 ± 0.7 U/mg protein) (Table 4).

Glutathione peroxidase concentration

Testicular GPx concentration was significantly ($p < 0.05$) the highest in group C (33.58 ± 2.9 U/mg protein), followed by group D (23.3 ± 2.9 U/mg protein) or G (29.8 ± 3.5 U/mg protein). The concentration was moderately ($p < 0.05$) significant compared to groups E (15.7 ± 2.4 U/mg protein) and F (14.5 ± 1.0 U/mg protein), but significantly ($p > 0.05$) the lowest in groups A (7.6 ± 1.3 U/mg protein) and B (7.3 ± 2.3 U/mg protein) (Table 4).

Table 4 Testicular SOD and catalase activities, glutathione peroxidase and malondialdehyde concentrations of rabbit bucks in the treatment groups A, B, C, D, E, F and G sampled at week 12 (day 90) of the study

Testicular Antioxidants	Group A	B	C	D	E	F	G
Superoxide dismutase (U/mg protein)	2.8±0.6[a]	1.5±0.08[b]	5.4±0.2[c]	3.5±0.5[a]	4.3±0.7[c]	2.9±0.7[a]	3.3±0.8[a]
Catalase (U/mg protein)	4.8±1.0[a]	2.2±0.7[b]	32.32±4.9[c]	12.2±0.5[d]	10.6±1.5[d]	6.1±0.8[e]	11.3± 2.1[d]
Glutathione peroxidase (U/mg protein)	7.6±1.3[a]	7.3±2.3[a]	33.58±2.9[b]	23.3±2.9[c]	15.7±2.4[d]	14.5±1.0[d]	29.8± 3.5[b]
Malondialdehyde (µM/mg protein)	20.4±4.9[a]	60.52±1.7[b]	7.16±1.8[c]	17.3±3.1[d]	19.5±2.7[d]	19.4±4.1[d]	17.8±1.2[d]

[a,b,c,d,e] Means on the same row with different superscript letters are significantly ($p < 0.05$) different from one another.

Testicular lipid peroxidation

Malondialdehyde concentration

Testicular MDA concentration was significantly ($p < 0.05$) higher in group B (60.52 ± 1.7 µM/mg protein), than group C (7.16 ± 1.8 µM/mg protein). It was moderately ($p < 0.05$) significant in groups A (20.4 ± 4.9 µM/mg protein), D (17.3 ± 3.1 µM/mg protein), E (19.5 ± 2.7 µM/mg protein), F (19.4 ± 4.1 µM/mg protein) and G (17.8 ± 1.2 µM/mg protein) than group C (7.16 ± 1.8 µM/mg protein).

Serum antioxidant enzyme activities

The serum SOD, catalase and glutathione peroxidase activities and concentration of and malondialdehyde of rabbit bucks in the treatment groups A, B, C, D, E, F and G, sampled at weeks 2, 4, 6, 8, 10 and 12 of the study (Tables 5-8).

Superoxide dismutase activity

Superoxide dismutase (SOD) activity was significantly ($p < 0.05$) highest in group C (4.3 ± 0.7 U/mL) and control (3.06 ± 0.5 U/mL), compared to rabbit bucks exposed to BPA in groups B (2.3 ± 0.6 U/mL), E (2.0 ± 0.3 U/mL), F (2.8 ± 0.7 U/mL) and G (2.8 ± 0.6 U/mL) at the 2nd week. This pattern of decrease of SOD activities continued till the 6th week in rabbit bucks exposed to BPA. However at the 12th week, there was an increase in the activity in groups treated with either AG pulp extracts, melatonin or both. The SOD activity was significantly ($p < 0.05$) higher in groups C (6.3 ± 0.3 U/mL) and D (7.0 ± 0.5 U/mL) rabbit bucks, when compared to the controls (3.52 ± 0.6 U/mL), moderately significant in groups E (7.2 ± 0.4 U/mL) F (5.4 ± 0.9 U/mL) and G (6.5 ± 0.5 U/mL) lowest in group B (0.5 ± 0.2 U/mL) (Table 5).

Table 5 Serum superoxide dismutase (U/L) activity of rabbit bucks in the treatment groups A, B, C, D, E, F and G sampled forth-nightly during the study

Week	Group A	B	C	D	E	F	G
2	3.1±0.5^{a}	2.3±0.6^{b}	4.3±0.7^{c}	3.2±0.4^{a}	2.0±0.3^{b}	2.8±0.7^{a}	2.8±0.6^{a}
4	3.6±0.6^{a}	2.1±0.5^{b}	4.9±0.8^{c}	4.1±0.4^{c}	1.3±0.2^{d}	1.9±0.5^{d}	1.6±0.3^{d}
6	3.5±0.6^{a}	1.4±0.4^{b}	5.4±0.8^{c}	4.4±0.5^{c}	1.2±0.2^{b}	2.3±0.9^{d}	1.3±0.3^{b}
8	3.0±0.4^{a}	1.1±0.3^{b}	6.7±0.9^{c}	5.9±0.5^{c}	6.5±0.9^{c}	6.6±1.1^{c}	5.8±0.4^{c}
10	3.2±0.3^{a}	1.2±0.2^{b}	7.4±0.4^{c}	7.8±0.6^{c}	6.2±0.3^{c}	5.4±0.7^{c}	6.6±0.5^{c}
12	3.2±0.5^{a}	0.5±0.2^{b}	6.3±0.3^{c}	7.0±0.5^{c}	7.2±0.4^{c}	5.4±0.9^{c}	6.5±0.5^{c}

^{abcde} Means on the same row with different superscript letters are significantly ($p < 0.05$) different from one another.

Catalase

Catalase activity (U/mL) was significantly ($p < 0.05$) higher in group C (9.8 ± 1.9 U/mL) and D (9.8 ± 1.9 U/mL), moderate in groups A (6.8 ± 0.8 U/mL), G (4.2 ± 2.1 U/mL), F (4.1±1.2 U/mL) and E (3.7 ± 1.1 U/mL), but significantly lowest in group B (3.6 ± 1.4 U/mL) at week 4. This pattern of activity continued till the 6th week. However at the 8th, there was an increase catalase activity in the groups treated with AG pulp extracts, melatonin or both. The catalase activity was significantly ($p < 0.05$) higher in groups C (11.5 ± 2.7 U/mL) and D (10.2 ± 1.6 U/mL) rabbit bucks, when compared to the controls (6.0 ± 2.4 U/mL), moderately significant in groups E (8.3 ± 1.7 U/mL) F (7.7 ± 1.5 U/mL) and G (19.5 ± 3.4 U/mL) and lowest in group B (1.3 ± 0.2 U/mL)

This pattern of increase continued till the 12th week of the experiment (Table 6).

Table 6 Catalase (U/L) activity of rabbit bucks in the treatment groups A, B, C, D, E, F and G sampled forth-nightly during the study

Week	Group A	B	C	D	E	F	G
2	5.5±2.1^{a}	5.6±0.9^{a}	11.2±3.6^{b}	3.6±1.1^{c}	5.1±1.0^{a}	5.1±1.3^{a}	6.7±0.8^{a}
4	6.8±0.8^{a}	3.6±1.4^{b}	9.8±1.9^{c}	9.8±1.9^{c}	3.7±1.1^{b}	4.1±1.2^{b}	4.2±2.1^{b}
6	5.6±1.4^{a}	2.6±1.5^{b}	11.3±1.7^{c}	10.2±1.6^{c}	2.6±2.4^{b}	2.5±0.9^{b}	2.3±2.2^{b}
8	6.0±2.4^{a}	1.3±0.2^{b}	11.5±2.7^{c}	10.2±1.6^{c}	8.3±1.7^{d}	7.7±1.5^{d}	19.5±3.4^{e}
10	5.0.2±2.7^{a}	0.8±0.2^{b}	21.6±3.2^{c}	20.7±3.9^{c}	14.5±0.9^{d}	12.8±1.5^{d}	18.1±1.9^{c}
12	6.9±1.0^{a}	0.7±0.2^{b}	24.2±3.5^{c}	21.2±2.5^{c}	16.7±1.3^{d}	14.7±1.5^{d}	20.6±2.3^{c}

^{a,b,c,d,e} Means on the same row with different superscript letters are significantly ($p < 0.05$) different from one another.

Glutathione peroxidase concentration

At week 6, glutathione peroxidase's concentration was significantly ($p < 0.05$) the highest in group C (4.7 ± 0.2 U/mL), followed by groups D (3.1 ± 0.1 U/mL) but significantly ($p > 0.05$) lowest in rabbit bucks exposed to BPA such as groups G (0.2 ± 0.9 U/mL) and B (0.6 ± 0.6 U/mL). At week 12, GPx concentration was significantly ($p < 0.05$) the highest in group C (15.4 ± 0.2 U/mL), followed by groups D (10.2 ± 0.1 U/mL) and G (7.1 ± 0.1 U/mL), moderately ($p < 0.05$) significant in groups E (7.2 ± 0.6 U/mL) and F (5.9 ± 1.5 U/mL), but significantly ($P > 0.05$) lowest in groups A (1.9 ± 1.4 U/mL) and B (0.4 ± 5.6 U/mL) (Table 7).

Table 7 Glutathione peroxidase (µU/mL) concentration of rabbit bucks in the treatment groups A, B, C, D, E, F and G sampled forth-nightly during the study

Week	Group A	B	C	D	E	F	G
2	1.6±0.1^{a}	0.8±0.1^{b}	2.8±0.1^{c}	1.8±0.1^{a}	1.1±1.6^{a}	1.7±0.3^{a}	0.5±0.9^{b}
4	1.9±0.1^{a}	0.8±0.4^{b}	3.3±0.1^{c}	2.8±0.0^{c}	1.1±1.3^{b}	1.6±0.2^{a}	0.4±0.6^{b}
6	1.8±0.3^{a}	0.6±0.6^{b}	4.7±0.2^{c}	3.1±0.1^{d}	1.0±0.8^{a}	1.2±0.4^{a}	0.2±0.9^{b}
8	1.6±0.4^{a}	0.5±1.4^{b}	5.9±0.2^{c}	3.5±0.1^{d}	2.1±0.9^{d}	3.9±1.9^{d}	3.8±0.9^{d}
10	1.7±1.5^{a}	0.4±1.5^{b}	8.6±0.2^{c}	7.8±0.1^{c}	3.4±0.7^{d}	4.7±1.6^{d}	5.6±0.5^{d}
12	1.9±1.4^{a}	0.4±5.6^{b}	15.4±0.2^{c}	10.2±0.1^{d}	7.2±0.6^{e}	5.9±1.5^{e}	7.1±0.1^{e}

^{a,b,c,d,e} Means on the same row with different superscript letters are significantly ($p < 0.05$) different from one another.

Serum lipid peroxidation

Malondialdehyde concentration

Malondialdehyde's (MDA) concentration was significantly ($p < 0.05$) higher in group B (55.5 ± 5.5 µ/mL), compared to rabbit bucks in group C (7.2 ± 1.8 µmol/mg) at the 6th week. This pattern of increase continued up till the 12th week, where MDA concentration was significantly ($p < 0.05$) higher in group B (65.9 ± 5.5 µ/mL) than groups G (4.2 ± 1.1 µ/mL), C (5.1 ± 0.5 µ/mL) and D (5.3 ± 0.3 µ/mL). The concentration was moderately ($p < 0.05$) significant in groups E (8.9 ± 0.8 µ/mL), F (14.28± 1.7 µ/mL) and A (20.4 ± 5.2 µ/mL) (Table 8).

Table 8 Malondialdehyde (µ/mL) concentration of rabbit bucks in the treatment groups A, B, C, D, E, F and G sampled forth-nightly during the study

Week	Group A	B	C	D	E	F	G
2	20.4±4.9^{a}	46.0±2.3^{b}	9.9±2.2^{c}	17.3±3.1^{d}	19.5±2.8^{d}	19.48±4.2^{d}	17.8±1.2^{d}
4	15.3±1.4^{a}	47.8±2.1^{b}	8.1±1.7^{c}	9.1±0.4^{c}	32.3±6.6^{d}	36.56±3.5^{d}	38.4±2.9^{d}
6	16.7±2.0^{a}	55.2±5.5^{b}	7.2±1.8^{c}	7.8±1.7^{c}	50.0±4.8^{b}	56.56±3.9^{b}	41.3±2.3^{d}
8	22.1±3.0^{a}	58.8±7.2^{b}	6.9±1.3^{c}	7.3±0.4^{c}	26.8±2.3^{d}	31.54±2.5^{e}	19.8±2.1^{a}
10	21.9±3.1^{a}	60.5±1.7^{b}	6.1±0.2^{c}	6.4±0.4^{c}	7.8±1.4^{c}	18.3±1.7^{a}	10.5±0.8^{d}
12	20.4±5.2^{a}	65.9±5.5^{b}	5.1±0.5^{c}	5.3±0.3^{c}	8.9±0.8^{d}	14.3±1.^{7}e	4.2±1.1^{c}

^{a,b,c,d,e} Means on the same row with different superscript letters are significantly ($p < 0.05$) different from one another.

Percentage of live and dead sperm ratio

The percentage of live and dead sperm ratio was not significantly ($p > 0.05$) different at the 2nd week. However, at week 6, the live sperm significantly ($p < 0.05$) decreased in the group B bucks (68.3 ± 9.3 %) when compared to rabbit bucks in groups D (83.3 ± 6.0 %), C (80.0 ± 0.0 %) and A (71.7 ± 13.3 %). At week 12, there was a significant ($p < 0.05$) increase percentage live sperm in the rabbit bucks exposed to BPA and treated with AG as seen in groups E (70.0 ± 2.9 %), melatonin (65.0 ± 0.0 %) or both (78.3 ± 4.4 %), when compared to those in group B (26.7 ± 1.6 %) (Table 9 and Figure 1a).

Table 9 Live spermatozoa (%) ratio of rabbit bucks in the treatment groups A, B, C, D, E, F and G sampled forth-nightly during the study

Week	Group A	B	C	D	E	F	G
2	83.3±7.3[a]	80.0±2.9[a]	88.3±1.7[b]	80.0±7.6[a]	86.7±1.7[b]	83.3±3.3[a]	80.0±15.3[a]
4	80.0±0.6[a]	70.3±2.7[b]	83.3±7.3[a]	80.3±2.4[a]	65.8±2.0[c]	63.0±2.0[c]	60.3±2.5[c]
6	71.7±13.3[a]	68.3±9.3[a]	80.0±0.0[b]	83.3±6.0[c]	51.7±13.[d]	61.7±6.0[d]	58.3±19.0[d]
8	90.0±0.0[a]	56.7±3.3[b]	85.0±8.3[c]	85.0±0.0[c]	40.7±10.[d]	43.3±3.0[d]	24.7±3.3[e]
10	70.0±0.0[a]	40.0±2.9[b]	85.0±6.0[c]	85.0±0.0[c]	65.7±1.7[d]	60.0±5.8[d]	88.3±6.7[c]
12	85.0±0.0[a]	26.7±1.6[b]	86.0±1.7[a]	80.0±2.9[a]	70.0±2.9[c]	65.0±0.0[d]	78.3±4.4[a]

[a,b,c,d,e] Means on the same row with different superscript letters are significantly ($p < 0.05$) different from one another.

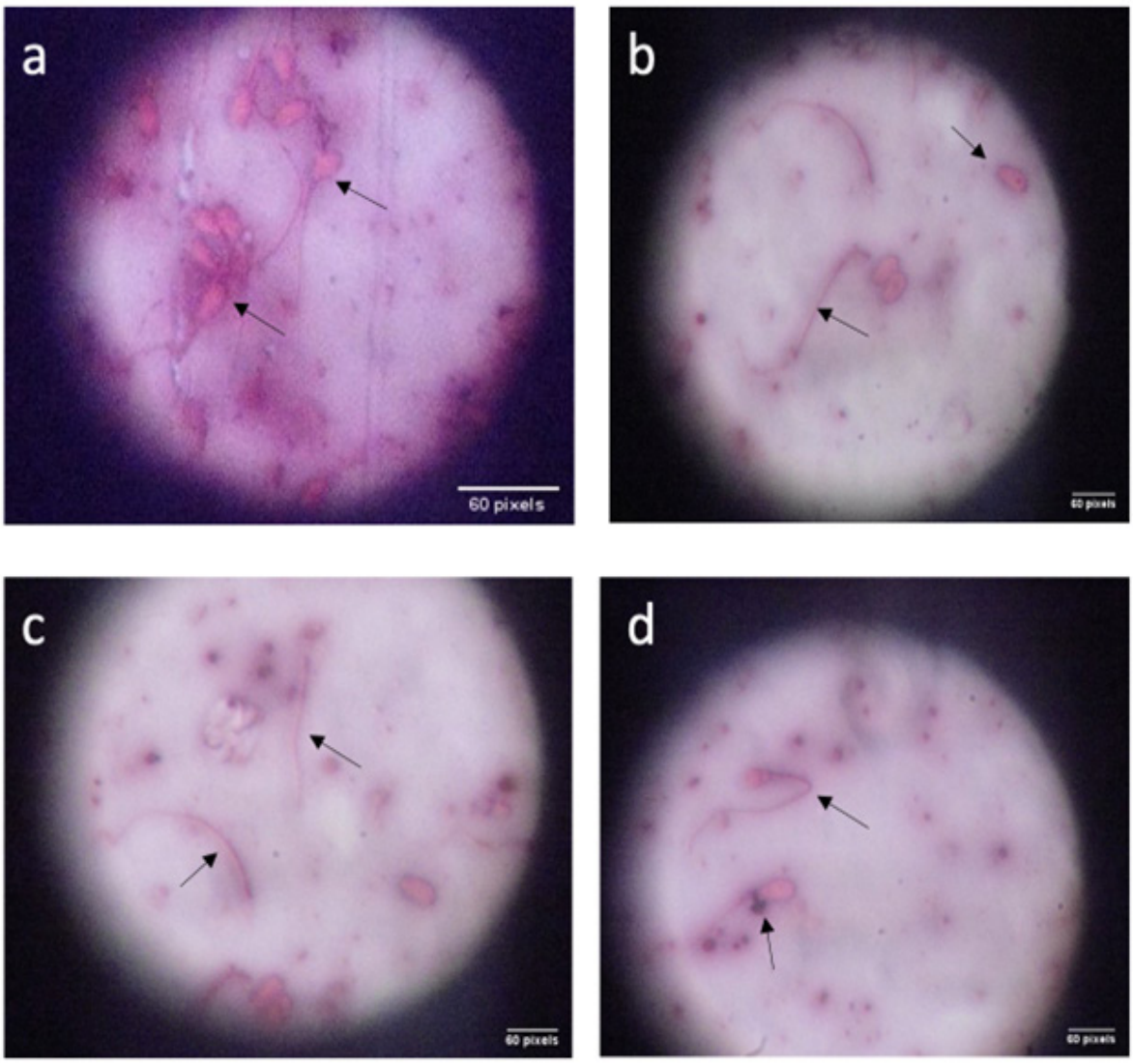

Figure 1 Photomicrograph showing morphological a; normal sperm (arrows) of rabbit bucks administered distilled water, b; detached sperm cell (arrows) of rabbit bucks administered BPA, c; free tails sperm (arrows) of rabbit buck BPA, d; bent tails sperm (arrows) of rabbit buck BPA (× 100).

The percentage dead sperm ratio was not significantly ($p > 0.05$) different at the 2nd week. However at week 6, the dead sperm significantly increased in the bucks exposed to BPA; groups B (32.3 ± 1.6 %), E (48.3 ± 13.0 %) and F (38.3 ± 6.0 %), when compared to rabbit bucks in groups, C (18.3 ± 8.3 %), D (16.7 ± 6.0 %) and A (28.3 ± 13.3 %). At week 12, there was a significant ($p < 0.05$) decrease in the percentage of dead sperms in rabbit bucks exposed to BPA and treated with AG in groups E (15.0 ± 2.9 %), melatonin F (30.0 ± 0.0 %) or both G (21.7 ± 4.4 %) and controls (15.0 ± 0.0 %), when compared to the rabbit bucks in group B (31.7 ± 1.7 % %) (Table 10).

Table 10 Dead spermatozoa (%) ratio of rabbit bucks in the treatment groups A, B, C, D, E, F and G sampled forth-nightly during the study

Week	Group A	B	C	D	E	F	G
2	16.7±7.3^{a}	20.0±2.9^{b}	12.0±5.0^{c}	20.0±7.6^{b}	13.3±1.6^{c}	16.7±3.3^{a}	40.0±15.3^{d}
4	22.0±2.0^{a}	28.0±1.6^{b}	10.0±2.6^{c}	18.0±1.9^{d}	29.0±2.1^{b}	32.1±2.4^{e}	45.0±1.8^{f}
6	28.3±13.3^{a}	32.3±1.6^{b}	18.3±8.3^{c}	16.7±6.0^{c}	48.3±13.0^{d}	38.3±6.0^{b}	38.3±23.2^{b}
8	10.0±0.0^{a}	44.0±2.9^{b}	6.7±3.3^{c}	5.0±0.0^{c}	18.3±10.9^{d}	16.7±3.3^{d}	13.3±3.3^{d}
10	30.0±0.0^{a}	60.3±3.3^{b}	16.7±6.0^{c}	5.0±0.0^{d}	8.3±1.7^{d}	20.0±5.8^{e}	11.7±6.7^{c}
12	15.0±0.0^{a}	21.7±9.3^{b}	31.7±1.7^{c}	20.0±2.9^{b}	15.0±2.9^{a}	30.0±0.0^{c}	21.7±4.4^{b}

^{a,b,c,d,e,f} Means on the same row with different superscript letters are significantly ($p < 0.05$) different from one another.

Detached head abnormalities

At week 2, the percentage of detached sperm heads significantly ($p < 0.05$) increased in group B bucks (8.3 ± 2.9 %), when compared to rabbit bucks in group C (4.0 ± 1.2 %). At weeks 12, there was a significant ($p < 0.05$) decrease in the detached head abnormalities of rabbit bucks exposed to BPA and treated with AG (6.0 ± 0.6 %), melatonin (2.0 ± 0.0 %) or both (3.7 ± 2.2 %), when compared to those in group B (11.0 ± 1.2 %) (Table 11 and Figure 1b).

Table 11 Detached head spermatozoa of rabbit bucks in the treatment groups A, B, C, D, E, F and G sampled forth-nightly during the study

Week	Group A	B	C	D	E	F	G
2	7.3±0.3^{a}	8.3±2.9^{a}	4.0±1.2^{b}	10.3±3.2^{c}	12.3±3.1^{d}	7.7±1.3^{a}	14.7±2.7^{d}
4	4.3±0.9^{a}	9.3±1.8^{b}	4.7±2.2^{a}	5.0±2.3^{a}	10.0±1.5^{b}	9.3±3.7^{b}	7.3±3.7^{b}
6	6.5±2.1^{a}	10.2±0.1^{b}	7.0±1.0^{a}	7.0±1.6^{a}	11.5±1.3^{b}	10.5±1.9^{b}	10.3±1.2^{b}
8	11.0±0.0^{a}	11.3±1.5^{a}	7.3±3.7^{b}	7.0±0.0^{b}	9.3±2.7^{a}	6.7±2.2^{b}	7.7±2.6^{a}
10	10.0±0.0^{a}	10.7±0.9^{a}	6.0±2.1^{a}	5.0±2.0^{b}	7.7±0.9^{c}	5.7±2.3^{b}	11.0±1.7^{a}
12	2.0±0.0^{a}	11.0±1.2^{b}	3.0±1.9^{c}	4.0±1.0^{c}	6.0±0.6^{d}	2.0±0.0^{a}	3.7±2.2^{c}

^{a,b,c,d,e} Means on the same row with different superscript letters are significantly ($p < 0.05$) different from one another.

Free tail abnormalities

The percentage of free tails significantly ($p < 0.05$) increased in the rabbit bucks exposed to BPA at week 6 in groups B (9.6 ± 1.8 %), E (7.6 ± 2.2 %), F (8.8 ± 1.6 %) and G (8.7 ± 1.5 %), when compared to rabbit bucks in groups C (4.0 ± 2.1 %), D (4.9 ± 4.2 %) and A (8.4 ± 2.6 %). However at week 12, there was a decrease ($p < 0.05$) in percentage of free tail abnormalities the rabbit bucks exposed to BPA and treated with either AG (3.0 ± 2.6 %), melatonin (3.0 ± 0.0 %) or both (5.5 ± 0.5 %), when compared to the rabbit bucks in group B (7.7 ± 2.6 %) (Table 12 and Figure 1c).

Table 12 Free tail spermatozoa of rabbit bucks in the treatment groups A, B, C, D, E, F and G sampled forth-nightly during the study

Week	Group A	B	C	D	E	F	G
2	9.3±0.7^{a}	6.3±3.8^{b}	2.7±0.9^{c}	10.0±4.0^{d}	12.7±4.9^{e}	6.3±3.4b	12.0±5.3^{e}
4	3.3±1.9^{a}	2.7±0.3^{b}	4.7±0.9^{a}	2.3±0.3^{b}	6.3±1.5^{c}	8.3±3.5^{d}	7.0±3.8^{d}
6	8.4±2.6^{a}	9.6±1.8^{b}	4.0±2.1^{c}	4.9±4.2^{c}	7.6±2.2^{a}	8.8±1.6^{a}	8.7±1.5^{a}
8	13.0±0.0^{a}	11.7±4.7^{a}	4.0±2.0^{b}	6.0±0.0^{c}	8.7±1.2^{d}	3.3±1.5^{b}	7.3±3.8^{d}
10	8.0±0.0^{a}	14.0±1.2^{b}	7.7±0.7^{a}	4.0±0.6^{c}	3.0±2.1^{c}	5.0±0.6^{c}	10.3±1.8^{b}
12	4.0±0.0^{a}	7.7±2.6^{b}	2.0±0.6^{c}	3.0±1.0^{a}	3.0±2.6^{a}	3.0±0.0^{a}	5.5±0.5^{b}

^{a,b,c,d,e} Means on the same row with different superscript letters are significantly ($p < 0.05$) different from one another.

Coiled tail abnormalities

The percentage coiled tail abnormalities significantly ($p < 0.05$) increased in the bucks exposed to BPA at week 6; groups B (2.0 ± 0.4 %), E (3.9 ± 1.0 %), F (10.4 ± 1.6 %) and G (5.0 ± 0.0 %), when compared to rabbit bucks in groups C (0.0 ± 0.0 %) and D (0.0 ± 0.0 %). However at week 12, there was a decrease ($p < 0.05$) in percentage of coiled tail abnormalities the rabbit bucks exposed to BPA and treated with either AG (1.0 ± 1.2 %), melatonin (1.0 ± 0.0 %) or both (0.3 ± 0.9 %), when compared to the rabbit bucks in group B (1.3 ± 0.9 %) (Table 13).

Table 13 Coiled tail spermatozoa of rabbit bucks in the treatment groups A, B, C, D, E, F and G sampled forth-nightly during the study

Week	Group A	B	C	D	E	F	G
2	1.0±0.6^{a}	0.3±0.3^{b}	1.7±1.7^{c}	1.3±1.5^{c}	0.0±0.0^{b}	1.3±1.3^{c}	1.3±1.2^{c}
4	2.0±1.5^{a}	0.7±0.0^{b}	0.0±0.6^{b}	1.0±0.6^{c}	2.7±1.5^{a}	3.7±1.5^{d}	2.3±2.3^{a}
6	2.0±0.0^{a}	2.0±0.4^{a}	0.0±0.0^{c}	0.0±0.0^{c}	3.9±1.0^{d}	10.4±1.6^{e}	2.5±1.8^{a}
8	2.0±0.0^{a}	1.0±0.6^{a}	0.0±0.0^{c}	0.0±0.0^{c}	3.0±1.7^{d}	8.3±0.7^{e}	2.0±0.6^{a}
10	0.0±0.0^{a}	2.7±0.7^{b}	1.7±0.7^{c}	1.0±0.6^{c}	2.3±0.3^{b}	11.3±9.4^{d}	1.3±0.3^{c}
12	5.0±0.0^{a}	1.3±0.9^{b}	0.0±0.0^{c}	0.3±0.0^{c}	1.0±1.2^{b}	1.0±0.0^{b}	0.3±0.9^{c}

^{a,b,c,d,e} Means on the same row with different superscript letters are significantly ($p < 0.05$) different from one another.

Bent tail abnormalities

The percentage bent tail abnormalities significantly ($p < 0.05$) increased in the bucks exposed to BPA at week 6; groups B (7.0 ± 2.1 %), E (14.9 ± 1.2 %), F (25.6 ± 1.7 %) and G (9.0 ± 1.8 %), when compared to rabbit bucks in groups C (7.2 ± 2.1 %), D (7.2 ± 1.4 %) and A (9.7 ± 0.9 %). However at week 12, there was a decrease ($p < 0.05$) in percentage of bent tail abnormalities the rabbit bucks exposed to BPA and treated with either AG (3.7 ± 0.9 %), melatonin (9.0 ± 0.0 %) or both (2.7 ± 1.8 %), when compared to the rabbit bucks in group B (10.0 ± 5.6 %) (Table 14 and Figure 1d).

Table 14 Bent tails spermatozoa of rabbit bucks in the treatment groups A, B, C, D, E, F and G sampled forth-nightly during the study

Week	Group A	B	C	D	E	F	G
2	7.0±2.0^{a}	2.7±1.8^{b}	4.3±2.4^{c}	6.0±1.5^{a}	3.3±0.3^{b}	5.7±2.0^{a}	6.7±2.3^{a}
4	3.7±1.5^{a}	7.3±2.3^{b}	5.7±2.7^{c}	2.0±0.6^{d}	6.7±2.9^{b}	8.0±1.0^{b}	2.7±1.8^{d}
6	9.7±0.9^{a}	7.0±2.1^{b}	7.2±1.4^{b}	17.5±2.0^{c}	14.9±1.2^{d}	25.6±1.7^{e}	9.0±1.8^{b}
8	8.0±0.0^{a}	8.0±0.1^{a}	6.7±3.3^{b}	17.0±0.0^{c}	13.3±0.3^{d}	22.3±6.0^{e}	8.3±1.5^{b}
10	6.0±0.0^{a}	11.7±1.3^{b}	9.3±1.7^{c}	7.0±0.6^{a}	2.3±0.7^{d}	17.0±8.6^{e}	4.3±0.3^{f}
12	5.0±3.5^{a}	10.0±5.6^{b}	2.3±1.2^{c}	3.7±0.3^{c}	3.7±0.9^{c}	9.0±0.0^{d}	2.7±1.8^{c}

^{a,b,c,d,e} Means on the same row with different superscript letters are significantly ($p < 0.05$) different from one another.

DISCUSSION

Folkloric medicine reported that *Azanza garckeana* fruit is currently used by indigenous Gombe State people and its northern environs as haematinic (Itodo et al., 2022a), an aphrodisiac and fertility stimulating agent (Maroyi, 2012; Itodo et al., 2022b; Itodo et al., 2022c). The animal model employed in this study has been used by numerous researchers in the past to evaluate the impact of various plant extracts from medicinal plants on male reproductive processes. The therapeutic and preventive effects of two distinct antioxidants (AG and melatonin) on BPA-induced oxidative stress biomarker alterations in bucks were observed in this experiment. The antioxidants were given orally for 90 days.

The preliminary quantitative and qualitative phytochemical screening is in concert with the results of Maroyi (2012). Medicinal plants are regarded as a rich source of bioactive compounds that have the potential to be used in the discovery and development of new drugs. Although there are different types of these bioactive metabolites, phenols, alkaloids, flavonoids, glycosides, and steroids are the most prevalent ones (Sakha et al., 2018). Generally, the pharmacological qualities of medicinal plants are significantly influenced by the quality and amount of their secondary metabolites (Otang et al., 2012). The phenolic compounds are widely distributed among different plant species and sections in plants (Harbone, 1993). The redox impact of phenolic compounds has been suggested as the source of the medicinal plants' antioxidant power that serve as reducing agents, donate protons, and scavenge single oxygen atoms (Birben et al., 2012).

The level of enzymatic antioxidant in serum provides general information about the whole body of the animal and cannot be attributed to a specific organ. The antioxidant levels from tissues of a localized organ may give an accurate picture of the antioxidant quantity in that organ. Testicular

tissue showed higher levels of LPO (malondialdehyde) concentrations than the serum, this could be attributed to the fact that testicular tissues are made up of fatty acids that can easily undergo redox reactions, making the testes prone to oxidative damages. The pathological lipid peroxidation of spermatozoa's membrane and decreased sperm motility have been attributed to elevated testicular and serum MDA concentrations (Zahak and Saraswat 2020). In this work, bucks that had been exposed to BPA showed a large decrease in the activity of antioxidant enzymes and a significant increase in the levels of ROS. Similar findings have been reported in chronic instances (Chitra et al., 2003) and adult rats that were given oral subacute dosages of BPA (Olukole et al., 2020). The decreased SOD activities by BPA exposure in this study may be due to the inability of cells to generate enough SOD, which may arise from severe damage to the cells or greater function in combating the oxidative stress. SOD protects tissues from oxidative stress and damage by speeding up the conversion of superoxide anion radical into the less toxic hydrogen peroxide, a more stable ROS (Fridovich, 1997). A rise in superoxide-free radical, as well as other ROS and an acceleration of the lipid peroxidation process, can be caused by a decrease in SOD levels in the bucks exposed to BPA in the first six weeks. It was concluded that in the testes of BPA-treated bucks, SOD transformed superoxide anion radicals into H_2O_2, which then accumulated in the testes and serum as a result of its decreased clearance.

A significant decline in CAT activity in BPA-administered bucks when compared to control was observed in the AG and melatonin treated groups. Thus, the excess ROS production caused by the decreased CAT activities in the cells and serum of BPA-exposed bucks exacerbated the harmful impact. The cells' failure to eliminate the hydrogen peroxide they make may be the reason for the decline in CAT activity. This could be as a result of excessive ROS generation in cells leading to enzyme inactivation (Pigoelet et al., 1990).

The most prevalent intracellular non-protein thiol in cells is GPx, it is an antioxidant molecule. The decrease in GPx, demonstrates the principal antioxidant system's inability to protect against oxidative damage (Huang et al., 2004). Consequently, the typical level of intracellular GPx, depletion is considered to be an indicator of oxidative stress. In the current study, there was a decrease in the levels of GPx, in the bucks exposed to BPA in the first six weeks of the study. This decrease in GPx, levels could be explained by the cells' inability to produce enough GPx, as a result of significant cellular damage or by their increased resistance to oxidative stress.

The BPA-induced decrease in the antioxidant enzymes' activities and the rise in MDA concentration in the serum and testes of rabbit bucks point to an improved framework for the inflammation and testicular dysfunction caused by the decline of spermatogenic cells as a result of excessive ROS production from peroxidation of the seminiferous tubule membranes. As a result, the elevated MDA levels found in the current investigation as a result of BPA exposure in multiple serum and testicular samples and points to an increase in ROS formation, which in turn leads to high LPO activity and, ultimately, increases DNA damage and membrane disruption. Studies have shown that BPA causes oxidative stress in the serum, testes and epididymal sperm of the rats by raising LPO and lowering the activity of antioxidant enzymes (Chitra et al., 2003).

In this investigation, AG performed better than melatonin alone at reversing the BPA-induced decline in the activity of antioxidant enzymes along with the higher levels of ROS. This outcome supports the findings of Yusuf et al. (2021) and El-Ratel et al. (2021), who reported that administration with the natural antioxidant such as turmeric or garlic and phytogenic extracts improves the antioxidant enzyme defense system of rabbit bucks. The results demonstrate AG's strength as an antioxidant, capable of reducing oxidative damage brought on by BPA.

The current results also revealed that BPA significantly increased testicular MDA concentration, and significantly decreased total antioxidant capacity (TAC). According to several research, natural antioxidants can prevent or mitigate the adverse health effects of oxidative stress and antioxidants (Sakha et al., 2018). According to various studies, natural antioxidants prevent or reverse abnormal health effects associated with antioxidants and oxidative stress (Sakha et al., 2018). A reduction in testicular oxidative stress-antioxidant status of BPA group was noticed. MDA was measured, as lipid peroxidation product, and the antioxidant enzymes SOD, CAT and GPx, in the testes. The current results agree with Hamden et al. (2008), who reported that oxidative stress decrease the antioxidant capacity of the testicles, thereby decreasing enzymatic antioxidants activities (SOD, catalase and glutathione peroxidase) and concentrations of non-enzymatic antioxidants (copper, magnesium and iron). SOD and catalase are the first line cellular defense enzymes against oxidative injury. In rabbit bucks treated with AG fruit pulp extracts, the activities of SOD and catalase increased while the concentrations of reduced GPx, and MDA decreased better than those treated with melatonin alone. However, the combination of both AG pulp extracts and melatonin gave the best optimum results in ameliorating the negative effects of BPA, compared to individual administrations. This finding is in agreement with the result of Zahak & Saraswat (2020) who reported that the combination of molecular and naturally occurring antioxidants may typically scavenge more ROS than single administrations, which attach to active free radicals and prevent chain reaction propagation (Amjad et al., 2020). Free radicals are eliminated by antioxidants by giving them an electron, which makes them less harmful and more easily neutralized by other antioxidants of the same class (Hornos-Carneiro, 2020).

The results of Silva et al. (2018), who showed that a high level of ROS in human seminal plasma has strong association with poor sperm motility, morphology, low concentration as well as aberrant and dead spermatozoa, are similar to the findings of the present study. For the effective elimination of oxidative stress in intracellular organelles, the equilibrium between the antioxidant enzymes is a critical step (Santiago et al., 2021). One sign of this oxidative stress was lower sperm counts, but other signs included more dead sperm, free, coiled, and bent tails, as well as free, detached heads (Ghosh, 2021).

Injuries brought on by oxidative stress may also contribute to lower counts of live % spermatozoa, in addition to changes in the hormonal profile (Santiago et al., 2021). To determine their relative impact on the reproductive potentials of bucks, herbal feed additives such *Moringa Oleifera*, *Phyllanthus amarus*, and *viscum album* leaves were analyzed (Jimoh et al., 2021). Melatonin (Olukole et al., 2020) and AG (Maroyi and Chiekh-Youssef 2017) both perform crucial functions in protecting spermatozoa from oxidative stress, and they also

serve as indicators of the enzyme's original testicular and epididymal origins. The current findings on oxidative stress are consistent with those of Aybek et al. (2008) who reported comparable findings regarding testicular SOD activity and reduced MDA in mature male Wistar rats treated with vitamin E. ROS, such as hydroxide (OH), can easily harm the polyunsaturated fatty acids that make up the mammalian sperm plasma membrane (Alvarez and Storey 1995). Since MDA level is the end-point reaction product of lipid peroxidation, this mechanism is commonly characterized as the lipid peroxidation reaction (Sikka, 2001; Agarawal et al., 2003). Lipid peroxidation is one of the primary signs of oxidative damage brought on by ROS. It has been connected to altered membrane structure, enzyme inactivation, excessive cellular macromolecular damage (protein, lipid, and nucleic acid damage), and has been shown to play a significant role in the toxicity of contaminants (Murugesan et al., 2005). It has been noted that a rise in LPO is associated with maturation arrest, a reduction in spermatozoa concentration, changes in morphology, and most notably, changes in motility brought on by changes in membrane potential (Benedetti et al., 2012). DNA damage in spermatozoa is strongly correlated with rising MDA levels as well (Atig et al., 2013).

CONCLUSIONS

BPA increased the production of ROS in the serum and testicular tissues, increased percentage of dead sperm cells and sperm abnormalities in rabbit bucks. However, the administration of *Azanza garckeana* only ameliorated the negative effects better than the administration of melatonin alone. The synergistic effects of *Azanza garckeana* and melatonin produced optimum results in the treatment of BPA-induced reproductive stress. It was therefore recommended that the methanol extract of Azanza garckeana fruit pulp should be administered as a supplement to breeding bucks kept in plastic and aluminum cages and fed with plastic feeders and drinkers for improved growth rate, semen quality and testicular functions. Food and animal feed industries should minimize the use of BPA as a plasticizer in food packaging. There should be increase sensitization of the general public on the proper handling and disposal of plastics in order to protect the environment.

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AUTHOR CONTRIBUTIONS

Joy Iyojo Itodo: Conceptualization, Investigation, Project administration, Formal Analysis, Writing - Original Draft. **Tagang Aluwong**: Supervision, Writing - Review & Editing. **Chidiebere Uchendu**: Conceptualization, Supervision, Writing - Review & Editing. **Ngozi Ejum Ogbuagu:** Editing**.** Investigation: **John Shiradiyi Bugau: Bode Abdulmojeeb Adewuyi**: Writing, **Felix Uchenna Samuel:** Data Curation, **Abah Kenneth Owoicho:** Formal Analysis. **Mathew Shinkut**: Experiment. **Peter Kanayo Ogbuagu**: Data Analysis

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