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Comparative Effects of *Azanza garckeana* Fruit Pulp and Melatonin on Chronic Bisphenol A- evoked Oxidative Stress Biomarkers and Semen Parameters Changes 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. Adult New Zealand White rabbit bucks (n = 42), with an average live weight of 1.2 ± 0.03 kg and aged 10-18 months 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 MDA concentration in treatment groups. The percentage of dead sperm, and spermatozoa abnormalities such as detached sperm heads, and free, coiled, and bent tails in the groups exposed to BPA increased significantly ($P < 0.05$) compared to controls. 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, Malondialdehyde, Glutathione peroxidase, Superoxide dismutase**,** Teratozoospermia

Introduction

Bisphenol A (BPA) is a high-production volume chemical, commonly used in food packaging materials, dental sealants, medical devices, and thermal receipts (1). Exposure to BPA is ubiquitous via ingestion, inhalation, and dermal contact (2,3). Bisphenol A (BPA) induces oxidative stress in mammals. It is transformed through oxidation into a variety of metabolites, many of which may have a more significant physiological impact than the compound itself. The most common cause of damage to the sperm is induced by ROS. Natural antioxidants play an important role in scavenging the damaging effect of ROS in humans and animals (4), by exerting protective effects against different medications or chemically-provoked toxicities (5).

Azanza garckeana (Professor August Garcke, 1819-1904). It is called snot apple Azanza, tree hibiscus, snot apple, quarters, wild hibiscus, and African chewing gum (English) (6). The plant is widely grown in Tula (latitude 9° 48’51"N, longitude, 11° 18′ 32″E an 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 (7).

The AG is very rich in antioxidants, scavenging ROS that damage cell membranes and DNA (8). It also demonstrates antioxidant properties in the reproductive system of males. *Azanza garckeana* contains nutrients, minerals, and phytochemical compounds, such as flavonoids, phenols, and tannins that are beneficial for human and animal health (9). Melatonin is a potent endogenous tryptophan-derived molecule with pleiotropic and broad-spectrum antioxidant activities. It is a sleep promoter (10), a chemical signal of light and darkness as well as a regulator of photo-dependent seasonal reproduction in some vertebrates (11). Exposure to BPA results in increased generation of ROS in the body including the testes, which decreases the antioxidant level of the body and impairs spermatogenesis (12). The stem bark of AG extract exhibit very high-scavenging properties, when compared to standard antioxidants like ascorbic acid and vitamin E (13).

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It has been suggested that AG may be used as an antioxidant, which may reduce the dependence on synthetic drugs as a ROS-scavenging agent (14).

There is a need to also compare the antioxidant properties of AG to a standard antioxidant like melatonin due to its availability and affordability. Currently, there is a global trend towards the use of natural phenolics as antioxidants and functional ingredients due to their perceived safety and prevalence in wild edible fruits (15-17). It has been widely documented that oxidative stress plays an important role in the pathophysiology of diseases in rabbit bucks (18). Optimal strategies to treat both idiopathic and BPA-induced toxicities may be achieved by using combination antioxidant therapy such as *Azanza garckeana* and melatonin (19).

Materials and Methods

Study Area

The research was conducted at the Department of Theriogenology and Production, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

Plant material

Azanza garckeana (AG) ripe fruits were sourced during February-April, 2021, which coincided with the harvest season from Tula, Nigeria. The sample was sent to the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria for authentication and identification, with voucher no: ABU07276. The authenticity of the plant was also confirmed by comparing it with the features available in databases (http://www.theplantlist.org/' and 'http://www.ipni.org/) (Plate 1).

Plate I. *Azanza garckeana* (gorun tula) from Tula area of Gombe State

Methanol Extraction

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. The whole nut of AG was rinsed under clean running water and the seed was removed. The pulp was air-dried for two weeks. The dried material was pulverized into a coarse powder using a grinder mill. Exactly 5 kg of the plant material was extracted with methanol, using the Soxhlet apparatus at 50°C. The solvent was recovered, concentrated, and finally dried to a constant weight on a rotary evaporator. Thereafter, it was stored in an air-tight container for subsequent use (15).

Determination of Percentage Yield from Methanol Extraction of Plant Material

The weight of the extract was recorded and the percentage extract yield was computed using the formula (20) below:

$$\% \text{ Yield} = \frac{\text{Weight of Extract (g)}}{\text{Weight of Dried powdered sample (g)}} \text{ x100}$$ (Table 1)

Phytochemical analysis

The methanol extract of the AG fruit pulp was screened for the presence of secondary metabolites like alkaloids, saponins, flavonoids, phenols, and tannins according to the standard tests described (21). The specific tests for the phytochemicals were done as follows (Table 2).

For alkaloids (Wagner's test)

Exactly 10 mg of the extract was dissolved in 1 ml of distilling water. With this solution, three drops of Wagner's reagent were added. The presence of alkaloids was confirmed by the formation of a reddish-brown colored solution (22).

Test for Tannin (Lead acetate and ferric chloride test)

Exactly, 0.1 mg of the extract was dissolved in 2 ml of distilling water. Then 1 ml of the solution was taken and 0.5 ml of 1% lead acetate was added to it. The formation of yellowish precipitate was observed for the presence of tannins.

The ferric chloride test was performed by adding 0.5 ml of 5% ferric chloride solution to the same solution used for the lead acetate test, and the development of dark bluish or black color was observed for the presence of tannins (23).

Test for flavonoids (Alkaline reagent or NaOH test)

The methanol extract (0.3 g) was dissolved in 2 ml of distilled water. To these, three drops of 20% sodium hydroxide solution were added. An intense yellow color was formed which turned colorless with the addition of three drops of 20 % hydrochloric acid which indicated the presence of flavonoids in each of the extracts. Besides, a lead acetate test was performed. To the same solution used above 3 drops of 10% lead acetate were added and the formation of a yellow precipitate was observed for the presence of flavonoids (22).

Test for saponins (Foam test)

About 0.3g of the methanol extract of Azanza garckeana fruit pulp was taken and dissolved in 20 ml of distilled water. After vigorous shaking, the formation of persistent foam observed for 30 min was taken as an indication of the presence of saponins (22).

Test for phenols (Ferric chloride test)

Exactly 10 mg of the extract was dissolved in 1 ml of water. Half ml of 5% ferric chloride solution was added to it and the development of deep blue or black color was taken as an indicator for the presence of phenols (22).

Test for steroids (Liebermann-Burchard test)

Exactly 0.5 g of the extract was dissolved in 0.5 mL dichloromethane to produce a dilute solution. To this solution 0.5 mL of acetic anhydride was added, followed by three drops of concentrated sulphuric acid. The formation of a blue-green coloration indicated the presence of steroids (21).

Phytochemical analysis and acute toxicity test of AG pulp extract

The phytochemical constituents of AG pulp extract were screened and those present were quantified using standard methods (22).

The acute toxicity profile of AG pulp extract was performed in two phases (24). Briefly, out of the 30 rabbit bucks used in the acute toxicity study, 15 rabbit bucks were divided into three groups of five rabbit bucks each was used in phase I. 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 rabbit bucks were monitored for 24 h. From the result of phase I, higher doses were chosen for phase II. In this phase, the remaining 15 rabbit bucks were divided into three groups of five rabbit bucks each: rabbit bucks in groups 4, 5, and 6 received 1600, 2900, and 5000 mg/kg body weight AG fruit pulp extracts, respectively. The rabbit bucks were then observed for 24 h for lethality or any morphological and behavioral signs of toxicity, including dullness, changes in eyes and fur appearance or color, hyperactivity, changes in feeding patterns, sedation, and mortality.

Animals

Forty-two (42) healthy New Zealand White rabbit bucks (Oryctolagus cuniculus), 10 - 18-month-olds with body weights of 1.20-2.00 kg were used for the study. The bucks were sourced from rabbit farms within Zaria and its environs. They were screened and treated against endoparasites and bacterial infection before the commencement of 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. The rabbit bucks were allowed to acclimatize for 14 days before the commencement of the study.

Experimental Diet

The proximate analysis of the diets was carried out according to the method of the American Organisation of Analytical Chemists (25). The diet was of isonitrogenous and isocaloric values (Table 3) (26).

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 (500 mg/kg) + melatonin (1.0 mg/kg) for six weeks.

The duration of the study was three months (90 days), during which serum samples were collected fortnightly (weeks 2, 4, 6, 8 10, and 12). A total of 252 serum samples were collected for laboratory analysis. At the end of the study, five bucks were sacrificed from each group, testicles were harvested for assessment of biomarkers of oxidative stress (27).

Exactly 3 mL of blood was collected from the marginal ear vein of each buck weekly using a 27- G needle and placed immediately on ice in heparinized tubes. Serum was collected from the blood by centrifugation at 3000 × g for 5 minutes and kept at a temperature of 25-26 ℃ (28).

Evaluation of Oxidative Stress Biomarkers in the Testes

The testes were collected and separated carefully from the epididymides, then weighed separately before analysis. One gram of testis was homogenized in 9 volumes of buffered saline and centrifuged at 3000 x g for 15 min. The supernatant was separated off and used for assessments of the oxidative stress biomarkers; malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) (29).

Evaluation of testicular and serum antioxidant enzyme activities

Superoxide dismutase

Superoxide dismutase (SOD) activity was determined by the method described by (30). The ability of SOD to inhibit the auto-oxidation of adrenaline at pH 10.2 forms the basis of the assay. Briefly, testicular tissue homogenate of 0.1 mL was diluted in 0.9 mL of distilled water to make 1;10 dilutions of microsome. An aliquot mixture of 0.2 mL of the diluted micro-some was added to 2.5 mL of 0.05 M carbonate buffer. The reaction was started with the addition of 0.3 mL of 0.3 mM adrenaline. The reference mixture contained 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 measured over 30 seconds up to 150 seconds at 480 nm, using a spectrophotometer (BIOXYTECH SOD-525).

Increase in absorbance per minute = $(A_2\text{-}A_1)$ /2.5

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

One unit of SOD activity was the quantity of SOD necessary to elicit 50% inhibition of the oxidation of adrenaline to adrenochrome in 1 minute. The SOD activity was expressed in IU/mg tissue protein concentration.

Catalase

Catalase (CAT) activity was determined by the method described by (31). The ability of one unit of CAT to decompose 1.0 µmole of H202 per minute at pH 7.0 at 25° C, while the H202 concentration falls from 10.3mM to 9.2 mM. The rate of

disappearance of H202 is followed by observing the rate of decrease in the absorbance at 240nm (32).

Glutathione peroxidase (GPx)

An assay of glutathione peroxidase (GPx) activity was performed according to (33) as described by (34). It was based on the reaction between 5, 5-dithiobis nitro benzoic acid (DNTB) and glutathione peroxidase (GPx). Briefly, to 150 µL of testicular tissue homogenate (in phosphate-saline buffer, pH 7.4), 1.5 mL of 10% Trichloroacetic acid (TCA) was added and centrifuged at 1500 g for 5 minutes. Exactly 1 mL of the supernatant was treated with 0.5 mL of Ellman's reagent and 3 mL of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm. The quantity of GPx was obtained from the graph of the GPx standard curve. The GPx concentration was expressed as IU/mg tissue protein concentration.

Evaluation of serum and testicular lipid peroxidation

The principle of the reaction was based on the ability of lipid peroxidation to generate peroxide intermediates, which upon cleavage releases MDA. The concentration of MDA served as an index of the intensity of lipid peroxidation, a product that reacted with thiobarbituric acid (TBA). The reaction yielded a complex color, which absorbed light at 535 nm and was measured by a spectrophotometer (BIOXYTECH -525).

Tissue homogenate of 150 µL was treated with 2 mL of 0.37 % TBA solution- 15% trichloroacetic acid solution-0.25N HCl reagent (1:1:1ratio) and placed in the water bath at 90°C for 60 minutes. The mixture was cooled and centrifuged at 3000 *x g* for 5 minutes and the absorbance of the pink supernatant (TBA-MDA complex was measured at 535 nm. The concentration of MDA formed was calculated using the molar extinction coefficient of $1.56 \times 10^{-5} cm1M^{-1}$.

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

Percentage of live and dead sperm cells

This was determined as described by (35). A thin smear of semen was made on a clean grease-free slide and stained with 2 drops of Eosin-Nigrosin stain. This technique was based on the principle that Eosin-Nigrosin penetrated and stained dead sperm cells, while live sperm cells repelled the stain. Dead spermatozoa stained pinkish or reddish, while live spermatozoa remained colorless. Stained and unstained sperm cells were counted when the slide was dried, using light microscopy at × 40 magnification, and the percentage of each was estimated.

Sperm abnormalities

Sperm abnormalities were determined by making a thin smear of the semen sample, on a clean grease-free glass slide and staining with 2 drops of Eosin-Nigrosin. Sperm cells were counted per slide using a hand counter under light microscopy at × 100 magnification using oil immersion. All abnormal cell types were recorded (35).

Statistical analyses

Data were expressed as mean ± SEM. The analyses were performed using a two-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. Values of $P < 0.05$ were considered significant. The analyses were carried out using GraphPad Prism version 5.0 for windows 2003 from GraphPad Prism Software, San Diego, California, USA. (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 (36.52 ± 3.69 %). Flavonoids and saponins were found to be present in the amounts of 25.50 ± 2.43 % and 18.90 ± 0.15%, respectively. Alkaloids occurred in smaller quantities (19.00 ± 0.07 %), and tannins had the least quantity (14.90 ± 0.43 %) (Table 1).

Acute toxicity profile of AG pulp

There was no mortality recorded up to the dose of 5000 mg/kg body weight of AG pulp administered. In addition, there were no significant body weight and behavioral changes recorded within 24 h of the acute toxicity study. The observations suggested that AG pulp was safe for consumption.

Testicular Antioxidant Biomarkers

The mean ± SEM of testicular SOD and catalase, glutathione peroxidase activities, and malondialdehyde concentration 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/mL), moderately compared to groups A (2.8 ± 0.6 U/mL), D (3.5 ± 0.5 U/mL), E (4.3 ± 0.7 U/mL), F (2.9 ± 0.7 U/mL), G (3.3 ± 0.8 U/mL) and B (1.5 ± 0.08 U/mL) (Table 4).

Catalase

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

Glutathione peroxidase activity

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

Malondialdehyde concentration

Testicular MDA concentration was significantly ($P < 0.05$) higher in group B (60.52 ± 1.7 µmol/mg protein), than in group C (7.16 ± 1.8 µmol/mg protein). It was moderately ($P < 0.05$) significant in groups A (20.4 ± 4.9 µmol/mg protein), D (17.3 ± 3.1 µmol/mg protein), E (19.5 ± 2.7 µmol/mg protein), F (19.4 ± 4.1 µmol/mg protein) and G (17.8 ± 1.2 µmol/mg protein) than group C (7.16 ± 1.8 µmol/mg protein) (Figure 2).

Serum antioxidant biomarkers

The SOD, catalase, glutathione peroxidase activities and concentration of 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 in SOD activities continued till the 6th week in rabbit bucks exposed to BPA. However, in the 12th week, there was an increase in the activity in groups treated with either AG pulp extracts and 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).

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, on the 8th, there was an increase in 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).

Glutathione peroxidase activity

At week 6, glutathione peroxidase activity 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, glutathione peroxidase activity 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)

Malondialdehyde concentration

Malondialdehyde (MDA) concentration was significantly ($P < 0.05$) higher in group B (55.5 ± 5.5 µmol/mg), compared to rabbit bucks in group C (7.2 ± 1.8 µmol/mg) in 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 µmol/mg protein) than in groups G (4.2 ± 1.1 µmol/mg protein), C (5.1 ± 0.5 µmol/mg protein) and D (5.3 ± 0.3 µmol/mg protein). The concentration was moderately ($P < 0.05$) significant in groups E (8.9 ± 0.8 µmol/mg protein), F (14.28± 1.7 µmol/mg protein), and A (20.4 ± 5.2 µmol/mg protein) (Table 8).

Percentage live and dead sperm ratio

The percentage of live and dead sperm ratio was not significantly ($P > 0.05$) different in 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 of 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).

The percentage dead sperm ratio was not significantly ($P > 0.05$) different in 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).

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 week 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).

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 the percentage of free tail abnormalities in 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).

Coiled tail abnormalities

The percentage of 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 the percentage of coiled tail abnormalities in 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).

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Bent tail abnormalities

The percentage of 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 the percentage of bent tail abnormalities in 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).

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

Phytoconstituents	(w/w) (%)
Tannins	14.90 ± 0.43
Flavonoids	25.50 ± 2.43
Saponins	18.90 ± 0.15
Phenols	36.52 ± 3.69
Alkaloids	19.00 ± 0.07

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

Table 3. Composition of Experimental Diet

Feedstuff	Composition (%)
Maize	30.16
Groundnut cake	28.12
Rice offals	35.32
Vitamin premix	0.5
Palm oil	1.0
Bone meal	4.0
Methionine	0.4
Salt	0.5
Total	100
Proximate Composition	
Dry Matter	89.50
Crude protein	16.81
Ether extract	1.27
Crude fiber	8.65
Nitrogen free extract	53.96
Ash	7.20
ME (kCal/kg)	2,640.42

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

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

Testicular Antioxidants	Group A	Group B	Group C	Group D	Group E	Group F	Group G
Superoxide dismutase (U/L)	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/L)	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/L)	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 (nMol/L/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]

[abcde] Means on the same row with different superscript letters are significantly (P < 0.05) different from one another.

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	Group B	Group C	Group D	Group E	Group F	Group 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.

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]

[abcde] Means on the same row with different superscript letters are significantly (P < 0.05) different from one another.

Table 7. Glutathione peroxidase (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	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]

[abcde] Means on the same row with different superscript letters are significantly (P < 0.05) different from one another.

Table 8. Malondialdehyde (nMol/L/protein) 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]

[abcde] Means on the same row with different superscript letters are significantly (P < 0.05) different from one another.

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]

[abcde] Means on the same row with different superscript letters are significantly (P < 0.05) different from one another.

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]

[abcdef] Means on the same row with different superscript letters are significantly (P < 0.05) different from one another.

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]

[abcde] Means on the same row with different superscript letters are significantly (P < 0.05) different from one another.

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±3.4[b]	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]

[abcde] Means on the same row with different superscript letters are significantly (P < 0.05) different from one another.

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]

[abcde] Means on the same row with different superscript letters are significantly (P < 0.05) different from one another.

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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]

[abcde] Means on

Discussion

Azanza garckeana fruit is currently used in folklore medicine by the people of Gombe State and its northern environs as a haematinic (36), an aphrodisiac, and fertility-promoting agent (37). The animal model used in this study has been used previously by several workers to assess the effects of different types of plant extracts, obtained from medicinal plants on reproductive functions in males. In the present study, we examined the therapeutic and prophylactic properties of two different antioxidants (AG and melatonin) on BPA-induced oxidative stress biomarker changes in bucks which were administered by oral gavage for 90 days.

The preliminary quantitative and qualitative phytochemical screening of the methanol extract of *Azanza garckeana* fruit pulp is in agreement with the results of (37). Medicinal plants are considered a rich source of bioactive metabolites with the potential for drug discovery and development. These bioactive metabolites are of different types, but the most common of these metabolites are phenols, alkaloids, flavonoids, glycosides, and steroids (38, 39). Generally, the quality and quantity of secondary metabolites strongly influence the pharmacological properties of medicinal plants (40). The phenolic compounds considerably occur in plants and vary across plant species and parts (41). The antioxidant potential of medicinal plants has been attributed to the redox effect of phenolic compounds which scavenge singlet oxygen, donate a proton, and act as reducing agents (42).

The level of serum antioxidants 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 or level of the antioxidant quantity in that organ. Testicular tissue showed an increase in antioxidant biomarker levels 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 damage. Elevated testicular and serum MDA concentrations have been suggested to be responsible for the pathologic lipid peroxidation of spermatozoa membrane and reduction of sperm motility (43). In this study, BPA - exposed bucks exhibited a significant reduction in the activities of antioxidant enzymes and significant elevations in the levels of ROS. Similar observations have been documented in adult rats treated with oral sub-acute doses of BPA (44) and chronic cases (45). The decreased SOD activities by BPA exposure in the present study may be due to the inability of the cells to generate enough SOD, which may arise from severe cellular damage or greater functioning in combating oxidative stress (46). Superoxide dismutase (SOD) protects tissues from oxidative stress and damage by catalyzing the conversion of superoxide anion radical into the less toxic hydrogen peroxide, a more stable ROS (30, 47). The decrease in SOD level in the bucks exposed to BPA in the first six weeks can result in an increase in superoxide-free radicals as well as an elevation of other ROS and intensification of the lipid peroxidation process. In the testes of the BPA-treated bucks, it could be inferred that SOD converted superoxide anion radicals into H_2O_2, which thereafter accumulated in the testes and serum due to its reduced elimination.

In the present study, we observed a significant decrease in CAT activity in BPA-administered bucks as compared to control, AG and melatonin-treated groups. Thus, the decrease in CAT activities in the cells and serum of BPA-exposed bucks increased the toxic effect due to excess ROS generation. The reduction in the activity of CAT may reflect the inability of cells to eliminate hydrogen peroxide generated in the cells. This may be due to enzyme inactivation caused by excess ROS production in cells (48).

Glutathione peroxidase (GPx) is an antioxidant molecule and the most abundant intracellular non-protein thiol present in cells. The reduction in GPx shows the failure of the primary antioxidant system to act against oxidative stress (49). Hence, the depletion of intracellular GPx is usually regarded as a measure of oxidative stress. In the present study, there was a decrease in the levels of GPx in the bucks exposed to BPA at the first six weeks of the study, which may be due to the inability of cells to generate enough GPx as a result of severe cellular damage or due to greater combat against the oxidative stress.

The BPA - induced reduction in the activities of the antioxidant enzymes and increase in MDA concentration in the testes and serum of rabbit bucks are suggestive of an improved platform for the inflammation and testicular dysfunction via the degeneration of spermatogenic cells due to excessive generation of ROS by peroxidation of the membranes of the seminiferous tubules. Thus, the increased MDA levels observed in the present study due to the exposure of BPA in various serum and testicular samples indicate an increase in the generation of ROS, leading to high LPO activity and thereby enhancing the membrane disruption and DNA damage. It is evident from the studies that BPA generates ROS by decreasing the activity of

antioxidant enzymes and increasing LPO, thereby causing oxidative stress in the serum, testes, and epididymal sperm of rats (45).

In this study, AG ameliorated the BPA-induced decrease in the activities of antioxidant enzymes together with the elevated levels of ROS better than melatonin only. This result confirms the findings of (50) and (51), who reported that the antioxidant enzyme defense system of rabbit bucks is enhanced after treatment with the antioxidant, turmeric, or garlic phytogenic extract. The findings show the ability of AG as a potent antioxidant, capable of ameliorating BPA - induced oxidative stress.

The current results also revealed that BPA significantly increased testicular MDA concentration, and significantly decreased total antioxidant capacity (TAC). Several studies have indicated that natural antioxidants prevent or reverse abnormal health effects associated with antioxidants and oxidative stress (39). The present investigation recorded a reduction in the testicular oxidative stress-antioxidant status of the BPA group. MDA was measured, as a lipid peroxidation product, and the antioxidant enzymes SOD, CAT, and GPx in the testes. The current results are in agreement with those of (52), who found that oxidative stress decreases testicular antioxidant capacity, by decreasing activities of both enzymatic antioxidants (SOD, catalase, and glutathione peroxidase) and concentrations of non-enzymatic antioxidants (copper, magnesium, and iron levels). Free radical scavenging enzymes such as 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, catalase, and GPx increased while the 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 (43), who reported that the combination of molecular and naturally occurring antioxidants may typically scavenge more ROS than single administrations that bind to active free radicals and disrupt chain propagation reactions (53). The antioxidants donate an electron to free radicals to neutralize them, becoming free radicals with reduced toxicity that are easily neutralized by other antioxidants in the same class (54).

The present findings are similar to the results of (55) who reported that a high level of ROS in human seminal plasma is related to poor sperm morphology, poor motility, and a low sperm concentration as well as abnormal and dead spermatozoa. The equilibrium between the antioxidant enzymes is an important process for the effective removal of oxidative stress in intracellular organelles (56). This state of oxidative stress was reflected not only by lower sperm counts, but also by a decrease in the viability of sperms such as increased dead sperm, detached head, and free, coiled, and bent tails (57).

Lower counts of live percent spermatozoa may not only be attributed to an alteration in hormonal profile but also to the injury induced by oxidative stress (56). Herbal feed supplements such as *Moringa Oleifera*, *Phyllanthus amarus*, and *viscum album* leaves were evaluated to ascertain their comparative effect on the reproductive potentials of bucks (57, 58). AG (9) and melatonin (44) play important roles in the protection of spermatozoa from oxidative stress and provides an indicator of a primary testicular and epididymal origin of this enzyme.

The present findings concerning the oxidative stress status agree with those of (59), who reported similar results concerning testicular SOD activity and decreased MDA in adult male Wistar treated with vitamin E. The mammalian sperm plasma membrane, which is rich in polyunsaturated fatty acids, can be easily damaged by ROS, such as OH (60). This mechanism is widely known as the lipid peroxidation reaction (61) and measured as MDA level, which is the end-point reaction product of lipid peroxidation (62). Lipid peroxidation is one of the main manifestations of oxidative damage initiated by ROS, and it has been linked to the altered membrane structure and enzyme inactivation as well as excessive damage of cellular macromolecules (protein, lipids, and nucleic acids), which is a major contributor to the toxicity of contaminants (63). It has been observed that an increase in the level of LPO has been correlated with maturation arrest, decreased spermatozoa concentration, and morphology, as well as most notably motility due to alterations in the membrane potential (64). An increase in MDA level also positively correlates with DNA damage in spermatozoa (65). Natural antioxidant compounds found in fruits, vegetables, and other dietary sources, alone or in combination with other antioxidants, were found to be effective in ameliorating oxidative stress-mediated infertility problems in both natural and assisted reproductive settings (66).

It was concluded that BPA increased the production of ROS in the serum and testicular tissues, increased percentage dead sperm cells and abnormalities of 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 is recommended that breeding bucks be kept in plastic and aluminum cages and fed with plastic feeders and drinkers should be given the methanol extract of *Azanza garckeana* fruit pulp as a supplement 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 products to prevent oxidative stress and reproductive toxicities. There should be increased sensitization of the populace on the proper use and disposal of plastic materials to safeguard the environment.

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Conflicting Interests

The authors declare that there is no conflict of interest.

Ethical Approval

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

Authors contributions

This work was conducted and approved in collaboration between all the authors. RPI, AT, AL designed the study; IJI sourced for funding and conducted the experiments; ONE did statistical analysis; IJI wrote the final manuscript; ABA assisted in the investigation; AJO proofread the manuscript.

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