

**NSAP****47th Annual
Conference
(JOS 2022)****CONFERENCE
PROCEEDINGS**THEME
**SECURING ANIMAL
AGRICULTURE AMIDST
GLOBAL CHALLENGES****COMPARATIVE ROLES OF AZANZA GARCKEANA (GORON TULA) AND MELATONIN IN AMELIORATING CHRONIC EXPOSURE TO BISPHENOL A ON TESTICULAR MORPHOMETRY AND SPERM RESERVES IN NEW ZEALAND WHITE BUCKS.**

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ABSTRACT

Azanza garckeana (AG) contains antioxidant properties that can provide a scavenging effect on reactive oxygen species. The current study aimed to compare the antioxidant properties of AG to a standard antioxidant melatonin on the testicular measurements and sperm reserves of rabbit bucks exposed to BPA. Twenty-one New Zealand White rabbit bucks were fed ad libitum on a commercial isocaloric and isonitrogenous diet for 90 days and randomly divided into seven groups of three bucks each. Group A was administered distilled water (1.5 ml), group B was administered only BPA (100 mg/kg), group C was administered only AG (500 mg), group D was administered only melatonin (1.0 mg/mL for 12 weeks), group E was pre-dosed for six weeks with BPA (100 mg/kg), then AG for another six weeks, Group F was pre-dosed for six weeks with BPA (100 mg/kg), then melatonin (1.0 mg/mL) for another six weeks and group G was pre-dosed for six weeks with BPA (100 mg/kg), then AG (500 mg/kg) + melatonin (1.0 mg/mL) for another six weeks. Bucks treated with AG had significantly ($P < 0.05$) higher morphometry and sperm reserves when compared to the bucks treated with melatonin. The synergistic effects of AG and melatonin was observed to exert the best therapeutic results in the treatment of BPA induced toxicities. It is concluded that BPA has deleterious effects on reproduction but administration of AG and melatonin may possess some therapeutic properties that can reverse and ameliorate such adverse effects to the barest minimum.

Key words: *Azanza garckeana*, Melatonin, Bisphenol A, Sperm reserves and Rabbit Bucks

INTRODUCTION

Rabbit (*Orytolagus cuniculus*) is defined as a micro-livestock with a very high potential in reproduction. One of its unique ability is that it serves as a flexible financial reserve and as a good laboratory animal, which allows for scientific studies and periodic sample (blood and semen) collections ante mortem, unlike other laboratory animals. Bisphenol A (BPA) is a high-production volume (HPV) chemical, commonly used in food packaging materials, dental sealants, medical devices and thermal receipts (Rochester, 2013). Exposure to BPA is ubiquitous via ingestion, inhalation, and dermal contact (Meli *et al.*, 2020). *Azanza garckeana* (AG) is locally called 'goron tula' (Hausa, Nigeria); variously it is called snot apple Azanza, tree hibiscus, snot apple, quarters, wild hibiscus, African chewing gum (English). In Nigeria, it is abundantly found in Tula, a town in Kaltungo Local Government Area, Gombe State, Nigeria. Melatonin is a known potent endogenous tryptophan-derived molecule with pleiotropic and broad-spectrum antioxidant activities. Subsequently it was found to be a sleep promoter (Lerner and Case, 1959), a chemical signal of light and darkness as well as a regulator of photo-dependent seasonal reproduction in some vertebrates (Tan *et al.*, 2007). Information to be obtained may shed more light on the possible synergistic/additive effects of AG with standard antioxidants like melatonin and the ability to reduce the dependency on synthetic drugs while embracing natural herbal remedies.

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The study was carried out at the Department of Theriogenology and Production, Ahmadu Bello University, Zaria. Twenty-one (21), New Zealand White rabbit bucks (*Oryctolagus cuniculus*), 10 - 18-month-old were used for the study. The proximate analysis of the various diets was carried out according to the method of American Organisation of Analytical Chemists (AOAC, 2012) (Table 1). The diet was of isonitrogenous and isocaloric values. *Azanza garkeana* ripe fruits were sourced from Tula area of Kaltungo Local Government Area of Gombe State, Nigeria. Sample was sent to the Department of Botany, Ahmadu Bello University, Zaria. Bisphenol A (BPA) of pure analytical grade of 100% purity was purchased from Sigma Aldrich USA. Melatonin (10 mg/Tablet, Nature made, South California, USA) was obtained from a reputable pharmaceutical outlet in Kaduna, Nigeria and dissolved in 10 mL of distilled water to make 1mg/mL suspension daily before administration to the animals. Approval for this study was sought from the Ahmadu Bello University Committee for Animal Use and Care (ABUCAUC) with the approval number: **ABUCAUC/2021/062**. Testicular sperm reserves were estimated using the homogenisation haemocytometric technique.

Table 1: 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
Dry matter	89.50
Crude protein	16.81
Ether extract	1.27
Crude fibre	8.65
Nitrogen-free extract	53.96
Ash	7.20
ME (kCal/kg)	2,640.42

Metabolisable energy was calculated according to formula of Ponzenga (1985): $ME = 37 \times \%CP + 81 \times \%EE + 35.5 \times \%NFE$

Experimental Design

Twenty-one (21) rabbit bucks in a completely randomized design (CRD) into seven (7) groups of three (3) 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 twelve weeks. Group B: BPA (100 mg/kg) for five consecutive days per week for twelve weeks. Group C: AG (500 mg/kg) for seven consecutive days per week for twelve weeks. Group D: Melatonin (1.0 mg/mL) seven consecutive days per week for twelve weeks. Group E: six weeks pre-dosed with BPA 100 (mg/kg) for five consecutive days per week, then AG (500mg/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/mL) 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/mL) for six weeks.

Statistical Analyses.

Data collected were expressed as mean $\pm$ standard error of the mean (SEM) and subjected to repeated-measures one-way analysis of variance (ANOVA) for repeated sampling. One-way analysis of variance

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was used for single sampling, followed by Tukey's multiple comparison test using GraphPad Prism version 5.0. Values of $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Testicular Measurements and Sperm Reserves of bucks.

There was no significant difference ($P > 0.05$) between the right and left testicular sperm reserves within same treatment group. However, the right and left testicular length, weight, volume, filtrate volume and concentration of bucks in groups B decreased significantly ($P < 0.05$) when compared to the other groups. The testicular sperm reserves were significantly ($P < 0.05$) higher in bucks administered AG only than when compared to the other treatment groups respectively. Groups E, F and G had significantly higher morphometry and sperm reserves than the control and the bucks exposed to BPA.

The results for testicular morphometry and sperm reserves shows that BPA has deleterious effects on the testicular architecture of the testes. This is in agreement with the works of Richter *et al.* (2007) who noted that rodents exposed to BPA during the prenatal or perinatal periods exhibited a large variety of adverse reproductive outcomes, including decreased testicular weights and daily sperm production. Regarding prepubertal or pubertal exposures, rodent studies have described a dramatic reduction in the testicular sperm reserves. Bucks exposed to BPA had the least morphometry and sperm reserves. This is in agreement with the findings of Takahashi and Oishi (2000) who reported that BPA can cause shrinkages and decrease in weight of testes, epididymis, prostates, and seminal vesicles and a decrease in sperm production. The bucks exposed to AG had higher testicular reserves when compared to the bucks in the MLT. This is in agreement with the findings of Attia *et al.* (2019), who reported increases in testicular reserves of roosters supplemented with phytogetic extracts. The administration of AG, or melatonin or the combination of both as seen in groups E, F and G respectively showed that the adverse effects of BPA exposure can be significantly ($P < 0.05$) ameliorated using both substances either singly or synergistically. Our results showed that combination of AG and melatonin elucidated the optimal results in the treatment of BPA induced reproductive toxicity.

CONCLUSIONS AND RECOMMENDATIONS

BPA caused a decrease in testicular weights, lengths and seminal volumes of testes of rabbit bucks. However, administration of *Azanza garckeana*, melatonin or both ameliorated the adverse effects.

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Table 2. Mean $\pm$ (SEM) of Testicular Measurements and Sperm Reserves of bucks sampled at week 12 of the study

Parameters	A	B	C	Groups D	E	F	G
Testicular Length (cm)							
Right	3.2 $\pm$ 0.02 ^a	2.2 $\pm$ 0.15 ^b	3.3 $\pm$ 0.25 ^a	3.4 $\pm$ 0.15 ^a	3.3 $\pm$ 0.20 ^a	2.4 $\pm$ 0.20 ^b	2.8 $\pm$ 0.00 ^b
Left	2.7 $\pm$ 0.08 ^a	2.2 $\pm$ 0.05 ^b	3.2 $\pm$ 0.05 ^c	3.1 $\pm$ 0.10 ^c	2.8 $\pm$ 0.07 ^a	3.2 $\pm$ 0.20 ^c	2.9 $\pm$ 0.20 ^c
Testicular Weight (g)							
Right	2.9 $\pm$ 0.05 ^a	2.1 $\pm$ 0.08 ^b	3.3 $\pm$ 0.18 ^b _c	3.8 $\pm$ 0.02 ^c	7.1 $\pm$ 2.60 ^d	2.9 $\pm$ 0.03 ^b	2.1 $\pm$ 0.1 ^b
Left	3.0 $\pm$ 0.01 ^a	2.2 $\pm$ 0.16 ^b	3.4 $\pm$ 0.10 ^a	4.0 $\pm$ 0.03 ^c	5.9 $\pm$ 3.25 ^d	1.9 $\pm$ 0.04 ^a	1.9 $\pm$ 0.10 ^a
Testicular Volume (ml)							
Right	2.4 $\pm$ 0.15 ^a	1.1 $\pm$ 0.10 ^b	5.0 $\pm$ 0.00 ^c	4.3 $\pm$ 0.25 ^d	4.3 $\pm$ 0.25 ^d	4.0 $\pm$ 0.00 ^d	4.1 $\pm$ 0.10 ^d
Left	2.5 $\pm$ 0.02 ^a	1.0 $\pm$ 0.00 ^b	5.0 $\pm$ 0.00 ^c	4.0 $\pm$ 0.00 ^d	4.4 $\pm$ 0.10 ^d	4.0 $\pm$ 0.00 ^d	4.3 $\pm$ 0.25 ^d
Testicular Filtrate Volume (ml)							
Right	9.0 $\pm$ 0.00 ^a	4.4 $\pm$ 0.40 ^b	9.1 $\pm$ 0.10 ^a	8.7 $\pm$ 0.20 ^a	9.6 $\pm$ 0.10 ^a _b	6.8 $\pm$ 0.25 ^c	9.6 $\pm$ 0.10 ^{ab}
Left	9.1 $\pm$ 0.10 ^a	4.5 $\pm$ 0.50 ^b	9.4 $\pm$ 0.15 ^a	9 $\pm$ 0.00 ^a	9.1 $\pm$ 0.10 ^a	6.4 $\pm$ 1.20 ^c	8.8 $\pm$ 0.10 ^a
Testicular Concentration (x10⁶/mls)							
Right	5.0 $\pm$ 0.00 ^a	0.0 $\pm$ 0.00 ^b	14 $\pm$ 0.00 ^c	9.0 $\pm$ 0.00 ^d	9.0 $\pm$ 0.00 ^d	4.5 $\pm$ 0.50 ^a	8.0 $\pm$ 0.00 ^e
Left	4.0 $\pm$ 0.00 ^a	0.0 $\pm$ 0.00 ^b	13.0 $\pm$ 0.0 ^c _{0^c}	10.0 $\pm$ 0.00 ^d _d	6.0 $\pm$ 0.00 ^e	3.5 $\pm$ 0.50 ^f	6.3 $\pm$ 0.25 ^e

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