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Evaluation of the Gonadoprotective Effects of *Allanblackia floribunda* Oliver (Clusiacea) On Testes and Accessory Organs of Wistar Rats

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ABSTRACT

This study investigated the effect of the crude ethanolic extract and fractions of the stem bark of *Allanblackia floribunda* on the testes and accessory organs of rats. Forty five (45) male rats of average weight between 150 to 200 g were distributed into nine groups of five rats per group. Rats in group 1 (control group) were administered distilled water, while groups 2 to 9 were orally administered 200 and 300 mg/kg body weight of the crude ethanolic extracts and aqueous, ethyl acetate and butanol fractions, once daily for 28 days. On the 29th day, the animals were sacrificed; weights of the testes, epididymis, seminal vesicle and prostate glands were taken and documented. Testicular antioxidant level was determined by measuring testicular catalase and glutathione peroxidise activity. Serum alkaline phosphatase, acid phosphatase, lactate dehydrogenase and glucose-6-phosphate dehydrogenase activities were also assayed for. Statistical analysis to test significant difference between mean values was performed using One-way Analysis of Variance (ANOVA).The results revealed that crude ethanolic extract of *A. floribunda* gave positive reactions for the presence of alkaloids, flavonoids, saponins, tanins, triterpenes and cardiac glycosides and the median lethal dose (LD_{50}) was higher than 5000 mg/kg body weight. The acid phosphatase (ACP) across all the groups showed an increase in activity when compared with control. However, a general decrease in alkaline phosphatase (glucose-6-phosphate dehydrogenase and catalase activities was observed in all the groups with the mean values of Group 2, Group 3 and Group 9 ACP; Group 6 and 7ALP; Group 2LDH and Groups 5, 6, 7, 8 and 9 G6PDH having a significant difference ($p<0.05$) when compared with control. Glutathione peroxidase activity (GPx) varied across all groups with most groups having slightly lower activity (0.19±0.01 U/L/mg protein, 0.15±0.01 U/L/mg protein, 0.18±0.01 U/L/mg protein, 0.17±0.01 U/L/mg protein, 0.16±0.01 U/L/mg protein, 0.15±0.01 U/L/mg protein) when compared with the control group (0.21±0.02 U/L/mg protein). With the exception of Group 7 (300 mg/kg ethyl acetate), all groups had slightly lower seminal vesicle, testicular and epididymis weights when compared with the control. The study concluded that the ethanolic extract and fractions of *A. floribunda* had deleterious effects on the testes and accessory organs of the animals, most especially at 300 mg/kg dosage of the crude ethanolic extract.

Key words: *Allanblackia floribunda*, Accessory organs, Testis, Ethanolic Extract.

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INTRODUCTION

Globally, plants are widely used for the production of medicinal agents. This usage is commonly referred to as traditional or herbal medicine and such plants used for their curative purposes are called herbs. Herbal

medicines encapsulates the synthesis of therapeutic experiences of generations of practicing physicians of indigenous systems of medicine for over hundreds of years (Pal and Shukla, 2003) and involves the use of plants for the promotion of healing and maintenance of health (Hussin, 2001). Out of the estimated 800,000 plant species on earth (von Reis, 1977); about a quarter have been categorized and only a small fraction of these have been examined for their pharmacological efficacy (Hussin, 2001). Although major use of herbal medicines is for health promotion and therapy for chronic, as opposed to life-threatening conditions, usage of traditional remedies increases when conventional medicine is ineffective in the treatment of disease, such as in advanced cancer and in the face of new infectious diseases (Canter and Ernst, 2004; Qato et al., 2008; Loya et al., 2009; Cohen and Ernst, 2010).

Furthermore, herbal medicines have been shown to have good values in treating many diseases including infectious diseases, hypertension, etc (Erah, 2002). In recent times, herbs are applied to the treatment of chronic and acute conditions and various ailments and problems such as cardiovascular disease, prostate problems, depression, inflammation, and to boost the immune system, to name but a few (Wachtel-Galor and Benzie, 2011). That they can save lives of many, particularly in the developing countries, is undisputable. However, the major challenges of any pharmaceutical scientist are serious problems with the overall quality, preservation, dosage measurement, safety and efficacy of herbal products; and that has really necessitated the continual and holistic studies being carried out by scientists in Asia and Africa especially in ascertaining the safety levels of these commonly used herbs in order to establish their potency or lethality upon consumption (Erah, 2002). *Allanblackia* is a family of medium-sized tree species of humid forest zone of Africa producing berry-like fruits that are suspended on long pedicels, and the seeds of the genus have a very low germination success {less than 5%} (Vivien and Faure, 1996). *A. floribunda* (Oliver or tallow-tree), is one out of nine identified species of the genus (Fobane et al., 2014) and it is a fruit tree of Clusiaceae family or Guttiferae.

It is found in the rain forest of South and Equatorial forest of East part of Nigeria areas, to the Central African Republic and East of the Democratic Republic of Congo, and South to Northern Angola (Orwa and Munjuga, 2007). The stem bark of *A. floribunda* has been found to significantly prevent increase in the mean blood pressure, serum lipid, total cholesterol, HDL-cholesterol and triglycerides in both alcohol-induced and sucrose-induced hypertensive rats. *A. floribunda* also prevented the increase in atherogenic index, bilirubin, urea, alanine transferase and aspartate transferase in alcohol-induced hypertensive rats and sucrose-induced hypertensive rats and it also significantly prevented the increase of superoxide dismutase (SOD), malondialdehyde (MDA) and catalase and the decrease of reduced glutathione (GSH) concentration in aorta, heart, kidney and liver of alcohol-induced hypertensive rats and sucrose-induced hypertensive rats (Bilanda et al., 2010). Dieudonné et al. (2013) also carried out a toxicological study on aqueous extract of the stem bark of *Allanblackia floribunda* on rats where they determined the acute toxicity both orally and intraperitoneally; and sub-acute toxicity orally alone; and discovered that in acute test, the oral administration did not cause any death treatment related signs.

The LD_{50} was estimated to be 125 mg kg^{-1} (intraperitoneal route). In sub-acute treatment, neither significant difference was observed on body weight, food and water consumption nor organs and haematological parameters. The biochemical analysis showed that the level of alanine transferase (ALT) dose dependently decreased at all doses in male and female rats while tissue creatinine decreased only in female rats (Dieudonné et al., 2013). Kada et al. (2012) in their investigation on the effect of aqueous extract of *A. floribunda* stem bark on sexual behavior in adult male rats, found that the administration of the extract resulted in the significant increase of mount frequency, intromission frequency, ejaculatory latency, penile licking frequency, computed indices of sexual behavior and wiring-touch frequency whereas the mount latency, Intromission latency and post-ejaculatory interval were significantly decreased throughout the experimental period establishing the stimulating potential of *A. floribunda* aqueous extract on sexual desire and potency of male rats. Kada et al. (2013) further investigated the effects of *A. floribunda* aqueous and ethanol stem bark extracts and their potential mechanism on fictive ejaculation in spinal male rats. Sequential intravenous treatments of rats with extracts significantly decreased the occurrence of ejaculation induced by dopamine; the oral pre-treatment with both extracts also significantly decreased the ejaculation induced by dopamine. All had inhibitory activities on ejaculation (Kada et al., 2013). Infertility issues affect approximately 15% of all couples globally, and male factor is the sole or contributing factor in roughly half of all these cases.

However, there has been an increased usage of natural herbs in the treatment of male sexual dysfunctions. Aside being widely used in West and Central Africa to treat myriads of ailments, *A. floribunda* has been reportedly used by some locals in boosting sexual performance (aphrodisiac). Despite the acclaimed sexual enhancing potentials of the stem and root decoction of this plant, there is dearth of information on the effects of this plant on the testicular function parameters as related to sexual enhancement. This study is thus set to investigate the effects of ethanolic extract of the stem bark of *A. floribunda* on the testicular function of male rats at similar doses used previously for the demonstration of its aphrodisiac potentials by previous authors. Several

researchers (Gonzales et al., 2001; Manna et al., 2004; Sharma et al., 2001; Gupta and Kumar, 2004) have used parameters like percentage testes–body weight ratio, activities of alkaline phosphatase, acidphosphatase, of the testes to assess the testicular function in male rats, hence, their use in this study.

MATERIAL AND METHODS

Collection and Identification of Plant Material

Fresh stem barks of *A. floribunda* were collected from J4 forest reserve in Ogun State, Nigeria. The plant was identified and authenticated by Mr. G. A. Ibhanesebor at Ife Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The voucher specimen was deposited in the Herbarium of the same department and the specimen identification number is IFE 7379.

Experimental Animals

The male wistar rats (weight range between 150 to 200 g) used in this study were obtained from the animal house, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria. The animals were acclimatized for two weeks, fed with standard commercial pellet diet (Ladokun Feeds, Ibadan) and given access to water *ad libitum*. The rats were housed under standard conditions, at natural light and dark cycle.

Preparation of Ethanolic Extract of *A. floribunda*

The dried barks of the *A. floribunda* (1.5 kg) was ground into fine smooth powder using impact mill. The powdered stem bark (500 g) was soaked in 70% ethanol (2.5 L) for 72 h, and filtered afterwards with a double layered cheese cloth and concentrated to dryness using a rotary evaporator Model ED-100.

Partitioning into Fractions

The ethanolic extract (50 g) was taken up in hot distilled water (200 ml). The filtrate was partitioned sequentially with hexane, ethyl acetate and butanol in capped separating funnel. The content was vigorously shaken, allowed to settle down and carefully separated. The process was repeated several times until the colour of the solvent remained unchanged. The same fractions were combined and concentrated in rotary evaporator separately. The fractions and aqueous residue were dried in a desiccator, weighed, labeled and kept in the deep freezer at -4^{O}C until needed for further analyses.

Phytochemical Screening of the Aqueous Extract

The phytochemical screening of the extract was carried out by a procedure that was based on the earlier reports of Trease and Evans, (1978) and Sofowora (2006).

Acute Toxicity Study (LD_{50} Determination)

Acute toxicity study was carried out according to the method of Lorke (1983) in two phases.

Sub-Chronic Toxicity Study

The study was carried out as described by Biswas et al. (2010). A total of forty five albino rats of average weight between 150 to 250 g were randomly distributed into nine groups of five rats per group. Group 1 served as control and were administered normal saline, while rats in groups 2 to 9 were orally administered 200 and 300 mg/kg body weight of the aqueous, ethyl acetate, butanol and crude extract respectively, once daily for 28 days. The rats were weighed before the commencement of treatment and thereafter weighed weekly throughout the duration (28 days) of the study. On the 29th day, after administration of the extract, the animals were sacrificed. The blood samples were collected by ocular puncture into plain bottles for estimation of biochemical parameters and the organs such as the testes, epididymis, liver, brain and kidney were excised, weighed, rinsed with normal saline and stored for further biochemical and histological studies.

Preparation of Blood Serum

The blood collected in plain bottle was allowed to clot for 30 min and later centrifuged at 3000 rpm for 10 min. The supernatant (serum) was stored in sterile vial and kept in freezer for biochemical analyses.

Preparation of Tissue Homogenate

Testes were surgically removed and a 10% (w/v) tissue homogenate was prepared by homogenizing the testes in phosphate buffer solution, pH 7.4 using mortar and pestle over ice. The homogenates were centrifuged at 10,000xg using a cold centrifuge and the supernatant was collected as a source for the determination of marker enzyme activity, and anti-oxidants.

Biochemical Assays

The activity of Alkaline Phosphatase in serum was assayed according to the method of Deutsche Gesellschaft KlinischeChemie (1972), using Randox Diagnostic Kit. The activity of Prostatic Acid Phosphatase in serum was assayed according to the method of Seiler, (1983), using Randox Diagnostic Kit. The Glucose – 6 - Phosphate Dehydrogenase Activity in testes was assayed according to the method of Seiler, (1983), using

Table 1. Phytochemical constituents of the ethanolic extract and fractions of *A. floribunda.*

Phytochemical constituents	Ethanolic extract	Aqueous fraction	Ethyl acetatefraction	Butanolfraction
Alkaloid	++	++	+	+
Cardiac glycoside	+++	+	+	+++
Flavonoids	++	++	++	+
Saponins	+++	+++	-	++
Tannins	+++	+	-	+++
Triterpenes	+++	+	+	+++
Phlobatanins	-	-	-	-

(+) represent positive result and (-) represent negative result.

Table 2. Acute toxicological effect of ethanolic Extract of *A. floribunda* in mice.

Dose (mg/kg	Mortality
First Phase	
10	0/3
100	0/3
1000	0/3
Second Phase	
1600	0/3
2600	0/3
5000	0/3

Randox Diagnostic Kit. The Lactate Dehydrogenase Activity in serum was assayed according to the recommendations of Deutsche Gesellschaft fur Klinische. Chemie (1970), using Randox Diagnostic Kit. The catalase activity was determined according to the direct UV assay method of Aebi (1973). The estimation of the serum total protein was carried out according to the biuret method, as reported by Tietz (1995) while the activity of glutathione peroxidase (GPx) was determined by the method of Rotruck et al. (1973).

Histopathological Analysis and Sperm Evaluation Test

Portions of the tissue from testes were used for histopathological examination by standard procedure. Sperm Motility was evaluated directly using the Neubauer's counting chamber, after mincing in drop of sperm suspension, microscopically. Non-motile sperm numbers were first determined, followed by counting of total sperm. Sperm motility was expressed as percent of motile sperm of the total sperm counted (Zemjanis, 1970). Sperm Viability by Eosin Stain technique was used to differentiate between live and dead sperms. A drop of the Eosin stain added into sperm suspension on the slide and allowed to stand for 5 min. at 37°C, then examined under microscope. The head of dead spermatozoa were stained red, while, the live spermatozoa remained unstained with Eosin (Zemjanis, 1970). Sperm viability was expressed as percentage of live sperm of the total sperm counted while in Sperm Morphology, a drop of Eosin stain was added to the sperm suspension and kept for 5 min. at 37°C. Following this, a drop of sperm suspension was placed on a clean slide and spread gently to make a thin film. The film was air dried and then observed under a microscope for changes in sperm morphology. The criteria chosen for head abnormality were; no hook, excessive hook, amorphous, pin and short head. For tail, the abnormalities recorded were; coiled flagellum, bent flagellum, bent flagellum tip. The result are the percentage overall abnormal form (Zemjanis, 1970).

Statistical Analysis

Data were expressed as Mean ± SEM, $n_{=}5$. The presence of significant differences among means of groups was determined by One-way Analysis of Variance (ANOVA), using GraphPad Prism (GraphPad Software, San Diego, CA, USA). Differences were considered to be significant if $p< 0.05$.

RESULTS AND DISCUSSION

Table 1 shows the results of the phytochemical constituents of the extracts and fractions while Table 2 shows the result of acute toxicological effect of the extract, this show that the extract is not acutely toxic up to 5000 mg/Kg body weight. Tables 3 and 4 show the

Table 3. Effect of ethanolic extract of *A. floribunda* and other fractions on the body weight of experimental rats.

Body Weight Change of Rats (g)			
Groups	**Initial**	**Final**	**%∆BWt.**
Control	158.46±5.13	211.04±8.52	33.54
200 mg/kg			
Aqueous	157.48±8.30199.	08±7.672	6.75
Ethyl Acetate	207.08±10.22	203.64±11.77	-1.93
Butanol	150.62±10.44	200.12±9.36	33.33
Crude	153.04±8.30	208.16±8.38	35.95
300 mg/kg			
Aqueous	164.32±19.19	170.36±14.44	3.66
Ethyl Acetate	182.46±13.90	179.98±15.07	-1.65
Butanol	159.65±12.73	182.50±14.95	14.47
Crude	169.36±28.40	163.76±24.23	-3.55

Values are presented as mean ± SEM of five (5) replicates. %∆BWt, percentage body weight.

Table 4. Relative organ:body weight ratio (%)

	Organ			
Groups	**Testes**	**Prostate**	**Seminal Vesicle**	**Epididymis**
Group 1 (Control)	0.16±0.02	0.14±0.01	0.59±0.09	0.16±0.02
Group 2 (AQF 200 mg/kg bwt)	0.14±0.12	0.18±0.01	0.53±0.08	0.14±0.01
Group 3 (EAF 200 mg/kg bwt)	0.15±0.01	0.17±0.01	0.53±0.04	0.15±0.01
Group 4 (BUT 200 mg/kg bwt)	0.13±0.01	0.17±0.01	0.46±0.01	0.13±0.01
Group 5 (CRU 200 mg/kg bwt)	0.13±0.01	0.16±0.01	0.56±0.06	0.13±0.01
Group 6 (AQF 300 mg/kg bwt)	0.15±0.01	0.18±0.02	0.45±0.07	0.15±0.01
Group 7 (EAF 300 mg/kg bwt)	0.16±0.01	0.18±0.01	0.39±0.04	0.16±0.01
Group 8 (BUT 300 mg/kg bwt)	0.14±0.01	0.16±0.02	0.39±0.04	0.14±0.01
Group 9 (CRU 300 mg/kg bwt)	0.12±0.01	0.19±0.02	0.34±0.07	0.11±0.01

Values are expressed as mean ±SEM. N = 5 replicates. Values with (*) are statistically significant at p< 0.05 when compared to the control group AQF represents (Aqueous fraction), EAF (Ethyl Acetate fraction), BUT (Butanol fraction) and CRU (Crude extract).

Table 5. Effects of aqueous, butanol, ethanolic and ethylacetate extract of *A. floribunda* on testicular marker enzymes in rats.

Group	**Parameter**				
	ACP (U/L)	**ALP (U/L)**	**LDH (U/L)**	**G6PDH (U/L)**	**Total protein (g/dl)**
Group 1 (Control)	2.05±0.86	362.66±21.53	103.62±14.21	179.47±19.69	5.46±0.29
Group 2 (AQF 200 mg/kg bwt)	10.45±1.96*	327.89±13.95	170.64±27.10*	98.71±5.94	6.02±0.24
Group 3 (EAF 200 mg/kg bwt)	7.33±0.10*	272.14±29.70	153.00±16.62	103.47±9.94	5.76±0.20
Group 4 (BUT 200 mg/kg bwt)	2.75±1.32	300.29±21.37	143.77±13.96	123.38±12.94	6.14±0.30
Group 5 (CRU 200 mg/kg bwt)	3.11±1.10	332.30±41.88	154.78±21.30	72.91±16.52*	4.60±0.16
Group 6 (AQF 300 mg/kg bwt)	4.77±0.37	230.18±18.89*	96.17±15.48	73.19±5.39*	5.05±0.74
Group 7 (EAF 300 mg/kg bwt)	7.13±0.70	222.46±14.46*	106.37±7.61	82.28±13.63*	5.47±0.23
Group 8 (BUT 300 mg/kg bwt)	8.30±1.70	249.23±14.24	96.94±11.81	90.86±15.78*	5.31± 0.36
Group 9 (CRU 300 mg/kg bwt)	18.89±1.41*	265.51±47.22	130.17±18.23	70.67±9.62*	5.86±0.24

Values are expressed as mean ±SEM. N = 5 replicates. Values with (*) are statistically significant at p< 0.05 when compared to the control group ACP represent (Acid phosphatase), ALP (Alkaline phosphatase), LDH (Lactate dehydrogenase), G6PDH (Glucose -6- phosphate Dehydrogenase). AQF represents (Aqueous fraction), EAF (Ethyl Acetate fraction), BUT (Butanol fraction) and CRU (Crude extract).

result of the effect of the extract on the body weight and relative organ body-weight ratio of the experimental animal, respectively while Tables 5 and 6 show the results of testicular marker enzymes, namely acid

Table 6. Effect of ethanolic extract and other fractions of *A. floribunda* on testicular.

Groups	Antioxidant enzymes	
	Catalase (U/L)	Glutathione peroxidase (U/L/mg protein)
Group 1 (Control)	0.62+0.36	0.21+0.02
Group 2 (AQF 200 mg/kg bwt)	0.28+0.05	0.19+0.01
Group 3 (EAF 200 mg/kg bwt)	0.25+0.04	0.15+0.01
Group 4 (BUT 200 mg/kg bwt)	0.53+0.21	0.18+0.01
Group 5 (CRU 200 mg/kg bwt)	0.21+0.05	0.21+0.02
Group 6 (AQF 300 mg/kg bwt	0.29+0.06	0.22+0.06
Group 7 (EAF 300 mg/kg bwt)	0.40+0.10	0.17+0.01
Group 8 (BUT 300 mg/kg bwt)	0.27+0.07	0.16+0.01
Group 9 (CRU 300 mg/kg bwt)	0.34+0.09	0.15+0.01

Values are expressed as mean + SEM, n = 5 replicates. Values with (*) are statistically significant at $p < 0.05$ when compared to the control group. AQF (Aqueous Fraction), EAF (Ethyl Acetate Fraction), BUT (Butanol Fraction), CRU (Crude Extract).

Table 7. Effects of ethanolic extract and other fractions of *A. floribunda* on sperm count, motility and virility.

Groups	Sperm count (millions)	Sperm motility	(%) Live:dead ratio
Group 1 (Control)	121.80+5.37	91.00+1.00	96.80+0.73
Group 2 (AQF 200 mg/kg bwt)	97.60+7.70	74.00+2.45	96.20+0.73
Group 3 (EAF 200 mg/kg bwt)	93.80+2.96	76.00+2.45	96.80+0.73
Group 4 (BUT 200 mg/kg bwt)	82.60+3.23	68.00+2.00	93.60+2.23
Group 5 (CRU 200 mg/kg bwt)	75.40+3.28	64.00+2.45	94.20+2.40
Group 6 (AQF 300 mg/kg bwt)	97.60+5.15	83.00+4.36	96.80+0.73
Group 7 (EAF 300 mg/kg bwt)	84.20+3.98	72.00+2.00	94.20+2.40
Group 8 (BUT 300 mg/kg bwt)	95.25+4.92	77.50+2.50	94.00+3.08
Group 9 (CRU 300 mg/kg bwt)	53.00+7.20	30.00+15.28	51.67+26.19

Values are expressed as mean + SEM, n = 5 replicates. Values with (*) are statistically significant at $p < 0.05$ when compared to the control group. AQF (Aqueous Fraction), EAF (Ethyl Acetate Fraction), BUT (Butanol Fraction), CRU (Crude Extract).

phosphatase,. alkaline phosphatase, lactate dehydrogenase, glucose 6 phosphatase, total protein and testicular antioxidant enzymes namely catalase, glutatione peroxidase, respectively, while Table 7 show the results of the effect or the ethanolic extract and fraction on the sperm count, sperm motility, and percentage live: dead ratio and Figure 1 show the photomicrograph of the testes showing the seminiferous tubules of the different groups. *A. floribunda* has been shown to display a wide spectrum of biological and pharmacological activities, which provide experimental support for the empiric ethno-pharmacological use of this plant in traditional medicine. Its stem bark has also been established to contain myriads of phytochemicals and metabolites such as benzophenones, xanthones, biflavonoids etc (Dieudonné et al., 2013).

In this study, the result of the phytochemical screening of the ethanolic extract of *A. floribunda* stem bark showed the presence of alkaloids, flavonoid, cardiac glycosides, tannins, saponin, terpenes, and steroids. The results obtained here are very similar to those earlier reported by Kada et al. (2013) where the thanol extract of *A. floribunda* was shown to contain phenolics, lipids, flavonoids, glucosides, cardiac glucosides, tannins, alkaloids,lipids and anthraquinones. The presence of these phytochemicals in the ethanolic extract of *A. floribunda* further supports its use and pharmacological significance in the treatment of ailments. The effect of ethanolic extract of *A.floribunda* when administered to mice acutely showed that no animal died within 48 h after treatment with extract. There were no signs of weakness and/or loss of appetite even up to doses of 5000 mg/kg body weight. Thus, the lethal dose (LD_{50}) was estimated to be higher than 5000 mg/kg body weight (Table 2). The LD_{50} being greater than 5000 mg/kg body weight is thought to be safe as suggested by Lorke (1983). Therefore, the ethanolic extract of *A.floribunda* is practically non-acutely toxic. This correlates with results of earlier works carried out on the plant.

The present investigation on the sub-chronic administration of the ethanolic extract and partitioned fractions of *A. floribunda* has provided information on the

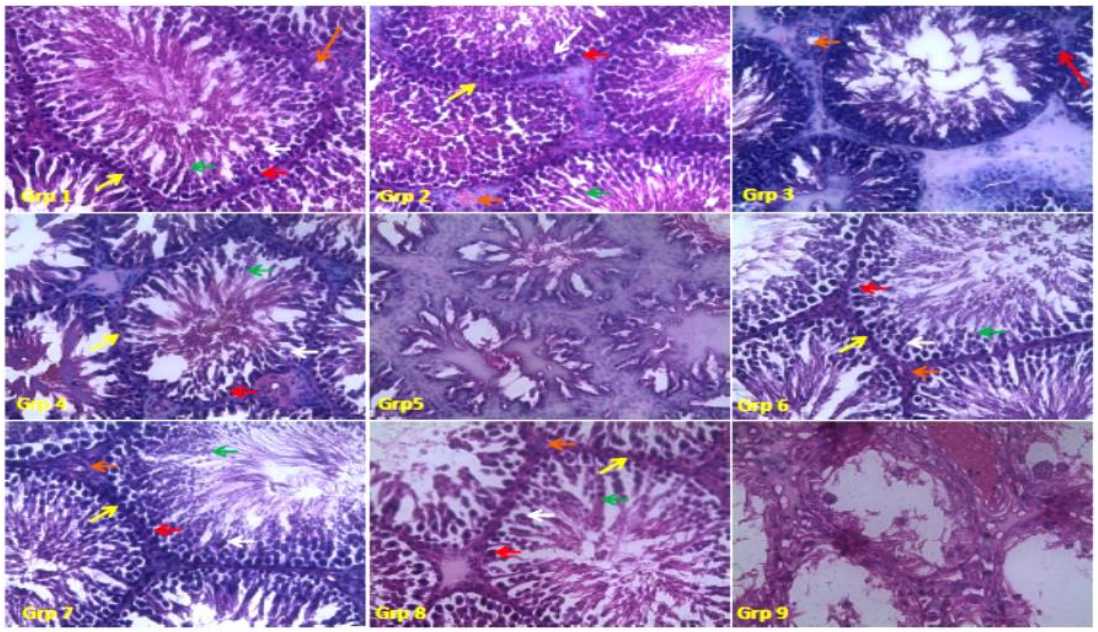

Figure 1. Photomicrographs of the testes showing the seminiferous tubules (ST) of the different groups. Stain H and E. Mag. X400. Normal seminiferous were noted in Groups 1, 6 and 7 with full complement of spermatogenic (Spermatogonia-Red arrow; Spermatocyte-White arrow; Spermatid-Green arrow) and Leydig cells (Brown arrow). Interstitial spaces (IS) were obscured by whitish deposit in Groups 2, 3, 4, 5, 8 and 9. Extension of whitish deposits was noted in the seminiferous tubule of Group 5 obscuring the epithelium (represented with Yellow arrow).

influence of the extract on testicular functions of the test animals. This is because alterations in some testicle enzyme activities have been widely used as biomarkers to evaluate the function of organs due to their important role in energy production and biotransformation (Zhang and Lin, 2009). The acid phosphatases (ACP) are a group of enzymes capable of hydrolyzing esters of orthophosphoric acid in an acid medium (El-Kashoury et al., 2009) Acid phosphatase activity is widely distributed in human tissues and acid phosphatases represent a heterogeneous group of enzymes containing many isoenzymes, each specific for one type of tissue. The human prostate is particularly rich in this enzyme and serum enzyme levels have been used as one of the markers of prostatic cancer (Uboh et al., 2010). Acid phosphatase activity/concentration has been reported to be elevated in the sera of males with metastatic prostatic cancer (Saito et al., 2006; Fang et al., 2008). The results of this investigation showed significant increase in ACP activity (Table 5) across groups Group 2 (200 mg/kg Aqueous Fraction), Group 3 (200 mg/kg Ethyl Acetate Fraction) and Group 9 (300 mg/kg Crude extract), when compared with control; with 2, 3 and 9 having a significant increase of 80.38, 72.03 and 89.15%, respectively. However, in contrast to Group 9, Group 4 (200 mg/kg Butanol Fraction) had comparatively similar activity with the control.

The increase in acid phosphatase activity is an indication of lytic activity in the prostate, causing prostate toxicity and predisposition to prostate cancer as similar results were reported by Zhang and Lin (2009) where administration of 3,4-dichloroaniline caused a significant increase in the acid phosphatase activity of rats. Furthermore, the increases inactivity of acid phosphatases and prostatic phosphatase represent specific toxicity in the prostate gland and a possible threat to the well-being of the testes and physiology of the sperm as this may result in autolysis and consequently cell death (Yakubu et al., 2008). Obianime and Roberts (2009) showed that there exists a correlation between the increases in prostatic acid phosphatases and histopathological damage, showing an indication of reproductive dysfunction, cell death and apoptosis leading to atrophy in testes and accessory sex organ tissues such as the prostate. Alkaline Phosphatase is a ‘marker’ enzyme for plasma membrane and endoplasmic reticulum and is frequently used to assess the integrity of the plasma membrane; such that any alteration in the activity of the enzyme in the tissue and serum would indicate likely damages to the external boundary of the cell (plasma membrane) (Ananthan and Kumaran, 2013). Testicular alkaline phosphatase is involved in the intra and intercellular transport, which is needed for the metabolic reactions to channelize the necessary inputs for steroidogenesis (Latchoumycandane et al., 1997).Specifically, it is involved in mobilizing carbohydrates and lipid metabolites to be utilized either within the cells of the accessory sex structure or by the

spermatozoa in the seminal fluid (Ramalingam and Vimaladevi, 2002); showing its importance ins teroidogenesis (Latchoumycandane et al., 1997). In this investigation, there was a general decrease in alkaline phosphatase (ALP) activity (Table 5) across the groups when compared with the control group, which had an activity of 362.66 $\pm$ 21.53 U/L. The decrease observed was dose dependent with Groups 6, 7, 8 and 9 having a lower activity when compared with Group 2, 3, 4 and 5. Group 6 and 7 has a significant decrease of 57.56 and 63.02%, respectively, compared with the control. The reduction in the testicular ALP activity observed in this investigation could be as a result of the disruption of the ordered lipid bilayer of the membrane, inhibition of the enzyme activity (Ananthan and Kumaran, 2013) or it may also be due to a reduction in concentration or total absence of specific phospholipids required by this membrane-bound enzyme to express its full activity (Yakubu et al., 2002).

This could hamper the normal transportation of required ions or molecules across the membrane. It may also affect other metabolic processes where the enzyme is involved; such as synthesis of nuclear proteins, nucleic acids and phospholipids as well as in the cleavage of phosphate esters to release metabolites for use by accessory sex structure (Yakubu et al., 2008). Moreover, this might affect the testicular mobilization of carbohydrates and lipids which are to be utilized either within the cells of the accessory sex structure or by spermatozoa in the seminal fluid (Ramalingam and Vimaladevi, 2002). Similar depletion in alkaline phosphatase was also observed in the testis of rats as reported by Chitra et al. (1999), Joshi et al. (2007) and El-Kashoury et al. (2010) who reported that the reduction in the activity of this enzyme when treated with chlorpyrifos, mancozeb and endosulphanis due to the decreased metabolic activities. El-Kashoury et al. (2010) further linked the reduction to decrease in steroidogenesis and reduction in intercellular transport.

The normal adult testis of man, rat, rabbit, mouse, dog, guinea-pig, bull, and pigeon, contain unusual isoenzymes of lactate dehydrogenase, designated LDH-X and distinct from other LDH isoenzymes. The testis-specific isoenzymes of LDH are a convenient metabolic marker for the spermatogenic activity of the testis, as they are associated with thespermatogenic cycle from the stage of the pachytene primary spermatocytes. Hence, Lactate dehydrogenase has been used as a marker for active spermatogenesis (Elkington et al., 1972). Lactate rather than glucose is the preferred substrate for glycolysis in primary spermatocyte and lactate is generated from glucose in the Sertoli cells under the influence of Follicle Stimulating Hormone (Jutte et al., 1983). The activity of LDH in the testes reflects the production and/or utilization of the substrate lactate. Thus, the changes in testicular lactate dehydrogenase could give an indication of testicular energy status, spermatogenesis and the rate of transformation of spermatocyte to spermatozoa (Nurudeen and Ajiboye, 2012). Except groups 6 and 8, all the groups had higher LDH values with group 2 having a significant increase of 39.28% in LDH activity. The elevated LDH activity in this study may reflect testicular degeneration and/or damage to seminiferous epithelium, since the enzyme is closely associated with spermatogenesis and testicular development.

This is in agreement with the observation of Olayinka and Ore (2014) who reported that increase in the activity of LDH in the chlorambucil-treated rats resulted from adaptation to improve spermatogenesis and testicular development after oxidative damage. Glucose-6-Phosphate Dehydrogenase (G6PDH) is an X chromosome-linked enzyme which catalyzes the first step in the pentose phosphate pathway in which D-glucose 6-phosphate is converted to 6-phosphogluconolactone (Salatiand Amir-Ahmady, 2001). This oxidation/reduction reaction transfers hydride from glucose 6-phosphate to $NADP^+$ in order to reduce the coenzyme $NADP^+$. As a result, pentose phosphate pathway will generate NADPH, a coenzyme that has an important role as an electron donor which restores reduced glutathione (GSH). NADPH and GSH will help in the maintenance of tissue integrity and protect body cells (such as testicular cells) from oxidative stress by converting harmful hydrogen peroxide to water with the help of glutathione peroxidase.

In the male, Leydig cells which are present in the interstitium of the testis express all of the enzymes essential for the conversion of cholesterol to androgens and estrogens andGlucose-6-phosphate dehydrogenase, present in leydig, sertoli, and spermatogenic cells, is more active in leydig cells .Actually, the activity of G6PDH is associated with the function of leydig cells in that it provides required reducing power (NADPH) necessary for steroidogenesis and biosynthesis of testosterone the male reproductive hormone. The decrease observed in the Glucose-6-phosphate dehydrogenase activity (G6PDH) could be as a result of injury to the function of leydig cells and free radical attack on the sulfhydryl groups of the enzyme as reported by Farombi et al. (2008); who reported that the diminishing enzymatic activity of G6PDH upon treatment with tetracycline may decrease the generation of NADPH to a level that is below the demand of the GSH cycle. This injury inflicted on the leydig cells would translate to an alteration or reduction in the biosynthesis of testosterone resulting in infertility in men. The male generative glands also contain Sertoli cells which require testosterone for spermatogenesis (Yakubu et al., 2008). Hence a disruption in the biosynthesis of testosterone affects the production of sperm calls in the Sertoli cells.

The testis is sensitive to a variety of stressors and exposure to agents, hence this organ has fairly high concentrations of antioxidant (Maneesh et al., 2005).

These fact sindicate that the defense against oxidative stress plays critical roles in the maintenance of spermatogenesis and prevention of testicular atrophy. Reactive Oxygen Species (ROS), are regularly formed during the process of normal respiration. However, the production is kept at physiologically low levels by intracellular free radical scavengers. It had demonstrated that the major sources of ROS in semen were derived from the spermatozoa and infiltrating leukocytes (Sharma et al., 2001). Spermatozoa and seminal plasma have their own anti-oxidative mechanisms to protect ROS-induced cellular damage. Catalase (CAT) is a heme protein which catalyses the reduction of hydrogen peroxides and protects the tissues from hydroxyl radicals. Glutathione peroxidase (GPx) also, plays a significant role in the peroxyl scavenging mechanism and in maintaining functional integration of the cell membranes, spermatogenesis, and sperm morphology and motility. It is suggested that the metabolic pathway of testosterone biosynthesis requires protection against peroxidation and will be affected by a decrease in the activity of this enzyme (Maneesh et al., 2005). Therefore reduction in the activity of these enzymes (GPx, CAT) (Table 6) may result in a number of deleterious effects due to the accumulation of superoxide anion and hydrogen peroxide (Sajeeth et al., 2011). Testicular proteins are required for spermatogenesis and sperm maturation (Nurudeen and Ajiboye, 2012).

The testicular fluid normally contains stimulatory and inhibitory factors that selectively alters the protein secretions. Thus, changes in protein suggests a reduction in the synthetic activity in testes (El-Kashoury et al., 2009). The increase in Total Protein concentration (TP) in Groups 2, 3, 4, 7 and 9 is as a result of accumulation of protein in testes due to androgen deprivation to target organs (El-Kashoury et al., 2009). This observation synced with that recorded by El-Kashoury et al. (2010), where elevation in testicular protein was found to cause a reduction in testicular and cauda epididymis sperm population, loss of motility and increase in the number of abnormal spermatozoa, thereby manifesting 100% failure in treated animals. However, decrease in Total Protein concentration observed in Groups 5, 6 and 8 might be due to decrease in synthesis of the protein as a consequence of the constituents of the extract (Yakubu et al., 2008). The weight of testes and reproductive accessory organs is largely dependent on the mass of differentiated spermatogenic cells and the reduction in the weight of testes may be due to reduced tubule size, decreased number of germ cells and elongated spermatids (Sanhez et al., 2004).

In this study, a general reduction in the testicular and seminal vesicle weight was observed in the groups (Table 4). The reduction in the testicular weight observed suggests the degenerating capacity of the extracts. Reduction in the weight of the testis could be due to inhibition of seminiferous tubule fluid formation and loss of germ cell by direct inhibition on spermatogenesis (Ananthan and Kumaran, 2013) and is in agreement with earlier studies on rats which have well established that the structural and functional integrity of the male accessory sex glands are androgen dependent (Ananthan and Kumaran, 2013). In the present study, the decreased weight of male accessory sex organs might be due to decreased bioavailability androgens and estrogenic and anti-androgenic activities of *A. floribunda* extract. The same reduction in weight was observed in the relative weights of the epididymis as well; all groups with the exception of Group 7 had lower relative epididymis weight when compared with the control.

The decrease in testicular, epididymis and seminal vesicle weights could be due to reduced tubular size which resulted from degeneration and atrophy of seminiferous tubules (Olorunshola et al., 2011). The varying degrees of distortion in the testicular seminiferous tubules following the administration of the plant extract at the doses when compared with the control further point to the toxicity risk of the plant extract as revealed by the biochemical parameters investigated in this study. However, the severe tubular distortion and degeneration of the testes corroborated by microscopic pictures of the sperm cells (Plate 4) observed in group 9 strongly suggest a deleterious influence of the crude ethanolic extract at 300 mg/kg body weight on the male fertility. Gametogenesis occurs in the seminiferous tubules while the interstitial cells secrete the testicular hormone, mainly testosterone. Therefore, any alteration in the seminiferous tubules as observed in the histopathological studies will have its consequential effect on gametogenesis (Yakubu et al., 2008). From the results of this study, it is concluded that ethanolic extract and fractions of *A. floribunda* are not safe at the doses investigated, most especially at the higher dose of the crude ethanolic extract. This could be the explanation for the reduction in libido observed by Kada et al. (2012) when the rats were given 300 mg/kg doses of the extract. Therefore, high doses should be avoided and users should not rule out completely the possibility of chronic toxicity to the reproductive organs developing with continual usage of the plant.

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