AMELIORATIVE EFFECT OF ETHANOLIC FRUIT EXTRACT OF *Persseaamericana* and *Prunusdulcis* ON CAFFEIN-TESTICUL AR DAMAGE IN MALE WISTAR ALBINO RATS

Abstract

This study investigated the effect if ethanolic fruit extracts of *Persseaamericana* and *Prunusdulcis* on caffeine-testicular damage in male albino rats. Sixtyrats weighing 120 and 170 g were used for this study. The rats were separated into 12 groups of five rats per group. Group 1 served as normal control, group 2 received 200mg/kg of caffeine, serving as negative control while group 3 served as positive control. Rats in group 4-6 were orally administered 200mg/kg caffeine + ethanolic 100, 200, and 400mg/kg of *Prunusdulcis,* group 7-12 received 200mg/kg caffeine + ethanolic 100, 200, and 400mg/kg, and Caffeine + ethanolic 100mg/kg *Perseaamericana* +100mg/kg *Prunusdulcis* fruit extract for 7, 14, and 21 days. Blood sample was collected from the tail of each rats at 7 days interval after treatment, for biochemical assays while one rats in each group was sacrificed and the testis was harvested for histological analysis. The plasma luteinizing hormone level of the negative control for 7, 14, and 21 days were 1.62 ± 0.01IU/ML, 1.71 ± 0.02IU/ML, and 1.05 ± 0.09IU/ML respectively. The plasma luteinizing hormone level of group 6 treated with 400mg/kg of *Prunusdulcis* fruit extract for 7, 14, and 21 days were 3.06. ± 0.05IU/ML, 3.41 ± 0.05IU/ML, and 3.63 ± 0.01IU/ML respectively, were significantly different from group 2 while those of group 9 treated with 400mg/kg of *Perseaamericana*fruit extract for 7, 14, and 21 days were 3.51 ± 0.05IU/ML, 3.82 ± 0.05IU/ML, and 3.90 ± 0.02IU/ML . Regeneration of damage testicular tissues occurred after treatment. The significant increases observed on the hormonal profile and regeneration of damaged testicular tissues of the extracts treated rats indicated testiculo-curative effect of *P. dulcis* and *P.americana*fruit on caffeine-induced testicular damage in rats.

Keywords:Hormonal assay,*Prunusdulcis,Perssea Americana*,testicular damage, Wistar albino rats

1.0 INTRODUCTION

Testicular toxicity or damage could arise as a result diseases affecting the testes, radiotherapeutic prescription, occurrence of adjacent tissues, surgery and systemic chemotherapy [1,2]. Hypothyroidism which is a chronic disorder that is connected with under-secretion of the thyroid hormones such as tetraiodothyronine (T4) and triiodothyronine (T3) usually occur due surgery, exposure to chemotherapeutic agents against cancer of the central nervous system [3,4,], cardiovascular disorder [3], neurological, musculoskeletal symptoms [5,6] and environmental iodine deficiency [7,8], is the root cause of testicular disorder.

Gonadotropin-releasing hormone (GnRH) stimulate the secretion of luteinising hormone (LH) and follicle-stimulating hormone (FSH) [9]. FSH stimulates the seminiferous tubules of testicles to produce sperms [10] while LH stimulates specialized cells in the testes (Leydig cells) to secrete the male hormone called testosterone whose concentration influences the rate of secretion of LH [11]. FSH secretion is controlled by inhibin while the rate of inhibin secretion is governed by the amount of sperm produced by the seminiferous tubules [12, 13]. Besides inducing the male characteristics, testosterone and inhibin are the two negative feedback loops whose biological relevance regulate the secretion of gonadotropins [13, 14], and any disruption of this system or dysfunction of its components may lead to gonadal damage and infertility [11,14].

From the inception of civilization, humans have relied on plants that could meet their basic necessities such as food, shelter, clothing, fuel and health [15]. Of all the uses ascribed to the plants, their curative abilities played an inevitable part in the lives of primitive societies, as plants comprised their sole source for healing ailments [16]. A wide majority of herbal plants possess pharmacological principles, which has rendered them useful as curatives for numerous ailments [16, 17]. *Perseaamericana* (avocado) is a tree, native to central America, cultivated in tropical and subtropical climates around the world, belonging to the family Lauraceae, is widely

used in Ayurveda and evidence-based phototherapy [18]. *Perseaamericana* (avocado) is reported to contain peptone, b-galactoside, glycosylated abscisic acid, alkaloids, cellulose, polygalacto urease, polyuronoids, cytochrome P-450, and volatile oils [19]. The almond (*Prunus*dulci(s) is an important nut native to Central Asia, but today is produced worldwide in hot–arid Mediterranean climate regions [20]. *Prunusdulcis* tree nuts are recognized as good source of protein, monosaturated fatty acids, dietary fiber, vitaminE, riboflavin and essential minerals (manganese, magnesium, copper and phosphorus) [21]. Both plants have been reported to elicit curative effects in Southern Nigerian traditional which have not been scientifically proven. This research seeks to determine the combined ameliorative effects of ethanolic fruit extracts of *Persea Americana* and *Prunusdulcis* oncaffein-testicular toxicity in male Wistar albino rats

2.0 MATERIALS AND METHODS

2.1 Chemical/Reagents

All chemical/reagents used for this study were purchased from commercial industries and the manufacturers' standard methods and procedure were strictly followed with regard to this study.

2.2 Source and Identification of Plant Material

The fruits of *Persea Americana* and *Prunusdulcis* fruits were harvested within the premises of University of Port Harcourt, Port Harcourt Rivers State, Nigeria. The fruits of both sample were identified and authenticated by Dr. Ekeke Chimezie at the Herbarium Unit of the Department of Plant Science and Biotechnology (PSB), University of Port Harcourt. The sample was registered with Voucher Number UPH/P/123.

2.3 Proximate Analysis

The moisture contents of the fruit samples were evaluated by drying at 105°C in an oven until a constant weight was reached. The moisture contents of both samples were evaluated by drying at 107°C in an oven until constants weights were attained. For total ash determination, the fruit samples were weighed and converted to dry ash in a muffle furnace at 450 and at 550°C for incineration. The crude fat contents of both samples were characterized through extraction with hexane, using a Soxhlet apparatus. All these determinations were carried out according to [22] method. Kjeldahl method was used to quantify the crude protein contents of both samples. Carbohydrate contents were estimated by calculating the difference between the sums of all the proximate compositions from 100%. Energy values were obtained by multiplying the carbohydrate, protein and fat by the Atwater conversion factors of 17, 17 and 37, respectively following the [23] method.

2.4 Qualitative Phytochemical Screening

The phytochemical screening was performed on the *Perseaamericana*, *Prunusdulcis* and Combined 50:50% fruit's pulp respectively. The tests were carried out to determine the active constituents of the different parameters according to procedures of method outlined by [24] and [25].

2.5 Test for Tannins

To ten milliliters (10mls) of each sample, few drops of 0.1% ferric chloride ($FeCl_3$) solution was added. Formation of a blue-black precipitation indicated the presence of tannins.

2.6Test for Phytate

To two milliliters of each sample, 20% of triacetic acid was added and then diluted with two millilitres of water then filtered. To an aliquot of the filtrate, brown cresol purple indicator was added with one gram of magnesia mixture reagent. The mixture was stirred and ammonium hydroxide (NH_4OH) was added drop wise. The formation of a fluorescent precipitate indicates the presence of phytate.

2.7 Test for Oxalate

To two grams of each sample, $30cm^3$ of distilled water was added then solution boiled and allowed for two hours. Then, the solution filtered with a filter paper (Whatman filter paper) then five milliliters of calcium nitrate ($CaNO_5$) and $KMnO_4$ solution was added to the filterate. The formation of purple colouration indicates the presence of oxalate using the [26] method

2.8 Test for Terpenoids

To five milliliters (5mls) of each sample, mixed with 2ml of chloroform, three milliliters (3mls) of concentrated (H_2SO_4) sulphuric acid added to the solution slowly along the test tube wall in other not to stir the solution of the test tube. Formation of a reddish-brown coloration at the junction of two liquid phases indicated the presence of terpenoids using the [26] method

2.9 Test for Steroids (Lieberman's Test)

To 0.2g of each sample with chloroform, added 1 ml of acetic anhydride. Two drops of concentrated sulphuric acid (H_2SO_4) was later added. The presence of steroids was indicated by the development of pink colour which changes to bluish green on standing using the [23] method

2.10 Test for Glycoside

To five milliliters (5mls) of each sample, a mixture of 2ml of glacial acetic acid with (2%) ferric chloride ($FeCl_3$) were added. One milliliter (1ml) of concentrated sulphuric acid (H_2SO_4) was

added slowly along the walls of the test-tube carefully by using a pipette. Formation of brown ring at the interphase indicated the presence of glycoside.

2.11 Test for Alkaloids

To two milliliters of each sample, 2mls of $2NH_4Cl$ was added. The solution was shaken vigorously to mix together and kept aside for five minutes (5mins). Then, few drops of Meyer's reagent added and shaken. Formation of creamy colored precipitation indicated the presence of alkaloid.

2.12 Test forFlavonoids

Two milliliters of each sample was treated with few drops of lead acetate solution. Formation of a yellow colour precipitate indicated the presence of flavonoids.

2.13 Test for Saponins (Foam Test)

To two milliliters of each sample, distilled water was added and made up to twenty milliliters (20mls). Then, the suspension was shaken for 15mins. A 2cm layer of foam indicated the presence of saponins.

2.14 Quantitative Phytochemical Composition

The quantitative phytochemical constituents of *Perseaamericana*, *Prunusdulcis* fruits and their and their combined 50:50% fruit's pulp were also determined respectively

2.15 Determination of Oxalate

Two grams of each sample was placed in a 250 ml volumetric flask suspended in 190 ml distilled water, following the [24] method. 10mls of 6MHCl solution was added to the sample and the suspension digested at 100ºC for 1h. Then, the sample was allowed to be cooled and made up to 250 ml mark of the flask. The sample was filtered and the duplicate portion of 125mls of the filtrate were measured into a beaker and four drops of methyl red indicator was added, followed by the addition of concentrated NH_4OH solution (drop wise) until the solution changed from

pink to yellow colour. Each portion was then heated to 90ºC, cooled and filtered to remove the precipitate containing ferrous ion. Each of the filtrate was again heated to 90ºC and 10 ml of 5% $CaCl_5$ solution was added to the sample with consistent stirring and left overnight to cool. The solution was then centrifuged at 2500rpm for 5 mins , the supernatant decanted and the precipitates completely dissolved in 10 mL 20% H_2SO_4. The total filtrate resulting from digestion of 2 g of the samples was made up to 200 ml. Aliquots of 125 ml of the filtrate was heated until near boiling and then titrated against 0.05 M standardized $KMnO_4$ solution to a pink colour which persisted for 30 sec. The oxalate content of each sample was calculated.

2.16 Determination of Phytate

Two grams of each sample was weighed and put into a 250 ml conical flask, and 100 ml of 2% Concentrated, following the [25] method. HCl was used to soak the sample in the conical flask for 3 hrs, then filtered through a double layer filter paper, 50ml of the sample filtrate was placed in a 250 ml beaker and 107 ml of distilled water was added to improve the proper acidity then, 10 ml of 0.3% ammonium thiocyanate solution was added to the sample solution as an indicator and titrated with standard iron chloride solution which contained 0.00195 g iron/ml and the end point was signified by brownish-yellow coloration that persisted for 5 minutes. The percentage phytic acid was calculated as:

% Phytic acid= Y x 1.9 x 10 x 100

Y: Titre Value x 0.00195g

2.17 Determination of Cyanogenic Glycoside

Five grams of each sample was dissolved in 50mls of distilled water and allowed to stand overnight and then filtered with a filter paper (Whatman), adopting the [26] method.Varied concentrations of Potassium Cyanide solution between 0.1mg/ml and 1.0mg/ml cyanide was

prepared. Four millilitre of alkaline picrate solution was put into a test tube containing one millilitre of the sample filterate and standard cyanide solution then, kept in water bath to incubate for 15mins. A blank Solution was prepared by adding 1ml of distilled water to the alkaline picrate solution. The absorbance of the test solution was taken at 490nm by a UV-Spectrophotometer against the prepared blank after a yellowish to reddish brown colour occurred. The cyanide(C) estimation was obtained from the cyanide standard curve.

$$\% \text{ Glycoside} = \frac{C_{(mg)}}{\text{Weight of sample(g)}} \times \frac{100}{1}$$

2.18 Determination of Water soluble tannins

Five hundred milligrams (500mg) of the bark sample with 75mls of distilled water were transferred to a 250ml capacity conical flask, following the [27] method. The flask was gently heated on a hot plate and material boiled for 30 minutes and centrifuged at 2000rpm for 20 minutes. The residue was discarded and the volume of supernatant was adjusted to 100ml with volumetric flask and the extract was used for the estimation of the tannin in the bark sample. One millilitre(1ml) of the reaction mixture was transferred into a 100ml capacity volumetric flask containing 75ml of distilled water then, 5mls of Folin-Denis reagent was added followed by 10ml of sodium carbonate solution and diluted to 100 ml with water. Contents in the flasks were thoroughly mixed and after 30 minutes absorbance was measured at 700nmon double beam UV-visible spectrophotometer (Shimadzu-190). A blank was prepared with water instead of the sample. Water soluble tannins were estimated and calculated with the help of standard curve of tannic acid (0.1mg/mL) and expressed as g.100g-1 of dry weight. Mathematically, it is calculated as;

$$\text{Tannic acid (mg/100g)} = \frac{\text{C x extract volume}}{\text{Volume X Weight of sample}} \times \frac{100}{1}$$

2.19 Determination of Total Flavonoids

Five hundred milligram (500mg) of the sample was extracted in 10 ml, acetone (80 %) using mortar and pestle, following the [28] method. The homogenate was filtered through Buckner's funnel using (Whatman No. 1) filter paper. The volume of filtrate was adjusted to50ml with 80 % acetone. The reaction mixture contained 1.5ml of the sample and 1.5ml of 2%MethanolicAluminum Chloride (i.e. 2g Aluminium chloride dissolved in 100ml pure methanol).A Blank was prepared with distilled water in place of sample. The absorbance of the reaction mixture was measured at 367.5nm on a UV-visible double beam spectrophotometer (Shimadzu-190). Total flavonoids contents was calculated using standard curve of rutin (0.3 mg/ml) and values were expressed as g.100g-1 of dry weight.

2.10 Determination of Total Alkaloids

One hundred milligram (100mg) of bark powder was extracted in 10ml 80% ethanol following the [29] method. This was filtered through muslin cloth and centrifuged at 5000rpm for 10 mins. Supernatant obtained was used for the further estimation total alkaloids. The reaction mixture contained 1ml plant extract, 1ml of 0.025M $FeCl_3$ in 0.5M HCl and 1ml of 0.05M of 1, 10-phenanthroline in ethanol. The mixture was incubated for 30 minutes in hot water bath with maintained temperature of 70 ± 20C. The absorbance of red colored complex was measured at 510nm against reagent blank. Alkaloid contents were estimated and it was calculated with the help of standard curve of colchicines (0.1mg/ml, 10mg dissolved in 10ml ethanol and diluted to 100ml with distilled water).

The values were expressed as g.100g-1of dry weight**.**

2.21 Determination of Steroid

Aliquots of each sample was transferred into various test tubes and allowed to evaporate to dryness on warming slightly under a stream of nitrogen, then 0.5ml of methanol was used for the dissolution of the residue, following the [30] method. Then, 0.5ml of DNPH reagent was added to each test tube and thoroughly mixed. The test tubes were heated to boiling in a water bath at 59^0C for 90mins thereby preventing direct light penetration into the test tubes and tubes allowed to be cooled, 0.5mls of 4N of NaOH and 5mls of methanol was then added also and shaken thoroughly and allowed to stand for 30mins at 27^0C. The absorbance of each test tube was taken against the blank reagent at 475nm using a spectrophotometer. The quantity of steroid present was determined in terms of cortisone by reference to a calibration curve which had been prepared in terms of cortisone by reference to a calibration curve which had been prepared from a series of0.5ml of methanol (0 – 20) of cortisone.

2.22 Determination of Saponins

Two grams of each sample was introduced into a soxhlet extractor with a reflux condenser fitted on top, following the [30] method. The process of extraction of the sample's crude lipid was achieved with acetone in a 250mls round bottom flask for four hours after which the apparatus was removed then; 100mls of the methanol was then introduced into another round bottom flask, and then fitted also to the soxhlet extractor for another three hours. The weight of the flask was measured before and after the second extraction. After the second extraction, the methanol was recovered by distillation and the flask was dried in the oven to ensure all solvents inside the flask were eliminated. Then the flask was kept at room temperature and allowed to be cooled, and then weighed. Thus, the sample saponin content can be determined by;

$$\% \text{ Saponin} = \frac{\text{weight of saponin}}{\text{Weight of sample (g)}} \times \frac{100}{1}$$

2.23 Source of Experimental Wistar Albino Rats

Sixty (60) adult male albino Wistar rats (Rattusnorvegicus) weighing 180 and 200 g were purchased from the Biochemistry Animal House, University of Port Harcourt. The rats were kept in clean plastic cages in well ventilated room, fed with standard animal feeds, produced by Grand Cereals and Oil Mills Ltd., Port Harcourt, and water *ad libitum*. The animals were handled with care, according to the principles and standard protocols for the use of laboratory animals for experiments.

2.24 Ethanolic Extraction of the Plant Samples

The air-dried *Perseaamericana* and *Prunusdulcis* fruit samples were pulverized into coarse powder. One hundred and fifty (150 g) of each powdered sample was macerated in 500 ml of 75% ethanol at room temperature for 72 hours. The mixtures were filtered using a Whatzman filter paper grade 1 (542 mm) and the filtrates were condensed and evaporated to dryness using a rotary evaporator (RM2235 LeicaBiosystems USA) and water bath at 50˚C. The extracts which weighed 30 g (*Persea Americana*) and 40 g (*Prunusdulcis*) were stored in air-tight containers in a refrigerator until when required for treatments.

2.25 Experimental Design

Sixty (60) Wistar albino rats weighing between 120 and 170g were used for this study. They were be purchased from the Biochemistry Animal House University of Port Harcourt. The rats were then grouped based on body weight into Twelve groups of five rats per group and treated as follows:

ONE

Group1. Normal control: Feed + H_2O for 7, 14, and 21 days

Group 2. Positive control: Caffeine + 50mg/kg Proviron for 7, 14, and 21 days

Group 3. Negative control: Feed+ H_2O +200mg/Kg of Caffeine for 7, 14, and 21 days

Group 4. Caffeine Induced + 100mg/kg *Prunusdulcis* Fruit Ext for 7, 14, and 21 days

Group 5.Caffeine + 200mg/kg *Prunusdulcis* Fruit Ext for 7, 14, and 21 days

Group 6.Caffeine+ 400mg/kg *Prunusdulcis*Fruit Ext for 7, 14, and 21 days

Group 7. Caffeine + 100mg/kg *Perseaamericana* Fruit Ext for 7, 14, and 21 days

Group 8.Caffeine + 200mg/kg *Perseaamericana* Fruit Ext for 7, 14, and 21 days
Group 9.Caffeine + 400mg/kg *Perseaamericana* Fruit Ext for 7, 14, and 21 days
Group 10.Caffeine + 100mg/kg *Perseaamericana* +100mg/kg *Prunusdulcis*Ext for 7, 14, and 21 days
Group 11. Caffeine + 200mg/kg *Perseaamericana* +200mg/kg *Prunusdulcis*Ext for 7, 14, and 21 days
Group 12. Caffeine + 400mg/kg *Perseaamericana* +400mg/kg *Prunusdulcis*Ext for 7, 14, and 21 days

Blood sample was collected from the tail at interval of 7 days after treatment, while one rats in each group was sacrificed and the testis was harvested for histological analysis

2.26 Histological Analyses of Testes

Histopathological examination was carried out based on standard laboratory method. The testicular tissue was fixed in 10% formalin, dehydrated embedded paraffin, sectioned and stained with hematoxlin and eosin. The glacial glass slides were viewed under the microscope at x 400 magnification

2.27 Statistical Analysis

The data were expressed as means ± standard error of the mean (SEM). The analysis was carried out using the Statistical Package for Social Science (SPSS version 20.0). One-way analysis of variance (ANOVA) was done followed by Tukey's post hoc test was applied to compare means among groups at $p \leq 0.05$ level of significance.

3.0 RESULTS AND DISCUSSION

The proximate compositions determined in the*P.dulcis* and *P.americana* fruits extracts are summarized in Table 1. The Mean ± Standard deviation of the moisture, ash, content of *P.americana*, fibre, lipid, protein, and carbohydrate were 75.33 ± 2.33 %, 1.93 ± 0.30 %, 0.90 ± 0.10 %, 11.47 ± 1.27 %, 4.65 ± 0.86 %, and 5.72 ± 1.40 % respectively while those of *P.dulcis* were 81.18 ± 0.64 %, 0 .88 ± 0.02 %, 2.14 ± 0.03 %, 2.89 ± 0.02 %, 2.24 ± 0.04, and 10.27 ± 0.06 % respectively (Table 1). The estimated carbohydrate contents in *P.dulcis* and *P.americana* fruits extracts were lower, meanwhile, high and carbohydrates are known to produce energy required for the body because they are essential nutrient required for adequate diet [31] and supplies energy to cells such as brain, muscle and blood [32]. The Mean ± Standard deviation of the moisture, ash, fibre, lipid, protein, and carbohydrate contents of the combined *P.dulcis* and *P.americana* fruits extracts, were 75.39 ± 0.64 %, 1.23 ± 0.01%, 1.74 ± 0.03%, 7.59 ± 0.07 %, 3.53 ± 0.03, and 10.54 ± 0.05 % respectively (Table 1). The moisture content (27.63±0. 12%) of the plants show the plants are not a good source of water from vegetables, hence do not agree with the report of [31]. The content of fat in the fruits of the plant extracts (11.47 ± 1.27 %, 2.89 ± 0.02 %, 7.59 ± 0.07%) of *Perseaamericana,* and *Prunusdulcis* and the combined fruit samples were within the range (8.3%-27.0%) reported for some leafy vegetables consumed in Nigeria [32] prottein is vital for various body functions such as body development, maintenance

of fluid balance, formation of hormones, enzymes and sustaining strong immune function [32], hence *Perseaamericana,* and *Prunusdulcis* and the combined fruit samples are excellent source of protein for body development, maintenance of fluid balance, formation of hormones, enzymes and sustaining strong immune function.

Table 1 Proximate Analysis of fruit Samples.

Parameters (%)	*Perseaamericana*	*Prunusdulcis*	Combined Sample
Moisture Content	75.33 ± 2.33	81.18 ± 0.64	75.39 ± 0.64

Ash	1.93 ± 0.30	0 .88 ± 0.02	1.23 ± 0.01
Fibre	0.90 ± 0.10	2.14 ± 0.03	1.74 ± 0.03
Lipid	11.47 ± 1.27	2.89 ± 0.02	7.59 ± 0.07
Protein	4.65 ± 0.86	2.24 ± 0.04	3.53 ± 0.03
Carbohydrate	5.72 ± 1.40	10.27 ± 0.06	10.54 ± 0.05

Results are expressed as Mean ± Standard deviation. N=3

Qualitative phytochemical screening of the fruits of the plant samples revealed the presence of nine (9) phytochemicals (Table 2). The degree of triterpenoids of *P.americana* and *P.dulcis* were same (+++) in degrees of presence and were higher than the combine fruit extract and the combined (++) (Table 2). The flavonoids content of *P.americana* (+++) was observed to be

higher than those of *P.dulcis* and the combined (++) while the steroids *P.americana* (+++) was higher than those of *P.dulcis*and the combined (++) (Table 2). The phytate contents of *P.americana, P.dulcis* and the combined were observed to be equal in degree of presence (++) while the samponin content of *P.americana* and the combine were equal in degree of presence and were slightly higher than that of *P.dulcis*(Table 2). The glycoside contents of *P.americana* and the combine were higher than that of *P.dulcis* while the oxalate contents of *P.dulcis* and the combined were higher than that of *P.americana* (Table 2). However, the tannin contents of *P.dulcis* was highest followed by the combine while the least was that of *P.americana* (Table 1).

The triterpenoid concentration of *Prunusdulcis* (2.74 ± 0.063mg/100g) higher than those of the combined extract (1.87 ± 0.07mg/100g) while the least was those of *Persea Americana* (1.00 ± 0.09mg/100g). The alkaloid content (23.56 ± 0.06mg/100g) of*Prunusdulcis* was observed to be highest in concentration followed by *Persea Americana* (15.87 ± 0.52mg/100g) while the least was that of *Persea Americana* (4.48 ± 0.46 mg/100g) (Table 3). The flavonoid content of *Prunusdulcis* was observed to be 34.80 ± 0.03mg/100g which was higher than those of the combined extract (16.73 ± 0.12mg/100g) and *Persea Americana* (2.25 ± 0.14mg/100g) while the steroid level of *Prunusdulcis* (1.64 ± 0.16mg/100g) was observed to be higher than those of the combined (1.15 ± 0.05mg/100g) and *Persea Americana* (Table 2). Also, the saponin content of *Persea Americana* (45.01 ± 0.53mg/100g) which as higher than those of the combined (24.18 ± 0.12mg/100g) and *Prunusdulcis* (4.53 ± 0.89mg/100g) (Table 3). The oxalate content of *Persea Americana* (8.14 ± 0.30mg/100g) was higher than those of the combined () and *Prunus*dulcis (2.19 ± 0.09mg/100g). More so, the tannin acid concentration of *Persea Americana* (9.14 ± 1.37mg/100g) was different from the combined (2.70 ± 0.01mg/100g) and those of *Prunusdulcis* (2.70 ± 0.01mg/100g) (Table 3). The results on the phytochemical

composition of *Prunusdulcis*, and *Persea Americana* were supportive to the publications Wellington *et al.,* [33, 34] on the chemical composition of the aerial parts of *Euphorbia heterophylla* and *Leonurus cardiaca*.

Table 2 Qualitative Phytochemical Screening of the Samples

Parameters (mg/kg)	*Perseaamericana*	*Prunusdulcis*	Combined (50:50%)
Triterpenoids	++	+++	++
Flavonoids	+++	++	++
Phytate	++	++	++

Steroids	+++	++	++
Saponins	+++	++	+++
Glycosides	+++	+	++
Oxalate	+	+++	+++
Tannins	+	+++	++
Alkaloids	+++	+	++

NB: + = Low

\+ + = Moderate

\+ + + = High

Table 3 Phytochemical Composition of the plant samples

Parameters (mg/kg)	*Perseaamericana*	*Prunusdulcis*	Combined
Triterpenoids	1.00 ± 0.09	2.74 ± 0.063	1.87 ± 0.07
Alkaloids	4.48 ± 0.46	23.56 ± 0.06	15.87 ± 0.52
Flavonoids	2.25 ± 0.14	34.80 ± 0.03	16.73 ± 0.12
Phytate	0.06 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
Steroids	1.64 ± 0.16	1.33 ± 0.07	1.15 ± 0.05
Saponins	4.53 ± 0.89	45.01 ± 0.53	24.18 ± 0.12
Glycosides	0.05 ± 0.02	4.88 ± 0.06	0 .83 ± 0.01
Oxalate	8.14 ± 0.30	2.19 ± 0.09	3.87 ± 0.07
Tannins	9.14 ± 1.37	0.08 ± 0.01	2.70 ± 0.01

Results are expressed as Mean ± Standard deviation. N=5

Oral administration of caffeine to rats in group 2 resulted in a significantly decreased the mean plasma luteinizing hormone level when compared to the normal control (group 4). The significantly decreased mean plasma concentration observed after administration of caffeine is suggestive of testicular damage in the rats which agrees with the report of Owolabi*et al*. [35]on excessive caffeine intake disrupts testicular architecture, spermatogenesis and hormonal levels in

experimental Wistar rats. Also, treatment with ethanolic*Prunusdulcis* fruit extract particularly at 200 and 400mg/kg b.w for 7, 14, and 21 days caused significantly increased mean plasma luteinizing in group 5 and 6 when compared to the negative control (Table 4). Similarly, treatment with ethanolic *Perseaamericana* fruit extract at 200 and 400mg/kg b.w for 7, 14, and 21 days, significantly resulted in increased mean plasma level of luteinizing hormone in group 8 and 9 in comparison to the negative control (Table 4), abs these results are in agreement with the report of Wafa*et al.* [36] on green *coffeaarabica* extract ameliorates testicular injury in high-fat diet/streptozotocin-induced diabetes in rats. More so, treatments of rats in 10-12 using the combine ethanolic *Perseaamericana*and *Prunusdulcis* fruit extracts for 7, 14, and 21 days, resulted in a significantly increased mean plasma luteinizing hormone levels when compared to the negative control (Table 4). The significant increases observed after treatment with the ethanolic *Perseaamericana*and *Prunusdulcis* fruit extracts for 7, 14, and 21 days are reflective of the ameliorative effects of the plant extract against caffeine-induced testicular damage in rats. These result support the report of Ekaluo*et al.* [37] on fruit extract on sperm toxicity induced by caffeine in albino rats.

Table 4 Effect of the fruits pulp sample extracts onluteinizing Hormone levels (IU/ ML)

Groups	Treatments	DAY 7	DAY 14	DAY 28
1	Normal control	5.33 ± 0.05[a]	5.09 ± 0.05 [a]	5.17 ± 0.03 [a]
2	Negative control	1.62 ± 0.01[b]	1.71 ± 0.02 [b]	1..05 ± 0.09 [b]
3	Positive control	8.41 ± 0.07 [c]	8.11 ± 0.08 [c]	8.00± 0.06 [c]

4	Caffeine induced + 100mg/kg of *Prunusdulcis* fruit extract	2.41 ± 0.05[sb]	2.63 ± 0.03[sb]	2.81 ± 0.05[sb]
5	Caffeine induced + 200mg/kg of *Prunusdulcis* fruit extract	2.81 ± 0.05 [d]	2.90 ± 0.6 [d]	2.92 ± 0.05 [d]
6	Caffeine induced + 400mg/kg of *Prunusdulcis* fruit extract	3.06. ± 0.05 [d]	3.41 ± 0.05 [d]	3.63 ± 0.01 [d]
7	Caffeine induced + 100mg/kg of *Perseaamericana* fruit extract	2.51 ± 0.03[ab]	2.72 ± 0.03[ab]	2.92 ± 0.06[ab]
8	Caffeine induced + 200mg/kg of *Perseaamericana* fruit extract	2.75± 0.05 [d]	2.83 ± 0.08 [d]	3.22 ± 0.02 [d]
9	Caffeine induced + 400mg/kg of *Perseaamericana*fruit extract	3.51 ± 0.05 [d]	3.82 ± 0.05 [d]	3.90 ± 0.02 [d]
1 0	Caffeine induced + 100mg/kg of *Perseaamericana&Prunusdulcis* extracts	4.02 ± 0.06 [d]	4.13 ± 0.06 [d]	4.43 ± 0.05 [d]
11	Caffeine induced + 200mg/kg of*Perseaamericana*&Prunusdulcis extracts	5.19 ± 0.06 [d]	5.41 ± 0.04[d]	5.71 ± 0.05 [d]
12	Caffeine induced + 400mg/kg of *Perseaamericana&Prunusdulcis*extracts	4.59 ± 0.05 [d]	4.20 ± 0.05 [d]	4.61 ± 0.06

Values are means ± Standard Error Mean (SEM). Values with different superscript are statistical significant at (P < 0.05). Values bearing superscript ([ab]) were not significantly different from the negative control down the groups. Values bearing superscripts ([d]) were significantly different from the normal and negative control down the groups.

Oral administration of 200mg/kg.b.w of caffeine for 21 days resulted in a significantly decreased mean plasma follicles stimulating hormone levels in the negative control when compared to the normal control (Table 5). The significantly decreased mean plasma follicles stimulating hormone concentration observed in the negative control is indicative of dysfunction of the testes arising from damage to the pituitary gland which is in line with the report of Esegbue*et al.* [38] on coffee and caffeine consumption in reproductive functions of adult Wistar rats. Oral

administration of ethanolic *Prunusdulcis* fruit extract at 100, 200 and 200mg/kg b.w for 7, 14, and 21 days to rats in group 4-6 caused significantly increased mean plasma follicle stimulating hormone concentration when compared to the negative control and similar improvement alo occur with administration using ethanolic *Perseaamericana* fruit extract at 200 and 400mg/kg b.w and combined ethanolic *Perseaamericana*and*Prunusdulcis* fruit extracts for 7, 14, and 21 days (Table 5). These significantly dose-dependent increases observed in rats in group 4-2 are suggestive of the curative effects of ethanolic *Prunusdulcis,Persea Americana, and* combined ethanolic *Perseaamericana*and*Prunusdulcis* fruit extracts against caffeine-induced testicular damage in rats which is in alignment with the report of Uno *et al*., [39] on the effect of *Annonamuricata* leaf extract on sperm toxicity induced by caffeine in albino rats.

Table 5 Effect of the fruits pulp sample extracts on follicle stimulating hormoneconcentration (IU/ML)

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Groups	Treatments	DAY 7	DAY 14	DAY 28
1	Normal control	6.18 ± 0.05[a]	7.09 ± 0.05[a]	6.92 ± 0.04[a]
2	Negative control	2.42 ± 0.05[b]	2.99 ± 0.06[b]	3.55 ± 0.05[b]
3	Positive control	8.17 ± 0.03[c]	8.40 ± 0.03[c]	8.67 ± 0.06 [c]
4	Caffeine induced + 100mg/kg of *Prunusdulcis* fruit extract	3.09 ± 0.05[d]	3.22± 0.06[d]	3.32 ± 0.04[d]

5	Caffeine induced + 200mg/kg of *Prunusdulcis* fruit extract	3.60 ± 0.06^{d}	3.72 ± 0.05^{d}	3.61 ± 0.05^{d}
6	Caffeine induced + 400mg/kg of *Prunusdulcis* fruit extract	3.84 ± 0.06^{d}	3.89 ± 0.06^{d}	3.97 ± 0.05^{d}
7	Caffeine induced + 100mg/kg of *Perseaamericana* fruit extract	4.09 ± 0.05^{d}	4.11 ± 0.06^{d}	4.21 ± 0.06^{d}
8	Caffeine induced + 200mg/kg of *Perseaamericana* fruit extract	4.26 ± 0.05^{d}	4.57 ± 0.04^{d}	4.61 ± 0.05^{d}
9	Caffeine induced + 400mg/kg of *Perseaamericana* fruit extract	4.75 ± 0.05^{d}	$4.83 \pm 0.05^{b,d}$	4.87 ± 0.07^{d}
1 0	Caffeine induced + 100mg/kg of *Perseaamericana&Prunusdulcis* extracts	5.09 ± 0.05^{d}	5.15 ± 0.05^{d}	5.30 ± 0.06^{d}
11	Caffeine induced + 200mg/kg of*Perseaamericana&Prunusdulcis* extracts	5.76 ± 0.06^{d}	5.83 ± 0.05^{d}	4.92 ± 0.05^{d}
12	Caffeine induced + 400mg/kg of*Perseaamericana&Prunusdulcis* extracts	7.20 ± 0.06^{d}	7.19 ± 0.05^{d}	7.33 ± 0.03^{d}

Values are means ± Standard Error Mean (SEM). Values with different superscript are statistical significant at ($P < 0.05$). Values bearing superscript (^{ab}) were not significantly different from the negative control down the groups. Values bearing superscripts (^{d}) were significantly different from the normal and negative control down the groups.

Oral administration of 200mg/kg b.w of caffeine to rats in group 2 for 21 days, resulted in significantly increased mean plasma level of prolactin when compared to the normal control (Table 6). The significantly increased mean plasma prolactin concentration observed in rats in group 2 is reflective of impairment in the posterior pituitary gland, leading to over-secretion of prolactin and this is in line with Dombrowski*et al.* [40] on Verapamil-induced hyperprolactinemia complicated by a pituitary incidentaloma, where they reported that elevate prolactin levels, and this may interfere with erectile function. Dombrowski*et al.* [41] explained that some medications belong to the dopamine antagonist group, primarily inhibits the secretion

of prolactin from the pituitary gland by the inhibiting hormone prolactin inhibitory factor [PIF]). However, treatment with ethanolic *Prunusdulcis* , *Perseaamericana* , and combined *Perseaamericana*and*Prunusdulcis* fruit extract at 100, 200, and 400mg/kg b.w for 7, 14, and 21 days resulted in significantly decreased mean plasma concentration of prolactin when compared to the negative control (Table 6). The dose-dependent significant decreases observed after treatment with extracts are suggestive of the curative effect of ethanolic *Prunusdulcis* ,*Perseaamericana* , and combined *Perseaamericana*and*Prunusdulcis* fruit extract against caffeine-induced testicular damage in rats. This result agrees with the report of Nkosinathi*et al.* [42] on testicular dysfunction ameliorative effect of the methanolic roots Extracts of *Maytenusprocumbens* and *Ozoroapaniculosa* in rats.

Table 6 Effect of the fruits pulp sample extracts onProlactin concentration (µg/l)

Groups	Treatments	DAY 7	DAY 14	DAY 28
1	Normal control	9.30 ± 0.04 [a]	9.03 ± 0.06 [a]	9.75 ± 0.05 [a]
2	Negative control	28.52 ± 0.12 [b]	28.19 ± 0.06 [b]	28.39 ± 0.06 [b]
3	Positive control	4.62 ± 0.06 [c]	4.15 ± 0.03 [c]	4.76 ± 0.05 [c]
4	Caffeine induced + 100mg/kg of *Prunusdulcis* fruit extract	25.14 ± 0.08 [d]	25.15 ± 0.02 [d]	25.20 ± 0.03 [d]
5	Caffeine induced + 200mg/kg of *Prunusdulcis* fruit extract	21.88 ± 0.06 [d]	21.03 ± 0.06 [d]	21.12 ± 0.05 [d]
6	Caffeine induced + 400mg/kg of *Prunusdulcis* fruit extract	17.20 ± 0.05 [d]	17.11 ± 0.05 [d]	17.75 ± 0.04 [d]

7	Caffeine induced + 100mg/kg of *Perseaamericana* fruit extract	24.52 ± 0.06	24.81 ± 0.05	24.42 ± 0.03
8	Caffeine induced + 200mg/kg of *Perseaamericana* fruit extract	21.89 ± 0.06 ^{d}	21.32 ± 0.04 ^{d}	21.47 ± 0.03 ^{d}
9	Caffeine induced + 400mg/kg of *Perseaamericana* fruit extract	14.20 ± 0.05 ^{d}	147.61 ± 0.04 ^{d}	14.27 ± 0.03 ^{d}
1 0	Caffeine induced + 100mg/kg of*Perseaamericana&Prunusdulcis* extracts	22.30 ± 0.03 ^{d}	22.75 ± 0.02 ^{d}	22.19 ± 0.06 ^{d}
11	Caffeine induced + 200mg/kg of *P Perseaamericana&Prunusdulcis*extracts	17.63 ± 0.01 ^{d}	17.78 ± 0.06 ^{d}	17.31 ± 0.03 ^{d}
12	Caffeine induced + 400mg/kg of *Perseaamericana&Prunusdulcis* extracts	14.14 ± 0.03 ^{d}	14.84 ± 0.03 ^{d}	14.46 ± 0.03 ^{d}

Values are means ± Standard Error Mean (SEM). Values with different superscript are statistical significant at ($P < 0.05$). Values bearing superscript (^{ab}) were not significantly different from the negative control down the groups. Values bearing superscripts (^{d}) were significantly different from the normal and negative control down the groups.

Smith, L.B., & Walker [43] reported that the Leydig cells are testosterone (T)-producing cells of the mammalian testis and it initiate and subsequently support spermatogenesis. Decreased serum testosterone levels lead to decreased muscle mass, increased weight gain, reduced bone density, cognitive changes, erectile dysfunction, increased fatigue, and low libido as reported by Kumar *et al.* [44] and Ullah*et al.* [45]. In this present study, oral administration 200mg/kg b.w of caffeine to rats in group 2 resulted in significant decreases on the mean plasma testosterone levels when compared to the normal control (Table 7). The significantly decreased mean plasma testosterone concentration speaks of testicular dysfunction and hypogonadism facilitated by caffeine administration which supports the report of Jin-Yong *et al.*[46] on the effects of

pharmacologically induced Leydig cell testosterone production on intratesticular testosterone and spermatogenesis in rats. However, treatment with ethanolic *Prunusdulcis* fruit extract at 100, 200, and 400mg.kg b.w for 7, 14 and 21 days yielded significant increases on the mean plasma testosterone level when compared to the negative control (Table 7). Also, treatment with ethanolic *Perseaamericana* fruit extract at 100, 200, and 400mg/kg b.w for 7, 14, and 21 days resulted in significantly increased mean plasma testosterone concentration in comparison to the negative control (Table 7). More so, treatment with the combine ethanolic *Perseaamericana*and *Prunus* fruit extracts yield a more significantly increased mean plasma testosterone levels when compared to the negative control values (Table 7). The significant increases observed on the mean plasma testosterone after treatment with ethanolic *Prunusdulcis,Persea Americana,* and *Perseaamericana*and *Prunus* fruit extracts is indicative of the testiculo-ameliorative effects of the plants against caffeine-induced testicular damage in rats. These results are in tandem with the reports of Nkosinathi*et al*. [42] on testicular dysfunction ameliorative effect of the methanolic roots extracts of *Maytenusprocumbens and Ozoroapaniculosa* in rats

Table 7 Effect of the fruits pulp sample extracts onTestosterone Hormone (ng/dl)

Groups	Treatments	DAY 7	DAY 14	DAY 28
1	Normal control	750.00 ± 8.66[a]	740.75 ± 5.20[a]	756.69 ± 2.83[a]
2	Negative control	277.09 ± 3.46[b]	242.75 ± 6.35[b]	209.75 ± 3.46[b]
3	Positive control	823..25 ± 8.08[c]	893.50 ± 5.20[c]	862.25 ± 4.04[c]
4	Caffeine induced + 100mg/kg of *Prunusdulcis* fruit extract	289.25 ± 5.20[d]	297.25 ± 4.62[d]	309.75 ± 4.62[d]
5	Caffeine induced + 200mg/kg of *Prunusdulcis* fruit extract	296.25 ± 5.20[d]	304.50 ± 8.66[d]	315.50 ± 1.73[d]
6	Caffeine induced + 400mg/kg of *Prunusdulcis* fruit extract	311.00 ± 3.46[d]	332.50 ± 2.31[d]	363.50 ± 4.04[d]
7	Caffeine induced + 100mg/kg of *Perseaamericana* fruit extract	259.50 ± 5.20[d]	271.75 ± 6.35[d]	285.00 ± 8.08[d]

8	Caffeine induced + 200mg/kg of *Perseaamericana* fruit extract	284.25 ± 5.20 [d]	295.00 ± 4.62 [d]	309.25 ± 5.20 [d]
9	Caffeine induced + 400mg/kg of *Perseaamericana* fruit extract	314.50 ± 5.78 [d]	336.25 ± 5.78 [d]	352.75 ± 3.46 [d]
1 0	Caffeine induced + 100mg/kg of *Perseaamericana&Prunusdulcis* extracts	511.00 ± 5.20 [d]	519.75 ± 4.62 [d]	531.50 ± 4.62 [d]
11	Caffeine induced + 200mg/kg of*Perseaamericana&Prunusdulcis* extracts	562.75 ± 6.35 [d]	584.75 ± 3.46 [d]	591.75 ± 6.93 [d]
12	Caffeine induced + 400mg/kg of *Perseaamericana&Prunusdulcis* extracts	592.20 ± 5.20 [d]	606.75 ± 4.04 [d]	611.75 ± 4.62 [d]

Values are means ± Standard Error Mean (SEM). Values with different superscript are statistical significant at ($P < 0.05$). Values bearing superscript ([ab]) were not significantly different from the negative control down the groups. Values bearing superscripts ([d]) were significantly different from the normal and negative control down the groups.

Histological analysis of the testis of the negative control rat (Plates 2) showed distorted testicular histology facilitated by caffeine exposure for 21 days when compared to the normal control testicular tissue (Plate 3). Treatment with ethanolic *P. dulcis* fruit extract for 7, 14, and 21 days caused almost normal seminiferous tubules containing spermatogeniccells and mature spermatozoa, interstitial space (ISS) containing laydic cells (Plates 4-6) and similar changes occurred for treatment with ethanolic *P.americana* fruit extract for 7, 14, and 21 days (Plates 7-9) when compared to the negative control testicular tissue (Plate 3). .More so, treatment with the combine ethanolic *P.americana*and *P. dulcis* fruit extract for 7, 14, and 21 days resulted in regeneration of damaged testicular tissue when compared to the negative control (Plates 10-12).

The regeneration of damaged testicular tissues observed after treatment with ethanolic *P. dulcis*, *P.americana,* and the combine ethanolic *P.americana*and *P. dulcis* fruit extract for 7, 14, and 21 days are suggestive of the ameliorative potential of the plant extracts against caffeine-induced testicular toxicity, which also agrees with the report of Nkosinathi*et al.* [42] on testicular dysfunction ameliorative effect of the methanolic roots extracts of *Maytenusprocumbens and Ozoroapaniculosa* and Uno *et al.* [39] on the effect of Soursop (Annonamuricata l.) leaf extract on sperm toxicity induced by caffeine in Albino rats

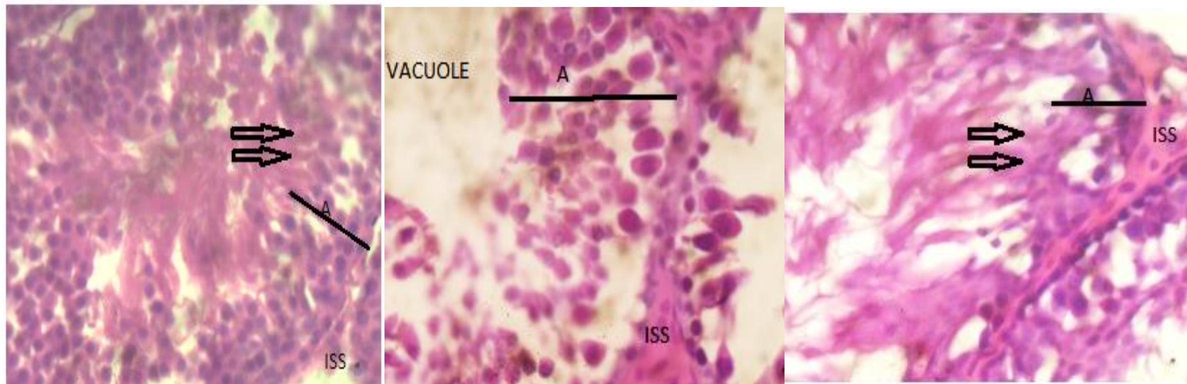

Plate 1: N/Control Plate 2: Ne/Control Plate 3: positive control

Plates 1, 2, and 3: Photomicrographs of testicular tissues of normal, negative and positive control. X 400 (H and S) staining

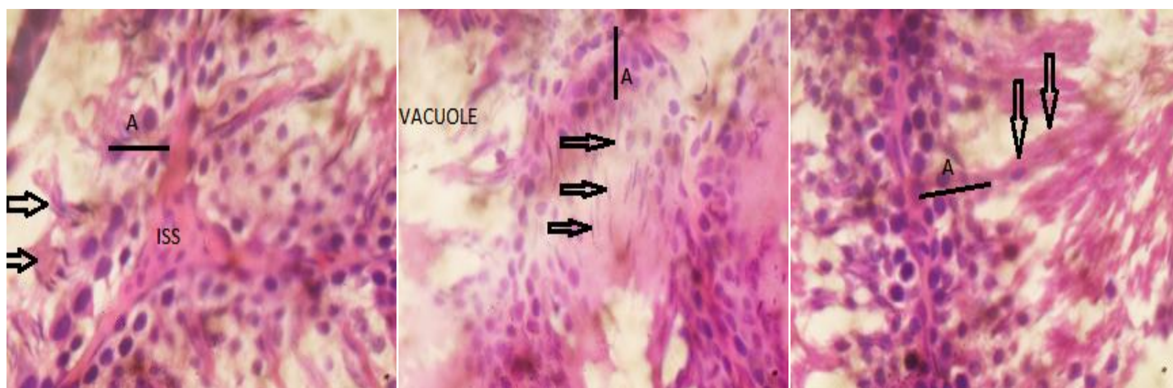

Plate 4: 100mg/kg 7 days Plate 5: 200mg/kg 14 days Plate 6: 400mg/kg 21 days treatment with ethanolic *P. dulcis*fruit extract

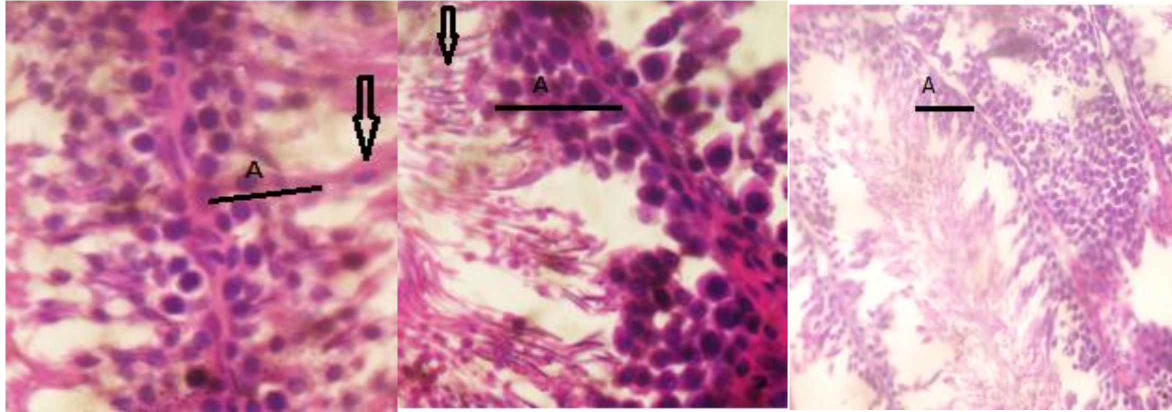

Plate 7: 100mg/kg 7 days Plate 8: 200mg/kg 14 days Plate 9: 400mg/kg 21 days treatment with ethanolic *P.americana*fruit extract

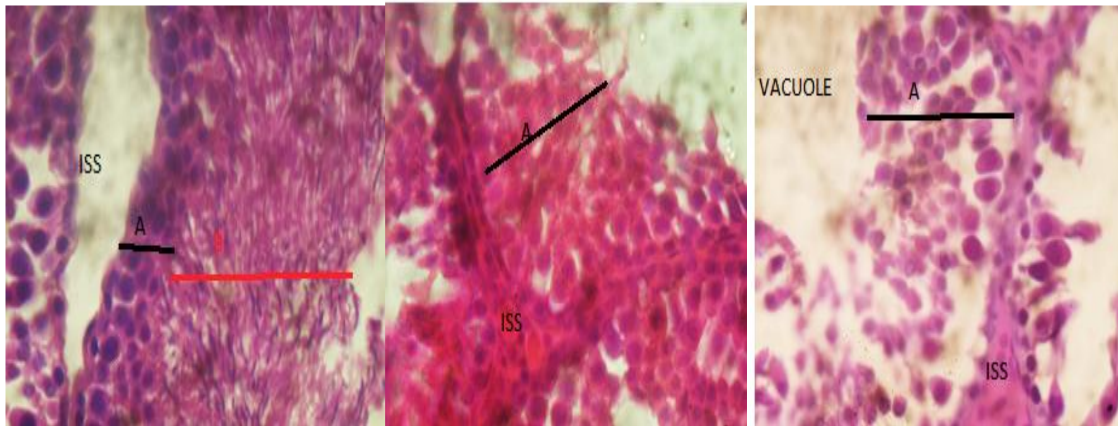

Plate 10: 100mg/kg 7 days Plate 11: 200mg/kg 14 days Plate 12: 400mg/kg 21 days treatment with the combine ethanolic *P. dulcis*and *P.americana*fruit extracts

CONCLUSION

This study revealed that extract ethanolic *P. dulcis*and *P.americana*fruit extracts meliorated the damage caused by overdose of caffeine on the testicular tissues through decreases on all assayed hormones and disruption of testicular tissue architecture.Significant increases occurred in all hormonal assays when compared to the negative control. Regeneration of damaged testicular tissues occurred after treatment with the extract for 7, 14, and 21 days. The significant increases observed on the hormonal profile and regeneration of damaged testicular tissues of the extracts treated rats indicated testicular-curative effect of *P. dulcis*and*P.americana*fruit on caffeine-induced testicular damage in rats.

ETHICAL APPROVAL

All authors hereby declared that the principles of laboratory animal care were followed as well as scientific national laws where applicable. All experiments and procedures were thoroughly examined and approved by the ethical committee on human and animal research University of Port Harcourt.

NOTE

This study highlighted the effectiveness of "traditional medicine" which is an ancient tradition practiced in some parts of India. This ancient concept should be carefully investigated in the light of modern clinical science and can be adopted partially if considered appropriate

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of

knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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