

Microstructural Tissue Assessment, Sex Hormones and Biochemical Investigations Following Acute Administration of *Piper guineense*Schumach&Thonn. on Female *RattusNovergicus*.

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Abstract: The effect of graded doses (50, 100 and 150mg/kg body weight) of crude ethanol extract of the dry seeds of *Piper guineense*Schumach&Thonn was investigated on the ovary, liver, hormonal and some biochemical parameters of female rats weighing between 150-190g. Twenty rats were randomly divided into four groups of five rats each per group, group A (control) were orally dosed once daily with 1ml of distilled water (vehicle) while the test groups i.e. B, C and D were orally dosed with 50, 100 and 150mg/kg body weight of the crude extract in 1ml of the vehicle. After twenty one (21) days of the extract dosing samples were collected for analysis. The results are expressed as the mean of five replicates ± standard deviation, means were analyzed using one way analysis of variance (ANOVA) followed by Posthoc Turkey. $p < 0.05$ was regarded as significant. All doses of the extract caused significant increase $p < 0.05$ in a dose related manner on testosterone, luteinizing hormone, follicle stimulating hormone, progesterone and estradiol and at the same time reducing the prolactin level. AST, ALT and ALP were not significantly influenced $p > 0.05$ except the 100 and 150mg/kg dose on ALP and only the 150mg/kg dose on the AST and ALT, all doses caused significant elevation ($p < 0.05$) in cholesterol and HDL while the 150mg/kg caused significant reduction in triglyceride and low density lipoprotein cholesterol, microstructural integrity assessment of the liver (H&E × 40) of the 150mg/kg group in comparison with the control group showed microvesicular steatosis (fatty change), in the same vein the ovary of this group reveals enlarged cells with poor follicle development.

Keywords: *Piper guineense*, biochemical, histology, hormones, tissue.

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I. Introduction

Sexuality, a key determinant of the quality of life, is an important element of emotional and physical intimacy that most men and women crave to experience throughout their life time [1], unfortunately this may sometimes be a mirage to many, most especially the female folk, due to female sexual dysfunction (FSD). Female sexual dysfunction is characterized by disturbances in the psychophysiological processes associated with sexual response cycle or by pain associated with sexual intercourse [2,3]. In women sexual disorder may appear as hypoactive sexual disorder, arousal disorder, orgasm disorder and sexual pain [4,5]. In a survey of 1500 women seeking gynaecological care, 65 % of the respondents reported one or more sexual concerns, most frequently described was hypoactive sexual disorder (HSD) 87.20 %, orgasmic disorders 83.30 %, difficulty in lubrication often resulting in sexual arousal disorder 74.79 % and dyspareunia indicative of sexual pain disorder in 71.70 % [6,7,8]. Ambler *et al.*[1] reported FSD prevalence between 25 % and 63 % with even a higher rate of prevalence in postmenopausal women with rates of between 68 % and 86.50 %. Pontiroli *et al.*,[9] and Nascimento *et al.*,[10] also reported high prevalent rate of FSD in patients with diabetes and cardiovascular diseases respectively.

Sexual function is a cyclic process that encompasses social, psychological, hormonal, environmental and biological factors[11], in a process that is highly complex but poorly understood and yet affects women of all ages. Although it is not life threatening and does not produce any serious impact on patient's physical health it may impair the quality of life and interpersonal relationships of the patient[12]. This is particularly worsened in the Niger Delta Geo - Ecological zone[13] whose land and water resources have been greatly affected by pollution resulting from oil exploration[14], the air quality also has been greatly compromised due to gas flaring, these factors acts synergistically affecting the sexual health and financial fortunes of the average Niger Delta.

Cultural norms and traditions have long defined the woman's sexuality for many years in Nigeria, making it a taboo for it to be discussed in the open thus making FSD a topic many women in Nigeria shy away from for fear of being labeled Promiscuous, the few that are bold enough to come out open are not financially buoyant to seek treatment in hospitals coupled with the rare availability of over the counter medication for FSD

[15,16], many thus resort to self-therapies that often involve the use of herbal medicines with little or no regulation and sufficient data on their toxicity and safe level despite their acclaimed safety when compared to synthetic orthodox medicines [17].

The extract of the seeds of *Piper guineense* is often used as sex tonic by women in the Niger Delta region of Nigeria, but with little or no data on its toxicity and safety margin. *P. guineense* belongs to the family *Piperaceae* and commonly called climbing pepper, it is a slender climber up to 12 m high with prominent nodes and clasping roots, the leaves are elliptic in shape about 15 cm long and 7 cm broad, the flowers are small, borne on common stalk as cluster opposite to the leaves, the fruits are red and turns black when dry [18,19]. The seeds are consumed after child birth by women to enhance uterine contraction for the expulsion of placenta and other remain from the womb [20], extracts of the seeds have been shown to exhibit antimicrobial activities [21, 22], as an adjuvant in the treatment of rheumatic pains and as an antiasthmatics[23], for weight control [24], rectification of female infertility [25] and as sex invigorator [25,19].

The objective of the present study is to investigate the acute toxicity of graded dose of ethanol extract of *P. guineense* on ovary, liver, hormonal and some biochemical indices of female albino rats.

II. Materials and Methods

2.1 Collection of plant material and preparation of extract

The seeds of *P. guineense* were bought from a local herb market in Warri, Delta State, Nigeria. The species was identified and confirmed at the Herbarium, Department of Plant Science and Biotechnology, University of Portharcourt, Rivers State. Voucher specimen was prepared and deposited in the herbarium of the same department with voucher No: UPH/V/1277. The seeds of *P. guineense* were thoroughly washed with distilled water to remove debris and other contaminants, they were sun dried until a constant weight was reached and then pulverized to fine powder with the aid of an electric blender (Saisho, Model S – 742). The extraction was done by soxhlet method. 200 g of the fine powder was extracted by wrapping 50 g per batch in a thimble and inserted into the extractor connected to 1 L Pyrex round bottom flask containing 500 ml of absolute ethanol and extracted at 60°C for 24 h, the extract was evaporated to dryness using a rotatory evaporator (Model RE52-1). The sample was further dried to remove any available moisture in a desiccator with constant changing of the self-indicating silica gel to give 6.24 g of solid extract, which was later reconstituted in 100 ml distilled water to give the respective working dose.

2.2 Experimental animals and experimental design

A total of twenty (20) healthy female rats (*Rattus norvergicus*) age 2 – 2.5 months and weighing between 150 – 180 g, were obtained from the animal house unit of the Department of biochemistry, University of Portharcourt. The animals were kept in a clean metabolic cage and housed in a well-ventilated room at temperature between 28 - 30°C, under natural light and dark cycle with free access to growers mash and water for a period of one week to acclimatize prior to the commencement of the experiment. All protocols were performed in accordance with the Institutional Animal Ethical Committee (IAEC) as per the directions of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA). The twenty rats were randomly divided into four groups labeled A – D with each group consisting of five rats. Group A which serve as the control were orally administered once daily with 1 ml of distilled water (vehicle), while groups B, C and D were orally administered with 50, 100 and 150 mg/kg body weight of the extract for 21 days.

2.3 Method of collection and handling of serum, liver and ovary

At the end of the treatment regimen, the animals were anaesthetized in a chloroform chamber and blood samples collected via cardiac puncture. The blood was allowed to clot for 10 minutes at room temperature and subsequently centrifuged to obtain serum for hormonal and biochemical assay. The liver and ovaries were also excised and kept in 10 % formaldehyde histological examination.

2.4 Assay kits

Testosterone, follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), progesterone (PRG), estrogen (EST) radioimmunoassay test kits are products of BYK – Sangtec Diagnostica, GmbH and Co. KG. While assay kits for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), serum total protein, albumin, total bilirubin, conjugated bilirubin, total cholesterol, triglyceride, high density lipoprotein cholesterol (HDLc), low density lipoprotein cholesterol (LDLc) are products Randox Laboratories Ltd, Co. Antrim, United Kingdom.

2.5 Hormonal assay

Serum samples were assayed for the following hormones testosterone, follicle stimulating hormone, luteinizing hormone, prolactin, progesterone, and estrogen by using the procedure described by BYK –

SangticDiagnostica. This was based on the principle of radioimmunoassay of competitive binding between the sample serum and the standards for a constant amount of the antisera [26].

2.6 Biochemical assay

Liver function test was conducted to investigate derangement in the liver of the animals used for the study. Aspartate aminotransferase, alanine aminotransferase were determined by colorimetric method of Reitman and Frankel [27]. Alkaline phosphatase was determined by colorimetric method of REC [28]. Serum total protein, and albumin were estimated by Biuret method and Bromocresol Green (BCG) binding method respectively. Serum globulin level was calculated as the difference between total protein and albumin, albumin/globulin ratio (A/G) was obtained from the division of the values of albumin and globulin. Total and conjugated bilirubin was determined by colorimetric method as described by Jendrassik and Grof[29].

2.7 Histopathological studies of ovary and liver

Microstructural integrity of the ovary and liver was examined by histopathological techniques using the method described by Krause [30].

2.8 Statistical analysis

The results are expressed as the mean of five replicates \pm standard deviation, means were analyzed using one way analysis of variance (ANOVA) followed by Posthoc Turkey. $P < 0.05$ was regarded as significant. The Statistical Package for Social Sciences (SPSS) version 16 was used for data analysis.

III. Results

3.1 The effects of *P. guineense* on hormonal, liver enzymes, serum chemistry and lipid profile are indicated in Tab. 3.1, 3.2, 3.3 and 3.4 respectively.

TABLE 3.1: Effect of ethanol extract of *P.guineense* on reproductive hormones after 3 weeks of administration.

HORMONE	GROUP A (CONTROL)	GROUP B (50 mg/kg B.W)	GROUP C (100 mg/kg B.W)	GROUP C (150 mg/kg B.W)
Testosterone (nmol/L)	0.06 \pm 0.02 ^a	0.12 \pm 0.01 ^b	0.15 \pm 0.02 ^c	0.19 \pm 0.01 ^d
Luteinizing hormone (IU/L)	4.10 \pm 0.34 ^a	4.96 \pm 0.40 ^b	5.20 \pm 0.32 ^c	6.00 \pm 0.31 ^d
Follicle Stimulating hormone (IU/L)	4.08 \pm 0.19 ^a	4.50 \pm 0.28 ^b	5.52 \pm 0.29 ^c	6.10 \pm 0.22 ^d
Progesterone (nmol/L)	11.20 \pm 0.42 ^a	12.24 \pm 0.40 ^b	13.16 \pm 0.46 ^c	13.42 \pm 0.45 ^d
Prolactin (ng/ml)	10.52 \pm 0.47 ^a	9.86 \pm 0.34 ^a	9.52 \pm 0.42 ^b	9.28 \pm 0.37 ^c
Estradiol	19.80 \pm 1.64 ^a	23.20 \pm 2.00 ^b	25.40 \pm 2.20 ^c	28.10 \pm 2.28 ^d

Values are mean of five replicate determinations \pm standard deviation. Test values in the same column carrying different superscript letters are statistically different ($P < 0.05$) from the control.

TABLE 3.2: Effect of ethanol extract of *P.guineense* on liver enzyme in U/L after 3 weeks of administration.

LIVER FUNCTION	GROUP A (CONTROL)	GROUP B (50 mg/kg B.W)	GROUP C (100 mg/kg B.W)	GROUP C (150 mg/kg B.W)
AST	18.40 \pm 1.82 ^a	18.90 \pm 1.64 ^a	19.10 \pm 1.74 ^a	21.20 \pm 2.24 ^b
ALT	37.10 \pm 1.64 ^a	39.30 \pm 2.24 ^a	38.50 \pm 2.34 ^a	41.30 \pm 2.30 ^b
ALP	29.00 \pm 2.16 ^a	30.20 \pm 2.55 ^a	35.00 \pm 2.45 ^b	41.00 \pm 2.60 ^c

Values are mean of five replicate determinations \pm standard deviation. Test values in the same column carrying different superscript letters from the control group are statistically different ($P < 0.05$) from the control

TABLE 3.3: Effect of ethanol extract of *P.guineense* on serum chemistry in U/L after 3 weeks of administration.

Serum chemistry	GROUP A (CONTROL)	GROUP B (50 mg/kg B.W)	GROUP C (100 mg/kg B.W)	GROUP C (150 mg/kg B.W)
Total protein	6.56 \pm 0.30 ^a	6.54 \pm 0.19 ^a	6.60 \pm 0.21 ^a	6.65 \pm 0.22 ^a
Albumin	4.13 \pm 0.27 ^a	3.98 \pm 0.29 ^a	4.20 \pm 0.23 ^a	4.30 \pm 0.31 ^a
Globulin	2.45 \pm 0.21 ^a	2.58 \pm 0.19 ^a	2.50 \pm 0.36 ^a	2.59 \pm 0.38 ^a
Albumin/Globulin	1.69	1.54	1.68	1.66
Total bilirubin	7.20 \pm 1.14 ^a	6.80 \pm 1.00 ^a	7.19 \pm 1.58 ^a	9.40 \pm 1.64 ^b
Conjugated bilirubin	1.08 \pm 0.16 ^a	1.13 \pm 0.11 ^a	1.12 \pm 0.16 ^a	1.60 \pm 0.20 ^b

Values are mean of five replicate determinations \pm standard deviation. Test values in the same column carrying different superscript letters are statistically different ($P < 0.05$) from the control.

TABLE 3.4: Effect of ethanol extract of *P.guineense* on lipid profile in mg/dL after 3 weeks of administration.

Lipid profile	GROUP A (CONTROL)	GROUP B (50 mg/kg B.W)	GROUP C (100 mg/kg B.W)	GROUP C (150 mg/kg B.W)
Total cholesterol	146.00 ± 3.39 ^a	154.20 ± 4.76 ^b	167.60 ± 4.56 ^c	171.80 ± 3.85 ^d
Triglycerides	112.00 ± 3.97 ^a	111.20 ± 2.39 ^a	109.00 ± 2.35 ^a	105.40 ± 3.05 ^b
Low density lipoprotein	109.00 ± 2.14 ^a	106.60 ± 2.20 ^a	104.80 ± 2.30 ^a	101.00 ± 2.45 ^b
High density lipoprotein	49.20 ± 2.77 ^a	49.70 ± 2.71 ^a	49.90 ± 2.41 ^a	50.40 ± 2.20 ^b
LDL/HDL	2.22	2.14	2.10	2.00

Values are mean of five replicate determinations ± standard deviation. Test values in the same column carrying different superscript letters are statistically different ($P < 0.05$) from the control.

3.2 Effect of ethanol extract of *P.guineense* on microstructural tissues (ovary and liver) after 3 weeks of administration.

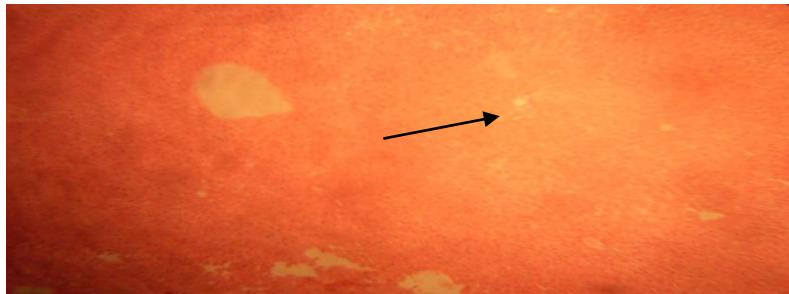


PLATE 3.1A: Photomicrograph female rat's liver administered with distilled water (control). Normal liver with well-preserved lobular architecture, normal hepatocytes, normal central vein, capsules with no indication of adhesion or inflammation, (H & E X 100).



PLATE 3.2A: Photomicrograph of female rat's liver administered with 50mg/kg body weight of *P. guineense*. Liver similar to that of control and shows well preserved lobular architecture, normal hepatocytes, normal central rain and capsules with no indication of adhesion and inflammation, (H & E X 100).



PLATE3.3A: Photomicrograph of female rat's liver administered with 100mg/kg body weight of *P. guineense*. Liver similar to that of control and shows well preserved lobular architecture, normal hepatocytes, normal central rain and capsules with no indication of adhesion and inflammation, (H & E X 100).



PLATE 3.4A: Photomicrograph of female rat liver administered with 150mg/kg body weight of *P. guineense*. Arrow showing microvesicular steatosis (fatty change), (H & E X 100).

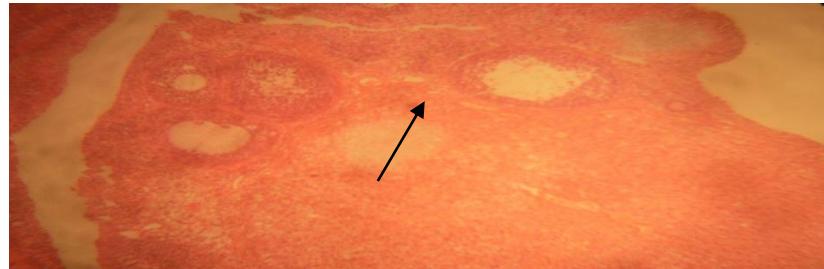


PLATE 3.1B: Photomicrograph of female rat ovary administered with distilled water (control). Arrow indicate normal ovary with follicles at different stages of maturation, there are pre antra and atra type ova, (H & E X 100).

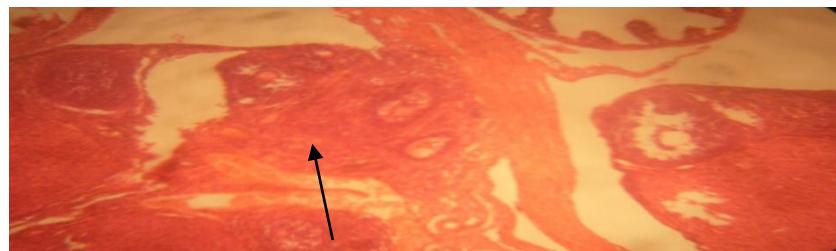


PLATE 3.2B: Photomicrograph of female rat ovary administered with 50mg/kg body weight *P. guineense*. Arrow indicates normal ovary with germ cells at different stages of development, (H & E X 100).

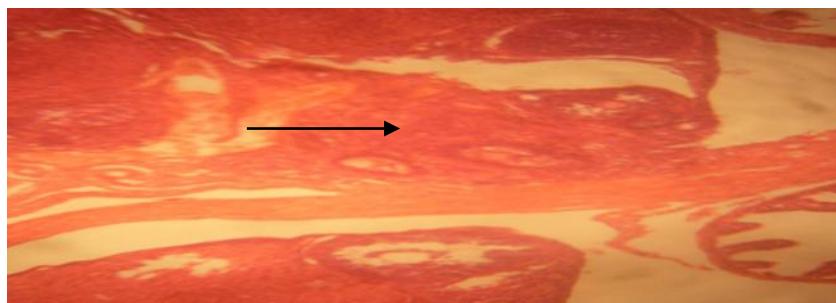


PLATE 3.3B: Photomicrograph of female rat ovary administered with 100mg/kg body weight extract of *P. guineense*. Arrow indicates normal ovary with germ cells at different stages of development, (H & E X 100).

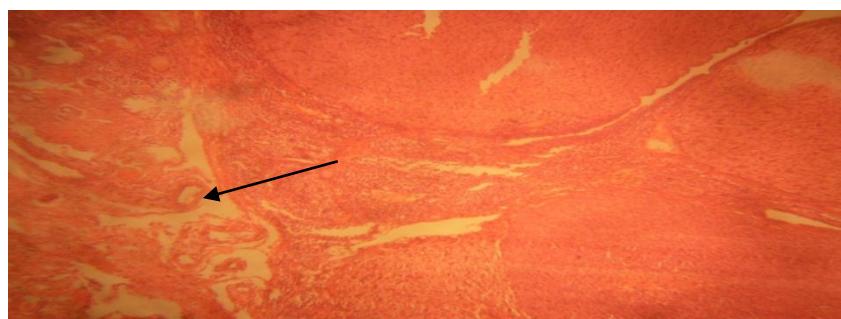


PLATE 3.4B: Photomicrograph of female rat ovary administered with 150mg/kg body weight extract of *P. guineense*. Arrow indicates ovary with enlarged cells, (H & E X 100).

IV. Discussion

The acute treatment of the female rats with the ethanol extracts of *P. guineense* at dose levels of 50, 100 and 150mg/kg body weight significantly ($p < 0.05$) stimulates steroidogenesis in a dose related manner as evidenced by the increase in testosterone level. The increase may be attributed to the presence of phytochemicals. Phytochemicals are biologically active compounds in plants that are responsible for colour, flavour, disease resistance and protection of the body against a number of biochemical, physiological and metabolic disorders. [31,32,33]. Phytochemicals found in *P. guineense* include saponin, alkaloids, steroids, glycosides, flavonoids, tannin and phenolic compounds[34]. Saponins have been implicated as possible bioactive

agent responsible for the aphrodisiac effects of many plants extract e.g *Tribulusterrestris*, *Massulariaacuminata*[35,36,37,38]. It exerts its effect due to its androgen increasing property [35]. The Saponin may have assisted in stimulating an increase in the body's natural endogenous testosterone levels by raising the level of luteinizing hormone (LH), this hormone released by the pituitary gland helps to maintain testosterone levels, as increase in LH triggers automatic increase in testosterone [35].The increase in testosterone level may also be attributed to the presence of zinc one of its major mineral composition [39], as studies have shown that zinc supplements can be used as therapeutic agent against androgenic deficiencies [40,41,42,43]. Testosterone augmentation have been shown to increase desire and initiation of sexual activity in hypoactive sexual disorder women [44,45,46,47,48,49]. The extract at all dose level investigated also significantly increased ($p < 0.05$) the levels of luteinizing hormone (LH) and follicle stimulating hormones (FSH). Both LH and FSH are produced by the anterior pituitary lobe and are often needed to maintain testosterone levels [19], thus an increase in LH and FSH automatically triggers an increase in testosterone levels [50,19]. The also had positive stimulatory effect on progesterone and estradiol, progesterone boast receptability to sexual activity and estrogen is associated with increased sexual desires and arousal[51,52] while deficiency is associated with vaginal atrophy, decreased lubrication, vasocongestion and reduced sensation[52]. The extract was able to reduce significantly ($p < 0.05$) the level of prolactin, as excess prolactin is often associated with inhibited arousal [51].

Alterations in the concentration of major lipids like cholesterol, high density lipoprotein, low density lipoprotein and triglyceride could give useful information on the lipid metabolism as well as predisposition of the heart to atherosclerosis and its associated coronary heart disease [53]. All doses of the extract under investigation was able to significantly ($p < 0.05$) increase the levels of cholesterol and this may be due to increase in concentration of cholesterol substrate like Acetyl CoA which may arise from β - oxidation of fatty acids, it should be noted however, that it is a major metabolic precursor for the biosynthesis of steroid hormones which include the male and female sex steroids (androgen & oestrogen). [54],hence the observed increase in the value of steroid hormone of female rats. Although increase above 200mg/dL may not be beneficial [National Cholesterol Education Programme (NCEP), [55], as it may predispose the animals to atherosclerosis and hypertension [56].LDL takes cholesterol from the liver to peripheral tissues while HDL carries cholesterol from the peripheral tissues to the liver where it is either broken down or expelled as waste [57]. Low density lipoproteins are the major arteriogenic lipoprotein and usually account for most of the coronary heart disease (CHD) associated with elevated plasma total cholesterol [58]. High density lipoprotein cholesterol protects against CHD, the risk of CHD from arteriosclerosis is inversely proportional to serum levels of HDL [59,60,57].The non-significant reduction ($p > 0.05$) in triglycerides and low density lipoprotein cholesterol by 50 and 100mg/kg body weight of *P. guineense*, and the significant decrease ($p < 0.05$) by the 150mg/kg of *P. guineense* is an affirmation of its antihyperlipidemic effect of *P. guineense* [34]. In the same vein the significant increase ($p < 0.05$) by the 150mg/kg body weight of *P. guineense* on HDL speaks volume of its cardio – protective effect because increased HDL correlates inversely with CHD [61].

The liver plays a key role in the metabolic process of itself as well as other tissues in maintaining the internal body environment (homeostasis). It is principally involved in the regulation, synthesis, storage and transport of nutrients (carbohydrates, lipids, protein) and chemicals as well as the biotransformation of xenobiotics [62,63]. The liver is thus susceptible to a variety of metabolic, toxic, microbial and circulatory insults [64], in the course of its function. Plasma enzyme (AST, ALT and ALP) assay is a vital toxicological tool in the assessment of tissue damage, increase in these enzyme values is proportional to the extent of the tissue damage which also is an indication of loss of functional integrity of tissue, cell membranes and consequently leakage into the blood stream. AST is present in high concentration in hepatic, renal, cardiac, skeletal muscles and erythrocytes, damage to any of these tissues may increase plasma levels of AST. ALP is present in high concentration in osteoblast of bone and the cells of hepatobiliary tract, intestinal walls, renal tubules and placenta, thus damage to any of these tissues can increase ALP levels. ALT is more specific for hepatic damage. The non-significant ($p > 0.05$) change in AST, ALT and ALP by both the 50 and 100mg/kg of *P. guineense* is an indication of its nontoxic nature at these dose margin however, the 150mg/kg caused significant change ($p < 0.05$) in all values of AST, ALT and ALP an indication of adverse effect on vital tissues.

Increased total protein concentration may be due to dehydration, increased immunoglobulin concentration due to infections, and decrease in concentration may occur as a result of over hydration, impaired protein synthesis due to malnutrition, malabsorption, liver disease, hypogammaglobulemia or increased protein loss due to renal, gastrointestinal and skin disorder [65]. Total protein of the female rats after 21 days treatment with 50 and 100mg/kg of *P. guineense* showed that total protein was not significant different ($P > 0.05$) from control an indication that the synthetic function of the liver of the rats were not altered [65].Albumin plays an important physiological role by maintaining osmotic pressure, transport of both endogenous and exogenous substance and serving as protein reserves[65]. The liver's ability to synthesize albumin, globulin is reduced if

the synthetic function of the liver is tempered with [66] and it is an indication of hepatitis and liver cirrhosis (liver damage). After 21 days of treatment regimen by all doses of the extract, albumin, globulin and albumin/globulin ratio were insignificantly influenced ($P>0.05$) except the 150mg/kg dose, an indication that liver function was not altered in any way. Bilirubin is formed by the breakdown of haemoglobin in the liver, spleen and bone marrow [67]. An increase in tissue or serum bilirubin concentration results in jaundice and it occurs in toxic or infectious disease of the liver e.g. hepatitis or bile obstruction [68]. Bilirubin measurement is also a useful index of determining the excretory function of the liver and assessment of haemolytic anaemia. Results obtained after 21 days of extract dosing on female rats showed that both total bilirubin and conjugated bilirubin were insignificantly affected ($P>0.05$), an indication of non-adverse effect on haemoglobin metabolism and normal liver function.

Histopathological examination of the ovary after the treatment regime by the graded doses of the extracts showed no major difference between the control group and the groups treated with 50 and 100mg/kg dose, although the group treated with 150mg/kg dose indicated mild inflammation and enlargement of cells. Histopathological examination of the liver after 21 days of extract treatment showed that there was no major difference between the control group and those treated with 50 and 100mg/kg dose, as all liver slides showed well preserved lobular architecture, normal hepatocytes and capsules with no evidence of adhesion and inflammation, however, the 150mg/kg dose showed slight difference between control groups, showing microvesicular steatosis (fatty change) an indication that the extracts at 150mg/kg dose might be toxic and may impair the normal functioning of the liver.

V. Conclusion

All doses of the crude extract under investigation except the 150mgkg⁻¹ had positive stimulatory effect on the sex hormone levels with little or no signs of toxicity as indicated by results of liver enzymes, serum chemistry and histopathological analysis of the liver and ovary, this study has thus lent credence to its usage as local sex invigorator among women in the South-South Geo – Ecological zone of Nigeria.

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