Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Aqueous Extract of *Phyllanthus amarus* Schum & Thonn Leaves Attenuated the Alterations in Fluoxetine-Induced Anti-Oestrogenic Activity in Female Wistar Rats

Quadri O. Nurudeen* and Musa T. Yakubu

Phytomedicine, Toxicology and Reproductive Biochemistry Research Laboratory, Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.

ARTICLE INFO

ABSTRACT

Phyllanthus amarus leaves is widely acclaimed to be significant in the management of female sexual inadequacies. This study aimed to investigate the effects of aqueous extract of *Phyllanthus amarus* (Schum & Thonn) leaves (AEPAL) on the oestrogenic and uterine functioning indices of fluoxetine-treated female rats. Thirty healthy, sexually-responsive female rats (141.52±6.26g) were divided into six groups (A–F) comprising of 5 rats each. The animals in Group A (control group) were administered distilled water only, whereas those in Groups B,C,D,E, and F received orally 15 mg/kg body weight (b.w) of fluoxetine prepared daily in distilled water for 14 days for the induction of anti-oestrogenicity and subsequently received 0.5ml of distilled water, 10 mg/kg b.w of a reference drug (Tadalafil) and 0.5 mL equivalent to 20, 40 and 80 mg/kg b.w of the extract respectively, orally, once daily (08:00-08:45h) for 7 days. The concentrations of total protein, total cholesterol, glycogen and oestrogen, along with the activities of alkaline phosphatase and acid phosphatase in the ovaries and uteri of the animals were significantly reduced ($p<0.05$) following the administration of fluoxetine. In contrast, these reductions were overturned by the AEPAL towards the control group. The extract at 80 mg/kg b.w ameliorated the reduced concentrations of these oestrogenic indices when compared with the non-sexually dysfunction control animals. The effect of *Phyllanthus amarus* leaves on endogenous estrogen's activity and induction of gonadotrophin synthesis or secretion may further lend backing to the widespread use of *Phyllanthus amarus* leaves in handling some sterility/infertility problems in women.

Keywords: Oestrogenic activity, *Phyllanthus amarus*, Euphorbiaceae, Fluoxetine, Progesterone.

Introduction

The ovaries, which are regularly found in pairs are the sites of production and periodical release of egg cells and are analogous to the testes in male individuals.[1] A number of different conditions, from cysts to tumours, genetic mutation in the DNA repair gene Breast Cancer Type 1 (BRCA1) and/or exposure to organic compounds including medications like fluoxetine can cause ovarian problems.[2] Fluoxetine (also known by the trade names; such as Prozac, Rapiflux, Seraphim, Animex-On, Sarafem, Adofen, Ladose, Fontex, Deprex) and other selective serotonin reuptake inhibitors (SSRIs) are among the most prescribed sets of antidepressants used in the treatment of neurological disorders, such as anorexia and depression.[2] Administration of fluoxetine delays the onset of puberty by increasing the concentration of serotonin in the ovaries and consequently decreases the number of ova shed. A recent study reported that fluoxetine acts on the ovary or hypothalamo-pituitary axis, and subsequently causes modifications of the follicular development and ovulation.[3] Some plants that have been acclaimed to be possible alternative remedies for the management of female sexual dysfunction and enhancing fertility problems include: *Cimicifuga racemosa* (black cohosh), *Glycyrrhiza glabra* (licorice), *Paeonia officinalis* (white

peony), *Angelica sinensis* (Dong Quai)**,** *Vitex agnus-castus* (Chaste tree berry)*, Lepidium meyenii* (Maca root), *Althea officinalis* (Marshmallow root), *Trifolium pratense* (Red Clover) and *Borago officinalis* (Borage seed oil).[4] *Phyllanthus amarus* is also on the list of medicinal plants widely acclaimed to be useful in the management of female sexual dysfunction among rural dwellers in Nigeria.[5]

Phyllanthus amarus Schum & Thonn (Euphorbiaceae), which is commonly called *geeron tsutsaayee* (Hausa), *eyin olobe* (Yoruba), and *Ngwu ite kwowa nasu* (Igbo) is widely distributed in Philippine, Cuba, India and Nigeria, as an unwanted plant in cultivated and wastelands.[5] It is a widely dispersed, small, erect tropical annual plant that grows up to 30 – 40 cm high and has slim, leaf-bearing branchlets, distichous leaves which are sub-sessile elliptic-oblong, obtuse, and a rounded base. Traditionally, *Phyllanthus amarus* leaves has been acclaimed to have diverse therapeutic uses such as in the management of urinogenital disorders, jaundice, intermittent fevers, dropsy, dysentery, diarrhoea, gonorrhea, pain, swelling, ophthalmopathy, sores, wounds, scabies, stomach pain, sexual disorders, ulcers, ringworm, colic, snake bite, menorrhagia, leucorrhoea, and constipation.[6-9] Primary screening of the secondary metabolites, mineral contents and amino acid profile of the aqueous extracts of *P. amarus* leaves showed the presence of alkaloids, flavonoids, saponins, steroids, tannins, calcium, potassium, iron, zinc, chromium, copper, glutamine and methionine.[10] In another study, Nurudeen and Yakubu[11] reported that the aqueous extract of *Phyllanthus amarus* leaves restored sexual adequacy in female rats induced into sexual dysfunction by the selective serotonin reuptake inhibitor (fluoxetine). It has also been reported that the aqueous extract possesses hypotensive,[12] antioxidant,[13] anti-viral,[14,15] analgesic,[16] anti-arthritic,[17] anti-plasmodial,[18] anti-nociceptive,[19] anti-hepatitis,[20] anti-amnesic[21] and anti-convulsant[22] activities.

With these myriads of pharmacological studies, there seems to be a scarcity of scientific data on the effects of the aqueous extract of *P.*

*Corresponding author. E mail: quadriolaide@yahoo.com
Tel: +234-803-4273-045

amarus leaves on fluoxetine-induced anti-oestrogenic activity in female Wistar rats regarding the synthetic and secretory functioning indices of the ovaries and the uterus. Consequently, this study seeks to investigate and elucidate the effects of the aqueous extract of *P. amarus* leaves on fluoxetine-induced anti-oestrogenic activity in female rats. This study is furtherance to the efficacious study of the *P. amarus* leaves as a sex-enhancing plant in fluoxetine-induced sexual dysfunction in female rats previously reported by Nurudeen and Yakubu.[11]

Materials and Methods

Plant material and authentication
Fresh *Phyllanthus amarus* leaves were collected within the premises of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria around July, 2016. The leaves were identified and authenticated by Mr. Bolu Ajayi of the University of Ilorin Herbarium, Ilorin, Nigeria. The plant was assigned a voucher number UIH 001/1109.

Experimental animals
Thirty healthy, in-bred, sexually active, female Wistar rats weighing 141.52 ± 6.26 g were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals which were contained in their respective cages were placed in a well-ventilated Animal House and maintained at a temperature of 22 ± 3°C, 12:12 h light and dark cycle, relative humidity of 45–50%. The animals were maintained on rat pellets (Premier Feeds, Ibadan, Nigeria) and tap water (pH 6.54 ± 0.20) *ad libitum*. The ethical review committee of the University of Ilorin, Ilorin, Nigeria approved this study in a letter referenced UERC/ASN/2015/210. All the animals were handled strictly according to prescribed guidelines. [23]

Assay kits
Fluoxetine and Tadalafil were products of Tillomed Laboratories Limited, Herdfordshire, UK and Tuyil Pharmaceuticals Industries Limited, Ilorin, Nigeria respectively. Assay kits for glycogen, cholesterol, acid phosphatase and alkaline phosphatase were products of Randox Laboratories, Co-Atrim, UK, while that of oestrogen was from Inteco Diagnostics UK Ltd., London, United Kingdom. All other reagents used were of analytical grade and were prepared in volumetric flask using all glass-distilled water.

Preparation of extracts
The method described by Nurudeen and Yakubu[11] was used in the preparation of leaf extracts of the test plant. Prior to use, leaf stalks of *Phyllanthus amarus* were removed. The leaves were thereafter rinsed in distilled water. The leaves were later oven-dried at 40°C for 48 h and pulverized in a blender (Master Chef Blender, Model MC-BL 1980, China). A known weight (50 g) of the powder was extracted in 500 mL of distilled water for 48 h at room temperature with constant shaking. The lyophilized (Vir Tis Benchtop K, Vir Tis Co., Gardiner, NY) filtrate yielded 5.25 g (percentage yield of 10.5%). This was reconstituted in distilled water to give the required doses of 20, 40, and 80 mg/kg body weight before administration to the experimental animals as previously used by Nurudeen and Yakubu.[11]

Experimental design
Thirty female rats, after two weeks of acclimatization were randomly assigned into six groups (A–F) of five animals each. Rats in group A (control group) were orally administered 0.5 mL of distilled water, once daily with the aid of a metal oropharyngeal cannula. Those in groups B, C, D, E, and F apart from being treated with 15 mg/kg of fluoxetine suspension (prepared daily in distilled water), once daily (08:00 - 08:45 h) for 14 days, also received 0.5 mL each of distilled water, 10 mg/kg body weight of Tadalafil, 20, 40 and 80 mg/kg body weight of the extract, respectively for 7 days.

Preparation of serum and tissue supernatants
The serum and tissue supernatants were prepared according to the procedure described by Yakubu *et al*.[24] Rats were anesthetized in diethyl ether fumes. When they became unconscious, the jugular veins were cut, and 5 mL of the blood was collected into clean, dry centrifuge tubes. The samples were left for 15 min at room temperature for the blood to clot. Clear serum was then collected using Pasteur pipette after centrifuging at $503 \times g$ for 10 min using Uniscope Laboratory Centrifuge (Model SM800B, Surgifriend Medicals, Essex, UK). Furthermore, the ovaries and uterus were blotted with blotting paper, cut very thinly with sterile scalpel blade and then homogenized in ice-cold 0.25 M sucrose solution (1:5 w/v) to maintain the integrity of the organs. The homogenates were centrifuged at $894 \times g$ for 15 min, while the resulting supernatant was frozen at $-20°C$ until use for the biochemical assays. The biochemical assays were carried out within 24 h of preparation.

Determination of oestrogenic parameters
The ovarian and uterine activities of acid phosphatase, alkaline phosphatase and the concentration of oestrogen were determined using standard methods.[25-27] Other biochemical parameters of the ovaries and uterus were determined for ovarian and uterine protein,[28] ovarian and uterine glycogen[29] and ovarian and uterine total cholesterol.[30]

Statistical analysis
Data were expressed as the mean $\pm$ SEM of five replicates. Means were analyzed using one-way analysis of variance followed by Duncan Multiple Range Test. Statistical Package for Social Sciences, version 20.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

Administration of fluoxetine to sexually active female rats significantly ($P < 0.05$) reduced the levels of oestrogen by 31.14%, when compared with the distilled water-treated animals (Figure 1). The reduced level of oestrogen in the fluoxetine-treated animals were significantly ($p < 0.05$) increased following the administration of the aqueous extract of *P. amarus*, but the significant increase was more pronounced in the fluoxetine-induced sexual dysfunction animals administered 80 mg/kg body weight of the extract, and compared favourably ($p > 0.05$) both with those administered the reference drug (tadalafil) and those of the distilled water-treated animals. The ovaries are the sites of production and periodical release of egg cells for possible fertilization. The ovaries also secrete hormones, principally oestrogen (which is responsible for the appearance of secondary sex characteristics for females at puberty and for the maturation and maintenance of the reproductive organs in their mature functional state) and progesterone (which prepares the uterus for an eventual implantation of the embryo).[1] Whereas, the reproductive function of the uterus is not only to accept a fertilized ovum and nourish the developing foetus prior to birth, but also essential in sexual response by directing blood flow to the pelvis and to the external genitalia, which is crucial for the maintenance of the female reproductive system.[31,32] The secretory constituents of the ovaries and uterus include total protein, glycogen, cholesterol, acid phosphatase and alkaline phosphatase. These constituents can be used to assess the normal functioning of the ovaries and uterus as well as the oestrogenic and anti-oestrogenic properties of a chemical compound in a living system.[33]

The administration of fluoxetine to sexually active female rats significantly reduced ($p < 0.05$) the concentrations of the ovarian secretory constituents: total protein, glycogen, cholesterol, alkaline phosphatase and acid phosphatase of the animals, when compared with the distilled water-treated animals (Table 1). The reduced levels of these ovarian secretory constituents in the fluoxetine-treated animals were significantly increased ($p < 0.05$) following the administration of the aqueous extract of *P. amarus* at all the doses evaluated, but only the fluoxetine-induced sexual dysfunction animals

that were administered with 80 mg/kg body weight of the extract compared favourably (p > 0.05) with those of the distilled water-treated animals (Table 1). Administration of fluoxetine to sexually active female rats significantly (p < 0.05) reduced the levels of the uterine secretory constituents: total protein, glycogen, cholesterol, alkaline phosphatase and acid phosphatase of the experimental animals when compared with the distilled water-treated animals (Table 2). In contrast, all the doses of the extract (20, 40 and 80 mg/kg body weight) evaluated produced a significant (p < 0.05) increase in the concentrations of the uterine total protein, glycogen, cholesterol, alkaline phosphatase and acid phosphatase of the sexual dysfunction female rats, when compared with the distilled water-treated fluoxetine-induced sexual dysfunction female rats (Table 2). Although, the significant increase produced by 20 and 40 mg/kg body weight of the extract did not compare favourably (p > 0.05) with those of the distilled water-treated animals, the experimental animals that were administered 80 mg/kg body weight of the extract showed a significant increase that does not only compared favourably (p > 0.05) with those administered with the reference drug (Tadalafil), but also with those administered with distilled water only. However, the extract at all the doses evaluated (20, 40 and 80 mg/kg body weight) produced a significant (p < 0.05) increase in the concentrations of uterine alkaline phosphatase, but did not compare favourably (p < 0.05) with those of the distilled water-treated animals (Table 2). Ovarian proteins are required for ovarian folliculogenesis, corpus luteum functions, oocyte maturation and fertility as well as for managing some reproductive disorders.[34] In the present study, the significant reversal in the concentration of ovarian protein in fluoxetine-treated female rats following oral administration of the aqueous extract of *P. amarus* might contribute to the enhanced maturation of the oocytes and improved control of ovarian functions.[34] Uterine proteins are required to enable the uterus provides an environment in which remarkable rates of growth are achieved and in the maintenance of pregnancy.[35] Thus, the significant reduction in the concentration of uterine protein in fluoxetine-treated rats could limit the growth of the conceptus. The restoration of the concentration of uterine protein following the administration of the aqueous extract of *P. amarus* leaves could play a part in directing the growth and enhancing the development of the foetus.[36]
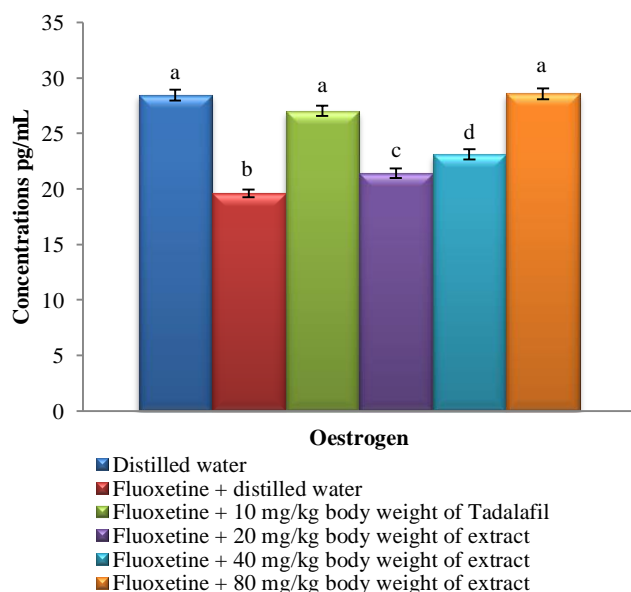
Glycogen, an energy source for general metabolism and constant supply of glucose, has been reported to be directly proportional to the steroid hormones.[37] Therefore the decrease in glycogen levels in the ovaries and uterus of the fluoxetine-treated rats may suggest depleted carbohydrate reserve.[38] The restoration of the concentration of uterine glycogen following the administration of the aqueous extract of *P. amarus* leaves may be an indication that hormonal secretions were increased since oestrogen levels were enhanced in the present study. Such increased uterine glycogen content may seem to enhance the availability of substrates of energy required for uterine and pre-embryonic growth through implantation and early pregnancy.[38] Furthermore, the significant reversal of the ovarian glycogen contents by the aqueous extract of *P. amarus* leaves suggests that the extract enhanced the utilization of available fuel by increasing hormonal secretion and improving ovulation reproductive behaviour.[39]

Cholesterol is the precursor in the synthesis of steroid hormone and its requirement for normal ovarian and uterine activity has been well established.[40-42] Thus, the significant reduction in the level of ovarian and uterine cholesterol in the fluoxetine-treated animals could be an indication of hindered oestrogenic activity in the present study. However, the significant reversal in the levels of ovarian and uterine cholesterol in all the animals treated with various doses of *P. amarus* suggests that the extract enhanced the biosynthesis of cholesterol required for the steroidogenesis, which could also be responsible for the elevated levels of oestrogen in the ovaries of the animals in the present study. Ovarian and uterine alkaline phosphatases are involved in mobilizing carbohydrates and lipid metabolites to be utilized either within the cells of the accessory sex structure or by the oocytes.[43,44] The decreased ovarian and uterine alkaline phosphatase in the fluoxetine-treated rats could compromise the transport of necessary materials needed for folliculogenesis. Administration of *P. amarus* significantly attenuated the fluoxetine-mediated decrease, indicating the capability of the extract to enhance mobilization of necessary materials needed for steroidogenesis. The restorations of the ovarian and uterine ALP levels also suggest that the extract could enhance the development of fertilized eggs and provide a niche for maturation.[43]

Acid phosphatase (ACP) is a lysosomal enzyme which contributes to the ovarian and uterine metabolic functions such as oocyte maturation, resumption of mitotic divisions, germinal vesicle breakdown, ovulation and preparation of the uterine lining for implantation.[45] The attenuation of fluoxetine-mediated decrease in acid phosphatase following the administration of the aqueous extract of *P. amarus* shows the capability of the plant extract to enhance the process of steroidogenesis.[45]

Figure 1: Oestrogen concentrations of sexual dysfunction female rats following oral administration of aqueous extract of *P. amarus* leaves.
Data are mean of five replicates ± SEM. Bars with superscripts different from the control across the group for each parameter are significantly different (*p* < 0.05).

Conclusion

In conclusion, this research has revealed that fluoxetine; a SSRIs can be used to induce anti-oestrogenicity in female rats. Besides, the potentiating effect of *Phyllanthus amarus* leaves on endogenous oestrogen's activity and induction of gonadotrophin synthesis or secretion which restored the alterations in the functioning parameters of the ovaries and the uterus has been established. This may further lend support to the popular use of *Phyllanthus amarus* leaves in handling sexual inadequacies in folkloric medicine in Nigeria.

Conflict of interest

The authors declare no conflicting interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Table 1: The levels of some ovarian parameters and specific activities of enzymes in sexual dysfunction female rats following the administration of aqueous extract of *P. amarus* leaves

Treatment	Protein (mg/mL)	Glycogen (mg/100 mg glucose)	Cholesterol (mmol/L)	Alkaline phosphatase (IU/mL)	Acid phosphatase (IU/mL)
Distilled water (control)	6.78 ± 0.07[a]	5.27 ± 0.05[a]	6.72 ± 0.06[a]	12.16 ± 0.10[a]	9.15 ± 0.08[a]
Fluoxetine-treated	2.14 ± 0.02[b]	2.43 ± 0.02[b]	3.26 ± 0.04[b]	7.35 ± 0.06[b]	4.65 ± 0.04[b]
Fluoxetine + Tadalafil	6.42 ± 0.06[a]	5.02 ± 0.05[a]	6.32 ± 0.05[a]	10.86 ± 0.09[c]	8.42 ± 0.08[a]
Fluoxetine + 20 mg/kg of extract	5.11 ± 0.05[c]	3.83 ± 0.04[c]	5.76 ± 0.05[c]	8.97 ± 0.10[d]	6.22 ± 0.05[c]
Fluoxetine + 40 mg/kg of extract	5.42 ± 0.05[c]	4.72 ± 0.05[d]	5.20 ± 0.04[c]	8.81 ± 0.09[d]	6.96 ± 0.06[c]
Fluoxetine + 80 mg/kg of extract	6.67 ± 0.06[a]	5.21 ± 0.05[a]	6.62 ± 0.05[a]	10.91 ± 0.09[c]	8.85 ± 0.08[a]

Data are mean of five replicates ± SEM. Values carrying superscripts different from the control down the group for each hormone are significantly different (P < 0.05).

Table 2: The levels of some uterine parameters and specific activities of enzymes in sexual dysfunction female rats following the administration of aqueous extract of *P. amarus* leaves

Treatment	Protein (mg/mL)	Glycogen (mg/100 mg glucose)	Cholesterol (mmol/L)	Alkaline phosphatase (IU/mL)	Acid phosphatase (IU/mL)
Distilled water (control)	8.49 ± 0.08[a]	6.46 ± 0.09[a]	13.86 ± 0.31[a]	16.24 ± 0.35[a]	13.46 ± 0.30[a]
Fluoxetine-treated	4.08 ± 0.03[b]	4.41 ± 0.06[b]	8.73 ± 0.19[b]	9.35 ± 0.21[b]	8.95 ± 0.17[b]
Fluoxetine + Tadalafil	8.03 ± 0.07[a]	6.31 ± 0.11[a]	12.52 ± 0.26[c]	15.38 ± 0.31[c]	13.02 ± 0.26[a]
Fluoxetine + 20 mg/kg of extract	6.72 ± 0.06[c]	5.85 ± 0.07[c]	10.21 ± 0.22[d]	14.16 ± 0.28[d]	12.22 ± 0.23[c]
Fluoxetine + 40 mg/kg of extract	7.31 ± 0.07[c]	6.02 ± 0.08[a]	11.04 ± 0.24[e]	13.94 ± 0.31[d]	12.06 ± 0.25[c]
Fluoxetine + 80 mg/kg of extract	8.26 ± 0.08[a]	6.38 ± 0.09[a]	13.05 ± 0.31[a]	15.45 ± 0.26[c]	13.35 ± 0.24[a]

Data are mean of five replicates ± SEM. Values carrying superscripts different from the control down the group for each hormone are significantly different (P < 0.05).

Acknowledgements

References

1. Brown HM and Russell DL. Blood and lymphatic vasculature in the ovary: Development, function and disease. Hum Reprod Update. 2013; 20(1):29-39.
2. Rzepka-Górska I, Tarnowski B, Chudecka-Głaz A, Górski B, Zielińska D, Tołoczko-Grabarek A. Premature menopause in patients with BRCA1 gene mutation. Breast Cancer Res Treat. 2006; 100(1):59-63.
3. Romero-Reyes J, Cárdenas M, Damián-Matsumura P, Domínguez R, Ayala ME. Inhibition of serotonin reuptake in the prepubertal rat ovary by fluoxetine and effects on ovarian functions. Reprod Toxicol. 2016; 59:80-88.
4. Mazaro-Costa R, Andersen ML, Hachul H, Tufik S. Medicinal plants as alternative treatments for female sexual dysfunction: utopian vision or possible treatment in climacteric women? J Sex Med. 2010; 7(11):3695-3714.
5. Kumar S, Choudhary H, Seniya C. *In vitro* antibacterial study of aqueous and methanolic extracts of some selected medicinal plants. J Chem Pharm Res. 2011; 3:854-860.
6. Khatoon S, Rai V, Rawat A. Comparative pharmacognostic studies of three *Phyllanthus* species. J Ethnopharmacol. 2004; 104:79-86.
7. Patel JR, Tripathi P, Sharma V, Chauhan NS. Dixit VK. *Phyllanthus amarus*: Ethnomedicinal uses phytochemistry and pharmacology: A review. J Ethnopharmacol. 2011; 138(2):286–313.

8. Dhongade H and Chandewar AV. Pharmacognostical and Phytochemical studies of *Phyllanthus amarus* leaves. Int J Biomed Adv Res. 2013; 4(6):382-389.
9. Sen A and Batra A. The study of *in vitro* and *in vivo* antioxidant activity and total phenolic content of *Phyllanthus amarus* Schum Thonn: A medicinally important plant. Int J Phar Pharm Sci. 2013; 5(3):942-947.
10. Nurudeen QO and Yakubu MT. Phytochemical Composition, Mineral Contents and Amino Acid Profile of *Phyllanthus amarus* leaves. Al-Hikmah J Pure Appl Sci. 2015; 1:1-7.
11. Nurudeen QO and Yakubu MT. Aqueous extract of *Phyllanthus amarus* leaves restores sexual competence in female rats induced into sexual dysfunction by fluoxetine. Nig J Biochem Mol Biol. 2016; 31(1):1-14.
12. Amaechina FC and Omogbai EK. Hypotensive effect of aqueous extract of the leaves of *Phyllanthus amarus* Schum and Thonn (Euphorbiaceae). Acta Polon Pharm Drug Res. 2007; 64(6):547-552.
13. Lim YY and Murtijaya J. Antioxidant properties of *Phyllanthus amarus* extracts as affected by different drying methods. LWT-Food Sci Tech. 2007; 40(9):1664-1669.
14. Harikumar KB, Kuttan G, Kuttan R. Inhibition of viral carcinogenesis by *Phyllanthus amarus*. Integ Cancer Ther. 2009; 8(3):254-260.
15. Ravikumar YS, Ray U, Nandhitha M, Perween A, Naika HR, Khanna N, Das S. Inhibition of hepatitis C virus replication by herbal extract: *Phyllanthus amarus* as potent natural source. Virus Res. 2011; 158(1-2):89-97.
16. Santos AR, Filho VC, Niero R, Viana AM, Moreno FN, Campos MM, Calixto JB. Analgesic effects of callus culture extracts from selected species of Phyllanthus in mice. J Pharm Pharmacol. 1994; 46(9):755-759.
17. Mali SM, Sinnathambi A, Kapase CU, Bodhankar SL, Mahadik KR. Anti-arthritic activity of standardised extract

313

of *Phyllanthus amarus* in Freund's complete adjuvant induced arthritis. Biomed Aging Pat. 2011; 1(3):185-190.

18. Appiah-Opong R, Nyarko AK, Dodoo D, Gyang FN, Koram KA, Ayisi NK. Antiplasmodial activity of extracts of *Tridax procumbens* and *Phyllanthus amarus* in *in vitro Plasmodium falciparum* culture systems. Ghana Med J. 2011; 45(4):143 – 150.

19. Santos AR, De Campos RO, Miguel OG, Filho VC, Siani AC, Yunes RA. Calixto JB. Antinociceptive properties of extracts of new species of plants of the genus *Phyllanthus* (Euphorbiaceae). J Ethnopharmacol. 2000; 72(1-2):229-238.

20. Cheng YA, Wang SD, Dang SS, Gao N, Yang Y. Clinical study of *Phyllanthus* pill on treating chronic hepatitis B. Zhongxiyi Jiehe Ganbing Zazhi. 2009; 19(17):195-197.

21. Joshi H and Parle M. Evaluation of the antiamnesic effects of *Phyllanthus amarus* in mice. Colomb Médica. 2007; 38(2):132 – 139.

22. Shyamjith M, Deepa B, Joy AE, Rao SN. Anticonvulsant activity of *Phyllanthus amarus* in experimental animal models. Int J App Biol Pharm Tech. 2011; 2(4):144 – 149.

23. National Research Council. Guide for the Care and Use of Laboratory Animals. 8th ed. Washington: National Academies Science Press; 2011. 161-196 p.

24. Yakubu MT, Oladiji AT, Akanji MA. Mode of cellular toxicity of aqueous extract of *Fadogia agrestis* (Schweinf. Ex Hiern) stem in male rat liver and kidney. Hum Exp Toxicol. 2009; 28(8):469-478.

25. Wright PJ, Leathwood PD, Plummer DT. Enzymes in rat urine. Acid phosphatase. Enzymol. 1972; 42(6):459-462.

26. Wright PJ, Leathwood PD, Plummer DT. Enzymes in rat urine: Alkaline phosphatase. Enzymol. 1972; 42(4):317-327.

27. Tietz NW. Clinical Guides to Laboratory Tests. 3rd ed, W.B. Saunders Company; Philadelphia, USA 1995. 554-556 p.

28. Gornall AC, Bardawill CJ, David MM. Determination of serum protein by means of biuret reaction. J Biol Chem. 1949; 177:751-756.

29. Kemp A and Van Heijningen AJ. A colorimetric micromethod for the determination of glycogen in tissues. Biochem J. 1954; 56(4):646-648.

30. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972; 18(6):499 –502.

31. Takacs P, De Santis T, Nicholas MC, Verma U, Strassberg R, Duthely L. Echogenic endometrial fluid collection in postmenopausal women is a significant risk factor for disease. J Ultra Med. 2005; 24(11):1477–1481.

32. Cotton S, Small J, Pomiankowski A. Sexual selection and condition- dependent mate preferences. Curr Biol. 2006; 16(17):R755–R765.

33. Mutreja A, Agarwal M, Kushwaha S, Chauhan A. Effect of *Nelumbo nucifera* seeds on the reproductive organs of female rats. Iran J Reprod Med. 2008; 6(1):7-11.

34. Sirotkin AV, Makarevich AV, Grosmann R. Protein kinases and ovarian functions. J Cell Physiol, 2011; 226(1):37-45.

35. Lee DC, Hassan SS, Romero R, Tarca AL, Bhatti G, Gervasi MT, Caruso JA, Stemmer PM, Kim CJ, Hansen LK, Bechner N, Uldbjerg N. Protein profiling underscores immunological functions of uterine cervical mucus plug in human pregnancy. J Proteomics. 2011; 74(6):817-828.

36. Roberts RM and Bazer FW. The functions of uterine secretions. J Reprod Fert. 1988; 82(2):875-892.

37. Pathak S and Prakash AO. Post-coital contraceptive effect of *Ferula jaeschkeana* Vatke and its hormonal properties. Phyto Res. 1989; 3:61–66.

38. Dean M, Hunt J, Mcdougall L, Rose J. Uterine glycogen metabolism in mink during estrus, embryonic diapause and pregnancy. J Reprod Develop. 2014; 60(6):438-446.

39. Schneider JE. Energy balance and reproduction. Physiol Behav. 2004; 81(2):289-317.

40. Schuler LA, Toaff ME, Strauss JF. Regulation of ovarian cholesterol metabolism: control of 3-hydroxy-3-methylglutaryl coenzyme A reductase and acyl coenzyme A:cholesterol acyltransferase. Endocrinol. 1981; 108(4):1476-1486.

41. Smith RD, Babiychuk EB, Noble K, Draeger A, Wray S. Increased cholesterol decreases uterine activity: functional effects of cholesterol alteration in pregnant rat myometrium. Am J Physiol Cell Physiol. 2005; 288(5):C982-988.

42. Elmes MJ, Tan DS-Y, Cheng Z, Wathes DC, Mcmullen S. The effects of a high-fat, high-cholesterol diet on markers of uterine contractility during parturition in the rat. Reprod. 2011; 141(2):283-290.

43. Bansode FW, Chauhan SC, Makker A, Singh MM. Uterine luminal epithelium alkaline phosphatase activity and pinopod development in relation to endometrial sensitivity in the rat. Contracep. 1998; 58(1):61-68.

44. Hamidabady GH, Salehnia M, Batahi Z. A comparative study of mouse ovarian alkaline phosphatase activity in normal and pseudopregnancies. Yakhteh Med J. 2006; 8(1):53-59.

45. Bojnordi MN, Salehnia M, Allameh A. Evaluation of acid phosphatase activity alterations in mouse ovary after ovarian hyperstimulation in early stages of pseudo and normal pregnancies until implantation time. J Reprod Infert. 2006; 7(1):17-24.