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Kigelia africana improves libido in phenyl hydrazine induced anaemic female Wistar rats

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Abstract

Background and Objective: Anaemia is a global public health challenge affecting adolescent girls, women of reproductive age, pregnant women and children in low and middle income countries. This study therefore was designed to evaluate the effect of extract of *Kigelia africana* on female sexual hormones following phenyl hydrazine induced anaemia.

Materials and Methods: This research work was carried out using twenty five (25) female Wistar rats. The experimental rats were randomly divided into five (5) groups, with five animals per group .Group A served as Normal control (non-anaemic control), group B: Anaemic control(induced with phenyhdrazine) without treatment ,group C; anaemic rats treated with feroton (Standard control), group D; Anaemic rats co-administered with 200mg/kg petroleum ether leaf extract *Kigelia africana* extract (PETLETKG1), and group E; Anaemic rats treated with 200mg/kg between petroleum ether leaf extract *Kigelia africana* extract (PETLETKG2) .All administrations were done orally using oropharyngeal cannula once per day for 14 days (2 week). Blood was collected by cardiac puncture using disposable syringe and needle to draw blood into plane sterile tubes. Thereafter, the samples were analyzed using standard methods.

Results: The result depicts the significant ($p<0.05$) increase in serum follicle stimulating hormone, prolactin, progesterone, oestrogen when compared with both normal and standard control. More so, the petroleum ether extract of K. *africana* showed no significant ($P<0.05$) difference on L.H hormone when compared with both the standard and the normal control. Contrastingly, the extract of *K. africana* decreases serum testosterone significantly ($P<0.05$) when compared with both the standard and normal control.

Conclusion: The result suggests that the extract might be useful in improving the sexual health and libido of female animals following phenyl hydrazine induced anaemia.

Keywords: Anaemia; *Kigelia africana*; Libido; Prolactin; Progesterone; Oestrogen; testosterone

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1. Introduction

Anaemia is defined as a reduction in haemoglobin (Hb) concentration, haematocrit, or red blood cells per litre below the reference interval for healthy individuals of similar age, sex, and race, under similar environmental conditions [1,2]. Anaemia is a common clinical condition characterized by decreased Hb levels which are insufficient for the body's demand [3].Anaemia, has been shown to be a public health problem that affects low, middle and high income countries at different degrees and the prevalence varies with socio-economic status [4]. The most reliable indicator of anaemia at the population level is blood haemoglobin concentration [5]. Anaemia is a serious global public health problem that particularly affects young children and pregnant women. In 2019, global prevalence of anaemia was 29.9% (95% uncertainty interval (UI) 27.0%, 32.8%) in women of reproductive age; equivalent to over half a billion women aged 15-49 years. Prevalence was 29.6% (95% UI 26.6%, 32.5%) in non-pregnant women of reproductive age, and 36.5% (95% UI 34.0%, 39.1%) in pregnant women [5].

The prevalence of anaemia among women in Turkey is 27.8% that 56% of them have iron deficiency anaemia (IDA) [13].According to a study among women aged 15–45 in Zanjan, Iran, 23.6% of women had anaemia [14]. Nigeria is one of the countries listed by the WHO to have a severe burden of this disease, with >40% of the population being anemic. The prevalence of anemia in Nigeria is as follows: children under (5years) 71%; non-pregnant women (15-49 years) 47.3%, and pregnant women 57.5%.

Anaemia resulting from iron deficiency has been reported to increases morbidity and mortality in preschool-aged children and pregnant women [6]. Several factors contribute to the occurrence of anaemia and nearly half of (43%) the anaemia cases in childhood are due to iron deficiency [7]. The deficiency may result from inadequate dietary intake of iron, malabsorption of iron, an increased iron demand during rapid growth in children and chronic blood loss. Other causes of anaemia include folate, vitamin B_{12} deficiencies, malaria, intestinal helminthes, viral infections among others [8, 9, 10]. In women of childbearing age, a common cause of iron-deficiency anaemia is a loss of iron in the blood due to heavy menstruation or pregnancy. A poor diet, or certain intestinal diseases that affect how the body absorbs iron, can also cause iron-deficiency anaemia. Anaemia is an indicator of both poor nutrition and poor health. The most dramatic health implication of anaemia is increased risk of maternal and child mortality which have been well documented [11].

In addition, anaemia should be built into the primary health care system and existing programmes. These strategies should be tailored to local conditions, taking into account the specific etiology and prevalence of anaemia in a given setting and population group [12].

Iron deficiency anaemia (IDA) may cause such symptoms as weakness, headache, restlessness, fatigue, anxiety, pallor, palpitation, reduced strength, impair learning and productivity, and reduced physical and mental capacity as well as fatigue, poor mental health, lack of concentration and poor pregnancy outcomes. Since IDA can cause anxiety and fatigue in women, and these can, in turn, be effective factors in sexual function, the IDA could be considered as a factor to reduce sexual function.

A study showed that after management of IDA in women, most of the sexual function domains were significantly enhanced. Also, studies showed that with increasing serum haemoglobin level, sexual performance score, level of energy, physical and social function increased, while anxiety and depression score decreased, which are directly related to better sexual function [15].

The use of traditional medicine and medicinal plants in most developing countries as a normative basis for the maintenance of good health has been widely observed [16]. The search for new pharmacologically active agents obtained by screening natural sources such as microbial fermentations and plant extracts has led to the discovery of many clinically useful drugs that play a major role in the management of human diseases [17]. One of such plant is *Kigelia africana*. The plant is used traditionally as a remedy for numerous diseases such as wounds healing, rheumatism, psoriasis, diarrhea and stomach ailments. It is also used as an aphrodisiac and for skin care. Therefore, this study was designed to access the effect of extract of *Kigelia africana* on serum sexual hormones in phenyl hydrazine induced anaemia in female Wistar rats.

2. Materials and methods

2.1. Study area

The study was carried out at the Medical Biochemistry Department, University of Cross River State, Okuku Campus,Cross River State, Nigeria from October, 2021-January, 2022**.**

2.2. Plant materials

Fresh leaves of *K. africana* were collected from the UNICROSS environment, Okuku, Cross River State, Nigeria. The leaves were taken to the University of Calabar, Department of Botany for identification and authentication. The voucher number of 205 has been deposited for future reference at the department's herbarium.

2.3. Chemicals and Reagents

All chemicals and reagents (Phenyl hydrazine, ethanol, sulphuric acid, ferric chloride, chloroform, hydrochloric acid, Mayer's reagent and Wagner's reagent) used were of analytical grade. Fresh distilled water was used throughout the experimental period. Assay kits used in the analysis in this study were products of Randox Laboratories (England).

2.4. Experimental animals

Twenty five (25) male Wistar rats were obtained from the animal holding unit of the Department of Medical Biochemistry, Cross River University of Technology. The animals was allowed to acclimatize for a period of 7 days, in a well-ventilated room at room temperature and relative humidity of 29 °C and 70% respectively with 12 hours natural light-dark cycle. They were allowed food and water *ad libitum*. Good hygiene was maintained by daily cleaning and removal of faeces and spills from their cages.

2.5. Preparation of extract of *K. africana* leaf

The leaves of *K. africana* were collected around UNICROSS and air dried at room temperature for a period of 21 days until constant weight was obtained. The dried leaves were then pulverized to powdered form by a machine blender and sieved. Thereafter, 400 g of the pulverized plant material (*K. africana*) was dissolved in 1200 ml of 70% petroleum ether for 72 hours. This was followed with vacuum filtration and extracts was concentrated using an evaporator water bath at 40 °C to obtain a solvent free extract, and stored in a refrigerator at 4°C.

2.6. Induction of haemolytic anaemia

Haemolytic anaemia was induced by intraperitoneal (I.P.) injection of phenyl hydrazine (PHZ) at 10 mg/kg for 7 days .Anaemia was considered to be induced by comparing the PCV of the PHZ-induced animals with that of the normal control (non-induced) animals after 24hours of the last induction. The PCV was carried out by the capillary tube method whose procedures are as follows; blood was collected from the tail into the capillary tube and one end of the tube was sealed with plastacin. The capillary tube was place inside a haematocrite centrifuge and spun for five minutes at 2000 rpm.

2.7. Treatment of animals

The experimental rats were randomly divided into five (5) groups, with five animals per group and treated for a period of fourteen (14) days.

- Group A: Normal control (non-anaemic control)
- Group B: Anaemic rats(induced with phenyhdrazine) without treatment (anaemic control)
- Group C:Anaemic rats treated with feroton (Standard control)
- Group D: Anaemic rats treated with 100 mg/kg bwt petroleum ether leaf extract of *Kigelia africana* (PETLETKG1)
- Group E: Anaemic rats treated with 200 mg/kg bwt petroleum ether leaf extract of *Kigelia africana* (PETLETKG2)

All administrations were done orally using oropharyngeal cannula once per day for 14days (2 week).

2.8. Blood sample collection

Blood was collected from all the test rats and control by cardiac puncture using disposable syringe and needle draw blood into plane sterile tubes. The specimens were labeled with the identification alphabets/ number. The samples were kept at room temperature until processing, which occurred within 30 minutes of collection.

2.9. Effect on sexual hormones

2.9.1. Determination of testosterone concentration

Serum testosterone concentration was determined by the method of [18].Using test kits procured from Monobind Inc., U.S.A.

2.9.2. Determination of serum follicle stimulating hormone concentration

Serum follicle stimulating hormone (FSH) concentration was determined by the method of[19] using test kits procured from monobindinc., U.S.A.

2.9.3. Determination of serum luteinizing hormone concentration

Serum luteinizing hormone (LH) concentration was determined by the method of [20]

Using test kits procured from Monobid Inc., U.S.A

2.10. Statistical analysis

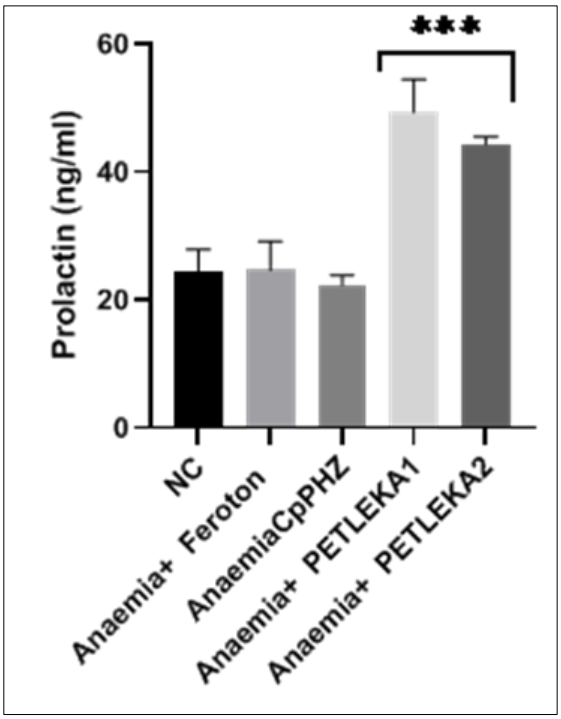
The data obtained were analyzed using One Way Analysisof Variance (ANOVA) followed by post hoc test at $P<0.05$. The Statistical Package for Scientific Solutions (SPSS) Software version 20.0 was used for the analysis.

3. Results

The result below indicates the effect of *K. africana* extract on serum sexual hormones following phenyl hydrazine induced anaemia on female Wistar rats. The extract was found to significantly ($P<0.05$) increased serum prolactin, progesterone, follicle stimulating hormone and oestrogen when compared with both the normal and standard control (fig 1-3).

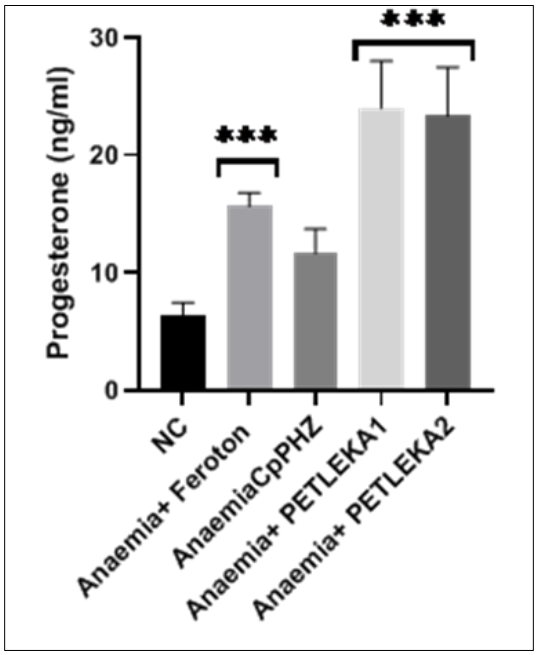
More so, the petroleum ether extract of *K. africana* shows no significant ($P>0.05$) difference on luteinizing hormone when compared with both the standard and the normal control (fig 4).

Contrastingly, the extract *K.africana* decreases serum testosterone significantly ($P>0.05$) when compared with both the standard and normal control (fig 5).



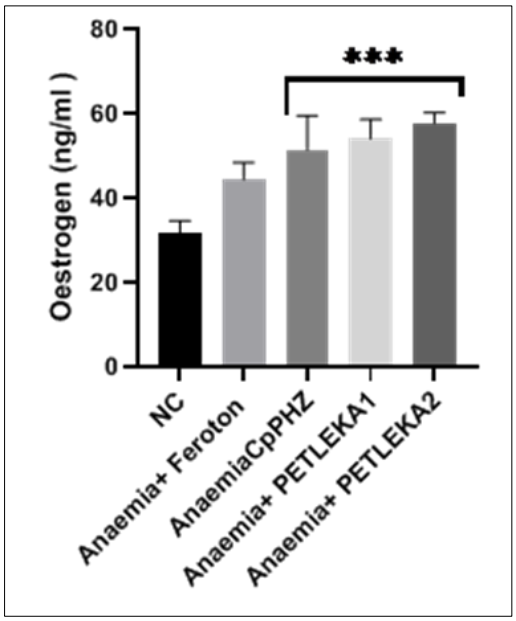
Results were expressed as mean ± SD (n=5) *** significant at P<0.05 compared with the control.NC: Normal Control, Standard: Anaemic rats+ Feroton (10mg/Kgbwt), AnaCpPHZ; Anaemic rats control, induced with phenyl hydrazine, Anaemia + PETLEKA1: Anaemic rats + Petroleum ether leaf extract of *Kigelia africana* (100mg/Kgbwt) , Anaemic rats + PETLEKA2: Anaemic rats + Petroleum ether leaf extract of *Kigelia africana* (200mg/Kgbwt).

Figure 1 Effect of petroleum ether extract on K. *africana* leaf on serum prolactin hormones following phenyl hydrazine induced anaemia in Wistar rat



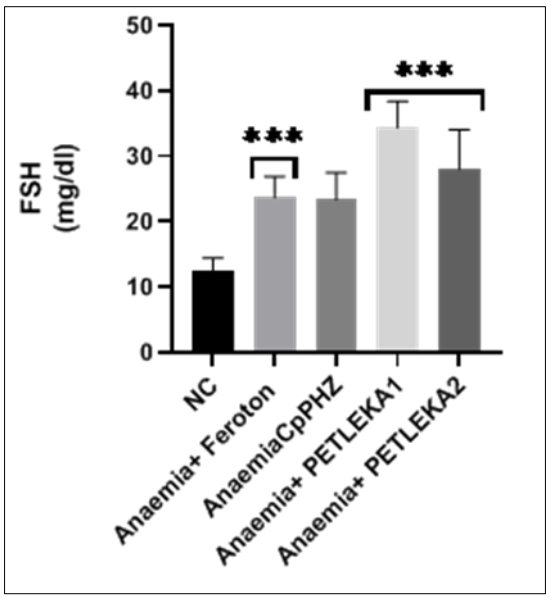
Results were expressed as mean ± SD (n=5) *** significant at P<0.05 compared with the control NC: Normal Control, Standard: Anaemic rats+ Feroton (10mg/Kgbwt), AnaCpPHZ; Anaemic rats control, induced with phenylhydrazine, Anaemia + PETLEKA1: Anaemic rats + Petroleum ether leaf extract of *Kigelia africana* (100mg/Kgbwt) , Anaemic rats + PETLEKA2: Anaemic rats + Petroleum ether leaf extract of *Kigelia africana* (200mg/Kgbwt).

Figure 2 Effect of petroleum ether extract on *K.africana* leaf on serum progesterone hormones following phenyl hydrazine induced anaemia in Wistar rat



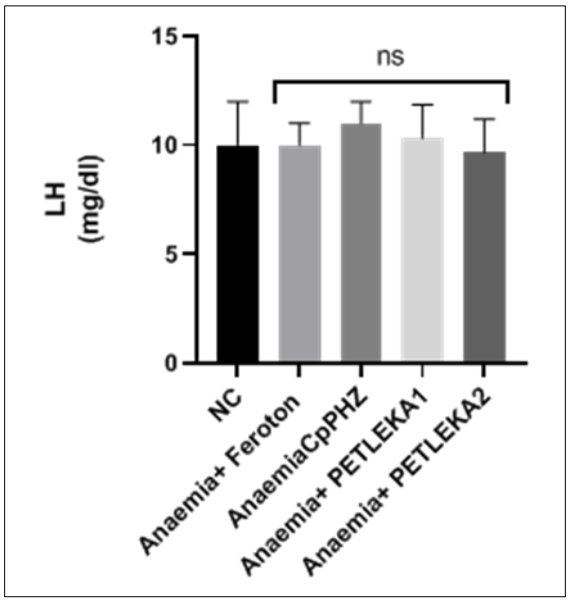
Results were expressed as mean ± SD (n=5) *** significant at P<0.05 compared with the control NC: Normal Control, Standard:Anaemic rats+ Feroton (10mg/Kgbwt), AnaCpPHZ; Anaemic rats control, induced with phenylhydrazine, Anaemia + PETLEKA1: Anaemic rats + Petroleum ether leaf extract of *Kigelia africana* (100mg/Kgbwt) , Anaemic rats + PETLEKA2: Anaemic rats + Petroleum ether leaf extract of *Kigelia africana* (200mg/Kgbwt).

Figure 3 Effect of petroleum ether extract on *K.africana* leaf on serum oestrogen hormones following phenyl hydrazine induced anaemia in Wistar rat



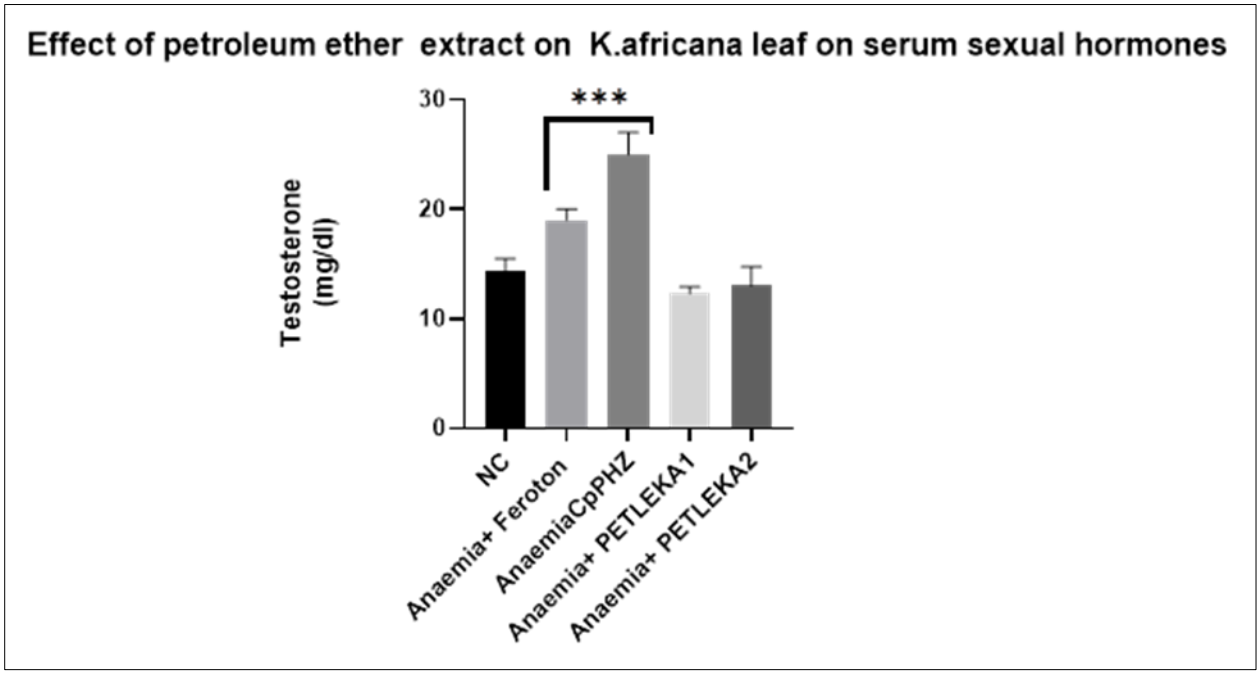
Results were expressed as mean ± SD (n=5) *** significant at P<0.05 compared with the control NC: Normal Control, Standard:Anaemic rats+ Feroton (10mg/Kgbwt), AnaCpPHZ; Anaemic rats control, induced with phenylhydrazine, Anaemia + PETLEKA1: Anaemic rats + Petroleum ether leaf extract of *Kigelia africana* (100mg/Kgbwt) , Anaemic rats + PETLEKA2: Anaemic rats + Petroleum ether leaf extract of *Kigelia africana* (200mg/Kgbwt).

Figure 4 Effect of petroleum ether extract on *K.africana* leaf on serum FSH hormones following phenyl hydrazine induced anaemia in Wistar rat



Results were expressed as mean ± SD (n=5) *** significant at P<0.05 compared with the control NC: Normal Control,ns: non-significant. Standard: Anaemic rats+ Feroton (10mg/Kgbwt), AnaCpPHZ; Anaemic rats control, induced with phenylhydrazine, Anaemia + PETLEKA1: Anaemic rats + Petroleum ether leaf extract of *Kigelia africana* (100mg/Kgbwt) , Anaemic rats + PETLEKA2: Anaemic rats + Petroleum ether leaf extract of *Kigelia africana* (200mg/Kgbwt).

Figure 5 Effect of petroleum ether extract on *K.africana* leaf on serum LH hormones following phenyl hydrazine induced anaemia in Wistar rat



Results were expressed as mean ± SD (n=5) *** significant at P<0.05 compared with the control NC: Normal Control, Standard: Anaemic rats+ Feroton (10mg/Kgbwt), AnaCpPHZ; Anaemic rats control, induced with phenyl hydrazine, Anaemia + PETLEKA1: Anaemic rats + Petroleum ether leaf extract of *Kigelia africana* (100mg/Kgbwt) , Anaemic rats + PETLEKA2: Anaemic rats + Petroleum ether leaf extract of *Kigelia africana* (200mg/Kgbwt).

Figure 6 Effect of petroleum ether extract on *K.africana* leaf on serum FSH hormones following phenyl hydrazine induced anaemia in Wistar rat

4. Discussion

In this present study, it was observed that phenylhydrazine significantly ($p<0.05$) decreased the level of prolactin. The decrease may be due to the inhibition of thyrotropin-releasing hormone (TRH) whose mechanism of action is to stimulates prolactin secretion from the anterior pituitary gland thus, necessitating milk production in the females and regulation of sex drive [21].Prolactin has a significant role in the physiology of the breast, especially in females. Lack of prolactin secretion or excessive prolactin secretion may results in clinically significant, pathologic processes. The level of prolactin hormone is imperative for normal lactational capabilities. Imbalances in prolactin levels may also compromise this ability [22]. Therefore, from this present work, there was a significant ($p<0.05$) increase in serum prolactin level following the administration of the extract. This increase may be due to the presence of bioactive compounds in *Kigelia africana* thus, activating galactorrhea (leaking of milk through the nipples) in non-breastfeeding females or males [23].

More so, this present study showed a significant ($p<0.05$) decrease in serum progesterone level following administration of phenyl hydrazine induced anaemia but co-administration of *Kigelia africana extracts,* significant ($p<0.05$) increased serum progesterone level. This increase may be due to the constituent of plant extract thus, preparing the endometrium for the potential of pregnancy after ovulation or contain some bio-constituents or phytonutrients or phyto androgens that might stimulate reproduction [24].Interestingly, this phytonutrient activates progesterone to readily cross the membrane of a target cell and bind to and activate progesterone receptors located within the cytoplasm. Thus. progesterone and receptor complex then transport to the nucleus and binds to DNA, specifically near the promoter regions of genes that contain enhancers, containing hormone response elements. This binding of the complex to the promoter can either enhance or repress transcription, which ultimately alters the production of proteins. In the line of this, progesterone is primarily known as the pregnancy hormone in females, and most of its function relates to maintaining pregnancy specifically by preparing the endometrium, decreasing myometrial contractions for implantation, promoting gestation, and inhibition of lactation during pregnancy [25]. Decrease in progesterone may indicate amenorrhea. The exploitation of plant bioactive components, which are nutraceuticals, can help to control the rise of a female positive factor in couples' infertility resulting from human exposure to toxicants [26].Therefore, from this work, the increase in serum progesterone following the administration of phenyl hydrazine may be due to the presence of the phytochemical constituents such as flavonoids, tannin and saponin thus, necessitating its mechanism of action.

In this present study, it was observed that phenylhydrazine induction significantly ($p<0.05$) decreased the activities of serum LH and FSH. This finding is in accordance with the work of [27] who reported that phenylhydrazine induced anaemia that ultimately lead to deleterious decrease in the level of LH, FSH and testosterone. Hence, FSH stimulates testosterone spermatozoa development and promotes seminiferous tubule formation. FSH is responsible for the development, growth, pubertal maturation and reproductive processes of the human body [28]. Excess secretion of FSH is responsible for early puberty, whereas deficiency causes infertility and underdevelopment of gonads. In furtherance to this, the production and release of follicle stimulating hormone is regulated by the levels of a number of circulating hormones released by the ovaries and testes [29]. This system is called the hypothalamic–pituitary–gonadal axis. Gonadotrophin-releasing hormone is released from the hypothalamus and binds to receptors in the anterior pituitary gland to stimulate both the synthesis and release of follicle stimulating hormone and luteinizing hormone [30]. The released follicle stimulating hormone is carried in the bloodstream where it binds to receptors in the testes and ovaries. Using this mechanism follicle stimulating hormone, along with luteinizing hormone, can control the functions of the testes and ovaries [31]. From this study, the observed increase in serum FSH and LH indicate that the extract may improve secondary sexual characteristics, sexual health and libido. Thus, this work is in accordance with previous study which reported an interaction of aluminium chloride thus, inducing toxicity that leads to decrease in LH and FSH and treatment with *Hymenocardia acida* stem bark extracts significantly ($p<0.05$) elevated the level of L.H, FSH and progesterone [32].

Conversely, the present study shows a significant ($p<0.05$) decrease in the level of oestrogen after the induction of phenyl hydrazine but co-treatment with *K. africana extracts* significantly ($p<0.05$) elevated and restored the serum level of oestrogen. This increase may be due to the presence of bio-active compounds of the extracts thus, enhancing puberty and stimulate the growth of egg follicle or improves sexual health[33].Oestrogen is a steroid hormone associated with the female reproductive organs and is responsible for developing female sexual characteristics [34].

The observed increase in the level of testosterone after the induction of phenyl hydrazine but co-administration of *K.africana extracts* significantly ($p<0.05$) decreased and restored the serum level of testosterone. Testosterone is a male hormone with significant impact on spermatogenesis [35]. Leydig cells of the testicles secrete testosterone, the adrenals and ovaries, and are the most important androgen secreted into the blood [36, 37].Testosterone, deficiency is presented

with delayed puberty or regression of previously established male characteristics that depend on testosterone, such as hair distribution, potency, and libido. An elevated level of testosterone has been associated with a moderate but significant increase in sexual desire and penile function [38]. Clinical data on testosterone revealed that a slightly increased testosterone level in adult males, may enhance sexual desire and arousability [39]. The level of testosterone has been reported to be related to LH and FSH such that increase in the levels of the gonadotropins results in a corresponding increase in testosterone [40]. However, decrease in serum level of testosterone in this study has no adverse effect and has not been clearly defined [41, 42].

5. Conclusion

The result suggests that the extract might contain some phyto androgens or secondary metabolites which might be useful in improving the sexual health and libido of female animals following phenyl hydrazine induced anaemia.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors have declared no conflict of interest.

Statement of ethical approval

Ethical approval for the study was obtained from the Faculty of Basic Medical Science Animal Research Ethical Committee of University of Cross State , Calabar, Nigeria (approval number FBMS/UNICROSS/21/029).

References

[1] Amanatkar, H.R., J.T. ChibnallSeo,J.N.Manepalli andG.T. Grossberg,2014. Impact of exogenous testosterone on mood: a systematic review and meta-analysis of randomized placebo-controlled trials. Annals of Clinical Psychiatry; 26(1): 19–32.

[2] Ayensu, J., R. Annan, H. Lutterodt, A. Edusei and L. S. Peng, 2020. Prevalence of anaemia and low intake of dietary nutrients in pregnant women living in rural and urban areas in the Ashanti region of Ghana. Plos one, 15(1), e0226026.

[3] Gafter-Gvili, A., A., Schechter and B. Rozen-Zvi ,2019. Iron deficiency anemia in chronic kidney disease. Actahaematologica, 142(1), 44-50.

[4] Gafter-Gvili, A., A. Schechter and B ,Rozen-Zvi ,2019. Iron deficiency anemia in chronic kidney disease. Actahaematologica, 142(1), 44-50.

[5] Tabi, K., A. S. Randhawa, F. Choi, Z. Mithani, F.Albers,M. Schnieder, and M. Krausz,2019. Mobile apps for medication management: review and analysis. JMIR mHealth and uHealth, 7(9), e13608.

[6] Melku, M., F. Asrie, E. Shiferaw, B. Woldu, Y. Yihunew, D. Asmelash, and B. Enawgaw,,2018. Knowledge, attitude and practice regarding blood donation among graduating undergraduate health science students at the University of Gondar, Northwest Ethiopia. Ethiopian Journal of Health Sciences, 28(5).

[7] Adish, A. A., S. A. Esrey, T. W. Gyorkos,T. Johns, 1999. Risk factors for iron deficiency anaemia in preschool children in northern Ethiopia. Public health nutrition, 2(3), 243-252.

[8] López, E. N., A. R. Medina, P. A. G Moreno, L. E. Cerdán and Grima E. M ,2016. Extraction of microalgal lipids and the influence of polar lipids on biodiesel production by lipase-catalyzed transesterification. Bioresource Technology, 216, 904-913.

[9] Crawley, E. M,2004. Emotion and performance: Prison officers and the presentation of self in prisons. Punishment & Society, 6(4), 411-427.

[10] Gebreweld, A., N. Ali, R. Ali and T Fisha, 2019. Prevalence of anemia and its associated factors among children under five years of age attending at Guguftu health center, South Wollo, Northeast Ethiopia. PloS one, 14(7), e0218961.

[11] Irwin J , J. T. Kirchner, 2001. Anemia in children. American Family Physician, 64(8), 1379.

[12] De Benoist B M, Cogswell I, EgliE, McLean,.Worldwide prevalence of anaemia 1993-2005; 2008,WHO Global Database of anaemia.

[13] Pellegrini A D, Huberty P D and Jones I,. The effects of recess timing on children's playground and classroom behaviors. American Educational Research Journal, 1995,32(4), 845-864.

[14] Fronticelli C M T, Sanna G C, Perez-Alvarado M, Karavitis A ,Lu L, Brinigar W S. Allosteric Modulation by Tertiary Structure in Mammalian Hemoglobins: introduction of the functional characteristics of bovine hemoglobin into human haemoglobin by five amino acid substitutions . Journal of Biological Chemistry, 1995,270(51), 30588-30592.

[15] Gulmez, H., Y., Akin,Savas, M. M. Gulum, H. Ciftci, S. YalcinkayaandE. Yeni, 2014). Impact of iron supplementation on sexual dysfunction of women with iron deficiency anemia in short term: a preliminary study. The Journal of Sexual Medicine, 11(4), 1042-1046.

[16] Dasofunjo, K., A.A Asuk.and C.I. Nku, 2020.Evaluating the effect of ethanol leaf extract of *Gongronemalatifolium* on some reproductive hormones of male Wistar rats. GSC Biological and Pharmaceutical Sciences 12(03),166-173.

[17] Kumar, V and S. K . Yadav, 2009. Plant-mediated synthesis of silver and gold nanoparticles and their applications. Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology, 84(2), 151-157.

[18] Horton, R and J. F. Tait, 1966. Androstenedione production and interconversion rates measured in peripheral blood and studies on the possible site of its conversion to testosterone. The Journal of Clinical Investigation, 45(3), 301-313.

[19] Odell, W. D., A. F. Parlow, C. M. Cargille and G. T. Ross, 1968. Radioimmunoassay for human follicle—stimulating hormone: physiological studies. The Journal of Clinical Investigation, 47(12), 2551-2562.

[20] Hale, R. W., T. Kosasa, J. Krieger and S. Pepper, 1983. A marathon: the immediate effect on female runners' luteinizing hormone, follicle-stimulating hormone, prolactin, testosterone, and cortisol levels. American journal of obstetrics and gynecology, 146(5), 550-554.

[21] Berger, R. G., J. Shaw and D. decatanzaro, 2008. Impact of acute bisphenol-A exposure upon intrauterine implantation of fertilized ova and urinary levels of progesterone and 17β-estradiol. Reproductive Toxicology, 26(2), 94-99.

[22] Matalliotakis, M., Koliarakis, I., Matalliotaki, C., Trivli, A, Hatzidaki E. (2019). Clinical manifestations, evaluation and management of hyperprolactinemia in adolescent and young girls: a brief review. Acta Biomedical. 90(1):149-157.

[23] Auriemma, R.S, Pirchio, R. De Alcubierre, D., Pivonello, R. and Colao, A. (2019) Dopamine Agonists:. Neuroendocrinology. 109(1):34-41.

[24] Lee, S., C. Kim , H. Youn andK. Choi, 2017. Thyroid hormone disrupting potentials of bisphenol A and its analogues: In vitro comparison study employing rat pituitary (GH3) and thyroid follicularcells. Journal of Toxicology,40:297–304.

[25] Gómez, J.M., C.Weil, M. Ollitrault, P.Y. Le Bail, B. Breton and F Le Gac,1999. Growth hormone (GH) and gonadotropin subunit gene expression and pituitary and plasma changes during spermatogenesis and oogenesis in rainbow trout. Endocrinology, 113:413–428.

[26] Goletiani, N.V., D.R Keith, and S.J. Gorsky, 2007. Progesterone: review of safety for clinical studies. ExpClinPsychopharmacol.15(5):427-44.

[27] Agu,P. C., P. M. Aja, E. U. Ezebuilo, H. A. Ogwoni, E. M.Ezeh, P. C. Oscar-Amobi, A. A. Agbor, O. G. Ani, J. N. Awoke, F. E. Nwite, O. U. Ukachi, O. U. Orji, P.C. Nweke,E. E. Ugbala,G. O. Ewa , I.O. Igwenyi,C. O. Egwu, E.U. Alum, D. C. Chukwu and Famurewa, A. C,2022. Cucumeropsismannii seed oil (CMSO) attenuates alterations in testicular biochemistry and histology against Bisphenol a-induced toxicity in male Wistar albino rats.Heliyon, DOI: 10.1016/j.heliyon.2022.e09162

[28] Lee, S., C. Kim, H Youn,.and K. Choi, ,2017. Thyroid hormone disrupting potentials of bisphenol A and its analogues: In vitro comparison study employing rat pituitary (GH3) and thyroid folliculARcells. Journal of Toxicology,40:297–304.

[29] Jiang, C., F. Zuo, Y. WangH. , Lu, Q. Yang ,and J. Wang, (2017).Progesterone Changes VEGF and BDNF Expression and Promotes Neurogenesis after Ischemic Stroke. Molecular Neurobiology. 54(1); 571–581.

[30] Dasofunjo. K, Nwodo OFC , Ezugwu H.C, Atunka E.A., Onah I.N .(2018). Impact of oral administration of ethanol leaf extract of *Piliostigma thonningii* on fertility hormones of male Wistar albino rats. *Perspectives in Medical Research* .6(2):16-20.

[31] Akingbemi, B.T., C.M. Sottas, A.I. Koulova, G.R. Klinefelter and M.P. Hardy, 2004. Inhibition of testicular steroidogenesis by the xenoestrogenbisphenol A is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat Leydig cells. Endocrinology, 145:592–603

[32] Berger, R. G and J. Shaw, 2008. Impact of acute bisphenol A exposure upon intrauterine implantation of fertilized ova and urinary levels of progesterone and 17β estradiol. Reproductive Toxicology, 26 (2):94– 99.

[33] Kutlubay, R., E.O. Oguz, B. Can, M.C. Guven., Z. Sinik and O.L. Tuncay .2007. Vitamin E protection from testicular damage caused by intraperitonealAluminium. International Journal of Toxicology. 26:297-306.

[34] Yakubu, O. E., O. F. C. Nwodo, C. Imo and H. A. Ogwoni, 2017. Spermatogenic and Haematological effects of Aqueous and Ethanolic Extracts of Hymenocardiaacida Stem Bark on Aluminium Induced Toxicity in Male Wistar Rats. Insights in Biomedicine, 2:1

[35] Cao, X. L., J. Corriveau , S. Popovic, G. Clement, F. Beraldin and G. Dufresne, 2009. Bisphenol A in baby food products in glass jars with metal lids from Canadian markets. *Journal of Agricultural and Food Chemistry*, 57(12): 5345– 5351.

[36] Vandenberg, L.N., T. Colborn and T.B. Hayes, 2012.Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocrinology Review, 33:1–78

[37] Amanatkar, H. R., J. T. Chibnall, B. W. Seo, J. N. Manepalli and G. T. Grossberg, 2014. Impact of exogenous testosterone on mood: a systematic review and meta-analysis of randomized placebo-controlled trials. Ann Clin Psychiatry, 26(1), 19-32.

[38] Mansoureh, E., Mehrdad, M., K. Akbar and P.D. Aliasghar,2016.The effects of frankincense's hydro alcoholic extract on pituitary-gonadal axis in female mice. Journal of Chemical and Pharmaceutical Research.8 (2): 697-700.

[39] Gauthaan, K., P.G. Adaikan and Prasada, R.N.V, 2002. Aphrodisiac properties of *Tribulu sterrestris* extract (Protodioscin) in normal and castrated rats. Life Science.71: 1385-1396.

[40] Vingren, J.L, W.J. Kraemer,Ratamess, N.A, Anderson, J.M, Volek, J.S and C.M Maresh, ,2010. Testosterone physiology in resistance exercise and training: the up-stream regulatory elements. Sports Medicine.40(12): 1037–1053.

[41] Valdes-Socin, H., A.F, Daly and A. Beckers, 2017.Luteinizing Hormone Deficiency: Historical Views and Future Perspectives. Austin Andrology.2(1): 1015-1020.

[42] Jameson, J.L and L.J. De Groot ,2015. Endocrinology: Adult and Pediatric E-Book. Elsevier Health Sciences. 2: 21-79.