Histological and Hormonal Study of the Effects of Ethanol Extract of *Chrysophyllum albidum* on Ovaries of Female Sprague-Dawley Rats

Idaguko C. Anna[1], Okoko E. Ini-Ibehe[2], Sanyaolu A. Omolara[2] and Abasiekong S. Richard[3]

[1]Department of Anatomy, Faculty of Basic Medical Sciences. Edo State University Uzairue, Edo State. Nigeria.

[2]Department of Anatomy, Faculty of Basic Medical Sciences. University of Lagos. Lagos State.

[3]Department of Anatomy, Faculty of Basic Medical Sciences, Madonna University. Elele. Rivers State. Nigeria

Corresponding author: Idaguko C. Anna (+ 234-(0)805-491-9134, annachi67@yahoo.com).

ABSTRACT

BACKGROUND: Some plants can affect female reproductive health either positively or negatively.

OBJECTIVE: To investigate the effects of ethanol leaf extract of *Chrysophyllum albidum* on the follicle-stimulating hormone, luteinising hormone, estrogen, progesterone, and histology of the ovary of adult female rats.

MATERIALS AND METHODS: Fifteen female rats were grouped into A, B and C. Group A was the control and the animals were given distilled water orally, while groups B, and C were administered with ethanol leaf extract of *Chrysophyllum albidum* 250 and 500 mg/kg/ body weight daily for 30 days respectively. On day 31, the animals were euthanised by diethyl ether anaesthesia and blood samples were collected for serum hormonal assay. The ovaries were processed for histology.

RESULTS: Levels of Progesterone, FSH, Estrogen, and LH of the extract-treated groups were significantly decreased ($P<0.05$) when compared with the control group. Furthermore, the decrease observed was in a dose-dependent manner. Histological sections of the ovary of the rats after treatment with *C. albidum* in groups B and C showed the granulosa cells were not well differentiated and there was delayed maturation of ovarian follicles.

CONCLUSION: This study showed that *C. albidum* may possess anti-fertility agents as observed by the decreased in reproductive hormones and delay in folliculogenesis. Therefore, *C. albidum* leaf extract could be explored as a potential source of contraceptive for female.

KEYWORDS *Chrysophyllum albidum*, ovary, histological, hormones, Sprague-Dawley

INTRODUCTION

The plant *Chrysophyllum albidum* (*C. albidum*) is widely distributed in the Southern part of Nigeria (Idowu *et al*., 2006) and it is well known as the African star apple. It belongs to the family of Sapotaceae and studies have reported on the potential health benefits of the different parts of this plant such as the bark in the treatment of malaria, the leaf for treating skin rash, stomach-ache, frequent and watery bowel, and the roots for sprains, bruises, and wounds (Idowu *et al*., 2006; Anang *et al.,* 2019). Its cotyledon, fruit peel, and leaf possess anti-hyperglycaemic, anti-hypolipidemic, antimicrobial, anti-nociceptive, anti-inflammatory, antioxidant and antiplatelet properties (Adebayo *et al*., 2006; Omoboyowa *et al*., 2016; Asagba *et al*., 2019; Ajayi *et al*., 2020). *C. albidum* exhibits its antioxidant potentials by decreasing lipid peroxidation and increasing endogenous antioxidant enzymes level thereby, making it a good scavenger of free radicals in the body (Burits and Bucar, 2002; George *et al*., 2018). In diabetic rats, the leaf extract of *C. albidum* reduces the blood glucose and lipid level (Idaguko *et al*., 2017; Idaguko *et al*., 2018). Reports showed that the root bark as well as the leaf extract inhibit the

production of FSH, Luteinizing and Testosterone hormones in albino male rats respectively (Onyeka *et al*., 2012; Onyeka *et al*., 2013), also the fruit of *C. albidum* altered the testicular functions in male rat (Ogunwole and Mangai, 2019).

Globally, about 20% of reproductive age couples are faced with issues related to infertility (Coussa *et al*., 2020) and female-related factors account for about 50% of causes of infertility in these couples (Agarwal *et al*., 2015). Hormones are key in the regulation of female reproductive functions and any imbalance in the production of these reproductive hormones can negatively impact sexual and fertility abilities (Naveed *et al*., 2015; Obi *et al*., 2018). The use of plants in the treatment of various medical ailments has enjoyed some level of positivity. However, studies have

also reported its negative effect on various organs in the body inclusive of reproductive organs. Example of such plants with antifertility potential in females includes *Nelumbo nucifera, Achyranthes aspera, Renshen (Radix Ginseng), Heracleum persicum, Mentha spicata, Camellia sinensis,* and *Foeniculum vulgar* (Shibeshil *et al*., 2006; Mutreja *et al*., 2008; Raj *et al*., 2011; Farahbod and Soureshjani, 2018; Ashkar *et al*., 2020). Hence, there is a need to screen medicinal plants for its toxicological outcome on fertility (Yama *et al*., 2011). This study aimed at evaluating the effect of ethanol leaf extract of *C. albidum* on the female reproductive hormones and ovarian folliculogenesis in *Sprague-Dawley* rats.

MATERIALS AND METHODS

Collection and Identification of Plant

This *Chrysophyllum albidum* fresh leaves were collected from the Madonna University Garden, Elele, Rivers State. These leaves were taken to the Department of Botany of the University of Lagos for identification and authentication. The voucher specimen was deposited in the herbarium of the Department of Botany of the University of Lagos for reference with accession number LUH- 7458.

Extraction of Plant Material

The leaf material of *C. albidum* was dried for two weeks under direct sunlight, grounded into a powder form, and weighed using Wiggen Hauser top loading balance (Model ES 2000-2 Serial no 12930, U.S.A). The grounded leaves of 1000 g were completely soaked in 5 L of 95 % ethanol in a covered glass jar for 4 days at room temperature to separate the soluble components of the extract. After the separation had occurred the extract was filtered using a muslin cloth and Whatman No. 1 filter paper. It was concentrated at 50 °C using a rotary evaporator (BUCHI R-215 Switzerland) and transferred into an oven at 40 °C for further concentration to give a yield extract of 84 g. The extract was kept in a sterilized universal glass bottle and stored in a refrigerator at 4°C until when needed.

Experimental animal

Fifteen female adult rats weighing between 170-200 g, were procured from the animal house of the University of Port Harcourt, Rivers State. The animals were kept in cages in a standard and well-ventilated animal house with a temperature range of 28-31 °C; a photoperiod of 12 h natural light and 12 h dark with humidity 50-55%. All rats were fed with standard rat chow and water was made available *ad libitum*. The Experiment was approved by Health Research Ethics Committee (HREC) of the College of Medicine, University of Lagos with ethical number CMUL/HREC/05/16/011. The animals were handled humanely in line with the Guide for the Care and Use of Laboratory Animals (2011).

Dosage, route and duration of administration of test solutions

The acute oral toxicity study of the leaf extracts of *C. albidum* was carried out to determine the LD50 according to the method described by Lorke (1983). The LD50 was far above the dosage used in this study. The dosage of the ethanol leave extract of *C. albidum* that was used was calculated based on a pilot study (Idaguko *et al*., 2017), and was administered orally via the use of cannula daily for thirty days.

Experimental Design

Animals were divided into three groups of five animals each. Group A is the control group and animals in this group received distilled water (1.5 ml kg/ of body weight). Group B and C are the

experimental groups that received *C. albidum* extract daily (250 and 500 mg/kg/ of body weight respectively) throughout the period of the experiment.

Experimental procedure

The rats were weighed before the commencement of the experiment and 24 hours before the expiration of the experimental period. All the rats from each group were starved for 12 hours before the time of sacrifice on the thirty-first day. Blood samples were obtained from the orbital venous sinus with a micro haematocrit tube inserted into the medial canthus of the eye between 7- 8 a.m. and were immediately spurned at 3,000 rpm for 10 minutes. Separation of the serum was done, and it was frozen at -20°C until needed for hormonal assays. Animals were euthanized after anaesthetising them with diethyl ether. The animals were carefully dissected through a ventral abdominal transverse incision to remove the reproductive tube where the ovaries were separated from the spiral oviduct tube and carefully trimmed of fat. Weights of the right and left ovaries were measured using Mettler Toledo weighing balance with model number-AL204, Switzerland.

Hormone assays

Assay kits for progesterone, estrogen, follicle-stimulating hormone, and luteinizing hormone were supplied by Wkea Med Supplies Corp, Changchun, Jilin, China. The protocol that was used for analysis was as described by the kit producers (Wkea Med Supplies Corp, China). The assays employed competitive inhibition enzyme immunoassay technique using enzyme immunoassay ELISA kits. All other reagents used were of analytical grade.

Histological studies

The ovary specimens were fixed in Bouin's solution for 24 hours at room temperature, followed by its transfer into an ascending grade of alcohol for dehydration. The tissues were treated by clearing in xylene before been embedded in molten paraffin wax. Tissue sections of 5µm in thickness were cut from each paraffin block using a microtome (Thermo scientific Micron HM 325 USA) in preparation for the staining process, using Haematoxylin and Eosin (H & E) stain.

Statistical analysis

Data obtained from this study were analysed using GraphPad Prism software, version 5.0 (GraphPad Software, La Jolla, CA, USA). A one-way analysis of variance (ANOVA) followed by Bonferroni post-test was done on the data. Statistically, the significant level was considered at $P < 0.05$ and the results obtained were represented as the mean ± SD ($n = 5$).

RESULTS

Effect on body and organ weight

The effect of ethanol leaf extract of *C. albidum* on the body and ovarian weight is illustrated in Table I and II, respectively. In the control animals, the final body weight was increased when compared to their initial body weight. Rats treated with *C. albidum* (250 and 500 mg /kg of body weight) showed a statistically significant increase in their final body weight ($P < 0.05$) compared to their initial body weight. No physical signs of toxicity were observed in rats of all the different groups. The relative ovarian weight of the group B and C treated with the extract (250 and 500 mg/kg of body respectively) was reduced when compared to the ovaries of rats in control group.

Serum hormonal levels

There was a statistically significant ($P<0.05$) decrease in the serum Progesterone, LH, FSH and Estrogen levels of animals in Group B and C when compared with the serum levels of the same hormones in rats from the control group as shown in Table III.

TABLE I. Effect of leaf extract of C. albidum on body weight of female rats

	Group A Control	Group B 250 mg of *C. albidum*	Group C 500 mg of *C. albidum*
Initial weight	170.2 ± 2.4	176.8± 1.6	178.3 ± 6.8
Final weight	195.0 ± 1.5	218.2± 3.2*	213.5 ± 3.9*

***: Statistically significant, P<0.05 compared with the control. Data recorded as (Mean ± Standard Deviation)**

TABLE II. Effect of leaf extract of C. albidum on body weight of female rats

Organ	Group A (control)	Group B (250 mg)	Group C (500 mg)
Right Ovary	1.02 ± 0.02	0.92 ± 0.07	0.80 ± 0.06
Left Ovary	1.04 ± 0.02	0.88 ± 0.07	0.84 ± 0.09

***: Statistically significant, P<0.05 compared with the control. Data recorded as (Mean ± Standard Deviation).**

TABLE III. Effect of leaf extract on *C. albidum* on hormonal profile

Hormones	Group A Control	Group B 250 mg of *C. albidum*	Group C 500 mg of *C. albidum*
FSH (miU/ml)	1.41 ± 0.06	0.88 ± 0.02*	0.66 ± 0.03*
LH (miU/ml)	3.34 ± 0.05	2.71 ± 0.10*	2.08 ± 0.05*
Progesterone (pg/mL)	50.27 ± 1.72	39.55 ± 1.20*	21.43 ± 1.06*
Estrogen (pg/mL)	129.08 ± 1.01	88.00 ± 2.87*	48.00 ± 7.89*

***: Statistically significant, P<0.05 compared with the control. Values represent (Mean ± Standard Deviation). FSH= Follicle Stimulating Hormone, LH = Luteinizing Hormone.**

Histological observation of the ovary

The ovarian histological examination of the control (Plate 1) shows a matured Graafian and primary follicle at the cortical edge is seen, and a matured follicle that has distinct theca arrangement around the follicle. The granulosa cells are well differentiated into corona radiata around the egg, cumulus oophorous, and membrane granulosa. Also, a large antrum is seen. There is a meshwork of connective tissue with a vascular network in pigmentation at the central medulla. At the dose of 250 mg/kg of body weight (Plate 2), the follicle is not so distinct, and this is a deviation from normal. Although there is a well-aligned theca arrangement, the granulosa cells are not well differentiated and at the dose of 500 mg/kg of body weight (Plate 3), the ovary has a few follicles at the

primary stage of development. There was no distinct mature follicle, hence folliculogenesis is slow.

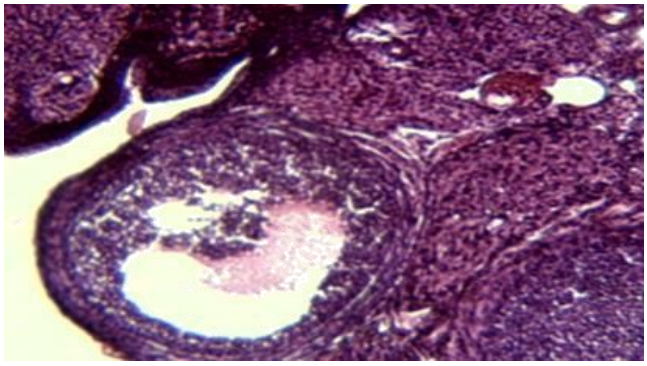

PLATE 1. Photomicrograph of ovary section from Normal control group showing matured graffian follicle

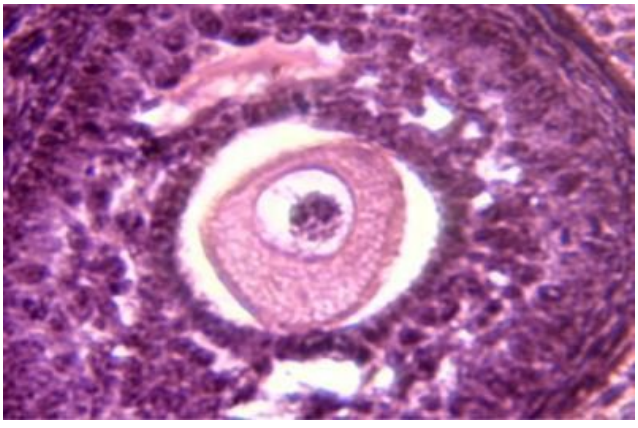

PLATE 2. Photomicrograph of ovary section from 250 mg/kg BW of ethanolic leaf extract of C. albidum treated group showing a deviation from normal; the follicle is not so distinct

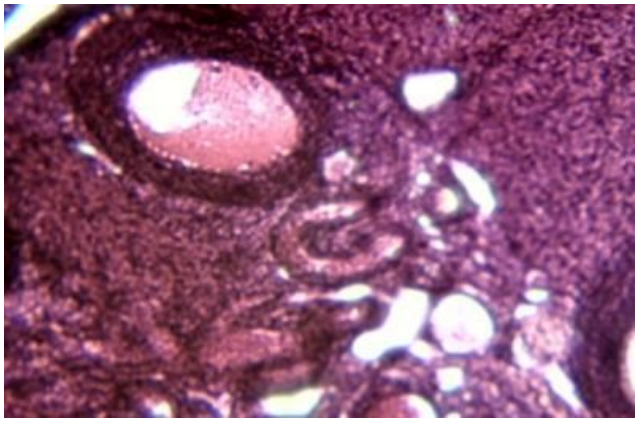

PLATE 3. Photomicrograph of ovary section from 500 mg/kg BW of ethanolic leaf extract of C. albidum treated group showing a number of follicles at the primary stage of development

DISCUSSION

The use of medicinal plant in the treatment of various diseases in different part of the world is well reported and about 80% of the world's population still depends majorly on traditional or herbal medicine for the treatment of diseases especially in African and other developing nations (Okoye *et al*., 2014). While most of these medicinal plants have great benefits to human health, some of these plants are potential toxic agents to both humans and animals with a significant adverse effect on some organs of the body. This study showed the toxicological impact of *C. albidum* on the serum reproductive hormones level in a dose-dependent manner. 7

The increase observed in the bodyweight of the treated groups disagree with the finding of *Shobo et al*., (2019) who reported a significant decrease in the body weight of rats treated with methanol leaf extract of *C. albidum.* The observed reduction in the organ weight in this study was in line with the findings of Osebhahiemen and Omoregie, (2018) that reported a decrease in the organ weight of animals treated with ethanol leaf extract of *C. albidum.* Shobo *et al*., (2019) also reported that the ethanolic and butanoic extract of *C. albidum* decreases the organ weight. The decrease in the ovarian weight can be associated with the low level of reproductive hormones in groups treated with *C. albidum* because changes in the weight of an organ is a good barometer of chemically induced changes in the body (Woldemeskel, 2017). Moreover, hormones are chemical compounds secreted by different endocrine glands of the body and are needed to maintain various metabolic processes in the body (Roop, 2018). 18

The gonadotrophins (FSH and LH) plays a major role in the complete maturation of ovarian follicles and its development, and any decrease in serum level of these hormones will affect the follicle development (Raju *et al*., 2013) as reported in this study. LH stimulates the secretion of steroid hormone (testosterone) and progesterone. Testosterone is converted to estrogen by the adjacent granulosa cells, and progesterone regulates the monthly menstrual cycle, and also get the body ready for conception, pregnancy and stimulate sexual desire (Montaserti *et al*., 2007; Yakubu *et al*., 2008). The estrogen that is been produced binds with the estrogen receptors which are key in the maintenance of ovarian granulosa cell differentiation, follicles development, oocyte growth and even ovulation (Taijima *et al*., 2007). 27

The female reproductive system is a complex system that involves a feedback mechanism in its hormonal regulation. The FSH and LH are released from the anterior pituitary gland via the influence of Gonadotropin-releasing hormone (GnRH) which is secreted from the hypothalamus. This GnRH is also been regulated by estrogen that is present in the ovary hence, being referred to as the hypothalamic- pituitary-ovary axis (Skinner *et al*., 2008; Stamatiades and Kaiser, 2018). Therefore, any imbalance in one of these hormones

that is being produced have a spiral effect on the activities of the others.

Studies have shown the presence of phytoestrogen in various medicinal plants and these phytoestrogens mimic the estrogen hormones in the body. Its activities in the body can sometimes be estrogenic or antiestrogenic, which can alter the reproductive activities at different regulatory levels (Benassayag *et al*., 2002). The phytochemical screening of the *C. albidum* revealed the presence of alkaloids, tannins (naturally occurring polyphenol), flavonoids, terpenes, and glycosides (Emudainohwo *et al*., 2015).

The result of this study shows a decrease in the level of LH, FSH, Estrogen, and Progesterone in the treated groups which could be a consequential impact of phytoestrogenic effect of *C. albidum* in all the treated groups. This hormonal imbalance may account for the poor follicular development as seen in plate 3. The reported effect is similar to that observed in the use of some other medicinal plant such as methanol extract of *Rumex steudelii* and aqueous extracts of *Allium porrum* leaves which significantly decrease the number and growth of the developing follicles (Solomon *et al*., 2010; Al-Shaibani *et al*., 2014). Flavonoids and alkaloids have been implicated in the reduction of the serum concentrations of Estrogen, LH and FSH (Asuquo *et al*., 2013). These results agreed with the effect of some other medicinal plants that causes reduction in the level of LH and FSH (Yakubu *et al*., 2008; Asuquo *et al*., 2013). Alkaloids are also known to inhibit cellular progesterone synthesis (Gucze *et al*., 1996). Plants that have estrogenic property may influence the action of the pituitary gland to reduce the secretion of these hormones (FSH and LH) by peripheral modulation thereby, obstructing ovulation (Banerjee *et al*., 1999; Al-Qarawi *et al*., 2000) as observed in this study.

In addition, the dose and duration of treatment may play a role in the further decreased that was observed in Progesterone, FSH, Estrogen, and LH levels in the extract treated groups. Further studies will be required to fully understand the mechanism of the action of *C. albidum* on the female reproductive organs.

CONCLUSION

The ethanol extract of the leaf of *C. albidum* alters the histomorphology of the ovary and reduced the serum levels of Follicle-Stimulating Hormones, Luteinizing Hormones, and Progesterone and Estrogen hormones in the female rats. There is a possibility that the effect of the *C. albidum* may have been at a level of the pituitary-gonadal axis and the activity of the leaf extracts on the reproductive system of the female may offer hope in its usage as a possible contraceptive.

AUTHOR'S CONTRIBUTION

Conception and the study design: ICA, OEI, and SAO. Experimental studies and data acquisition: ICA. ASR, and OEI. Data acquisition, data analysis, and statistical analysis: ICA, ASR, and SAO. Manuscript preparation, manuscript editing, and manuscript review: ICA, OEI, SAO, and ASR. Finally, the manuscript was approved by all the authors. Responsibility for the integrity of the work as a whole from inception to published article: ICA

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

REFERENCES

1. Adebayo H.A., Aliyu R., Gatsing D., Garba H.I. (2006). The effects of ethanolic leaf extract of *Commiphora africana* (Burseraceae) on lipid profile in rats. Int. J. Pharmacol, 2(6), 618-622.
2. Agarwal A., Mulgund A., Hamada A., Chyatte M.R. (2015). A unique view on male infertility around the globe. Reprod. Biol & Endo, 13(1), 37.
3. Ajayi A.M., Diya O.O., Adedapo A.D.A. (2020). Hypolipidemic Effect of Chrysophyllum albidum Peel Extract and Its Underlying Antioxidant Mechanisms in Normal and Triton-X-100-Induced Hyperlipidemic Rats. J. dietary Suppl, 17(4), 365-383.
4. Al-Qarawi A.A., Abdel-Rahman H.A., El-Badry A.A., Harraz F., Razig N.A., Abdel-Magied E.M. (2000). The effect of *Cynomorium coccineum* and *Withania somnifera* on gonadotrophins and ovarian follicles of immature Wistar rats. Phytother Res, 14: 288-290.
5. Al-Shaibani S.W., AL-hashimhi W.H., Bshibash H.S. (2014). Histological Study of Aqueous Extracts Leek *Allium porrum L*. in Female Reproductive System of Laboratory White Rats. J. Nat Sci Res, 4:19
6. Anang M.A., Oteng-Peprah M., Opoku-Boadu K. (2019). Extraction and characterisation of African Star Apple (Chrysophyllum albidum) seed oil and the adsorptive properties of the fruit shell in Ghana. Int. J. Food Sci., 2019.
7. Asuquo O.R., Oko, O.O. K., Brownson E.S., Umoetuk G.B., Utin I.S. (2013). Effects of ethanolic leaf extract of Spondias mombin on the pituitary–gonadal axis of female Wistar rats. Asian Pacific J. Reprod, 2(3), 169-173.
8. Asagba S.O., Kadiri H.E., Ezedom T. (2019). Biochemical changes in diabetic rats treated with ethanolic extract of Chysophyllum albidum fruit-skin. JoBAZ 80(1): 42.
9. Coussa A., Hasan H.A., Barber T.M. (2020). Impact of contraception and IVF hormones on metabolic, endocrine, and inflammatory status. J. Assisted Reprod. & Gene. 1-6.
10. Banerjee R., Pal A.K., Kabir S.N., Pakrashi A. (1999). Antiovulatory faculty of the flower of Malvaviscus conzattii. Phytotherapy Research: An Int. J. Devoted to Pharma and Toxic Evaluation of Nat Prod Derivatives, 13(2), 169-171.
11. Benassayag C., Perrot-Applanat M., Ferre F. (2002). Phytoestrogens as modulators of steroid action in target cells. J. Chromatogr B, 777(1-2), 233-248.
12. Burits M., Bucar F. (2002). Antioxidant activity of Chrysophyllum albidum essential oil. Phtother Res, 14, 323-328.
13. Emudainohwo JOT., Erhirhie E.O., Moke E.G., Edje K.E. (2015). A comprehensive review on ethno-medicine, phytochemistry and ethnopharmacology of Chrysophyllum albidum. J. Adv in Med & Pharma Sci, 147-154.

14. Farahbod F., Soureshjani H. (2018). Medicinal Herbs Affecting Gonadotropin Hormones in Women: An Updated Systematic Review. Review. Int. J. Life Sci & Pharma Res, 8(1): 20-28.
15. Fatemeh A., Shahla R., Sara S., Farhad V., Maryam G., Samaneh M. D., Saeid D. (2020). The Role of medicinal herbs in treatment of insulin resistance in patients with Polycystic Ovary Syndrome: A literature review. BioMol Concepts, 11: 57–75
16. Ashkar F., Rezaei S., Salahshoornezhad S., Vahid F., Gholamalizadeh M., Dahka S.M., Doaei, S. (2020). The Role of medicinal herbs in treatment of insulin resistance in patients with Polycystic Ovary Syndrome: A literature review. Biomol Concepts, 11(1), 57-75.
17. George O.A., Adenipekun E.O., Fasogbon S.A., Oparanozie J.A. (2018). Antimicrobial activities of Chrysophyllum albidum leaves, fruits and seeds. American J. Biomed Sci, 10(1).
18. Gucze P.M., Porpaczy Z., Freeman D.A. (1996). Effect of alkaloids in cigarette smoke on human granulosa cell progesterone synthesis and cell viability. Gynecol Endocrinol, 10(4), 223-228.
19. Raju G.A.R., Chavan R., Deenadayal M., Gunasheela D., Gutgutia R., Haripriya G., *et al.,* (2013). Luteinizing hormone and follicle stimulating hormone synergy: A review of role in controlled ovarian hyper-stimulation. J Hum Reprod Sci, 6(4): 227-234
20. Idaguko C.A., Duru F.I.O, Oremosu A.A. (2017). Antioxidant, hypolipidemic and Hypoglycemic Effect of Ethanol Leaf Extract of *Chrysophyllum albidum* on Streptozotocin- Induced Diabetic Rats. J Anat Sci, 8(1) 67-73
21. Idaguko C.A., Oremosu A.A., Duru F.I.O., Awopetu P.I. (2018). Protective Effect of Ethanolic Leaf Extract of *Chrysophyllum albidum* (Sapotaeae G. Don) on Histological Changes in the Pancreas of Streptozotocin-induced Diabetic *Sprague Dawley* Rats. J Anat. Sci, 9(1): 1-6
22. Idowu T.O., Iwalewa E.O., Aderogba M.A., Akinpelu B.A., Ogundaini, A.O. (2006). Biochemical and behavioural effects of eleagnine from Chrysophyllum albidum. J. Biol. Sci, 6, 1029-1034.
23. Lorke D. (1983). A new approach to practical acute toxicity testing. Archives of toxicology, 54(4), 275-287.
24. Montaseri A., Pourheydar M., Khazaei M., Ghorbani R. (2007). Anti-fertility effects of Physalis alkekengi alcoholic extract in female rat. Iranian J Reprod, 5: 13- 16.
25. Mutreja A., Agarwal M., Kushwaha S., Chauhan A. (2008). Effect of Nelumbo nucifera seeds on the reproductive organs of female rats. Iran J Reprod Med, 2008; 6:7–11.
26. Institute for Laboratory Animal Research. (2011) Guide for the Care and Use of Laboratory Animals. 8th Ed. pg. 15. (National Academies Press, Washington, DC).
27. Naveed S., Ghayas S., Hameed A. (2015). Hormonal imbalance and its causes in young females. JIPBS, 2(1), 12-16.
28. Obi C.C., Patrick-Iwuanyanwu K.C., Onyeike E N. (2018). Effect of Ethanol Extract of Leaves of Chrysophyllum albidum on the Reproductive Hormones of Lead-Exposed Female Wistar Albino Rats. J Appl Sci & Environ Manage, 22(9), 1503-1509.
29. Ogunwole E., Mangai E.U. (2019). The effect of Chrysophyllum albidum fruit on testicular functions in rats. *Andrologia*, *51*(7), e13307.
30. Okoye T.C., Uzor P.F., Onyeto C.A., Okereke E.K. (2014). Safe African medicinal plants for Clinical studies. In Toxicological Survey of African Medicinal Plants (pp. 535-555). Elsevier.
31. Omoboyowa A.D., Ephraim C.I., Glory O.C., Kalu D.O. (2016). Anti-diabetic activity of methanolic extract of seed cotyledon of Chrysophyllum albidum in alloxan-induced diabetic rats. Nigerian Soc for Exp Biol. 28(2): 88–95
32. Onyeka C.A., Aligwekwe A.U., Olawuyi T.S., Nwakanma A.A., Kalu E.C., Oyeyemi A. W. (2012). Antifertility effects of ethanolic root bark extract of Chrysophyllum albidum in male albino rats. Int. J Appl Res Nat Prod, 5(1), 12-17.
33. Onyeka C.A., Fabunmi O.O., Aligwekwe A.U., Ofoego U.C., Leko B., Anthony O.A. (2013). Ethanolic Leaf Extract of *Chrysophyllum albidum* on sperm analysis, hormonal profile, SOD and testicular histology of adult male wistar rats. Agric. Biol. J. N. Am, 4(3): 160-165
34. Osebhahiemen O.I., Omoregie E.S. (2018). The Antihyperlipidaemic Property of Methanol Leaf Extract of Chrysophyllum albidum in Albino Rats (Wistar Strain) Fed on High Fat Diet. J. Adv. Bio, 11(1):2146-2154
35. Raj A., Singh A., Sharma A., Singh N., Kumar P., Bhatia V. (2011). Antifertility activity of medicinal plants on reproductive system of female rat. Int. J. Bio-Eng. Sci. Technol, 2(3): 44-50
36. Roop J. K. (2018). Hormone imbalance—A cause for concern in women. RJLBPCS, 4(2): 237.
37. Shibeshil W., Makonnen E., Debella A., Zerihun L. (2006). Phytochemical, contraceptive efficacy and safety evaluations of the methanolic leaves extract of Achyranthes aspera L. in rats. Pharmacol, 3: 217-224.
38. Shobo A.A., Daniyan M.O., Olayiwola G., Idowu T.O., Ogundaini A.O., Adesanya S.A. (2019). Toxicity Evaluation of the Extract and Fraction of Chrysophyllum Albidum Seed Cotyledons in Rats. SOJ Pharm Sci, 6(1) 1-12.
39. Skinner M.K., Schmidt M., Savenkova M.I., Sadler-Riggleman I., Nilsson E.E. (2008). Regulation of granulosa and theca cell transcriptomes during ovarian antral follicle development. Mol Reprod Dev, 75(9), 1457-1472.
40. Solomon T., Largesse Z., Mekbeb A., Eyasu M., Asfaw D. (2010). Effect of Rumex steudelii methanolic root extract on ovarian folliculogenesis and uterine histology in female albino rats. Afr Health Sci, 10(4): 353-361
41. Stamatiades G.A., Kaiser U.B. (2018). Gonadotropin regulation by pulsatile GnRH: signaling and gene expression. Mol. Cell Endocrinol, 463, 131-141.
42. Tajima K., Orisaka M., Mori T., Kotsuji F. (2007). Ovarian theca cells in follicular function. Reprod. Biomedicine online, 15(5), 591-609.
43. Woldemeskel, M. (2017). Toxicologic pathology of the reproductive system. In Reprod Develop. Toxicol. Academic Press. (pp. 1209-1241).
44. Yakubu M.T., Akanji M.A., Oladiji A.T., Olatinwo A.O., Adesokan A.A., Yakubu M.O., *et al*., (2008). Effect of Cnidoscolous aconitifolius (Miller) IM Johnston leaf extract on reproductive hormones of female rats. Iranian J. Reprod Med, 6 (3):149-155
45. Yama O.E., Duru F.I., Oremosu A.A., Noronha C.C., Okanlawon A.O. (2011). Stereological evaluation of the effects of Momordica charantia, antioxidants and testosterone on seminiferous tubules of rat. Int. J. Morphol, 29(3), 1062-8.