International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 11, Issue 1-2024

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2024.11.01.007

Variations in Serum Progesterone and Estradiol Levels in Proestrus Rats Administered Extracts from *Alstonia boonei* Stem Bark

Spencer. C. NWANGWU[*1], OMOREGIE E.S[2], Sunday J. JOSIAH[1]., Isaac S. MOMOH[*3].

[1]Department of Biochemistry, College of Health Sciences, Igbinedion University, Okada, Edo State.

[2] Department of Biochemistry, University of Benin, Benin City, Edo State.

3. Department of Biochemistry, Faculty of Science Confluence University of Science and Technology, Osara Kogi State.

[*]Corresponding author:

Spencer. C. Nwangwu., e-mail: *nwangwu.spencer@iuokada.edu.ng* Mobile phone: +2348037511008;

MOMOH, I.S., e-mail: *momohis@custech.edu.ng* Mobile phone: +2348169333248

Abstract

In this part of the world, traditional healers enjoy patronage for treatment of infertility and the use *Alstonia boonei* stem bark amongst others is prevalent. The effects of aqueous and ethanol extracts of *A. boonei* stem bark on serum progesterone, estradiol, uterine and ovarian weights of proestrus rats were evaluated as indices for infertility treatment. Rats with regular estrous cycle were randomly selected into eight (8) cages of five rats. Extracts of *A. boonei* stem bark were administered orally at concentrations of 250mg/kg, 150mg/kg and 75mg/kg, for fifteen (15) days. The control groups were administered normal saline. The animals were sacrificed at the proestrus phase after 15 days of extracts administration and blood samples collected for hormonal assays. The results obtained showed a concentration dependent increase in serum progesterone levels of animals administered aqueous extract of *Alstonia boonei* stem bark that was significant ($P<0.05$) in the 250mg/kg group (14.82ng/ml) when compared with the control. The ethanol extract group also revealed concentration dependent increase in serum estradiol levels significant ($P<0.05$) in the $150mgkg^{-1}$ and $250mgkg^{-1}$ ethanol extract administered animals with 28.14 pg/ml and 33.40pg/ml serum estradiol respectively. There were no significant changes in the ovary and uterine weights. The results suggest that the plant extracts increased the serum progesterone and estradiol in proestrus rats, thus may contain ingredients for infertility treatment.

Keywords: *Alstonia boonei*, Infertility, Progesterone, Estradiol, proestrus

Introduction

Infertility is the inability of a woman to achieve conception after 12months of frequent unprotected sexual intercourse [1]. Infertility also includes the inability to carry a pregnancy to the delivery of a live baby [2] and could either be primary or secondary [3]. In Nigeria, the common causes of infertility include pelvic infection disease (PID), endometriosis, cervical factor, ovarian factor, uterine factor and male factor [2]. Some factors that are more often not emphasized such as age, lifestyle and physical condition also play considerable role to the problem of fertility[1]. Nutrition is another infertility factor that has been accorded little attention. Acute fasting has been found to alter both rabbit's metabolic and endocrine markers and embryo development, though the follicle and oocyte and embryo gene expression were affected [4].

In Nigeria, the belief in the natural and supernatural causes of infertility is widespread as women are frequently ostracized because of fear of their jealouse anger or acclaimed witches and that curse can be placed on either or both of the couple extends from the illiterate to the most educated and elite members of the society. As a consequence of this deep conviction on the natural and supernatural causes of infertility, infertile people often patronize traditional healers and spiritualists very early [5], while the orthodox medical practitioners are mostly consulted only when the religious, spiritual and traditional methods fail to produce the desired results. The role of traditional healer cannot be over emphasized in fertility treatment in Nigeria.

Plants are also used for health conditions notably reproductive health conditions including infertility, abortion, delivery complications, menstrual disorders, miscarriages, family planning and many gynaecological disorders. In this part of the world, medicinal plants play a significant role before and during pregnancy, birth and postpartum care in many rural areas of the world [6]. The use of plants to ensure good development of pregnancy and facilitate labour is a particularly well established practice in Africa [7].

Myriad of plants have been in use for the treatment of infertility in Nigeria from the time of our fore fathers. Such plants include: *Elytraria marginata, Khaya ivorensis, Morinda lucida, Picralima nitida, Spathodea companulata, Spondias mombin, Alstonia boonei, Whitefieldia elongate, Xylopia aethopica* and *Paullinia pinnata. Alstonia boonei* have been cited for infertility treatment in female, [8, 9, 10]. .*Alstonia boonei* is a deciduous plant that is native to the tropics and sub-tropical regions of the world [11]. *Alstonia boonei* De Wild belongs to the family Apocynaceae which consists of about 50 species widely distributed in the continents of Africa, Asia and America. *Alstonia boonei* is known by different names in different cultures and tribal settings. It is commonly known as stool wood, as *Ahun* in Yoruba, *Egbu-ora* in Igbo, *Ukhu* in Edo and *Ukpukunu* in Urhobo. It is not edible as food but possesses root, stems, barks, leaves, fruits, seeds and latex which are claimed to have medicinal properties [12].

Alstonia boonei is extensively used in folk medicine by a reasonable percentage of the population especially in developing nations to treat diseases [12]. It is a widely distributed plant in the lowlands and rain-forest areas of Nigeria. It has also been reported that ethanol extract from *A. boonei* possess antimicrobial properties against *E. coli*, *Salmonella typhi*, *Salmonella paratyphi*, *Shigella disentrae*, *Staphylococcus aureus*, *Klebsiella pneumonae* and *Proteus aureus* [11]. [13] reported that the pulverized stem bark of *A. boonei* reduces the rate of fermentation. The plant showed antipyretic, analgesic and anti – inflammatory properties [14]. The anti-inflammatory activity was widely reported in different animal models demonstrating the ability of the plant extract to inhibit carageanan-induced paw oedema, cotton-pallet granuloma and other rheumatoid arthritis models [15, 16, 17].

This research focuses on the extracts of *Alstonia boonei* stem bark commonly used in the treatment of infertility across the geopolitical zones in Nigeria. The changes in serum estradiol and progesterone levels in proestrus female rats administered aqueous and ethanolic extracts of

Alstonia boonei and stem bark were evaluated as indices in treatment of infertility.

Materials and Methods

Plant Materials

The *Alstonia boonei* stem bark was collected from a farmlands Okada, Benin City, Edo State. The plants was identified and confirmed at the Department of Botany, Igbinedion University Okada, Benin City, Edo state.

Preparation of Extracts

The *Alstonia boonei* stem bark was air-dried, blended and stored until subsequent use. The powdered plant material was soaked in 95% ethanol (1:10w/v) for 24 hours. The resulting supernatant was filtered using Whatman (No.1) filter paper, concentrated in a rotary evaporator to 10% its volume and then lyophilized to give the crude ethanol extract. The powdered plant material was soaked in (1:10w/v) of distilled water for 24 hours. The mixture was then filtered using Whatman (No.1) filter paper and then lyophilized to give the crude aqueous extract.

Experimental Animals

A total of forty (40) female Wistar rats that weighed 120 – 220g were obtained from the animal care facility of the School of Basic Medical Sciences, Igbinedion University, Okada, Nigeria and used for the study. The animals were acclimatized for two weeks and allowed 12 hour light cycle.

Experimental Design

Rats with regular estrous cycle were then randomly selected into 8 cages of five rats each. The ethanolic and aqueous extract of *Alstonia boonei* stem bark were administered to the animals at concentrations of 250mg/kg, 150mg/kg and 75mg/kg for fifteen (15) days. The last two cages with five animals each served as control for the two extracts and were administered normal saline. The animals were sacrificed at the proestrus phase of the rat's estrous cycle after the 15 days duration of extract administration and blood samples were collected for hormonal assays after fasting.

Determination of Estrous Cycle

The estrous cycle of the female rats was determined using method of [18]. While the proportion of epithelial cells, cornified cells and leukocytes were used for the determination of the estrous cycle phases [19].

Hormonal Assay for Progesterone and Estradiol

The serum progesterone and estradiol level were determined using assay kits (Fortress Diagnostics BT41 1QS, United Kigdom).

Statistical Analysis

The results were expressed as mean ± SEM (n = number of animals). One-way Analysis of variance (ANOVA) was used. Significant differences between groups were detected in ANOVA using Bonferroni-Holm posthoc at $p<0.05$.

Results

Effect *Alstonia boonei* extracts on Serum Progesterone Level in Proestrus Female Rats

Figure I represents the serum levels of progesterone in proestrus female rats administered ethanol and aqueous extracts of *Alstonia boonei* stem bark. The results showed a concentration dependent increase in progesterone levels of animals administered aqueous extract of *Alstonia boonei* stem bark. The increase was significant ($P<0.05$) in the 250mg/kg group (14.82ng/ml) when compared with the control. Though there was a significant ($P<0.05$) reductions in serum progesterone level (09.26ng/ml) in animals administered 75mg/kg of ethanol extract of *Alstonia boonei* stem bark when compared with the control, the progesterone levels increased marginally with increasing concentrations of extract administration. The results also showed that the aqueous extract of *A. boonei* increased the serum progesterone the most when compared with the control and the ethanol counterpart.

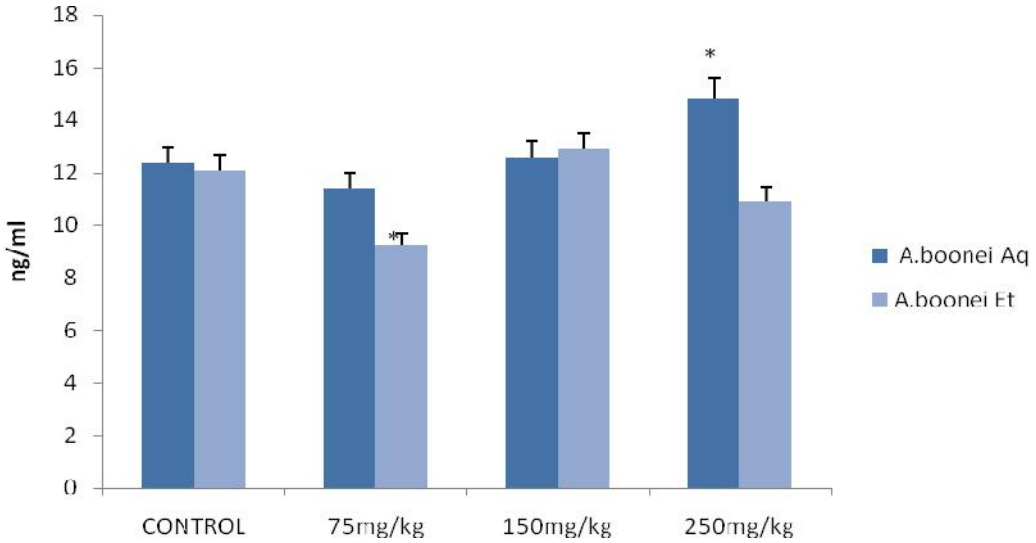

Figure 1: **Serum Levels of Progesterone in Proestrus Female Rats Exposed to Extracts of *Alstonia boonei* Stem Bark**

Values are Mean ± SEM (* = P < 0.05). A.boonei Aq. = Aqueous extract of *Alstonia boonei*; A.boonei Et. = Ethanol extract of *Alstonia boonei.*

Effect of *Alstonia boonei* extracts in Serum Estradiol Level in Proestrus Female Rats

Serum levels of estradiol in proestrus female rats administered ethanol and aqueous extracts of *Alstonia boonei* stem bark are shown in Figure 2. There was concentration dependent increase in serum estradiol levels in animals administered ethanol extracts of *Alstonia boonei* stem bark. The concentration dependent increase in serum estradiol levels were significant (P<0.05) in the 150mgkg^{-1} and 250mgkg^{-1} ethanol extract administered animals with 28.14 pg/ml and 33.40pg/ml serum estradiol respectively. The aqueous extract group revealed similar pattern of concentration dependent increase but was significant (P<0.05) only in the 250mgkg^{-1} group with 27.64pg/ml when compared with the control.

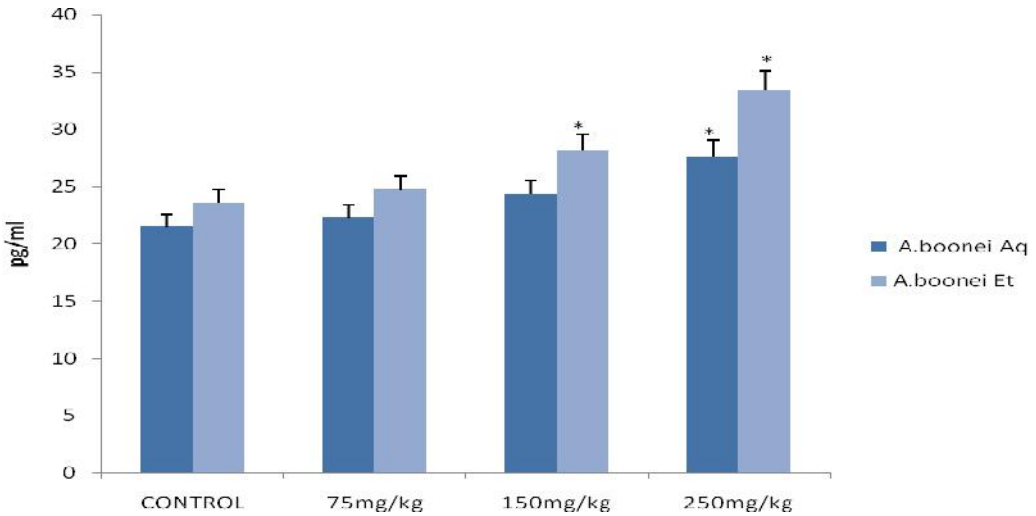

Figure 2: Serum Levels of Estradiol in Proestrus Female Rats Exposed to Extracts of *Alstonia boonei* Stem Bark

Values are Mean ± SEM (* = P < 0.05). A.boonei Aq. = Aqueous extract of *Alstonia boonei*; A.boonei Et. = Ethanol extract of *Alstonia boonei.*

Effect of *Alstonia boonei* extracts on Uterine Weights in Proestrus Female Rats

The weights of uterus of proestrus female rats administered ethanol and aqueous extracts of *Alstonia boonei* stem bark are represented in Figure 3. The uterine weights of animals administered *Alstonia boonei* aqueous and ethanol extracts did not reveal any pattern of change as shown in Figure 3.

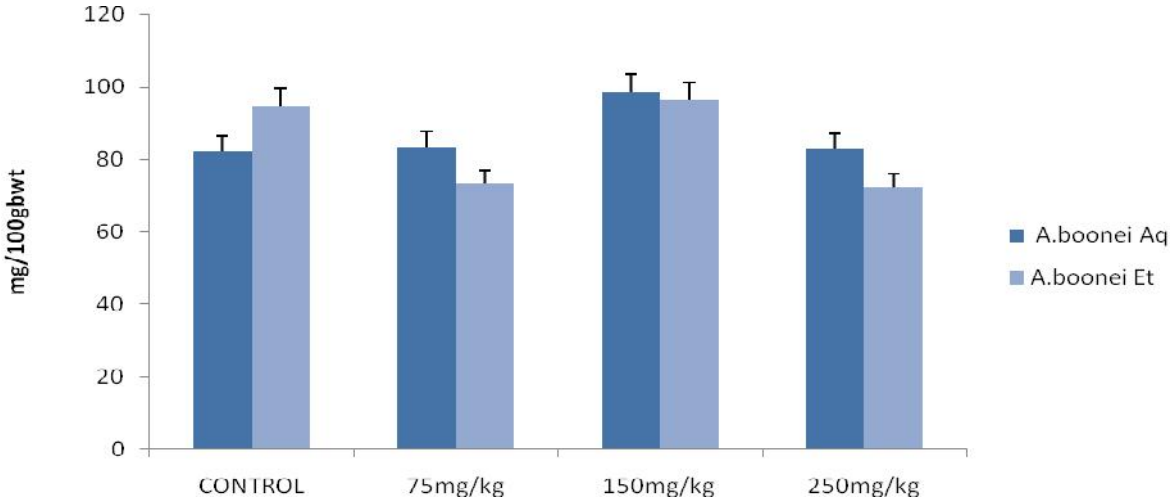

Figure 3: Uterine Weights of Proestrus Female Rats Administered Extracts *Alstonia boonei* Stem Bark

Values are Mean ± SEM (* = P < 0.05). A.boonei Aq. = Aqueous extract of *Alstonia boonei*; A.boonei Et. = Ethanol extract of *Alstonia boonei.*

Effect of *Alstonia boonei* extracts on Ovarian Weights in Proestrus Female Rats

The ovarian weights of proestrus female rats administered ethanolic and aqueous extracts of *Alstonia boonei* stem bark are shown in Figure 4. The results which are represented in Figure 4 showed no change (P<0.05) in the ovarian weight of rats administered aqueous and ethanol the extracts when compared with the control. Though there were marginal increases that seemed concentration dependent, it was not significant (P<0.05) when compared with the control in the two extracts.

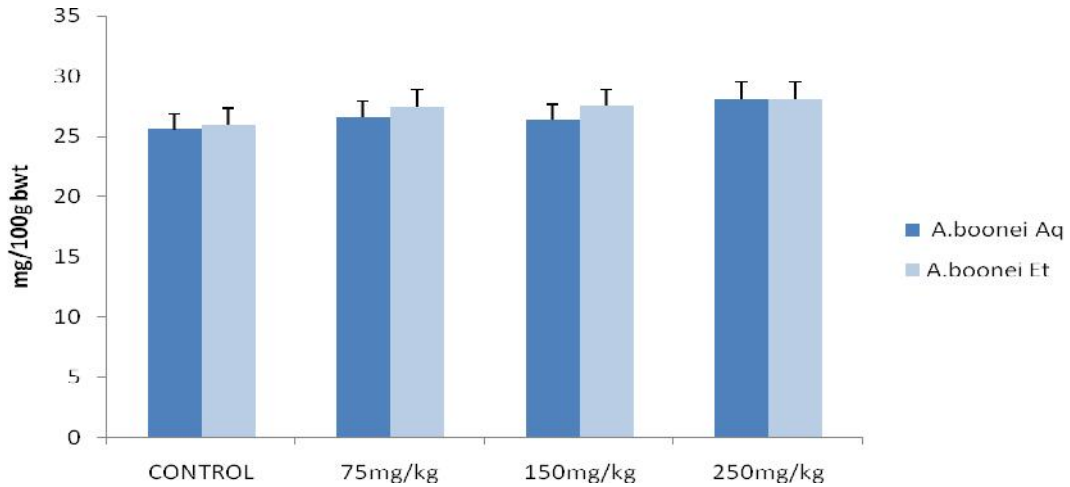

Figure 4: Ovarian Weights of Proestrus Female Rats Administered Extracts *Alstonia boonei* Stem Bark

Values are Mean ± SEM (* = P < 0.05). A.boonei Aq. = Aqueous extract of *Alstonia boonei*; A.boonei Et. = Ethanol extract of *Alstonia boonei.*

Discussion

During the estrous cycle, prolactin, leuteinizing hormone (LH) and follicle stimulating hormone (FSH) remain low and increase in the afternoon of the proestrus phase. Estradiol levels begin to increase at metestrus, reaching peak levels during proestrus and returning to baseline at estrus. Progesterone secretion also increases during metestrus and diestrus with a decrease afterwards. Then the progesterone value rises to reach its second peak towards the end of proestrus [20].

In this study, the results obtained suggest that the administration of aqueous and ethanolic extracts of *Alstonia boonei* to female proestrus rats was able to induce steroidogenesis which was reflected in the observed elevated serum estradiol and progesterone levels in the proestrus rats. The *Alstonia boonei* aqueous extract significantly ($P<0.05$) increased the serum progesterone level with increase in concentration as demonstrated in figure 1. The serum estradiol levels on the other hand were increased in concentration dependent pattern by the aqueous and ethanol extracts of *Alstonia boonei*.

The active principles in the extracts may directly induce the up-regulation of FSH and LH receptors leading to increase in steroidogenesis. The binding of LH to its receptor leads to a cascade of event that regulates inter-organelle communication and fate of *corpus luteum* [21, 22].Studies have demonstrated the involvement of reactive oxygen species in the follicular-fliud environment, folliculogenesis and steroidogenesis [23]. These reactive oxygen species have been implicated in oocyte maturation by leaving them susceptible to damage, specifically in environments such as in vitro where protective marternal factors are absent[24], progesterone production by the corpus luteum [21].Therefore, the ability of the aqueous and ethanolic extracts of *Alstonia boonei* to increase serum progesterone and estradiol may be dependent on the antioxidant potential of the extracts and this agrees with the work of [12].

Also, the bioactive principles present in the extracts of the plants might have sustained the hormonal levels of progesterone and estradiol in the proestrus rats by mopping up the concomitant reactive species generated as a result of steroidogenesis. Besides, the phytochemicals present in the extracts may up-regulate the SR-BI scavenger receptor that mediates the selective uptake of cholesterol esters from HDL by the *corpus luteum* [25]. It has been previously demonstrated that acute fasting before conception affects metabolic endocrine status without impaction on follicle and oocyte development [4]. It is possible that the fasting of the proestrus rats prior to the hormonal assays might have reduced the availability of cholesterol to the *corpus luteum* which can trigger increase in the generation of nitrosative and or oxidative stress that will lead to lipid peroxidation of membrane with concomitant release of cholesterol. The released cholesterol is mobilized for the biosynthesis of steroid hormones in the ovary [26].

The pr oestrus rats administered aqueous and ethanolic extracts of *Alstonia boonei* showed non-significant changes in the ovary weights of at the various concentrations relative to the control. This may be indicative of normalcy in stroma, follicle and corpus luteum [27].

The effects of the ovarian steroid hormones, estrogen and progesterone, on growth and regression of the non-pregnant uterus are reflected in the cyclic patterns of cellular proliferation, vascular growth, and blood flow that occur in the endometrium during the estrous cycle [28]. In the non-pregnant uterus, estradiol effects predominate during the follicular phase, whereas progesterone dominates during the proliferative phase of the estrus cycle. These steroids can influence uterine cell proliferation and tissue remodelling, which are necessary to prepare the uterus for successful implantation and support of embryonic growth. The non-significant changes in uterine weights observed in this study may not have been a function of estrogen or progesterone [28].

Conclusion

Serum progesterone measurement is widely used to assess corpus luteum function in fertility studies. Also, the estrogenic activity can be determined by estradiol via estradiol-receptor complexes which trigger ovulation. In this study, proestrus female rats administered the aqueous and ethanolic extracts of *Alstonia boonei* stem bark revealed increased level of progesterone. The *Alstonia boonei* stem bark aqueous extract had more influence on the serum progesterone level, while the ethanolic extract mostly affected the serum estradiol. Therefore, the claims that the plant extracts are ingredients for infertility treatment deserve more attention.

Acknowledgments

Conceptualization, Spencer. C. NWANGWU, methodology Spencer. C. NWANGWU and Sunday J. JOSIAH.; validation Spencer. C. NWANGWU, OMOREGIE E.S., and Isaac S. MOMOH.; formal analysis, Spencer. C. NWANGWU.; investigation Spencer. C. NWANGWU, OMOREGIE E.S and Sunday J. JOSIAH.; resources, Spencer. C. NWANGWU, OMOREGIE E.S and Sunday J. JOSIAH.; data curation, Spencer. C. NWANGWU and Isaac S. MOMOH.; writing original draft preparation, Isaac S. MOMOH.; editing, Isaac S. MOMOH.; supervision, C. NWANGWU and Sunday J. JOSIAH.; project administration, Spencer. C. NWANGWU.; funding acquisition, Spencer. C. NWANGWU, OMOREGIE E.S and Sunday J. JOSIAH.; All authors have read and agreed to the published version of the manuscript.

Funding Statement

The authors received no financial support for the research, authorship, and/ publication of article

Conflict of Interest

No potential conflict of interest was reported by the author(s)

Data availability Statement

This is original data and not in any repository for now.

Ethical Approval:

All animals used for the study were handled strictly in accordance with the Igbinedion University Ethics Committee guidelines for the use of experimental animals in research.

References

1. Carson, S.A., and Kallen, A.N. 2021. Diagnosis and Management of Infertility. *JAMA*, 326(1):65-76.
2. Sule, J.O., Erigbali, P., Eruom, L. 2008. Prevalence of infertility in women in a southwestern Nigerian Community. *Afr J Biomedical Res*, 11, 225-27.
3 World Health Organization. Infertility. Available from: https://www.who.int/news.room/fact.sheets/detail/infertility
4. Garcia-Garcia, R.M., Rebollar, O.P.G., Arias-Alvarez, M., Sakr, O.G., Bermejo-Alvarez, P., Brecchia, G., Gutierrez-Adan, A., Zerani, M., Bioti, C., Lorenzo, P.L. 2011.Acute fasting before conception affects metabolic and endocrine status without impacting follicle and oocye development and embryo gene expression in rabbit. *Reprod, Fert and Develop*, 23, 759 – 68.
5. Cornwall, A.2007. Taking chances, making choices: The Tactical Dimensions of “Reproductive Strategies” in Southwestern Nigeria. *Medical Anthropology*, 26:229-254.
6. De Boer, H., Lamxay, V. 2009. Plant used during pregnancy, childbirth and postpartum in Lao PDR: A comparative study of the Brou, Saek and Kry ethnic groups. *J Ethnobiol and Ethnomed*, 5, 25-9.
7. Van Der Kooi, R., Theobald, S. 2006. Traditional medicine in late pregnancy and labour: perception of Kgaba remedies amongst the Tswana in South Africa. *Afr J Traditional, Complementary and Alternative Medicine*, 3(1), 11-22.
8. Omobuwajo, O.R, Alade, G.O, Sowemimo, A. 2008. Indigenous Knowledge and practice of women herb sellers of Southwestern Nigeris. *Indian J trad knowledge*, 7(3), 505-10.

9. Okigbo, R.N., Anuagasi, C.L., Amadi, J.E.2009. Advances in selected medicinal and aromatic plants indigenous to Africa. *J Med Plant Res*, 3(2), 086-095.
10. Malam, F.D., Neuba, F.R.D. 2011. Traditional practices and medicinal plants use during pregnancy by Anyi-Ndenye women (Eastern Cote d'Ivoire). *Afr J Reprod Health*, 15(1), 85-94.
11. Elijah, A.I., Ojimelukwe, P.C., Ekong, U.S., Asamudo, N.U. 2010. Effect of *Sacoglottis gabonensis* and *Alstonia boonei* on the kinetics of *Saccharomyces cerevisiae* isolated from palm wine. *Afr. J. Biotechnol*, 9(35),5730-34.
12. Oze, G., Onyeze, G.O., Ojiako, O., Abanobi, S., Ozims, S., Ohiri, A.2012. Raised serum estrogen and progesterone concentrations in female rats treated with extract of Alstonia boonei (De wild). *Int. Res. J. Biochem. Bioinform*, 2(6), 135-41.
13. Elijah, A.I., Ojimelukwe, P.C., Ezeronye, O.U. 2007. Preliminary investigations on the effect of incorporation of *Alstonia boonei* bark powder on the fermentation of palm wine. *J Food Sci. Technol*, 44(2), 190-94.
14. Olajide, O.O., Awe, S.O, Makinde, M., Ekhelar, A.I., Olusola, A., Morebise, O., Okpako, D.T. 2000. Studies on the anti-inflammatory, antipyretic and analgesic properties of *Alstonia boonei* stem bark. *J. Ethnopharmacol*, 71, 179-86,.
15. Raji, A., Kwefio-Okai, G., Mcrides, T., Sandeman, M., Chandler, D.S., Playa, G.M. 2000. Inhibition of serine protease by anti-inflammatory Triterpenoids, *Planta Med*, 66, 206 –10.
16. Salahedeen, H.M., Yemitoan, D.K., Alada, A.R. 2003.Effect of aqueous leaf extract of Tridax procumbens in blood pressure and heart rate in rats. *Nig. J. Physical Sci*, 18(1-2), 50-55.
17. Owoyele, B.V., Alabio, T., Adelayo., J.O, Aboye, A. 2004. Hematological evaluation of ethanolic extract of Allinu assalonicum in male Albino Rats. *Fitoterapia*, 75, 322 -26.
18. Marcondes, F.K., Bianchi, F.J., Tanno, A.P. 2002. Determination of the estrous cycle phases of Rats: some helpful considerations. *Braz. J. Biol*, 62(4a), 609-14.
19. Ajayi, A.F., Akhigbe, R.E. 2020. Staging of the estrous cycle and induction of estrus in experimental rodrnts: an update. *Fertil Res Pract*, 6:5.
20. Islam, A., Naskar, S., Mazumder, U.K, Gupta., M, Ghosal., S. 2008. Estrogenic properties of Phyllanthin and Hypophyllanthin from *phyllanthus amarus* against carbofuran induced toxicity in female rats. *Pharmacologyonlin*e, 3, 1006-1016.
21. Emilia, P., Michele R.P., John, S.D. 2021. Luteinizing Hormone Regulation of Inter-Organelle Communication and Fate of the Corpus Luteum. *Int J Mol Sci*, 22(18):9972.
22. Fujii, J., Iuchi, Y., Okada, F. 2005. Fundamental roles of reactive oxygen species and protective mechanisms in the female reproductive system. *Reprod Biol Endocrinol*, 3(43), 1-10.
23. Agarwal, A., Said, T.M., Bedaiwy, M.A., Banerjee, J., Alvarez, J.G. 2006. Oxidative stress in an assisted reproductive techniques setting. *Fertil steril*: 86(3), 503 -12.
24. Hardy, M.L.M., Day, M.L., Morris, M.B. 2021. Redox Regulation and Oxidative Stress in Mammalian Oocytes and Embryos Developed In Vivo and in Vitro. *Int J Environ Res Public Health.* 18(21):11374.
25. Christenson, K.L., Devoto, L. 2003**.** Cholesterol transport and steroidogenesis by the corpus luteum. *Reprod Biol and Endo***,** 1**,**90-8**.**
26. Bochem, A.E., Holleboon A.G., Romijn J.A. 2013. High-density lipoprotein as a cholesterol for adrenal steroidogenesis. *J Lipid Res*, 5-6.
27. Shivalingappa, H.., Satyanarayan, N..D.,., Purohit, M..G.., Sharanabasappa ,A ., Patil, S.B..2002. Effect of ethanol extract of Rivea hypocrateriformis on the estrous cycle of the rat. *J. Ethnopharmacol*, 82(1), 11-7.

28. Johnson, M.L., Redmer, D.A., Reynolds, L.P.1997. Uterine growth, cell proliferation, and *c-fos* proto-oncogene expression throughout the estrous cycle in ewes. *Biol Reprod*, 56, 393-401.

Access this Article in Online	
Quick Response Code	Website: www.ijarbs.com
	Subject: Biochemistry
DOI:10.22192/ijarbs.2024.11.01.007	

How to cite this article:
Spencer. C. NWANGWU, OMOREGIE E.S, Sunday J. JOSIAH., Isaac S. MOMOH. (2024). Variations in Serum Progesterone and Estradiol Levels in Proestrus Rats Administered Extracts from *Alstonia boonei* Stem Bark. Int. J. Adv. Res. Biol. Sci. 11(1): 62-70.
DOI: http://dx.doi.org/10.22192/ijarbs.2024.11.01.007