# Effect of Hippocratea Africana Root Bark Extract on the Pituitary-Gonadal Hormones of Female Wistar Rats

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***Abstract***

*The problem of drug- induced reproductive failure termed infertility necessitated this study on the effect of Hippocratea africana (HA) root bark extract used traditionally in the treatment of malaria in the South-Eastern part of Nigeria on the pituitary-gonadal hormones. Twenty-four sexually matured female rats of Wistar strain weighing between 100 - 190g were used for this study. They were randomly divided into four groups of six rats each and allowed to acclimatize for one week. They were fed standard grower mash and water was allowed ad libitum. Group 1 (control) were administered 1ml of distilled water. Groups II, III and IV (test groups) were administered 100, 200 and 300 mg/kg body weight of HA root bark extract respectively for fourteen days orally using a cannula. There was a dose dependent increase concentration that was not significant (P>0.05) compared with the control for Follicle Stimulating Hormone (FSH). Prolactin recorded a dose dependent non-significant (P>0.05) decrease concentration between test groups II and III and a non-significant (P>0.05) increase in test group IV compared with the control. There was a non-dose dependent increase in the concentrations of Estradiol and Progesterone for all test groups, with statistical significance (P<0.05) recorded in group IV compared with the control. The ovaries of all the test groups did not show any pathological condition. There was significant (P<0.05) increase in ovary weight of test groups III and IV compared with the control but the ovaries weight as percentage body weight for all the test groups animals were not significant (P>0.05) compared with the control. The result showed that Hippocreatea africana root bark extract boosted the pituitary gland to produce the necessary reproductive hormones. It can therefore be concluded that there was no negative distortion of the pituitary gonadal hormones suggesting a nourishing effect of the herb on the endocrine system. The intact reproductive tissues in all the test groups' ovaries also suggest that the herb did not induce any oxidative stress on the ovaries. This may be due to the rich phytochemical present in the herb. A worrisome observed increase concentration of prolactin at higher dose though not significant raises a concern that very high concentration may regulate the oestrus cycle, conception and reproduction. Its use therefore as antiplasmodial agent is advised but with caution.*

**Keywords** - *Malaria, Hippocratea africana Root Bark, Pituitary-Gonadal Hormones, Fertility.*

## I. INTRODUCTION

Plant medicine is the oldest and most popular form of treatment for many diseases in the world and more than eighty per cent of the populace depend directly or indirectly on it. One of such diseases is malaria, a tropical scourge that is prevalent among the poor and the peasant.

Malaria affects about five million people annually and has been reported to be the leading mortality and morbidity disease in the developing world [1]. Effective treatment of malaria has been a great challenge to the world of medicine and this in turn affects man's health and the economy [2]. Treatment failure is linked majorly to the development of resistance of the malaria parasite to standard antimalarial agents. The resistance created a need for new drugs and the discovery of artemisinin and its derivatives gave renewed hope for combating resistant malaria [3,4].

The unaffordability and unavailability as well as the emerging resistance of the World Health Organisation (WHO) approved artemisinin combination therapy (ACT) has led to a fall back on phyto-remedies for the treatment of malaria which are usually over exploited without proper scientific evaluation(s). There is also a problem of no vaccine for permanent cure of the disease till date.

Hippocratea africana root bark extract is one of such phyto-remedies used in the South-Eastern part of Nigeria for the treatment of malaria. Its schizontocidal property with LD50 of 2.45 mg/kg in mice and rich phytochemical property have been reported [5,6]. Many herbs contain active ingredients, which have been examined and isolated by pharmaceutical industries and made into drugs. Heywood *et al.* [7] have reported that the gonads are affected by series of factors which include exposure

to certain drug types and physical agents, irradiation and hypoxia.

The key to reproductive health in virtually all of the woman's sexual life are hormones. This is because they regulate menstruation, fertility, menopause and even libido. The main hormones that affect menstrual cycle and fertility are produced by glands present in the brain and by the ovaries. The hypothalamus in the brain produces gonadotropin releasing hormone (GnRH) that triggers the pituitary gland to release both follicle stimulating hormone (FSH) and luteinizing hormone (LH) that starts the process of ovulation in the ovaries. The ovaries also produce estrogen and progesterone during this process that help in the preparation of the uterus for pregnancy [8]. When there is derangement in this endocrine balance either as a result of hyper or hypo secretion of these hormones, it could adversely affect ovulation thereby making it challenging for pregnancy to be achieved, a condition widely termed infertility. For example, some medications which are necessary to treat certain conditions may leave women with both the possibility of not being able to conceive and also with difficult feelings, including sadness, isolation, frustration, and anger. Women with infertility issues have equivalent levels of anxiety and depression to those with cancer, HIV, or heart disease.

Drugs in general have been reported to affect the female hormonal system, for example antidepressants can increase levels of the hormone prolactin, possibly suppressing ovulation; antimalarial drugs like proguanil and ulcer medications for peptic ulcers-cimetidine can increase prolactin stopping ovulation. Also, blood pressure medications including ACE inhibitors and epilepsy drug–carbamazepine, suppresses luteinizing hormone (LH) and estrogen in female [9].

The current trend in the problem of reproductive failure termed infertility in Nigeria necessitated this study, as some antimalarials have been reported to be teratogenic. For example, amodiaquine hydrochloride has been reported to disrupt the oestrus cycle and ovulation and thus reduces the number of ova release [10]. Artesunate have been reported to cause significant embryo-foetal toxicity resulting in embryo deaths and malformations [11]. Lou *et al.* [12] also reports that, it significantly reduces serum progesterone and degenerate the deudual cells and foetus of pregnant rats. Chlorquine have been reported to disrupt the oestrus cycle by inducing estradiol hormone, block ovulation and consequently reduces fertility [10]. Halfan (Halofanantrien hydrochroride) has been reported to have embryotoxic effect [13].

## II. MATERIALS AND METHODS

### *A. Sample Collection and Preparation of Extract*

Fresh roots of *Hippocratea africana* were harvested from Afaha Etok village in Ibesikpo-Asutan LGA, Akwa Ibom State. The roots were identified and authenticated by a taxonomist in the Department of Botany, Faculty of Science, University of Uyo, Uyo. They were washed with clean water to remove sand. The bark was scrapped, cut into very tiny pieces and air dried. The dried sample was pulverized with the aid of electric blender. 1500g of the pulverized root bark was macerated in 5000ml of 80% ethanol and allowed to stand for 72 hours to obtain a clear orange colour crude extract. The crude extract was carefully siphoned off the filtrate and concentrated to dryness in vacuum at $40^{o}$ C.

### *B. Experimental Animal and Experimental Design*

Twenty-four sexually matured female rats of Wistar strain weighing between 100- 190g obtained from the Department of Pharmacology and Toxicology, University of Uyo were used for this study. They were housed in a cross ventilated room in standard cages under standard Laboratory conditions. They were randomly divided into six groups of four rats to a group and allow acclimatizing for one week. All the experimental animals were fed standard grower mash and water was allowed ad libitum.

Group 1 animals served as the control and were administered 1ml of distilled water. Groups II, III and IV served as the test groups and were administered 100, 200 and 300 mg/kg body weight of *Hippocratea africana* root bark extract respectively for fourteen days orally using a cannula.

### *C. Collection of Blood Sample*

At the end of the treatment period, the animals were denied food for 24 hours but still had water *ad libitum.* They were chloroform anaesthetized and dissected. Blood sample was collected by cardiac puncture using sterile syringes and needles into EDTA bottles. The serum was obtained by centrifugation using an MES top centrifuge at 4,000 rpm for 10 minutes

### *D. Biochemical Analyses*

Commercial immunoassay kits manufactured by Randox Laboratories Ltd, United Kingdom were used for the determination of the concentrations of Progesterone, Estradiol (E2), Follicle stimulating hormone (FSH) and Prolactin.

### *E. Histological Examination*

The ovaries were extracted by use of forceps and scissors and excess blood mopped off. They were preserved in 10% buffered formaldehyde solution embedded with paraffin. After routine processing, paraffin sections of each tissue were cut into 5µm thickness and stained with haematoxylin and eosin [14]. The photomicrographs were taken with a digital camera (Canon Powershot A520) attached to a light microscope (Leitz Wetzlar, German36y).

### *F. Statistical Analysis*

The results of all determination are expressed as mean ± standard deviation. Data between groups were analysed using one analysis of variance (ANOVA). Pair wise comparison was done using the Dunnett test. A probability level of 0.05 was considered significant.

## III. RESULTS

The effect of Hippocratea africana root bark extract on some pituitary-gonadal hormones of albino Wistar rats and on the histomorphology of the ovary are presented on Figure 1 and Figure 2 respectively while the weight of the ovaries and relative weight of the ovaries as per body weight is represented in Figure 3.

The Follicle Stimulating Hormone (FSH) of the test group's animals recorded a dose dependent increase concentration that was not significant ($P>0.05$) compared with the control. Estradiol concentration of the test groups' animal were increased but not in a dose dependent manner compared with the control. Group IV increase alone was very significant ($P<0.05$) compared with the control. Prolactin recorded a dose dependent non-significant ($P>0.05$) decrease concentration between test groups II and III and a non-significant ($P>0.05$) increase in test group IV compared with the control. There was a non-dose dependent increase in the concentration of Progesterone of the test groups but only test group IV increase was significant ($P<0.05$) compared with the control.

There was no pathology in the all ovaries of the test groups animals following administration of varying dosage of the herb compared with the control as evidence in the intact granulosa cells (GC), corona radiata, zona pellucida (ZP) and oocytes (OC) (Figure 2). The weight of the ovaries also recorded favourable results. There was significant ($P<0.05$) increase in ovary weight of test groups III and IV compared with the control. The ovaries weight as percentage BW for all the test groups not significant ($P>0.05$) compared with the control (Figure 3).

## IV. DISCUSSION

Malaria has been defined as the disease of the poor [4] and the World Health Organization recommended artemisinin combination therapy (ACT) is out of reach for the poor as it is unavailable and unaffordable in the region of high prevalence of the disease. Many herbs have been explored for the treatment of malaria and documented for their antiplasmodial properties. Even with the promising antiplasmodial properties observed by the many explored abundant herbs in nature, it is worth mentioning that a lot of ignorance still exists on the side effects following their usage. Drugs in general are known to exhibit both beneficial and deleterious effects. Some drugs when abused or used in over dosed concentrations may have significant effect(s) on biochemical parameters. Some drugs (agonist) may mimic hormones while the antagonist drugs block them. Many common used drugs are potentially toxic to the gonads and may cause infertility. Infertility whether permanent or temporary, resulting from drug-induced injury is an important clinical problem and may be due to the duration of therapy, sex or age [15].

Most common causes of infertility in female are hormonal imbalance, commonly associated with ovulation, polycystic ovarian syndrome and premature ovarian failure, damage to the fallopian tube or uterus or cervical problems etc. Endocrine disorders occur when there is excessive production of hormones or insufficient production of one or more hormones or lack of tissues response to normal circulating hormones [16]. The interplay between the Luteinizing hormone, follicle stimulating hormone, progesterone and estradiol determines the functionality of the female reproductive cycle [17]. Factors such as genetic, environmental and psychological have also been reported to affect the ovary like any other organ in the body. Emotion and stress have been found to affect both menstrual and ovarian cycles but drugs constitute a substantial environmental factor, which could affect the hormone [18].

This study investigated the effect of Hippocratea africana root bark extract used traditionally in the treatment of malaria on the pituitary gonadal hormones. FSH is gonadotropic hormone that regulates the development, growth, pubertal maturation and reproductive process of the body. It initiates growth, specially affecting granulosa cells with the concomitant rise in Inhibin B and declines in the follicular phase in females.

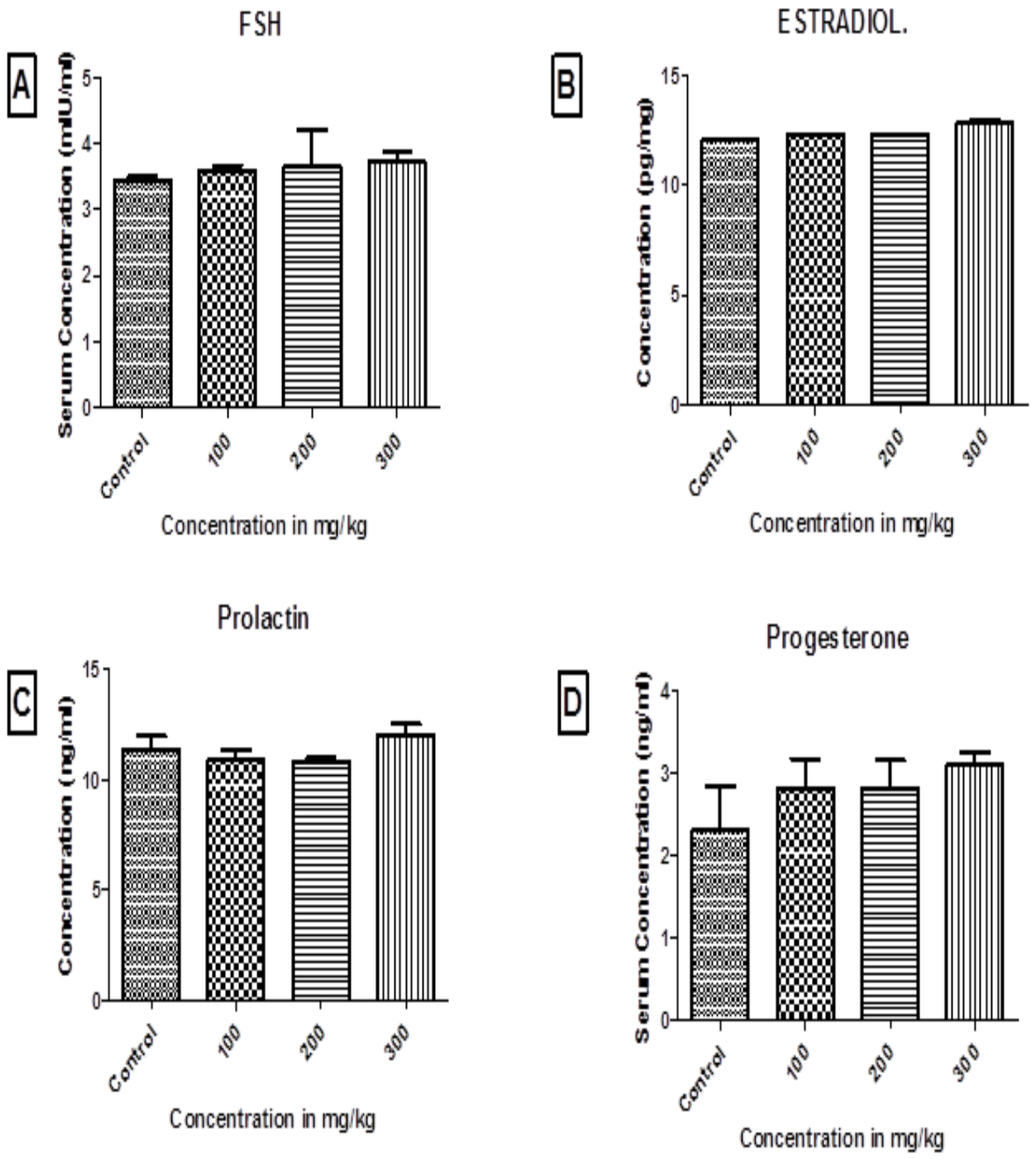


**Figure I: Showing Serum Concentrations for Follicle Stimulating Hormone-FSH (A); Estradiol (B); Prolactin (C) and Progesterone (D) of Albino Wistar Rats Administered Root Bark Extract of *Hippocratea africana* (H.A). (Group 1- Control; Group II - 100 mg/kg body Weight H. A; Group III - 200 mg/kg body weight H.A and Group IV - 300 mg/kg body weight H.A).**

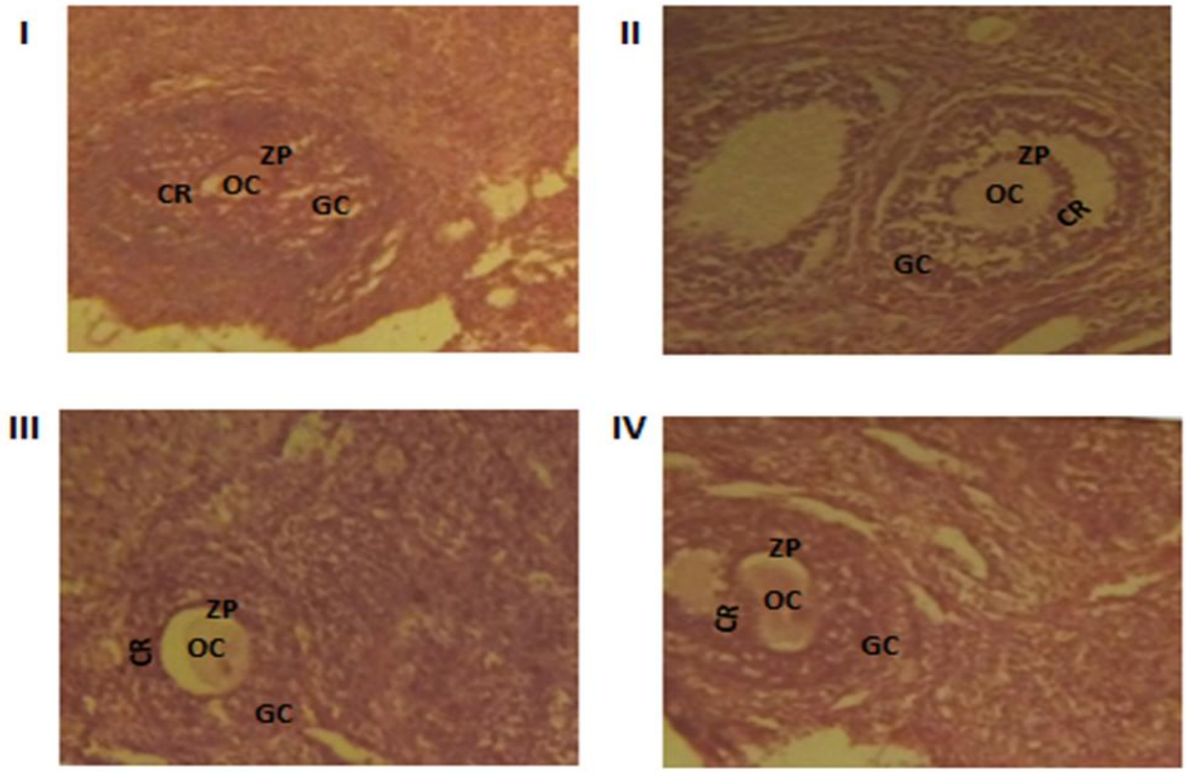


**Figure 2: Photomicrographs of Ovaries of albino Wistar Rats administered Root bark Extract of Hippocratea africana (H.A). Plate I (Group 1 - Control); Plate II (Group II - 100 mg/kg body weight H. A); Plate III (Group III - 200 mg/kg body weight H.A); Plate IV (Group IV - 300 mg/kg body weight H.A) (Mag. X 160)**

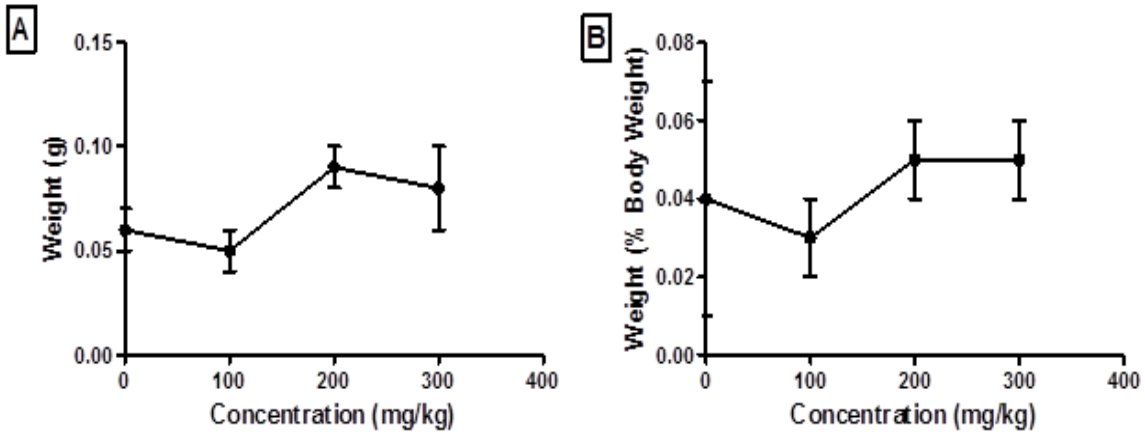


**Figure 3: Weight of Ovary (A) and Ovary weight expressed as % body weight (BW) (B) of albino Wistar rats administered root bark extract of *Hippocratea africana* (H.A). (Group 1- Control; Group II - 100 mg/kg body weight H. A; Group III - 200 mg/kg body weight H.A and Group IV - 300 mg/kg body weight H.A).**

Gonadodotropin releasing hormone (GnRH) has also been shown to play an important role in the secretion of FSH as it is subject to oestrogen feedback from the gonads via the hypothalamic pituitary axis [19].FSH stimulates the growth and recruitment of immature ovarian follicle in the ovary causing an egg to grow. It also triggers the production of estrogen in the follicle. Increase in serum estradiol levels cause decrease in FSH production by inhibiting GnRH production in the

hypothalamus. A high level of FSH in reproductive years is known to be abnormal and may be due to poor ovarian reserve, gonadal dysgenesis, systemic erythmatosus etc, which are conditions associated with subfertility and/ or infertility. Low concentration may be due to dysfunctions including hypothalamic suppression; gonadotropin deficiency etc [19]. The dose dependent increases in the concentration of FSH observed in this study suggest that the phytochemicals present did not have a suppressive effect on the hypothalamus. Arteunate and amodiaquine have been reported to moderately decrease serum concentration of FSH [20].

Prolactin stimulates the mammary glands to produce milk. It also decreases normal levels of sex hormone - estrogen in women. The non-significant decrease observed in this study at lower doses with corresponding increase in the estradiol and FSH concentrations suggest that the herb will not have suppressing effect on the ovulatory cycle. High levels of prolactin tend to suppress the ovulatory cycle by inhibiting the secretion of both FSH and Gonadotropin-releasing hormone [18]. This property may be attributed to the phytochemicals present in the herb [6]. There is a report that phytochemical agents present in plant extracts can regulate oestrus cycle, conception and reproduction [21,22}. Ikpeme *et al.* [23] also reported a non-significant difference in prolactin concentration of treated animals compared with the control following administration of Enantia chlorantha bark extract used traditionally in the treatment of malaria, suggesting it to be a fertility booster. The raised concentration of prolactin, although not significant at 300mg/ kg body weight of the extract suggest that at very high concentration, the ovulatory cycle may be compromised, the effect may be ovulation inhibition which may lead to loss of menstrual periods thereby hindering conception. Asuquo *et al.* [24] however, reported that ethanol extract of *S.* mombin used traditionally in the treatment of malaria may have anti-fertility property which confirmed it's used as local contraceptive. The herb reduces progesterone and estrogen concentrations in a dose dependent manner and was deleterious to the ovaries of the treated animals but reverse of this effect was observed in our study. In our study, the Granulosa cells which convert androgenic steroids precursor into estrogen; Oocyte- the mature female gamete; Zona

pellucida- a thick homogenous layer of glycoprotein and acid proteoglycans that develops between the oocyte and the follicular cells that facilitates and maintains sperm binding and induces the acrosome reaction as well as Corona radiate- the single layer of granulosa cells immediately adjacent to the primary oocyte which forms a barrier protecting the female gamete are all intact in the ovary section of the test groups animal as with the control (Figure 2).

The slight weight increase of the ovary of test groups III and IV observed in this study did not generally suggest toxicity of the herb as no deleterious effect/s was observed in the histopathology of the ovaries of the test groups animals compared with the control. It may be described as initial response of organs to foreign substances. Lovati *et al.* [25] have reported that exposure to drugs and other xenobiotics can affect weight and relative weight of organs or tissues.

Progesterone is synthesized from both tissue and circulating cholesterol. It exhibits a wide variety of end organ effects with the primary role being in the reproductive organs. Its measurement in female is done to determine ovulation as well as to characterize luteal phase defect [26]. Administration of varying concentration of 100, 200 and 300 mg/kg body weight of HA recorded increases in the concentration of progesterone that was significant at 300mg/kg body weight of the extract compared with control. Lou *et al.* [12] however reported significantly reduced serum progesterone concentration following the administration of artesunate that resulted in the degeneration of decidual cells and foetus of pregnant rats. It has also been reported to cause significant embryo-faetal toxicity causing embryo deaths and malformation [11,27]. The increase concentration of progesterone observed in this study suggest that the herb contains phytochemicals which may affected the FSH and LH concentration in the animals, thus eliciting a corresponding and proportional effect on other reproductive hormones.

## V. CONCLUSION

Hippocreatea africana root bark extract did not distort the pituitary gonadal hormones negatively. Increasing concentrations of the herb boosted the pituitary gland to produce the necessary reproductive hormones, suggesting a nourishing effect of the herb on the endocrine system. It also suggests that the herb did not induce any oxidative stress on the ovary as evidence the intact reproductive tissues. However, the observed increase concentration of prolactin though not significant raises a concern that very high concentration may not be advised in females as it may regulate the oestrus cycle, conception and reproduction. Its use therefore as antiplasmodial agent is advised but with caution.

***Conflict Of Interest:*** No conflict of interest exists.

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