EFFECTS OF HYDROETHANOLIC EXTRACT OF THE SEEDS OF*Sesamumindicum* ON SOME REPRODUCTIVE PARAMETERS OF FEMALE ALBINO RATS

BY

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Declaration

I declare that the work in this thesis titled "Effects of Hydroethanolic Extract of the Seeds of *Sesamumindicum* on Some Reproductive Parameters of Female Albino Rats" has been performed by me in the Department of Human Physiology under the supervision of Dr. Aliyu Mohammed, and Dr. Yusuf Tanko. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

______________________ ______________________

Umar Baraka **Date**

CERTIFICATION

This thesis titled "Effects of Hydroethanolic Seeds Extract of *Sesamumindicum* on Some Reproductive Parameters of Female Albino Rats" by Umar Baraka meets the regulations governing the award of the degree of Master of science of Ahmadu Bello University Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This research work is dedicated to all women who in one way or the other had reproductive complications during their lifetime.

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ABSTRACT

Phytoestrogens are natural estrogenic agents present in plants. Phytoestrogens are any plant compounds structurally and/or functionally similar to ovarian and placental estrogens and their active metabolites. *Sesamumindicum* is one of the phytoestrogen containing seeds used by man for food and medicinal purposes. Researches have shown that phytoestrogens present in plants might have both fertility-enhancing and anti-fertility effects. Present studies evaluated the mean lethal dose of the hydroethanolic seed extract of sesame and the effects of the extract on some reproductive parameters of female albino rats. Cycling female rats weighing 120-140g were used for the studies. For the ovulation study, twenty (20) female rats were divided into four (4) groups of five (5) rats groups II, III and IV received 100, 300 and 500 mg/kg of seeds extract of *S. indicum*orally respectively. The animals were dosed at 4 hourly interval for 24 hours. Animals were sacrificed the following morning. For the hormonal level study, the extract was administered orally to three (3) experimental groups of five (5) female rats at doses of 100, 300 and 500 mg/kg once daily while control group received distilled water at dose of 1ml/kg for 30 days. The animals were subsequently sacrificed in the morning of the day after the last administration. For studies on pregnancy outcome, the extract was administered orally four (4) groups of five (5) pregnant rats each. Administration was done once daily from day 1-7 of pregnancy as follows: group I: distilled water (1 ml/kg), group II: 100 mg/kg, group III: 300 mg/kg and group IV 500 mg/kg respectively, with seeds extract of *S. indicum*.

The number of ovarian follicles was significantly higher ($p < 0.05$) in group IV than control rats (31.40 $\pm$ 3.20 vs 22.80 $\pm$ 4.22), while groups II and III had lower number of follicles than control rats (14.80 $\pm$ 2.40, 19.80 $\pm$ 3.40 vs 22.80 $\pm$ 4.22).

The values of gonadosomatic index were similar in group III and control rats (0.83 ± 0.12% vs 0.83 ± 0.11%). However, the gonadosomatic indices in groups II and IV were significantly lower ($p < 0.05$) than control rats (0.66 ± 0.03, 0.75 ± 0.14 vs 0.83 ± 0.12, respectively).

The mean (± SEM) serum estrogen concentration was significantly lower ($p<0.05$) in group III than in groups II, IV and control rats (349.60 ± 10.20 pg/ml vs 422.40 ± 28.19 pg/ml, 414.17 ± 17.49pg/ml, 431. 80 ± 9.47 pg/ml respectively). The mean serum progesterone followed a similar pattern, with relatively lower values in group III than groups II, IV and control rats (14.70 ± 1.39 ng/ml vs 15.10 ± 0.74 ng/ml, 19.12 ± 1.91 ng/ml and 17.22 ± 4.22, respectively).

The number of fetuses delivered by pregnant rats was not significantly different ($p < 0.05$) between treated and control rats (group I: 6.0 ± 0.71, group II: 7.00 ± 0.90, group III: 6.20 ± 1.07 and group IV: 7.40 ± 1.78, respectively; $p<0.05$).

It is therefore concluded that hydroethanolic seeds extract of *S. indicum* possess both anti- and pro-estrogenic properties in a dose-dependent fashion in adult female albino rats.

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17ß-HSD	17ß-Hydroxysteroid
ANOVA	Analysis of Variance
DES	Diethylstilbesterol
DHEAS	Dehydroepiandrosteron Sulfate
DHS	Demographic and Health Survey
DNA	Deoxyribonucleic Acid
ELISA	Enzyme – Linked Immunosorbent Assay
ENL	Enterolactone
ER	Estrogen Receptor
FSH	Follicle-Stimulating Hormone
GnRH	Gonadotropin-Releasing Hormone
GV	Gentine Violet
HDL	High Density Lipoprotein
ICMART	International Community for Monitoring Assisted Reproductive Technology
LD_{50}	Mean Lethal Dose
LDL	Low Density Lipoprotein
LH	Luteinizing Hormone
MR	Mineralocorticoid Receptor
NICHD	National Institute of Child Health and Human Development
OPKs	Ovulation Predictor Kits
PCOS	Polycystic Ovarian Syndrome
PID	Pelvic Inflammatory Disease

PR	Progesterone Receptor
SEM	Standard Error of Mean
SHBG	Sex Hormone-Binding Globulin
SPSS	Statistical Package for the Social Sciences
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

Hormones are intimately involved in our sex lives, as well as all other aspects of ourlives, from intrauterine life to death. Of these, the most important are the socalledsex hormones. Mammalian sexual behavior is controlled by these gonadal steroids actingat the level of the central nervous system. They are divided into the male hormones (orandrogens) and the female hormones. While, both sexes produce both types, those corresponding to the appropriate sex predominate (in most cases) (Wierman, 2007). Women produce a number of hormones specific to them, particularly during their childbearing years. The two best-known female hormones are estrogen and progesterone, sometimes also referred to as sex hormones. These are produced predominantly in the ovaries, although the adrenal glands and the placenta of pregnant women also secrete them. Some research has indicated that estrogen can be manufactured in the brain as well (Haybach, 2010).

The main female hormone is estrogen. Estrogens display one common biologic activity, the ability to stimulate growth and maintain the female sex characteristics (Goldzieher and Castracane, 2008). Estrogen stimulates the development of the female sex organs both within the fetus and at puberty, creating her femaleness, her rounded breasts andcurvy hips. It also enhances pheromone secretion and 'invitational' behavior and her yearning for sexual contact. Estrogen levels peak just prior to ovulation. They drop off precipitously during menopause, with consequent vaginal dryness and atrophy and increased risk of osteoporosis and heart disease (Wierman, 2007).The actions of estrogen are mediated by the estrogen receptor (ER), a dimericnuclear protein that binds to DNA and controls gene expression (Cooke*et al.*1998;Psarra and Sekeris, 2008.).

Progesterone is an essential regulator of normal human female reproductive function in the uterus, ovary, mammary gland and brain, and also plays an important role in non-reproductive tissues such as the cardiovascular system, bone and the central nervous system, highlighting the widespread role of this hormone in normal physiology. The effects of progesterone are mediated through the nuclear progesterone receptor (PR), which interacts with transcriptional co-regulators, moves into nuclear aggregates and regulates gene expression. Although, progesterone plays a pivotal role in normal physiology, exposure to its analogues in exogenous hormone formulations is associated with deleterious effects, most notably an increase in breast cancer risk (Scarpin*et al.,* 2009).

Sesame, *Sesamumindicum*L., is an annual herb native tothe tropics, which was formerly classified as *Sesamumorientale*L.It belongs to the Pedaliaceae family (Crews*et al.*, 2010; Mahabadi*et al.* 2013). All parts of the sesame plant such as the seed, oil and leaves are useful and are locally consumed as a staple food by subsistence farmers in the Northern, South-west and Middle - belt regions of Nigeria and celebrated also in folkloric medicine in Asia and Africa (Shittu and Shittu, 2012). The local names of the plants depend on the source areas of cultivations in the world, such as *ekuku–gogoro* (Yoruba- *Sesamumradiatum*), *yanmoti*(Yoruba-*S. indicum), ridi* (Hausa) and *beni* (Tiv/Idoma and English) or gingelly (English) (Shittu*et al*, 2009).

Sesame lignans, such as: sesamin, sesamolin, sesaminol, sesamolinol, pinorsinol, sesamol and gamma-tocopherol are isolated from *S.indicum*and

*S.radiatum*seeds. They have more tumorigenic, estrogenic or anti-estrogenic and antioxidant features compared with other plant species. The plant is rich in trace elements/minerals such as calcium, iron, magnesium, zinc, copper and phosphorus (Shittu and Shittu, 2012; Mahabadi*et al.*, 2013). In terms of phytochemistry, these seeds have glycosides, alkaloids, flavonoids, tannins, polyphenols, steroids, and saponins (Sireesha*et al.*, 2013); non-protein amino acids, unsaturated fats and lipids with multiple double bonds, glazes, phospholipids and vitamins E, B1 and B2 (Mahabadi*et al.*, 2013). Approximate analysis of sesame seed has made it clear that the seeds contain 50-60% oil, 8% protein, 5.8% water, 3.2% crude fiber, 18% carbohydrate and 5.7% ash (Crews*et al.*, 2010; Mahabadi*et al.*, 2013).

Sesaminwas reported to be converted by intestinal microflora to enterolactone, a compoundwith estrogenic activity and also an enterometabolite of flaxseed lignans, which are known to be phytoestrogenic (Wen-Huey*et al.*,2006). The phytoestrogens have attracted so much attention in the last decade in view of their reported health benefits and they include four broad classes of phytochemicals namely the lignans (grains e.g sesame), isoflavones (soybeans), stillbenes and coumestrol (Pradhan*et al.*, 2012; Shittu and Shittu, 2012). These agents mimic endogenous estrogens depending on their concentrations, they either act agonistically or antagonistically by displacing theendogenous estrogens from their binding sites on the estrogen receptors (ER1 and ER2), among its other mechanisms of action (Shittu*et al.,* 2009; Shittu and Shittu, 2012).Lignans have beneficial effects on serum lipid levels and liver function and give sesame seed oil a marked antioxidant activity. The lignans are also responsible for the great stability of sesame seed oil to oxidation (Crews*et al.*, 2010). Based on the fact that they are consumed in large amounts in the diet, the effects noticed are usually antiestrogenic, thus, competing with the much more potent endogenous estradiol for the estrogen receptor binding sites and ultimately block its estrogenic activity (Shittu and Shittu, 2012).This led to the quest to undertake this research on the effects of *Sesamum*indicum on female fertility

Studies have investigated the multiple beneficial functions of sesame which includes antiaging, anticancer, antioxidation, antihypertensive, modulation of lipid metabolism and lipid peroxidation. Their effects on enhancement of liver function, and immunoregulatory and antithrombosis properties have also been investigated.These properties have made sesame seeds and their byproducts functional foods beneficial in the prevention and treatment of chronic diseases and metabolic disorders (Mirmiran*et al.*, 2013). Sesamin, the major lignan found in sesame oil has been demonstrated to enhance hepatic detoxification, to protect against oxidative stress, and to prevent the development of hypertension (Fu-Chou*et al.*, 2006).*Ewe atura* (one of the local names in southeast Nigeria) means leaves that provide comfort and health for the body because of being useful for treatment of constipation and digestive disorders. It has been reported that boiling a mixture of roots and leaves has antiviral and antifungal activity (Mahabadi*et al.*, 2013). Shittu*et al.* (2007) evaluated the effects of fluid extract ofsesame leaves on epididymal spermatocyte reserves inadult rats and concluded that sesame leaves increase male fertility.Also researchers, who evaluated the effects of leavesextract of this plant on the fertility of hypoglycemic rats,found that this extract improves parameters related to thetestis and increases reproductive potential in mice (Shittu *et al.*, 2009).

Figure 1.1: Sesame Plant (Source: www.shutterstock.com)

Figure 1.2: Sesame seeds (Chemonics International Corporation, 2012)

1.1 Statement of Research Problem

Plant-derived chemicals that influence endocrine activities in both humans and animals have received a great deal of attention due to their possible beneficial as well as adverse effects (Yakubu*et al.,* 2008). Since ancient times, mankind has used plants to cure diseases and relieve physical sufferings. The better cultural acceptability, better compatibility with the human body, lesser side effects and effectiveness of many, traditional medicine is now an accepted fact (Pradhan, *et al.*, 2012). More than 35,000 plant species are being used in various human cultures around the world for medicinal purposes. Nearly 80% of the world population rely on traditional medicine for primary health care, most of which involve the use of plant extracts (Pradhan*et al.*, 2012).

Fertility regulation with plants or plant preparations has been reported in the ancient literature of indigenous systems of medicine (Kaur*et al.*,2011). The role of estrogen in female reproduction makes it to be a very important hormone whose level in the body has to be carefully regulated. Some traditional herbs consumed by females knowingly or unknowingly alter the levels of this hormone and other important aspects of the reproductive system. This might lead to beneficial effects by boosting the fertility level or produce anti-fertility effects.*Sesamumindicum*is one of the phytoestrogen containing edible seeds consumed by man as food and for medicinal purposes. Its high phytoestrogen content, about 373mg/100g (Peterson *et al.*, 2010), makes it necessary to investigate its effects on the body, particularly the female reproductive system.

1.2 Justification

There are over 186 million couples in developing countries alone (excluding China) today who are affected by infertility (both primary and secondary). Rates of infertility vary considerably from country to country; in the worst-affected areas, over 25% of couples may be unable to have children (WHO, 2003). However, in developing countries of the world like Africa for example, with an infertility rate of more than 30% in some countries (WHO, 2011), there are more infertile cases with limited or no treatment options especially assisted reproductive technology available for the management of this medical condition (Shittu and Shittu, 2012). Hence, expressed worldwide is the search for an ideal folkloric phyto-chemical medicinal agent with a broad spectrum and proven potential of treating infertility/ subfertility conditions with minimal or no side effects as compared to their synthetic counterparts (Shittu*et al*, 2009).

The phytoestrogens appeared to be one of such natural estrogenic agents that have attracted so much attention in the last decade in view of their reported health benefits (Mahabadi*et al.*, 2013). Sesame ingestion has been shown to improve blood lipids in humans and anti - oxidative ability in animals (Shittu and Shittu, 2012). The methanolic extract of the plant has antimicrobial effects on the male reproductive system (Shittu and Shittu, 2012). Sesame phytoestrogeniclignans tend to promote aromatization of testosterone to estradiol to favor spermatogenesis (Shittu and Shittu, 2012). In addition, sesame leaves extract consumption enhances the quality of the spermatozoa produced with improvement in the storage capacity of the epididymis for these spermatozoa in a dose related manner (Shittu*et al.*, 2007). A research conducted by Wen-Huey*et al.*(2006) showed that sesame seeds tend to improve the conditions of post-menopausal women because of its estrogenic effects on the serum levels of dehydroepiandrosterone sulfate (DHEAS), sex hormone-binding globulin (SHBG) and follicle-stimulating hormone (FSH).

1.3 Aim andObjectives

This study was aimed at evaluating the effects of the hydroethanolic extract of seeds of *Sesamunindicum*on some reproductive parameters of matured female albino rats.

Specific Objectives were:

1. To determine the mean lethal dose (LD_{50}) of the hydroethanolic extract of seeds of*Sesamumindicum*
2. To evaluate the effects of the hydroethanolic seed extract on ovulation and gonadosomatic index of female albino rats.
3. To determine the effects of the extract on serum levels of estrogen and progesterone in femalealbino rats.
4. To evaluate the effect of the extract on pregnancy outcome.

1.4 Research Hypothesis

H_0: The hydroethanolic extract of *Sesamumindicum* seeds does not have pro-fertility effects on matured female albino rats.

H_A:The hydroethanolic extract of *Sesamumindicum* seeds has pro-fertility effects inmatured female albino rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

Reproduction can be defined as the process by which an organism continues its species. In the human reproductive process, two kinds of sex cells (gametes) are involved: the male gamete (sperm), and the female gamete (egg or ovum). These two gametes meet within the female's uterine tubes located one on each side of the upper pelvic cavity, and begin to create a new individual. The female needs a male to fertilize her egg; she then carries offspring through pregnancy and childbirth (Bergerson, 2010).

A woman's reproductive system produces sex hormones and functional gametes and also must be able to protect and support a developing embryo and nourish the newborn infant. The ovaries and the uterus are among the most important organs of the reproductive system. The ovaries perform three main functions: produce immature female gametes, or oocytes, secrete female sex hormones, including estrogens and progestins, and secrete inhibin, that is involved in the feedback control of pituitary FSH production (Bergerson, 2010; Taylor, 2013).

2.1.1 Processes taking place in the ovary

a. Oogenesis Ovum production or oogenesis begins before a woman's birth, accelerates at puberty, and ends at menopause. Between puberty and menopause, oogenesis occurs on a monthly basis as part of the ovarian cycle. Unlike spermatogonia, the oogonia, or stem cells of females, complete their mitotic divisions before birth. Between the third and seventh months of fetal development, the daughter cells, or primary oocytes, prepare to undergo meiosis. They proceed as far as the prophase of meiosis I, but at that time the process comes to a halt. The primary oocytes then remain in a state of suspended development until the individual reaches puberty, when rising levels of FSH trigger the start of the ovarian cycle. Each month thereafter, some of the primary oocytes will be stimulated to undergo further development. Not all primary oocytes produced during development survive until puberty. The ovaries have roughly 2 million primordial follicles at birth, each containing a primary oocyte. By the time of puberty, the number has dropped to about 400,000. The rest of the primordial follicles degenerate in a process called atresia (Gray, 1918; Whitehead and Nussey, 2001).

b. Steroidogenesis Ovaries secrete estrogen, testosterone and progesterone. Estrogen is responsible for the appearance of secondary sex characteristics for females at puberty and for the maturation and maintenance of the reproductive organs in their mature functional state. Progesterone prepares the uterus for pregnancy, and the mammary glands for lactation. Progesterone functions with estrogen by promoting menstrual cycle changes in the endometrium (Gray, 1918).

2.1.1.1 Estrogen

The three major naturally occurring estrogens in women are estrone (E1), estradiol (E2), and estriol (E3). They are a group of hormones that primarily influence the female reproductive tract in its development, maturation, and function. Estradiol is the predominant one (Wierman, 2007). Estrogens, in females, are produced primarily by the ovaries, and during pregnancy, the placenta (Mandal, 2014). Follicle-stimulating hormone (FSH) stimulates the ovarian production of estrogens by the granulosa cells of the ovarian follicles and corpora lutea (Wierman, 2007). Some estrogens are also produced in smaller amounts by other tissues such as the liver, adrenal glands, and the breasts. These secondary sources of estrogens are especially important in postmenopausal women (Mandal, 2014).

In females, synthesis of estrogens starts in theca interna cells in the ovary, by the synthesis of androstenedione from cholesterol. Androstenedione is a substance of weak androgenic activity which serves predominantly as a precursor for more potent androgens such as testosterone as well as estrogen. This compound crosses the basal membrane into the surrounding granulosa cells, where it is converted either immediately into estrone, or into testosterone and then estradiol in an additional step (Mandal, 2014). The conversion of androstenedione to testosterone is catalyzed by 17β-hydroxysteroid dehydrogenase (17β-HSD), whereas the conversion of androstenedione and testosterone into estrone and estradiol, respectively is catalyzed by aromatase, enzymes which are both expressed in granulosa cells. In contrast, granulosa cells lack 17α-hydroxylase and 17, 20-lyase, whereas theca cells express these enzymes and 17β-HSD but lack aromatase. Hence, both granulosa and theca cells are essential for the production of estrogen in the ovaries (Ryan, 1982;Goldzieher and Castracane, 2008).

The actions of estrogen are mediated by the estrogen receptor (ER), a dimeric nuclear protein that binds to DNA and controls gene expression. Like other steroid hormones, estrogen enters passively into the cell where it binds to and activates the estrogen receptor. The estrogen-receptor complex binds to specific DNA sequences called a hormone response element to activate the transcription of target genes (in a study using an estrogen-dependent breast cancer cell line as model, 89 such genes were identified). Since estrogen enters all cells, its actions are dependent on the presence of the ER in the cell. The ER is expressed in specific tissues including the ovary, uterus and breast (Goldzieher and Castracane, 2008; Wierman, 2007).

While estrogens are present in both men and women, they are usually present at significantly higher concentrations in women of reproductive age. They promote the development of female secondary sexual characteristics, such as breasts, and are also involved in the thickening of the endometrium and other aspects of regulating the menstrual cycle. In males, estrogen regulates certain functions of the reproductive system important to the maturation of sperm and may be necessary for a healthy libido. Furthermore, there are several other structural changes induced by estrogen in addition to other functions (Goldzieher and Castracane, 2008).

One of the most important actions of estrogen is its effect on the cardiovascular system. Estrogens alter plasma fibrinolytic activity and plasma aggregation, resulting in a detrimental increase on thrombosis. Conversely, they also confer protection against atherosclerosis by decreasing total cholesterol and low-density lipoprotein (LDL) levels and increasing high-density lipoprotein (HDL) levels. Also, estrogens have vasodilatory effect on vascular smooth muscle of coronary arteries and peripheral vascular beds. Estrogen has been found to control the production and release of nitric oxide (endothelium-derived relaxation factor) (Frankovich and Lebrun, 2000). Another mechanism for the vasodilatory effect of estrogen is its ability to act as a calcium channel blocker (Frankovich and Lebrun, 2000).

Estrogen affects several processes important to the regulation of substrate metabolism and metabolic rate. Intramuscular and hepatic glycogen storage and uptake are increased by the estrogens. Increased lipid synthesis, enhanced lipolysis in muscles, and greater use of free fatty acids result in a glycogen-sparing effect. In response to elevated levels of estrogen, insulin-binding capacity is decreased, resulting in deterioration in glucose tolerance and insulin resistance. Transdermal estradiol has also been shown to change glucose metabolism through decreased gluconeogenesis and changes in epinephrine secretion and glucose transport (Frankovich and Lebrun, 2000).

Other actions of estrogen include deposition of fat in the breasts, buttocks and thighs; sodium and chloride retention resulting in edema, weight gain and an increase in blood pressure; and facilitation of sodium uptake into bone (Ryan, 1982; Frankovich and Lebrun, 2000)

2.1.1.2 Progesterone

Progesteroneis a C-21 steroid hormone involved in the femalemenstrual cycle, pregnancy (supports *gestation*) and embryogenesis of humans and other species. Progesterone belongs to a class of hormones called progestogens, and is the major naturally occurring human progestogen (Goldzieher and Csatracane, 2010).

Progestogens, as indicated by the name, are hormones whose major function is the maintenance of pregnancy. They have been specifically defined as agents that induce secretory changes in the proliferative endometrium and maintain pregnancy. Although there are several natural progestogens, progesterone is the only natural progestogen of major biologic significance. This hormone does not display the multiplicity of systemic effects characteristic of the estrogens; its action is limited predominantly to the reproductive organs. The elevation of the basal body temperature after ovulation is due to the thermogenic effect of progesterone at the level of the hypothalamus (Goldzieher and Castracane, 2010).

Progesterone is produced by all tissues that have steroidogenic activity, and is a major component in the pathway of synthesis of many steroid hormones; therefore, low levels of progesterone are always produced by steroidogenic tissues. The weak progestogen 17-hydroxy progesterone is also produced by the ovary in a predictable pattern in relation to follicular and luteal function. The only dramatic increase in non-pregnant women occurs after ovulation, when the corpus luteum produces large amounts of progesterone, which in turn bring about the development of the secretory endometrium. The placenta assumes the function of progesterone production between the fifth and seventh weeks of gestation, and it produces increasing amounts during the course of pregnancy. The maintenance of pregnancy is dependent on the continued production of progesterone, first from the corpus luteum and, after a gradual shift, from the placenta for the remainder of pregnancy (Goldzieher and Castracane, 2010).

In women, progesterone levels are relatively low during the preovulatory phase of the menstrual cycle, rise after ovulation, and are elevated during the luteal phase. Progesterone levels tend to be < 2 ng/ml prior to ovulation and > 5 ng/ml after ovulation. If pregnancy occurs, human chorionic gonadotropin is released maintaining the corpus luteum and allowing it to maintain high levels of progesterone. At around 12 weeks the placenta begins to produce progesterone in place of the corpus luteum, this process is named the luteal-placental shift. After the luteal-placental shift progesterone levels start to rise further and may reach 100-200 ng/ml at term. Whether a decrease in progesterone levels is critical for the initiation of labor has been argued and may be species-specific. After delivery of the placenta and during lactation, progesterone levels are very low (Goldzieher and Castracane, 2010).

Progesterone exerts its primary action through the intracellular progesterone receptor although a distinct, membrane bound progesterone receptor has also been postulated. In addition, progesterone is a highly potent antagonist of the mineralocorticoid receptor (MR, the receptor for aldosterone and other mineralocorticosteroids). It prevents MR activation by binding to this receptor with an affinity exceeding even those of aldosterone and other corticosteroids such as cortisol and corticosterone (Scarpin*et al.*, 2009).

Progesterone has a number of physiological effects that are amplified in the presence of estrogen. Estrogen through its receptors upregulates the expression of progesterone receptors. Also, elevated levels of progesterone potently reduce the sodium-retaining activity of aldosterone, resulting in natriuresis and a reduction in extracellular fluid volume. Progesterone withdrawal, on the other hand, is associated with a temporary increase in sodium retention (reduced natriuresis, with an increase in extracellular fluid volume) due to the compensatory increase in aldosterone production, which combats the blockade of the mineralocorticoid receptor by the previously elevated level of progesterone (Goldzieher and Castracane, 2010).

2.1.2 The physiological changes of the ovaries (The Ovarian Cycle)

Ovarian follicles are specialized structures in which both oocyte growth and meiosis I occur. The ovarian follicles are located in the cortex of the ovaries. Primary oocytes are located in the outer portion of the ovarian cortex, near the tunica albuginea in clusters called egg nests. After sexual maturation, a different group of primordial follicles is activated each month. This monthly process is known as the ovarian cycle (Silverthorn, 2013). The ovarian cycle can be divided into a follicular phase(or preovulatory phase), ovulation (or ovulatory phase) and a luteal phase(or postovulatory phase) (Shanyar, 2010).

Follicular phase: Follicle formation is stimulated by FSH from the anterior pituitary gland. The ovarian cycle begins as activated primordial follicles develop into primary follicles. In a primary follicle, the follicular cells enlarge and undergo repeated divisions that create several layers of follicular cells around the oocyte. These follicle cells are now called granulosa cells (Fortune, 1994).As layers of granulosa cells develop around the primary oocyte, microvilli from the surrounding granulosa cells intermingle with those of the primary oocyte. The microvilli are surrounded by a layer of glycoproteins; the entire region is called the zonapellucida. The microvilli increase the surface area available for the transfer of materials from the granulosa cells to the rapidly enlarging oocyte(Shanyar, 2010; Silverthon, 2013). As the granulosa cells enlarge and multiply, adjacent cells in the ovarian stroma form a layer of thecal cells around the follicle (Sokol, 2011). Thecal cells and granulosa cells work together to produce sex hormones called estrogens (Fortune, 1994; Sadler, 2012).

The Formation of Secondary Follicles: Although many primordial follicles develop into primary follicles, only a few will proceed to the next step. The transformation begins as the wall of the follicle thickens and the granulosa cells begin secreting small amounts of fluid. This follicular fluid, or liquor folliculi, accumulates in small pockets that gradually expand and separate the inner and outer layers of the follicle. At this stage, the complex is known as a secondary follicle (Silverthon, 2013).

The Formation of a Tertiary Follicle: Eight to 10 days after the start of the ovarian cycle, the ovaries generally contain only a single secondary follicle destined for further development. By the 10th to the 14th day of the cycle, that follicle has formed a tertiary follicle, or mature Graafian follicle, roughly 15mm in diameter. This complex spans the entire width of the ovarian cortex and distorts the ovarian capsule, creating a prominent bulge in the surface of the ovary. The oocyte projects into the antrum, or expanded central chamber of the follicle (Silverthon, 2013).

Instead of producing two secondary oocytes, the first meiotic division yields a secondary oocyte and a small, nonfunctional polar body. The secondary oocyte then enters meiosis II, but stops once again on reaching metaphase. Meiosis II will not be completed unless fertilization occurs. Generally, on day 14 of a 28–day cycle, the secondary oocyte and the surrounding granulosa cells lose their connections with the follicular wall. The granulosa cells immediately surrounding the secondary oocyte now drift free within the antrum and are known as the corona radiate (Sadler, 2012; Silverthon, 2013).

Ovulation:In the days immediately preceeding ovulation, under the influence of FSH and LH, the vesicular follicle grows rapidly to a diameter of 25mm to become a mature (vesicular) graafianfollicle. Coincident with final development ofthe vesicular follicle, there is an abrupt increasein LH that causes the primary oocyte to completemeiosis I and the follicle to enter thepreovulatory mature vesicular stage (Shanyar, 2010; Sadler, 2012). Meiosis IIis also initiated, but the oocyte is arrested inmetaphase approximately 3 hours before ovulation.In the meantime, the surface of the ovarybegins to bulge locally, and at the apex, an avascularspot, the stigma, appears. The high concentrationof LH increases collagenase activity,resulting in digestion of collagen fibers surroundingthe follicle. Prostaglandin levels alsoincrease in response to the LH surge and causelocal muscular contractions in the ovarian wall.Those contractions extrude the oocyte, whichtogether with its surrounding granulosa cellsfrom the region of the cumulus oophorus breaks free (ovulation) and floats out of the ovary. Some of the cumulus oophoruscells then rearrange themselves around thezonapellucida to form the corona radiata (Sadler, 2012).

The Formation and Degeneration of the Corpus Luteum (Luteal phase): The empty tertiary follicle initially collapses, and ruptured vessels bleed into the antrum. The remaining granulosa cells then invade the area, proliferating to create an endocrine structure known as the corpus luteum, named for its yellow color. This process occurs under LH stimulation. The lipids contained in the corpus luteum are used to manufacture steroid hormones known as progestins principally the steroid progesterone (Sadler, 2012; Shanyar, 2010; Silverthon, 2013). Although moderate amounts of estrogens are also secreted by the corpus luteum, levels are not as high as they were at ovulation, and progesterone is the principal hormone in the interval after ovulation. Its primary function is to prepare the uterus for pregnancy by stimulating the maturation of the uterine lining and the secretions of uterine glands. Unless fertilization occurs, the corpus luteum begins to degenerate roughly 12 days after ovulation. Progesterone and estrogen levels then fall markedly. Fibroblasts invade the nonfunctional corpus luteum, producing a knot of pale scar tissue called a corpus albicans. The disintegration, or involution, of the corpus luteum marks the end of the ovarian cycle. A new ovarian cycle then begins with the activation of another group of primordial follicles

(Silverthorn, 2013).

2.2 Fertile Window

The most fertile period (the time with the highest likelihood of pregnancy resulting from sexual intercourse) covers the time from some 5 days before until 1 to 2 days after ovulation. In a 28-day cycle with a 14-day luteal phase, this corresponds to the second and the beginning of the third week. A variety of methods have been developed to help individual women estimate the relatively fertile and the relatively infertile days in the cycle; these systems are called fertility awareness (Wilcox, *et al.,* 2000).

Fertility awareness methods that rely on cycle length records alone are called calendar-based methods. Methods that require observation of one or more of the three primary fertility signs (basal body temperature, cervical mucus, and cervical position) are known as symptoms-based methods.Urine test kits are available that detect the LH surge that occurs 24 to 36 hours before ovulation; these are known as ovulation predictor kits (OPKs). Computerized devices that interpret basal body temperatures, urinary test results, or changes in saliva are called fertility monitors (Wilcox, *et al.,* 2000).

A woman's fertility is also affected by her age. As a woman's total egg supply is formed in fetal life, to be ovulated decades later, it has been suggested that this long lifetime may make the chromatin of eggs more vulnerable to division problems, breakage, and mutation than the chromatin of sperm, which are produced continuously during a man's reproductive life (Krock, 2001; American Society for Reproductive Medicine, 2012).

A woman’s best reproductive years are in her 20s. Fertility gradually declines in the 30s, particularly after age 35. Each month that she tries, a healthy, fertile 30-year-old woman has a 20% chance of getting pregnant. That means that for every 100 fertile 30-year-old women trying to get pregnant in one cycle, 20 will be successful and the other 80 will have to try again. By age 40, a woman’s chance is less than 5% per cycle, so fewer than 5 out of every 100 women are expected to be successful each month (American Society for Reproductive Medicine, 2012).

2.3 Infertility

Infertility is the inability to naturally conceive a child or to carry a pregnancy to full term. Thereare many reasons why a couple may not be able to conceive, or may not be able to conceivewithout medical assistance. The chance ofgetting pregnant in each menstrual cycle (each month) is very high. However, it may take a longtime to conceive, even if everything is normal, because some may just be a bit weak. Eightout of every ten women trying for a baby will fall pregnant within the first six months. Womenwho do become pregnant without any medical assistance generally do so within 8 months of trying. It is common for couples to seek help and advice if there is difficulty conceiving (Gaware, *et al.*, 2009).

According to the World Health Organization (WHO), infertility can be described as the inability to become pregnant, maintain a pregnancy, or carry a pregnancy to live birth. A clinical definition of infertility by the WHO and International Community for Monitoring Assisted Reproductive Technology (ICMART) is "a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse" (Zegers-Hochschild, 2009; WHO, 2013). Infertility can further be broken down into primary and secondary infertility. Primary infertility refers to the inability to give birth either because of not being able to become pregnant, or carry a child to live birth, which may include miscarriage or a stillborn child. Secondary infertility refers to the inability to conceive or give birth when there was a previous pregnancy or live birth (WHO, 2013).

2.3.1 Prevalence

Female infertility varies widely by geographic location around the world. In 2010, there was an estimated 48.5 million infertile couples worldwide, and from 1990 to 2010 there was little change in levels of infertility in most of the world. In 2010, the countries with the lowest rates of female infertility included the South American countries of Peru, Bolivia, Ecuador and Bolivia, as well as in Poland, Kenya, and Republic of Korea. The highest rate regions included Eastern Europe, North Africa, the Middle East, Oceania, and Sub-Saharan Africa. The prevalence of primary infertility has increased since 1990, but secondary infertility has decreased overall. Rates decreased (although not prevalence) of female infertility in high-income, Central/Eastern Europe, and Central Asia regions (Mascarenhas*et al.*, 2012).

Sub-Saharan Africa has had decreasing levels of primary infertility from 1990 to 2010. Within the Sub-Saharan region, rates were lowest in Kenya, Zimbabwe, and Rwanda, while the highest rates were in Guinea, Mozambique, Angola, Gabon, and Cameroon along with Northern Africa near the Middle East. According to a 2004 DHS report, rates in Africa were highest in Middle and Sub-Saharan Africa, with East Africa's rates close behind (Rutstein and Iqbal, 2004).

2.3.2 Causes andfactors

Risk factors for female infertility include:

i. Age: Fertility begins to decline when a woman reaches her mid-30s, and rapidly declines after her late 30s.
ii. Weight: Although most of a woman's estrogen is manufactured in her ovaries, 30% is produced by fat cells, which transform male hormones produced by the adrenal glands into estrogen. Because a normal hormonal balance is essential for the process of conception, extreme weight levels (either high or low) can contribute to infertility (Medical Reference Guide, 2013).

Being Overweight: Being overweight or obese (fat levels that are 10 - 15% above normal) can contribute to infertility in various ways. Obesity is also associated with polycystic ovarian syndrome (PCOS), an endocrinologic disorder that can cause infertility.

Being Underweight. Body fat levels 10 - 15% below normal can completely shut down the reproductive process. Women at risk include:

i. Women with eating disorders, such as anorexia or bulimia nervosa.
ii. Women on very low-calorie or restrictive diets are at risk, especially if their periods are irregular.
iii. Strict vegetarians might have difficulties if they lack important nutrients, such as vitamin B12, zinc, iron, and folic acid.
iv. Marathon runners, dancers, and others who exercise very intensely.
v. Smoking. Cigarette smoking can impair a woman's fertility (Medical Reference Guide, 2013)

Infertility may be caused by an underlying medical condition that damages the fallopian tubes, interferes with ovulation, or causes hormonal complications. These medical conditions include:

i. Pelvic inflammatory disease
ii. Endometriosis
iii. Polycystic ovary syndrome
iv. Premature ovarian failure
v. Uterine fibroids (Medical Reference Guide, 2013)

Causes of female infertility can basically be classified regarding whether they are acquired or genetic, or strictly by location. Although factors that affect female infertility can be classified as either acquired or genetic, female infertility is usually more or less a combination of nature and nurture. Also, the presence of any single risk factor of female infertility (such as smoking) does not necessarily cause infertility, and even if a woman is definitely infertile, the infertility cannot definitely be blamed on any single risk factor even if the risk factor is (or has been) present (Hughes *et al.*,2004).

Most cases of female infertility are due to medical conditions that cause:

i. Ovulation problems
ii. Blocked fallopian tubes
iii. Structural problems in the reproductive system
iv. Problems with quality of cervical mucus or eggs (Hughes *et al.*, 2004)

Ovulation Problems

Ovulation is the release of the egg that occurs during the monthly menstrual cycle. Problems that affect ovulation, and the hormones involved with ovulation, are the most common cause of female infertility. They include:

i. *Polycystic Ovarian Syndrome (PCOS).* Women with PCOS do not ovulate regularly and they experience infrequent or absent menstrual cycles. In PCOS, the ovaries produce excessive amounts of androgens (male hormones), particularly testosterone. Increased androgen production results in high levels of luteinizing hormone (LH) and low levels of follicle-stimulating hormone (FSH), so that follicles are prevented from producing a mature egg. Without egg production, the follicles swell with fluid and form into cysts. The elevated levels of androgens can also cause obesity, facial hair, and acne. PCOS poses a high risk for insulin resistance, which is associated with type 2 diabetes.

ii. *Premature Ovarian Failure (Early Menopause):* Premature ovarian failure is the early depletion of follicles before age 40, which, in most cases, leads to premature menopause. It is often caused by hormonal issues such as adrenal, pituitary, or thyroid gland deficiencies. It can also be caused by genetic disorders such as Turner syndrome and fragile X syndrome. Certain types of autoimmune disorders (type 1 diabetes, systemic lupus erythematosus, Addison's disease) are associated with premature ovarian failure. Radiation and chemotherapy cancer treatments can also cause early menopause.

iii. *Hormonal Imbalances*: Imbalances with reproductive hormones such as FSH, LH, estrogen, and progesterone can interfere with ovulation.

Blocked Fallopian Tubes

A blocked fallopian tube can prevent sperm from reaching and fertilizing the egg. Blockage in the fallopian tubes can also prevent a fertilized egg from traveling to the uterus for implantation. Conditions that can block or damage fallopian tubes include:

i. *Pelvic Inflammatory Disease (PID):* PID refers to infection in the pelvic area and reproductive tract, including the fallopian tubes. PID is a complication of bacterial infection. The most common causes are sexually transmitted diseases, especially chlamydia and gonorrhea. In addition to infertility, PID can increase the risk for ectopic pregnancy, where the embryo implants in the fallopian tube or another location outside of the uterus.

ii. *Endometriosis*: Endometriosis is a condition in which cells that line the uterus grow in areas outside of the uterus, such as the ovaries. Endometriosis rarely causes a complete inability to conceive, but it can reduce fertility both directly and indirectly. Implants in the fallopian tubes may block the egg's passage, while implants that occur in the ovaries can prevent the release of the egg. Severe endometriosis can form bands of scar tissue (adhesions) between the uterus, ovaries, and fallopian tubes that prevent egg transfer.

iii. *Uterine or Abnormal Scarring*: Adhesions can be caused by conditions besides endometriosis. Adhesions that form after abdominal or pelvic surgery or infection can restrict the movement of ovaries and fallopian tubes and may cause infertility. Asherman syndrome, for example, is scarring in the uterus that may be caused by surgery, repeated injury, or unknown factors.

iv. *Uterine Fibroids*: Uterine fibroids may contribute to infertility by blocking the fallopian tube, or by distorting the shape of the uterine cavity. Fibroids may also impair blood flow to the uterine lining.

Other Causes of Female Infertility

Other possible causes of female infertility include:

i. *Elevated Prolactin Level:* Prolactin is a hormone produced in the pituitary gland that stimulates breast development and milk production in association with pregnancy. High levels of prolactin (hyperprolactinemia) reduce gonadotropin hormones and inhibit ovulation. Hyperprolactinemia in women who are not pregnant or nursing can be caused by an underactive thyroid gland or pituitary adenoma. (Pituitary adenomas are benign tumors that secrete prolactin.) Some drugs, including oral contraceptives and some antipsychotic drugs, can also elevate levels of prolactin.

ii. *Congenital Structural Abnormalities:* Congenital reproductive tract abnormalities may cause infertility. These malformations typically affect the uterus or vagina. Daughters born to women who took the drug diethylstilbestrol (DES) during pregnancy are at increased risk of having uterine or fallopian tube structural abnormalities associated with infertility. Some women who are born with uterine or other reproductive tract malformations are still able to have successful pregnancies. Surgery can correct some of these problems.

iii. *Cervical Mucus:* Low amounts of cervical mucus or poor quality cervical mucus can contribute to infertility. Cervical mucus problems may be related to hormonal imbalances, prior surgeries, and certain medications.

iv. *Egg Quality*: As women age, the number and quality of their eggs diminish. Younger women can also have problems with egg quality, usually because of medical conditions or treatments that impair the ovaries. Radiation and chemotherapy cancer treatments, for example, can damage ovaries and affect egg development.

2.3.3 Treatment

Treatment for infertility should first address any underlying medical condition that may be contributing to fertility problems. Drugs, surgery, or both may be used to treat these conditions. Surgery may also be used to repair blockage in fallopian tubes (Hughes *et al.,* 2004).Fertility treatments are most likely to benefit women whose infertility is due to problems with ovulation. Treatment is least likely to benefit infertility caused by damage to the fallopian tubes or severe endometriosis (NICHD, 2013).

Infertility associated with infrequent or absent ovulation often can be treated with hormone medications called fertility drugs (Harvard Mental Health Letter, 2009).Fertility drugs are often used alone as initial treatment to induce ovulation. If they fail as sole therapy, they may be used with assisted reproductive procedures, such as in vitro infertilization, to produce multiple eggs, a process called *superovulation*. According to the American Society for Reproductive Medicine, fertility drugs can be divided into three main categories:

i. *Medications for Ovarian Stimulation*. Clomiphene (Clomid, Serophene, generic); letrozole (Femara), follicle stimulating hormone (FSH) [Follistim, Gonal-F, Bravelle]; human menopausal gonadotrophin (hMG) [Humegon, Repronex, Menopur]; luteneizing hormone (LH) [Luveris]
ii. *Medications for Oocyte Maturation*. Human chorionic gonadotropin (hCG) [Profasi, APL, Pregnyl, Novarel, Ovidrel]
iii. *Medications to Prevent Premature Ovulation*. GnRh agonists [leuprolide (Lupron, generic), nafarelin (Synarel), and goserelin

(Zoladex)]; Gn RH antagonists [ganirelix (Antagon), cetrorelix (Cetrotide)] (Hughes *et al.*, 2004).

2.4 HERBAL TREATMENT FOR INFERTILITY

Herbs have been used since the beginning of time to aid in many different ailments. Of these ailments, fertility has been enhanced and even corrected by the use of certain herbs (Etuk and Muhammad, 2009). Plant drugs have been used since time immemorial for their effects upon sex hormones particularly for suppressing fertility, regularizing menstrual cycle, relieving dysmennoroea, treating enlarged prostate, menopausal symptoms, breast pain and during and after childhood. Specific biological effects under the division of fertility regulating category are non- specific contraceptive or antifertility effects, abortifacient, uterine stimulant and uterine relaxants, labour induction and labour inhibition oxytocic and anti- oxytocic, estrogenic and anti- estrogenic, progestrogenic and anti- progesterogenic, ovulatory and anti- ovulatory, androgenic and anti- androgenic, spermicidal and anti- spermatogenic effects (Pradhan*et al.*, 2012).

Sesamumindicum is one of the most widely used plants for the treatment of infertility in Northern Nigeria. This is considered to be beneficial in view of the presence of phytoestrogens (particularly lignans) in the seeds. Up to 1.5% of the weight of sesame seed or oil is made up of lignans, the majority of which are sesamin and sesamolin. The level of lignans in sesame is even higher than in flaxseed, which was previously considered the richest source of lignans. Flaxseed lignans, isoflavones, and coumestans, have generally been categorized as the 3 major groups of phytoestrogens. Enterolactone (ENL) and enterodiol converted from flaxseed lignans by intestinal microflora, are considered the agents responsible for estrogenic activity (Wen-Huey *et al.* 2006). However, phytochemical screening of the aqueous and ethanolic extracts has revealed the presence of toxic agents such as glycosides, alkaloids, flavonoids, tannins, steroids and saponins (Sireesha*et al.*, 2013). These agents can affect regulation of estrus cycle, conception and reproduction. Alkaloids and flavonoids have been shown to reduce plasma concentrations of LH, estradiol and FSH. The enhanced levels tend to suppress the ovulatory cycle by inhibiting the secretion of both follicle-stimulating and gonadotropic-releasing hormones (GnRH), which are necessary for ovulation (Raj. *et al.*, 2011).

Many common plants can be used to influence fertility, including red clover, partridge berry, liferoot, wild carrot, and wild yam (Weed, 1999). Most of these plants contain phytoestrogens, responsible for their fertility-enhancing effects.The herbs and foods most often implicated with being estrogenic include *Trifolium pretense* (red clover), *Cimicifugaracemosa* (black cohosh), *Humuluslupulus* (hops), *Angelica sinensis* (dong quai, Dang Gui), *Glycyrrhizaglabra* (licorice)*Vitexagnuscastus* (chasteberry), *Polygonummultiflorum* (fo-ti), *Panax ginseng* (Asian ginseng), and *Panaxquinquefolius* (North American ginseng) (Sperber, 2014).

However, increased exposure to hormones - such as those used in the cattle industry, those given to women during menopause, those taken by women engaged in hi-tech pregnancy efforts, and even those naturally produced by the body - increases the risk of cancer, especially breast cancer. And many believe that hormone-like chemicals - xenoestrogens - increasingly found in our food and water, contribute to cancer as well (Weed, 2002). Hence, the use of herbs for the treatment of infertility should be done with great caution.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant collection

Plant seeds were obtained in May 2014 from a farm in Lere Local Government Area of Kaduna State, Nigeria. The seeds were identified at the Herbarium Section of the Department of Biological Sciences, Ahmadu Bello University Zaria, with a voucher number of 04 by Malam Musa Muhammad.

3.1.2 Experimental animals

Sixty (n=60) matured femalealbino rats were obtained from the animal house of the Department of Human Physiology, Ahmadu Bello University, Zaria. The animals were kept under well-ventilated housing conditions and provided with feed and water *ad libitum* throughout the experimental period.Matured female albino rats of about 10-12 weeks old (120-140g) were selected for the experiment.The vaginal smear of the rats was obtained from the rats' vagina using a microbiology pasteur loop before commencement of the research. The vaginal swab was observed after staining with Giemsa stain under a light microscope(Marcondes*et al.*2002; Aydin*et al.* 2011)

3.1.3 Laboratory materials

ELISA kits (Hinotek Group Ltd, Japan; Dist. by Bio Rapid Diagnostics, Nig. Ltd.), light microscope, dissecting microscope, digital weighing balance, centrifugemachine, micropipette, microplate reader, microscopic slides, petri dishes, microbiology pasteur loop, dissection kit, dissecting board, surgical gloves, disposable gloves, catgut material, cotton wool, oro-pharyngeal cannula, syringes, 1ml rubber pipette, anaesthesia box, sample bottles, mortar and pestle were used for the experiment.

3.1.4 Chemicals and solutions

Ethanol, methanol, Giemsa stain, oil immersion, chloroform, distilled water, normal saline, methylated spirit, disinfectant (Dettol) andGentine violet (GV) solution.

3.2 Methodology

3.2.1 Preparation of extract

The dried seeds were pounded into powder using mortar and pestle. Five hundred (500g) of the powdered seeds was dissolved in 70% ethanol and 30% water at room temperature for 48 hours. The solution was filtered using muslin cloth. The filtrate was allowed to stand for 1-2 hours. It was then decanted gently and evaporated to dryness on water bath at 70^{o}C. A brownish semi-solid oily residue of about 45.3g was obtained and was kept in air-tight container at room temperature.

3.2.2 Acute toxicity study

Determination of the median lethal dose (LD_{50}) was done using Lorke's method (1983). Thirteen (n=13) rats of both sexes were used. This method was carried out in two phases. In phase I, nine animals were divided into three groups of three animals each. The groups were administered 10, 100 and 1000 mg/kg extract of *S.indicum*, respectively. The animals were observed for 24 hours for any sign of toxicity and mortality.

In the second phase, three animals were used (i.e 1 rat per group). The rats were administered 1600, 2900 and 5000 mg/kg extract of *S. indicum* respectively. The animals were subsequently observed for signs of toxicity, discomfort or mortality. The $LD_{50}$was calculated as the geometric mean of the maximumdose producing 0% mortality and the minimum doseproducing 100% mortality.

LD_{50}= $\sqrt{D0}/D100$

Where: D0 = Maximum dose producing 0% mortality

D100 = Minimum dose producing 100% mortality

3.3 Experimental Design

3.3.1 Ovulation studies

Castrated male rats were introduced into cages of isolated female rats at a ratio of 4:1 and left together for two (2) days. Four (4) pre-treatment estrus cycles of the female rats were established by vaginal smears. The female rats that have undergone four successive 4- day cycle were selected for this study. Twenty (20) matured female albino rats were divided into four (4) groups, each group consisting of five (5) rats each (Oyesola*et al*, 2010). The rats were treated as follows

Group I: control group (1ml/kg distilled water)

Group II: 100mg/kg of extract

Group III: 300mg/kg of extract

Group IV: 500mg/kg of extract

The extract administration was doneorally atfour (4) hour interval (8am, 12noon, and 4pm). The animals were sacrificed the morning after the last administration. The uteri and ovaries of the rats were removed by abdominal incision (Akpantah*et al.*, 2005).

Weight measurements

The uteri and ovarian weights were measured immediately after dissection using digital weighing balance. Mean (± SEM) were calculated forthe gonadosomatic index ([gonad weight/body weight] x 100%)(Oluwatoyin*et al.*, 2013).

Ovulation count

The uterus and ovaries of the rats were removed, placed on a petri dish suspended in normal saline. The tissues were viewed under a dissecting microscope and the number of ovulation points and matured follicles were counted (Akpantah *et al.*, 2005).

3.3.2. Effect of extract on serum concentration of estrogen and progesterone

Twenty (n = 20) matured female albino rats were divided into four (4) groups, each n=5 and treated as follows:

Group I: Control group (1 ml/kg distilled water)

Group II: 100mg/kg of extract

Group III: 300mg/kg of extract

Group IV: 500mg/kg of extract

The animals received treatment orally using plastic syringes attached to oropharygeal cannula for a period of 30 days. The animals were sacrificed 24hrs after the last dose. Blood samples were collected by cardiac puncture for biochemical analysis. The blood was centrifuged and the sera aspirated and used for assayof estrogen and progesterone (Thakur *et al.,* 2009).

Determination of serum estrogen and progesterone levels

The serum estrogen and progesterone levels were analysed from the serum of the sacrificed rats at the Department of Chemical Pathology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria using ELISA hormone assay kits by the method of Tietz (1995) following the Manufacturer's manual.

3.3.3. Effect of extract on fetal number

Rats at stage of proestrus and estrus were left overnight with mature male albino rats at a ratio of 2:1. The vaginal smear was examined for presence of spermatozoa and/or vaginal plug morning of the next day. The day on which the spermatozoa are found is considered day one of pregnancy. The pregnant rats were divided into four (4) groups of 5 animals each. The animals received extract for seven days i.e., days 1-7 of pregnancy as follow:

Group I: Control group (1 ml/kg distilled water)

Group II: 100mg/kg of extract

Group III: 300mg/kg of extract

Group IV: 500mg/kg of extract

The animals were allowed to deliver at term (Montaserti*et al.*, 2007).

Litter count

The number of fetus was counted and fetal sizes observed.

3.4. Statistical Analysis

Data obtained were expressed as mean ± SEM and statistically analyzed using one-way analysis of variance (ANOVA) with Tukey's post – hoc test to compare the level of significance between the test groups.The statistical package used was SPSS version 17. Values with $p<0.05$ were considered significant.

CHAPTER FOUR

4.0 RESULTS

Table 4.1.: Results of acute toxicity study; showing doses of *Sesamumindicum*extract, recorded number of deaths and percentage mortality

Phase one

Group (N=3)	Dose (mg/kg) body weight	Deaths	Percentage mortality (%)
1	10	0/3	0
2	100	0/3	0
3	1000	0/3	0

Phase two

Group (N=1)	Dose (mg/kg) body weight	Deaths	Percentage mortality (%)
1	1600	0/1	0
2	2900	0/1	0
3	5000	0/1	0

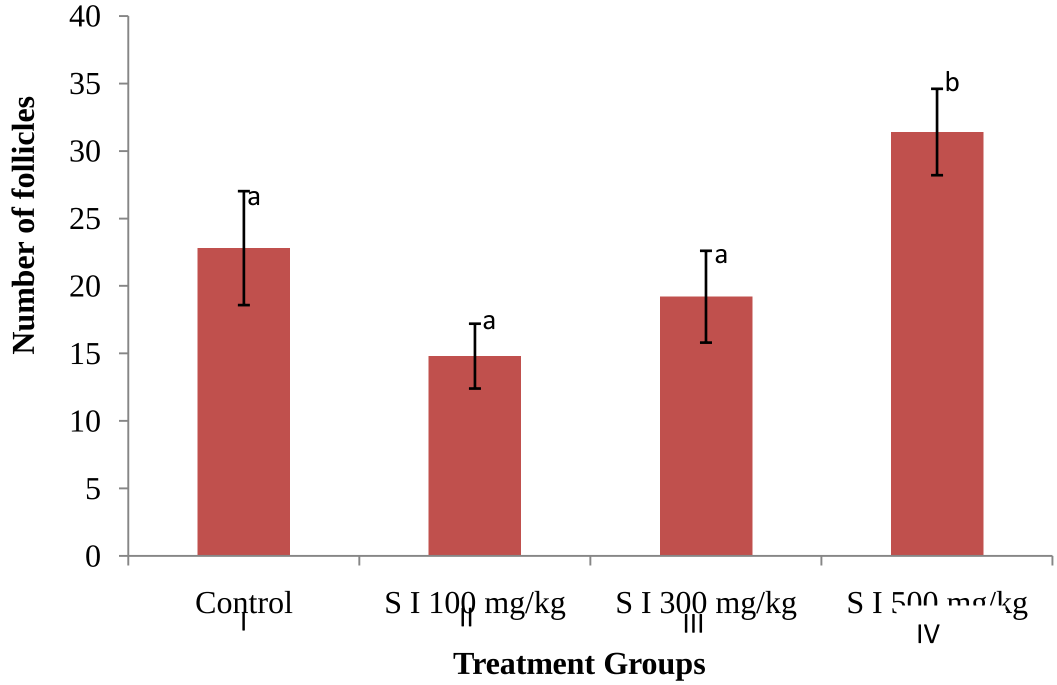

Figure 4.1: Mean values (± SEM) of number of ova in control and *Sesamumindicum* treatedmatured female albino rats

Number of follicles was significantly higher in group IV.

[a, b] level of significance at $p<0.05$

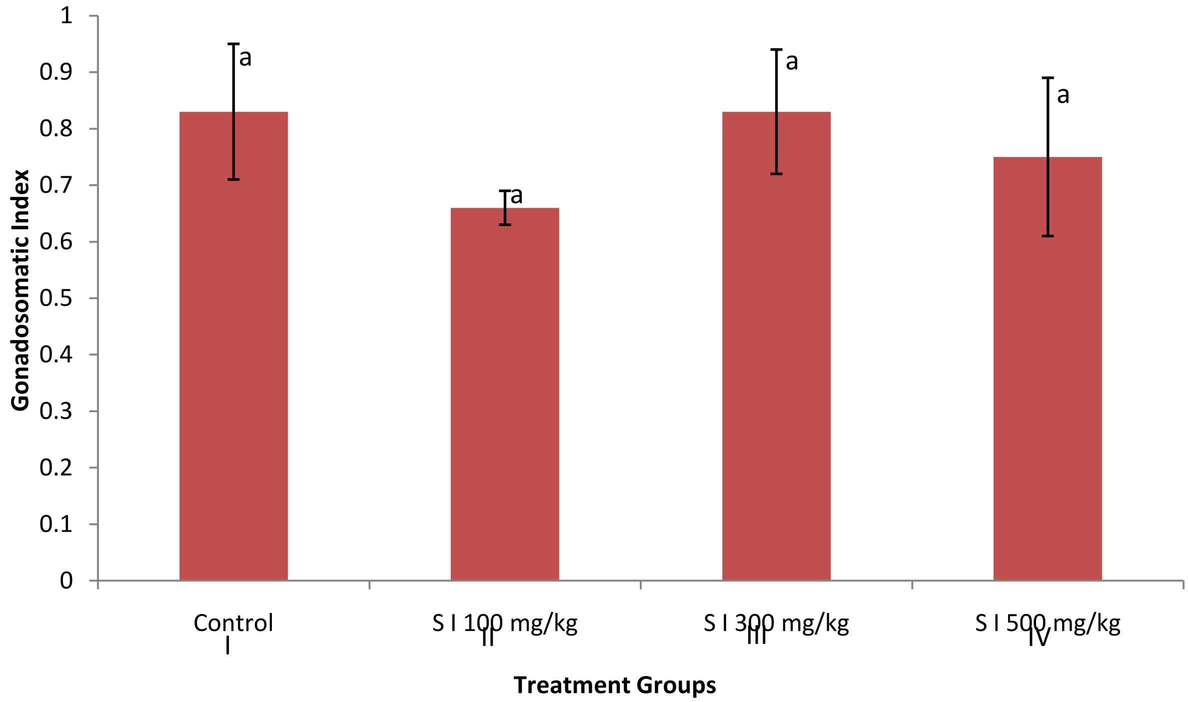

Figure 4.2 Mean values (+ SEM) of gonadosomatic indexin control and *Sesamumindicum* seeds extract treated matured female albino rats

[a, b] level of significance at $p<0.05$

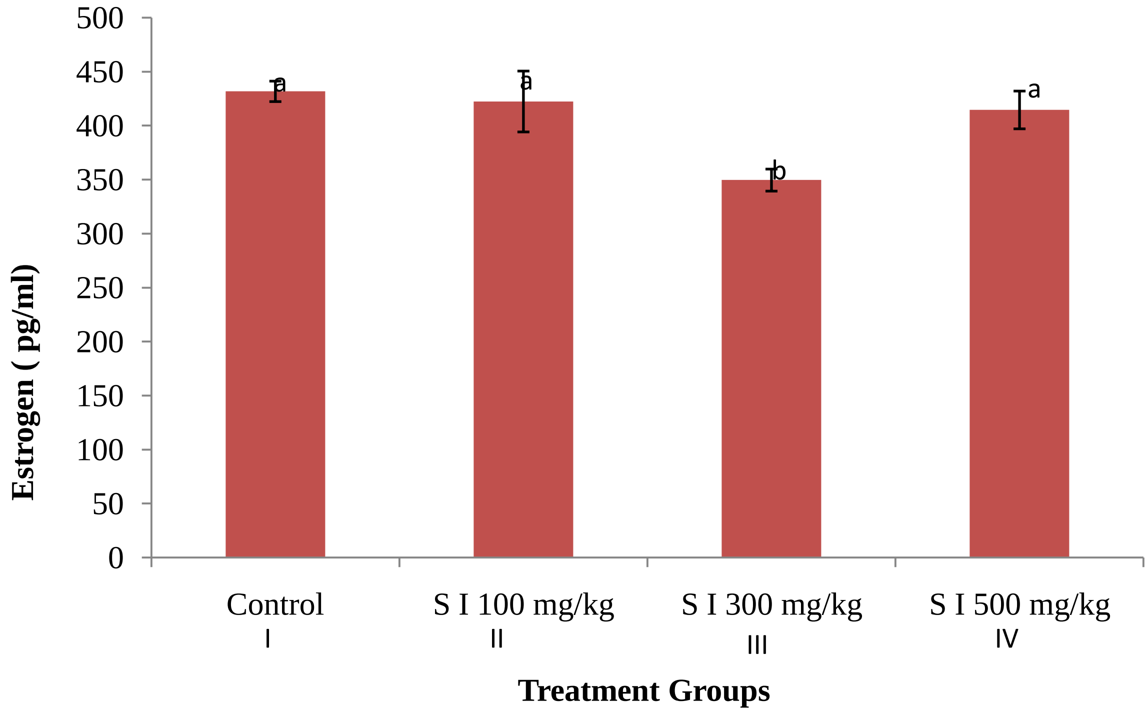

Figure 4.3 Mean (+ SEM) of serum concentration of estrogen in control and *Sesamumindicum*treated matured female albino rats

Serum estrogen was significantly lower ($p<0.05$) in group III.

[a, b] level of significance at $p<0.05$

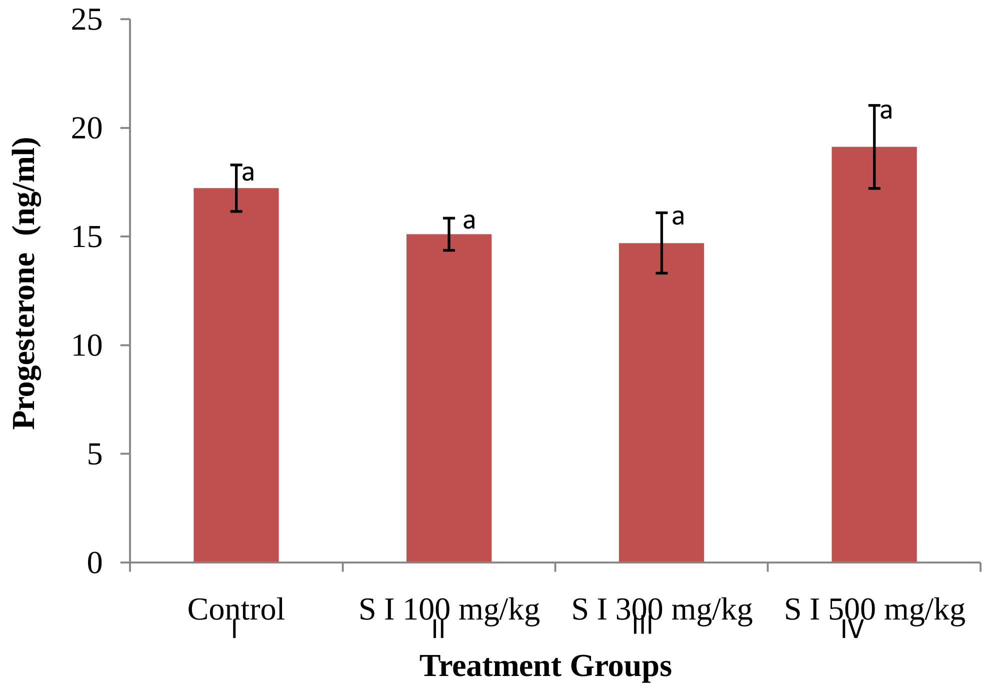

Figure 4.4 Mean (+ SEM) of serum concentration of progesterone in control and *Sesamumindicum* treated matured female albino rats

[a, b] level of significance at p<0.05

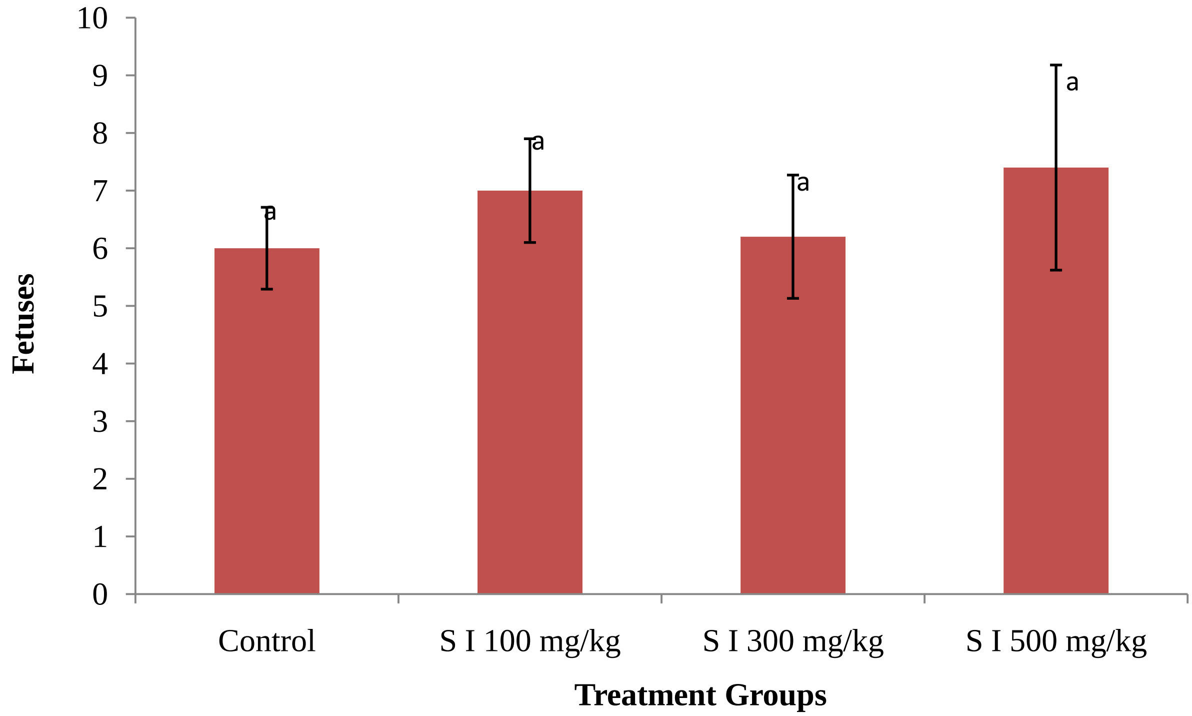

Figure 4.5 Mean (± SEM) of number of fetuses delivered by pregnant rats in control and *Sesamumindicum* treated matured female albino rats

[a, b] level of significance at $p<0.05$

CHAPTER FIVE

5.0 DISCUSSIONS

The result of acute toxicity study showed that the ethanolic seeds extract is relatively safe up to 5000mg/kg. This is not surprising because the plant has been consumed as food by man and other animals for ages without any deleterious effect.

5.1 Effects of S*esamumindicum* on Follicular Numbers and Ovulation

The results showed a slight non-significant decrease in the number of follicles inthe two groups that received 100 and 300 mg/kg of extract as compared with the controlgroup. However, there was a highly significant increase in number of follicles observed at high dose of extract (500 mg/kg) as compared with the control which could be attributed to the effect of the extract. The seeds of *S. indicum*are known to containphytoestrogens. Phytoestrogens are any plant compounds structurally and/ or functionally similar to ovarian and placental estrogens and their active metabolites. They include a vast variety of structurally diverse compound such as isoflavones found in soy, lignans found in grains, stilbenes found in the skin of grapes and fungal metabolites like macrolides. Phytoestrogens have been found to bind to the estrogen receptors in the hypothalamus and pituitary glands, indicating that the compound can interfere with the normal feed-back loop regulating the hormonal axis (Eriksen, 2006). Plants with estrogenic property can directly influence pituitary action by peripheral modulation of LH and

FSHdecreasing secretion of these hormones and blocking ovulation. The decrease in LH and FSH could explain the blockage of estrous cycle and ovulation by some plant extracts. All substances that are able to inhibit this release could provoke an ovulation disruption by decreasing the number of matured follicles (Pradhan*et al.,* 2012). FSH stimulates the growth and maturation of ovarian follicles by acting directly on the receptors located on the granulosa cells. It is possible that *S. indicum* extract might have exerted its effect on the anterior pituitary or the hypothalamus since the secretion of FSH is regulated by the gonadotropic releasing hormone secreted by the hypothalamus. This study agrees with the findings of Devendra*et al.* (2009), but however disagrees with that of Yakubu*et al.* (2008), who observed that the leaf extract of *C.aconitifolius* exert effect on ovulation by increasing the FSH level and decreasing the LH level. The high significant increase in the number of ova in the highest dose group is as a result of the negative feedback effect of the low estrogen level at the lower doses on GnRH (Frankovich and Lebrun, 2000), thereby increasing further release of FSH and LH that promote follicular maturation and ovulation respectively. This is in line with findings by Thompson and Rickard in 1997. They showed that lignans tend to act in an agonistic manner when the estrogen levels are low.

5.2 Effect of *Sesamumindicum*seeds extract on Gonadosomatic Index

There was no significant difference in the gonadosomatic index of the treatment groups as compared with the control. This shows that the extract possibly does not have effect on gonadotropin-releasing hormone (GnRH). Generally, sexual maturation and reproductive functions in mammals are mainly regulated by the GnRH which is released from the hypothalamus in a pulsatile way and this triggers the production and release of the gonadotropins, FSH and LH. The action of FSH on the ovary varies and the developmental stage of the follicles determines the nature of this effect. The non-reduction in the gonadosomatic index indicates that phytoestrogenic content of the extract had no significant effect on the ovarian mass and follicular atresia. This is in contrast to the report of Oluwatoyin*et al.* (2013), which showed that *Aloe vera gel*affectedgonadosomatic index (an index of ovarian mass).

5.3 Effect of *Sesamumindicum* extract on serum concentration of estrogen and progesterone

The results of this study showed a decrease in serum estrogen levels in treated as compared to control group. In lowest and highest doses, the decrease was low, while it was highly significant ($p < 0.05$) at 300 mg/kg dose. The decrease in estrogen level can be attributed to the decrease in folliculogenesis. Studies by Dusza*et al.*, (2006) revealed that phytoestrogens affect estrogen production by the granulosa cells. The same observation was reported on the effect of aqueous extract of *Bougainvillea spectabilis*leaves by Mishra *et al.,* 2009. When phytoestrogens bind to estrogen receptors in the cells, they translocate to the nucleus and stimulate cell growth in a manner similar to estradiol. Despite the apparently weak relative binding capacity of the phytoestrogens they have significant hormonal effects (Thakur *et al.*, 2009). This is due to their lower affinity for the serum estrogen binding proteins, and resulting in a net effect of enhancing the concentration of available phytoestrogen at the target tissue sites. Plants estrogens bind directly to estrogen receptors and produced estrogenic effect. Estrogens secreted by the granulose cells have a positive feedback effect on the ovary to produce more estrogens. *S. indicum*may also affect FSH release from pituitary gland or inhibit the positive feedback secretion of estrogens by the ovary resulting in decreased estrogen secretion at all doses of the extract used. The slight increase in the estrogen level in group IV that received 500 mg/kg of extract as compared to group III that received 300 mg/kg can be as a result of an increase in follicle numbers. It may also be attributed to an increase in FSH level at that dose. FSH and ovarian estrogens stimulate the ovarian production of more estrogens by the granulosa cells of the ovarian follicles and corpora lutea (Homburg, 2014).

Progesterone which is produced in the ovaries, placenta, and adrenal glands, helps to regulate the monthly menstrual cycle, prepare the uterus for conception and pregnancy as well as stimulate sexual desire(Goldzieher and Castracane, 2008). In the present study, there was a slight non-significant decrease in the serum progesterone levels ingroups II and III compared to control and group IV rats. This increase in progesterone concentration observed in group IV as compared to control rats may be as a result of the increase in follicular numbers observed at that dose. The weak progesterone derivative, 17-hydroxy progesterone is produced by the ovarian follicles. On the other hand, alkaloids present in the extract might be responsible for the slight decrease in the serum progesterone levels observed in groups II and III rats. This finding agrees with the report ofYakubu*et al.* (2008), who observed that extract of *C.aconitifolius* caused an decrease in serum progesterone level of female rats.

5.4 Effect of *Sesamumindicum*seeds extract on Fetal Number

The present study recorded no any significant difference in the number of fetuses between treated and control rats. This shows that the extract did not have any pro- or antifertility effect on fertilization and implantation at all dose levels used. This findingdoes not agree with the report of other researchers that showed that plants that possess estrogenic compounds interfere with pregnancy by possibly affecting the uterine estrogen receptors (Badami, *et al.*, 2003; Hazarika*et al.,* 2007; Sripriya*et al.*, 2011).

CHAPTER SIX

6.0 CONCLUSION, RECOMMENDATIONS AND CONTRIBUTIONS

6.1 Conclusion

This study has demonstrated that the hydroethanolic seeds extract of *S. indicum* possess both anti- and pro-estrogenic properties in a dose-dependent fashion in female albino rats.

6.2 Recommendations

Based on the findings of this study, it is recommended that:

1. The effects of the extract of *S. indicum*should be carried out at different phases of the estrus cycle, pregnancy and lactation.
2. Isolation of the active components of *S. indicum* and study their effects on isolated uterine tissue to determine the uterotrophic effects of the components.

6.3 Contributions to Knowledge

1. The hydroethanolic seeds extract of *S. indicum*is safe for oral consumption.
2. The hydroethalonic seeds extract has a dose dependent effect on serum levels of estrogen.
3. The seeds extract of *S. indicum*has both competitive inhibitory and stimulatory effect on ovarian secretion of estrogens.

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Appendix I

Toxicity Study

Phase 1

Weights of Rats, Volume of Extract Administered and Number of Death at 10mg/kg Dose

s/no	Weight (g)	Volume of extract administered (ml)	Status; Death (X), Live (A)
I	216	0.21	A
II	200	0.20	A
III	192	0.19	A

Weights of Rats, Volume of Extract Administered and Number of Death at 100mg/kg Dose

s/no	Weight (g)	Volume of extract administered (ml)	Status; Death (X), Live (A)
I	180	0.18	A
II	180	0.18	A
III	182	0.18	A

Weights of Rats, Volume of Extract Administered and Number of Death at 1000 mg/kg Dose

s/no	**Weight (g)**	**Volume of extract administered (ml)**	**Status; Death (X), Live (A)**
I	188	0.18	A
II	200	0.20	A
III	154	0.15	A

Phase 2

Weights of Rats, Doses and Volume of Extract Administered and Status of Death

s/no	**Doses (mg/kg)**	**Volume administered (ml)**	**Weight (g)**	**Death Status**
I	1,600	188	0.18	No
II	2,900	200	0.20	No
III	5,000	154	0.15	No

Appendix 2

Ovulation study

Record of animal weights, matured follicles, ovulation points and total number of ova in experimental groups of ovulation study

Group I: Animal weights, number of matured follicles (MF), number of ovulation points (OP) and total number of ova for control group

s/no	Weights (g)	Left ovary (MF/OP)	Right ovary (MF/ OP)	Total
Blue 1	122	4/6	0/9	19
Blue 2	131	4/0	5/0	9
Blue 3	121	3/11	8/9	31
Blue 4	140	5/6	5/7	23
Blue 5	131	10/10	4/8	32

Group II: Animal weights, number of matured follicles (MF), ovulation points (OP) and total number of ova for 100mg/kg extract group

s/no	**Weights (g)**	**Left ovary (MF/OP)**	**Right ovary (MF/OP)**	**Total**
Green 1	130	6/3	1/4	14
Green 2	124	0/3	10/6	19
Green 3	130	0/4	9/6	19
Green 4	130	0/2	3/1	6
Green 5	125	3/4	1/8	16

Group III: Animal weights, number of matured follicles (MF), number of ovulation points (OP) and total number of ova for 300mg/kg extract group

s/no	**Weights (g)**	**Left ovary (MF/OP)**	**Right ovary (MF/OP)**	**Total**
Black 1	130	0/7	5/7	19
Black 2	130	6/2	4/4	16
Black 3	120	6/1	1/4	12
Black 4	134	1/6	3/7	17
Black 5	122	6/9	5/12	32

Group IV: Animal weights, number of matured follicles (MF), number of ovulation points (OP) and total number of ova for 500mg/kg extract group

s/no	**Weights (g)**	**Left ovary (MF/OP)**	**Right ovary (MF/OP)**	**Total**
Red 1	123	7/5	3/10	25
Red 2	130	1/9	3/13	26
Red 3	130	12/12	11/6	41
Red 4	120	7/6	8/7	28
Red 5	131	2/12	3/20	37

Record of animal weights, uterine/ovarian weights and gonadosomatic index for ovulation study

Group I: Weight of animals, uterine/ovarian, and gonadosomatic index administered distilled water (control group)

s/no	Weights (g)	Uterine/ovarian weights (g)	Gonadosomatic index (%)
Blue I	122	1.55	1.27
Blue 2	131	0.84	0.64
Blue	121	1.00	0.83
Blue 4	140	0.89	0.64
Blue	131	1.00	0.74

Group II: Weight of animals, uterine/ovarian weights and gonadosomatic index for 100mg/kg extract group

s/no	**Weights (g)**	**Uterine/Ovarian weight (g)**	**Gonadosomaticindex (%)**
Green 1130		0.92	0.71
Green 2124		0.95	0.77
Green 3130		0.80	0.62
Green 4130		0.82	0.61
Green 5125		0.76	0.61

Group III: Weights of animals, uterine/ovarian eights and gonadosomatic index for 300mg/kg extract group

s/no WeightsUterine/ovarian weight (g)	**(g)**	**Gonadosomaticindex (%)**
Black 1130	1.57	1.21
Black 2130	1.01	0.78
Black 3120	0.75	0.63
Black 4134	0.78	0.58
Black 5122	1.16	0.95

Group IV: Weights of animals, uterine/ovarian weights and gonadosomatic index for 500mg/kg extract group

s/no	**Weights (g)**	**Uterine/ovarian weights (g)**	**Gonadosomaticindex (%)**
Red 1	123	0.81	0.66
Red 2	130	0.89	0.68
Red 3	130	0.52	0.40
Red 4	120	1.47	1.23
Red 5	131	1.02	0.78

Records of the effects of *Sesamumindicum* extract on follicular number and ovulation

Treatment	Dose (mg/kg)	No. of ova
I (Control)	Distilled water	22.80 ± 4.22
II	SI 100	14.80 ± 2.40
III	SI 300	19.80 ± 3.40
IV	SI 500	31.40 ± 3.20

Records of the effects of *Sesamumindicum*extract on gonadosomatic index of female albino rats

Treatment	Dose (mg/kg)	Gonadosomatic index (%)
I (Control)	Distilled water	0.83 ± 0.12
II	SI 100	0.66 ± 0.03
III	SI 300	0.83 ± 0.11
IV	SI 500	0.75 ± 0.14*

Appendix 3

Hormonal Level

Records of Hormonal levels in Experimental Animals

1. Estrogen Levels

Estrogen and Progesterone levels for control group

s/no	Estrogen (pg/ml)	Progesterone (ng/ml)
Blue 1	420	13.8
Blue 2	461	17.8
Blue 3	441	20.2
Blue 4	405	18.1
Blue 5	432	16.2

Group II: Estrogen and Progesterone levels in 100mg/kg extract group

s/no	**Estrogen (pg/ml)**	**Progesterone (ng/ml)**
Green 1	337	13.3
Green 2	443	16.8
Green 3	406	13.3
Green 4	511	16.2
Green 5	415	16.4

Group III: Estrogen and Progesterone levels for 300mg/kg extract group

s/no	**Estrogen (pg/ml)**	**Progesterone (ng/ml)**
Black 1	380	13.8
Black 2	337	12.6
Black 3	324	13.6
Black 4	366	20.2
Black 5	341	13.3

Group IV: Estrogen and Progesterone levels for 500mg/kg extract group

s/no	**Estrogen (pg/ml)**	**Progesterone (ng/ml)**
Red 1	461	17.6
Red 2	380	13.3
Red 3	420	24.1
Red 4	442	18.2
Red 5	370	22.4

Records of the effect of *Sesamumindicum*extract on serum concentration of estrogen and progesterone

Treatment	Dose (mg/kg)	Estrogen (pg/ml)	Progesterone (ng/ml)
I (Control)	Distilled water	431.80 $\pm$ 9.47	17.22 $\pm$ 4.22
II	SI 100	422.40 $\pm$ 28.19	15.10 $\pm$ 0.74
III	SI 300	349.60 $\pm$ 10.20*	14.70 $\pm$ 1.39
IV	SI 500	414.60 $\pm$ 17.49	19.12 $\pm$ 1.91

Appendix 4

Fertility study

Records for number of fetuses delivered by experimental animals

Group I: Number of fetuses delivered by control group

s/no	No. of fetuses
Blue 1	4
Blue 2	8
Blue 3	7
Blue 4	6
Blue 5	5

Group II: Number of fetuses delivered by rats administered 100mg/kg extract

s/no	**No. of fetuses**
Green 1	10
Green 2	6
Green 3	8
Green 4	5
Green 5	6

Group III: Number of fetuses delivered by rats administered 300mg/kg extract

s/no	**No. of fetuses**
Black 1	8
Black 2	6
Black 3	3
Black 4	5
Black 5	9

Group IV: Number of fetuses delivered by rats administered 500mg/kg extract

s/no	No. of fetuses
Red 1	14
Red 2	6
Red 3	5
Red 4	8
Red 5	4

Records of effect of *Sesamumindicum*extract on fetal number

Treatment	Dose (mg/kg)	Fetuses
I (Control)	Distilled water	6.0 ± 0.71
II	SI 100	7.00 ± 0.90
III	SI 300	6.20 ± 1.07
IV	SI 500	7.40 ± 1.78