EFFECTS OF HYDROETHANOLIC EXTRACT OF THE SEEDS OF *SESAMUM INDICUM* ON HORMONAL LEVELS OF FEMALE WISTAR RATS

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ABSTRACT

Fertility regulation with plants or plant preparations has been reported in the ancient literature of indigenous systems of medicine. 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. *Sesamum indicum* is one of the phytoestrogen containing edible seeds consumed by man as food and for medicinal purposes. Present study evaluated the mean lethal dose of the hydroethanolic seed extract of *Sesamum indicum* and the effects of the extract on some hormonal levels of female Wistar rats. Cycling female rats weighing 120-140g were used for the studies. 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 for 30 days while control group received distilled water at dose of 1ml/kg for 30 days. The animals were subsequently euthanized in the morning of the day after the last administration. The serum estrogen concentration was significantly lower ($p<0.05$) in 300 mg/kg group than in 100 mg/kg and 500 mg/kg groups and the control rats. The mean serum progesterone level showed relatively lower values in 300 mg/kg group than the 100 and 500 mg/kg groups and the control rats. 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 Wistar rats.

Keywords: Estrogen, phytoestrogens, progesterone, *Sesamum indicum*.
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INTRODUCTION

Hormones are intimately involved in our sex lives, as well as all other aspects of our lives, from intrauterine life to death. Mammalian sexual behavior is controlled by these gonadal steroids acting at the level of the central nervous system. They are divided into the male hormones (or androgens) and the female hormones. While, both sexes produce both types, those corresponding to the appropriate sex predominate (in most cases) [1]. Women produce a number of hormones specific to them, particularly during their childbearing years. The two best-known female hormones are estrogen and progesterone. 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 synthesized in the brain as well [2].

Sesame, *Sesamum indicum* L., is an annual herb native to the tropics, which was formerly classified as *Sesamum orientale* L. It belongs to the Pedaliaceae family [3, 4]. 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 [5]. The local names of the plants depend on the source areas of cultivations in the world, such as *ekuku–gogoro* (Yoruba - *Sesamum radiatum*), *yanmoti* (Yoruba - *S. indicum)*, *ridi* (Hausa) and *beni* (Tiv/Idoma and English) or gingelly (English) [6]. Sesame lignans, such as: sesamin, sesamolin, sesaminol, sesamolinol, pinorsinol, sesamol and gamma-tocopherol are isolated from *S. indicum* seeds. They have more tumorigenic, estrogenic or anti-estrogenic and antioxidant features compared with other plant species. Sesamin was reported to be converted by intestinal microflora to enterolactone, a compound with estrogenic activity and also an enterometabolite of flaxseed lignans, which are known to be phytoestrogenic [7]. 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 [8, 5]. These agents mimic endogenous estrogens depending on their concentrations, they either act agonistically or antagonistically by displacing the endogenous estrogens from their binding sites on the estrogen receptors (ER1 and ER2), among its other mechanisms of action [6, 5].

Fertility regulation with plants or plant preparations has been reported in the ancient literature of indigenous systems of medicine [9]. 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. *Sesamum indicum* is one of the phytoestrogen containing edible seeds consumed by man as food and for medicinal purposes. Its high phytoestrogen content, about 373 mg/100 g [10], makes it necessary to investigate its effects on the body, particularly the female reproductive system. This study aims at evaluating the effects of the hydroethanolic extract of seeds of *Sesamun indicum* on serum levels of estrogen and progesterone in matured female Wistar rats.

MATERIALS

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

Experimental animals

Thirty - three (n=33) matured female Wistar 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 Wistar rats of about 10-12 weeks old (120-140 g) were selected for the experiment. The stage of the estrus cycle of the rats was determined from their vaginal smear. 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 [11, 12].

Laboratory materials and instruments

ELISA kits (Hinotek Group Ltd, Japan) for estrogen and progesterone were purchased from Bio Rapid Diagnostics, Nig. Ltd. and was used to determine the Estradiol and Progesterone concentrations in the rat serum. Light microscope, dissecting microscope, digital weighing balance, centrifuge machine, 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.

Chemicals and solutions

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

METHODOLOGY

Preparation of extract

The dried seeds were pounded into powder using mortar and pestle. Five hundred (500 g) 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 45^{o}C. A brownish semi-solid oily residue of about 45.3g was obtained and was kept in air-tight container at room temperature.

Acute toxicity study

Determination of the median lethal dose (LD_{50}) was done using Lorke's method (1983) [13]. 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 orally administered with 1600, 2900 and 5000 mg/kg extract of *S. indicum* once daily respectively. The animals were subsequently observed for signs of toxicity, discomfort or mortality. The LD_{50} was calculated as the geometric mean of the maximum dose producing 0% mortality and the minimum dose producing 100% mortality.

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

Where: D0 = Maximum dose producing 0% mortality

D100 = Minimum dose producing 100% mortality

Experimental protocol

Twenty (n = 20) matured female Wistar rats were divided into four (4) groups, each n=5 and treated as follows: Control group (1 ml/kg distilled water)**,** Group I, II and III received 100, 300 and 500mg/kg of extract respectively. The animals received treatment orally using plastic syringes attached to oropharygeal cannula for a period of 30 days. The animals were sacrificed 24 hrs 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 assay of estrogen and progesterone [14].

Biochemical assay 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) [15] using the manufacturer's manual. The test was run based on the principle of competitive binding between E2 in the test specimen and E2-HRP conjugate for a constant amount of rat anti-estradiol. In the incubation, goat anti-rat IgG - coated wells were incubated with 25 µl E2 standards, controls, rat samples, 100 µl Estradiol-HRP Conjugate Reagent and 50 µl rat anti-Estradiol reagent at room temperature (18-25ºC) for 90 minutes. The microwells were rinsed 5 times with washing buffer. 100 µl of TMB Reagent was added into each well and incubated for 20 minutes resulting in the development of a blue color. The reaction was stopped by adding 100 µl of Stop Solution to each well and mixed for 30 seconds until the blue color changes to yellow color completely. The absorbance was read at 450 nm with a microtiter well reader within 15 minutes.

Statistical analysis

Data obtained were expressed as mean $\pm$ 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 20. Values with $p<0.05$ were considered significant.

RESULTS

Acute toxicity studies

All animals did not show any sign of toxicity, discomfort or mortality throughout the period of observation in phases I and II of the study. Thus, the extract is safe up to the 5000 mg/kg dose.

Table 1: Median Lethal dose of *Sesamum indicum* extract, recorded number of deaths and percentage mortality of female Wistar rats

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

two			
(N=1)	**Dose (mg/kg) body weight**	**Deaths**	**Percentage mortality (%)**
1	1600	0/1	0
	2900	0/1	0
	5000	0/1	0

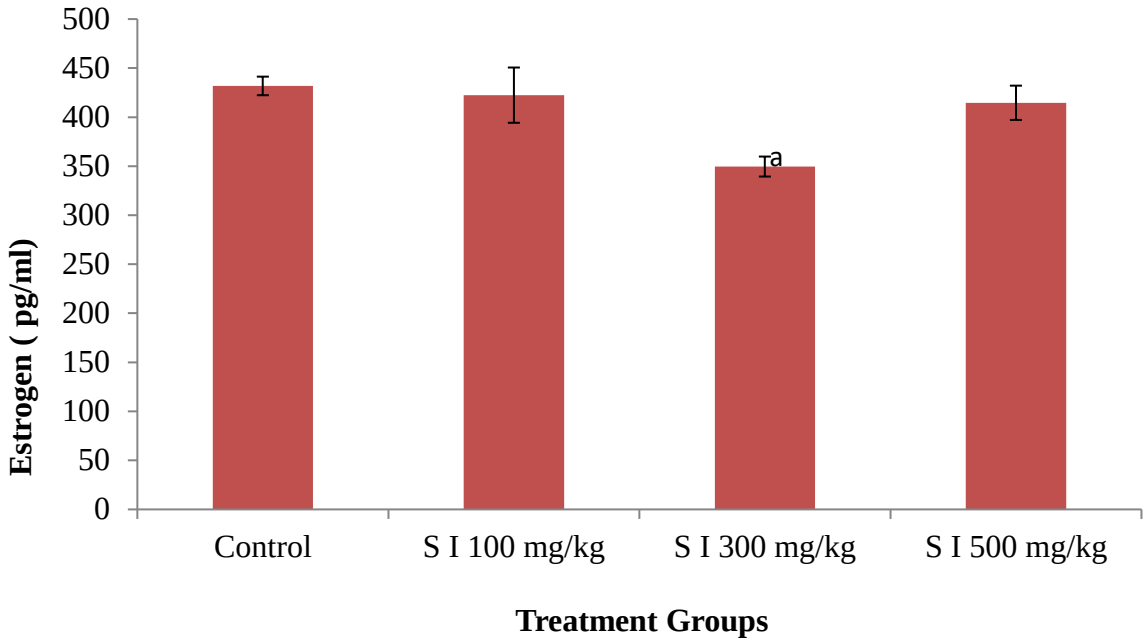

Figure 1: Effects of *Sesamum indicum* extract on serum concentration of estrogen in female Wistar rats
Mean (± SEM) of serum concentration of estrogen in control and *Sesamum indicum* treated matured female Wistar rats. The mean serum estrogen level was significantly decreased ($p<0.05$) in the group treated with S I 300 mg/kg when compared with control. [a] level of significance at $p<0.05$ when compared with control SPSS Version 20. SI: *Sesamum indicum*

The result of the effect of SI on serum progesterone level shows no statistically significant difference when compared with control at $p<0.05$ (n=5) (Figure 2).

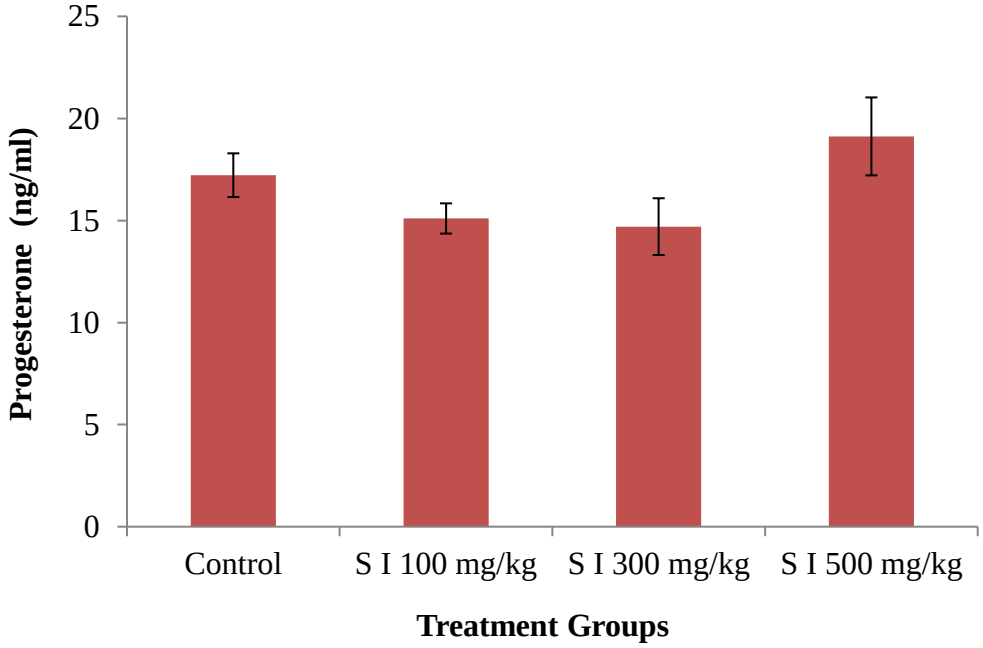

Figure 2: Effects of *Sesamum indicum* extract on serum concentration of progesterone in female Wistar rats

Mean (± SEM) of serum concentration of progesterone in control and *Sesamum indicum* treated matured female albino rats. The mean serum estrogen level was not statistically significant (p<0.05) at all doses when compared with control, SPSS Version 20. SI: *Sesamum indicum*

DISCUSSIONS

The result of acute toxicity study showed that the hydroethanolic seeds extract is relatively safe up to 5000 mg/kg. This is not surprising because the plant has been consumed as food by man and other animals for ages without any deleterious effect. The results showed a decrease in serum estrogen levels in treated groups as compared to control group. In lowest (100 mg/kg) and highest doses (500 mg/kg), 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) [16] 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 [17]. 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 [14]. 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 granulosa 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 [18].

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 [19]. In the present study, there was a slight non-significant decrease in the serum progesterone levels in groups II and III compared to control and group IV rats. This 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 of Yakubu *et al.*, (2008) [20], who observed that extract of *C. aconitifolius* caused a decrease in serum progesterone level of female rats.

CONCLUSION

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

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