# Effect of Aqueous Extract of Soya Bean (*Glycine Max*) on the Reproductive Hormones and the Ovaries of Female Albino Wistar Rats

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***Abstract*:**

***Introduction:***

**Soya bean is a legume that has been extensively used as an important source of dietary proteins, dietary fiber and oil throughout the world. It has also been identified to possess phytoestrogen components like isoflavones, coumestans and ligans, though rich in isoflavones. This has been shown to posse weak estrogenic and antiestrogenic activities at various concentrations. The aim of this study was to determine the effect of soya bean aqueous extract on the ovary and female reproductive hormones using adult female albino Wistar rats.**

***Methodology:***

**Fifteen adult female rats were assigned into three different groups of 5rats each. Group 1 was the negative control. Both groups 2 and 3 were the treatment groups. They were treated with 100mg/kg and 200mg/kg of soya bean aqueous extract respectively. The treatment of the animals lasted for 4weeks and the feeding was done orally. Blood samples were collected and assayed for FSH, LH and estrogen hormones. The ovaries were also processed for histological studies.**

***Result:***

**Increases in the hormonal levels were observed in both group 2 and 3, when compared with group 1. There was significant increase in LH in group 3 and estrogen in groups 2 and 3 when compared to group 1. Histological examination of the ovary showed a progressive enhancement in the ovarian follicles population and maturation in the treated groups.**

***Conclusion:***

**The research showed that soya beans had obvious positive effects on the ovary and female reproductive hormones. A further study in human is recommended especially in the aspect of the management of menopause.**

***Keywords*: soya bean, glycine max, reproductive hormones, FSH, LH, estrogen, ovary.**

## I. INTRODUCTION

Soya beans belong to the family of legumes and are biological named as *Glycinemax* (Singh, 2014). Most of the components of soybean have health benefits (Sugano, 2006). It is a popular food additive because it is cheap, cholesterol-free, vegetable protein rich in complex carbohydrates and unsaturated fats, high in fiber, and free of lactose (Patisaul and Jefferson, 2010). It is considered to be the richest source of plant estrogens (Dadon and Reifen, 2010). Oestrogen is the principal female hormone that induces folliculogenesis under the influence of FSH and LH (Zhao, 2011). Once all are working harmoniously, fertility is preserved (Zhao, 2011).

Phytoestrogens are compounds derived from plants thatare present in foods like leafy greens, soy, whole grains, garlic and beans (Wong, 2017). It is defined to be plant-derived xenoestrogens that are produced within the endocrine system rather gotten by consumption of phytoestrogenic plants (Yildiz, 2005). Phytoestrogens are also called “dietary estrogens”. They are natural nonsteroidal plant compounds of diverse origins but due to their similarity in structure with estradiol (17-β-estradiol), they have the strength of causing estrogenic or /and antiestrogenic effects (Yildiz, 2005). This is achieved due to its ability to sit in and block receptor sites against estrogen (Crenshaw and Goldberg, 1996).

Three main classes of phytoestrogen are known to occur in plants, they are isoflavones, coumestans and lignans (Murkies *et al.*, 2009). However, soy bean is rich in isoflavones (Price and Fenwick, 1985). Genistein and daidzein as major isoflavone commonly exist as inactive glucosides (Axelson *et al.*, 1984). They are obtained after breakdown by intestinal glucosides (Setchell and Adlercreutz, 1988). Female gender modify phytoestrogen metabolism though dietary phytoestrogen metabolism is majorly determined by the gastrointestinal flora, use of antibiotic or bowel disease (Kelly *et al.*, 1995).

Isoflavones have hormone-like activities as they contain diphenolic ring that allows them to bind to the estrogen receptor exerting weak estrogenic activity (Dinsdale and Ward, 2010; Patisaul and Jefferson, 2010). Also, the higher weight of the gonads may indicate estrogenic effects due to higher rates of cell proliferation within the organ. It may alter the concentration of the reproductive hormones (LH, FSH, estradiol). There may be elevated transcription of the beta-estrogen receptor (ER-ß) or alpha-estrogen receptors (ER-α) which is indicative of higher estrogenic activity and the length of time spent in each phase of the estrous cycle in any species is the estrous cycle, can be used to understand if fertility may be altered (Dinsdale and Ward, 2010). This effect enhances female fertility.

Controversially, some studies showed the harmful effects of phytoestrogens. Impaired fertility in females has been documented after soy Isoflavones exposure in a mouse model (Wendy*et al*., 2007; Dinsdale and Ward, 2010). Administration of genistein an Isoflavone found in soya beanhas been shown to affect the development of the reproductive system (Chen and Rogan, 2004; Dinsdale and Ward, 2010; Patisaul and Jefferson, 2010). This necessitated the importance of this research as soya bean products are widely used in Nigeria and other African Countries. To this, we aimed to study the effect of the aqueous extract of soya bean on the reproductive hormones (estrogen, FSH and LH) and the gonads (histological features) of female albino wistar rats.

## II. METHODOLOGY

Healthy 15 adult Female Albino Wister rats weighing 140-180g were purchased from animal house, Department of Anatomy, Faculty of Medicine, Ebonyi State University, Abakaliki. The rats were housed in a well-ventilated cage (aluminum cage) and allowed to acclimatize for one week under regulated environmental conditions of temperature (25± 5ºC), relative humidity (50± 5%) and 12 hours light /dark cycle. The experimental animals ate Vital Growers Feed all through the period of the experiment. The treatment of the animals lasted for 28 days, between 15th March to 14th April, 2018. The extract was administered orally to the animals. All the animals used for the experiment were sacrificed at the end of the study and their ovaries removed surgically for histology, while blood sample was collected for hormonal assay. Their bodies were respectfully buried. All animals were housed in accordance with international acceptable guideline for laboratory animal use and care as found in European Community guidelines (ECC, 1986).

Aqueous Extraction of Soya Bean was by the method described by Taziebou*et al.,* (2007). Fresh seeds of soya beans were cleaned, washed and boiled for over 30minutes using an ovum at $60^{oC}$to cause detoxification of some poisonous constituents and aid removal of the hustle. It was air-dried and ground with a blender. This soya bean powder was mixed in a proportion of 100gto 500ml of distilled water. The mixture was allowed to stand for 72 hours with 12hrs interval of shaking of the mixture so that the Isoflavones will settle. It was filtered with NO 1 Whatman filter paper. The filtrate was placed in a rotatory evaporator at a temperature of $40^{0}$C until it forms a semisolid. This was stored in a refrigerator. The extract was collected from the refrigerator, weighed and reconstituted at the concentration of 1g/10ml of distilled water and then administered to group 2 and group 3 at the concentration of 100mg/kg body weight and 200mg/kg body weight respectively.

Weight matched rats were randomly divided into 3 groups consisting of 5rats per group

***Group 1*****:** Served as control group and was given Vital Growers Feed and distilled water

***Group 2*****:** Received100mg/kg live body weight daily of the soya bean extract, Vital Growers Feed and distilled water

***Group 3*****:** Received200mg/kg live body weight daily of the soya bean extract, Vital Growers Feed and distilled water.

The treatment of the animals lasted for 28 days. The extract was administered orally to the animals. After 28th day of the experiment, the animals were sacrificed after anesthetizing with ketamine following the standard procedures for handling experimental animals. Blood was collected through the orbital sinus using glass capillary tube into a plain tube free of anticoagulants for hormonal assay. Hormonal assay was done using automated machine. Their gonads were removed surgically, weighed and fixed in 10% formalin solution and processed for histology using Hematoxylin and eosin stain.

Data obtained from this study were analyzed using SPSS Ver. 20.0 statistical package and values were expressed as mean ± SEM. Groups were compared using one-way analysis of variance (ANOVA) followed by a post hoc Newmannkeul`s multiple comparison tests. Statistical significance was considered at $P<0.05$.

## III. RESULTS

Table: Comparisons Among FSH, LH, And Estradiol-17 Beta Concentration In Albino

*Wistar rats fed two different levels of soya bean extract*

| Groups | FSH (miµ/ml) | LH (miµ/ml) | ES (Pg/ml) |
|---|---|---|---|
| (Control) | $8.85 \pm 2.76^{da,db}$ | $3.60 \pm 0.28^{da,b}$ | $69.00 \pm 5.37^{a,b}$ |
| (l00mg/kg) | $7.95 \pm 3.04^{da,dc}$ | $5.05 \pm 0.21^{d8,dc}$ | $114.85 \pm 7.71^{a,dc}$ |
| (200mg/kg) | $13.45 \pm 3.75^{db,dc}$ | $8.35 \pm 1.34^{b,dc}$ | $103.60 \pm 6.65^{b,dc}$ |

Source: Field Work, 2018

a - significant difference between group 1 & 2, b - significant difference between group 1 & 3, c - significant difference between group 2 & 3, da - no significant difference between group 1 & 2, db - no significant difference between group 1 & 3, dc - no significant difference between group 2 & 3.

The result showed that the female Albino Wister rats demonstrated increase in the concentration of LH with increase in the quantity of the aqueous extract of soya bean administered in a dose dependent manner and it was statistically significant. The FSH only showed increase in concentration in group 3 when compared to group 1, but was not statistically significant. The ES showed increase in concentration in group 2 and 3; and were statistically significant, but was not statistically significant, when group 2 and 3 were compared.

*Plates: Histological Effect of Aqueous Extract of Soya Bean on the Ovary*

Group 1 (Plate 1) showed healthy ovarian follicles with developing ovule (5 follicles/hpf). When compared, group 2 (Plate 2) showed enhanced ovarian follicle proliferation (11 follicles/hpf) and increase in the number of follicle. However, Group 3 (Plate 3) showed better enhancement of ovarian follicle proliferation (15 follicles/hpf) and more number of the follicle present in the ovary

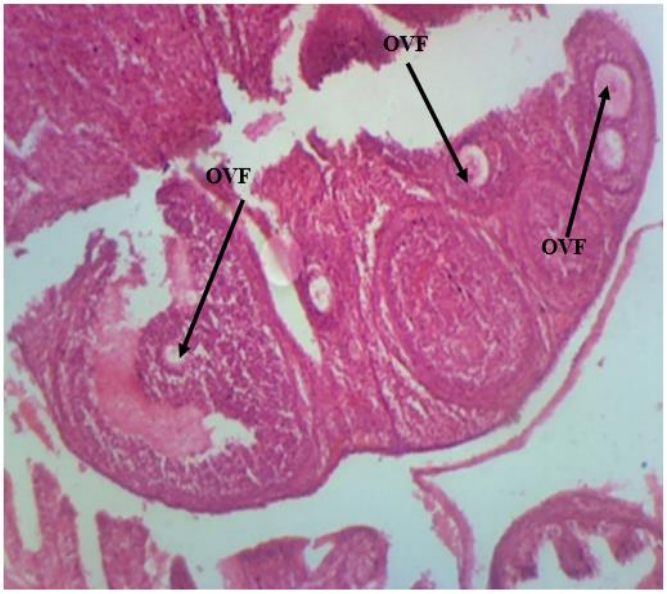


*Plate 1*: Ovarian section of rat not treated with soya bean extract (negative control) showing healthy ovary with developing egg. H & E; X60.

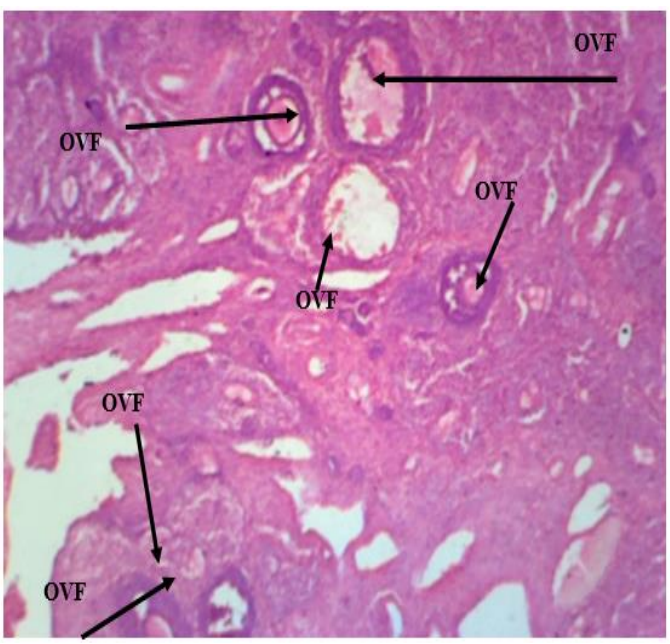


*Plate 2*: Ovarian section of rat treated with soya bean extract (100mg/kg) showing increased proliferation and maturation of ovarian follicles (OVF). H & E; X60.

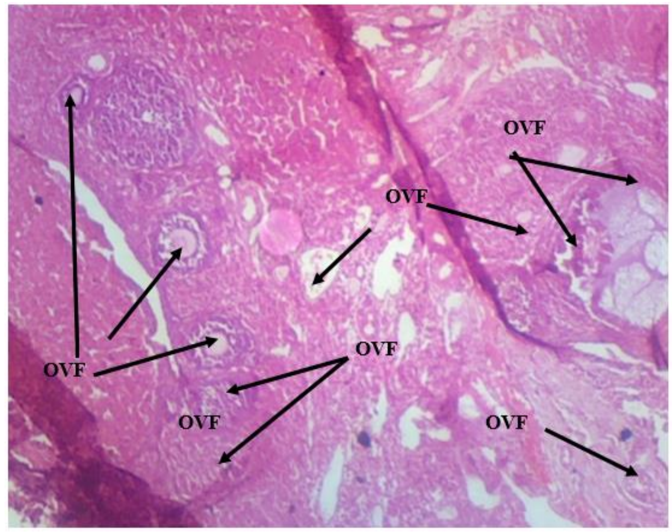


*Plate 3*: Ovarian section of rat treated with soya bean extract (200mg/kg) showing progressive increased proliferation and maturation of ovarian follicles (OVF). H & E; X60.

## IV. DISCUSSION

The hypothalamic-pituitary-ovarian axis is the principal endocrine system pathway that regulates female reproduction (Zhao, 2011). The Gonadotropin (FSH and LH) are released into the general circulation to stimulate sex steroid hormones production and to control ovulation in females (Zhao, 2011). This was evident in our study that showed enhanced folliculogenesis in a dose dependent manner.

Berrino et al. in 2001 reported that phytoestrogens lead favourable changes on reproductive hormones. This was noted in this study as most of the assayed hormones were elevated compared to control. The result demonstrated increases in the concentration of LH with increase in the concentration of the aqueous extract of soya bean administered but only in the comparisn of group 1 and 3 that LH was statistically significant. However, comparing LH level in group 2 and 3, it was marginally significant.

The FSH showed increase in concentration in group 3 when compared to group 1, but was not statistically significant. Also, comparisn of FSH level in group 2 and 3 was still not statistically significant. Important to note is the decrease in the level of FSH in group 2 when related to group 1. This is as a result of high level of oestrogen in group 2 which resulted in a negative feedback mechanism as described by Ezeilo, 2009. Van Putte et al., (2016) documented that this negative feedback mechanism is the response of GnRH from the hypothalamus towards the high level oestrogen which then gets to the anterior pituitary gland so as to lower the amount of FSH it releases.

Oestrogen showed increase in concentration in group 2 and 3, when compared to group 1 but the level of ES in group 2 was higher compared to group 3. This was noted by Singh, (2014) who showed that excessive consumption of soy apparently seems to contribute too much oestrogen into our bodies. Also as the ovarian follicles are maturing, it triggers the secretion of high oestrogens level (Van Putte et al., 2016). Wu et al (2000) observed a decrease in serum oestradiol in Asian human subjects on isoflavones. This outcome was different from our study. This maybe that only the isoflavones present in soya bean were isolated and studied but ours used soybean as a whole, the natural way is been consumed at home by human. There were statistical differences when group 1 was compared with groups 2 and 3. However, not significant when group 2 and 3 were compared. This is in line with a randomized dietary intervention study by Nagata et al, (200 Ib) which showed no statistical differences in oestradiol level between the treatment groups.

The histology of the ovary showed enhanced ovarian follicles proliferation which causes increased number of follicles [group I:(5 follicles/hpf), group II:(11 follicles/hpf) and group III:(15 follicles/hpf)]. This was more obvious in group 3 resulting in a more number of the follicle present in the ovary. This was observed by Van Putte et al., (2016) that noted that increase in the levels of FSH seen during the maturation of the ovarian follicle before ovulation is as a result of the enhanced folliculogenesis at the ovary. However it contradicts study by Jefferson et al., (2006) who documented that in the ovary, phytoestrogen genistein inhibits oocyte nest breakdown and attenuates cell death during mouse oocyte development. This was not seen in our study as genistein a chemical component of soya isoflavones documented as an endocrine disrupter was not used as the extract for the study.

## V. CONCLUSION

Our research showed that soya bean increased estrogen hormone, follicle stimulating hormones (FSH) and luteinizing hormones (LH). Though not all the increased in the hormone level were significant. However, the histological outcome of the ovary reported enhancement of the follicular growth after the treatment with the soya beans which was dose dependent. We therefore recommend it as a regular food additive in females.

*Ethical Approval*

Ethical approval for animal studies was obtained from the Faculty of Medicine Animal Ethical Committee, Ebonyi State University, Abakaliki.

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