

Effects Of Glycine Max And Sesamum Indicum On Reproductive Parameters In Male Wistar Rat Offsprings

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Abstract

Glycine max and Sesamum indicum are generally perceived to be safe and so they are normally consumed freely especially during the critical period of gestation and lactation. The disruptive properties of Glycine max and Sesamum indicum were investigated on the offsprings of pregnant Wistar rats. Fifteen (15) Male and thirty (30) female Wistar rats were paired to procreate 30 male pups. The adult rats were divided into 6 groups: Group 1 served as Control, Group 2 was treated with Clomiphene, Group 3 had 50mg Soya Beans, Group 4 had 100mg Soya Beans, group 5 had 50mg Sesame Seeds and group 6 had 100mg Sesame seeds. After a 9week experimental period, the study animals were sacrificed and blood samples taken as well as tissues harvested under standard procedures and analyzed. The results showed that Soya Beans and Sesame Seeds caused changes in male reproductive hormones- Testosterone-High dose Sesame seed group (2.54 ± 0.21 and 3.58 ± 0.57) ng/ml, when compared to the control, at $p < 0.01$; FSH ($0.19 \pm 0.03/0.28 \pm 0.01$) mlu/ml and LH ($0.43 \pm 0.02/0.94 \pm 0.18$) mlu/ml for Low dose Sesame seed when compared to the control at $p < 0.05$, Low dose Soya group also experienced a fall in FSH levels ($0.19 \pm 0.01/0.28 \pm 0.01$) mlu/ml, when compared to the control at $p < 0.05$. The decrease in the FSH and LH hormonal levels could lead to infertility, low sex drive, delayed onset of puberty while increased level of testosterone can increase and improve sperm quality. The study therefore, concludes that Glycine max and Sesamum indicum could alter reproductive hormones in male Wistar rat offspring.

Keywords: Soya Bean (Glycine max), Sesame Seed (Sesamum indicum), phyto-oestrogens.

Introduction

The use of therapeutic plants as dietary supplements and herbal medicine to prevent and cure diseases is on the increase. This may be due to awareness of the benefits of these medicinal plants, their efficacy and some adverse effects posed by many synthetic drugs hence people proscribe to natural remedies. Soya bean, Soy or Soybean (*Glycine max*) is a legume specie indigenous to East Asia generally cultivated for its numerous uses such as soy milk, (from which tofu and tofu skin are made), fermented foods such as soy sauce, fermented bean paste, natto, tempeh and soy oil, animal feeds and it is the main protein source in many packed meals such as textured vegetable protein (TVP) used in numerous meat and dairy alternatives for vegetarians (Riaz, 2006). Some newborns that are not strictly breast fed are sometimes given Soy-based infant formula (SBIF) especially those infants that are hypersensitive to pasteurized cow milk or who have vegetarian parents. The formula can be purchased as dry powder, ready to feed and condensed liquefied forms (US Food and Drug Administration, 1999). Industrially Soy bean is used for oils, solvents, crayons, cosmetics, resins, plastics, inks, soap, clothing, biodiesel and in the production of Vodka. Soya bean can trigger reactions in some persons especially in children.

Sesame seed (*Sesamum indicum*) is cultivated in tropical areas round the world; top producers are Tanzania, Myanmar, India and Sudan. Sesame seed is an oil seed crop famous for tolerating drought conditions or other vagaries of weather that other plants do not thrive in (Ogasawara *et al.*, 1988; Raghav *et al.*, 1997). Sesame seed has one of the richest oil contents of any seed, with a nice nuts flavor; it is generally used in cuisines for salads, bread topping, grain products, crackers, sushi, cakes, and soups or as breading for fish and meat. Sesame seeds are very small about 3-4mm long and 2mm wide. About 3.85million metric tons are produced yearly worldwide. Sesame can also spark hypersensitive responses in some individuals (Gangur *et al.*, 2005)). Phyto-oestrogens-one of the active components of soy and sesame may mimic natural hormones in the body causing the body to respond to their stimuli either by stimulating or inhibiting a natural hormone. This disruptive property is not highlighted to the public and most people still believe that phyto-oestrogens have therepeutic outcomes, including prevention or therapy against cancer, atherosclerosis, and osteoporosis, (Crain, *et al.*, 2008; Patisaul & Jefferson, 2010; McLachlan, *et al.*, 1980; Messina, *et al.*, 2002; Cooke, 2006; Cassidy, *et al.*, 2006; Kim, 2008). An increasing number of scholars and clinicians now caution that the health benefits often accredited to soya bean may be exaggerated (Balk *et. al*, 2005; Jacobs *et al.*, 2009; Sacks *et al.*, 2006).

Materials and methods

Research design

The research area was the animal house of the faculty of Basic Medical Sciences, University of Port Harcourt, and Rivers State, Nigeria.

Study animals

Male and female Wistar rats with weights between (130g-200g) were procured from the animal house of the Faculty of Basic Medical Sciences, University of Port Harcourt.

The animals were kept in an environment of normal room temperature and fed with standard feed and water ad libitum for 14 days to allow for acclimatization before the experimental process began. After acclimatization the animals were weighed weekly, grouped and paired for mating during the oestrous period (heat) of females so pregnancy can be achieved. The mature female animals were divided into 6 groups: a control group, the clomiphene group, the low dose Soya bean group, the High dose Soya bean group, low dose Sesame seed groups and High dose Sesame seed. Two females were paired with a male rat in a cage and allowed to mate. On the commencement of pregnancy, validated by the presence of sperm in the vaginal fluid and the development of the vaginal plug, the males were removed from the cage and the Wistar rats in each group were fed with normal animal feed and water, group 2 to 5 were force fed their designated substrate according to force feeding technique described by M. E Fowler (1981) with the slight modification of using a syringe instead of a speculum to intubate the test substance daily until the animals gave birth. Group 1 control were fed animal feed and water ad libitum, Group 2- Clomiphene group were force fed clomiphene citrate tablet dissolved in water for three days after which normal feed and water were administered daily, Group 3-Low dose Soya bean group: were force fed 50mg/kg weight soya beans powder, dissolved in water daily, Group 4- high dose Soya bean were force fed 100mg/kg body weight soya beans powder, dissolved in water, Group 5- Low dose Sesame seed group were force fed roasted ground sesame seeds, 50mg/kg weight dissolved in water and Group 6-the high dose Sesame seed group, were force fed roasted ground sesame seeds, 100mg/kg weight dissolved in water. Clomiphene is an ovulatory stimulant used to induce ovulation in women who do not release mature ova monthly, or in women with polycystic ovary syndrome who intend to get pregnant. It works like oestrogen by causing the ovaries to release eggs. Clomiphene is a 50mg/70kg weight tablet taken orally on or about day 5 of the human cycle. 50mg was dissolved in 50ml and 0.01mg was administered for 3 days, (WHO, 2009).

Preparation of Soya bean and Sesame seed.

Soya bean and Sesame seed were bought from the Mile 1 market, Diobu Port Harcourt, animal feed was also purchased from Choba market and fed to the animals. The Soya bean was washed and dried by roasting it dry. The outer coat was removed and the Soya bean was ground to fine powder. The sesame seed was also washed, roasted dry and milled to powder using the open pan technique described by Makinde *et. al.*, (2016).

Sample collection

Five weeks after the birth of the offsprings, blood samples were randomly taken from 5 males (a total of 30) of the offsprings of each group for serum hormonal assay tests of Follicle Stimulating Hormone, Luteinizing Hormone and Testosterone. The procedure involved anesthetizing the rats in desiccators using cotton wool infused with chloroform. The animals were laid in a supine position and their limbs were pinned. Blood samples were collected by venipuncture, pelvic incisions were also made to expose the testes. A

sharp scalpel was used to excise the organ (testes) for histological profiling to ascertain if there is any evidence of cellular disruptions during development.

Data Analysis

Data was analyzed using GraphPad prism 6.0 statistical software. Data was presented as Mean \pm SEM. Significant differences was considered using one-way analysis of variance (ANOVA) followed by Tukey's post- hot test. Significance was considered at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

Results

Table 1 Values of reproductive Hormones in male study offsprings.

	Group	N	FSH (mlu/ml)	Change (%)	LH (mlu/ml)	Change (%)	TET (ng/ml)	Change (%)
1	Control	5	0.28 \pm 0.01	0.00	0.94 \pm 0.18	0.00	2.54 \pm 0.21	0.00
2	Clomiphe ne (mg)	5	0.26 \pm 0.01	-7.69	0.59 \pm 0.14	-59.32	2.30 \pm 0.01	-10.43
3	50mg SB	5	0.19 \pm 0.01 *	-47.37	0.87 \pm 0.05	-8.05	2.63 \pm 0.07	3.42
4	100mg SB	5	0.28 \pm 0.02	0.00	1.16 \pm 0.10	18.96	2.38 \pm 0.04	-6.72
5	50mg SS	5	0.19 \pm 0.03 *	-47.37	0.43 \pm 0.02 *	-118.6	2.18 \pm 0.07	-16.51
6	100mg SS	5	0.21 \pm 0.04	-0.33	0.78 \pm 0.11	-20.51	3.58 \pm 0.57 **	29.05

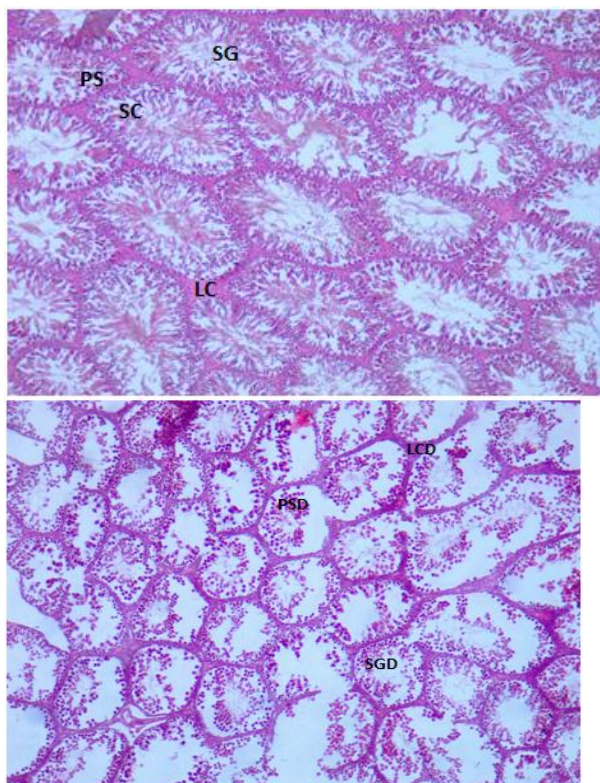
Values are expressed as Mean \pm SEM, * $p < 0.05$ when compared to control; ** $p < 0.01$ when compared to control. FSH: Follicle stimulating hormone; LH: Luteinizing Hormone; TET: Testosterone; SB: Soya Bean; SS: Sesame Seed. The results of the effect of Soya Bean and Sesame seed on the reproductive hormones of male offsprings of Wistar rats that were administered the test substances are presented in Table 1. There was a significant decrease in FSH for the 50mg Soya bean (0.19 \pm 0.01/0.28 \pm 0.01) mlu/ml and Sesame group (0.19 \pm 0.03) (0.28 \pm 0.01) mlu/ml when compared to control at $p < 0.05$; with the same percentage change of -47.37% and a significant decrease in LH (0.43 \pm 0.02/ 0.94 \pm 0.18) mlu/ml for 50mg Sesame group (-118.6% change) when compared to control and the level of significance was considered at $p < 0.05$. It was observed that there was significant increase in Testosterone for the 100mg Sesame Seed group with 29.05% change when compared to the control (2.54 \pm 0.21 and 3.58 \pm 0.57)ng/ml, the level of significance was considered at * $p < 0.05$ and ** $p < 0.01$. Dose dependent developmental exposures of male rats to phyto-oestrogens have shown disruptions in serum hormonal levels of neonates. It was observed that 100mg Sesame significantly increased Testosterone levels when compared to the control. This could be attributed to increased sensitivity to gonadotropin releasing

hormone which stimulates the synthesis of FSH and LH, which ultimately leads to increased Testosterone secretion (Wallace, 2019).

Ironically 50mg of Soya Beans and 50mg of Sesame seeds had an inhibitory effect on FSH levels. A significant decrease in FSH values was observed (0.19 ± 0.01 and 0.28 ± 0.01), in the 50mg Soya Bean group and (0.19 ± 0.21 and 0.28 ± 0.01) in the 50mg Sesame Seed group with a percentage decrease of -47.37% when compared to the control, significance was considered at $p < 0.01$ respectively. These results show reduced pituitary responsiveness and hence suppressed levels of FSH. This could delay the onset of puberty in the pups, low sex drive and possibly associated oligospermia (limited sperm). Another significant decrease in LH levels was recorded (0.43 ± 0.02 and 0.94 ± 0.18), in the 50mg Sesame seed group with a percentage decrease of -118.6% when compared to the control, significance was considered at $*p < 0.01$. The result of this present study is contrary to one study carried out by Julie *et. al.* (2001) that concluded that the phyto-estrogen low dose consumed had no observable effect on endocrine measurements.

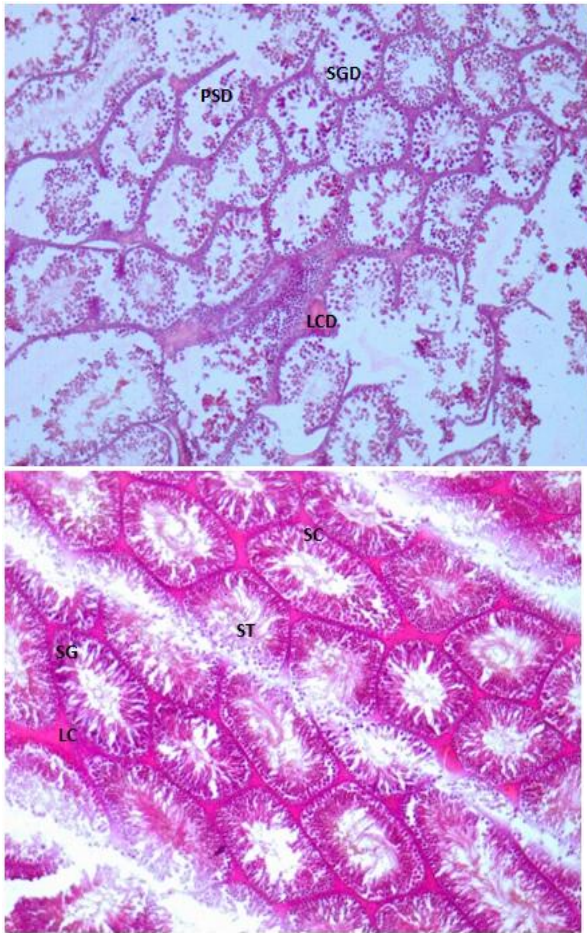
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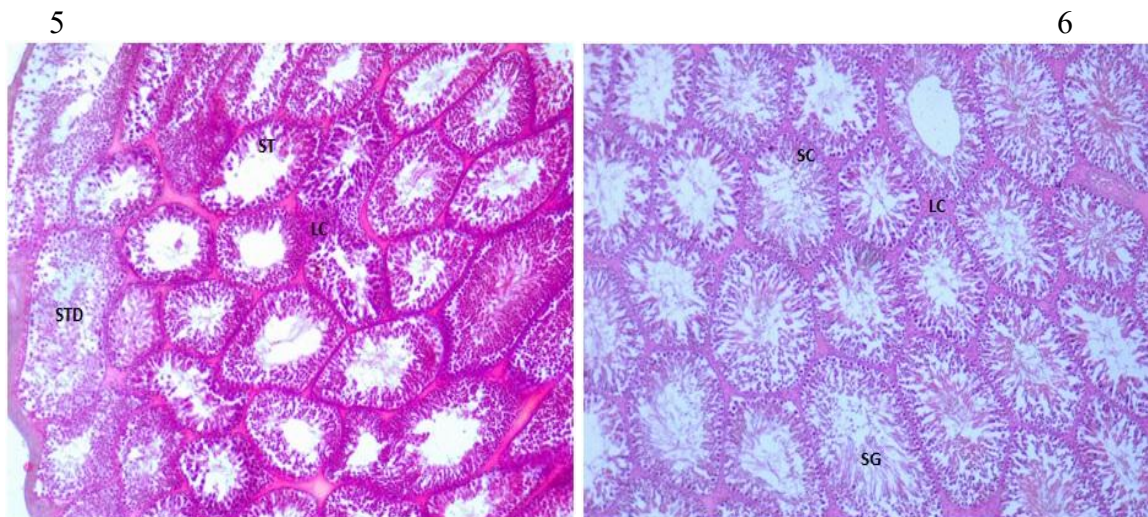
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Photomicrograph sections of testis, Mag.X100, H & E stain. Slide 1: Normal Testis : Seminiferous tubules containing Sertoli cells (SC), spermatogonia (SG), primary spermatocytes (PS), and spermatids. Interstitial spaces contain leydig cells (LC). Slide 2: (Clomiphene group). Histologically impaired Testis showing: Seminiferous tubules containing impaired spermatogonial cells (SGD), primary spermatids (PSD) and sertoli cells. Impaired Leydig cells (LCD) also present. Spermatozoa are reduced.

Distorted morphology (hexagonal shape of the seminiferous tubules). Slide 3: (50mg SB group): Histologically impaired Testis showing: Seminiferous tubules containing impaired spermatogonial cells (SGD), primary spermatids (PSD) and sertoli cells. Interstitial spaces containing leydig cells with tissue damage (LCD). Spermatozoa are reduced. Distorted morphology and orientation (hexagonal shape of the seminiferous tubules). Slide 4 (100mg SB group): Histologically normal Testis showing: Seminiferous tubules containing Sertoli cells (SC), spermatogonia cells (SG), and mature spermatozoa (ST). Interstitial spaces contain leydig cells (LC).



Photomicrograph sections of testis, Mag.X100, H & E stain. Plate 5: (50mgSS group). Seminiferous tubules (ST) containing spermatogenic cells (SG), with some normal (ST) and abnormally oriented mature spermatozoa (STD). Interstitial spaces contain Leydig cells (LC). Spermatozoa are reduced. Distorted morphology and orientation (hexagonal shape of the seminiferous tubules). Slide 6 (100mg SS group): Histologically normal Testis showing: Seminiferous tubules containing spermatogenic cells (SC), and mature spermatozoa (SG). Interstitial spaces contain Leydig cells (LC). The histological profiling of the Testis was normal for the control group, with the presence of seminiferous tubules containing Sertoli cells, spermatozoa, primary spermatocytes and interstitial spaces contained Leydig cells. The Testis was distorted in the Clomiphene Citrate group with the presence of seminiferous tubules with distorted morphology, they appeared nearly oval rather than hexagonal in shape, and spermatozoa were reduced when compared to control. The 50mg Soya bean group contained seminiferous tubules with impaired spermatogonial cells, primary spermatids and Sertoli cells with distorted morphology and orientation (hexagonal shape of the seminiferous tubules became nearly spherical). Spermatozoa were reduced when compared to control. The 50mg Sesame Seed group had seminiferous tubules containing spermatogenic cells, with some abnormally oriented mature spermatozoa and distorted morphology of the tubules. The interstitial spaces contained Leydig cells. Spermatozoa were reduced but appeared more in number than their 50mg Soya bean group. These reduced spermatozoa agree with the significant decrease in FSH and LH levels observed in the 50mg Soya Bean and Sesame Seed groups. The 100mg Soya Bean group, and the 100mg Sesame seed groups with the presence of seminiferous tubules containing normal spermatogenic cells and mature spermatozoa. The interstitial spaces contained Leydig cells. The spermatozoa in the 100mg Sesame group appeared to be more in number than those of the other groups. This agrees with the significantly increased levels of testosterone recorded in the 100mg Sesame seed group.

Fig 1 Hormonal Analysis for Male offsprings (FSH).

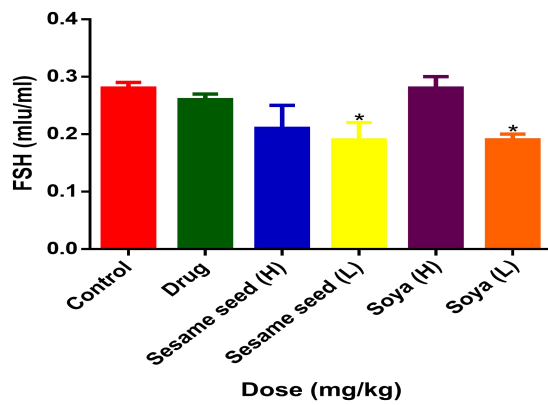


Fig 2 Hormonal analysis for male offsprings (LH).

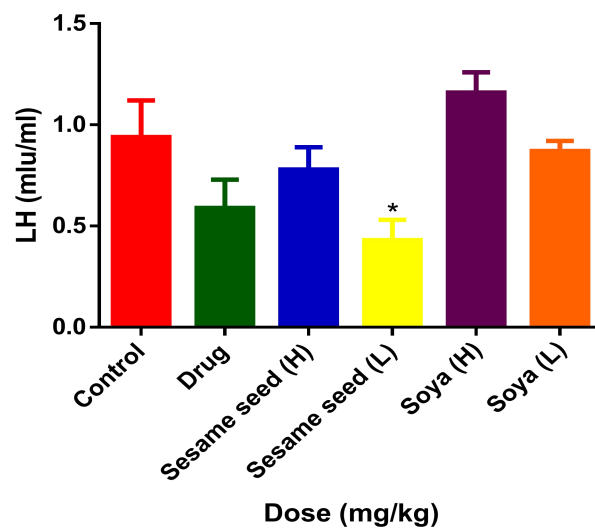
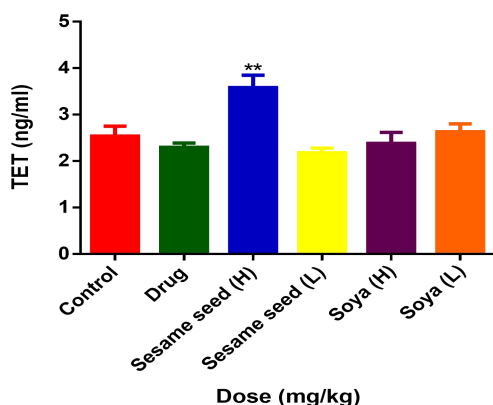


Fig 3 Hormonal analysis for male offsprings (testosterone).



Conclusions

The phytoestrogens present in Soya bean and Sesame seeds fed to pregnant Wistar rats could be the cause of endocrine disruptions in their male offsprings by interfering with Follicle stimulating hormone, Luteinizing hormone and testosterone. These phytoestrogens are also the probable cause of fluctuations in the weight of the offsprings and the damaged morphology of the testis in males.

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