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Original Research Article

Effects of Soy-flour Enriched Diet on the Testis and Gonadal Hormone Status of Male Wistar Rats

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Abstract: This study was designed to determine the effects of soy-flour enriched diet on the testis morphology, gonadal hormones (Testosterone and FSH) and accessory sex glands (prostate and seminal vesicle) semen count and motility in rats. Fifteen male rats were fed raw soy-flour enriched diet at 0% W: W, 10% W: W 20% W: W (soy flour / milled standard feed by weight) for eight weeks. The sperm count and the weight of accessory sex glands were measured. There was a significant decrease in the weight of the testis, seminal vesicle, and the prostate ($P < 0.05$) compared with the control. There was also significant decrease in the sperm count and testosterone level in a dose dependent manner. The testis histo-architecture was normal and the seminferous tubules were shrunken compared to the control in both the high dose and low dose. Raw soy-flour supplementation with standard feed produces a form of mild toxic effect on the testis histo-architecture.
Keywords: Soy-flour, Testosterone, FSH, LH, Accessory Sex Glands, Testis.

INTRODUCTION:

Soybean has been variously described as a "miracle bean" or a "golden bean" because it is a cheap protein-rich grain. It contains 40% high quality protein, 20% edible vegetable oil, and a good balance of amino acids [1, 2]. Legumes- beans, peas, soya beans and other legumes contain isoflavones notably genistein which binds oestrogen receptors in the body and thus tone down effects of excessive quantities of oestrogen by rendering the body relatively insensitive to synthesized oestrogen [3].

Phytoestrogens are secondary metabolite produced in a wide variety of plants that induce biologic responses in vertebrates and can mimic or modulate the actions of endogenous estrogens usually by binding to estrogen receptors [4]. Phytoestrogens assumed biological and economic importance in the 1940's, with the outbreak of infertility in sheep grazing on pastures rich in subterranean clover in western Australian, later known as "clover disease" [5]. Concentration of isoflavone fractions daidzein and genistein in raw soy have been found to vary between 25.2 diadzein mg/100g and genistein 34.3 mg/100g seed [6]. Soybeans also contain omega-three fatty acids, which have cardiovascular (antihyperlipidemic, antithrombotic) and cancer, arthritus, diabetes (antiinflammatory) disease advantages via prostaglandin effects, i.e. encouraging PGE1 and PGE3 formation [7]. Soybeans are quite uniquely rich in phytoestrogens called isoflavones resemble estrogens greatly in structure acting as strong competitive estrogen receptor weak agonists-estrogenomimetics. In vying for receptors yet eliciting little signal potency in high estrogen females, they act as antiestrogens and in fact mute administered estrogens in test subjects. Isoflavones are orally absorbed, achieve good blood levels and genistein is excreted renally as equal in the urine of most people [7].

There have been so many studies in the female rats [8, 9, 10] regarding the effects of soybean. Most of the studies reported significant reduction in absolute weight of ovary and uterus, as well as plasma estradiol and progesterone [10]. However, Santell *et al.;* [11] reported that soy fractions had no effect on the vaginal

opening, uterine weight and ovarian histopathology. We do not know the reason why there are limited numbers of studies in the male animal and many of the available studies used the subcutaneous administration of daidzein or Genistein. This route by-passes gut microflora and hepatic first pass metabolism which has a major impact on the biological potency of phytoestrogens. The use of soy containing infant foods is increasing, due to the publicity about the health promoting properties of soy. Recently, concern has been expressed that exposure to soy isoflavones may pose a developmental hazard to infants [12], particularly the reproductive system. Life time exposure to soy containing diet delay male reproductive development with manifestation detected in adulthood in rats [13]. This study was therefore, designed to study the effects of soy-flour enriched diet on testis and semen count and motility in male Wistar rats. The gonadotrophic hormones- Testosterome, Sperm count, and motility in male rats were measured.

MATERIALS AND METHODS

PREPARATION OF SOYBEAN:

The Soybean was obtained from Mile-12 market, Lagos state Nigeria and authenticated at the Botany Department of Lagos State University, Ojo, Lagos state, Nigeria. The outer coats of the beans were removed by light pounding in a mortar and winnowing the shaft. The seed without the coat were grounded using super blender (Nakai brand, model MX-735 Japan) and mixed with standard rat feed at a concentration of 10% and 20% W/W Soy flour/standard feed.

ANIMALS PROTOCOL AND DRUG ADMINISTRATION

Fifteen Wistar rats weighing between 120-200g were obtained from the animal house of Lagos State University College of Medicine (LASUCOM), Ikeja, Lagos. They were divided into control (A), high dose (B) and low dose diet (C) groups with five rats per group. Controls rats were given standard diet 0% W: W, while the treated were soy-flour enriched meal- low dose 10% W: W, high dose 20% W: W Soy flour (soy-flour /ground standard diet) five days of the week for eight weeks. All animals were allowed access to the different feed and water ad libitum. The standard feed was a product of Bendel Feed Nigeria Limited, Edo State, Nigeria. The animal rooms were well ventilated with a temperature of range of 25-37^0 C under day/night 12-12 hour photoperiodicity.

The rats were given the compounded feed with different concentration of soya bean flour. After the treatment, the animals were sacrificed by cervical dislocation, the paired testis; epididymis, prostate and seminal vesicle were dissected free, weighed in a torsion balance. One-half of dissected organs were fixed in Bouins solution, and stained with Haematoxylin and Eosin for histology studies. The caudal epididymis of the testis were exposed and excised, placed in a bottle containing 1ml saline (0.9% NaCl in H_2O) and homogenized to release the spermatozoa from the caudal epididymis. The bottles with their contents were incubated at 37^{0}C for 15 minutes for the spermatozoa to become motile. Motility score was performed on the semen samples withdrawn from the specimen bottle under light microscope (x400). The gross motility score in percentage was scored three times for each sample and the mean calculated.

Sperm count was determined using the new improved Neubaurs' counting chamber under light microscope (x400). Blood samples were obtained by cardiac puncture after cervical dislocation, centrifuged at 3000 rpm for sserum Testosterone level assay using enzyme immunoassay. All samples were assays in duplicate and sensitivity to T was 0.2 ng/ml to 16 ng/ml.

Statistics

All data for control and experimental animals were subjected to statistical evaluation using the student's t-test for significant differences, between control and experimental groups at values of $P < 0.05$.

RESULTS

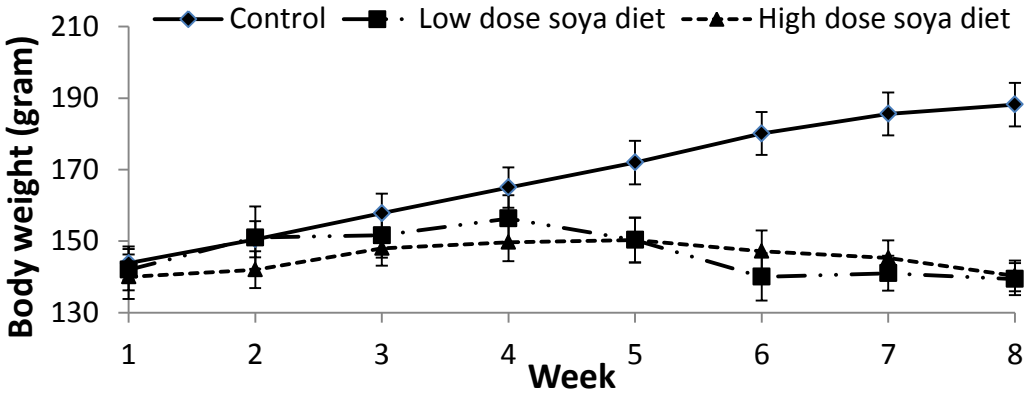

Fig 1: Effects of Soy-flour enriched feed on body weight

Table-1: Effects of Soy-flour enriched feed on Testosterone level, FSH, testicular weight and accessory sex glands

Factors	Control	10% w:w of Soy flour feed(low)	20% by w:w Soy flour feed (high)
Epididymal sperm count (10^6/ml)	59.6 ± 1.44	50.1± 2.4 [β]	46.2 ± 1.5 [β]
Gross motility (%)	64.00± 2.45	58 ± 4.2 [β]	52 ± 3.74 [β]
Testosterone (ng/ml)	2.1 ± 0.15	00.9 ± 0.2[a]	0.4 ± 0.06 [a]
FSH (mIu/ml)	6.53 ± 0.34	6.00 ± 1.5	6.37 ± 1.07
Testis (g/ 100g bw)	1.12 ± 0.03	0.85 ± 0.05 [a]	0.62 ± 0.08 [a]
Seminal vesicle (g/100g/ bw)	0.35±0.03	0.07± 0.06	0.85±0.05 [a]
Prostate (g/100g bw)	0.09±0.01	0.05±0.02 [a]	0.05±0.01 [a]

[a] significantly higher than control (P< 0.05)

β- Signifitcantly lower than control (P<0.05)

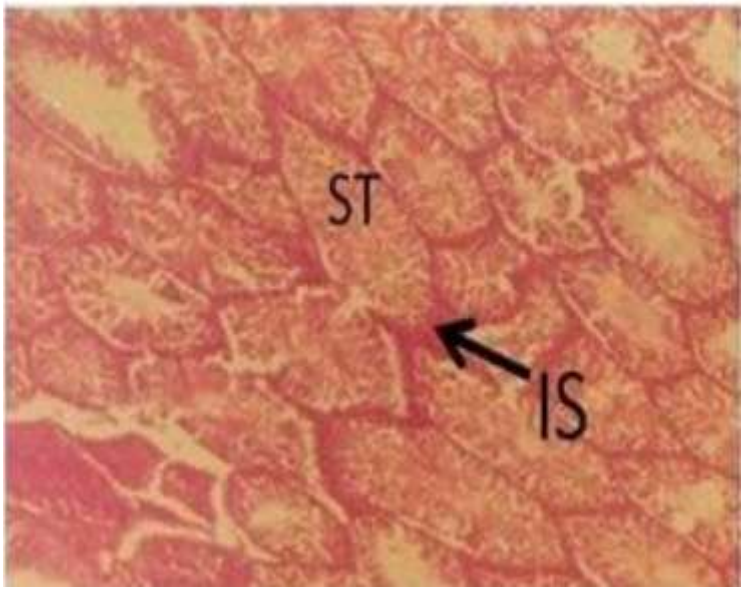

Plate-1: Normal histoarchitecture and large diameter ST. Wider interstitial space in control animal (40X). (ST- seminiferous tubules, IS- interstitial space)

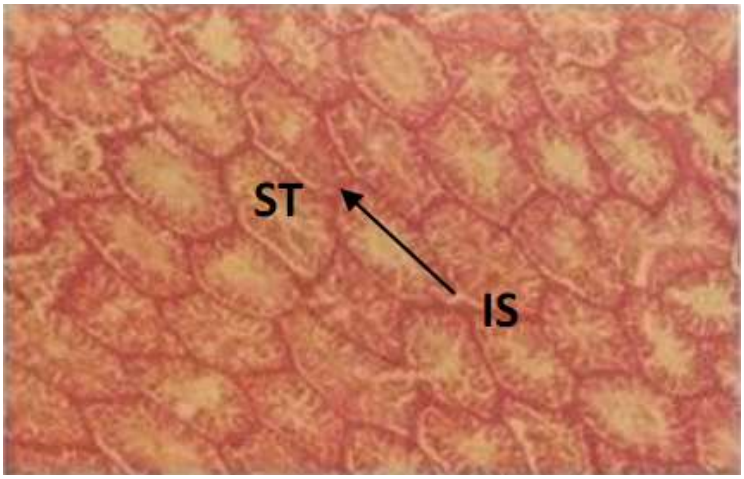

Plate-2: Low dose soy flour treated group showing normal architecture similar to control but reduced size of the seminiferous tubules(40X) (ST- seminiferous tubules, IS- interstitial space)

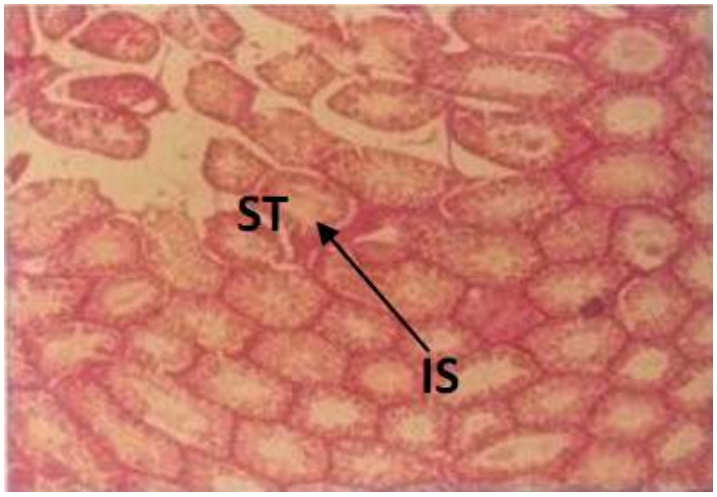

Plate-3: High dose soy flour treated group showing narrowing and disruption of lumen architecture, reduced size of the seminiferous tubules(40X) (ST- seminiferous tubules, IS- interstitial space)

Plates showing histological slides of testis of rats fed with soy flour supplemented.

DISCUSSION

The results of the present study indicate that soy rich diet may affect the growth and /or developments of reproductive tissues and also change the secretion of testosterone. The most significant changes is the concentration of hormone testosterone which was drastically lowered. At high soy dose the testicular weight was reduced by 45% and 24% at low dose. In support of these findings are arrays of publication confirming the biological activity of soy derived phytoestrogens were widely described as reproductive disturbances resulted in the action of these compound within and /or peripheral tissues [14,15,16,17].

Soybeans and soy based foods i.e. soybean are an excellent source of protein and are used extensively in feeds for all classes of livestock and in the preparation of the lab animal diets The influenced of such diets is diverse and dependent on the age and stage in which the animals were exposed. The most significant changes in the concentrations studied were recorded before and during puberty [18].

The animals used in this study were young adult male rats, soy-fed rats both low and high dose went through a gradual and consistent drop in body as the treatment progresses compared to the control (Figure 1). Although, soybean is called the 'miracle bean' containing almost all the amino acids you can name and a host of other nutrient, this nutrient may not be be available for body use and conversion except the soybean is roasted/toasted before processing to degrade some of the anti-nutrients present in the beans [19].

The epididymal sperm reserve was significantly reduced ($p<0.05$) both at high and low doses which may be due to the toxic impact on the testis as a whole, as unprocessed soy flour used as supplement in this study have anti-nutritive factors which has affected negatively on the testis and its function. In support of our results, some data exist, showing adverse effects of soy diet or soy-derived isoflavones on the genital organs and testosterone secretion in adult rodent males [20, 6, 21]. According to Odum *et al.*; [22], administration of different soy diets to rats can affect in a different way the timing of both male and female sexual development.

The motility of the sperm cell was also significantly reduced compared to the treated, probably due to the reactive oxygen species or oxidative stress damage induced by raw soy flour [23].

The histological slides showed a similar picture both in the treated and control, the histo-architecture was intact but the seminiferous tubular diameter were reduced in size in the soy treated. This reduction in diameter may indirectly translate to reduce testicular function. The reasons for this observation may be due phytoestrogens present in soy flour, which competitively bind to receptor sites for testosterone (T), there limiting the amount of T converted to DHT; hence, leading to reduction of germ cell maturation and indirectly reduced epididymal sperm reserve observed among the treated animals [24].

CONCLUSION:

Soybean the 'miracle bean' is a very rich source of plant protein but the protein can only be available in a form that body can absorb after the beans must have been processed to reduce the anti-nutritive factor present in the raw form. Simple treatment like roasting or toasting or fermenting processed form

before being used as supplement will do the magic. Only then then can the bean be put beneficial use.

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