Hormonal and Histoarchitectural Changes in the Prostate and Testis of Wistar Rats Fed with Soy-Flour Enriched Diet

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Abstract

Several epidemiological studies have suggested an inverse association between the risk of prostate problem and intake of soybean and their products. Asian men who consumed a lot of soy products have lower incidence of prostate problems. This study, therefore, was designed to determine the effects of soy-flour enriched diet on the prostate morphology, gonadal hormones (Testosterone and FSH) and semen count and motility in rats. Fifteen male rats were fed soy-flour enriched diet at 0% W:W, 10% W:W, 20% W:W (soyflour / 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. Prostate histo-architecture was disrupted; displaying increased inter-acinar connective tissue that is diffused, with fewer acini and reduced glandular size, atrophy of the epithelium compared to the control prostate. It also produce a form of mild toxic effect on the testis histo-architecture.

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 (antiinflamatory) 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 equol in the urine of most people [8]. There has been so many study in the female rats [9,10,11] 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 [11]. However, Santell et al.,[9] 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 Genisten. 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 accessory sex glands and semen count and motility of male Wistar rats. The gonadotrophic hormones- T, and FSH, Sperm count, and motility in male rats were...
considered.

Material 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°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 H2O) and homogenized to release the spermatozoa from the caudal epididymis. The bottles with their contents were incubated at 370C 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 Neubaur's counting chamber under light microscope (x400). Blood samples were obtained by cardiac puncture after cervical dislocation, centrifuged at 3000 rpm for serum 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.

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

Figure 1: The average body weight of animals fed soy-flour enriched diet gradually increased between the 1st and 4th weeks of treatment, after which there a was a continuous drop in the body weight of the treated until the end of the study. The treated showed the body weight gain on a weekly basis for the animals over the period of eight weeks of treatment. There was a steady weight gain the first three weeks of treatment after the commencement of treatment after which the gains stopped in the high soy treated and the low soy treated (in the latter end of third week). The control animals continue to gain weight throughout the study but the treated ceased to gain weight from the early third week and later third week for the high soy and low soy treated respectively.

Prostate: The histological observation of the prostate (Plates 1-3), control prostate showed normal histo-architecture of the prostate, the gland and stroma are obvious, while the treated exhibited shrinking of the gland and the individual gland cannot be figured out clearly.

Testis: The histological observation of the testes showed no distortion in cell architectures of the treated groups. The seminiferous epithelium (SE) appeared reduced in diameter, interstitial tissue (IT) was also reduced in size compared the control and clearing of the lumen at high dose (Plates 4-6).

Discussion

The plates showed a reduced acinar size and fewer glands in the rats fed soyflour enriched diet, the prostate of the treated displayed distortion of the glands size in the soy treated showing a prostate gland with a reduce activity compared to the control where the gland acini size are larger and indicative of a more active and secreting prostate. The reasons for the observed effects may be due high amount of phytoestrogen present in soy-bean(a legume). Phytoestrogen have been reported to have weak oestrogenic activity, which interferes with productions
of androgen, by lowering the production and causing a disruption of the balance between androgen levels and loss of androgen receptors [14]. Hence, the poor performance of the treated prostate in terms of gland size and growth in the above study. The significant drop in the body weight of the animal (Figure 1) and accessory sex organs (P < 0.05) Table 3, may be due to the presence of anti-nutrient in raw soy flour used in this study which may cause mal-absorption of amino acid present in the rich soy protein. These anti-nutrient include the glycosides, phytic acid, trypsin inhibitor and hemaglutinins which are partially destroyed during mechanical handling of soy but required heat treatment in the form of cooking or roasting to inactivate and make this protein readily available for bioconversion and body usage [15]. The presence of anti-nutrient in unprocessed soy also exposes the reproductive system of treated animals to oxidative stress, oxidative stress has been linked to almost half of the diagnosed cases of oligospermia in human [16] and reduction in the overall sperm production and loss of motility due to impairment of energy production and abnormal sperm morphology [17]. Oxidative stress in semen occur when the level of reactive oxygen species (ROS), is greater than (TAC) total antioxidant capacity, although ROS are needed for normal sperm function, high level of ROS clearly impair fertility. Furthermore, oxidative stress impairs on the sperm plasma membrane, sperms having defective plasma membrane are unlikely to be motile [18].

The result of the sperm count and motility was also significantly reduced when compared with the control both in the rat fed high soy and low soy-flour enriched diet in dose dependent manner (P > 0.05) Table 1. This may be due partly to anti-nutrient effects of raw soy as explained above; on the other hand soy is a rich source of phytoestrogen, a component that interacts with the testis or modulates the plasma gonadotrophin or sex hormone concentration thereby disrupting spermatogenesis. Substance that affect the plasma gonadotrophin or the sex hormone milieu are usually evaluated by measuring sperm number and motility and the histopathological examination of the testis and epididymis [19]. Spermatogenesis may have been disrupted via the hypothalamo-pituitary axis which reduces the amount of FSH impacting on the Sertoli cells and so there is less stimulation of the spermatogenetic germ cell. FSH is also needed to stimulate inhibin, which together with testosterone are involved in regulating GnRH from the hypotalamus hence the lowered sperm count and reduced motility of the sperm cell observed in this study. It has also been said to have anti androgen effect which has been linked to the development of aberrant germ cell in testis in fetal life [20], prostate being an androgen responsive cell.

The result of hormone assay shows a dose dependent reduction in the value of testosterone and FSH compared with the control (P > 0.05) Table 2. The decrement in a dose dependent manner when compared with the control (P > 0.05). The reason is because soy-bean is a legume, rich in phytoestrogen which has been reported to have weak oestrogenic activity, which interferes with productions of androgen, by lowering the production and causing a disruption of the balance between androgen levels and loss of androgen receptors [14].

The accessory sex gland result and testis were also negatively affected compared with the control rat (P < 0.05). There was a significance decrease in the weight of the testis, seminal vesicle and prostate in a dose dependent manner (P < 0.05%) Table 2. The reduction in the weight the accessory sex gland observed in this study may be due to the disruption of testosterone production which is a necessary factor for growth, and by the weak oestrogenic activity of soy-phytoestrogen which destroys the androgen receptors sites in these organs [21]. Effects of soyflour on the testis were investigated to highlights the possible histological implications that could result following its consumption. The study reveals that raw soy-flour in animal feeds has mild toxic effect on the testis histo-architecture. The pharmacological mechanism behind this result may be traced to some anti-nutrient in soy-flour enriched diet. Despite the fact that soybean has a rich source of almost all the amino acids required for proper testicular function, unprocessed form in animal diet may affect proper body growth and testicular functions. The observed effect showed a promising effects regarding problem of prostate. Active ingredients like isoflavones present in soybean can be extracted and used to ameliorate problem of prostate enlargement and cancer. Isoflavones are polyphenolic compounds that are capable of exerting estrogen-like effects. For this reason, they are classified as phytoestrogens—plant-derived compounds with estrogenic activity [22]. Legumes, particularly soybeans, are the richest sources of isoflavones in the human diet. In soybeans, isoflavones are present as glycosides (bound to a sugar molecule). Fermentation or digestion of soybeans or soy products results in the release of the sugar molecule from the isoflavone glycoside, leaving an isoflavone aglycone.

Conclusion
Soy flour has promising future regarding the management of prostate ailments and women pre-menopausal conditions. We advised the use of raw unprocessed soy as animal feeds should be minimised as it body weight gain in animals. Processed soybean has a lot of benefit to offer than the raw unprocessed form. The anti-nutrients present in raw form have been proven to have a lot of beneficial effects on prostate, while the isoflavone contents are good for women pre-menopausal conditions.

References

testosterone levels and prostate weight without altering LH, prostate 5α-reductase or testicular steroidogenic acute regulatory peptide levels in adult male Sprague-Dawley rats. J Endocrinol 170: 591-599.

Illustrations

Illustration 1

Figure 1: Effects of Soy-flour enriched diet on body weight

Treatment with soy-flour at both high and low doses caused a steady growth of the animal for the first four weeks after which there was a continuous decrease in the weight of the treated animals till the end of the study. The treatment beyond the 4th week caused a further decrease in animal weight below the starting weight of the animals.
Illustration 2

Table 1: Effects of Soyflour enriched diet on epididymal Sperm count, sperm Motility, serum FSH and Testosterone level

<table>
<thead>
<tr>
<th>Factors</th>
<th>Control</th>
<th>Feed with 20% by weight of Soy flour (high)</th>
<th>Feed with 10% weight of Soy flour (low)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epididymal sperm count (10^6/ml)</td>
<td>59.6 ± 1.44</td>
<td>46.2 ± 1.5 β</td>
<td>50.1 ± 2.4 β</td>
</tr>
<tr>
<td>Gross motility (%)</td>
<td>64.00 ± 2.45</td>
<td>52 ± 3.74 β</td>
<td>58 ± 4.2 β</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>2.1 ± 0.15</td>
<td>0.4 ± 0.06 β</td>
<td>0.09 ± 0.2 β</td>
</tr>
<tr>
<td>FSH (mlu/ml)</td>
<td>6.53 ± 0.34</td>
<td>6.37 ± 1.07 β</td>
<td>6.00 ± 1.5 β</td>
</tr>
<tr>
<td>Testis (g/100g body weight)</td>
<td>1.12 ± 0.03</td>
<td>0.62 ± 0.08 β</td>
<td>0.85 ± 0.05 β</td>
</tr>
<tr>
<td>Seminal vesicle (g/100g body weight)</td>
<td>0.35 ± 0.03</td>
<td>0.07 ± 0.06 β</td>
<td>0.85 ± 0.05 β</td>
</tr>
<tr>
<td>Prostate (g/100g body weight)</td>
<td>0.09 ± 0.01</td>
<td>0.04 ± 0.05 β</td>
<td>0.05 ± 0.01 β</td>
</tr>
</tbody>
</table>

β significantly decreased compare with control (P< 0.05)
Plate 1
Control prostate (Mag x 400), showed distinct acini, with prostatic concretions (A), with moderate interacinar connective tissue (B).

Plate 2
Soy flour low dose treated rat prostate (Mag x 400) showing less proliferation of gland, increased interacinar connective tissue

Plate 3
Soy flour high dose treated rat prostate (Mag x 400), showing diffuse prostatic acini (A), wide fibromuscular stroma

Prostate slides of Control, High dose and low dose soya flour treated rats
Illustration 3

Plate 4

Soy Control testis

Normal histo-architecture and large diameter ST.
Illustration 4

Plate 5

Soy Testis low dose

Normal architecture similar to control, reduced seminiferous tubules compared to control (X 100).
Illustration 5

Plate 6

Soy Testis High dose

Normal architecture similar to control. Narrowing of lumen at low dose and clearing of lumen, reduced size of the seminiferous tubules and clearing of the lumen (X 100)
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