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RESEARCH PAPER

THE EFFECT OF SOYA BEAN ON SPERM CHARACTERISTICS AND TESTICULAR HISTOLOGY IN RABBITS

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ABSTRACT

This study investigated the effect of Soya bean meal on sperm concentration, motility and morphology, body and organ weights, and testicular histology, in 20 male Chinchilla rabbits (6±1 months-old),weighing between 1.4 - 1.9kg and classified into four groups (A,B.C and D; n=5 each). Group A was control, while B, C and D, were test groups. For 4 weeks after a 2-week acclimatization period, group A received normal feed, while test groups B,C and D, received 30g, 60g and 90g of Soya bean seeds each respectively, and based on a documented LD_{50} of 170g. Water was given *ad libitum*. The weights of the rabbits were monitored and recorded prior to the commencement of Soya bean administration and at the end of the experiment. After Soya bean administration, the rabbits were prepared for semen and testes tissue sample collection and subsequent analysis using standard procedures. The results showed comparative body and testicular organ weight changes, but no statistically significant differences in sperm cell concentration and morphology ($P>0.05$).The histological findings showed that the lowest dose (30g) presented the best histological picture indicating that excessive consumption of Soya beans can be deleterious to testicular cytoarchitecture and by extension, male fertility.

Keywords: Chinchilla rabbits, Soya bean, Sperm cell, Testes

INTRODUCTION

Soya bean (*Glycine Max*) belongs to the plant family *Papihonaceae.* Its protein content is well known and as such, has been widely recommended to populations as a reliable source of essential amino acids. It has also been considered as a medicinal food for diabetic patients, with suggestions that it has ability to prevent human cancer and other diseases (Messina*et al.,* 1994). Its phytochemicals include saponins, phytic acid and isoflavones with characteristics similar to 17β-oestradiol compounds like genistein, daidzein, formononetin, biochanin A and equol (Ogbuewu *et al.,* 2010) and lignans like enterolactone and enterodiol derived from precursors in diet by gut microflora (Martini *et al.,* 1993). Studies in non-human primates (Adams *et al*., 2005*)* and rabbits (Yamakoshi *et al.,* 2000), have demonstrated retardation of atherogenesis during dietary isoflavone-phytoestrogen administration. The black hull (seed coat) of Soya beans contains various polyphenols, such as anthocyanins, procyanidins and catechins (Kanamoto *et al*., 2011).

Available literature indicates that dietary cyanidin 3-glucoside-rich purple corn prevents high-fat diet induced obesity in mice (Tsuda *et al*., 2003), and that procyanidin from the chardonnay grape seed extract prevents high-fat diet-induced

obesity in hamsters (Décordé *et al.,* 2009). However, attention has been drawn to the potential toxicological effects of anthocyanins (Bentivegna and Whitney, 2002; Soulimani *et al*., 2001), procyanidins (Bentivegna andWhitney, 2002; Hanamura and Aoki*, 2008;* Ray *et al.,* 2001a*, b;* Yamakoshi *et al.,* 2002) and catechins (Chengelis *et al*., 2008; Fujii *et al*., 2007; Isbrucker *et al*., 2006a, b; Lambert *et al*., 2010).

Of course, the nutritious potentials of Soya bean have been hyped over and over again and the consequent reaction to the enlightenment campaigns by populations has been that massive reliance on soya bean as a reliable and cheap source of protein. However, there has not been a concurrent campaign against its excessive consumption. Toxicologists have recognized that "*everything is a poison*; *it all depends on the dose consumed*". Thus, it is likely that an excessive consumption of Soya bean portends deleterious consequences that needs to be determined; for instance, on the reproductive system.

This study was therefore considered significant as it could provide information on:

1. The dosage ranges of Soya bean that can significantly influence spermatogenesis in males, since there are suggestions that isoflavones may be involved in regulating the renewal of spermatogonial stem cells (Miura *et al.,* 2003), and male reproductive tissue with estrogen receptors (Amin *et al.,* 1969).
2. The dangers or benefits of indiscriminate Soya bean consumption on the fertility status of males, since there are evidence that soya bean seed contains estrogenic materials like gonad stimulating compounds that improve male fertility (Oyeyemi and Okediran*,* 2007*;* Mitchell *et al.,* 2001*;* Ogbuewu *et al.,* 2010).
3. The possible influence of Soya bean consumption on sperm morphology since there are suggestions that it has capacity to improve sperm motility in rabbits (Oyeyemi and Okediran, 2007).

On the other hand, these set of facts justify the need for this study:

1. The fact that Soya bean contain various biological active compounds that includes saponins, phytates, protease inhibitors, phenolic acids, lecithin, phytosterols, isoflavones cyanidin 3-glucoside and omega-3 fatty acids (Omoni and Aluko*,* 2005).
2. The fact that some of the active ingredients like anthocyanins (Soulimani *et al.,* 2001*,* Bentivegna and Whitney*,* 2002*;*), procyanidins (Ray et al., 2001a, b; Yamakoshi et al., 2002; Bentivegna and Whitney, 2002; Hanamura and Aoki, 2008)*, and catechins* (Isbrucker *et al.,* 2006a, b; Fujii *et al.,* 2007*;* Chengelis *et al*., 2008; Lambert *et al.,* 2010) have toxicological effects.
3. The fact that oral toxicity of Soybean has being reported in a 19-year old boy and 55-yearold woman who fell into coma (Morais *et al.,* 2010; Gillian, 2013).

Some questions of concerns include:

1. What then could be the consequence of soyabean consumption if the effective dose is doubled or tripled?
2. What could be the effect of soyabean consumption abuse on weight, sperm quality and testicular histology?
3. Can excessive consumption of soybean lead to acquired male infertility?

This study therefore, is designed to determine the effect of soya beans on sperm characteristics and testicular histology in male Chinchilla Rabbits.

MATERIALS AND METHOD

Experimental Animals: Twenty (20) male Chinchilla rabbits weighing 1.4 -1.9kg and 6±1 months of age, which were purchased from the Animal House of the college of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria, were used for this research work.

Substance of Study: Soya beans seeds used for this study and locally called *Esoya ere'bo*, were purchased from the Royal Market, Ekpoma, Edo State, Nigeria. The Soybeans were appropriately authenticated in the Department of Botany, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

Animal Grouping: The animals were divided into four (4) groups A, B, C and D. Group A served as control, while groups B, C and D served as test groups. Each group had 5 rabbits respectively.

Substance Administration: The substance of study was administered to the animals as follows:

1. Group A (Control) received only normal feed (Growers Mash) from Ewu Flour Mill, Ewu, Edo State.
2. Groups B, C, and D received 30g, 60g and 90g of soybeans per rabbit respectively.
3. The administered doses were determined based on an LD50 of 170g (Gillian, 2013).
   All the groups received water *ad libitum*

Sample Collection: Samples were collected at the end of the test period of four weeks in the Physiology laboratory within the premises of the Faculty of Basic Medical Sciences, College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria. Semen samples were collected by making an incision on the left testis/epididymis and sperm cells were subsequently squeezed out as described by Brackett *et al*. (1978). The right testes samples were obtained via dissection under chloroform anesthesia and immediately fixed in 10% formalin.

Semen Analysis: Semen Analysis was carried out in the Diagnostic and Research Laboratory of the Department of Medical Laboratory Sciences, College of Medicine, Ambrose Alli University Ekpoma, Edo State, Nigeria.

Determination of Sperm Motility: Sperm motility was determined by procedures described by Ochei and Kolhatkar (2008), using a drop of semen mixed with two drops of 2.9% sodium citrate and placed on a clean glass slide and covered with cover slip before examining it using x40 objective of a binocular microscope. The forward progression made by the largest members of the spermatozoa was graduated as follows

1. % quick and actively motile –Excellent Forward progression
2. % moderately motile- Moderate forward progression
3. % sluggishly motile- Weak forward progression
4. % non-motile –Absence of forward progression

Examination of Sperm Morphology: The morphological characteristics of the sperm cells are important for the complete assessment of the seminal fluid. This was determined using the carbol fuchsin and staining technique. For the purpose of this, a smear of the semen was made on glass slide and fixed in a fixative for 5-10 minutes. It was then washed with sodium bicarbonate –formalin solution to remove any mucus. The smear was covered with dilute carbol fuchsin for 3 minutes and then washed with water. This was counter stained with polychrome methylene blue for 2-3 minutes and washed with water and air dried. The cells were subsequently examined under x40 objective of a binocular microscope.

Sperm Count/Concentration: The determination of sperm count/concentration was done using a counting chamber (the improved Neubauer counting chamber). First, the specimen was diluted 1:20 using the white blood cell (WBC) pipette by drawing the semen to the "0.5" mark and diluted with diluting fluid to the eleven (11) mark and mixed. The counting chamber was charged and the cells were counted. The dilution fluid contains:

1. Sodium bicarbonate 5g
2. Formalin (40%) 1ml
3. Distilled water 100ml
4. Tinge (little) drop-wise of gentian violet or methylene blue was added to the fluid

Calculations were performed as follows:

Sperm count per ml = n x 50,000 x 20 = n x 10^6 (sperm concentration)

Where:
n = No of sperm cells counted
Multiplication factor = 50,000
Dilution factor = 20

Generally, normal sperm count range is 50-150 million/ml. But the World Health Organization (WHO) recommended that men with sperm counts ranging from 20-200 million/ml are in the fertile range.

Tissue preparation: The testes tissue samples were transported to the Histopathology Laboratory at Irrua Specialist Teaching Hospital, Irrua, Edo State, for tissue processing. The fixed testes tissues were processed using the 18-hour automatic tissue processor (LEICA TP 1020). The processed testes tissues were then embedded in molten paraffin wax with the use of plastic embedding moulds. The embedded tissues were placed inside the refrigerator for effective solidification. They were trimmed after removing them from the embedding mould to expose the surface of the tissues. The tissues were then placed on an ice block to cool the surface of the tissue so as to allow easy and effective cutting of ribbon of sections from the tissues. The tissue sections were cut at 5um with the use of rotary microtome. The tissues were placed on clean grease free slides and floated in the water bath (400C) with the use of 20% alcohol. The sections were picked from the water bath, well labeled using a diamond pencil and were placed on hot plate to allow the section to stick properly to the slide so as to avoid lifting during staining. Haematoxylin and Eosin technique was used to stain the tissue sections for the demonstration general tissue structure. The mounted slides were then examined using a binocular light microscope at x100 magnification. Microscopy was done for all the sections and the results were compared with that of the control sections before photomicrographs were taken.

Statistical Analysis: The Statistical Package for Social Sciences (SPSS version 16) was used for Statistical analysis. Analysis of Variance (Anova) and the t-test statistical tools were used to analyze the data obtained. All values were expressed as Mean ± Standard Deviation (SD) and Mean ±Standard Error of Mean (SEM), with level of significance set at $P<0.05$.

Study duration: This study lasted for 18weeks: 1) acclimatization - *2 weeks*; 2) Substance Administration -*4 weeks*; 3) Sample analysis and Statistics -*8 weeks*; and 4) Micrography and Results' interpretation -*4 weeks*.

RESULTS

On weight changes, sperm concentration and morphology, there were changes in body and testicular-organ weights between the test and control groups. There were also changes in sperm cell concentration between the test and control groups. The changes in weights (table 1) were particularly significant in test group B for body weight (1.99±0.16kg) and test group D for testicular organ weight(2.78±0.89g); as compared to the control values ($P<0.05$). However, there were no statistically significant difference in sperm cell morphology and concentration (tables 2 and 3) between the test and control groups ($P>0.05$).

TABLE 1: MEAN VALUES OF BODY WEIGHT, ORGAN WEIGHT AND SPERM CONCENTRATION

Groups	BODY WEIGHT (kg)	ORGAN WEIGHT (TESTES) (g)	SPERM CONCENTRATION ($x10^6$)/ml
A	1.58±0.07	2.47±0.88	1.82±0.28
B	1.99±0.16*	2.58±0.76	1.73±0.40
C	1.55±0.05	2.42±0.84	1.23±0.27
D	1.66±0.16	2.78±0.89*	1.08±0.22

Values are expressed as mean ± standard deviation. *Significantly different compared to control ($P<0.05$)

TABLE 2: MEAN VALUES OF NORMAL AND ABNORMAL SPERM CELLS (MORPHOLOGY)

GROUPS	NORMAL HEAD %	LARGE HEAD %	TAPERING HEAD %	DOUBLE HEAD %	AMORPHOUS HEAD %	DOUBLE TAIL %	COILED TAIL %	BENT TAIL %
GRP A	73.80 ±4.64	0.00 ±0.00	0.00±0.00	3.20 ±0.58	0.80 ±0.37	1.60 ±0.75	1.20 ±0.58	3.40 ±0.75
GRP B	68.40 ±1.54	1.20±0.73	0.60±0.40	3.20 ±0.58	0.60 ±0.40	0.80 ±0.80	10.40 ±1.05	3.40 ±1.03
GRP C	79.20 ±1.46	0.00 ±0.00	5.60 ±1.81	5.80 ±1.81	0.00 ±0.00	0.80 ±0.37	3.00 ±1.50	3.80 ±0.86
GRP D	83.20 ±1.46	0.40 ±0.24	1.00 ±0.55	3.40 ±0.55	0.2 0±0.02	1.00 ±0.44	2.20 ±0.75	3.00 ±1.00

Values are expressed as mean ± standard deviation

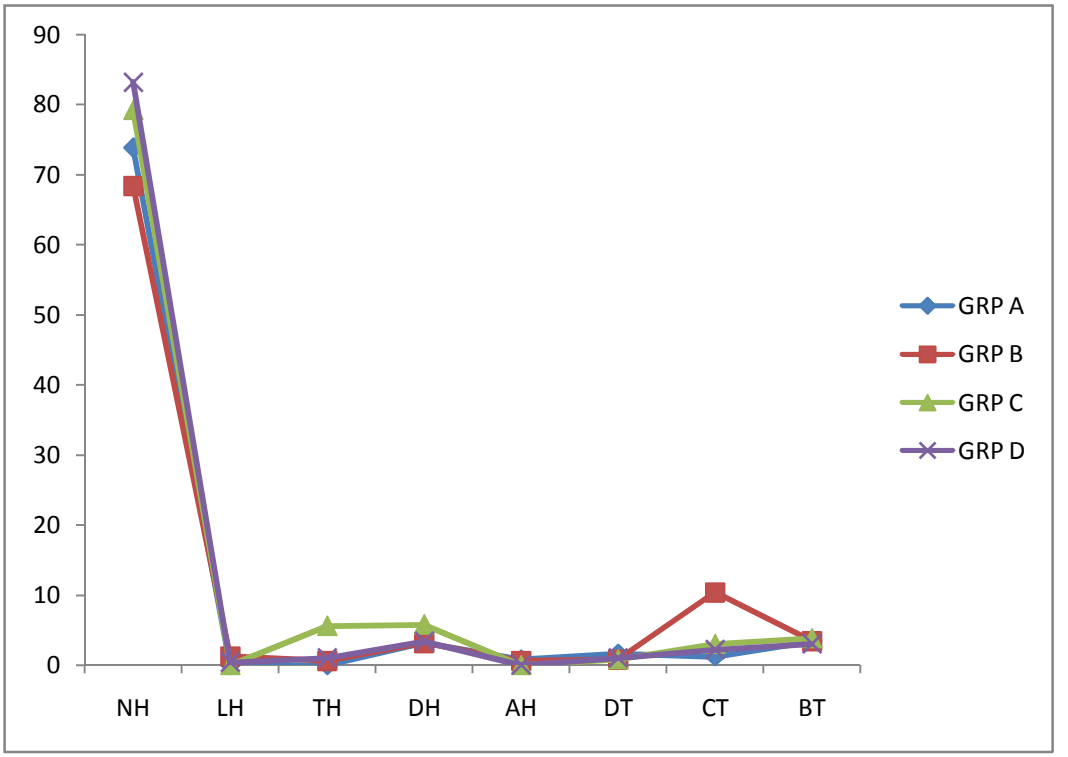

Figure 1: Morphological distribution of the sperm cells
(NH=Normal Head; LH=Large Head; TH=Tapering Head; DH=Double Head; AmH =Amorphous Head; DT=Double Tail; CT=Coiled Tail; BT =Bent tail)

TABLE 3: MEAN VALUES OF ACTIVE, SLUGGISH AND NON MOTILE SPERM CELLS.

GROUP	ACTIVELY MOTILE	SLUGISHLY MOTILE	NON MOTILE
A (Control)	74.00 ± 3.96	13.00 ±3.57	8.00 ± 1.22
B (30g)	82.00 ±1.00	10.00 ±2.26	6.00 ± 1.00
C (60g)	80.00 ±1.29	10.00 ±2.24	8.75 ± 1.25
D (90g)	86.00 ±1.52	7.30 ±2.14	5.00 ± 0.00

Values are expressed as mean ± standard error of mean (SEM)

HISTOLOGICAL FINDINGS

The histological examination of the testes tissue sections showed that:

1. The control testes tissue sections presented normal cytoarchitecture with intact and distinct seminiferous tubules and interstitial spaces respectively (See Plate 1).
2. Test group B tissue sections presented intact, distinct, and densely populated seminiferous tubules (See Plate 2).
3. Test group C testes tissue sections showed intact and distinct seminiferous tubules with reducing population of sperm cells as compared to group B above; though with persistent density of the tails of sperm cells (See Plate 3).
4. Test group D testes tissue sections showed seminiferous with necrotic sperm cell populations as indicated by densely staining basophilic sperm cell nuclei (*See* Plate 4).

These findings indicate that the lowest dose presented the best histological picture; suggesting therefore, that excessive consumption of Soya beans is deleterious to the testicular cytoarchitecture.

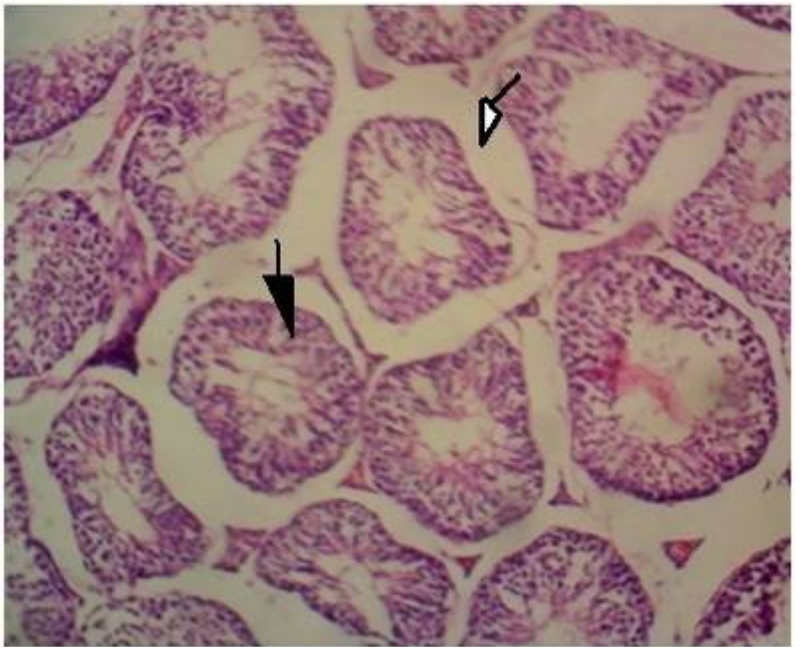

Plate 1: Photomicrograph of control testes tissue section (H&E; x100) showing normal cytoarchitecture with intact and distinct seminiferous tubules (black arrow) and interstitial spaces (white arrow) respectively

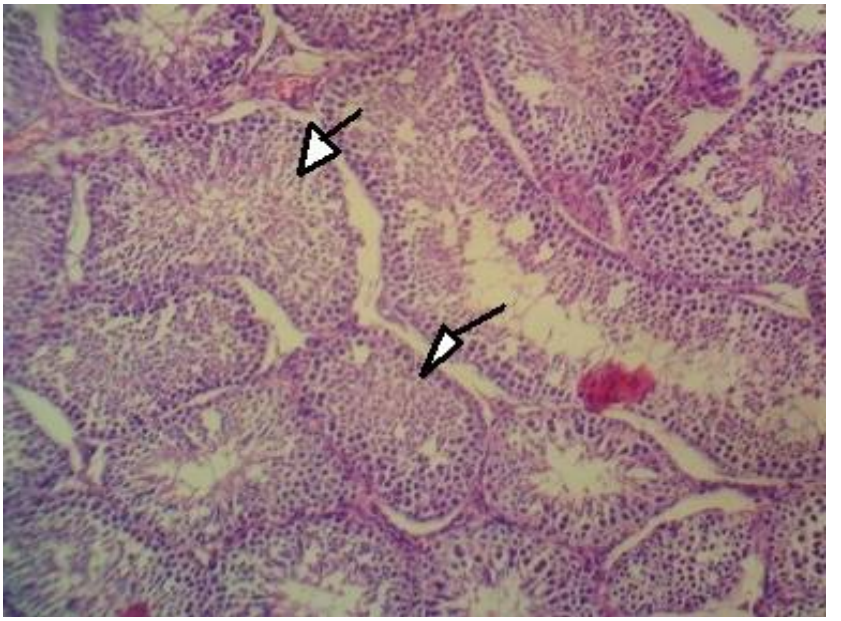

Plate 2: Photomicrograph of test group B testes tissue section (H&E; x100) showing with intact, distinct and densely populated seminiferous tubules (white arrows).

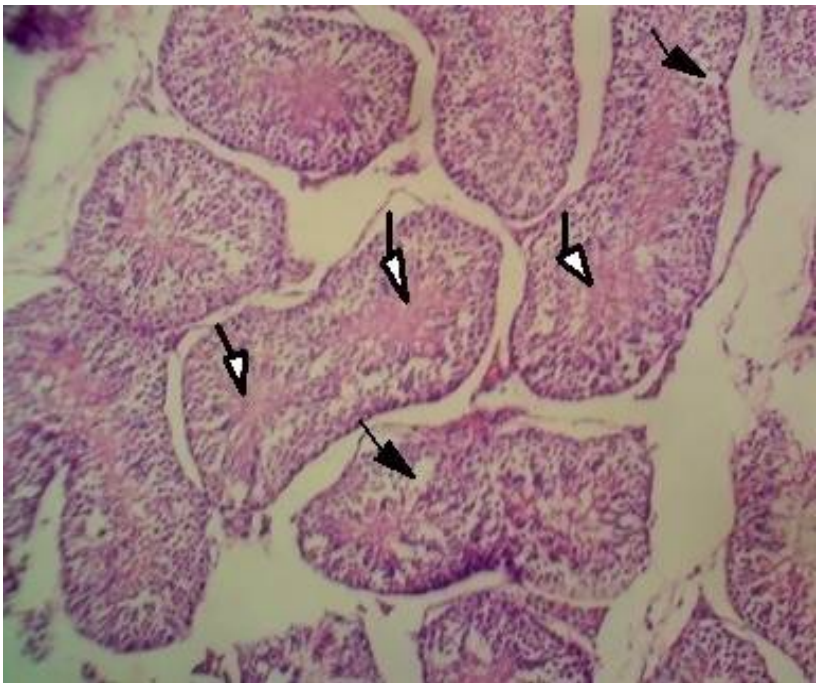

Plate 3: Photomicrograph of test group C testes tissue section (H&E; x100) showing with intact and distinct seminiferous tubules with reducing population of sperm cells (black arrows) as compared to group B above. Note also the density of the tails of sperm cells (white arrows)

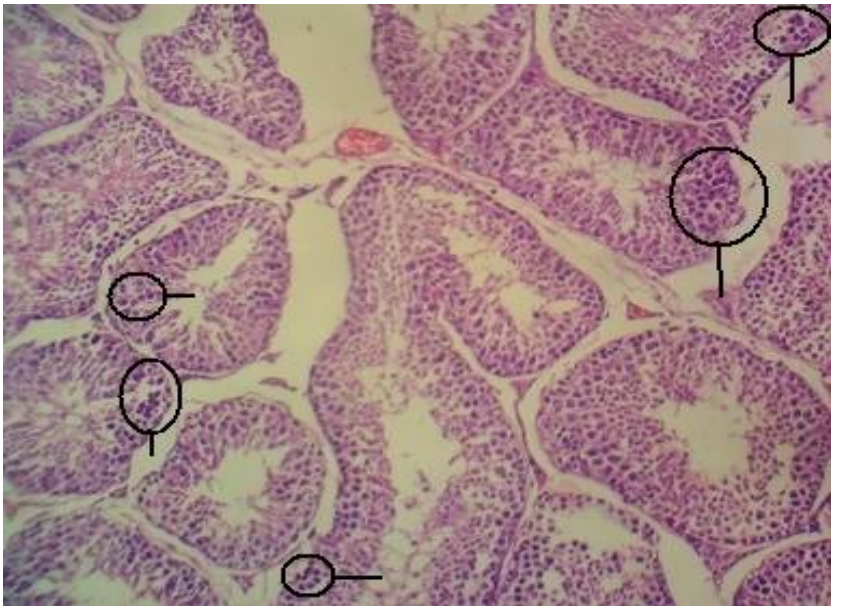

Plate 4: Photomicrograph of test group D testes tissue section (H&E; x100) showing seminiferous with necrotic sperm cell populations (encircled). Note densely basophilic cell nuclei

DISCUSSION

The observed sharp increase in the mean body weight of group B rabbits, mirrors the several assertions that soya bean enhances growth and development, but the comparative follow-up reductions in the mean body weights of groups C and D rabbits suggests that controlled moderate doses of soya bean would produce the best outcome.

Secondly, the changes observed in testicular organ weights, is in line with the reports by Ogbuewu *et al.* (2009) that there exists a high correlation between testicular weight, sperm reserve in the testis or epididymis, and sperm production following soya bean seed consumption. Specifically, Ogbuewu *et al.* (2009) asserted that such an increase in testicular weight of rabbits is a pointer that toasted soya bean seed meal promotes testicular growth.

Although, the testicular weight gain observed in this study can be said to align with the reproductive potentials of soya beans highlighted earlier by Ogbuewu *et al.* (2009), it is important however, to point out that there is a sharp difference in the nature of the Soya beans used in this study and that of Ogbuewu *et al.* (2009) -toasted and raw soya beans respectively. This obviously, may account for any difference in the overall findings of this study, as it is obvious that raw Soya bean would surely be more toxic than toasted Soya bean. This is evident in the histological findings indicating a degenerative trend in testicular histology as the dosage increased.

The histological findings in the lowest dose (30g) group, is reflective of the assertion by Oyeyemi and Okediran (2007) that soya bean seed meal positively affects testicular development in rabbits (increased proliferation of many types of testis cells involved in spermatogenesis). This however, was contradicted in the observed histological findings in groups C (60g) and D (90g) rabbits; suggesting that the above assertion is applicable to low or moderate doses only.

These findings of this study in groups C and D, corroborate the assertions on the potentials of phytoestrogens such as soy isoflavones in soya bean (Hymowitz and Newell, 1978), to induce low semen concentrations, poor semen quality, lack of sperm motility and eventually a reduced libido (Derosa *et al*., 1998), severe reproductive defects and infertility has also been reported by (Mitchell *et al*., 2001; Hess, 2003). More so, Hess (2003) and Glover and Assinder (2006), had argued that the distortions observed in the fertility of male mammals are directly correlated to the distortions in spermatogenesis. Such reduction in spermatogenic activities has also been reported to result in infertility, reproductive toxicity and dysfunctions (Gelain, 2005). Auger *et al*. (1975) and Carlsen *et al.* (1992) have also shown that there has been significant decline in sperm quality and quantity worldwide among fertile men following overall reduction in androgens

(Sharpe *et al*., 2002), that might occur with significant increase in the levels of estrogens in the male body (Ekaluo *et al*., 2011a, 2011b).

Other studies have also shown the capacity of soya bean to significantly reduce sperm viability and count, while increasing sperm head abnormality in a dose-dependent manner (Ekaluo *et al*.,2008; 2009; 2011a; 2011b; 2011c, 2011d); signifying the reproductive toxicity of soya beans. The study by Ekaluo *et al*., (2013) showed that the administration of soybean to rats at different doses caused significant dose-dependent toxicity effects to testicular integrity, ranging from mild degeneration of sperm in testicular tubules to excessive necrosis and haemorrhages; which might be the underlying cause of the effects on sperm parameters.

In addition, soya beans capacity to act as an endocrine disruptor in males has been highlighted by Ekaluo *et al*(2011a) and as such, can disrupt the synergy between testosterone and follicle stimulating hormone during the process of spermatogenesis (Ekaluo *et al*, 2011b; Ikpeme *et al*., 2010). Even Ikpeme *et al*. (2010) assertively revealed that the distortion in fertility in male mammals is directly correlated with the disruption of spermatogenesis and the hormone regulatory machineries. Such reduction in spermatogenic activities (Glover and Assinder, 2006; Sharpe *et al*., 1993) usually results from reproductive toxicity (Gelain *et al*.2005; Greenspan and Stawler, 1997).

CONCLUSION

The findings of this study, suggest that irrespective of the acclaimed nutritive benefits of Soya beans, its excessive consumption should be avoided to prevent acquired male infertility.

RECOMMENDATIONS

Based on the results of this study, the following recommendations are declared:

1. Individuals are advised that despite the acclaimed nutritive value of Soya bean, its consumption must be moderate and properly processed.
2. There is an urgent need for public health enlightenment campaign on the need to regulate the consumption of Soya bean.
3. The treatment of male infertility should incorporate diet control.
4. Further studies are required to fully situate the role of diet in the incidence of acquired male infertility among populations.

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AUTHORS CONTRIBUTIONS

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