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Reproductive toxicity of soybean (*Glycine max* L.) in rats

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Abstract: *Effect of soybean on weight of testes and epididymes, histology of testes, sperm count, sperm viability, sperm head abnormality and conception rate was investigated in eighty mature albino rats (20 males and 60 females) of 12 weeks old with similar body weights. The rats were assigned to four groups of 5 male rats each and treated with soybean meal at 0, 100, 200 and 300 mg/kg body weight (BW) respectively daily for 65 days. The treated and the control males were made to sire the untreated female rats in the ratio of 1:3 for conception rate. The male rats were then sacrificed and the testes and epididymes were dissected out and weighed. The testes were processed for testicular histology and the epididymes were processed for epididymal sperm count, viability and sperm head abnormality test. There were dose-dependent effects of soybean on sperm count, sperm viability and sperm head abnormality, as well as toxicity effects to testicular integrity and conception rate. Soybean had strong capability to reduce spermatogenic activities and sperm quality that could result in reproductive toxicity, reproductive dysfunctions and infertility in male animals.*
Keywords: *Soybean, male fertility, sperm head abnormality, sperm count, conception rate.*

I. Introduction

Soybean (*Glycine max* L.) is popularly known as the "miracle beans" because; it is a cheap source of high quality protein and amino acids. *Glycine max* is the modern cultivar of soybean developed from the ancestry and traditionally consumed "wild" soybean (*Glycine soja*), found in northern and central China, adjacent areas of the former USSR, Korea, Taiwan and Japan. The modern soybean (*G. max*) contains large amounts of phytoestrogens such as soy isoflavones when compared to the ancestry "wild" soybean (*G. soja*) [1].

G. max is consumed as soybean cake, fried beans, soy milk, soy flour and soy oil, as well as in many other forms; either solely or in combination with other food products by human beings and as animal feed.

Ingestion of phytoestrogens is said to result in low semen concentrations, poor semen quality, lack of sperm motility and eventually a reduced libido [2], severe reproductive defects and infertility has also been reported by [3,4]. According to Hess [4] and Glover and Assinder [5], distortions in the fertility of male mammals is directly correlated to the distortions in spermatogenesis. Reduction in spermatogenic activities has also been reported to result in infertility, reproductive toxicity and dysfunctions [6].

Auger *et al*. [7] and Carlson *et al*. [8] have shown evidence to confirm that, there has been significant decline in sperm quality and quantity worldwide among fertile men; decreasing by fifty per cent from levels measured in the 1930's. The scientific explanation for these reductions has to do with an overall reduction in androgens [9], that occurs when there is significant increase in the levels of estrogens in the male body [9,10,11].

This study set out to further explore the effect of soybean on reproductive toxicity, reproductive endpoints and fertility of male rat as a mammalian model.

II. Materials And Methods

2.1 Treatment

Processed soybean meal was obtained from the feed mill of the Livestock Farm, University of Calabar, Calabar. It was sun-dried for two days and pulverized into the treatment for the study.

2.2 Animals

Eighty mature albino rats (20 males and 60 females) of 12 weeks old were obtained from the Animal House of Department of Zoology and Environmental Biology, University of Calabar, Calabar, Nigeria for this study. The male rats were divided into four groups with five rats per group and housed in conventional wire mesh cages under standard laboratory conditions. They were allowed free access to water and commercial feed throughout the period of the experiment. Generally, the study was conducted in accordance with the recommendation from the declarations of Helsinki on guiding principles in care and use of animals.

2.3 Experimental design and procedure

Four experimental groups of five male albino rats each with about the same body weights were constituted in a Completely Randomized Design (CRD). The rats were treated with soybean meal at 0 (control),

100, 200 and 300 mg/kg body weight respectively daily for 65 days. The soybean meal was mixed with about 10-30% of the daily feed consumption and given in the morning, to ensure the consumption of the daily treatment dose [12], before the remaining feed was given later in the afternoon.

At the end of the treatment period, the treated and the control males were made to sire the untreated female rats in the ratio of 1:3 for the fertility test. The male rats were then sacrificed under chloroform anaesthesia. The testes and epididymes were dissected out, weighed using Scout Pro SPU 601 electronic weighing balance. The testes were processed for testicular histology of testes and the caudal epididymes were processed for epididymal sperm count, viability and sperm head abnormality.

2.3.1 Fertility test

Male rats from the treated and the control groups were introduced to parous females in the ratio of 1:3 for a period of four days. The conception rate was calculated according to Ikpeme *et al*. [13,14].

2.3.2 Histology of testes

The testes were fixed in Bouin's fluid for 24 hours then processed and stained using the hematoxylin and eosin (H&E) differential staining technique [15].

2.3.3 Sperm count

The epididymal sperm samples were obtained by macerating known weights of epididymes in physiological saline in the ratio of 1:10 weight by volume. After vigorous pipetting to release the sperm cells. The suspension was filtered using an 80µm stainless mesh. Caudal epididymal sperm count was obtained by cytometry using the improved Neubauer cytometer and was expressed as million/ml of the sperm suspension [15,16].

2.3.4 Sperm viability

The sperm viability test was determined using Eosin-Nigrosin staining technique. A portion of the sperm suspension was mixed with equal volume of Eosin-Nigrosin stain and five air-dried smears were prepared on glass slides for each sample. The slides were examined for percentage viability. Normal live sperm cells excluded the stain and appeared whitish, whereas dead sperm cells took up stain and appeared pinkish. Percentage viability was calculated based on the number of live sperm cells out of the total number of sperm cells observed [17,18].

2.3.5 Sperm head abnormality test

A portion of the sperm suspension was mixed with 1% eosin Y solution (10:1) for 30 minutes and air-dried smears were prepared on glass slides for the sperm head abnormality test. The slides were examined for percentage sperm head abnormalities in every 200 spermatozoa observed on each slide and five air-dried smears were prepared on glass slides for each sample. The percentage of sperm head abnormality was calculated according to Ekaluo *et al*. [12,19].

2.4 Statistical analysis

Data from conception rate, weight of testes and epididymes, and seminal analyses were subjected to the analyses of variance (ANOVA) while differences in means were separated using least significant difference (LSD) according to Obi [20].

III. Results

General observations showed that all the rats in the study looked healthy and there was a general increase in body weights of all rats in both treatment and control groups during the treatment period. The increases in body weights of the rats indicated that soybean meal had no adverse effect on growth and body weight of the rats.

Table 1 shows that there was no significant ($P<0.05$) effect of soybean meal on the weight of testes and epididymes at the various levels of the treatment. Sperm viability and sperm count were significantly ($P<0.05$) reduced in dose-dependent manner in the treatment groups when compared with the control. Sperm head abnormality was significantly increased in a dose-dependent manner.

Histological examination of the testicular sections in Plate 1 showed rats in the control group (A); had seminiferous tubules at various stages of development. The 100mg/Kg soybean meal treated group (B); had testicular tissues with compacted interstitial spaces, mild hemorrhage along the Sertoli's cells and slight degeneration of spermatids. Rats in 200mg/Kg group (C); showed testicular tissues with slight hemorrhage along the Sertoli's cells and mild degeneration of spermatids. While the rats in 300mg/Kg group (D); showed

testicular tissues with inflammation of interstitial cells, severe hemorrhage along the Sertoli's cells, excessive degeneration of spermatids and necrosis.

The conception rate of the female rats that were sired by males in the control and other treatment groups showed reduction in a dose-dependent manner; from 100% in the control group to 50% in 300 mg/kg BW group. The conception rates were also directly proportional to the values of sperm viability and sperm count; and inversely proportional to the values of sperm head abnormality as is shown in Fig. 1.

Table 1: Effect of soybean on sperm parameters, weight of the testes and epididymes in male rats.

Parameters	Soybean meal (mg/kg BW)			
	Control (0)	100	200	300
Testes (g)	$1.12^{a} \pm 0.02$	$1.20^{a} \pm 0.06$	$1.10^{a} \pm 0.05$	$1.13^{a} \pm 0.03$
Epididymes (g)	$0.32^{a} \pm 0.07$	$0.37^{a} \pm 0.05$	$0.37^{a} \pm 0.05$	$0.30^{a} \pm 0.08$
Sperm Viability (%)	$94.25^{d} \pm 2.02$	$82.08^{c} \pm 2.26$	$77.25^{b} \pm 1.32$	$73.17^{a} \pm 1.04$
Sperm Count (x 10^6/ml)	$6.86^{d} \pm 0.05$	$5.30^{c} \pm 0.04$	$4.60^{b} \pm 0.05$	$4.06^{a} \pm 0.01$
Sperm head abnormality (%)	$1.57^{a} \pm 0.15$	$5.39^{b} \pm 0.10$	$8.84^{c} \pm 0.17$	$11.32^{d} \pm 0.20$

^{abcd} [Values across the table with similar superscript are not significantly different at 5% based on ANOVA]

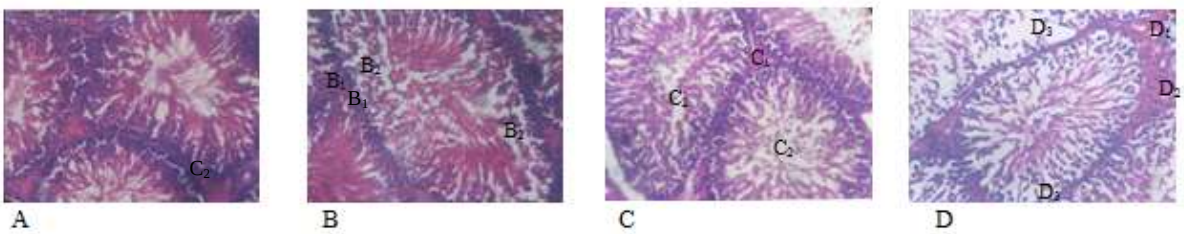

Plate 1: Effect of soybean on testicular integrity of rat. (H&E X100)

Plate 1A: Testicular section of control rat.
Plate 1B: Testicular section of rats treated with *Glycine max* at 100mg/Kg BW showing compacted interstitial spaces, mild hemorrhage along the interstitial cells (B_1) and slight degeneration of spermatids (B_2).
Plate 1C: Testicular section of rats treated with *Glycine max at* 200mg /Kg BW showing slight hemorrhage along the Sertoli's cells (C_1) and mild degeneration of spermatids (C_2).
Plate 1D: Testicular section of rats treated with *Glycine max* at 300mg /Kg BW showing inflammation of interstitial cells (D_1), severe hemorrhage along the Sertoli's cells (D_2), excessive degeneration of spermatids and necrosis (D_3).

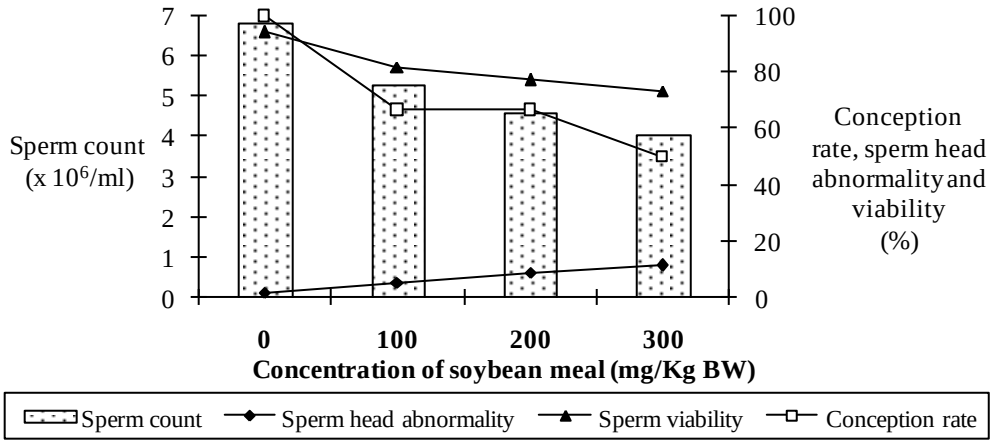

Figure 1: Relationship between effect of soybean on sperm count, conception rate, sperm viability and sperm head abnormality in rats

IV. Discussion

Soybean meal did not have any significant effect on weight of testes and epididymes which was not contrary to the earlier reports of Ikpeme *et al*. [15];Ekaluo *et al.* [18]. However, it significantly reduced sperm viability and sperm count, while increasing sperm head abnormality in a dose-dependent manner; and their relationships agreed with the reports of Ekaluo *et al*. [10,12,16,18] on sperm parameters which show reproductive toxicity on the reproductive endpoints of sperm count, sperm viability and sperm head abnormality.

Our results also revealed 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. Soybean is an endocrine disruptor in males[11] and it is rather probable that soybean may have disrupted the synergy between testosterone and follicle stimulating hormone during the process of spermatogenesis [6,11,21]. Ikpeme *et al*. [21] assertively revealed that the distortion in fertility in male mammals is directly correlated with the disruption of spermatogenesis and the hormone regulatory machineries.

The reductions in conception rate, sperm viability, sperm count and increase in sperm head abnormality agrees with World Health Organization report [22] that, sperm head abnormality correlates more closely with fertilization rather than sperm count and sperm viability. Distortions in the fertility of male mammals are directly correlated to the distortions in spermatogenesis [5,23]. Reduction in spermatogenic activities has also been reported to result in infertility, reproductive dysfunction and reproductive toxicity [6,24].

This study shows that soybean has some toxic effect on reproductive endpoints such as sperm count, sperm viability and sperm head abnormality, as well as toxicity effects to testicular integrity and conception rate. Hence the utilization of soy products should be done with caution since reduction in spermatogenic activities and sperm quality could result in reproductive toxicity, reproductive dysfunctions and infertility in male animals.

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