Effect of Roasted Soybean (*Glycine max*) diet on the Histology of Selected Rat Tissues

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Abstract

The effect of roasted soybean diet on the histology of the liver, kidney and testis of Wistar rats was examined. Roasted soybean was ground and incorporated into the normal rat diet at levels of 10%, 25% and 50%. Twenty four male Wistar rats of 100-140g body weight were equilibrated for seven days and randomly divided into four groups of six rats each, housed in separate cages and fed with control, 10%, 25% and 50% soybean incorporated diets respectively for fourteen days. The animals had free access to food and water ad libitum. No histological changes were observed in the liver and kidney of test animals fed with soybean. Testis of rats fed with 25% and 50% soybean showed increased and enhanced cellularity of the sertoli and spermatogenic cells. Seminiferous tubules showing spermatogenic cells undergoing various stages of spermatogenesis and spermiogenesis were observed. The effect was observed to be dose dependent. The results show that roasted soybean has no pathological effects on rat liver and kidney tissues, but promotes intensive proliferation of spermatogenic cells of the testis, and may therefore be beneficial to the male reproductive organ of rats.

Keywords: Roasted soybean, Rat tissue, Histology

Introduction

Soybean (*Glycine max*) is a legume which has been reported to have numerous health promoting effects; most of which are attributed to its high nutrient and isoflavone content (Messina, 1999; Soulsby *et al.*, 2004). It contains about 40% protein, 18% fat, and has a high concentration of minerals such as phosphorus, calcium and iron. It is also rich in fibre (Muller and Tobin, 1980). Soybean is rich in the isoflavones, genistein and daidzein (Kaufman *et al.*, 1997). It is used in the production of vegetable oil, soy protein, soy flours, soy grit, soy milk, soy beverages, and protein concentrates. Due to its unique nutrient composition, and functional properties, soybean is used in the production of baked goods, simulated meats, confectioneries and frozen desserts, beverages, dry cereals, pasta and macaroni, flakes and infant foods (Wolf and Cowan, 1971).

Studies have shown that people whose diet was rich in soybean protein and isoflavone, had a significant reduction in total serum cholesterol, Low Density Lipoprotein (LDL) and triglycerides (Anderson *et al.*, 1995; Dewell *et al.*, 2006; McVeigh *et al.*, 2006), and thus, lowered risk of cardiovascular diseases (Sagara *et al.*, 2004). Epidemiological studies have also suggested that soybean isoflavones are associated with a lower risk of prostate cancer and cancers of the other organs such as breast and colon (Barnes *et al.*, 1995; Yan and Spitznagel, 2005). Despite the high nutrient content of soybean, its utilization is impaired by the presence of several antinutritive and toxic compounds which exert deleterious effects in man and animals when ingested without proper heating and processing. These antinutrients include protease inhibitors, heamagglutin, goitrogenic substances, hydrogen cyanide (HCN) and ammonia. These can be detoxified by proper heat treatment (Osho, 1989; Liener, 1994). The aim of this study is to elucidate the histological changes and toxic effects, if any, in some organs of rats due to the incorporation of roasted soybean in the diet.

Materials and Methods

Animals: The animals used in this study were adult male Wistar rats with body weight of 100-140g. They were obtained from the animal house of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

Soybean seeds: The soybean seeds were purchased from the local market in Nsukka and identified by Mr. Alfred Ozioko of the Department of Botany, University of Nigeria, Nsukka. Voucher specimen was deposited in the herbarium unit of the Department of Botany.

Chemicals: All chemicals used in this study were of analytical grade and products of either May and Baker, England or BDH, England.

Experimental design: Soybean seeds were dehulled and roasted at 85°C ±10°C for 30mins, ground to a powdery form and incorporated into the normal rat chow at 10%, 25%, and 50% concentrations. Twenty four (24) adult male Wistar rats were equilibrated for 7 days and randomly divided into four groups of six rats each, housed in separate cages and fed for fourteen days. Group I served as the control and was fed with normal rat chow. Group II was fed with a diet consisting of 10% soybean and 90% normal rat chow. Group III was fed with a diet consisting of 25% soybean and 75% normal rat chow. Group IV was fed with a diet consisting of 50% soybean and 50% normal rat chow. The rats had free access to food and water *ad libitum*. The rats were weighed at the beginning (zero day) and at the end of the feeding period. The

animals were sacrificed by anesthesia with chloroform at the end of the experiment.

Histology: The organ specimens viz- liver, kidney and testis were quickly excised from the animals and fixed in 10% formaldehyde. This was allowed to stay for 3-4 days in 10 times (10x) the volume of the specimen. Processing started with the dehydration in which graded levels of ethanol, 70-100% (in ascending order) were used. The alcohols were changed after steeping the tissues in them for 1.5-2hrs. The tissues were cleared in chloroform and impregnated with paraffin wax and sectioned at 5 microns thickness using rotary microtome. The sections were floated on a water bath maintained at 2-3°C above the mid point of the paraffin wax used. When properly dried (15-30 min) they were stained with haematoxylin and eosin (H & E), dehydrated, cleared and mounted (D.C.M.) in D.P.X. mountant avoiding air bubbles.

Result and Discussion

No visual changes were observed in the visceral organs (liver and kidney) of test animals fed with 10%, 25%, 50% roasted soybean. Liver sections of control and test animals fed with soybean were normal. The central vein, hepatocytes and sinusoids were well preserved (Plates 1- 2).

Kidney sections from control and test animals fed with roasted soybean were normal and showed no histological change. Glomeruli and active renal convoluted tubules were preserved (Plates 3 – 4). Experimental report show the presence of antinutritive and toxic components such as protease inhibitors, goitrogenic substances, cyanogenic substances in raw and unprocessed soybean, which are very toxic to man and animals (Osho, 1987; Liener, 1994) Report from this study showing well preserved hepatic cells of the liver, glomerulus and renal tubules of the kidney of test animals fed with roasted soybean suggests that the antinutritive and toxic components in raw soybean were eliminated by roasting and therefore had no adverse effect on the organs.

There was increased cellularity of both the sertoli and spermatogenic cells of testis of the test animals fed with soybean (Plates 5 - 8). Seminiferous tubules showing spermatogenic cells undergoing intensive proliferative activity; various stages of spermatogenesis and spermiogenesis were observed. This effect was observed to increase with increased concentration of soybean in the diet. Spermatogenic cells of test animals fed with 10% soybean were not intensively proliferative as compared to that of test animals fed with 25% and 50% soybean. Spermatogenic cells of control were well preserved, but not actively mitotic as compared to those of the test animals. Seminiferous tubules lined by stratified epithelium consist of the spermatogenic series - spermatogenic cells in various stages of spermatogenesis and spermiogenesis, Sertoli cells which support and nourish the developing cells of the spermatogenic series, and Leydig cells found in the interstitial spaces with an endocrine function (Wheater *et al.*, 1982).

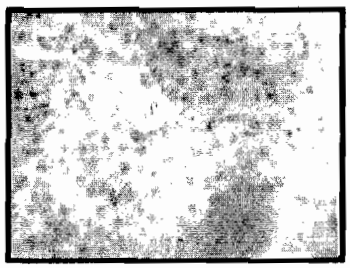

Plate 1: Liver section of rat fed with 25% soybean showing well preserved central vein and hepatic sinusoids (P). (H and E stain x 400).

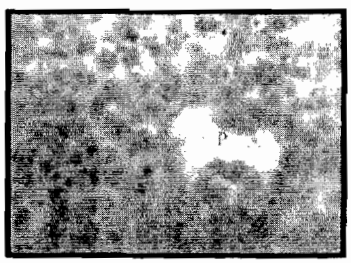

Plate 2: Liver section of rat fed with 50% soybean showing well preserved hepatic cells (P). (H and E stain x 400)

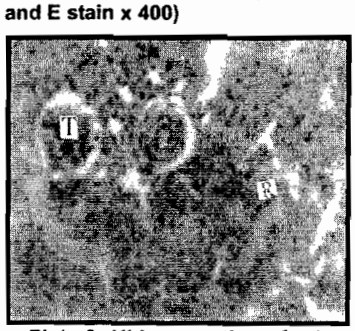

Plate 3: Kidney section of rat fed with 25% soybean showing normal glomerulus (T) and active tubular cells (R). (Hand E stain x 400)

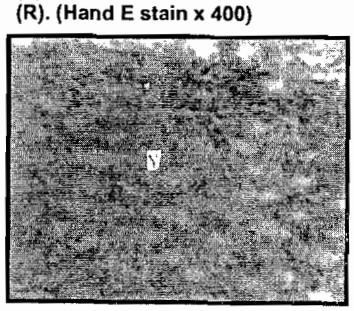

Plate 4: Kidney section of rat fed with 50 % soybean showing straight portions of nephrons and collecting tubules (Y) (H and E stain x 400)

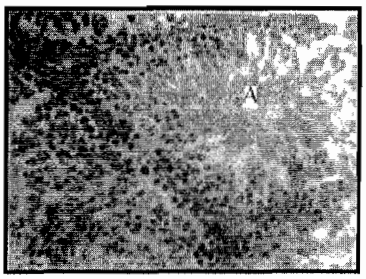

Plate 5: Testis section of rat fed with 50 % soybean showing intensive proliferative spermatogenic cells (A). (H and E stain x 400).

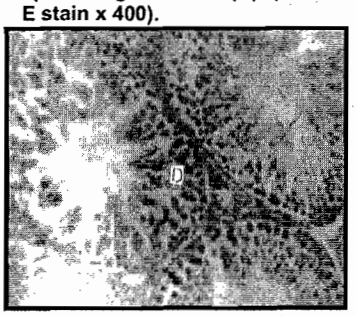

Plate 6: Testis section of rat fed with 25 % soybean showing active and enhanced spermatogenic and sertoli cells (D). (H and E stain x 400).

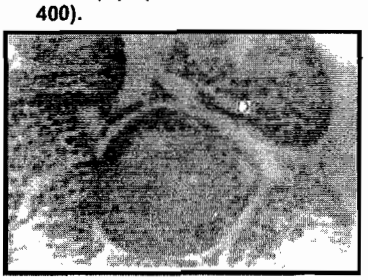

Plate 7: Testis section of rat fed with 10% soybean showing sertoli and spermatogenic cells (D), but not actively mitotic and meiotic as that of rat fed with 50% soybean. (H and E stain x 400).

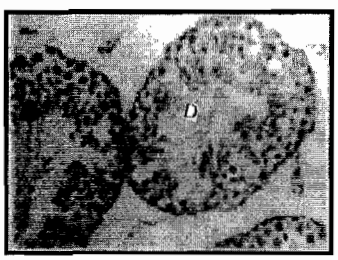

Plate 8: Testis section of control showing well preserved spermatogenic and sertoli cells (D), but not intensively proliferative as that of the test animals. (H and E stain x 400).

Seminiferous tubules of test animals fed with 25% and 50% soybean showing spermatogenic cells undergoing active mitosis and meiosis suggest that soybean is essential to, and promotes the growth of actively diving spermatogenic cells. Various reports show that isoflavones from soybean decreased the growth of human benign prostate hypertrophy (BPH) and prostate cancer tissues (Evans *et al.*, 1995; Yan and Spitznagel 2005). These reports support the finding in the present study of a beneficial and health promoting effect of soybean on the male reproductive organ of rats.

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