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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Beneficial Effects of Soybean Diet on Serum Marker Enzymes, Lipid Profile and Relative Organ Weights of Wistar Rats

C.A. Anosike, O. Obidoa and L.U.S. Ezeanyika
Department of Biochemistry, University of Nigeria, Nsukka, Nigeria

Abstract: The effect of dietary incorporation of soybean, a high isoflavone-containing legume, on serum marker enzymes, lipid profile and relative organ weights of wistar rats was studied. Male wistar rats weighing between 100-200g were divided into four groups. Group 1 was fed normal rat chow while groups 2, 3 and 4 had normal rat chow supplemented with 10, 25 and 50% soybean, respectively, for fourteen days. At the end of the feeding experiment, the rats were euthanised by chloroform anesthesia and the serum obtained were used for the biochemical assays. In all cases, the rats had free access to feed and water. The results of the study showed that high intake of soybean (25% and 50%) in the diet significantly ($P<0.05$) reduced the level of the serum enzymes; glutamate – oxaloacetate and glutamate pyruvate transaminases, alkaline and acid phosphatases and serum glucose. Supplementation of soybean at these levels also increased serum vitamin C. Insignificant decreases were found in the liver, kidney and testis relative weights, serum low density lipoprotein and lipid peroxidation products (Malondialdehyde) levels. Soybean incorporation at the 50% level significantly reduced total serum cholesterol. These findings support the reports of the beneficial and health promoting effects of soybean.

Key words: Soybean, isoflavone, rats, serum marker enzymes

Introduction

Foods with minimal processing offer enormous nutritional advantages over industrially processed foods. Factory processed food in most cases have lost the many naturally occurring components known to be health protective to animals (Meserole, 2002). There are enormous health benefits of fresh whole foods and medicinal plants in comparison to those that have been processed or stored for long periods and therefore have altered chemical and nutrient profiles. The presence of beneficial nutrients and active constituents in the diet may provide the ultimate prophylactic approach in disease risk reduction and anti-ageing medicine (Iwu, 1986).

Soybean seeds have been shown to have numerous health promoting effects mostly attributed to their high nutrient (Kaufman *et al*., 1997) and phytochemical (isoflavone) content (Messina, 1999). Soybean is not only protein rich, but also a good source of minerals like phosphorous, calcium, iron and soluble fiber. Soybean proteins complement cereal proteins to provide an ideal source of dietary protein of vegetable origin for human beings (Messina, 1999). Isoflavones present in Soybean include genistein and daidzein. Soybean proteins and isoflavones have been shown to reduce the risk of cardiovascular diseases by lowering blood pressure, blood cholesterol and triglycerides (Sagara *et al*., 2004; McVeigh *et al*., 2006). Soybean isoflavones act as anti-cancer agents (phytoestrogens), blocking the growth of hormone dependent cancers such as breast and prostate cancers (Messina *et al*., 2006; Allresd *et al*., 2004; Kurahashi *et al*., 2007). Studies have also shown that soy isoflavones in diet inhibit bone loss and increases bone mineral density in menopausal women (Ma *et al*., 2008). Soybean - rich diets reduces LDL cholesterol in the arteries, thus lowering the incidence of atherosclerosis (Wagner, 2003) and are also linked to the reduction of diabetes mellitus (Villegas *et al*., 2008). Most of the health benefit of soybean attributed to its high protein and isoflavone content are observed in people that consume large amounts of dietary soybean (Omoni and Aluko, 2005).

This study screened soybeans for isoflavone content using Thin Layer Chromatography (TLC) and studied the biochemical effects of its consumption for fourteen days in wistar rats.

Materials and Methods

Animals: Twenty four male wistar rats weighing between 100-200g, obtained from the animal house of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka were used for this work. The procedure for using the animals for the experiment was approved by the Faculty of Biological Sciences Animal Use in Scientific Experiment Committee, University of Nigeria, Nsukka. The method of euthanasia for the rats was by chloroform anaesthesia.

Plant: Soybean (*Glycine max*) were purchased from the local market at Nsukka and identified by the Department of Botany, University of Nigeria, Nsukka.

Voucher specimen of the soybean was deposited in the herbarium unit of the Department of Botany, University of Nigeria, Nsukka.

Chemicals: All chemicals used in this study were of analytical grade. They were products of May and Baker, England and Merck, Darmstadt, Germany. Reagents used for all the assays were commercial kits and products of Randox, USA; QCA, Spain, Biosystem Reagents and Instruments, Spain. Two pure samples of the isoflavoves – genistein and daidzein used were purchased from Sigma-Aldrich Chemie GMbH, Germany.

Experimental design: Soybean was screened for its isoflavone content using standard methods as in Harbone (1973) and Wandi *et al.*, 1994. Methanolic extraction of the ground /powdered soybean seeds was carried out using soxhlet extractor. Thin layer chromatography (TLC) of the methanol, chloroform and ethylacetate fractions of the extract was carried out with pure isoflavone samples – genistein and daidzein as standard markers using various solvent systems such as chloroform-acetone-methanol (5:4:1) and chloroform-benzene-methanol (4:5:1) (Harbone, 1973). Soybean was roasted for 30min at 50°C, ground into fine powder and incorporated into normal rat diet at 10, 25 and 50% concentrations (weight/ weight).

The rats used in this work were equilibrated for seven days, randomly divided into four groups of six rats each and housed in separate cages. They were fed with the soybean incorporated diet for 14 days as follows: group 1 was the control group fed with the normal rat diet. Group 2 was fed with a diet consisting of a mixture of 10% soybean and 90% normal rat diet. Group 3 was fed with a diet composed of 25% soybean and 75% normal rat diet while group 4 was fed with a diet consisting of 50% soybean and 50% normal rat diet. The proximate composition of the normal rat diet was crude protein 14.5%, fat 4.8%, fiber 7.2% and ash 8%. The animals were exposed to normal day-night cycle and had free access to food and water. They were euthanized on the fourteenth day of the experiment.

Body weight changes: The rats were weighed on the zero and fourteenth day of the experiment. The changes in the body weights were noted.

Weight of visceral organs: The rats were euthanised on the last day of the experiment following anaesthesia with chloroform and the relative weights of the liver, kidney and testis taken (as a percentage of their body weight).

Preparation of serum: Blood was collected from each of the rats by cardiac puncture, allowed to clot, and then followed by centrifugation at 3000rpm for 10mins. Clear serum was collected separately for each sample.

Assay of glutamate-oxaloacetate and glutamate-pyruvate transaminases: These were estimated calorimetrically using Randox reagent enzyme kits based on the method of Reitman and Frankel (1957). GOT and GPT substrate buffers (0.5ml) pipetted into sample blank (B)and sample test (T) test tubes were warmed for 5 minutes, and (0.1ml) serum added to sample test tube, mixed thoroughly, then incubated in a water bath for 30 minutes at 37°C. 2, 4 - dinitrophenylhydrazine (0.5ml) was added to both B and T. To B was added 0.1ml of serum. The medium was allowed to stand for 20 minutes at 25°C. Sodium hydroxide solution (NaOH) was added to both B and T and mixed thoroughly. Absorbance was read at 550nm against blank.

Assay of alkaline and acid phosphatases: These were carried out using Randox commercial enzyme kits according to the method of King and Kind (1954). Carbonate buffer and citric acid buffers were used for the alkaline and acid phosphatase respectively. The enzyme hydrolyses a colorless substrate of phenolphthalein monophosphate to give a pink color that can be photometrically determined at 520nm.

Assay of total cholesterol and low density lipoprotein (β-lipoprotein): Total cholesterol and low density lipoprotein (β-lipoprotein) were assayed according to the methods of King and Wootton (1959) and Burstein and Samaille (1958) respectively using Biosystems reagent kits.

Assay of lipid peroxidation product and Vitamin C: Lipid peroxidation products and Vitamin C were assayed using the methods of Wallin *et al.* (1993) and Baker *et al.* (1971) respectively. Lipid peroxidation was assayed as thiobarbituric acid reacting substances (TBARS). To 1 ml each of serum was added 0.05ml of 50% TCA and 0.075ml of 1.3% TBA (w/v) dissolved in 0.3% NAOH. The mixture was incubated in a water bath at 90°C for 40 minutes, allowed to cool on iced water. 20% (w/v) sodium dodecyl sulphate (SDS) (0.01ml) was added to each test tube to reduce turbidity. Absorbance at 532nm was read in a spectrophotometer against the blank.

Assay of serum fructose level: Serum fructose level was assayed with the method of Roe (1934) after deproteinization by the method of Somogyi (1945).

(a) Deproteinization of biological material:

This method is used to remove protein from biological materials such as blood or tissue extract. To serum (0.1m) pipetted into a test tube was added distilled water (1.9ml), $Ba(OH)_2$(1ml) and $ZnSO_4$ (1ml). The solution was thoroughly shaken after each addition. The resultant solution was centrifuged at 2,000rpm for 30 minutes and the clear supernatant used for assays.

(b) To deprotenised sample (0.2ml) pipetted into a test tube was added resorcinol solution (0.2ml) and conc. HCl (0.6ml). The solution was shaken thoroughly after each addition and allowed to stand in a water bath at 80°C for 8 minutes. The tube was cooled at room temperature and absorbance read at 530nm. Fructose in the presence of conc. HCl and when heated, forms furfuraldehyde, which condenses with resorcinol to give a pink colour that is proportional to the concentration of fructose in the sample.

Assay of blood glucose: The ONE-TOUCH(TM) blood glucose monitoring meter and test-strips (Life Scan Inc., Johnson – Johnson Company, Milpitas California, USA) which is based on the method of Marks and Dawson (1965) was used to monitor blood glucose level.

Statistical analysis: All values are expressed as means and standard deviations. Data were analyzed by one-way ANOVA and significant differences between groups were determined by Duncan's multiple range test and least significant difference (LSD). Statistical analyses were done using SPSS, the statistical package for Windows, version 11.0. (SPSS Inc. Chicago, IL. USA). The acceptable level of significance was $p<0.05$.

Results and Discussion

Thin layer chromatographic separation of the ethyl acetate fraction of soybean extract: Table 1 shows the thin layer chromatographic profile (with chloroform-acetone-methanol (5:4:1) as solvent system) of soybean in this study. The result shows that *Glycine max* (soybean) gave spots with the same Rf values as the pure daidzein and genistein reference standards.

Effect of soybean on mean body weight changes: The results of this study are shown in Table 2. Rats fed 10% soybean supplemented diet had significantly reduced body weight gain (g) relative to those fed control and 50% soybean supplemented diets.

Effect of soybean on relative organ weights (as percentage of body weight): The results of this study are shown in Table 2. There were no significant changes ($p>0.05$) in the relative liver and testis weights of the rats after 14 days. The rats fed the diet supplemented with 25% soybean had a significantly reduced ($p<0.05$) kidney weight relative to the control group on normal rat chow.

Effects of soybean supplementation in diets on some serum enzymes

Acid phosphatase activity: The acid phosphatase activity of the test animals fed 25% and 50% soybean supplemented diets was significantly lower ($p<0.05$) than those of the control animals. The acid phosphatase level of the test animals fed with 10% soybean showed no marked difference from the control. (Table 3).

Table 1: Thin layer chromatographic separation of the ethyl acetate fraction of the soybean extract

S/No	Legume	Spots	Hr_f
1	*Glycine max*	1	34.1
		2	38.6
		3	79.5
		4	85.2
D	Daidzein	1	79.5
G	Genistein	1	85.2

Alkaline phosphatase activity: The results of the effect of soybean supplementation on alkaline phosphatase activity (shown in Table 3) were similar to those for acid phosphatase.

Glutamate-oxaloacetate transaminase (GOT) activity: As shown in Table 3, the GOT level of test groups fed with 10%, 25% and 50% soybean supplemented diets were significantly lower ($p<0.05$) than that of the control group. The GOT level of the 50% soybean supplemented group was also significantly lower ($p<0.05$) than that of the test group fed with 10% soybean

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Glutamate-pyruvate transaminase (GPT) activity: The GPT level of the test groups fed with 10%, 25% and 50% soybean were significantly lower ($p<0.01$) than that of the control. The GPT level of test groups fed with 25% and 50% soybean were also significantly lower ($p<0.05$) than that of the test group fed with 10% soybean. These results are depicted in Table 3.

Effect of soybean supplementation in the diet on total serum cholesterol: There were no significant differences between the test and control groups except for the group fed on 50% soybean supplemented diet, which showed a significant decrease relative to the 10% soybean supplemented group. These results are presented in Table 4.

Effect of soybean on low density lipoprotein (LDL): As shown in Table 4, there were no significant differences in low density lipoprotein concentration of the test and control animals after 14 days.

Effect of soybean on vitamin C level: The results of the study on the effect of soybean supplementation on serum vitamin C concentration show that the vitamin C concentration of the test groups fed with 25% and 50% soybean for 14 days were significantly higher ($p<0.05$) than that of the control. No marked difference was observed between the vitamin C level of control and test animals fed with 10% soybean for 14 days. (Table 4).

Effect of soybean supplementation on lipid peroxidation products (MDA concn): The results of this

Table 2: Effect of soybean supplementation in the diet on mean body weight gain (g) and relative organ weights

	Treatment			
Parameter	Control	10% soybean	25% soybean	50% soybean
Body weight Gain (%)	32.33±5.51[a]	19.33±0.51[b]	26.38±3.88[a,b]	32.67±5.00[a]
Liver (% of body weight)	4.00±0.22	4.14±0.11	3.87±0.15	3.90±0.04
Kidney (% of body weight)	0.46±0.01[a]	0.42±0.06[a,b]	0.37±0.02[b]	0.40±0.02[a]
Testis (% of body weight)	0.84±0.03	0.73±0.10	0.71±0.11	0.72±0.03

N = 6. Means with different superscripts [(a, b, c)] in a row are statistically significant ($p<0.05$)

Table 3: Effect of soybean supplementation in the diet on some serum enzymes of wistar rats after 14 days

	Treatment			
Parameter	Control	10% soybean	25% soybean	50% soybean
Acid phosphatase (K.A.U/100ml)	26.23±2.14[a]	25.68±1.52[a]	18.95±1.21[b]	16.46±2.16[b]
Alkaline phosphatase (K-A.U/100ml)	72.19±2.72[a]	72.94±2.12[a]	61.01±2.27[b]	62.66±7.11[b]
Glutamate-oxaloacetate transaminase(GOT) (1U)	90.50±2.78[a]	80.00±4.77[b]	76.00±1.70[b,c]	73.83±1.89[c]
Glutamate-pyruvate transaminase(GPT) (1U)	75.83±0.76[a]	68.83±2.47[b]	64.67±0.76[c]	61.83±2.02[c]

N = 6. Means with different superscripts [(a, b, c)] in a row are statistically significant ($p<0.05$)

study which is presented in Table 4, show that the MDA concentration of the soybean supplemented groups fed with 10%, 25% and 50% soybean was non-significantly lower ($p>0.05$) than that of the control group after 14 days.

Effect of soybean supplementation on serum glucose and fructose levels: As shown in Table 4, in all cases, the glucose levels of the soybean supplemented diets were significantly lower ($p<0.05$) than those for the control. The increase in the fructose level of the 50% soybean supplemented group was not statistically significant.

Soybean was screened for isoflavone content before incorporation into the diet of the rats. It showed spots on TLC with the same Rf values as the isoflavone reference standards (daidzein and genistein). This result agrees with the report of the high isoflavone content in soybean (Kaufman *et al.*, 1997). Soybean has been credited with numerous health promoting effects. Most of these health benefits are attributed to its high isoflavone content (Omoni and Aluko, 2005).

We are unable to explain the decrease in the body weight gain of the 10% soybean supplemented group. There was no significant change in the relative organ weights of rats fed with soybean supplemented diets as compared to control, except for the relative kidney weight of rats fed with 25% soybean supplemented diet which decreased compared to control. This suggests that soybean incorporation in the diet had no adverse effect on the organs. Earlier study on the histology of the liver and kidney of rats fed with soybean supplemented diet showed normal and well preserved sections of the organs (Anosike *et al.*, 2007).

Acid phosphatase is abundant in prostate and seminal fluid. It also occurs significantly in the red cells, liver, spleen, kidney and bone (Modder, 1973). In this study, there was a significant reduction ($p<0.05$) in the acid phosphatase level of test groups fed with 25% and 50% soybean supplementation relative to the control group. Serum acid phosphatase determinations are important in both detection and following the progress of metastatic cancer of the prostate (Lam *et al.*, 1973). Enhanced reduction in the acid phosphatase level observed with increased supplementation of soybean in the diet suggests that increased soybean consumption may be reducing or suppressing the release of the enzyme from the prostate into the bloodstream and thus may lower the risk of prostate cancer. Thus, soybean incorporation in the diet may enhance prostate function. Histological studies on the testis of rats fed with soybean incorporated diet showed increased cellularity of the sertoli cells and spermatogenic cells undergoing intensive proliferative activity (Anosike *et al.*, 2007), thus supporting the report of the beneficial effect of soybean on the male reproductive system. Soy isoflavones have also been reported to reduce the risk of prostate cancer by acting as anti-cancer agents blocking the growth of hormone dependent cancers such as prostate cancer (Kurahashi *et al.*, 2007; Heald *et al.*, 2007).

Alkaline phosphatase activity of the 25% and 50% soybean supplemented diets were significantly decreased ($p<0.05$) compared to control. Either inanition or a high protein diet may cause the serum alkaline phosphatase to fall into the lower range of normal (Eastham, 1985). The observed decrease in serum alkaline phosphatase may be ascribed to the protein content of the high soy diets. A dose or concentration-dependent decrease in the levels of GOT and GPT were observed with increased soybean in the diets of the experimental rats. This perhaps suggests that soybean confers protection on the liver tissues against injury, damage or disease, which are often the direct cause of elevation of the enzymes in the bloodstream (Sanjiv, 2002).

Results from this study showed an insignificant

Table 4: Effect of soybean supplementation in the diet on some serum biochemical parameters of wistar rats after 14 days

	Treatment			
Parameter	Control	10% soybean	25% soybean	50% soybean
Cholesterol (mg/100ml)	403.75±41.12[a,b]	546.25±85.63[a]	407.71±27.42[a,b]	85.97±112.83[b]
Low density lipoprotein (mg/100ml)	174.93±13.87	186.07±15.84	172.13±8.50	165.26±15.80
Vitamin C level (mg/100ml)	1.97±0.15[a]	2.12±0.30[a]	2.49±0.40[b]	2.86±0.24[b]
Glucose level (mg/100ml)	250.67±13.65[a]	208.67±3.21[b]	136.33±6.51[c]	142.00±51.03[c]
Lipid peroxidation products (MDA)(mg/100ml)	0.20±0.07	0.16±0.07	0.16±0.04	0.18±0.08
Fructose level (mg/100ml)	106.67±30.55	93.33±23.09	106.67±46.19	113.33±30.55

N = 6. Means with different superscripts [(a, b, c)] in a row are statistically significant ($p<0.05$)

decrease ($p>0.05$) in total serum cholesterol and low density lipoprotein (LDL) of the test groups fed with 25% and 50% soybean compared to the control. The levels of total serum cholesterol and LDL decreased with increase in the concentration of soybean in the diet of the test animals. Earlier reports (McVeigh *et al.*, 2006) showed that people whose diet was rich in soybean protein had a significant reduction in total serum cholesterol and LDL. Low serum cholesterol and triacylglycerols reduce the risk of cardiovascular diseases and are among the health benefits attributed to soybean (Sagara *et al.*, 2004).

Lipid peroxidation products (as malondialdehyde concentration) of the test groups fed soybean supplemented diet showed a non-significant decrease. The high vitamin C level in all the test animals relative to the control supports this. The level of this antioxidant vitamin in the serum increased with increase in percentage concentration of soybean. There is an inverse relationship between serum levels of vitamin C and LDL. Vitamin C synergistically enhances the antioxidant potential of isoflavones in LDL oxidation (Miller *et al.*, 1998). Mezzetti *et al.* (2000) reported that lipid peroxidation, a consequence of free radical oxidation of LDL, protein and DNA, results in the formation of unstable hydroperoxides, which break down to malondialdehyde, leading to cellular injury and damage. Soybean isoflavones - genistein and diadzein, possess antioxidant properties that help combat cell damage by free radicals (Wei *et al.*, 1995).

A reduction in the serum glucose levels of the groups fed with soybean-supplemented diets was observed in this work. This may imply that soybean could reduce the incidence of hyperglycemia. Soluble fibers from soybean resist digestion and absorption and are thus used to regulate glucose absorption and elevation in diabetes (Villegas *et al.*, 2008).

The apparent increase in the fructose level of the 50% soybean fed rats is supportive of the effect of soybean in the prostate. Serum fructose is a biochemical marker of normal seminal vesicle function. Fructose in the serum originates from the seminal vesicle and provides energy for sperm motility. Its absence suggests obstruction of the vas deferens or the absence of seminal vesicle (Malpani, 2000). The increase in fructose level observed at 50% soybean concentration in this study suggests that soybean may induce increased fructose level in serum and thus may increase the energy needed for sperm motility.

The results of this work suggest that high intake of soybean may confer significant advantages on the health of the animal model used.

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