Effects Of Soy Protein On Serum Levels Of Cardiovascular Disease Diagnostic Enzymes In Cholesterol-Fed Rats

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Citation

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Abstract

The effect of soy protein on the activity of Serum Lactate dehydrogenase (LD), Alanine aminotransaminase (ALT), Aspartate aminotransaminase (AST) and Gamma-glutamyl transpeptidase (-GT) in rats fed cholesterol-diet was investigated. Rats were subjected to feeding over a period of six weeks on formulated diets containing: 20% soy protein with no cholesterol (group A); 20% soy protein with 5% cholesterol (group B); 20% soy protein with 10% cholesterol(group C): 0% soy protein with 20% cholesterol (group D); and 5% soy protein with 20% cholesterol (group E). The serum levels of these enzymes were determined weekly for the six weeks treatment period. LDH, ALT, AST and -GT activities were observed to be significantly elevated ($p < 0.01$) in groups D and E compared to groups B and C though the enzymes activities in group B and C were significantly higher ($p < 0.05$) when compared with the control. The activities of the enzymes were highest in group D. It is considered that consumption of soy protein-rich diets as opposed to those high in animal protein may help reduce oxidative damage to tissues (such as heart, liver, and kidney) and hence reduce cardiovascular disease risk due to the presence of soy isoflavones and its hypolipaemic attributes.

INTRODUCTION

As a legume, soy is a plant protein rich in soluble and insoluble fibre. Soy has a healthier mixture of fats than animal protein [low in saturated fat, contains omega-3-fatty acids (8%) and monounsaturated fatty acids (25%)] Soy is also phytochemically rich in isoflavones.[1]

There is a large body of literature supporting claims that soy protein is an effective cholesterol lowering agent [1,2,3,4]. Studies have shown that consumption of products containing soy protein reduced blood total cholesterol, LDL-cholesterol,VLDL-cholesterol and triglyceride concentrations. [1,2,3,4]. Potter[3] and Puska et al[5] have demonstrated the hypocholesterolemic effect of soy protein in experimental animals and humans. A significant factor underlying the high continuing incidence of coronary heart disease (CHD) is a typical diet high in saturated fat and cholesterol both of which contribute to elevated serum cholesterol.[6] Elevated blood total cholesterol concentrations and other lipid abnormalities are part of a number of risk factors identified for cardiovascular diseases (CVD) [7]. Cardiovascular disease is the dominant single cause of premature mortality in the world. [8] The effect of dietary changes on serum lipid levels differ significantly between individuals and species [9]. Humans and animals however show a certain consistency in the response of their serum lipids to fat-modified diets [9]. The difference in response may be caused by variation in genes regulating serum lipid levels [10].

Lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyltranspeptidase (-GT) in addition to cholesterol and triglyceride are demonstrated to be associated with cardiovascular risk factors [11,12,13]. The role of -GT in replenishing intracellular glutathione, and possibly in controlling apoptosis and proliferation in atheromatous plaques [14,15] add more credence to its significance.

In Nigeria (and in most under-developed nations of the world) most people subsist on diets high in saturated fatty acids because the prevailing economic hardships leave them with no choice. This observation has been of great concern and even more of concern is the increased rate of sudden deaths arising from CHD [8]. In the light of this, the authors

are interested in examining the effects of soy protein in diets containing different proportions of cholesterol on the activity of some serum enzymes used in the diagnosis of CHD.

MATERIALS AND METHODS

Materials: All chemicals and reagents used were of analytical grade and are all products of BDH chemicals Ltd, Pool, England.

Soy protein: Matured soy beans (uncooked) was purchased from Iwaro market, Oka Akoko Ondo State, Nigeria and was identified as Glycine maximus (soy bean) by a taxonomist in the Department of Crop Science, Faculty of Agriculture, University of Benin, Nigeria. This was ground into powder and used in diet formulation. Some of the soy protein was dropped at the herbarium of the faculty.

Animals and diets: Thirty five (35) twelve-weeks old albino rats (Wister strain) weighing between 60-70g, purchased from the animal house, Department of Biochemistry, University of Ilorin, Nigeria were used for the study .The rats were housed in stainless steel cages with raised wire floors at a temperature of about 30° C and fed on rat chow and water ad libitum for a period of two weeks to acclimatize. Rats were then divided into five groups of seven animals each designated: A (control), B, C, D and E (experimentals) were then placed on five different dietary regimens as shown in Table 1. The composition of diet fed each group is shown in Table 1. Before the commencement of feeding, the animals were fasted overnight but allowed access to water ad libitum. One rat from each group was sacrificed on day zero and its serum collected to determine the baseline level of the test parameters studied.

Figure 1

Table 1: Feeding regimen for each group

Feed composition\Groups	A	B	C	D	E
Maize Flour	70	65	60	70	65
Fish meal	10	10	10	10	10
Soy protein	20	20	20	-	5
Cholesterol	-	5	10	20	20
Total	100	100	100	100	100
Calorie equivalent	450	475	500	510	530

Serum preparation: At weekly intervals one rat from each group was sacrificed and 2ml of blood was collected from the animal by cardiac puncture. The blood was allowed to stand at room temperature to clot and centrifuged at 10,000g for 5minutes using Hettich (universal II) centrifuge to separate serum from the cells. The supernatant (serum) was carefully decanted and analyzed immediately.

Assays: The activity of lactate dehydrogenase (LDH) was measured using the method of Kubowitz and Otti[16]. Alanine aminotransaminase (ALT) and aspartate minotransaminase (AST) activities were determined using the methods described by Reitman and Frankel [17]. The gamma glutamyl transpeptidase (-GT) activity was measured according to the method described by Szaz [18]. Protein concentration was determined by the Biuret reaction as described by Gornall et al. [19]

Statistical Analysis: Statistical analysis was by one way analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT).

RESULTS

The animals consumed their daily rations satisfactorily and showed increase in body weight. The percentage increase in body weight observed for the respective groups over the feeding period is shown in table 2.

Figure 2

Table 2: % change in mean body weights

Time on diet(Weeks)	GROUPS				
	A	B	C	D	E
0	0	0	0	0	0
1.	6.58	4.13	6.90	8.68	9.00
2.	8.38	14.57	11.59	13.15	9.06
3.	18.77	19.18	22.90	19.78	19.66
4	25.33	20.54	23.82	35.75	30.80
5.	19.29	13.89	9.54	5.56	6.19
6.	14.58	14.41	12.39	11.90	6.25

Figure 3

Table 3: Serum LDH activity (U/L)

Time on diet (weeks)	GROUPS				
	A	B	C	D	E
0	217± 5 ^{a}	222±3^a	219±4^a	220±4^a	217±6^a
1	250±3^a	242±4^b	237±4^b	300±11^c	294±8^c
2	268±6^a	270±6^a	262±5^a	382±9^b	362±13^c
3	291±5^a	281±7^b	282±4^b	446±12^c	488±11^d
4	300±8^a	297±6^a	300±5^a	609±19^b	517±14^c
5	312±6^a	314±5^a	319±3^a	714±14^b	589±10^c
6	330±3^a	328±3^a	330±4^a	807±13^b	677±14^c

Tabulated results are means of five determinations ± SEM. Values in the same row carrying different superscripts are significantly different ($p<0.05$; $p<0.01$)

Table 3 shows the weekly changes in the activity of LDH from the respective groups. Our results indicate that the

animals in groups D and E had about 2.5 and 2.0 folds increase respectively in their enzyme activities at the end of the feeding trial when compared with the control. These differences were statistically significant. On the contrary, the LDH activity in groups B and C did not differ significantly from that of the control. (p>0.05)

Figure 4

Table 4: Changes in Serum ALT activity (U/L)

Time on diet (weeks)	GROUPS				
	A	B	C	D	E
0	3.2 ± 0.6 ^{a}	4.1±0.8^{a}	4.4±1.0^{a}	3.8±0.6^{a}	4.6±0.9^{a}
1	3.9±0.2^{a}	6.0±1.2^{bc}	5.1±0.6^{b}	5.4±0.2^{b}	7.4±0.6^{c}
2	4.7±0.4^{a}	6.7±0.5^{bc}	6.0±0.4^{b}	7.3±0.1^{c}	9.8±0.4^{d}
3	5.6±0.6^{a}	7.8±0.4^{b}	7.4±0.2^{b}	10.1±0.6^{c}	13.9±0.8^{d}
4	6.4±0.3^{a}	8.9±0.5^{b}	8.6±0.1^{b}	15.7±1.1^{c}	18.4±0.9^{d}
5	7.1±0.4^{a}	10.0±0.8^{b}	9.9±0.2^{b}	27.0±1.1^{c}	29.6±2.2^{c}
6	8.4±0.3^{a}	11.2±0.6^{b}	11.6±0.4^{b}	36.8±1.2^{c}	37.0±0.8^{c}

Tabulated results are means of five determinations ± SEM. Values in the same row carrying different superscripts are significantly different (p<0.05;p<0.01)

Table 4 shows the serum levels of ALT for the respective groups over the six weeks period. Rats in groups B,C,D and E showed elevated levels of ALT activity when compared with the control (group A).These increases were statististically significant (p < 0.01) and were greater in groups D and E (p<0.01) than in groups B and C.(p<0.05)

Figure 5

Table 5: Changes in Serum AST activity (U/L)

Time on diet (weeks)	GROUPS				
	A	B	C	D	E
0	4.0 ± 0.5 ^{a}	3.7±0.4^{a}	4.1±0.3^{a}	5.0±1.2^{a}	4.2±0.3^{a}
1	4.8±0.1^{a}	4.8±0.2^{a}	6.1±0.4^{a}	7.1±0.6^{b}	6.9±1.1^{b}
2	5.6±0.2^{a}	6.0±0.3^{a}	7.2±0.6^{b}	9.0±1.0^{c}	8.4±0.1^{c}
3	5.8±0.1^{a}	6.9±0.1^{b}	8.1±0.2^{c}	14.9±0.2^{d}	15.8±0.3^{e}
4	6.7±0.2^{a}	7.8±0.3^{b}	9.0±0.1^{c}	22.9±0.5^{d}	21.6±0.4^{e}
5	7.8±0.4^{a}	9.4±0.9^{b}	10.6±0.6^{b}	27.8±1.5^{c}	26.8±1.0^{c}
6	9.8±0.2^{a}	10.2±0.5^{ab}	11.4±0.9^{b}	31.3±3.1^{c}	27.9±0.7^{c}

Tabulated results are means of five determinations ± SEM. Values in the same row carrying different superscripts are significantly different (p<0.05;p<0.01).

The activity of serum AST is as shown in Table 5. The results obtained showed that rats in groups B, C, D and E showed significantly higher (p < 0.01) serum AST levels when compared the control (group A) The enzyme activities in groups D and E were however significantly raised than those in groups Band C at the end of the feeding period.

Figure 6

Table 6: Changes in Serum -GT activity (U/L)

Time on diet (weeks)	GROUPS				
	A	B	C	D	E
0	17.0 ±1.0^{a}	21.0±3.2^{a}	18.0±3.8^{a}	20.0±2.1^{a}	19.0±2.6^{a}
1	19.0±2.1^{a}	24.0±19^{b}	22.0±2.1^{ab}	23.0±3.1^{ab}	24.0±1.8^{b}
2	18.0±0.9^{a}	23.0±1.2^{b}	22.0±1.4^{b}	31.0±3.0^{c}	30.0±1.1^{c}
3	21.0±1.1^{a}	25.0±0.9^{b}	26.0±1.4^{b}	47.0±2.8^{c}	44.0±1.8^{c}
4	24.0±1.6^{a}	27.0±1.5^{b}	27.0±0.8^{b}	54.0±4.2^{c}	56.0±0.6^{c}
5	27.0±0.9^{a}	31.0±1.0^{b}	29.0±1.6^{b}	68.0±3.8^{c}	62.0±1.8^{c}
6	28.0±1.1^{a}	33.0±1.0^{b}	31.0±0.9^{b}	72.0±4.2^{c}	67.0±3.2^{c}

Tabulated results are means of five determinations ± SEM. Values in the same row carrying different superscripts are significantly different (p<0.05;p<0.01).

The weekly changes in serum -GT activity for the respective groups are presented in Table 6. The results obtained showed that serum -GT activities in groups B, C, D and E were significantly elevated (p < 0.05) when compared with the control.

DISCUSSION

Tissue enzyme activity as well as cholesterol and triglyceride concentrations in different animals species have been extensively investigated by several workers [20, 21]. However, limited data on the effect of soy protein on serum levels of these enzymes are available. Lactate dehydrogenase (LDH) is less specific than AST and ALT as a marker of hepatocyte injury. However, it is noteworthy that LDH is disproportionately elevated after an ischemic liver injury [22]. AST and ALT values are higher in obese patients, probably because these persons commonly have fatty livers[23]. LDH is an intracellular enzyme found particularly in the kidney, heart, liver, lungs and skeletal muscle. Increased serum level of LDH is usually found in cellular death and/or leakage from cells or in some cases it is a useful marker of myocardial or pulmonary infarction. Although -GT is considered to be an index of hepatobilliary dysfunction and alcohol abuse[11], recent epidemiological and pathological studies have suggested its independent role in the pathogenesis and clinical evolution of cardiovascular diseases brought on by atherosclerosis[11, 24]. A Seventeen (17) years study of 163944 Australian adults by Ruthaman et al shows that -GT is independently associated with cardiovascular mortality[25]. Serum -GT had a prognostic impact on fatal events of chronic forms of coronary heart disease, congestive heart failure, and ischemic or hemorrhagic stroke.

Although all the four enzyme (LDH, ALT, AST, and -GT) investigated in this study are associated with cardiovascular

risk.factors[26],[27], the role of -GT in replenishing intracellular glutathione, and possibly in controlling apoptosis and proliferation of antheromatous plaques,[14],[15] may give it added significance.Because it is possible that -GT plays a role in the proliferation of atheromatous plaques, some of the circulating -GT may come from such plaques.

The results of our present study shows that consumption of soy protein leads to reduction to baseline in the serum levels of lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotranferase (AST) and gamma-glutamyl transpetidase (-GT). This is observed in the significantly ($p < 0.01$) low serum levels of these enzymes in groups A, B, and C (contaning20% soy protein) when compared with group D (without soy protein) and group E (with only 5% soy protein). Even the 5% soy protein in group E diet still produced a non significant ($p > 0.05$) reduction in the activity of the enzymes.

In addition to its hypocholesterolemic properties, soy protein as earlier suggested may reduce kidney and liver damage by a second mechanism involving soy isoflavones. Isolated soy protein provides approximately 2mg isoflavones /g. The main isoflavones present in soy protein, genistein and daidzein, may reduced glomerular damage during nephrosis by protecting LDL particles from oxidation[28], although theitr antioxidant capacity is limited[29]. Also, isoflavones can react with reactive oxygen species.

Amando et al [30] from a study on the effect of soy protein diet on the development of fatty liver associated with diabetic using Zucker diabetic rats that develop hyperinsulinemia and hepatic steatosis observed that soy protein prevented the accumulation of triglyceride and cholesterol in the liver despite the development of obesity and hyperinsulinemia in the rats[30]. This effect, they observed were due to a low expression of genes involved in the synthesis of fatty acids and triglyceride in the liver. In addition, they also found that the levels of a transcriptional factor involve in controlling genes involved in fatty acid breakdown, as well as its target genes were increased in rats fed soy protein[30].Thus , soy protein not only reduce the amount of fatty acids in the liver by reducing its production but also by increasing its breakdown.

Soy protein has additional advantages over animal protein. As little as 25g of soy protein is all that is required to reduce cholesterol in hypercholesterolemic subjects[31] .Thus ,soy protein represents a safe ,viable and practical non-phamacologic approach to lowering serum cholesterol.

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