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CHOLESTEROL-LOWERING EFFECT OF SOY BEAN PRODUCTS ON HYPERCHOLESTEROLEMIC RATS

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ABSTRACT

This investigation was carried out to study the effect of soy bean products as a hypocholesterolemic agent. For this purpose an experiment was carried out by using 60 rats which were divided into 10 groups (6 rats for each group). The daily gain in body weight, food intake and food efficiency were determined. It was found that the addition of 10% soy bean to hypercholesterolemic diet decreased total lipids, total cholesterol and triglycerides by 3.75%, 341.1% and 164.2% respectively as compared with the hypercholesterolemic rats. The other soy bean diets also significantly decreased all these parameters but in different degrees. HDL-cholesterol, LDL-cholesterol and the ratio of total cholesterol to HDL-cholesterol were also calculated in rat serum. The ratio of total cholesterol/HDL-cholesterol was decreased in hypercholesterolemic rats by giving soy bean diets that proved the beneficial effect of this seeds as hypocholesterolemic agent.

INTRODUCTION

There is increasing evidence that consumption of soy protein and isoflavones in place of casein lowers low-density lipoprotein (LDL) (Sirtori *et al.*, 1995 and Nagata *et al.*, 1998) cholesterol and may provide other cardiovascular benefits such as antioxidant properties. (Kapiotis *et al.*, 1997 and Exner 2001) However, even after a meta-analysis of 38 controlled clinical studies suggesting its potential role in the reduction of total and LDL cholesterol (Anderson *et al.*, 1995), no recommendation was made to include soy protein in the American Heart Association dietary guidelines (Krauss *et al.*, 1996 and Erdman, 2000).

Soy protein is an edible component of soybeans and contains all the essential amino acids in sufficient amounts to be considered as a complete protein. (Bricarello *et al.*, 1999 and Houston, 1996) In addition to protein, other components occur naturally, such as isoflavones, saponins, and fibers.

Many attempts have been made to elucidate the mechanisms involved in the multiple beneficial effects when following soy diets. These studies have suggested an inhibition of cholesterol absorption at the small intestine by soy protein (Huff and Carroll, 1980).

A reduced rate of saponins-mediated bile salt absorption, and an antioxidant effect on lipids associated with soy protein with or without isoflavones (Kapiotis *et al.*, 1997 and Damasceno *et al.*, 2000) as well as the higher increase of high-density lipoprotein (HDL) cholesterol serum levels appears to be associated with the presence of isoflavones in the soy protein. (Anthony *et al.*, 1998) In addition, an activation of peroxisome proliferator-activated receptors (PPARs) was recently reported, which may explain these interesting effects of soy protein on lipid metabolism (Mezei *et al.*, 2003).

Nagata *et al.,* (1998) studied a hypercholesterolemic effect of soy protein, a substantial increase in HDL cholesterol and a reduced lipid peroxidation. In fact there were different results with the soy protein diet, which can be attributed to variations in soy components such as the protein content and the amount of isoflavones, (Anthony *et al.,* 1998) saponin, (Sidhu and Oakenfull, 1986) and fiber (Potter *et al.,* 1993 and Potter, 1995). Interestingly, on the basis of the meta-analysis reported by Anderson *et al.,* 1995, hypercholesterolemic patients appeared to have the highest benefit after the use of soy protein. However, this finding was not confirmed by another study (Wong *et al.,* 1998).

After comparing the effects of soy milk with those of cow milk in severe hypercholesterolemic patients, Sirtori *et al.,* 1999 found a significant reduction in total and LDL cholesterol levels, thus confirming the benefits of soy milk even in patients with substantially elevated plasma cholesterol and only partly replacing the animal protein in the diet.

These abovementioned researches suggest a potential role of soy milk and its derivatives in the prevention of cardiovascular disease.

Soy protein consumption has been shown significantly decrease in serum concentrations of total and LDL cholesterol and triacylglycerids (Vigna *et al.,* 2000 and Scheiber *et al.,* 2001). Many components associated with soy protein, eg, isoflavones (Jenkins et al., 2002), saponins (Oakenfull, Sidhu, 1990), and β-conglycinin (7Sglobulin), are reported to have a lipid-lowering effect (Adams *et al.,* 2004).

The aim of the present work was to evaluate the hypocholesterolemic effects of soy bean products (soy flour, soy bean, soy tofu and soy milk) on the hypercholesterolemic rats.

MATERIALS AND METHODS

1-Soy bean products

Soy bean seeds and other products where obtained from Food Technology Research Institute (FTRI), Agriculture Research Center (ARC).

2-Biological experiment

Sixty male albino rats (100-110g) were obtained from the National Research Center, Dokki, Giza. Animals were housed in individual cages with screen bottoms and fed on basal diet (60% starch, 20% casein, 10% corn oil, 5% cellulose, 4% salt mixtures and 1% vitamin mixtures) for eight days (6 for each). The first group was fed on the basal diet for 4 weeks and was considered as a positive control group. The second group was fed on hypercholesterolemic diet (H-diet) for 4 weeks (basal diet supplemented with 1% cholesterol + 0.5% bile salts + 15% sheep tail fat). It was the control of hypercholesterolemia (negative control). The third group was fed on H-diet, containing 10% soy flour. The fourth group was fed on H_1-diet, containing 5% soy flour. The fifth group was fed on H_2-diet, containing 10% soy bean. The sixth group was fed on H_3-diet, containing 5% soy bean. The seventh group was fed on H_4-diet, containing 10% soy tofu. The eighth group was fed on H_5-diet, containing 5% soy tofu. The ninth group was fed on H_6-diet, containing

10% soy milk. The tenth group was fed on H_7-diet, containing 5% soy milk. During the whole experiments, rats were kept separately in well aerated cages, diet and water were supplied *ad libitum*. Each rat was weighed every two days and the food intake was also recorded. At the end of experiment, rats were fasted for 18 h and killed by decapitation, and the blood of each rat was collected in tubes and centrifuged at 3000 rpm for 10 minutes to obtain the serum. Serum sample was stored at -20 °C until analysis.

3-Biochemical analysis

Total lipids in serum were determined according to Kinght *et al.*, (1972). Cholesterol and triglycerides were estimated by standard methods according to Allain *et al.*, (1974) and Fossati and Prencipe (1982), respectively. Serum HDL-cholesterol was determined in the supernatant after treatment with a mixture of phosphotungstic acid and magnesium chloride (Lopes – Virella *et al.*, 1977). Serum LDL-cholesterol was determined by the method of Steinberg (1981).

4-Statistical analysis

Statistical analysis was carried out according to Fisher (1970). LSD (Least squares difference) test was used to compare the significant differences between means of treatment (Waller and Duncan, 1969).

RESULTS AND DISCUSSION

1-Food intake

Food intakes of rats fed normal and hypercholesterolemic diet supplemented or not with soy bean diets are shown in Table (1). Food intake of rats fed hypercholesterolemic diet was significantly lower than that of positive control. Results concerning food intake supported the hypothesis that the digestion of food constituents may be inhibited by addition of bile salts (Aoyama *et al.*, 1975 and Hamama *et al.*, 1988).

2-Body weight gain and food efficiency

Rats fed on a normal diet (Table 1) showed the highest daily gain in body weight (2.3g), while rats fed on hypercholesterolemic diet (Negative group) showed a significantly lower daily gain in body weight (1.59g)

Addition of soy bean products to the hypercholesterolemic diet significantly increased body weight gain from 21.73g in negative control to 44.52, 36.95, 33.13 and 27.17g by addition 10% soy flour, 10% soy bean, 10% soy tofu and 10% soy milk respectively. Also, the daily in body weight was increased in the same period from 0.78g (negative control) to 1.59g (by addition 10% soy flour) which is in close value to that of positive control (2.3g).

As it is well known that the hypercholesterolemic diets disturb the metabolic process in animal organism, which led to decrease gain in body weight and food efficiency (Esmaiel *et al.*, 1993).

In the present experiments, the diets containing soy flour, soy bean or soy tofu stimulated both body weight gains and food efficiency.

It is worthy to mention that food efficiency is strongly correlated with daily gain of body weight and food consumption as cited by Squibb (1959), who calculated it according to the equation:

Food efficiency = gain in body weight /food intake.

Food efficiency also followed the same trend where increased from 0.44g (negative control) to 0.64g (by addition 10% soy flour) which is a value close to that of positive control 0.74g. It is clear that the addition of soy flour to the hypercholesterolemic diet has a beneficial effect on daily gain of body weight and food efficiency as compared with the negative control.

The present results concerning food efficiency are in agreement with those reported by Kahlon *et al.*, (1993) who found that food efficiency was affected by diet containing hypercholesterolemic agents.

Table (1): Body weight gain (g) and food efficiency on rats fed hypercholesterolemic diet supplemented with Soy Bean Products

Treatment	Daily food intake	Initial body weight(g)	Final body weight(g)	Body weight gain(g)	Daily body weight gain(g)	Food efficiency values
Untreated group (Positive control)	87.45^{a} ±0.038	101.39^{a} ±1.325	165.83^{b} ±1.22	64.44^{a} ±2.538	2.3^{a} ±0.09	0.74^{a} ±0.032
Hypercholesterolemic group (Negative control)	49.72^{i} ±0.04	100.14^{e} ±1.193	113.07^{i} ±1.106	21.73^{ij} ±2.29	0.78^{ij} ±0.08	0.44^{f} ±0.05
Soy flour 10%	69.33^{b} ±0.056	126.83^{a} ±0.929	171.36^{a} ±1.179	44.52^{b} ±1.705	1.59^{b} ±0.06	0.64^{b} ±0.025
Soy flour 5%	67.63^{c} ±0.168	121.25^{b} ±1.117	162.29^{c} ±0.836	41.04^{c} ±1.951	1.47^{c} ±0.07	0.605^{bc} ±0.031
Soy bean 10%	66.18^{d} ±0.075	119.03^{b} ±0.451	156.15^{d} ±0.312	36.95^{d} ±0.477	1.32^{d} ±0.02	0.56^{cd} ±0.01
Soy bean 5%	65.55^{e} ±2.3	114.54^{c} ±0.0656	148.15^{e} ±1.141	33.61^{e} ±1.785	1.2^{e} ±0.07	0.55^{cd} ±0.025
Soy tofu 10%	61.62^{f} ±0.02	114.08^{c} ±0.661	147.21^{e} ±1.584	33.13^{e} ±0.141	1.18^{f} ±0.2	0.52^{de} 0
Soy tofu 5%	61.1^{g} ±0.02	111.77^{cd} ±1.029	142.05^{f} ±0.979	30.34^{f} ±0.834	1.087^{f} ±0.03	0.52^{de} ±0.01
Soy milk 10%	60.04^{h} ±0.036	109^{d} ±1.054	136.17^{g} ±1.815	27.17^{g} ±1.861	0.97^{g} ±0.07	0.51^{de} ±0.03
Soy milk 5%	58.37^{i} ±0.047	104.34^{e} ±1.064	127.89^{h} ±0.865	23.55^{h} ±1.581	0.83^{h} ±0.08	0.48^{ef} ±0.025

LSD (p<0.01)

a, b, c, d and e the means different superscript in each column are significantly different at (p<0.01). Each value represents the mean of 6 rats ± S.E.

3-Total lipids, triglycerides and total cholesterol

Total lipids, triglycerides and total cholesterol were determined in the rat serum to evaluate the role of soy bean diets on lipid metabolism. The results are shown in Tables (2&3).

Table (2): Effect of soya bean products on total lipids, triglycerides by feeding rats for 4 weeks

Treatment	Total lipids		Triglycerides	
	mg/dL	%	mg/dL	%
Untreated group (Positive control)	470.74^{f} ± 6.62	100	73.92^{d} ± 0.678	100
Hypercholesterolemic Group (Negative group)	630.59^{a} ± 6.564	133.95	252.4^{a} ± 1	341.45
Soy Flour 10%	510.72^{f} ± 6.528	108.49	83.87^{cd} ± 0.874	113.46
Soy Flour 5%	531.51^{e} ± 6.606	112.9	97.33^{cd} ± 1.45	131.67
Soy Bean 10%	518.79^{ef} ± 6.638	110.2	86.2^{cd} ± 6.169	116.6
Soy Bean 5%	543.3^{f} ± 5.49	115.4	114.5^{bcd} ± 10.16	190.98
Soy Tofu 10%	560^{c} ± 5.81	119.57	141.17^{bc} ± 0.945	191.1
Soy Tofu 5%	562.88^{b} ± 6.31	120.54	141.27^{b} ± 1.344	241.48
Soy milk 10%	550.99^{e} ± 6.42	117.48	142.2^{bc} ± 0.757	192.37
Soy milk 5%	561.29^{d} ± 5.43	119.24	129.2^{bcd} ± 1.266	174.78

LSD (p<0.01)

a, b, c, d and e the means different superscript in each column are significantly different at (p<0.01). Each value represents the mean of 6 rats ± S.E.

The amounts of total lipids, triglycerides and total cholesterol, in positive control were given the arbitrary value 100 and the increase and decrease in lipid fraction in negative control and after addition of soy bean diets were related to 100. Data of total lipids showed an increase in serum of hypercholesterolemic rats from 470.74 mg/dL in positive control to 630.59 mg/dL in negative control. By given the value 100 to positive control, the increase in total lipids was 133.95% for negative control.

On the other hand, serum triglycerides and total cholesterol were also significantly increased in negative control by (341.45%) and (308.01%) respectively as compared with positive control (Table 2&3).

The effect of soy flour diets administration during 4 weeks on lipids pattern is recorded also in Tables (2 & 3). The present results indicated that, the levels of serum total lipids and triglycerides were significantly decreased by 25.46% and 227.99% in rats fed 10% soy flour, respectively. Also, a significant decrease (355.15%) in total cholesterol by 10% soy flour was observed. A concentration of 10% soy bean, 10% soy tofu and 10% soy milk caused also a significant decrease by percentage of (23.75%), (14.38%) and (16.47%) for total lipids, (224.85%), (150.35%) and (149.08%) for

triglycerides and (341.09%), (330.64%) and (254.91%) for total cholesterol respectively, relatively to negative control.

Data in Table (2 & 3) reveal the effect of soy bean diets treatment on serum total lipids, triglycerides and total cholesterol of hypercholesterolemic rats. It is clearly shown that the administration of soy flour (5%) for 4 weeks caused a significant decrease in serum total lipids by 21.05% in serum triglycerides by 209.78% and in cholesterol by 352.53% relatively to negative control.

These results agreed with that findings reported by Liliana *et al.*, (2004).

4-Serum lipoprotein cholesterol

The changes in high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) and the ratio of total cholesterol to HDL-C (risk ratio) of hypercholesterolemic rats fed on different soy bean diets were investigated. The results are summarized in Table (3). The data revealed that no significant differences were found between high density lipoprotein-cholesterol (HDL-C) values in all treatments even in negative control.

Table (3): Effect of soy bean products on total, HDL and LDL-cholesterol and risk ratio by feeding rats for 4 weeks

Treatment	Total cholesterol		HDL-cholesterol		LDL-cholesterol		Risk ratio
	mg/dL	%	mg/dL	%	mg/dL	%	%
Untreated group (Positive control)	$97.93^{f} \pm 0.86$	100	$51.47^{a} \pm 0.74$	100	$41.53^{h} \pm 0.9$	100	1.9
Hypercholesterolemic Group (Negative group)	$301.63^{a} \pm 1.18$	308.01	$18.73^{f} \pm 0.47$	36.39	$219.4^{a} \pm 1.2$	528.29	16.10
Soy Flour 10%	$107.87^{f} \pm 1.23$	110.15	$32.8^{b} \pm 1.18$	63.73	$71.23^{g} \pm 0.7$	171.51	3.29
Soy Flour 5%	$110.4^{ef} \pm 1.7$	112.73	$29.73^{c} \pm 1.2$	57.76	$73.77^{g} \pm 1.47$	177.69	3.7
Soy Bean 10%	$121.69^{g} \pm 0.95$	124.17	$32^{bc} \pm 1.23$	62.17	$86.6^{f} \pm 1.1$	208.52	4.67
Soy Bean 5%	$125.77^{f} \pm 1.17$	128.43	$31.23^{bc} \pm 0.76$	60.68	$121.23^{e} \pm 1.24$	291.9	4.03
Soy Tofu 10%	$131.83^{e} \pm 0.83$	134.62	$30.47^{bc} \pm 0.87$	59.2	$124.93^{de} \pm 10.9$	300.82	4.33
Soy Tofu 5%	$149.37^{d} \pm 1.15$	152.53	$27.27^{d} \pm 1.07$	52.98	$127.63^{d} \pm 1.18$	307.32	5.48
Soy milk 10%	$206.23^{c} \pm 0.7$	210.35	$26.3^{b} \pm 1.35$	51	$136.3^{c} \pm 0.9$	328.2	7.84
Soy milk 5%	$233.87^{b} \pm 1.12$	128.43	$22.7^{e} \pm 1.1$	44.1	$162.03^{b} \pm 0.99$	308.15	10.3

LSD ($p<0.01$)

a, b, c, d and e the means different superscript in each column are significantly different at ($p<0.01$). Each value represents the mean of 6 rats ± S.E.

In the present experiment, values of HDL alone could not be taken as criteria for hypercholesterolemia. Values of LDL must also be taken into consideration. The serum LDL-C was increased from 41.53 mg/dL in positive

control to 219.4 mg/dL in negative control. It could be noticed that in hypercholesterolemic rats LDL-C was increased to 528.29%.

Addition of soy bean products on diets significantly decreased LDL-C in rats. It was increased to 219.4 mg/dL in negative control and was decreased again to 148.17, 132.8, 94.47 and 83.1 mg/dL in rats fed 10% soy flour, 10% soy bean, 10% soy tofu and 10% soy milk, respectively. These values are in close to the positive control value. The risk ratio which equal to the ratio of total-cholesterol/HDL value, is a good indication for hypercholesterolemia. This ratio was increased to 16.1 in the negative control as compared with the positive control 1.9. It was decreased to 3.29, 4.67, 4.33 and 7.84 in rats fed 10% soy flour, 10% soy bean, 10% soy tofu and 10% soy milk, respectively. The increase in LDL-cholesterol may be due to the increase in hepatic VLDL production and significant decrease in hepatic LDL receptor activity. These results are in agreement with those of Sirtori, *et al.*, (1995); Nagata, *et al.*, (1998); Anderson, *et al.*, (1995); Liliana, *et al.*, (2004) and Siyan Zhan and Suzanne, (2005).

Hypercholesterolemia and atherosclerosis result from the accumulation of LDL in the arterial wall and from the cellular response of wall compounds to injury. Oxidative modification of LDL is a key and early event in the pathogenesis of atherosclerosis (Stenberg, *et al.*, 1981). Several dietary components of soy bean compounds had been shown to lower LDL-C level and inhabit the oxidative modification of LDL (Huff and Carroll, 1980).

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تأثير خفض مستوى الكوليسترول بواسطة وجبات مختلفة من وجبات فول الصويا و ذلك مع فئران التجارب المرتفعة فى مستوى الكوليسترول

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أجريت الدراسة بهدف دراسة تأثير استخدام وجبات مختلفة تحتوى على بعض منتجات فول الصويا كعامل خفض مستوى كوليسترول الدم . حيث أجريت تجربة بيولوجية باستخدام ٦٠ فأر قسمت الى ١٠ مجموعات (٦ فئران فى كل مجموعة): المجموعة الاولى غذيت على عليقة قياسية لمدة ٤ أسابيع و المجموعة الثانية تم تغذيتها على عليقة مرتفعة فى محتوى الكوليسترول لمدة ٤ اسابيع و ذلك لزيادة مستوى الكوليسترول فى الدم، المجموعة الثالثة غذيت على عليقة مرتفعة فى الكوليسترول و تحتوى على ١٠٪ دقيق صويا و المجموعة الرابعة غذيت على عليقة مرتفعة الكوليسترول و تحتوى على ٥٪ من دقيق الصويا و المجموعة الخامسة غذيت على عليقة مرتفعة الكوليسترول وتحتوى على ١٠٪ من فول الصويا و المجموعة السادسة غذيت على عليقة مرتفعة الكوليسترول و تحتوى على ٥٪ من فول الصويا و المجموعة السابعة غذيت على عليقة مرتفعة الكوليسترول و تحتوى على ١٠٪ من توفو الصويا و المجموعة الثامنة غذيت على عليقة مرتفعة الكوليسترول و تحتوى على ٥٪ من توفو الصويا و المجموعة التاسعة غذيت على عليقة مرتفعة الكوليسترول وتحتوى على ١٠٪ من لبن الصويا و المجموعة العاشرة غذيت على عليقة مرتفعة الكوليسترول و تحتوى على ٥٪ من لبن الصويا. تم تقدير الزيادة فى وزن الجسم و الغذاء المأكول و مدى الاستفادة منه . تم تقدير محتوى الليبيدات الكلية و محتوى الكوليسترول الكلى و الجليسريدات الثلاثية فى مصل الدم ، وجد ان المجموعة التى تناولت فول الصويا بنسبة ١٠٪ أعطت انخفاض معنوى فى محتوى الليبيدات بنسبة ٣,٧٥٪ و الكوليسترول بنسبة ٣٤١,١٪ و الجلسريدات الثلاثية بنسبة ١٦٤,٢٪ مقارنة بالفئران المرتفعة فى مستوى الكوليسترول. و ايضا حدث انخفاض معنوى كباقى المجموعات التى تناولت وجبات فول الصويا الاخرى و لكن بدرجات مختلفة. كذلك تم تقدير كوليسترول الليبوبروتينات المرتفعة الكثافة وكوليسترول الليبوبروتينات منخفضة الكثافة و النسبة من (الكوليسترول الكلى/كوليسترول الليبوبروتينات مرتفعة الكثافة)، أظهرت النتائج انخفاض معنوى فى النسبة بين الكوليسترول الكلى و الكوليسترول الليبوبروتيبات مرتفعة الكثافة. لذلك يوصى البحث باستخدام وجبات فول الصويا التى اثبتت تأثيرها النافع كعامل خافض لمستوى كوليسترول الدم .