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The Effect of Soybean Extracts on Serum Lipid Profile and the Accumulation of Free Cholesterol and Cholesteryl Ester in the Aorta, Carotid Artery and Iliac Artery-Experimental Study

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Abstract

Background: We evaluated the effect of soybean extracts on serum lipoprotein profile and cholesterol accumulation on the arterial walls.

Objective and design: Sixty-four female Sprague-Dawley rats were randomly divided into eight groups. Soybean extracts were given to the rats via oral gavage every day for eight weeks, after which serum was collected. In the thoracic aorta, left carotid artery, and right iliac artery, we measured the lipoprotein fractions in the serum and the accumulation of free cholesterol and cholesteryl ester, which are predictors of subclinical atherosclerosis.

Results: After eight weeks of a continuous soybean diet, only two groups showed a lipid-lowering effect (n-hexane extract for 200 mg/kg dose and ethyl acetate extract for 200 mg/kg dose). We found lower free cholesterol and cholesteryl ester accumulation in the aortas and iliac arterial walls only in these two groups.

Conclusion: The results indicated that soybean extract intake leads to weight change and may influence lipid metabolism. The positive effects of the soybean diet involved not only serum lipids but also aortic wall cholesterol accumulation.

Keywords: Cholesterol; Soybean extracts; Experimental model; Cholesterol accumulation; Arterial wall

Introduction

It has been known for many years that dietary soybeans have positive health benefits, particular for atherosclerosis-related diseases. Many experimental studies have shown that a diet high in soy protein reduces hyperlipoproteinemia and atherosclerosis [1-3]. However, the role of individual soy components and the mechanisms underlying the beneficial effects of soybeans are not fully understood. The increased amount of research over the past 20 to 25 years has resulted in the theory that soy protein consumption may improve cardiovascular health [2,4]. Soy protein extract has been commonly used in experimental studies. The major components of soy protein extract are protein/peptide fractions. The estrogen-like compounds in soybeans are called isoflavones, which are also present in many legumes and grains [5]. The aim of the present study is to evaluate the effects of soybean extracts on the serum lipoprotein profile and atherosclerosis in the aortic, iliac, and carotid arterial walls of female Sprague-Dawley rats.

Materials and Methods

Animals: Sixty-four female Sprague-Dawley rats (Afyon Kocatepe University in Turkey) were randomly assigned to eight groups of eight rats each. The animals were two months old and weighed 200 ± 15 grams. During the experiment, they were kept in a standard controlled environment (12 hours light-dark cycle, temperature 23°C ± 2°C, and relative humidity 60% ± 5%) in individual metabolic cages for approximately 15 days in order to adapt to the surroundings. They were given free access to standard feed and water. During the study period, the gratings were cleaned regularly every two days, and feed and water containers were kept filled. The total study period was eight weeks. Soybean extracts were given to each group via oral gavage every day for eight weeks. The daily dose was adjusted to 1 ml in volume, and the soybean extract density was 100 or 200 mg/kg/dose. Variations in body weight and food intake were checked daily during the eight-week period. All procedures were conducted in compliance with the Republic of Turkey's state laws. The Institutional Animal Care and Use Committee and the ethical committee of experimental study of Afyon Kocatepe University approved all procedures involving the animals. The procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals. The experimental procedure was approved by the Research Ethics Committee (Res. No. 49533702/341/2013) [6].

Soybean extracts of n-hexane, ethyl acetate, and ethanol were prepared as described by Carrao-Panizzi et al. [7]. The collected soybeans were gradually extracted with n-hexane, ethyl acetate, and ethanol. Soybeans weighing 25 grams were extracted two times by using 500 ml of n-hexane, ethyl acetate, and ethanol at room temperature. The samples were then shaken for 48 hours. The combined extracts were concentrated under low pressure at 40°C in a Rotavapor® and then dehydrated under low pressure in a glass vacuum-controlled desiccator dryer. After extraction, the extract was transferred to an Eppendorff tube and stored at 5°C.

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Solvent removal process

This process was conducted using an ultimate vacuum system. To achieve optimal distillation conditions, the distillation energy supplied by the heating bath was removed by the condenser. All procedures were done according to the D20 5°C principle, which is mainly known as the solvent-removing method. The vacuum was adjusted to the rotary evaporation system appropriately for each solvent with an operating bath temperature of 60°C to yield a solvent vapor temperature of 40°C, which was subsequently condensed at 20°C.

At the end of the eight-week period, after 12 hours of fasting, the animals were anesthetized with ketamine HCl (40 mg/kg) and xylazine (8 mg/kg), and then 1 ml of blood was collected via heart puncture. The blood was collected into tubes containing SST gel separator II (BD Vacutainer® B, Franklin Lakes, NJ, USA), and the serum was promptly separated from the cells via centrifugation at 4500 RPM for 15 minutes at 4°C. Serum was collected and stored at -20°C for subsequent biochemical analysis. The rats were then euthanized via cervical dislocation. The carotid artery, aorta, and iliac artery, were promptly removed and placed in a 10% neutral buffered formalin solution for subsequent examination.

Measurement of serum lipid and lipoprotein

Serum lipoproteins were separated as described by Haug et al. [8] and isolated lipoprotein fractions were used for the enzymatic determination of cholesterol. Total serum cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), non-high-density lipoprotein cholesterol (non HDL-C) and serum triglycerides (TG) were determined in the Clinical Chemistry and Hormone Laboratory of the Department of Biochemistry at Sifa University Hospital. Non HDL-C was calculated by subtracting the HDL-C value from the TC value. The TC, non-HDL-C, HDL-C, and TG concentrations in the serum were measured enzymatically by using commercially available reagent kits (HDL and LDL/VLDL Quantification Colorimetric/Fluorometric Kit, BioVision Incorporated, Milpitas Boulevard, Milpitas California, USA). We measured the lipoprotein fractions at 570 wavelengths by using an Epoch Micro-Volume Spectrophotometer System (Bio Tec Instruments, Inc., Tigan Street Winooski, VT, USA).

Histochemical examination

The levels of free cholesterol and cholesteryl ester concentrations in the aorta, carotid, and iliac arteries levels were analyzed. The analysis was performed as described in the commercial kit prospectus (Cholesterol Colorimetric Assay Kit Cell Biolabs Inc., San Diego, USA). The aorta, iliac, and carotid arteries were placed on the platform of a dissecting microscope, and the adventitia was carefully dissected and removed.

Measurement of free cholesterol and cholesteryl ester concentrations

We used 10 mg of tissue that was extracted with 200 µL of a mixture of chloroform and isopropanol in a micro-homogenizer. The extracts were centrifuged for 10 minutes at 15,000 g. Air drying was performed at 50°C to remove the chloroform. Samples were then placed in a vacuum for 30 minutes to remove any trace amounts of organic solvent. The dried lipids were dissolved in 200 µL of 1 X Assay Diluent and then treated by sonicating and vortexing until the solution was homogeneous. After sample preparation, we assayed at 570 wavelengths by using an Epoch Micro-Volume Spectrophotometer System (Bio Tec Instruments, Inc., Tigan Street Winooski, VT, USA), using a commercial kit (Cholesterol Colorimetric Assay Kit Cell Biolabs Inc., San Diego, USA).

Statistical analysis

All data were assessed by a one-way ANOVA and a Tukey's test in order to detect the main effects of diet type on serum lipoproteins and atherosclerosis. Multiple linear regressions were used to assess the relationship between the effects of the treatment on serum lipoproteins and the effects on atherosclerosis. We selected covariates for the analysis of covariance. The analyses were conducted using R Statistical Analysis V. 3.0.2 Statistical Software. The results were presented as the mean value ± standard deviation. Differences were considered significant at $p < 0.05$.

Results

The initial ages and body weights of the all animals were similar. The experimental groups and diets are shown in Table 1. Some statistical differences ($p < 0.05$) in the body weight gain and growth of the animals showed after the eight-week experiment. The effects of soybean extracts on the growth and weight gain of the study animals are shown in Table 2. The average daily gains (ADGs) and feed/gain ratios of the rats in Groups 4, 6, and 8 were higher than those in other groups ($P < 0.05$). Comparisons among Groups 3, 5, and 7 and the

	N	Dıet	Doses
Group 1	8	Commercial diet	Negative Control
Group 2	8	0.5% Carboxymethylcellulose	Positive Control
Group 3	8	N-hexane extract	100 mg/kg
Group 4	8	N-hexane extract	200 mg/kg
Group 5	8	Ethyl acetate extract	100 mg/kg
Group 6	8	Ethyl acetate extract	200 mg/kg
Group 7	8	Ethanol extract	100 mg/kg
Group 8	8	Ethanol extract	200 mg/kg

Table 1: The experimental groups and diets.

Groups/ Parameters	Group 1 (n=8)	Group 2 (n=8)	Group 3 (n=8)	Group 4 (n=8)	Group 5 (n=8)	Group 6 (n=8)	Group 7 (n=8)	Group 8 (n=8)
Age (week)	8	7	8	8	7	8	8	8
Initial Body weight, g	208.9 ± 1.4	207.4 ± 1.6	206.6 ± 2.0	207.2 ± 2.7	207.7 ± 2.8	207.1 ± 1.8	207.9 ± 2.7	207.7 ± 2.2
Final Body weight, g	216.6 ± 1.9	215.9 ± 1.4	213.8 ± 2.3	226.9 ± 4.2*	215.3 ± 2.2	222.8 ± 25.1*	214.2 ± 2.9	223.1 ± 9.8*
Body Weight gain g/8week	7.7 ± 1.4	8.5 ± .6	7.2 ± 1.2	19.7 ± 1.2*	7.6 ± 2.6	15.7 ± 1.5*	6.3 ± 25.9	15.4 ± 1.4*
Food consumption	481.5 ± 19.1	492.3 ± 11.4	482.8 ± 18.8	491.4 ± 15.2	493.2 ± 11.1	492.9 ± 15.2	494.3 ± 12.9	489.1 ± 11.5
Each value is presented as the mean value ± standard deviation; g, gram. *Mean value is significantly different when compared to control groups ($p < 0.05$).								

Table 2: Baseline values, total food intake, and body weight gain of rats fed on the soybean for 8 weeks.

mmol/l	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
T Chol	1.63 ± 0.05	1.59 ± 0.04	1.59 ± 0.04	1.49 ± 0.02*	1.62 ± 0.02	1.40 ± 0.03*	1.62 ± 0.03	1.59 ± 0.03
Non-HDL Chol	0.93 ± 0.03	0.95 ± 0.02	0.99 ± 0.29	0.77 ± 0.03*	1.1 ± 0.16	0.78 ± 0.03*	1.06 ± 0.13	0.89 ± 0.5
HDL-Chol	0.66 ± 0.04	0.67 ± 0.03	0.67 ± 0.04	1.02 ± 0.07*	0.68 ± 0.4	1.14 ± 0.12*	0.65 ± 0.02	0.66 ± 0.02
Triglyceride	0.67 ± 0.04	0.70 ± 0.03	0.68 ± 0.02	0.47 ± 0.03*	0.69 ± 0.06	0.50 ± 0.04*	0.70 ± 0.05	0.68 ± 0.03
Atherogenic Index	1.40 ± 0.8	1.42 ± 0.08	1.47 ± 0.07	0.75 ± 0.04*	1.61 ± 0.18	0.68 ± 0.9*	1.63 ± 0.19	1.34 ± 0.14

Values are represented as mean value ± standard deviation and mmol/L for each group.
T Chol, total cholesterol; HDL-Chol, high density lipoprotein; non-HDL-Chol, difference between T Chol and HDL- Chol; atherogenic index, non-HDL- Chol /HDL- Chol.
*Mean value is significantly different when compared to control groups (p <0.05).

Table 3: Serum total cholesterol, HDL-cholesterol, non-hdl-cholesterol, and triglyceride concentrations in rats fed on the soybean for 8 weeks.

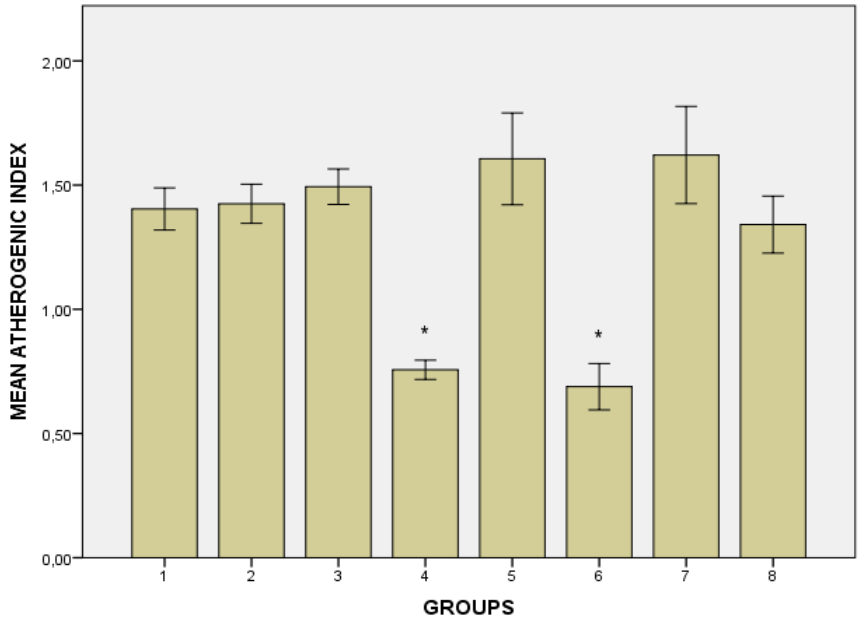

Figure 1: The atherogenic index values were significantly lower in Group 4 and 6 when compared to other groups. (*Mean value is significantly different when compared to other groups (p < 0.05).

control groups, there was no significant difference in terms of growth performance (P > 0.05).

After eight weeks of treatment, the distributions of cholesterol and triacylglycerol among the lipoprotein fractions was determined in mmol/L. The effects of diets supplemented with soybean extracts on serum lipoprotein profile are shown in Table 3. The serum lipoprotein levels were the same in Groups 1 and 2 (the negative and positive control groups, respectively). Between Groups 1 and 2, there were many differences in terms of all parameters. Lower serum TC, TG, and non-HDL-C levels were observed in Groups 4 and 6. When they were compared to other groups, this difference was significant (P < 0.01). The HDL-C concentrations of Groups 4 and 6 were higher than those of other groups (P < 0.05). Serum HDL-C levels in Groups 4 and 6 were 1.02 ± 0.07 and 1.14 ± 0.12 mmol/L, respectively, whereas those of the controls were 0.66 ± 0.04 and 0.67 ± 0.03 mmol/L, respectively. However, serum TC, TG, non-HDL-C and HDL-C levels were not significantly different in Groups 3, 5, 7, and 8 (P > 0.05). The control groups did not have higher serum cholesterol levels than Groups 3, 5, 7, and 8 did (P > 0.05). The differences between the control groups and Groups 3, 5, 7, and 8 were not significant. The highest HDL-C level was seen in Group 6, whereas the HDL-C level was lower in Group 7 than in the control groups. In this group, the TC and TG levels were similar to those in the control groups. The atherogenic index values were significantly lower in Groups 4 and 6. compared to other groups (Figure 1).

We measured the accumulation of free cholesterol and cholesteryl ester, which are *predictors of subclinical atherosclerosis,* in the thoracic aorta, the left carotid artery, and the right iliac artery. The atherogenic response, as measured by aortic cholesteryl ester concentration, is considered the primary indicator of the beneficial or detrimental effects of any diet supplementation on atherosclerosis. The analyses were performed in miligram/gram (mg/g) on cholesterol and cholesteryl ester. Table 4 shows the results of the effects of diets supplemented with soybean extracts on the arterial wall. Aortic and iliac artery triglyceride, free cholesterol, and cholesteryl ester concentrations were significantly lower in Groups 4 and 6 (P < 0.05). The lowest triglyceride values were found in Groups 4 and 6, which differed greatly from those found in the control groups (Table 4). Comparison showed no significant differences between other groups. The measurement results for the cholesteryl ester and free cholesterol concentrations in Groups 4 and 6 were significantly lower (P < 0.05) than those in Groups 1 and 2 (control groups). In addition, the aortic cholesteryl ester values in Groups 3, 5, 7, and 8 were similar to those in the control groups (Figures 2 and 3).

The aortic measurements yielded similar results for the iliac arteries. The greatest accumulation of both forms of cholesterol in the iliac artery was in the control groups. The cholesteryl ester values were the lowest in Groups 4 and 6. Interestingly, the results of the carotid artery measurements were different from those of the aorta and iliac artery. The carotid artery measurements showed no differences among

Values	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
Aortıc Values, mg/g tissue								
TG	4.50 ± 0.87	4.58 ± 0.87	4.41 ± 0.96	3.7 ± 1.05	4.45 ± 1.08	3.58 ± 0.95	4.39 ± 0.88	4.47 ± 1.02
TC	1.92 ± 0.16	1.81 ± 0.15	1.72 ± 0.26	1.11 ± 0.23	1.83 ± 0.32	1.21 ± 0.23	1.89 ± 0.22	1.95 ± 0.24
FC	1.62 ± 0.07	1.78 ± 0.07	1.69 ± 0.07	1.19 ± 0.08	1.71 ± 0.07	1.21 ± 0.13	1.79 ± 0.11	1.69 ± 0.11
CE	0.25 ± 0.03	0.27 ± 0.04	0.21 ± 0.03	0.11 ± 0.02	0.24 ± 0.04	0.11 ± 0.01	0.19 ± 0.02	0.26 ± 0.03
Iliac Artery Values, mg/g tissue								
TG	4.58 ± 0.07	4.50 ± 0.35	3.99 ± 0.42	3.58 ± 0.59	4.51 ± 0.20	3.51 ± 0.16	4.79 ± 0.58	4.51 ± 0.19
TC	1.92 ± 0.18	1.93 ± 0.27	1.81 ± 0.23	1.03 ± 0.26	1.72 ± 0.26	1.01 ± 0.17	1.83 ± 0.22	1.82 ± 0.14
FC	1.67 ± 0.09	1.69 ± 0.12	1.62 ± 0.14	1.10 ± 0.08	1.50 ± 0.11	1.04 ± 0.06	1.79 ± 0.12	1.70 ± 0.09
CE	0.22 ± 0.02	0.27 ± 0.03	0.20 ± 0.04	0.02 ± 0.01	0.20 ± 0.03	0.07 ± 0.01	0.21 ± 0.03	0.30 ± 0.07
Carotıd Artery Values, mg/g tissue								
TG	3.51 ± 0.10	4.01 ± 0.52	3.58 ± 0.10	3.48 ± 0.05	3.63 ± 0.09	3.69 ± 0.58	3.58 ± .0.34	3.60 ± .0.52
TC	1.12 ± 0.12	1.20 ± 0.29	1.02 ± 0.16	1.22 ± 0.16	1.13 ± 0.06	1.15 ± 0.16	1.02 ± 0.12	1.03 ± 0.11
FC	1.01 ± 0.03	1.01 ± 0.08	1.02 ± 0.03	1.11 ± 0.06	1.11 ± 0.05	1.21 ± 0.08	1.12 ± 0.07	1.01 ± 0.03
CE	0.20 ± 0.05	0.13 ± 0.05	0.09 ± 0.03	0.12 ± 0.02	0.09 ± 0.02	0.12 ± 0.03	0.12 ± 0.02	0.13 ± 0.02

Values are represented as the mean value ± standard deviation; g, gram; mg, milligram; TG, triglycerides; TC, total cholesterol; FC, free cholesterol; CE, cholesteryl ester. The bold digits, different from control groups ($p < 0.05$).

Table 4: Aortic, Iliac and Carotid arterial values of triglyceride, total cholesterol, free cholesterol and cholesteryl ester of rats fed on the soybean for 8 weeks (mg/g tissue).

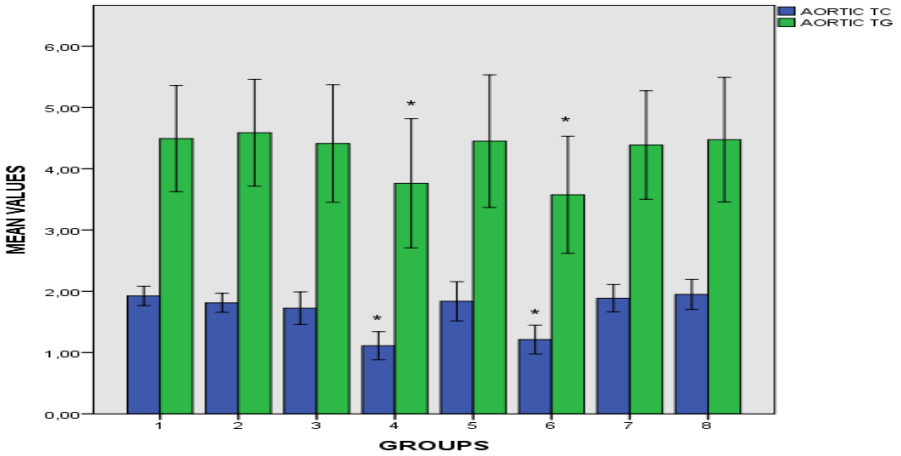

Figure 2: The mean values of total cholesterol and triglyceride in the thoracic aortic wall. (*Mean value is significantly different when compared to other groups ($p < 0.05$). TC: Total cholesterol; TG: Triglycerides. (mg/g tissue). Mean Values are represented as the value ± standard deviation and mg/g tissue).

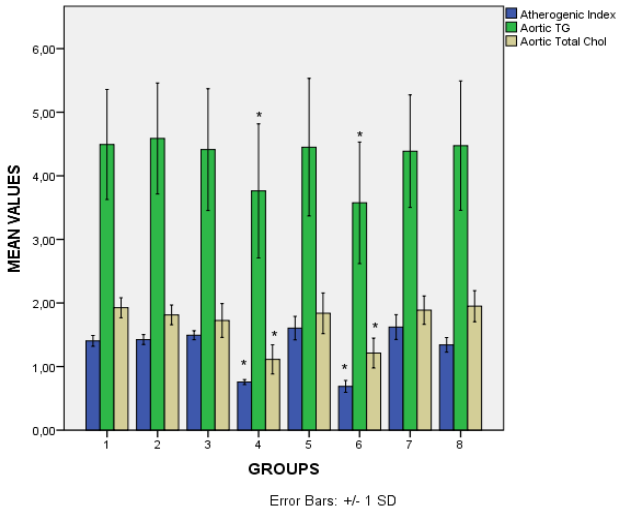

Figure 3: The mean values of total cholesterol and triglyceride in the thoracic aortic wall and the atherogenic index values were significantly lower in Group 4 and 6 when compared to other groups. (*Mean value is significantly different when compared to control groups ($p < 0.05$). Atherogenic index, non-HDL- Chol /HDL- Chol; TG: Triglycerides; T Chol: Total cholesterol. Values are represented as mean value ± standard deviation and mg/g tissue for each group).

all groups. The results obtained from all study rats were similar, and there was no statistically significant difference between the control and study groups.

Discussion

The beneficial effects of soybean protein on serum lipid and lipoprotein concentrations and thus on cardiovascular diseases have been well-documented. Many studies have reported a correlation between the oral intake of soybean protein and serum lipid profile [8-10]. The increased number of studies performed over the past 10 to 12 years have provided evidence that soybean consumption has a positive effect on serum lipid profile and that it might protect against the accumulation of cholesterol on the vascular walls and thus improve cardiovascular health. Consequently, it may also inhibit the early progression of coronary artery atherosclerosis [11,12]. Several studies have investigated the soybean's effects on serum lipid and lipoprotein metabolism. These studies showed that soy protein intake was positively associated with HDL-C and negatively associated with total cholesterol, non-HDL-C, and TG [13-15]. Clarkson et al. [2] reported that soybean usage in human subjects caused a reduction in LDL-C by approximately 13%, a reduction in serum TG by approximately 10%, and an increase in HDL-C by approximately 2%. It has been shown that cholesterol accumulation in the vascular wall is associated with structural and functional changes in the vessels. Experimental data suggested that the alteration of cellular phospholipid metabolism is an adaptive response to prevent the ratio of cellular-free cholesterol and phospholipids from reaching cytotoxic levels in the macrophage foam cells of atherosclerotic lesions [16].

In this study, we showed that after eight weeks of continuous soybean diet consumption, only two groups displayed a lipid-lowering effect (n hexane extract at 200 mg/kg dose and ethyl acetate extract at 200 mg/kg dose). Our results showed that soybean extract intake led to weight change; thus, it may be considered a nutritional supplement and could influence lipid metabolism. In addition, we found that only Groups 4 and 6 had less free cholesterol and cholesteryl ester accumulation in the aorta and iliac arterial wall. The histochemical and biochemical analyses of other groups were similar to those of the control groups. However, we found no effects of the lower doses. Interestingly, the results of the carotid arterial wall analyses were similar in all groups; there were no significant differences among the groups. The results indicated that carotid artery atherosclerosis could not be significantly reduced by a soybean diet, given these doses and this period of study. These findings were similar to the literature [1,4,13]. In this respect, the carotid artery differed from the aorta and the iliac arteries. These findings showed that the positive effects of a soybean diet appear not only in lowered serum lipids but also less cholesterol accumulation in the aortic wall.

Many reports in the literature have indicated that soybean extracts may prevent coronary heart disease. Our findings suggested that the favorable cardiovascular effects of soybean may be enhanced with a 200 mg/kg dose of ethyl acetate extract and n hexane extract. We did not investigate higher doses. Nevertheless, in light of these findings, we can say that doses of ethanol extracts below 200 mg/kg did not affect the blood lipid profile and did not prevent free cholesterol and cholesteryl ester accumulation on the aortic wall. The aortic cholesteryl ester values in all groups were similar to those of the control groups, except for Groups 4 and 6. We interpret these findings to indicate that the ethyl acetate or n hexane extracts of the soybean diet may act to prevent atherosclerosis in a rat model at 200 mg/kg doses. However, the components of the soybean diet that are responsible for this effect and the mechanisms involved remain uncertain. Recent research has focused primarily on efforts to identify the components of soybean protein that are responsible for its beneficial effects on the cardiovascular system. However, some experimental studies have shown that the isoflavones contained in soybeans and many soy-based products are responsible for these effects [4,5]. Conversely, various studies reported that isoflavone-free soy protein preparations reduce serum cholesterol [17]. How do soybeans exert these effects on the aortic wall and blood lipid profile? The pathways of the effects remain unclear. Adams et al. [1] concluded that the consumption of peptides from purified soybean beta-conglycinin has an inhibitory effect on the development of atherosclerosis, which greatly exceeds the effect of whole-isoflavone soy protein isolate and does not depend on low-density lipoprotein cholesterol (LDL-C) receptors or effects on serum lipoproteins. Cavallini et al. [18] emphasized that atherosclerosis is a chronic immune inflammatory disease, and they attributed the biological effects of soybeans to their antioxidant and anti-inflammatory effects. Because the initiation and progression of atherosclerosis are known to involve the oxidation of serum lipoproteins in the arterial intima, cell proliferation, and localized inflammatory reaction, these represent potential pathways by which soy peptides could directly inhibit atherosclerosis [19].

Some authors [3] determined that an undigested, insoluble high-molecular-weight Fraction (HMF), which bound bile acids, increased the excretion of acidic and neutral steroids and caused a marked reduction in serum and hepatic cholesterol concentrations in rats. Wang et al. [20] showed that the increased consumption of HMF of soybean by human subjects gave rise to bile acid secretion, raised serum HDL-C concentration, and decreased serum LDL-C concentration. Higher HDL-C and lower LDL-C concentrations have a preventive effect on cardiovascular diseases. Adams et al. [1] observed that soy protein isolate had a potent atheroinhibitory effect in female mice but that it had no effect in male rats. Conversely, experimental studies have demonstrated the inhibitory effects of dietary soy protein isolate on atherosclerosis in both male and female rats [16,17]. Therefore, we investigated only female rats. Some investigations in the literature reported that serum total cholesterol and lipoprotein cholesterol concentrations were unaffected by dietary soy protein isolate in animals and humans [21,22]. In contrast, in this study, we found a positive effect on blood lipid profile, the aorta, and the iliac arterial wall. However, we did not observe an atheroinhibitory effect on the carotid artery wall. In addition, we hypothesize that an effective dose of soybeans must be administered; otherwise, there is no atheroprotective effect [23]. This is supported by the findings of Clarkson et al. [24], who found that serum isoflavone concentrations must be in the optimal range for the inhibition of atherosclerosis to occur.

Conclusion

The present study supports the lipid-lowering and the atheropreventative effects of soybean extracts. We observed significantly decreased serum TC, non-HDL-C, and TG levels only in Groups 6 and 8 ($P < 0.05$). The results obtained for other groups were similar. The reason for this finding was probably that the dose was too low or that the duration of experiment was too short. After eight weeks of a soybean diet, rats had decreased levels of free cholesterol and cholesteryl ester accumulation in the aorta and the iliac arteries, but not in the carotid arteries. In the light of these findings, we can say that the soybean diet has a positive effect on the cardiovascular system; however, further long-term studies are needed.

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