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The Effect of Cost Effective and Useful Diets on Blood Parameters in Female Mice

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Abstract

The high rate of cardiovascular disease is due to excessive intake of animal protein. The aim of this study was to investigate the effects of the replacement of the soybean as a cost effective protein on the blood parameters. 110 immature female Balb/c mice three weeks of age were randomized, into eleven groups of 10 animals each based on following diets: 1) low protein, 2) full protein without soybean, 3) full protein with 20% soybean, and 4) full protein with 40% soybean,. The animals received their diet orally on a daily basis for three and six months. At the end of the study period, the blood samples were collected and blood parameters were measured. The data were analyzed with SPSS software using one-way ANOVA and Tukey's test. Results: In the groups that received 20% and 40% soybean diet, the cholesterol, LDH, glucose, creatinine and urea levels showed meaningful decrease, and total protein level showed a significant increase in comparison with the other groups ($P < 0.01$). However, the mean values of ALT, AST, ALP, triglyceride, calcium and phosphorus did not show significant change among experimental groups ($P < 0.01$). Results of the present study indicated that soybean as a cost effective protein could be suitable replacement for animal protein and soybean may have beneficial effects on health like reduction of cholesterol, LDH and urea.

Keywords

Soybean, Blood Parameter, Liver Enzymes, Diet

1. Introduction

Nowadays, advances in the medical field have controlled many diseases and has ended up rise of life expectancy. Since a large number of plants are used in herbal medicine, the study of the effects of plants could be very beneficial in the treatment of diseases [1].

In recent years, consumption of plants and vegetable proteins instead of animal proteins has been taken into consideration because of their fewer side effects. Soybean bears many benefits and can form a basic diet. Russian researchers were among the first to realize the benefits of soybean. They found that soybean bears high protein, essential amino acids, calcium, iron, phosphorus, magnesium, zinc, B vitamins and fiber. It can also be used to reduce heart disease [1], for bone health [2] [3], to prevent the cancer and to reduce the symptoms of menopause [4] [5]. In addition, soybean is a rich source of phytoestrogens with antioxidant properties, anti-carcinogenic and estrogenic effects. The diets containing soybean play an important role in preventing diseases and controlling blood lipids and lipoproteins.

The effects of several compounds in the diet, including beta-glucan, sterols and ethanol plant, and garlic extract on reduction of blood cholesterol have been studied, however, the beneficial effects of soybean protein on cardiovascular diseases and diabetes type II has attracted more attention. In addition, soybean protein via lowering the serum total cholesterol and LDL can reduce the incidence of diseases caused by the consumption of the diets rich in saturated fats [6].

The beneficial effect of soybean on blood lipids is due to its specific amino acid. Methionine and low ratio of lysine to arginine, a parameter that decreases cholesterol, are constituent components of soybean protein [7]. Also some non-protein compounds of soybean are reported to have beneficial effects on improvement of plasma cholesterol [8]. In addition, soybean contains the highest level of isoflavones among vegetable proteins. Isoflavones, estrogen and cyclic compounds are found in abundantly in soybeans. It has been shown that isoflavones of soybean, especially genistin with effects on the expression of estrogen receptor's gene and serum insulin concentration, can greatly influence the metabolism of lipids and blood lipoproteins [9]. Soybean protein is also rich in isoflavones with reduced susceptibility to oxidation of LDL. The increased expression of LDL receptor's gene plays a significant role in the prevention of heart diseases and cancers [10].

However, the main cause of the positive effects of soybean protein has not been conclusively established and needs further investigation. Thus, due to the lack of scientific research and to introduce far-reaching effects of medicinal plants by scientific study, this study was designed to evaluate the effects of soybean meal on blood parameters in females mice, and also comparison of cholesterol lowering effects of soybean protein as a source of plant protein with animal protein in mice.

2. Materials and Methods Work

2.1. Laboratory Animals and Diets

110 immature female mice of Balb/C initially weighing 12 - 15 g, were purchased

from Tehran Pasteur Institute and maintained in the animal room under controlled temperature (22˚C ± 1˚C), humidity and air flow condition, with a fixed 12 h light-dark cycle with adequate access to food and water. The mice were randomly randomized into 11 groups of 10 animals each (Table 1). Four types of diet were set by nutritionists based on the Table 2. Soybean were obtained from Asan Govar co. (Tehran, Iran) with the name of Herta brand and along with other materials used again according to Table 2. The soybean was blended with water and converted the paste form. Afterward it was placed on a platform and after drying, were administered orally as follows:

Table 1. The experimental groups were randomly divided into 11 groups of 10 animals each.

Row	Groups	Duration of treatment with diet	Diet
1	A	3 months	Protein deficiency (Food 1)
2	B	3 months	Complete protein (Food 2)
3	C	3 months	Complete protein-Soybean protein with 20% (Food 3)
4	D	3 months	Complete protein-Soybean protein with 40% (Food 4)
5	A	6 months	Protein deficiency-Soybean protein 13.5% (Food 1)
6	B	6 months	Complete protein-23% soybean protein (Food 2)
7	C	6 months	Complete protein-Soybean protein accounted for 20% (Food 3)
8	D	6 months	Soybean protein is a complete protein as it accounted for 40% (Food 4)
9	E	6 months	3 months Diet A (Food 1) 3 months of diet B (Food 2)
10	F	6 months	3 months Diet A (Food 1) 3 months of diet C (Food 3)
11	G	6 months	3 months Diet A (Food 1) 3 months of diet D (Food 4)

Table 2. The rations were programmed based on four basic diets.

Diet	Protein deficiency (13.5% protein) (gr)	Complete protein (23% protein) (gr)	20% of soybeanbeans (gr)	40% of soybeanbeans (gr)
Corn	700	450	562	562
Soybeanbean meal	-	-	200	400
Rapeseed meal	150	229	100	-
Sunflower Meal	112	283	100	-
Oyster shell	17	17	17	17
Calcium phosphate	15	15	15	15
Mineral supplements	2.5	2.5	2.5	2.5
Vitamin supplements	2.5	2.5	2.5	2.5
Salt	1	1	1	1
Total	1000	1000	1000	1000

Diet 1: Diet deficient in protein—the protein content of 13/5% (Food 1).
Diet 2: Diet with complete full protein—the protein content of 23% (Food 2).
Diet 3: Diet containing 20% of soybean protein (Food 3).
Diet 4: Diet containing 40% of soybean protein (Food 4).

2.2. Biochemical Analyses

After 24 hours from the last food intake, the blood samples were taken from heart. The samples undergone centrifugation in 1000 rpm and the sera were isolated from blood, stored at −20˚C. the blood parameters such as glucose, cholesterol, triglycerides, phosphorus, calcium, ALP (Alkaline Phosphatase), LDH (lactate dehydrogenase), AST (Aspartate transaminase), ALT (Alanine aminotransferase), UREA, CR (Creatinine), TOP (Total Protein) were determined in the laboratory using the kit by Elisa and enzymatic methods.

2.3. Statistical Analysis

Experimental results were expressed as means ± SD. Statistical analyses were performed using PASW 18.0 (SPSS Inc., Chicago, IL, USA). Model assumptions were evaluated by examining the residual plot. Bonferroni test for pairwise comparisons was used. The differences were considered significant when $P < 0.05$.

3. Results

During the study period, the rats physiological conditions in terms of appearance, body weight, exercise and intake of food and water were controlled and specific differences were observed among different groups.

3.1. The First Experimental Group: Treated with 4 Types of Diet for Three Months

The results of changes in triglycerides, glucose, AST, ALT, ALP, TOP and phosphorus in the blood sera of rats treated with Food 1, Food 2, Food 3 and Food 4 (respectively called groups of three months A, B , C and D) did not show significant changes ($P > 0.05$). The serum cholesterol mean values in group D was significantly lower than the A and B groups ($P < 0.01$). The mean values of glucose, urea and LDH levels were significantly lower in groups D and c compared to the A and B groups ($P < 0.01$). Also the mean value of creatinine was significantly decreased in group D compared to group B ($P < 0.01$). In addition, calcium level in group C, showed significant increase compared to group B ($P < 0.01$) Table 3).

3.2. The Second Experimental Group: Treated with 4 Types of Diet for Six Months

The results of changes in triglycerides, AST, ALT, ALP, calcium and phosphorus in the blood sera of rats treated with Food 1, Food 2, Food 3 and Food 4 (respectively called Group of six months a, b, c and d) a did not show significant change

Table 3. The mean values of blood parameters and serum liver enzymes of immature female rats in different groups for three months. Data are expressed as Mean ± S.E.M.

Parameters	Group A Dietary protein deficiency	Group B Complete Protein Diet	Group C 20% soybean diet	Group D 40% soybean diet
Glucose (mg/dl)	68.58 ± 17.34	62.58 ± 6.47	53.90 ± 5.48*	55.08 ± 1.54*
Total protein (g/dl)	5.18 ± 0.22	5.32 ± 1.17	5.95 ± 0.54	5.93 ± 0.60
Creatinine (mg/dl)	0.20 ± .08	.53 ± 0.13	0.40 ± 0.08	0.26 ± 0.160*
Cholesterol(mg/dl)	112.28 ± 14.05	117.70 ± 20.71	86.77 ± 20.53	83.03 ± 16.06*
LDH (U/L)	6372.0 ± 58.26	6907.1 ± 37.64	5581.6 ± 47.90*	5689.1 ± 47.79*
Tri glyceride (mg/dl)	112.28 ± 14.05	112.17 ± 26.72	111.43 ± 15.50	112.53 ± 25.73
Urea (mg/dl)	48.63 ± 4.07	65.33 ± 7.28	36.23 ± 10.43*	40.58 ± 12.14*
ALT (U/L)	201.00 ± 49.78	378.83 ± 30.84	206.50 ± 10.86	263.33 ± 98.03
AST (U/L)	1119.0 ± 43.44	3003.3 ± 97.67	2574.6 ± 37.7	1237.1 ± 26.53
ALP (U/L)	269.8 ± 78.76	139.0 ± 53.54	220.5 ± 121.60	225.0 ± 10.26
Calcium (mg/dl)	5.82 ± .91	3.73 ± 2.92	6.48 ± 0.30*	5.85 ± 1.11

*P <0.01 vs. other groups.

(P > 0.05). The mean values of cholesterol and LDH in the sera of mice of groups C and D were significantly decreased compared to groups A and B (P < 0.01). The mean values of urea and creatinine in groups C and D were significantly lower than those of group B (P < 0.01).The mean value of glucose in groups C and D showed significant reduction compared to that of group B (P < 0.01). While TOP mean value in group D significantly was higher than groups A and C (P < 0.01) (Table 4).

3.3. The Third Experimental Group: Treated with a Combination of Diets for Six Months

The results of changes in cholesterol, triglycerides, glucose, urea, creatinine, LDH, AST, ALT, serum calcium and phosphorus in the blood of mice treated with F1 + F2, F1 + F3 and F1 + F4 (respectively called Groups of combined six months E, F and G) did not show significant differences (P < 0.05). The ALP showed significant decrease in group G compared to that of Group E (p < 0.01), and serum TOP value of groups F and G showed significant decrease compared to that of group E (P < 0.01) (Table 5).

4. Discussion

Medicinal plants due to their easier access, fewer side effects, cost effectiveness and less toxic effects are considered as an ideal alternative for chemical agents [11].

The large number of deaths occurs due to—cardiovascular diseases that could be because of animal fat consumption rich in cholesterol and LDH. Therefore, consumption of vegetable protein is being increased every day. Soybean is a widely used vegetable seed [8]. In this study, diets containing soybean meal

Table 4. The Mean values of blood parameters and serum liver enzymes of immature female rats in different groups studied for six months. Data are expressed as Mean ± S.E.M.

Parameters	Group a Dietary protein deficiency	Group b Complete Protein Diet	Group c 20% soybean diet	Group d 40% soybean diet
Glucose (mg/dl)	38.00 ± 12.86	78.00 ± 32.61	39.00 ± 11.69*	33.00 ± 7.97*
Total protein (g/dl)	8.52 ± 1.50	6.03 ± 0.19	7.15 ± .99	12.07 ± 5.15*
Creatinine (mg/dl)	.0.28 ± 0.07	0.53 ± 0.13	0.40 ± 0.08*	0.27 ± 0.16*
Cholesterol (mg/dl)	123.83 ± 9.98	157.50 ± 14.22	116.00 ± 15.87*	120.83 ± 5.74*
LDH (U/L)	6511.0 ± 43.8	6540.0 ± 33.22	6233.8 ± 56.18*	164.83 ± 72.23*
Tri glyceride (mg/dl)	118.66 ± 5.27	155.66 ± 59.46	159.08 ± 47.70	164.83 ± 45.90
Urea (mg/dl)	48.78 ± 3.27	67.25 ± 7.38	39.88 ± 14.62*	40.33 ± 12.71*
ALT (U/L)	200.33 ± 46.50	451.00 ± 57.57	203.50 ± 94.33	289.17 ± 10.50
AST (U/L)	1129.8 ± 63.76	3113.17 ± 85.02	2089.83 ± 14.79	1326.0 ± 30.78
ALP (U/L)	235.8 ± 21.9	103.2 ± 18.78	203.0 ± 13.82	274.0 ± 14.43
Calcium (mg/dl)	6.17 ± 0.93	5.57 ± .96	6.25 ± 1.21	6.48 ± 1.14
Phosphorus (mg/dl)	8.82 ± 1.85	11.42 ± 3.37	12.300 ± 4.22	11.85 ± 2.44

*P <0.01 vs. other groups.

Table 5. Mean blood parameters and serum liver enzymes of immature female rats in different groups studied in combination with diet for six months. Data are expressed as Mean ± S.E.M.

Parameters	Group E Full-protein diet + protein deficiency	Group F +20% soybean protein deficient diet	Group G +40% soybean protein deficient diet
Glucose (mg/dl)	35.67 ± 8.73	40.00 ± 11.64	35.67 ± 12.35
Total protein (g/dl)	9.78 ± 1.48	7.23 ± 0.30*	7.00 ± 0.40*
Creatinine (mg/dl)	0.4667 ± .10	0.4167 ± 0.09	0.3667 ± 0.19
Cholesterol (mg/dl)	142.67 ± 21.05	146.50 ± 56.28	115.66 ± 18.55
LDH (U/L)	6040.8 ± 74.38	6129.3 ± 56903	5791.0 ± 35.62
Tri glyceride (mg/dl)	50.20 ± 50.61	178.17 ± 39.12	171.17 ± 33.67
Urea (mg/dl)	946.67 ± 11.32	61.29 ± 5.00	49.13 ± 13.27
ALT (U/L)	117.50 ± 77.56	809.16 ± 79.93	855.83 ± 83.75
AST (U/L)	1853.8 ± 83.13	1560.5 ± 91.25	1487.6 ± 56.57
ALP (U/L)	234.5 ± 21.65	181.83 ± 57.57	163.83 ± 21.53*
Calcium (mg/dl)	5.91 ± 1.02	6.57 ± .87	6.33 ± 1.37
Phosphorus (mg/dl)	15.13 ± 2.69	14.0 ± 2.37	13.23 ± 1.54

*P <0.01 vs. other groups

showed a significant decrease in blood cholesterol levels than the control group. The soybean as a cost effective protein, can be substituted for animal proteins because it reduces blood serum cholesterol. The cardiovascular diseases are growing worldwide due to increase in the cholesterol and LDL as the main parameters of mortality [12]. This study confirmed that soybean di*et al.*so plays an important role in reducing these two parameters. Other studies have shown that low cholesterol levels as a result of soybean consumption is significantly reduced compared to milk casein [13]. The researchers stated that existing methionine and low ratio of lysine to arginine in soybean may be one factor that reduces blood cholesterol [14]. Isoflavones have been mentioned to influence blood lipid and protein metabolism [15].

In this, it has been demonstrated that that lecithin reduced total cholesterol, and significant reduction in levels of HDL cholesterol were also observed [15]. This difference may be due to experimental conditions, genetic, diet and intrinsic differences between lipoproteins of animals in within individuals of the same species among different species [16]. In a recent study cholesterol and HDL levels in the soy groups were significantly reduced compared to the animal protein group.

In another study, it was found that soy diet can significantly reduce the serum triglycerides value. However, the consumption of animal protein raised the triglyceride level [16]. Some studies have shown that significant reduction in total cholesterol, LDL and triglycerides using soy was because of lesser intake of cholesterol and saturated fatty acids and higher intake of the fiber [11]. The findings of other studies have shown that intake of soybean meal did not show significant changes in triglycerides [17] [18] [19].

It had also been stated that consumption of diets containing soy proteins significantly reduced urea. They concluded that consumption of soy protein could modify the risk factors for heart disease, reduce protein excretion in 24 h and reduce urinary urea nitrogen [16].

They also found that diets containing soy protein caused significant reduction in the serum levels of urea that may be due to certain amino acids which reduced urea production. The fact that deamination and conversion of co_2 to urea occurs in liver, it could be concluded that less deamination and conversion of co_2 to urea take place, hence, mean value of blood urea nitrogen is reduced. This reduction in urea values was in agreement with findings of others [21].

Due to presence of low amounts of the methionine in soybean protein, it was expected that following consumption of soy, the serum creatinine levels would reduce, however, no significant change in serum creatinine was observed. In contrary, animal proteins increased blood serum creatinine among different groups.

In the studies of Soroka [20], Anderson [21] and Hanna *et al.* [22] the effect of soy consumption did not decrease serum creatinine in the blood. The reduction of urinary creatinine level was somewhat unexpected, and this may approve the theory of researchers that reported the urine creatinine excretion [23]. However, in the present study, the amount of creatinine in groups consuming soy diet for 3 or 6 months was significantly decreased compared to the group consuming total protein without soybean. The serum creatinine levels are influenced by functional factors of kidney as well as various factors such as energy intake, metabolic acidosis, impaired fat metabolism, physical activity, weight and blood pressure changes [24]. Nevertheless, our results were consistent with those of other studies that showed that replacement of isoflavones by soy protein in the diet for 4 years ended up the significant decrease in proteinuria and urinary creatinine [25] [26].

Others indicated that consuming the soy because of its isoflavones can cause a significant decrease in serum glucose of diabetic patients. One reason for the effect of soy isoflavones can be due to increase or activation of mitochondria in skeletal muscle that cause reduction in blood sugar or can increase insulin as an antidiabetic hormone [27]. In a recent study it was also found that the use of dietary soy protein in the short term, did not result in reduction in blood glucose, however, in the long term, different doses of soy protein diet could impact blood sugar levels and reduce it. Therefore, effect of soy on blood glucose levels could be time-dependent.

AlT and AST enzymes are released from the liver parenchyma cells and are the most reliable markers of liver disease due to chronic injury of liver cells and necrosis [28] [29] [30]. In the present study, different diets did not show significant change in the level of these enzyme indicating that soy protein cannot cause liver damage.

Some studies also show an increase in serum alkaline phosphatase (ALP) level in the group receiving soy diet for 6 months [31] [32]. However, in the present study there was no significant change in ALP levels.

The increase in values of the liver enzymes AST is not an specific marker for the hepatic damage [28] [30]. Therefore, in the present study three types of liver enzymes were tested and they did not show significant changes in experimental groups indicating that soy protein did not influence hepatic enzymes. Numerous studies have reported AST increase in acute liver injuries [28] [30]. Others studied the effects of black soya beans on AST liver and found that serum AST levels showed significant decrease compared to the groups without soya diets and found out that total protein concentration in various diseases was greater than normal [33] [34]. The mean value of total protein has been assessed in liver disease, kidney and bone marrow [35]. In the present study, TOP level in the soy diet for 3 months was not changed significantly compared to the control group, however the consumption of soya for longer than 6 months, significantly increased the value of TOP. Globulin is one of the most important types of serum total protein, therefore, it could be concluded that soy consumption has played a role in strengthening the immune system.

The results of the present study showed that phosphorus intake in diets containing soy foods were lesser than in diets containing the animal protein. This might be due to insufficient intake of phosphorus in the study period or low levels of phosphorus in soy products [36]. However, in the present study, the men value of phosphorus was not changed significantly compared to that of the control group. Therefore, consumption of soy did not affect serum levels of phosphorus.

Various studies suggest the effects of soy on the absorption of minerals such as calcium from the intestines. Numerous studies have shown that soy bears the highest phytic acid that could inhibit the absorption of calcium in the intestine, however, where fermented soy is consumed in long-term, it almost could reduce the phytate soybean and increase calcium absorption from the intestine. The present study showed that the amount of calcium in the group of 20% soybean consumer, within three months was increased compared to the group of free soy protein consumption. This finding was consistent with the those of others [37]. Therefore, where soy diet is recommended, it should be supplemented with vitamin D3.

5. Conclusion

Based on the findings of the present study, soy as an inexpensive protein can be replaced by animal protein. In addition, the effects of soy on reduction of blood serum cholesterol, LDH and urea levels as the harmful factors that can cause various diseases, seems essential in the diet. However, Overuse of soy is not recommended.

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