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Original article

Effect of soy protein on hypercholesterolemia and hypertension to reduce the risk of cardiovascular diseases

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Summary. High blood cholesterol and hypertension are the leading causes of cardiovascular diseases which are one of the leading causes of death worldwide. The current research study was designed to isolate soy protein to be used as nutraceutical agents against hypercholesterolemia and hypertension. Soy protein was extracted and isolate was given to rats for 4 weeks after inducing hypercholesterolemic and hypertensive conditions. Blood samples were collected and analyzed for lipid profile (cholesterol, triglycerides, low density lipoprotein and high density lipoprotein) and serum nitric oxide on 14th and 42nd days of study. The results re vealed significant decrease in total cholesterol (120.00 to 110.00 ml/dL), triglycerides (87.66 to 74.00 mg/dL), and low density lipoprotein (67.00 to 47.66 mg/dL) while significant increase in high density lipoprotein (29.33 to 50.00 mg/dL) and nitric oxide (24.00 to 50.33 mg/dL) due to the uptake of soy protein isolate for a period of 28 days was observed.

Key words: soy protein, hypercholesterolemia, hypertension, cardiovascular diseases

Introduction

Globally most of the deaths are caused by heart diseases, liver diseases and cancers. Among these, cardiac ailments are major contributors (1). Main CVD risk factors are high blood pressure, diabetes, hyperlipidemia, obesity and inflammation. Dietary modifications are advised for cardio-protective effects, for that plant foods are recommended because they can regulate blood plasma concentrations (2). Healthy changes in diet can lower the incidence of cardiovascular disease by lowering risk factors and in specific, legumes are highlighted as part of heart friendly diet, so augmented ingestion can lower weight and blood glucose, high blood pressure, and can manage dyslipidemia (3).

Soybeans are certainly the topmost oilseed produced globally as it is extensively cultured for their lipid content. Moreover, soybeans are familiar as a valued source of nutrients as they comprise high-quality protein (~40%); carbohydrates, poly unsaturated fatty acids (PUSFA, 18%) and dietary fibers. Soybean is a cherished legume because it contains all essential amino acids which human body can't produce; but it is comparatively deficient in sulfur containing amino acids, cysteine and methionine. It can be ingested as a complete protein. Soybean contains about 37 to 42% of protein. The two chief proteins of soybean are 11S glycinin, and 7S β-conglycinin, both have globular structure (4).

Due to having all essential nutrients, soybeans are known to be health friendly. These health benefits are attributed to essential amino acids, bioactive peptides, unsaturated fatty acids, secondary metabolites such as isoflavones, anthocyanins etc. The protein part of soybeans is comprised of 37-45%, of which 70 to 83 are glycinin and beta-conglycinin that is storage proteins. Besides providing basic functions such as maintenance, healing, soy derived proteins also prevent from developing dangerous diseases. Some proteolytic enzyme inhibitors prevent and treat the cancers of colon and rectum without developing toxicity in normal body cells (5).

Soybeans can reduce bad cholesterol and this effect is proven by several research studies. It can lower 12.9% of this bad cholesterol. Food and drug administration has approved a health claim regarding soybean consumption which states that daily consumption of 25g of soy prevent can lower the incidence of heart diseases (2). Soy protein is usually used to substitute animal proteins in diet. The ingestion of soy protein is considered to lower down high blood cholesterol and LDL-cholesterol levels (6). Soy protein hence prevents from cardiovascular diseases as it reduces intestinal cholesterol absorption and increases fecal cholesterol excretion (7).

The proposed mechanisms for decreasing the incidence of cardiovascular diseases are dilation of blood vessels, inhibition of platelets accumulation with vessels walls, check the relocation and propagation of smooth muscle cells, and lessening the sticking of some compounds to the vascular endothelium such as cholesterol by enhancing the excretion of bile through feces or reducing the absorption of cholesterol in small intestine. Undigested soy peptides carry with them the bile acids out of the human body and prevent cholesterol absorption. Beta- conglycinin, one of the major proteins of soy, lowers blood triglyceride level and keep body fat mass lowers. These biologically active peptides also possess effects against high blood pressure, oxidative stress, obesity, immune system diseases, diabetes, high blood cholesterol and cancer (8-10)

Keeping in view the above described themes, this research study was preliminary designed to isolate soy protein to be used as nutraceutical agents and to conduct in vivo studies for effectiveness of soy protein against hypercholesterolemia and hypertension

Materials and Methods

The study was conducted in Food Microbiology and Biotechnology Laboratory, National Institute of Food Science and Technology (NIFSAT), University of Agriculture, Faisalabad (UAF). Soy beans were obtained from local market. NaOH, HCl, H_2SO_4, digestion tablets, Hexane and Cholesterol were obtained from Food Microbiology Lab, NIFSAT, UAF.

Preparation of soy protein isolates

Soy protein was isolated from soy beans following the protocol given by L'hocine (11). Intact soybeans were first broken down into three or four pieces to separate the husk and then ground into fine pieces. Then ground soy meal 250g were mixed with hexane in the ratio of 1:2. The meal and solvent mixture was stirred for half an hour and then allowed to get settled after which the solvent layer was drained off. The hexane was used to de fat the soy meal. After removal of hexane, the mixture was washed with fresh water and then left overnight to dry at room temperature.

Water was added in defatted meal in the ratio of 1:15, heated up at 55°C, and the pH 9.0 was adjusted by adding 2N NaOH and stirred continuously for 40 minutes. The mixture was then allowed to cool down at room temperature. The slurry was then centrifuged at 14300g for half an hour and the temperature of centrifuge was maintained at 4°C. The supernatant was collected in a separate flask and the pH was set at 4.5 by using 2N HCl and was stirred for 45 minutes at 25°C. It was again centrifuged at 2830g for 15 minutes at 4°C. The precipitate thus obtained was washed with water and again centrifuged at 2830g for 10 minutes twice. The washed precipitate was suspended in water and pH was adjusted at 7.0 by using 2N NaOH. The precipitates were then freeze dried and stored at refrigeration temperature.

Crude protein of soy powder and soy protein isolate

Protein content of soy powder was determined by Kjeldhal method by using Kjeltech Apparatus (Model: D-40599, Behr Labor Technik, Gmbh-Germany, Method No: 984-13) as per procedure described in AOAC (12).

Efficacy Study/Experimental protocol

To evaluate the role of soy protein on hypercholesterolemia and hypertension, rats as an experimental model were used. Purposely, fifteen rats were acquired and housed in animal room of National Institute of Food Science and Technology, University of Agriculture, Faisalabad. Rats were acclimatized for a period of 1 week by the provision of regular diet and water ad libitum. Afterwards rats were divided into three groups; each comprising of five rats. The group G_1 was given normal diet, G_2

and G_3 were given high cholesterol diet for specific period to induce hypercholesterolemia and hypertension. After induction of hypercholesterolemia and hypertension, the baseline values for different biomarkers (total cholesterol, triglycerides, low density lipoprotein, high density lipoprotein and nitric oxide) were recorded. Then, the G_2 was started to feed normal diet whereas G_3 was given soy protein isolate diet for a period of one month. After one month, the above mentioned parameters were again recorded. During entire experimental period, animal room was maintained at a temperature and relative humidity of 23±2°C and 55±5% respectively, with 12:12 hours light: dark cycle (13).

Study parameters

Body weight and feed intake were measured on weekly basis. Lipid profile and nitric oxide levels were observed twice during whole study *i.e.* at 14th and 42nd days. For this purpose, rats were fasted overnight. Blood samples of rats were collected through cardiac puncture in EDTA coated tubes for study and non-coated tubes to measure serum lipid profile and nitric oxide level through Microlab-300, Merck, Germany.

Body weight gain

Increase in body weight of rats from all experimental groups was measured weekly throughout the study period to analyze the effect of soy diet on body weight.

Feed intake

Feed intake of rats of all groups was measured weekly throughout the study period to analyze any effect of high cholesterol diet or soy protein diet.

Determination of serum lipid profile

Serum lipid profile of rats including cholesterol, high density lipoproteins (HDL), low density lipoproteins (LDL), and triglycerides were measured by following protocols.

Estimation of triglycerides

Triglycerides in serum sample were estimated by liquid triglycerides (GPO-PAP) method as illustrated by (14). Three test tubes were taken and labeled as blank, standard and sample. Triglyceride reagent (1mL) was added in these tubes and heated at 37°C for 4 minutes. Then 0.01 mL of blood sample was added. The mixture of reagent and blood was mixed properly and then placed in incubator at 37°C for 5 minutes. Then absorbance of all solutions was observed. For this, spectrophotometer was used and its wavelength was adjusted at 520 nm.

Estimation of total cholesterol

Serum cholesterol level of rats was measured using CHOD-PAP method following the protocol of Kim (15). Three test tubes were taken and labeled as blank, standard and sample. One mL of reagent was added in these tubes and heated up to 37°C for 2 min. Then blood sample (0.01ml) was transferred in these tubes, shaken and placed in incubator at 37°C for 10 min. The absorbance by contents of all tubes was then observed by using spectrophotometer whose wavelength was adjusted at 250nm.

HDL

High density lipoproteins (HDL) in serum samples were calculated by method as mentioned by Alshatwi (16). Two tubes were taken and labeled control and sample. Equal amounts of serum and HDL cholesterol reagent was added in these tubes and mixed them thoroughly. Then the mixture was centrifuged at 1500-2000 rpm for 10 min. and separated the supernatant. The supernatant was considered as sample and processed further to calculate high density lipoprotein cholesterol. The absorbance by supernatant was measured by placing in spectrophotometer whose wavelength was adjusted at 520nm.

LDL

Low density lipoproteins (LDL) in serum samples were calculated by method as mentioned by Alshatwi

Table 1. Composition of diet g/100g

Ingredient	Normal diet	High fat diet
Wheat flour	65 g	45.5 g
Chickpea flour	20 g	14 g
Milk powder	15 g	10.5 g
Fat	----	30 g *
Energy	360.5 kcal	522.4 kcal

** Animal fat 20g, vegetable fat 10g*

(16). Blank, Standard, and Sample tubes were labeled appropriately. Reagent (1000µL) was transferred to all tubes and pre-warmed at 37°C for 2 min. Then sample (100 µL) was added to all the tubes, mixed and returned to 37°C. All the tubes were incubated at 37°C for 5 min. After that, absorbance of all tubes was measured by using spectrophotometer whose wavelength was set at 546 nm.

Nitric oxide (NO)

NO determination was carried out by Griess assay presented by (17). Firstly, serum proteins were removed by ultrafiltration with 10 kd rnicron at 4°C, 130,000 rpm. Then, nitrate in the serum was reduced with nitrate reductase and β-NADPH for 3 h. After that, β-NADPH was removed by 10 min incubation with 80 mM α-ketoglutaric acid and 1 M NH_4Cl. The amount of nitrite was measured by adding 150 µL of Griess reagent (Promega, USA). At last, the absorbance of the chromophores formed was read by using spectrophotometer at wavelength 540 nm.

Statistical analysis

All the data attained during the study was analyzed using a software Statistic 8.1 as described by Montgomery (18).

Results and Discussion

Crude protein of soy meal and soy protein isolate

The concentration of protein was 34.32% in soy powder as it contained all other nutrients such as carbohydrates, fats, minerals and vitamins. When soy protein was isolated from soy meal, the percentage of protein got enhanced as it was only protein. The concentration of protein in soy protein isolate was 81.2% as shown in Figure 3. It indicates that percentage of protein got enhanced by two folds in soy protein isolate as compared to that of soy meal.

Weight of rats

The weight of rats was observed on weekly basis. Rats who were fed normal diet gained more weight from 131.00g to 196.20g which was 65.2g during six weeks of study, then comes the group of rats who fed high cho-

Figure 1. Preparation of soy protein isolates

Figure 2. Efficacy study to determine the effect of protein isolates on health.

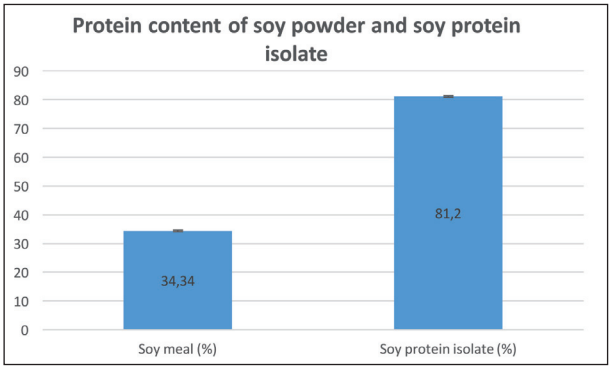

Figure 3. Protein content of soy powder and soy protein isolate

lesterol diet whose weight increased from 121.00g (on 1st week) to 201.00g (on 2nd week) which was 80g. The lowest body weight was observed in the group who was treated with soy protein isolate (Table 2). These readings have shown that soy protein isolate had significant effect in reducing weight and high cholesterol diet had remarkable effect in gaining weight.

The rats given normal diet, high cholesterol diet and high cholesterol plus soy protein isolate shown highly significant increase in weight. But the trend of increase was same with the passage of time that may be due to the increase in body size. The change in weight was consistent. This study has shown that as rats get older with days their weight got increased. But the effect of treatment on weight of rats shows that rats who fed normal diet gain more weight, then comes the group of rats who fed high cholesterol diet and the least weight gain was recorded in group which was fed soy protein isolate diet. It indicates that G_2 who took soy protein isolate diet gained less weight as compared to those who fed normal and high cholesterol diet.

Result of this study can't be co-related with previous research work mentioned by Kobayashi (19). Work by this scientist showed no significant change in body weight of rats either by consuming high cholesterol diet or soy protein isolate diet.

Feed intake

The purpose of this observation was to find out the effect of cholesterol and soy protein isolate supplementation in normal diet on diet consumption pattern. The values of feed intake of rats are given in Table 3. Effects of days and combined effect of days and treatment are highly significant as they increased the uptake of feed but effect of treatment on feed intake of rats is non-significant as it remained almost constant in all groups.

First group who received normal diet had minimum consumption of 19.800g at day 1 and maximum consumption of 23.400g at day 42 which showed increment of 3.6g during six weeks of study. Second group who received high cholesterol diet had minimum consumption of 21.20g recorded at day 1 and maximum of 22.80g on day 42 which showed increase of 1.6g during entire study. Third group who received soy protein isolate diet had minimum consumption of 22.80g on day 21 and maximum of 23.00g on day 42 which showed increase of 0.2 g during 4 weeks. The effect of treatment on diet consumption pattern was non-significant which indicates that high cholesterol, soy protein isolate diet has no effect on consumption pattern.

A slight increase in feed intake of rats was observed as they get older day by day. The results regarding effect of treatments on rats feed intake shows that there was no change in feed intake of rats either the diet was sup-

Table 2. Effect of treatments and days on weight of rats

Treatment	Days							Mean
	0	7	14	21	28	35	42	
G_0	131.00±1.00^{o}	146.00±1.58^{m}	167.00±1.58^{i}	182.80±1.30^{f}	188.80±1.30^{d}	196.20±0.83^{c}	211.20±0.83^{a}	174.71^{a}
G_1	121.00±1.58^{p}	136.00±1.58^{n}	154.00±1.58^{l}	160.00±1.58^{k}	173.00±1.58^{h}	186.00±1.58^{e}	201.00±1.58^{b}	161.57^{b}
G_2	118.80±5.01^{p}	131.80±2.58^{o}	144.00±1.58^{m}	162.40±2.70^{j}	165.40±1.51^{i}	175.80±1.09^{g}	191.00±0.70^{d}	155.60^{c}
Mean	123.60^{g}	137.93^{f}	155.00^{e}	168.40^{d}	175.73^{c}	186.00^{b}	201.07^{a}	

Values are given as Mean ± Standard deviation, Different small letters in rows and columns show significance

Table 3. Effect of treatments and days on feed intake of rats

Treatments	Days							Mean
	0	7	14	21	28	35	42	
G_0	19.80±0.83^{hi}	20.60±0.54^{gh}	20.20±0.83^{g-i}	3.40±0.54^{ab}	23.400±0.54^{ab}	23.800±0.83^{a}	23.400±1.14^{ab}	22.200^{a}
G_1	21.20±0.83^{e-g}	21.00±0.70^{fg}	21.80±0.83^{d-f}	23.200±0.83^{a-c}	22.400±1.14^{b-d}	23.000±0.70^{abc}	22.800±0.83^{abcd}	22.086^{a}
G_2	19.20±0.83^{i}	22.20±0.83^{c-e}	21.80±0.83^{d-f}	22.800±0.83^{a-d}	22.800±0.83^{a-d}	22.800±0.83^{abcd}	23.000±0.70^{abc}	22.086^{a}
Mean	20.06^{c}	21.26^{b}	21.26^{b}	23.133^{a}	22.867a	23.200^{a}	23.067^{a}	

Values are given as Mean ± Standard deviation, Different small letters in rows and columns show significance

plemented with cholesterol or soy protein isolate. Current results highly correlate with earlier research findings of Kobayashi who reported non-significant effect on food intake, food efficiency and total energy intake after supplementing the diet with either cholesterol or soy protein isolate (9).

Total cholesterol

Soy protein possesses hypocholesterolemic properties in both normal and hypertensive subjects. Serum levels of total cholesterol are presented in Table 4. The highest value after 14 days for cholesterol 123.33ml/dl and 120.00ml/dl was measured in groups provided with high cholesterol diet trailed along by normal cholesterol level 90.00ml/dl in rats given normal diet. A decrease from 123.33±3.055 to 121.33±3.055 (2ml/dl) was observed in second group and decrease from 120.00±5.00 to 110.00±3.00 (ml/dl) was observed in third group which was fed soy protein isolate. Results revealed 8.51% decrease in total cholesterol of rats of third group due to intake of soy protein isolate.

After inducing hypercholesterolemia, when rats were given soy protein diet there was reduction in their serum cholesterol levels. By the 2nd week of study, group 2nd and 3rd had highest blood cholesterol levels but by the 6th week the serum cholesterol of third group i.e. the group given soy protein diet reduced noticeably while that of members of second group remained same.

Cholesterol level must be below 200mg/dl as this concentration is considered as ideal. People having cholesterol 240mg/dl or more are at high risk of developing heart diseases. Current results are highly correlate with earlier research findings presented by kawakami (19) which showed a decrease in plasma cholesterol level by 10% in hypercholesterolemic subjects after ingesting soy protein isolate. Another finding by Kawakami (19) showed decrease in serum cholesterol which was attributed to soy protein isolate intake. Kobayashi (9) supported the current investigation of total cholesterol depended on the amount of isoflavone aglycones ingested along with soy protein. Wang (20) found significant reductions in plasma total cholesterol (8.4%, $P<0.001$) when subjects having high cholesterol levels ingested the diets having soy protein compared with the animal protein diets.

Triglycerides

The highest value of triglycerides was 87.66 mg/dl in group third after that second highest value was 85.00 mg/dl in group second as both these groups were fed high cholesterol diet. The lowest value of triglyceride was 72.66 mg/dl in group first because it was fed with normal diet. After 28 days, the lowest value for TG was 74.00 and 74.66 mg/dl in first and third groups respectively. This decrease from 87.66 mg/dl to 74.00 mg/dl in third group was due to treatment with soy protein isolate. Triglyceride was also decrease in second group from 85.00 mg/dl to 84.00 mg/dl. Results revealed that triglycerides level was decreased by 9.60% in third group due to consumption of soy protein isolate (Table 5).

Rats given high cholesterol diet had more triglycerides while the rats given treatment after inducing hypercholesterolemia showed decrease in TG levels almost near to group consumed normal diet. Trend of increase or decrease in serum TG due to effect of days is shown in Graph 4.8. The TGs of 2nd group members remained highest at the 2nd and 6th week but that of the animals of 3rd group got decreased at week 6 after getting treatment.

Current data is in collaboration with previous research work illustrated by Yoon (21) which showed

Table 4. Effect of treatments and days on total cholesterol (TC) of rats

Treatments	Days		Means
	14	42	
G_0	90.00±5.00^{d}	99.67±4.50^{c}	94.83^{c}
G_1	123.33±3.05^{a}	121.33±3.05^{a}	122.33a
G_2	120.00±5.00^{a}	110.00±3.00^{b}	115.00^{b}
Means	111.11^{a}	110.33^{a}	

Values are given as Mean ± Standard deviation, Different small letters in rows and columns show significance

Table 5. Effect of treatments and days on triglycerides (TG) contents

Treatments	Days		Means
	14	42	
G_0	72.667±2.51^{b}	74.000±1.00^{b}	73.333^{b}
G_1	85.000±5.00^{a}	84.000±5.00^{a}	84.500^{a}
G_2	87.667±5.03^{a}	74.667±1.52^{b}	81.167^{a}
Means	81.778^{a}	77.556^{b}	

Values are given as Mean ± Standard deviation, Different small letters in rows and columns show significance

that plasma TG levels were significantly decrease in the group given soy protein isolate. When subjects were provided soy protein isolate for consumption by quitting high cholesterol diet the serum triglycerides level decreased significantly (11.9 %, P<0.0001). Another study presented by Wang (20) showed decrease in serum triacylglycerol by 12% when subjects were given soy diet. The reduction in plasma TG levels was consistent, decreased by 12.3% (P = 0.018) after subjects consumed the soy protein diet relative to animal protein containing diets.

High density lipoprotein (HDL)

There is an inverse relationship between high density lipoprotein cholesterol (HDL) concentration and development of coronary artery disease. More the amount of HDL in plasma, lesser will be the chances of getting cardiac ailments. In the current study, at 14th day, the highest HDL was 52.00 mg/dl in 1st group. The second highest was 29.33 mg/dl in 3rd group while lowest was 27.66 mg/dl in 2nd group. Third group showed 22.66% decline in HDL while second group showed 24.33% decline in HDL after consuming high cholesterol diet. As HDL is termed as good cholesterol, so consumption of high cholesterol diet declined its level in Group 2 and Group 3 as members of both groups ingested high cholesterol diet. But HDL remained normal in Group 1 because they were on normal diet. At 42nd day, the group 3 showed remarkable increase in HDL from 29.33 mg/dl to 50.00 mg/dl which was 20.67%. The Group 2 also showed increase in HDL from 27.66 mg/dl to 30.00 mg/dl which was 2.33% and may be due to cessation of high cholesterol diet and shifting to normal diet (Table 6).

In the current study, the group three after getting treatment for hypercholesterolemia shown increase in serum HDL while that of group two remained same because this group didn't get any treatment. Group one also showed normal range as it was on normal diet balance in all nutrients. After giving high cholesterol diet, the HDL levels of both group second and group third got decreased as compared to that of group first. But by the 6th week, group 3 shown remarkable increase in HDL comparable with that of normal group.

Kobayashi found that daily soy beans consumption alleviates bad cholesterol due to the formation of equol from soy isoflavone daidzein (9). The ingestion of high soy protein diet for a period of one month resulted in 17% increase in HDL level in hypercholesterolemic rats.

Table 6. Effect of treatments and days on high density lipoprotein (HDL) contents

Treatments	Days		Means
	14	42	
G_0	52.00±3.00[a]	52.00±2.64[a]	52.00[a]
G_1	27.66±2.51[b]	30.00±2.00[b]	28.83[c]
G_2	29.333\±2.51[b]	50.00±2.00[a]	39.66[b]
Means	36.33[b]	44.00[a]	

Values are given as Mean ± Standard deviation, Different small letters in rows and columns show significance

Low density lipoprotein (LDL)

Soy protein decreases low density lipoprotein in hypercholesterolemic subjects. The results showed a highly significant decrease in low density lipoprotein. The effect of days, treatment and combined effect of days and treatment was highly significant in reducing low density lipoprotein in hypercholesterolemic subjects in which soy protein isolate was ingested (Table 7). At 14th day, the highest LDL level 67.00 mg/dl was observed in third group, the second highest 64.66 mg/dl was observed in second group and lowest 48.00 mg/dl was observed in first group. The first two highest values were due to consumption of high cholesterol diet. Then at 42nd day, the highest mean 64.33 mg/dl was observed in 2nd group while lowest means 47.66 mg/dl and 47.66 mg/dl were observed in 1st and 3rd group respectively. Third group showed a remarkable decrease from 67.00 mg/dl to 47.66 mg/dl and this was due to shift from high cholesterol diet to diet containing soy protein iso-

Table 7. Effect of treatments and days on low density lipoprotein (LDL) content

Treatments	Days		Means
	14	42	
G_0	48.00±1.00[b]	47.66±2.08[b]	47.83[c]
G_1	64.66±5.50[a]	64.33±4.50[a]	64.50[a]
G_2	67.00±6.24[a]	47.66±1.52[b]	57.33[a]
Means	59.88[a]	53.22[b]	

Values are given as Mean ± Standard deviation, Different small letters in rows and columns show significance

late. Means of Group 2 at 42nd day were remained almost same 64.66 mg/dl and 64.33 mg/dl because it was not given soy protein isolate diet. 14.42% decrease was observed after treatment with soy protein isolate diet in group 3.

The ingestion of high cholesterol diet increased the level of LDL in both Group 2 and Group 3. The level dropped down in Group 3 when given soy protein while remained high in Group 2. The results further showed that at 2nd week, LDL was high in Group 2 and group 3. At 6th week, the level got decreased in Group 3 after getting treatment for 4 weeks.

Results of present project are comparable to earlier studies of Wang (20) who reported that when subjects having high cholesterol were fed with soy diet the serum low density lipoprotein showed a significant decrease (17%, P = 0.003). Kobayashi found that equol, a metabolite of daidzein decreased plasma low density lipoprotein cholesterol (9). But this reduction was depended upon the amount of soy foods consumed daily. In his study, serum LDL cholesterol got elevated after provision of high cholesterol diet. But when treated with diet containing 10% and 15% soy protein its level got decreased by 16% and 18% respectively.

Serum nitric oxide (NO)

Nitric oxide is an inter and intra-cellular signaling molecule that plays important roles in many physiological and pathological processes, including vasodilation, transmission of signals along neurons, modulation of immune system, cardiac contraction, inhibition of platelet accumulation, stem cell differentiation into macrophages and their proliferation (22).

Serum nitric oxide levels are given in Table 8. At day 14th, the lowest mean 24.00 mg/dl was observed in Group 3, the second lowest 27.66 mg/dl was observed in Group 2 while highest 49.33 mg/dl was observed in Group 1. The lowest means were due to high cholesterol diet which decreased serum NO level and highest was due to normal diet as it didn't contain high cholesterol. At day 42nd, the highest mean 50.66 mg/dl was observed in first group which was on normal diet throughout the project. The 2nd highest 50.33 mg/dl was observed in third group. The significant increase from 24.00 mg/dl to 50.33 mg/dl was due to provision of diet containing soy protein isolate and decline in level of high cholesterol in diet. The means of 2nd group were changed from 27.66 mg/dl to 29.00 mg/dl which was due to shifting on normal diet from high cholesterol diet. This indicates that normal cholesterol in diet can also improve serum NO but significant increase happened when diet was high in soy protein. Almost 23.9% increase in serum NO was observed as compared to that of control group after feeding soy protein isolate. Till 2nd week, serum NO levels fell down in Group 2 and group 3 but at 6th week the level increased in Group 2. When Group 2 and Group 3 were given high cholesterol diet serum NO levels got decreased, but when Group 3 was given soy protein diet its level increased comparable to that of normal group.

Hypertension is caused when systolic blood pressure (SBP) get increased to 140 mm·Hg and/or diastolic blood pressure (DBP) to 90 mm·Hg. Primary hypertension accounts for 90% of all cases and although the root cause is unclear, the vital issues are unhealthy diet, smoking, stress, obesity, and possibly genetics. Hypertension increases the risk of blood vessels injury through Inflammation, hence aggregate the risk for cardiovascular diseases. Angiotensin II causes constriction of blood vessels; its level gets increased due to high blood pressure and elevates the peroxidation of lipids, which in turn generates low density lipoprotein cholesterol and free radicals. Soy beans have some constituents who can alleviate the chances of developing high blood pressure by producing vasodilator. Soy isoflavone is considered to form nitric oxide which is a key vasodilator (3).

Results of present project are comparable to earlier studies. Park (17) found that NO concentration was significantly elevated in the soy protein isolate group, compared to the control group, a difference of 40% (48.4±8.9 v 29.8±2.0, $p<0.05$).

Table 8. Effect of treatments and days on Nitric Oxide (NO) contents

Treatments	Days		Means
	14	42	
G_0	49.333±1.52[a]	50.667±1.52[a]	50.000[a]
G_1	27.667±2.51[b]	29.000±2.00[b]	28.333[c]
G_2	24.000±2.00[c]	50.333±2.51[a]	37.167[b]
Means	33.667[b]	43.333[a]	

Values are given as Mean ± Standard deviation, Different small letters in rows and columns show significance

Conclusion

Serum cholesterol, triglycerides and low density lipoprotein levels were significantly reduced while serum high density lipoprotein and nitric oxide levels were significantly increased after getting treatment. From present research work it is concluded that high cholesterol diet raises blood cholesterol and blood pressure but when consumption of cholesterol is decreased and diet is supplemented with soy protein isolate it causes decrease in blood cholesterol and blood pressure near normal levels.

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