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EFFECT OF SOY PROTEIN ON ORGANS WEIGHT, SERUM LIPIDS, GLUCOSE , LIVER FANCTION,UREA AND CREATININE IN RATS FED HIGH – CHOLESTEROL DIETS .

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ABSTRACT

Effect of soy portion (10% & 20%) on serum lipids, glucose , function of liver and kidney in rats fed high – cholesterol diets (hypercholestrolemic rats). The body weight gain of rats fed on diets containing hypercholestrolemic rats +soy protein (10 % &20%) were significantly increased than rats fed on standard diets and hypercholestrolemic rats. There were significantly increased in weight of liver and kidney of rats fed on 10% & 20% soy protein. Results indicated that serum hypercholestrolemic rats + soy protein (10% & 20%) total cholesterol , triglycerides, low-density lipoprotein (LDL),high-density lipoprotein (HDL) and very low-density lipoprotein (vLDL) significantly decreased as compared with hypercholestrolemic rats. Also, results showed a significant increase in high-density lipoprotein (HDL),whereas hypercholestrolemic rats had a significant increase as compared with control. Also, results showed significantly decreased in low-density lipoprotein (LDL) as compared with control. Whereas serum hypercholestrolemic rats +soy protein (10 % &20%) serum glutamate pyruvate oxaloacetate transaminase (SGOT), glutamate pyruvate transaminase (SGPT) significantly decreased as compared with hypercholestrolemic rats and control groups, while had non significant as hypercholestrolemic rats and control. The urea & creatinine level were significant increased in hypercholestrolemic rats + soy protein (10 % &20%) as compared with hypercholestrolemic rats and control. Treatment of hypercholestrolemic rats with 10 % &20% soy protein diet showed an improvement in serum lipid profile, liver function and histopathology (liver& kidney). Encouraging people to increase their dietary intake of soy protein specially Atherosclerosis and hypercholesterolemia.

INTRODUCTION

Soy is derived from a soybean plant and has both protein and fat components as well as polysaccharide component (Araya *et al.*, 2004). Soy protein contains differing amounts of some amino acids when compared with both casein and whey protein. In two different studies, soy protein amino acid content was analyzed and then compared to either casein or whey protein (Aoyama *et al* 2000 ,Damasceno *et al.,*2001). When compared with casein, soy protein had higher amounts of arginine and lysine as well as a higher arginine to lysine ratio, but had lower amounts of glycine and methionine (Damasceno *et al.*,2001). When compared to whey protein soy protein had larger amounts of arginine, glycine and a higher ratio of arginine to lysine, but had smaller amounts of lysine and methionine. In both studies the differences in amino acid content of the proteins was believed to play a role in health effects from soy (Aoyama *et al.*, 2000). Soy protein, unlike casein and whey, also contains isoflavones, which are phytochemicals that could possibly play a role in the beneficial health effects seen from consumption of soy (Banz, *et*

al.,2004). Soy oil however does not contain significant amounts of isoflavones (Murphy, *et al.*, 1999).

Prospective, epidemiological studies have established that lipid and lipoproteins play a role as risk factors for atherosclerotic cardiovascular diseases (CVD). It is generally recognized that the higher the total cholesterol, the higher the mortalities of CVD.1-4In recent years, a great deal of interest has emerged in the role of soy-bean isoflavones in reducing heart diseases, and isoflavones might be responsible, in part, for the ability of soybean to lower the risk of CVD and Atherosclerosis(Munro *et al.*,2003). Review of 38 trials suggested that about 60-70% of the cholesterol lowering effect of soy protein may be due to its isoflavone content(Potter 1998). Isoflavones are a group of phytoestrogens which occur mainly in soy and its products receiving great scrutiny as food supplements for the purpose of both enhancing health and preventing several chronic diseases, including coronary heart disease, cancers of reproductive organs and osteoporosis (Lichtenstein 1998).

Dietary proteins have long been understood to be involved in the development of coronary artery disease (CAD), and, in the past 20 y, research has shown that plasma cholesterol decreases when soy protein is substituted for animal protein in the diets of hypercholesterolemic individuals Anderson, *et al.*,(1995). Some investigations have reported up to a 35% reduction in total plasma cholesterol, whereas others have reported no change or even slight increases (Carroll 1991).There are many different soy products suitable for human consumption and, depending on the range of conditions under which they were manufactured, they contain markedly different components. When equivalent amounts of protein are fed, isolated soy protein results in larger depressions in plasma lipids than does defatted soy protein . Furthermore, cholesterol concentrations are higher when amino acids patterned after soy are fed than when intact soy proteins are fed, although cholesterol concentrations are reduced compared with when amino acids patterned after casein or intact casein are fed (Potter, *et al.*, 1993).

MATERIALS AND METHODS

Materials:

Rats of 28 weanling male white albino rats of the sprague Dawly strain weighting about 114 - 120 gm were used All animals were put on balanced diet under the same condition of ventilation, temperature and care for 4 , 8 weeks and soy protein (table 1) Determination of body weight gain, food intake and relative organs weight: Daily food intake and total food intake per group were calculated throughout the experimental period. Weight gain of rats was calculated by the following [final weight (gm) – initial weight (gm)and the organs (Liver, kidney heart and spleen) . At the end of the experimental period, rats from each group were sacrificed by decapitation. Blood samples were collected into clean dry centrifuge tubes, and were left at room temperature until the clot is formed. The blood was centrifuged for 10 minutes at 3000 r.p.m to separate the serum. Serum was carefully aspirated and transferred into clean quite fit plastic tubes and kept frozen at -20º C until analysis.

Table 1:Nutritional composition of the tested diets (g/Kg).

Component	Standard diet (control G_1)	Hyperchole sterolemia (G2)	Hyperch. +soy protein 10 % (4Weeks) (G3)	Hyperch. +soy protein 20% (4Weeks) (G4)
Casein	100	100	-	-
Corn starch	350	340	350	250
Albumin	100	100	100	100
Glucose	298	298	298	298
Corn oil	50	50	50	50
Cellulose	50	50	50	50
Vitamin mixture	10	10	10	10
Mineral mixture	40	40	40	40
Choline cholirde	2	2	2	2
Soy protein	-	-	100	200
Cholesterol	-	10	-	-

Salt and vitamin mixture according to Horwitz(1980)

Methods:

Determination of total cholesterol was carried out according to Richmond (1973).Enzymatic colorimetric determination of triglycerides was carried out according to Wahalefeld (1974). Determination of HDL cholesterol was carried out according to the method of Lopes-Virella (1977). Determination of LDL cholesterol was carried out according to the method of Assmann, *et al.*, (1984). The vLDL was calculated according to the equation given by Lee and Nieman, (1996) as follow : vLDL= triglycerides ÷ 5 . Determination of serum glutamic pyruvic transaminase (SGPT)& glutamic oxaloacetic transalinase (SGOT) were carried out according to Bergmeyer. *et al.*,(1985).Enzymatic colorimetric method for glucose was determined according to Trinder (1969). Creatinine (mg/dl) by using sigma diagnostics Reagent kits were determined (Patton and Crouch,1977. Teitz, 1978).

Method of Histopathology

At selected time of surgery, rats were an anesthetized by surgically dissented and the liver and kidney was cut out of the body by surgical sterile blades into small pieces (1× 1× 0.3 cm) and immersed in 10% buffered formalin.

Fixation in formalin was kept overnight in three different changes. Tissues were then dehydrated in ascending grades of ethanol (70%, 75%, 80%, 90%, 100%) then kept in pure ethanol 100% for two changes, then in xylene in ethanol then in pure xylene. Tissues were then infiltrated by paraffin in xylene then embedded in pure paraffin.

Five thick sections were cut, placed on glass slides, stained by hemato xylene & eosin & were kept ready for histopathological examination and evaluation (Frankel and Reitman, 1963).

Statistical analysis:

Data from each diet were combined and analyzed using a computer. Two-ways analysis of variance (Statistical Package) was used to evaluate the effects of tested sweeteners and herbs on body weight gain, feed intake. Analysis of variance (ANOVA) of the data was performed with the MATAT-C

(Statistical Package) " A microcomputer program for the Design", Management and Analysis of Agronomic Research Experiments Michigan State. University, USA. Results are reported as mean ± SD by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

As shown in table (2) body weight and food intake increased significantly after addition of soy portion (10% & 20%) as compared with the hypercholestrolemic rats and control. This result agreed with these obtained by Hasegawa, *et al.*,(2000)and Armando, *et al.*, (2005) who indicated that there was significant increase in body weight gain of rats fed on soy portion diet.

Table2: Effect of soy protein diets on body weight and food intake as compared with control & hypercholestorlemic rats) (g/28day).

Groups	Body weight (g)		Body weight gain (g) B.W.G	Food intake / rat (g)	
	initial	Final		Daily Food intake	Total food intake g/ 28 day
G1Control Mean±S.D	115.5 ± 4.21	140.13 ± 6.12	24.62 ± 3.76	11.23 ± 3.63	308.41 ± 8.52
G2Hyperch. Mean±S.D	114.32 ± 5.85	173.12 ± 5.12	58.81 ± 3.89*	12.99 ± 3.26*	363.71± 7.61*
G3Hyperch.+soy protein 10% Mean±S.D	118.53 ± 3.14	186.76 ± 8.19	68.23 ± 6.32*	16.61 ± 4.12*	465.o8 ± 8.24*
G4Hyperch.+sooy protein (20% Mean±S.D)	120.41 ± 4.22	185.84 ± 7.14	75.11 ± 5.96*	17.13 ± 3.86*	479.64 ± 9.82*

Values are mean ± SD. * significant

Results presented in table (3) show that the treatment with soy portion (10% & 20%) were significant increase the liver and kidney weight as compared with, G2 hypercholestrolemic rats. On the other hand G2 hypercholestrolemic rats significantly decreased as compared with G1(the control). Although, heart and spleen did not significantly change than standard diet and hypercholestrolemic rats. These are an agreement with those obtained byU.S National Institutes of Health and National Heart. Lung, Liver and Blood Institute, (2002) and (Munro *et al.,*2003).

Table (4) show that feeding on hypercholestrolemic rats + soy portion (10% & 20%) were significantly decreased of the mean values of serum total cholesterol , triglycerides , low density lipoprotein cholesterol (LDL-C) and VLDL-C as compared with the hypercholestrolemic rats and control, while HDL-C increased significantly as compared with the hypercholestrolemic rats and control. This result agreed with those obtained by Setchell, *et al.,* (1998) and Weggemans and Trautwein (2003). Wangen*, et al* .,(2001) reported that soy protein has hypercholestrolemic effect as lowering total cholesterol and LDL-C, also, it has hypolipidemic effect as reducing total lipid. . Zhan and Ho(2005) reported that soy protein lowered low-density lipoprotein cholesterol (LDLC) by 5.25 percent.

Table 3: Effect of soy protein diets on relative organs weight as compared with the control and hypercholestorlemic rats.

Organs / Groups	Liver weight	Kidney weight	Heart weight	spleen weight
G1 Control Mean ± S.D	4.03 ± 0.11	3.96 ± 0.03	0.30 ± 0.02	0.28 ± 0.03
G2 Hyperch. Mean ± S.D	3.02 ± 0.90*	2.98 ± 0.01*	0.29 ± 0.05	0.28 ± 0.04
G3 Hyperch. + soy protein 10% Mean ± S.D	4.02 ± 0.92*	3.01± 0.29*	0.30 ± 0.01	0.29 ± 0.04
G4 Hyperch. + soy protein 20% Mean ± S.D	4.39± 0.49*	3.03 ± 0.41*	0.30± 0.03	0.30 ± 0.06

*** significant.**

Table 4. Effect of soy protein diets on serum lipid profile as compared with control and hypercholstorlmic rats

Lipid profile / Groups	Total cholesterol (Tch) mg/dl	Triglycerides (Tr.) mg/dl	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
G1 Control Mean ± S.D	93.96 ± 0.72	52.89 ± 1.08	46.04 ± 0.64	36.96 ± 0.62	10.48 ± 0.31
G2 Hyperch. Mean ± S.D	193.89 ± 1.13*	96.82 ± 0.39 *	38.69 ± 0.56 *	135.86 ± 0.42*	19.71 ± 0.46*
G3 Hyperch. + soy protein 10 % Mean ± S.D	87.82 ± 0.62 *	49.92 ± 0.53 *	49.05 ± 0.17 *	28.81 ± 0.09 *	9.95 ± 0.43 *
G4 Hyperch. + soy protein 20% Mean ± S.D	83.13 ± 0.75 *	46.75 ± 0.15 *	51.84 ± 0.47 *	21.91 ± 0.24 *	9.33 ± 0.75 *

***significant -low-density lipoprotein (LDL),high-density lipoprotein (HDL) and very low-density lipoprotein (vLDL) .**

Table (5)show that feeding on hypercholestrolemic rats + soy portion (10% & 20%) were significantly decreased of the mean SGOT and SGPT as compared with the hypercholestrolemic rats and the control . In this study it is shown that soy protein have non significant effete on serum glucose concentrations that it may be due to its isoflavone content. This result agreed with these obtained by Vedavanam, *et al.*,(1974).

Table (6)show that feeding on hypercholestrolemic rats + soy portion (10% & 20%) were increased significantly of the mean values of urea and creatinine as compared with the hypercholestrolemic rats and control . Gretz, *et al.*, (1989) indicated that a vegetarian diet seems to be superior to meat containing diet in delaying the progression of chronic renal failure. Agadzhanov (1984) studied the effect of two diets containing different proteins on the time course of clinical and characteristic in 60 patients with incipient chronic renal failure, One of the diets included protein of vegetable origin (85%). while the other one was animal protein (75%), the diet with vegetable protein produced a beneficial effect of the patients demonstrated as blood pressure and azotemia reduction.

Table 5: Effect of soy protein diets on liver enzymes as compared with control and hypercholestorlemic rats.

Lipid profile / Groups	SGOT U/l	SGPT U/l	Glucose mg/dl
G1 Control Mean ± S.D	8.46 ± 0.54	69.68 ± 0.48	135.48 ± 2.06
G2 Hyperch. Mean ± S.D	33.62 ± 0.47 *	73.45 ± 0.68*	135.46 ± 2.16 *
G3 Hyperch. + soy protein10% Mean ± S.D	6.98 ± 0.44 *	63.95 ± 0.39 *	135.01 ± 3.11 *
G4 Hyperch. + soy protein 20% Mean ± S.D	6.12 ± 0.34 *	61.84 ± 0.46 *	135.27 ± 0.44*

*** significant - serum glutamate pyruvate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) .**

Table 6: Effect of soy protein diets on urea and creatinine as compared with control and hypercholestorlemic rats.

Kidney function / Groups	Urea mg/dl	Creatinine mg/dl
G1 Control Mean ± S.D	18.14± 0.45	0.48 ± 0.39
G2 Hyperch. Mean ± S.D	22.82 ±0.36*	0.51 ± 0.24 *
G3 Hyperch. + soy protein10% Mean ± S.D	26.22 ± 0.29 *	0.59 ± O.53 *
G4 Hyperch. + soy protein 20 % Mean ± S.D	27.67 ± 0.16 *	0.62 ± 0.02 *

*** significant**

Results of histopathology:

Control (No hypercholesterolemia) revealed liver & kidney tissue displaying preserved lobular architecture, no inflammation, no fibrosis, no steatosis, liver tissue appeared normal and healthy. (Fig. 1&5).

Rats fed with hypercholestrolemic diet revealed fat infiltration in liver&kidny tissue. There were differences between rats in the same group but all revealed steatosis ,mild inflammation in portal tracts were also seen some and hepatocytes exhibited glycogen and protein nuclei.(Fig. 2 &6) .

The hypercholestrolemic rats treated with 10% and 20% soy protein diets revealed normal liver&kidny tissue displaying preserved lobular architecture, no steatosis, no inflammation, and all signs developed as result of hypercholesterolemia has disappeared in those groups. (Fig. 3,4,7 &8). In conclusion, results showed that soy protein increase the levels of HDL-c, decrease total cholesterol, triglycerides, LDL-c and VLAL-c significantly . Results suggest that using soy protein can decrease the risk of hypercholesterolemia and function of liver & kidney.

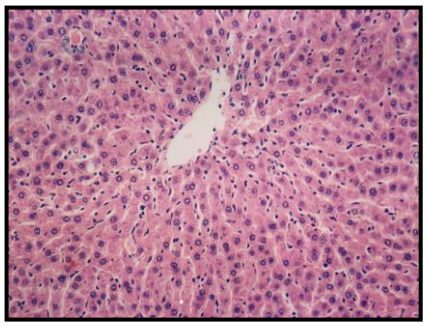

Fig. (1): Liver of rat as normal tissue showing no histopathological changes.

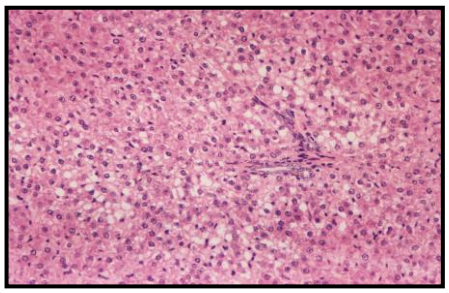

Fig. (2): Liver of rat from hypercholstorlmiegroup showing infiltration, steatosis and mild inflammation in portal tracts.

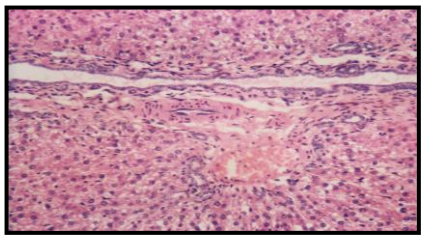

Fig. (3): Liver of hypercholstorlmic rat from group treated with 10% soy protein diet, showing mild improvement.

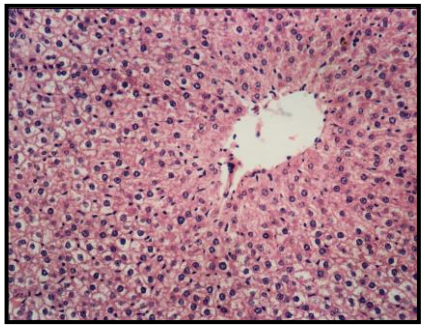

Fig. (4): Liver of hypercholstorlmic rat from group 4 treated with 20% soy protein diet showing mild improvement, fat infiltration and inflammation.

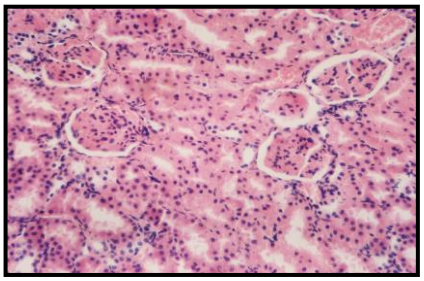

Fig. (5): Kidney of control group 1 showing the normal histological structure.

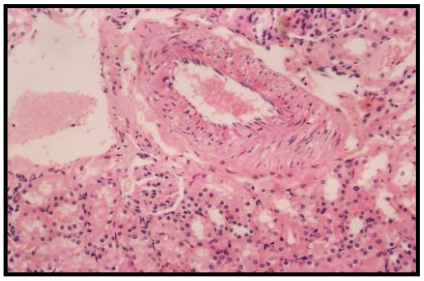

Fig. (6): Kidney of rat from hypercholstorlmie group, showing macrobiotic of renal tubular epithelium as well as intraluminal roteinaceous eosinophilic casts.

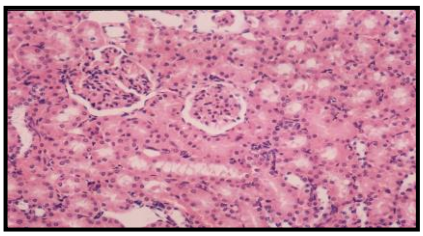

Fig. (7): Kidney of rat hypercholstorlmic rat from group 3 treated with 10% soy protein diet, showing apparent structure.

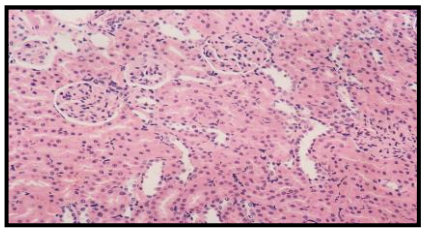

Fig. (8): Kidney of rat hypercholstorlmic rat from group 4 treated with20% soy protein diet Showing apparent mild improvement histological structure.

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تـأثير بـروتين الصـويا علـي ليبـدات السـيرم و الجلوكـوز وظـائف الكبـد و الكلـي فـي الفئران التي تغذت علي وجبات مرتفعة في الكوليسترول

جميل حبيب محمد حبيب
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استهدفت هذه الدراسة تـأثيربروتين الصـويا بنسبة (١٠%-٢٠%) علـي ليبـدات السـيرم ووظـائف الكبدوالكلي في فئران التجارب التي تغذت علي وجبات مرتفعة الكوليستيرول .
وقد ا وضحت النتائج الأتي :
-زيادة متوسط وزن الجسم المكتسب بدرجة معنوية في المجموعات المصابه بارتفاع الكولسترول+ بروتين الصويا بنسبة (١٠%-٢٠%) بالمقارنة بالمجموعة المصابة بارتفاع الكوليستيرول.يجد تغير معنوي في الطعام المتناول يوميا في المجموعة المرتفعة الكوليسترول وكذلك المجموعة بارتفاع الكولسترول+ بروتين الصويا بنسبة (١٠%-٢٠%) بالمقارنة بالعينة الضابطة.
- متوسط وزن الكبد و الكلي اقل في فئران التجارب المصاب بارتفاع الكوليسترول بالمقارنة بالعينة الضابط واعلي معنويا في العينات المصاب بارتفاع الكوليسترول+ بروتين الصويا بنسبة (١٠%-٢٠%) بالمقارنة بالعينة المرتفعة الكوليسترول .
-حدث نقص ملموس عند المعالجة بيروتين الصويا بنسبة (١٠%-٢٠%) في كل من الكولسترول الكلي و الجلسريدات الثلاثية والليبوبروتينات المنخفضة الكثافة والمنخفضة الكثافة جدا بالمقارنة بالمجموعة المرتفعة الكوليسترول و ارتفاع ملموس في مستوي الليبوبروتينات العالية الكثافة بالمقارنة بالمجموعة المرتفعة الكولسترول كما يوجد ارتفاع في الكولسترول الكلي و الجلسريدات الثلاثية والليبوبروتينات المنخفضة الكثافة والمنخفضة الكثافة جدا وانخفاض الليبوبروتينات العالية الكثافة بالمقارنة بالعينة الضابطة.
- يوجد انخفاض ملموس في وظائف الكبد عند المعالجة ببروتين الصويا بنسبة (١٠%-٢٠%) في المجموعة المصابة بارتفاع الكوليسترول بالمقارنة بالعينة الضابطة كما لا يوجد أي تغير معنوي في الجلوكوز.
-حدوث ارتفاع ملموس في وظائف الكلي (اليورياو الكريتينين) عند المعالجة ببروتين الصويا بنسبة(١٠%-٢٠%) بالمقارنة بالعينة المرتفعة الكوليسترول و العينة الضابطة.
- أظهرت النتائج الهستوباثولوجية للكبدوالكلي في المجموعة المصابة بارتفاع الكوليستيرول تغير في خلايا الكبدوالكلي من حيث ترسب الدهون والتهاب الخلايا الكبدية وخلابا الكلي . كما أوضحت المجموعات التي تم علاجها ببروتين الصويا بنسبة (١٠%-٢٠%) تحسن في نسيج الكبد و الكلي بدرجة معنوية بالمقارنة بالمجموعة المصابة بارتفاع الكوليستيرول و العينة الضابطة .
وتوصي الدراسة بإعطاء ببروتين الصويا بالنسب ألمقرره لمرض ارتفاع الكوليستيرول.