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BIOLOGICAL EFFECT OF PHYTOESTEROGEN ON HYPERCHOLESTEROLEMIC RATS

Abd El-M.H. Darwish[(1)] , K. A. Shaheen[(2)] and Eman M. S. A. Zaid[(2)]

[(1)] Chemistry Department of Nutrition and Metabolism,Institue of Nutrition-Cairo

[(2)] Nutrition and Food Science Dept., Faculty of Home Economics, Minufiya University, Shebin El- Kom, Egypt.

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ABSTRACT: *The present work was conducted to study the effect of different levels (5%, 10%,15%,20%, 25% and 30%) of Defatted soy flour and estrogen hormone on liver, kidney function and blood lipid profile in rats feeding with diet containing cholesterol.Fifty four female albino rats weighing of 130±5g were used and divided into night equl groups. One was kept as a control–ve group, while the other groups were fed on diet containing cholesterol(1%) for 3 weeks to obtain hypercholesteral rats. Body weight and food intake were recorded weekly. At the end of the experimental, all rats were weighted for calculation of body weight gain%, feed efficiency ratio and blood serum samples were used for estimation of liver and heart functions. Serum analysis showed a significant decrease in Cholesterol, Triglyceride and LDL in rat groups consumed different concentrations of defatted soy flour and estrogen hormone. While HDL was significantly increased in all rats groups comparing with control positive group and serum liver functions were significantly decreased. The obtained results concluded that an improvement of all chemical analysis as compared to positive control group.*

It can be recommended that dietary intake of defatted soy flour to decrease the side effects and reach to healthy condition. Further study should be conducted to carry out the pathological and safety investigation in concern to consume of defatted soya flour and esterogen hormone for a long period of time.

Key words: *Defatted soya flour, Rats, estrogen hormone, HDL, LDL cholesterol.*

INTRODUCTION

Phytoestrogens, or plant-based sources of estrogen, have received a great deal of attention as a way to treat and manage the symptoms of menopause. Research involving the phytoestrogens has increased consumer interest and subsequent demand for phytoestrogen-rich supplements and foods. This review focuses on the clinical evidence available on the phytoestrogens and their role in the management of menopausal symptoms and associated disease. Many types of phytoestrogens have been described, including coumestans, lignans, and isoflavones. The isoflavones are the best-researched phytoestrogens and are found in soy products, namely soybeans, tofu, and other soy derivatives. The two most prevalent isoflavones in soy foods are genistin and daidzein. Biochanin A and formononetin, precursors for genistin and daidzein, , are found in a variety of legumes and plant sources chickpeas, broad beans, green split peas respectively. The soybean is the richest source of isoflavones *(Candy, 2001)*.

Soybeans is a rich source of isoflavones.so defatted soya flour was used as isoflavone source in this experiment (every 100g.of soya contains 164.5mg) *(Bakker, 2004)*.

Coward et al., (1993), defined the phytoestrogen as: any plant substance or metabolite that induces biological responses in vertebrates and can mimic or modulate the actions of endogenous oestrogens usually by binding to oestrogen receptors.

Soybeans contain phytoestrogens, called isoflavones, which are believed responsible for soy's protective effect *(Rebecca,and Carol 2002).*

In person at high risk of cardiovascular events, a greater isoflavone intake is associated with better vascular endothelial

function and lower carotid atherosclerotic burden *(Yab-Hang, et al., 2007).*

Anderson et al., (1995) showed that, Soy protein (mean of 47 g/day)with isoflavonoids was associated with reductions in total cholesterol (9.3%), LDL cholesterol (12.9%) and triglycerides (10.5%), whereas the levels of HDL cholesterol showed no significant change.

Rebecca and Carol (2002), showed that Soy protein improves blood lipid levels. A meta-analysis of 38 controlled clinical trials regarding soy and cholesterol showed that consumption of soy protein is associated with a 9.3% decrease in total cholesterol, a 12.9% decrease in low-density lipoprotein (LDL) cholesterol, a 10.5% decrease in triglycerides, and a nonsignificant 2.4% increase in the high-density lipoprotein (HDL) cholesterol. The decreases in total and LDLcholesterol concentrations were strongly related to subjects' initial serum cholesterol concentration (ie, the higher the baseline cholesterol, the greater the reduction in cholesterol with soy protein). Soy protein intake in these studies averaged 47 g/day, and 37% of studies used 31 g/day or less.

MATERIALS AND METHODS

Materials:

1- Defatted soy flour was purchased from the Experimental Station, of Agriculture Research Center, Giza, Egypt
2- Diet cholesterol, casein, bile acids and cholin cholerid were purchased from El-Gomhoria Co, Cairo
3- Minerals and vitamins were purchased from El-Gomhoria Co.

Animals:

Fifty four female albino rats were obtaind from The Research Institute Ophtha Lmology and divided to nine groups of six rats , each rat housed individually in cylindncal metabolic wire cages .

-numbers from 1 to 6 groups were treated with several portions of defatted soya flour (5% - 10% - 15% - 20% - 25% - 30%). Group 7 is negative control, group 8 is positive control and group 9 is hormone group.

Animals and Experimental Design:

Rats were housed individually in well aerated cages under hygienic laboratory conditions. In animals house of Faculty of Home Economics, Minufiya University, and fed for one week on standard died for adaptation before the begining of the experiment according to NRC (1995).

Rats were divided into 9 groups, every 6 rats in each group fed on certain diet for 28 days as following :

Group (1): hypercholesterolemic rats fed on 5% defatted soya flour.
Group (2): hypercholesterolemic rats fed on 10% defatted soya flour.
Group (3): hypercholesterolemic rats fed on 15% defatted soya flour.
Group (4): hypercholesterolemic rats fed on 20% defatted soya flour.
Group (5): hypercholesterolemic rats fed on 25% defatted soya flour.
Group (6): hypercholesterolemic rats fed on 30% defatted soya flour.
Group (7): Rats fed on basal as diet negative control .
Group (8): Rats fed on hypercholesterolemic diet for 21 days and after that fed on basal diet as positive control.
Group (9): Rats fed on hypercholesterolemic diet for 21 days and after that fed on 0.5% estrogen hormone diet.

Methods

Food intake was calculated daily and rats were weighed weekly. Feeding and growth performance were carried out by determination of food intake, body weight gain and food efficiency ratio (FER) according to *Chapman et al., (1959)* using the following formula:

$$FER = \frac{\text{Body weight gain (g)}}{\text{Food intake (g)}} .$$

$$\text{Body weight gain (BWG\%)} = \frac{\text{Final weight} - \text{initial weight}}{\text{Initial weight}} \times 100.$$

At the end of the experiment peried (8 weeks) rats were starved for 12hr., then sacrificed under ether for anesthesia. Blood samples were collected into clean dry

centrifuge tubes and stored at room temperature for 15 minutes, then put into a refrigerator for 2 hour; after that centrifuged for 10 minutes at 3000 rpm to separate serum. Serum was carefully aspirated and transferred into dry clean Wasserman tubes using a Pasteur pipette and then kept frozen at (- 20C^{o}) till analysis.

Biochemical Analysis:

Serum total cholesterol, triglyceride (TG) and high density liprotein cholesterol (HDLc) were determined by using enzymatic colorimetric methods of *NIHP, (1987); Young and Pestaner,(1975) and Gordon and Amer,(1977)*, respectively. The determination of low density lipoprotein cholesterol (LDLc) and very low density lipoprotein cholesterol (VLDLc) were carried out according to the method of *Lee and Nieman (1996)* as follows:
VLDLc = TG/5 and LDL$_C$= Total Cholesterol – HDLc – VLDLc.

Atherogenic indices were calculated as Cholesterol/HDL-c and LDL-c /HDL-c *(Casterlli and Levitar, 1977)*.

Serum glucose and serum insulin were estimated according to *Asatoor and King (1954) and Wilson and Miles (1977).* Serum Aspartate and Alanine amino transferases (AST, ALT) and alkaline phosphatas (ALP) were determined by using enzymatic colorimetric methods *(Reitman and Frankel, 1957 and Haussement 1977)*.

Statistical Analysis:

Statistical analysis was performed by using computer program, Statistical Package for Social Science and compared with each other using the suitable tests *(SPSS, 1998)*.

RESULTS AND DISCCUTION

Data present in Table (1) show the effect of feeding with different concentrations (5%,10%, 15%, 20%, 25% and 30%) of defatted soya flour and esterogen hormone on food intake, body weight gain% and feed efficiency ratio in hypercholesterolemic rats.

The mean value of food intake of control positive group was 14.92g while the mean value of control negative group was 14.08g. The obtained results showed that there were non- significant differences except for 5%,10%, 15% and 20% tested groups as compared to positive groups.

The same table showed the highest mean value of BWG% was 63.11 for rats fed on control positive group. While the lowest mean value was 39.69 and 45.23g of rats fed on diet containing of 5% and 10% of defatted soya four. However, feeding rats on 30% gave the highest values (59.50) as compared with the other treatments.

There were a significant decrease in BWG% among all tested groups as compared to positive group.

As For FER, the results show that the mean value of all group were a non-significantly differences among all tested groups as compared to positive groups except for rats feeding 5% were significant ($P \leq 0.05$)

The mean values of food intake were almost the same in most of cases, food efficiency ratio showed a pronounced decrease as compared with the negative control. This reduction may be due to the decrease of body weight during the experimental period

The results agreed with Owiss (1999) who reported that cholesterol supplemented can decrease the body weight gain than that of control group but the difference was not significant

Table (2) illustrate the festing serum lipids and the effect of feeding with different concentration (5%,10%, 15%, 20%, 25% and 30%) of defatted soya flour and esterogen hormone on serum lipids in hyper cholesterolemic rats.

It could be observed that total cholesterol, TG and LDL decreased as the percentage of defatted soya flour in the diet increased there was very high significant difference in TC and TG of estrogen hormone group compared to positive group. As for HDL, the mean value of negative control was higher than that of positive control. While the mean values of groups fed on diets with 25%, 30% and estrogen hormone were more significantly higher than that of positive control.

Table 1 , 2

Concerning LDL, the mean value of negative control was lower than that of positive control. The mean values of all the groups were significantly lower than control positive.

The results agreed with *Anderson et al. , (1995)* they showed that, Soy protein (mean of 47 g/day) with isoflavonoids was associated with reductions in total cholesterol (9.3%), LDL cholesterol (12.9%) and triglycerides (10.5%)

Wong, et al. (2012), showed that soy isoflavones significantly lowered serum total and LDL cholesterol but did not change HDL cholesterol and triacylglycerol. Soy protein with or without isoflavones also significantly improved lipid profiles

Rebecca and Carol,(2002), showed that Soy protein improved blood lipid levels. A meta-analysis of 38 controlled clinical trials regarding soy and cholesterol showed that consumption of soy protein is associated with a 9.3% decrease in total cholesterol, a 12.9% decrease in low-density lipoprotein (LDL) cholesterol, a 10.5% decrease in triglycerides, and a nonsignificant 2.4% increase in the high-density lipoprotein (HDL) cholesterol.

But study of *Alicia et al., (2008),* showed that not in agreement, they reported that no significant effect on plasma LDL cholesterol from regular consumption of foods providing 24g soy protein/d from soy isoflavone in mildly hypercholesterolemic subjects.

Table (3) shows testing serum ALT, AST and ALP for control positive and different groups of hypercholestrolemic rats fed on different concentration (5%,10%, 15%, 20%, 25% and 30%) of defated soya flour and estrogen hormone. It's clear that ALT and AST for control positive was higher than control negative.

It could be observed the improvement of ALT and AST was highest for 30% diet (41.32 mg/dl) while was lowest for 5% diet (53.5 mg/dl) difference were high significant ($P \leq 0.05$) when compared with control positive group.

As regards the (ALP) activity, the highest improvement was estrogen hormone (138.18) being lowest was 5% diet (defatted soya flour) the values of serum ALT, AST and ALP were significantly decreased in all rats groups.

The results in Table (3) agreed with those reported by *Lucivalda et al., (2012),* who reported that protein supplementation diet reduced hepatic steatosis in both groups; however, a significant reductions in ALT levels occurred in the soy group however, not in agreement with those reported by *Dong et al., (2013)* they reported that the treatments with high dose of isoflovones significantly increased in body weight and lipoprotein level as compared to the control group without elevating the serum level of the liver enzyme AST and ALT.

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التأثير البيولوجي للاستروجين النباتي علي الفئران المصابة بارتفاع مستوى الكوليسترول في الدم

عبد المنعم حسن درويش[1] ، خالد على شاهين[2] ، إيمان محمد سيد أحمد زيد[2]

(1) قسم كيمياء التغذية والتمثيل الغذائي – المعهد القومي للتغذية القاهرة

(2) قسم التغذية وعلوم الأطعمة – كلية الاقتصاد المنزلي . جامعة المنوفية

الملخص العربى

استهدف هذا البحث لتقييم تأثير النسب المختلفة (5%، 10%، 15%، 20%، 25%، 30%) لدقيق الصويا منزوع الدهن ومجموعة هرمون الاستروجين علي وظائف الكبد والكلى وصور دهون الدم في الفئران التي تتغذى على وجبات تجريبية تحتوي علي الكوليسترول حيث تم استخدام 54 فأر من إناث الألبينو يتراوح وزن كل منهم ما بين (130±5جرام) وتم تقسيمهم إلي 9 مجموعة أحداهما المجموعة الضابطة السالبة أما المجموعات الأخرى تم تغذيتهم علي وجبات تحتوي علي الكوليسترول(1%) لمدة 3 أسابيع لرفع مستوى الكوليسترول في الدم.

وسجل وزن الجسم والغذاء المتناول أسبوعيا وفي نهاية التجربة تم حساب معدل الزيادة في وزن الجسم ومعدل كفاءة الغذاء وقدرت وظائف الكبد والقلب عن طريق سيرم الدم وقد أوضحت النتائج المتحصل عليها بالمقارنة بالمجموعة الضابطة الموجبة. سجلت نقص معنوي فى كلا من الكولسترول والجليسيريدات الثلاثية واللليبوبروتينات منخفضة الكثافة فى كل المجموعات بينما ظهر زيادة معنوية فى اللليبوبروتينات عالية الكثافة فى كل مجموعات الدراسة ووجود تحسن معنوي فى وظائف الكبد.

وتوصى الدراسة بضرورة تناول دقيق الصويا منزوع الدهن حتى نتجنب الآثار الجانبية والمحافظة على كفاءة القلب و الكبد. مع إجراء أبحاث مستقبلية وهستوباثولوجية لمعرفة سلامة الأثر الناتج لاستعمال دقيق الصويا المنزوع الدهن وهرمون الاستروجين لفترات طويلة.

الكلمات الكشافة:

دقيق الصويا منزوع الدهن – هرمون استروجين – فئران التجارب – بروتينات الدهون مرتفعة الكثافة – بروتينات الدهون منخفضة الكثافة – الكوليسترول

Table (1): Food intake (FI) g, body weight gain (BWG%) and Feed efficiency ratio (FER) of control and hypercholesterolemic rats treated fed on with defatted soya flour levels and estrogen hormone.

Groups / parameters	Control		defatted soya flour.						Estrogen hormone
	Negative	Positive	5%	10%	15%	20%	25%	30%	
Food intake (g)	14.08±0.85	14.92±1.02	12.87±0.98*	13.15±1.06*	13.64±0.87*	13.82±0.93*	14.35±2.76	14.51±1.18	14.46±1.32
Body weight gain%	53.98±3.6*	63.11±4.18	39.69±2.73	45.23±3.19*	46.86±3.96*	50.37±4.10**	53.22±3.18**	59.50±4.22**	54.15±3.39**
FER[1]	4.85±0.27**	4.79±0.36	3.97±0.29*	4.51±0.36	4.55±0.45	4.82±0.26	4.82±0.22	4.95±0.38	4.53±0.41

* Differences are significant at P≤0.05 **Differences are highly significant at P≤0.01 ***Differences are highly significant at P≤0.001
1 Feed efficiency ratio

Table (2): Mean values of some lipids parameters of Control and hypercholesterolemic rats treated fed on with defatted soya flour levels and estrogen hormone.

Groups / Parameters	Control		defatted soya flour.						Estrogen hormone
	Negative	Positive	5%	10%	15%	20%	25%	30%	
TC mg/dl	93.53±5.21	170.31±6.16*	154.82±5.15**	144.54±8.13*	138.25±5.38**	153.32±7.12*	146.41±3.91*	140.67±4.33**	123.75±8.76**
TG mg/dl	86.38±7.23	125.25±5.88	119.72±9.17*	107.16±7.46*	99.18±3.49**	118.33±5.13*	112.63±7.36*	102.54*±9.11*	95.37±4.71***
HDL mg/dl	47.15±2.18*	29.34±1.83	36.98±0.93*	39.321.18*	43.16±3.24**	36.33±1.26*	39.87±2.28*	41.12±1.11**	40.18±2.39**
LDL mg/dl	29.11±1.33	115.92±5.72	93.90±3.38	83.79±2.21*	75.26±2.42**	93.33±5.63	84.02±3.16*	79.05±2.34*	64.5±5.16**
VLDL mg/dl	17.27±0.87	25.05±1.77	23.94±1.91	21.43±1.74*	19.83±0.89*	23.66±1.11	22.52±1.37*	20.50±1.34**	19.07±1.84***

Table (3): Mean values of liver function parameters of control and hypercholesterolemic rats treated fed on with defatted soya flour levels and estrogen hormone

Groups / parameters	Control		defatted soya flour.						Estrogen hormone
	Negative	Positive	5%	10%	15%	20%	25%	30%	
ALT u/l	35.72±2.31f	62.25±5.16a	53.50±3.50b	52.60±2.66b	50.21±3.17c	48.90±2.37cd	43.75±4.11de	41.32±3.32e	44.63±2.29de
AST u/l	130.21±9.42f	185.34±13.12a	174.18±6.15b	165.25±12.71c	165.32±8.26c	157.40±4.38d	159.53±11.83d	146.44±9.67e	147.11±12.18e
ALB u/l	135.42±10.01h	183.36±14.43a	172.27±15.40b	168.18±9.53c	161.41±10.55d	157.11±14.1e	145.50±11.43f	143.22±7.07f	138.18±5.39g