Dietary Phytoestrogens Reduce the Leptin Level in Ovariectomized Female Rats

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Abstract— Soy isoflavonoids have many useful properties. Daidzein and genistein are the main aglycones of soy isoflavonoid, and have many useful activities in vitro and in vivo. However, Evidence is emerging that dietary phytoestrogens play a beneficial role in obesity. Soy phytoestrogens, has estrogenic activity and is used as a natural substitute for estrogen replacement therapy in postmenopausal women. The primary objective of this study was to determine the effect of soy phytoestrogens on feed intake and body weight gain, serum leptin hormone level, abdominal fat mass , serum high density lipoproteins (HDL), triglycerides (TG), total cholesterol (TC) and low density lipoproteins (LDL). A total of 30 ovariectomized female Albino rats were divided into three groups (10 female / group) and they were given phytoestrogen-free casein-based diet for the control group, moderate dose of phytoestrogens (7% soybean) and high phytoestrogens diet containing (27% soybean) for the other group. These diets were given for 45 days. The high phytoestrogens in diet decreased feed intake significantly ($P<0.05$) in high and low groups than control. Body weight gain showed significant ($P<0.05$) increase in low dose while the high group decreased significantly than both other groups. Abdominal fat mass was significantly ($P<0.05$) lower in high group than low and control one Level of HDL was significantly ($P<0.05$) increased in the high and low phytoestrogens group than control but TC, and TG were significantly ($p<0.05$) decreased in both moderate and high phytoestrogens group than the control one. The results of LDL showed non significant difference among the three groups. The serum leptin level was significantly ($P<0.05$) decreased in high group than low and control groups. These findings show the high dietary phytoestrogens interfere with adiposity and reduce leptin production in ovariectomized female rats.

Keywords—Dietary phytoestrogens, leptin, lipid profile, overiectomized rat.

I. Introduction

Recently, adipose tissue was shown to be a major endocrine system that plays a role in energy homeostasis, lipid metabolism, immune response, and reproduction **(Badman & Flier 2005 and Kershaw &Flier 2004)**. Estrogens promote, maintain, and control the typical distribution of body fat and adipose tissue metabolism, through a still unknown mechanism. These steroids are known to regulate fat mass by increasing lipolysis through the modulation of the expression of genes that regulate adipose deposition (lipogenesis) and differentiation and adipocyte metabolism **(Cooke *et al.*, 2001; Cooke & Naaz, 2004).** This regulation mainly occurs through estrogen receptors (ERα and ERβ), which also mediate the action of several nutritional compounds such as lignans, stilbenes, and a variety of different polyphenols.

Phytoestrogens are bioactive molecules present as nutritional constituents of commonly consumed vegetables. Their name derives from the fact that they can bind to estrogen receptors and induce an estrogenic/antiestrogenic response in target tissues (**Kuiper *et al.*, 1998**). The isoflavones genistein and daidzein are among the most abundant phytoestrogens in human diets and are found predominantly in legumes like soy. Because of its estrogenic potential, genistein was proposed to have a role in the maintenance of health status by acting in several organs and to prevent cardiovascular risk by regulating lipid and carbohydrate homeostasis (Kreijkamp-Kaspers *et al*., 2004). Thus, its consumption is suggested to improve human health. Goodman-Gruen and Kritz-**Silverstein (2001)** revealed that the consumption of isoflavones – genistein and daidzein were known by their ability to reduce body mass indexes, fasting insulin concentration, increased HDL cholesterol (**Nogowski, *et al*., 1998; Potter *et al*., 1998 and Sanders *et al*., 2002**) lower total cholesterol, LDL cholesterol (**Potter *et al*., 1998; Merz-Demlow *et al*., 2000; Teede *et al*., 2001; Wangen *et al*., 2001; Jayagopal *et al*., 2002; Lemay *et al*., 2002**).

The estrogenic activity of genistein is reported to depend on its concentration (**Wilson *et al*., 2004**), endogenous estrogen levels (**Ratna 2002**), and gender (Faughnan *et al*., 2004). In vitro studies show that, at low doses, genistein efficiently binds both estrogen receptors, although ERβ is bound with higher affinity (**Kuiper *et al*., 1998)**. At high doses, genistein was reported to act as a tyrosine kinase inhibitor (**Huang *et al*., 1992 and Hong *et al*., 2005),** an antioxidant (**Hwang *et al*., 2003)**, and a steroid-metabolizing enzyme modulator (**Atkinson *et al*., 2003**). In addition, at high concentration, genistein may inhibit the action of estrogen receptors by acting through nuclear receptors such as the peroxisome proliferator-activated receptors (PPARs) (**Dang and Lowik 2004**). These data suggest that genistein may activate or inhibit estrogen-dependent pathways depending on the extent of intake. Furthermore, recent studies on adipose tissue in women (**Goodman-Gruen and, Kritz-Silverstein 2003**) and female mice (**Naaz *et al*., 2003**) indicate that genistein inhibits adipose deposition and decreases adipose mass, and that this activity occurs through regulation of the expression of specific genes (**Penza *et al*, 2006**). Genistein and daidzein were also found to inhibit lipogenesis and stimulate lipolysis in rat adipocytes. This activity was manifested by the direct effect of

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these compounds on isolated fat cells (**Szkudelska *et al*., 2000**). The previously documented direct influence of genistein and daidzein on metabolism of fat cells suggests the possibility of its effect on leptin secretion. This problem seems to be important since the proper leptin secretion constitutes an important factor regulating the energetic status of the whole organism (**Szkudelski *et al*., 2005**). Accordingly the aim of this study was to understand the effect of dietary phytoestrogens in estrogen deprived condition on feed intake, weight gain, abdominal fat mass, lipid profile and serum leptin hormone level as factor regulating the energy status and at low and high doses.

II. Materials And Methods

Animal care

Thirty female Albino rats with 13 weeks age and mean weight 140.52±10.35 were used in this study and housed 4 females/ cage under standard laboratory conditions. They were kept at room temperature (28±2°C) under natural day light rhythm at least 2 weeks prior to surgery The animals were accessed to casein based diet and tap water freely. The animals received human care and experiments were carried out according to the criteria outlinend by Faculty of Science, Suez Canal University.

Ovariectomy

Thirty female Albino rats (5 month) weighing approximately 180.9±11.4 g were anathaesized by general inhalation anaethesiausing diethyl ether ovariectomy was preceded by a midline dorsal skin incision, 3 cm long, approximately half way between the middle of the back and the base of the tail after placing an animal on its ventral surface. Incision of the muscles was made at linea Alba. the ovary was found, surrounded by a variable amount of fat after accessing to peritoneal cavity. The blood vessels were ligated at the connection between the Fallopian tube and the uterine horn was cut and the ovary moved out. Suturing to muscle layer then to skin was performed by simple continuous suture using vicryl 4/0 (**Lasota and Danowska-Klonowska 2004).** Animals were injected with broad spectrum antibiotic for 3 successive days after ovariectomy and fed casein based diet

Experimental design

After 3 weeks from ovariectomy The ovariectomized female rats were divided randomly into three groups: **Group I,** control group, n= 10, they were fed on a casein based diet, **Group II,** receive moderate phytoestrogens diet, n= 10. and **Group III,** receive high phytoestrogens diet, n = 10. All diets were formulated to fulfill all the nutritional requirements of adult rat (**Table 1**) according to **NRC (1995)** and were offered for 45 days.

Daily food intake and weekly body weight gain were recorded. At the end of experiment the overiectomized females were weighed then sacrificed under effect of light anaethesia for obtaining blood. Whole blood was allowed to clot and then centrifuged at 3000 *g* for 20 min to obtain serum then stored at -20°c.Immediately after blood sample collection, visceral fat was collected from the superficial area covering the alimentary tract and the uterus, removed and weighed.

Table (1): Diet composition

INGREDIENTS	CONTROL %	LOW PHYTO-ESTROGEN %	HIGH PYTO-ESTROGEN %
Yellow corn	40.59	35.04	35.04
Corn gluten	15.00	11.82	-
Soybean*	-	6.60	26.41
Casein	5.00	5.00	5.00
Sucrose	22.43	23.08	22.32
Starch	7.63	9.08	4.16
Cellulose	1.30	1.10	0.17
Corn oil	5.00	-	-
Soybean oil	-	5.00	5.00
Ground limestone	1.02	1.00	1.04
Dicalcium phosphate	0.34	0.31	-
Common salt	0.13	0.13	0.13
Premix	0.30	0.30	0.30
Methionine	0.30	0.33	0.43
Lysine	0.26	1.16	-
Tryptophan	0.70	0.05	-
Total	100.00	100.00	100.00

*Soybean was autoclaved at 110^{0}c for 30 minutes according to **(Westfall and Hauge, 1948)** to inactivate trypsin inhibitor, tannins, saponins, phytate, protease inhibitors, lectins and goitrogens**.**

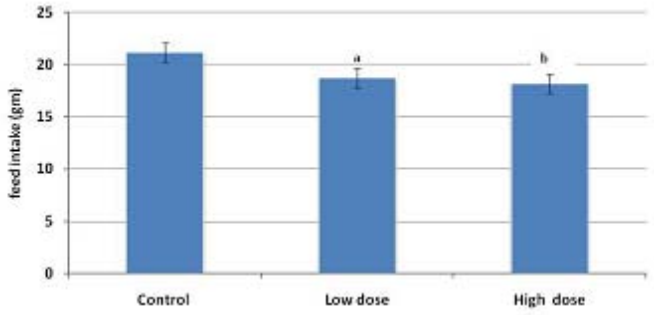
Serum levels of high-density lipoprotein cholesterol (HDL), total cholesterol (TC), triglycerides (TG) and low-density lipoprotein cholesterol (LDL) were measured using enzymatic calorimetric kits(Biodiagnostic Co., Egypt) according to **(Wieland & Seidel 1983)**. Serum leptin level was determined using specific enzyme linked immunosorbant assay kit (cat # 27295A, TakaRa Bio, Inc., Japan) according to manufacturer instructions.

Statistical analysis

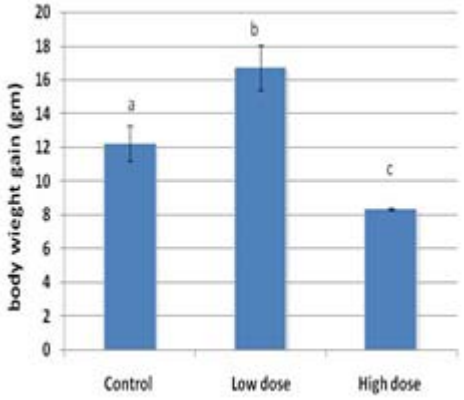
All values were expressed as the mean ± SE. Differences among groups were determined using a one-way analysis of variance (ANOVA) followed by Duncan test using SPSS program version 16.0. A value of $P < 0.05$ was considered to be statistically significant **(Field, 2000).**

III. Results

The performed experiment demonstrated that dietary phytoestrogens was significantly (P<0.05) lower the food intake in high 18.18± 0.51 g/ day and low 18.71± 0.54 g/ day groups than control **(Figure 1)**. Body weight gain was significantly (P<0.05) lower in high phytoestrogens-fed group 8.34± 0.07 g than low 16.72± 1.36 g and control one 12.23±1.04 g.



Figure(1): Effect of dietary phytoestrogens on daily feed intake of ovariectomized albino female rats



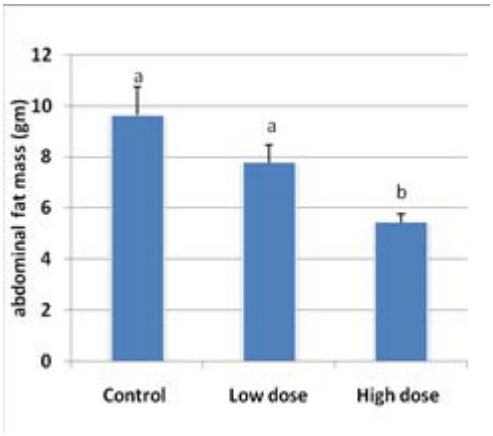
Figure(2):Effect of dietary phytoestrogens on weekly body weight gain of ovariectomized albino female

The abdominal fat mass showed a significant ($p<0.05$) reduction in high group than those of low and control groups 9.64± 1.12 g versus 7.78±0.69 g and 5.44±0.31 g respectively as shown in **Figure 3**.The values of lipid profile that shown in **Table (2)** revealed that HDL, was significantly ($P<0.05$) higher in both treated groups than control. While LDL showed only non significant numerical decrease in its value among the three groups.

TABLE (2): EFFECT OF DIETARY PHYTOESTROGENS ON LIPID PROFILE MG/ DL

Parameters /group	Control group	Low dose group	High dose group
HDL mg/ dl	12.29±0.37[a]	13.21±0.66 [b]	15.58±0.59 [c]
TG mg/ dl	114.74±7.30[a]	84.94±3.75 [b]	88.28±7.70 [c]
TC mg/ dl	69.58± 2.37 [a]	61.06±1.43 [b]	58.75±2.03 [c]
LDL mg/ dl	74.89±1.93[a]	69.67±2.23 [a]	68.75±1.72 [a]

[a, b, c] Duncan multiple range test ($p< 0.05$)



Figure(3):Effect of dietary phytoestrogens on abdominal fat mass/ g of ovariectomized albino female rats.

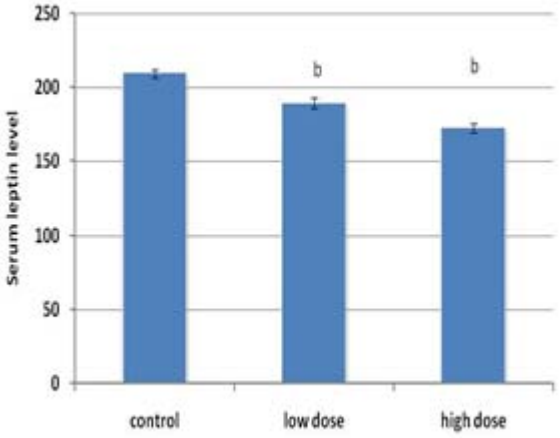


Figure (4): Effect of dietary phytoestrogens on serum leptin level ng/ dl of ovariectomized albino female rats.

IV. DISCUSSION

The present study demonstrated the protective effects of *soy phytoestrogens* against the risks of obesity in rats under estrogen-deprivation conditions. Dietary soy phytoestrogens administered to ovariectomized female albino rats affect food intake and substantially diminished the body weight gain in high group than the control and low one. These results are consistent with previous records **(Kim *et al.*, 2005**). Phytoestrogens are structurally similar to endogenous estrogens, they can act as a weak estrogen and bind to the ER in various tissues(**Naaz *et al.* 2003),** thus reduction in feed intake may be due to the appetite repressing action of estrogen **(Roy and Wade, 1975)** as dietary phytostrogens decrease feed intake and hence decrease body weight. Also the decrease in abdominal fat mass that demonstrated in the current study in the high group could be attributed to that phytoestrogens are capable of inducing apoptosis of adipocytes, suggesting that at least part of the weight loss is due to ablation of fat cells, which could result in better maintenance of weight loss **(Kim *et al.*, 2005).** These in vivo data are further supported by in vitro studies showing that

genistein induced lipolysis and inhibited de novo lipid synthesis in 3T3-L1 adipocytes **(Harmon & Harp 2001 and Harmon *et al*., 2002)** and rat adipocytes **(Szkudelska *et al*., 2000).** Phytoestrrogens also affect fat growth and development, which is the main source of leptin, through PPAR **(Anderson *et al*., 2004)** which is a major factor involved in *de novo* fatty acid synthesis, adipocyte differentiation, lipid accumulation, and adipocyte survival/maintenance **(Jump *et al*., 2005).**

Dietary phytoestrogens was also shown to have direct effects on lipid metabolism as it decreased TC & TG and increased HDL significantly (P<0.05) which are consistent with previous record of **Kirk *et al*., 1998**; **Nogowski *et al*., 1998; Wangen *et al*., 2001 and Uesugi *et al*., 2002**. Because they affect lipid metabolism in liver and adipose tissue, decreasing triglycerides while increasing free fatty acids in serum **(Nogowski *et al*., 1998).** phytoestrogens might lower cholesterol levels by increasing LDL receptor activity, and the reduction in cholesterol may offer some protection against atherosclerosis **(Kirk *et al*., 1998)**. Another explaination is that soy phytoestrogens decrease intestinal cholesterol absorption increase in bile acid excretion that mediate the lipid-lowering effect of soy protein **(Greaves *et al*., 2000).**

The biological activity of isoflavones in animals and humans is partly ascribed to the structural similarities between the isoflavones and E2. E2 is biosynthesized by the cytochrome P450 enzyme complex called aromatase and acts predominantly via two distinct nuclear ERs, ERα and ERβ, defined as ligand-inducible transcription factors **(Rosen *et al*., 2000)**. Binding of E2 to ERs inhibits lipogenesis primarily through decreasing activity of lipoprotein lipase (LPL), an enzyme that regulates lipid uptake by adipocytes **(Misso *et al*., 2003)**, and the isoflavone genistein has recently been shown to cause decreases in LPL mRNA in adipose tissue with concomitant decreases in lipid filling of adipocytes **(Naaz *et al*., 2003 and Heim *et al*.,2004)**. These result suggests that the hypolipidemic effect of genistein could be ascribed in part to the upregulation of genes involved in fatty acid catabolism which is consistent with results from *in vitro* studies **(Owen *et al*., 2004, Mullen *et al*., 2004).**

The observation that dietary phytoestrogens depressed significantly (p<0.05) serum leptin concentration in high group than low and control groups in combination with the depression in abdominal fat mass allowed to believe that this effect of genistein was due to its direct influence on adipocytes which are the main source of leptin **(Szkudelski *et al*., 2005)**. The effect of phytoestroges specially genistein inhibit some enzymes in adipocytes substantially abates leptin secretion **(Bradley and Cheatham 1999)** in spite of unchanged expression of its gene **(Szkudelski *et al*., 2005)**.

V. CONCLUSION

The results of the current study show that high dietary phytoestrogens interfere with adiposity, reduce lipid parameters and leptin hormone in blood of ovariectomized female rats.

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