Effect of SoyBeans on Lipid Profile of Female and Male Albino Rats

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Abstract: Isoflavones has the largest impact due to the extensive consumption of soy-foods. Although many studies have focused on the role of soy on human health the results remain controversial. Consequently, the question of whether or not phytoestrogens are beneficial or harmful to human health(lipid profile) remains unresolved. Male and female Wister albino rats were used in the present study. Each sex was randomly divided into four groups control group fed on the basal diet (AIN93 G), three treated groups given 30; 60 and 90 gm cooked soybeans/70 kg human body weight (b.w.) for three month. At the end of the experimental period female and male rats showed that the administration of soybean significantly decreased serum total cholesterol (TC), low density lipoproteins (LDL), triglycerides (TG), leptin hormone and net body weight gain however it showed a significant increase of high density lipoproteins (HDL). These results suggest that soybean play a protective role from cardiovascular diseases by decreasing lipid profile.

Key words: Soybean, Lipid profile, Leptin hormone, Albino rats.

INTRODUCTION

Coronary heart disease is a major health problem in many countries. Diet has received considerable attention as a cardiovascular disease (CVD) prevention strategy, and studies have linked the varied CVD incidences in different countries to dietary differences and focused on the role of soy in improving lipid profile and subsequent cardioprotective effects. This interest has stemmed from numerous studies in humans and animals which showed that consumption of soy components significantly lowered total and LDL-cholesterol and TG as well as elevated HDL-cholesterol which are the major risk factors of CVD (Anderson et al., 1999; Clarkson, 2002; Rosell et al., 2004 and Hoie et al., 2005).

However, other results did not support this hypothesis and proved no effect of soy on lipid profile (Lichtenstein et al., 2002; Meinertz et al., 2002; Weggemans and Trautwein 2003; Zittermann et al., 2004 and Sacks et al., 2006). However, Anthony et al. (1997) observed that soy increased TC and LDL cholesterol in monkeys; decreased good cholesterol (HDL) in healthy subjects (Ashton and Ball, 2000); in rats (Madani et al., 2000); in rabbits (Amani et al., 2005); in mice (Ryokkynen et al., 2006) and increased serum TG levels in rabbits (Amani et al., 2005).

In addition, other authors studied the effect of soy on the polypeptide leptin hormone. It has been shown that leptin play an important role in food intake regulation and energy metabolism. Leptin is secreted by mammalian adipocytes and functions as a hormonal sensing mechanism for fat deposition (Brunner et al., 1997). Adipocytes produce and secrete more leptin as fat storage increases, signaling the brain to reduce food intake and increase energy expenditure.

Some studies showed that soy increased serum level of leptin hormone and decreased body weight in rats (Chen et al., 2006). However, other results did not support this hypothesis and showed that soy decreased leptin hormone (Cederroth et al., 2008), while Maskarinec etal(2009) noted that soy foods had no significant effect on serum level of leptin hormone in premenopausal women.

So the aim of the present study was to investigate the role of soybeans on lipid profile.

MATERIAL AND METHODS

Experimental Animals:

Male and female Wister albino rats with average body weight 120 gm obtained from the private market Abou-Rawash, Giza, Egypt, were used in the present study. They were kept on vegetables and water ad libitum for one week prior to the experiment to remove any traces of previous soybean. Following this brief adjustment...
period, each sex was divided into four groups (n = 12 per group).

Control group: rats were kept on the basal diet (AIN 93 G) according to (Reeves, 1997) and water ad libitum for three months.

First group: Each rat was fed individually on 30 gm cooked soybean/70 kg human b.w. daily for three months.

Second group: Rats were fed individually on 60 gm cooked soybean/70kg human daily for three months.

Third group: Rat was fed individually on 90 gm cooked soybean/70kg human dialy for three months. Doses used in this study are according to (Messina, et al., 1999 and Chang et al., 2008).

All three treated groups were then given the basal diet (AIN 93G) and water ad libitum through out the experimental period.

Soybean Diet:

Commercial soybean seeds sample (Giza 22) obtained from the Agriculture Research Center; Giza, Egypt was used in the present study because it's the common soybean used in the manufacture of most soy foods present in local markets. Also it's used as a dietary source of proteins for poultry and livestock.

It contains 40 % protein, 20 % fat, 5 % ash and 35 % carbohydrates (soluble sugars and insoluble sugars).

Food Technology Research Institute Agriculture Research Center, Giza, 2008.

Oligosaccharides are soluble sugars but are not broken down by the enzymes of the digestive tract and are fermented by the micro-organisms present in the intestine, with the formation of the intestinal gas flatulence. That's why raw soybean was soaked for 12 hours at room temperature to get rid of these oligosaccharides. Also soybean was cooked at 120°C for 18 minutes in attempt to decrease the amount of the anti-nutrients present such as trypsin inhibitors, phytin, lectins, saponins, and hemagglutinins (Sat and Keles, 2002).

At the end of experiment, animals were sacrificed after 24 hours of starvation and blood was collected from the orbit plexus into clean centrifuge tubes. Serum was separated and stored for biochemical analysis.

1- Body Weight:

Body weights of rats were recorded before and after the experimental period from which net body weight gain was calculated.

2- Biochemical Analysis:

Determination of total cholesterol (TC) in serum samples were measured by Synchron CX4 according to the method of (Roeschlau et al., 1974). High density lipoprotein cholesterol (HDL-C) was measured according to (Gordon et al., 1977) also, triglycerides (TG) was estimated depending on assay depicted by (Stein, 1987).

Then, LDL-C was calculated from the TC, HDL-C and TG according to (Friedewald et al., 1972) equation:

\[ \text{LDL-C} = \text{TC} - \text{HDL-C} - \frac{\text{TG}}{5} \text{ (mg/dl)} \]

Serum leptin hormone was measured by EIA ELISA Kits according to Clement et al. (1998)

Statistical Analysis:

Data were analyzed using the SPSS for windows (version 12.0). Analysis of variance (one-way ANOVA) was performed to test for any significant differences among groups and independent sample t-test was used to calculate statistical significance between the control group and each treated group. The level of significance was set as \( P < 0.05 \) for all statistical tests (Tello et al., 2003).

Results:

Table (I) and Figs. (1a-f): Show the effect of soybean on serum total cholesterol, lipoproteins, triglyceride, leptin hormone and body weight gain of female rats treated for three months with three different doses.

Serum levels of TC, LDL, TG and body weight gain significantly decreased in three treated groups with (-7.13% -11.35% and -12.35%) for TC and (-21.12%, -30.42% and -34.70%) for LDL, and (-3.62%, -6.11% and -14.12%) for TG and (-14.51%, -18.00% and -23.36%) for body weight gain respectively. This was accompanied by highly significant decrease in leptin hormone concentration in rats supplemented with 60 and 90 gm / 70 human b.w. with (-20.28% and -35.23%) respectively. In addition serum level of HDL significantly increased in all treated groups with (8.65%, 6.53% and 12.68%) respectively.
Fig. (1a): Effect of 30, 60 and 90 gm soybean/70 kg b.w. on serum TC level of female rats

Fig. (1b): Effect of 30, 60 and 90 gm soybean/70 kg b.w. on serum LDL level of female rats

Fig. (1c): Effect of 30, 60 and 90 gm soybean/70 kg b.w. on serum HDL level of female rats

Fig. (1d): Effect of 30, 60 and 90 gm soybean/70 kg b.w. on serum TG level of female rats

Fig. (1e): Effect of 30, 60 and 90 gm soybean/70 kg b.w. on serum leptin hormone level of female.

Fig. (1f): Effect of 30, 60 and 90 gm soybean/70 kg b.w. on body weight gain

Table I: And Figs. (1a-f): Show the effect of soybean on serum total cholesterol, lipoproteins, triglyceride, leptin hormone and body weight gain of female rats treated for three months with three different doses.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TC (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>TG (ng/ml)</th>
<th>Leptin hormone (mg/dl)</th>
<th>Body weight gain (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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<tr>
<td>Range</td>
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<td>38.63—48.05</td>
<td>38.00—43.00</td>
<td>90.20—108.80</td>
<td>2.00—3.41</td>
<td>74.02—19.75</td>
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<td>Mean ± S.E</td>
<td>104.09 ± 1.04</td>
<td>43.23 ± 0.96</td>
<td>40.00 ± 0.46</td>
<td>104.29 ± 1.36</td>
<td>2.81 ± 0.15</td>
<td>94.26 ± 4.86</td>
</tr>
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<tr>
<td>Range</td>
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<td>25.17—38.10</td>
<td>41.00—47.50</td>
<td>97.00—104.10</td>
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<td>96.67 ± 0.86</td>
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<td>P = 0.05</td>
<td>P = 0.05</td>
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<td>Range</td>
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<td>17.33—39.40</td>
<td>40.00—44.30</td>
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<td>64.06—92.72</td>
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<td>92.28 ± 2.16</td>
<td>30.08 ± 2.14</td>
<td>42.61 ± 0.47</td>
<td>97.92 ± 0.92</td>
<td>2.24 ± 0.18</td>
<td>77.23 ± 2.75</td>
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<td>Range</td>
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<td>42.20—46.70</td>
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<td>89.36 ± 1.15</td>
<td>1.82 ± 0.21</td>
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N.S: Non significant. S.E: Standard error. P: Probability
Table II: And Figs. (2a-f): Show the effect of soybean on serum total cholesterol, lipoproteins, triglyceride, leptin hormone and body weight gain of male rats treated for three months with three different doses.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>TC (mg/dl) Range</th>
<th>LDL (mg/dl) Range</th>
<th>HDL (mg/dl) Range</th>
<th>TG (ng/ml) Range</th>
<th>Leptin hormone (ng/ml) Range</th>
<th>Body weight gain (gm) Range</th>
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<td>80.90 — 100</td>
<td>3.99 — 4.35</td>
<td>78.72 — 21.55</td>
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<td></td>
<td>Mean ± S.E</td>
<td>105.53 ± 2.06</td>
<td>51.35 ± 1.40</td>
<td>38.91 ± 0.84</td>
<td>85.58 ± 1.70</td>
<td>4.17 ± 0.08</td>
<td>90.02 ± 3.93</td>
</tr>
<tr>
<td>First</td>
<td>Range</td>
<td>90.00 — 100.00</td>
<td>32.35 — 42.58</td>
<td>40.00 — 43.50</td>
<td>77.00 — 83.00</td>
<td>3.62 ± 0.26</td>
<td>56.47 — 98.75</td>
</tr>
<tr>
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<td>Mean ± S.E</td>
<td>95.77 ± 0.97</td>
<td>38.15 ± 1.06</td>
<td>41.63 ± 0.30</td>
<td>79.95 ± 0.44</td>
<td>3.57 ± 0.28</td>
<td>79.15 ± 3.81</td>
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<tr>
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<td>% of change</td>
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<td>-6.36</td>
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<td>Range</td>
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<td>61.94 — 90.00</td>
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<td>Mean ± S.E</td>
<td>91.92 ± 1.90</td>
<td>32.96 ± 2.34</td>
<td>43.29 ± 0.83</td>
<td>78.31 ± 1.88</td>
<td>5.44 ± 0.13</td>
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<td>% of change</td>
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<td>Range</td>
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<td>57.94 — 82.16</td>
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<td>Mean ± S.E</td>
<td>88.35 ± 0.76</td>
<td>31.24 ± 1.26</td>
<td>42.59 ± 0.58</td>
<td>72.64 ± 2.67</td>
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<td>71.10 ± 2.78</td>
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<td>% of change</td>
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<td>-39.16</td>
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<td>-14.92</td>
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<td>F = 21.42</td>
<td>F = 31.48</td>
<td>F = 10.33</td>
<td>F = 6.64</td>
<td>F = 7.25</td>
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</tr>
</tbody>
</table>

N.S.: Non significant  S.E: Standard error  P: Probability

Fig. (2a): Effect of 30, 60 and 90 gm soybean/70 kg b.w. on serum TC level of male rats

Fig. (2b): Effect of 30, 60 and 90 gm soybean/70 kg b.w. on serum LDL level of male rats

Fig. (2c): Effect of 30, 60 and 90 gm soybean/70 kg b.w. on serum HDL level of male rats

Fig. (2d): Effect of 30, 60 and 90 gm soybean/70 kg b.w. on serum TG level of male rats

Fig. (2e): Effect of 30, 60 and 90 gm soybean/70 kg b.w. on serum leptin level of male rats

Fig. (2f): Effect of 30, 60 and 90 gm soybean/70 kg b.w. on body weight gain of male rats
Serum levels of TC, LDL, TG and body weight gain significantly decreased in three treated groups with (-9.25%, -12.90% and -16.28%) for TC and (-25.71%, -35.81% and -39.16%) for LDL and (-6.36%, -8.38% and -14.92%) for TG and (-12.08%, -17.25% and -21.01%) for body weight gain respectively. This was accompanied by highly significant decrease in leptin hormone concentration in rats supplemented with 60 and 90 gm /70 human b.w. with (-15.10% and -29.02%) respectively. Meanwhile serum level of HDL significantly increased in three treated groups with (6.99%, 11.26% and 9.46%), respectively.

**Discussion:**

In the present study, female and male rats fed with 30, 60 and 90 gm soybean/70 kg b.w. resulted in significant decrease of serum TC; also there was a significant decrease in serum level of LDL and TG, leptin and net gain body weight Moreover serum level of HDL significantly increased.

These results agree with previous studies on rodents which showed significant decrease in serum TC and LDL (Zhong et al., 2007 and Onyeneke et al., 2008); significant decrease in serum TG (Onyeneke et al., 2008 and Zhang et al., 2009) and significant increase in serum HDL (Tovar-Palacio et al., 1998 and Zhang et al., 2009) after consumption of soy protein or isoflavones or both.

Studies on humans also correlate with the present study showing that soy and its constituents significantly decreased TC (Liao et al., 2007; Clerici et al., 2007 and Torres et al., 2009); LDL (Clerici et al., 2007 and Mourad et al., 2010); TG (Torres et al., 2009) and significant increase serum HDL (Sanders et al., 2002 and Hernansen et al., 2003).

It has been suggested that the hypocholesterolemic effect of soybean may be due to its ability to reduce cholesterol and bile acid absorption from the gastrointestinal tract (Demonty et al., 2002 and Lin et al, 2004) and increase bile acid excretion (Lin et al., 2004 and Lee et al., 2005).

Soy isoflavones are also believed to reduce the risk of heart disease by reducing the susceptibility of LDL to oxidation via antioxidant action (Jenkins et al., 2002; 2003 and Zhang et al., 2003). In addition soy stimulates sterol regulatory element binding protein-2 which increase serum cholesterol clearance (Mullen et al., 2004).

Also Torres et al. (2006) interpreted hypocholesterolemic effect of soy is due to the ability of soy protein to reduce insulin level which in turn down-regulates the expression of the hepatic transcription factors sterol regulatory element binding protein (SREBP)-1. The reduction of this factor decreases the expression of several lipogenic enzymes, causing decreased serum and hepatic triglycerides as well as LDL and VLDL.

Another theory to explain the cholesterol-lowering properties of soy has been attributed to the fiber component of the soybean. It is known that fiber binds to bile acids in the intestinal tract. The fiber and bile acid combination is then excreted causing the reabsorption of fewer bile acids. The liver is then forced to remove circulated LDL-C to make new bile acids, thus lowering blood LDL-C concentrations and subsequently reducing the risk for the development of CVD (Jones, 2008).

Moreover, hypcholesterolemic effect of soy may be due to its high content of phytosterols which are similar in structure to cholesterol, so they have the ability to enhance excretion of cholesterol, interfering with cholesterol synthesis and competing for cholesterol acceptor sites in the intestinal walls (Lin et al., 2009).

However, the present study conflicts those of Hodgson et al. (1998) who reported that isoflavones tablets had no effect on serum cholesterol. Also Dent et al. (2001) showed that hamsters fed isoflavones (0.02 g/100 g diet) for 5 weeks did not differ in TC, TG, or HDL-C from control. Also the present results contradict those given by Ma et al. (2005) who found that soy protein with isoflavones fed for 5 weeks has no effect on TC, LDL, and HDL when compared to milk-protein in moderately hypercholesterolemic subjects.

These authors did not find any effect of soy or its constituents on lipid profile which may be due to short duration of treatment or alcohol washed method which is used in the manufacture of isoflavones tablets, which lead to the depletion of isoflavone as well as other components associated with the soy protein that are known to lower cholesterol concentrations (e.g. saponins, phytic acid, and other alcohol-extractable phytochemicals) Gardner et al. (2001).

The present study also showed, significant decrease in body weight of treated rats. The significant decrease in TC, LDL, triglyceride and significant increase in serum HDL may explain the loss of body weight.

The present data on rat's body weight coincide with McClain et al. (2006) who reported that after 52 weeks’ treatment with genistein at doses of 5, 50, and 500 mg/kg b.w. /day in male rats, food consumption and body weight gain significantly decreased; and on human studies by Allison et al. (2003) and Deibert et al. (2004) who noted greater weight loss with soy-protein consumption compared to control.

Another possible explanation of decreased body weight may be due to the decrease in serum leptin hormone. Leptin hormone in the present work significantly decreased in rats fed only on 60 and 90 gm
soybean/70kg human for female and males rats. These results run in agreement with Szkudelski et al. (2005) who cited that the concentration of blood leptin was reduced as early as 3 days after phytoestrogen administration in adult male rats receiving genistein (5 mg/kg body weight).

Also, Jung et al. (2004) reported that the plasma leptin level was positively correlated with body weight in db/db mice.

In addition vitro studies showed that soy isoflavones decreased leptin secretion from adipocytes (Niwa et al., 2010).

Leptin was originally isolated from the obese gene as an endogenous inhibitor of appetite (Zhang et al., 1994), and also secreted by adipocytes. Leptin supplementation has been successfully used to control the body fat mass (Halaas et al., 1997), but the serum leptin level in an obese person is higher than that of normal-weight subjects (Maffei et al., 1995) and the controversial results of the leptin concentration in the serum were explained by the leptin resistance (Halaas et al., 1997). Many studies suggested that the role of leptin is not only dietary control, but also concerns many functions including the health risks (Onuma et al., 2003 and Pai et al., 2005). Thus, the reduction of the serum leptin seems to be important for human health, especially for an obese person.

In conclusion, this study recommend benefits of soybean in the protection from cardiovascular diseases by decreasing lipid profile except good HDL level.

REFERENCE


