ABSTRACT

Introduction: Great concern has been raised recently concerning the therapeutic impact of soybean. The present study aims to investigate the effects of soybean on bone health and metabolic parameters in postmenopausal women. Methods: In this clinical study, 72 healthy postmenopausal women aged between 45-65 years were given soybean bioactive fraction 2 capsules (500mg each) daily for 24 weeks. Each capsule contained 31.25 mg proteins, 3.2 mg carbohydrates and 4.84 mg isoflavones. Blood pressure, bone mineral density, plasma osteocalcin (OCN), telopeptides of collagen type I (CTX), fasting insulin and blood glucose, lipid profile, serum creatinine, alanine transaminase (ALT), aspartate transaminase (AST), and TSH were assessed prior and after the period of the study. Insulin resistance was calculated by homeostatic model assessment-IR formula (HOMA-IR). Results: Soy ingestion resulted in a significant increase in T score of the hip and OCN; recording -1.97±0.13/-1.76±0.12 and 22.44±0.60ng/ml/20.93±0.57ng/ml before/after treatment, respectively. A marked decrease was also detected in CTX from 2.22±0.10ng/ml to 1.48±0.08ng/ml. With regard to metabolic parameters, there was a significant decrease in fasting insulin (5.40±0.62uIU/ml vs 4.15±0.45uIU/ml), however, fasting glucose and HOMA-IR showed no significant alterations. Lipid profile displayed remarkable decline in total cholesterol (188.86±72.38mg/dl vs 159.60±4.72mg/dl), triglycerides (97.09±5.23mg/dl vs 83.56±4.27mg/dl), LDL-c (75.60±3.06mg/dl vs 63.95±1.86mg/dl) accompanied with a significant elevation in HDL-c (53.09±0.88 vs 65.81mg/dl/0.80mg/dl). A significant decrease in both TSH (1.97±0.13 uIU/ml vs 1.40±0.08 uIU/ml) and serum creatinine (0.82±0.02mg/dl vs 0.77±0.02mg/dl) was also noticed. Conclusion: Consumption of soy improves bone health, reduces cardiovascular risk with no adverse effects on kidney, liver or thyroid functions.

Key words: Bone health, Bone mineral density, Hypocholesterolemic effect, Insulin resistance, Metabolic parameters, Soybean.

INTRODUCTION

Menopause is a natural biological process resulting from loss of ovarian follicle development and decreasing level of circulating estrogen. It is associated with increased risk of cardiovascular diseases and osteoporotic fractures 1. The manifestations of low estrogen level though commonly treated by hormone replacement therapy (HRT), the latter is evidently related with increased risk of breast cancer, thromboembolic conditions, gall bladder and liver disease 2, which provokes the search for complementary medicine with minimal side effects. Over the recent decades, researchers have been interested in the health benefits of soy and soy-products. They have postulated that soy consumption may improve cardiovascular and bone health 3.

Soybeans (Glycine max L. Merrill) were first grown as a crop in China about 5000 years ago and have been widely consumed as folk medicines in China, India, Japan and Korea for hundreds of years. It is a rich source for protein and isoflavones. Isoflavones are classified as phytoestrogens with structural similarity to 17 β-estradiol. Isoflavones exhibit weak estrogenic activity as they interact with estrogen receptor (ER) ER-β and to a lesser extent ER-α, so they were considered as selective ER modulators, and potential alternatives to HRT 4. There are 12 different isoﬂavones detected in soybean: three aglycones: genistein, daidzcin and glycitein, their respective β-glycosides; genistin, daidzin and glycitein, their respective β-glycosides; genistin, daidzin and glycititin along with the 3 β-glucosides each esterified with either malonic or acetic acid 5.

The aim of the current study was to investigate the beneficial effects of the oral administration of the bioactive fraction of soybean prepared in the form of hard gelatinous capsules containing a dose of (500mg) to be taken one capsule 2 times daily for 24 weeks on bone mineral density, bone turnover markers and some metabolic parameters in postmenopausal women.

Phytochemical study

Plant material
Seeds of *Glycine max* L. were purchased from Harraz herbal store, Cairo, Egypt and identified by the vice-head of the partial experimental unit of the faculty of Agriculture Cairo University, Mr. Eid Yossef Mohammed.

Preparation of the polar fraction of Soy

The finely grinded powder of the seeds of *G. max* L. was macerated in a 70% aqueous-ethanol (Fisher Scientific, Loughborough, Leics, UK) solvent system at 50°C several times till exhaustion. The solvents were removed from the collected fractions by evaporation in a rotatory evaporator under reduced pressure at a temperature not exceeding 60°C. The final product was introduced into a lyophilizer for the removal of moisture content. A friable powder was obtained which was used for the phytochemical analysis. The lyophilized biologically active and safe fraction of Soybean, rich in isoflavones, which revealed previously a potent and safe estrogen-like activity in ovariectomized rats [Project No. 1190401, NRC, Egypt, 2017paper in press] was encapsulated in hard gelatinous capsules according to the British Pharmacopoeia, 1993 and the National Formulary, 1975.

Estimation of total proteins, total carbohydrates and total isoflavones were done according to Horwitz, 2005 6 Masuko et al., 2005 7 and César et al., 2008 8.

LC-DAD/ESI-MS analysis of the bioactive fraction of *Glycine max* L. was done according to Kamo et al., 2014. 9

Clinical study

Patients

In the present study, seventy-two postmenopausal women aged between 45 and 65 years were recruited from internal medicine and complementary outpatient clinics of Medical Services Unit at National Research Centre (NRC). A detailed questionnaire was taken from every participant including age at menopause, history of tobacco intake, dietary habits, drug history, medical history and family history of breast cancer. Clinical examination was done including height and weight measurements and calculation of body mass index (BMI) and breast examination. Inclusion criteria were women with normal menopause, age: 45-65 years and with abnormal bone mass density. Clinical examination was done every 2 weeks to check for compliance and any adverse events. Measurement of blood pressure, assessment of height and weight and calculation of BMI and all laboratory tests were repeated at the end of the study, T score was used for evaluating bone density. A T score of -1 and above is considered normal. A T score between -1.1 and more than -2.5 is classified as osteopenia. A T score of -2.5 and below is classified as osteoporosis.

Exclusion criteria: Women with normal bone mineral density (BMD) by DEXA (osteopenia or osteoporosis) at the lumbar spine and/ or proximal femur.

Statistical analysis

The results were expressed as Mean ± SE. The results were analyzed statistically using Student’s t-test (2-tailed) and a significance level of p < 0.05 was used as the criterion of statistical significance.

RESULTS

A number of seventy-two women were subjected to clinical examination, laboratory investigations and BMD assessment by DEXA. Seventeen women were excluded as they had normal BMD at hip and lumbar spine. Fifty-five women fulfilled the inclusion criteria and took the treatment. Twelve women dropped out during the study, two of them reported epigastric pain and the other ten did not return for follow up although they were called several times and did not report any adverse effects. Forty-three women completed the trial for 24 weeks. Their ages ranged from 45 to 65 years, mean: 53.93± 5.24 years. None of them was a smoker. Nineteen of them (44.2%) reported history of caffeine intake and 7 (16.3%) of gaseous beverages.

Phytochemical analysis of soy bioactive fraction

The phytochemical analysis of Soy (*G. max*) L. bioactive fraction revealed the presence of 31.25 mg total proteins, 3.2 mg total carbohydrates and 4.84 mg total isoflavones. LC-DAD/ESI-MS analysis resulted in the separation and identification of 12 compounds 8 of which belong to the class of isoflavones to which the estrogenic activity is attributed. Additionally, 4 soyasaponins were identified although they were present in minute percentages (Figure 1) (Table 1).

The proportions of genistin, daidzein and glycitein are 15.19, 9.56 and 4.19% respectively present either free or as their glucoside conjugates.
Effect of soy on bone mineral density (BMD) and bone turnover markers

There was a significant improvement in BMD at the left upper femur after soy intake for 24 weeks with no significant effect at lumbar spine. Osteocalcin, a bone forming marker, showed significant increase and CTX, a marker of bone degradation, decreased significantly after soy intake (Table 2).

Effect of soy on metabolic parameters

A significant decrease in ALT, AST, serum creatinine, fasting insulin and TSH was indicated after soy intake. The lipid profile also showed improvement as TC, TG and LDL-c decreased and HDL-c increased after soy intake. No significant change in blood pressure, BMI, fasting glucose was recorded (Table 3).
Table 1: Compounds identified by LC-DAD/ESI-MS in the bioactive fraction of *Glycine max* L, their retention times (RT), major ion peaks and percentages of area under the curve.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rt</th>
<th>m/z</th>
<th>AUC %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  Soyasaponin Bb’ (III)</td>
<td>2.21</td>
<td>795, 633</td>
<td>trace</td>
</tr>
<tr>
<td>2  Soyasaponin E-II</td>
<td>6.77</td>
<td>909, 884</td>
<td>0.06%</td>
</tr>
<tr>
<td>3  Soyasaponin Bc’ (IV)</td>
<td>7.89</td>
<td>765, 457</td>
<td>0.02%</td>
</tr>
<tr>
<td>4  daidzein 7-glucoside</td>
<td>10.23</td>
<td>415, 253</td>
<td>6.46%</td>
</tr>
<tr>
<td>5  glycine 7-glucoside</td>
<td>10.86</td>
<td>445, 283</td>
<td>4.02%</td>
</tr>
<tr>
<td>6  Soyasaponin Ba (V)</td>
<td>10.89</td>
<td>939 [M-H-H₂O]</td>
<td>0.03%</td>
</tr>
<tr>
<td>7  genistein-7-glucoside</td>
<td>12.318</td>
<td>431, 269</td>
<td>12.73%</td>
</tr>
<tr>
<td>8  daidzein-7-malonylglycoside</td>
<td>13.63</td>
<td>457, 253</td>
<td>1.95%</td>
</tr>
<tr>
<td>9  genistein 7-malonylglycoside</td>
<td>15.256</td>
<td>473, 269</td>
<td>1.23%</td>
</tr>
<tr>
<td>10 Daidzein</td>
<td>13.643</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 Glycitein</td>
<td>14.15</td>
<td>283</td>
<td>0.17%</td>
</tr>
<tr>
<td>12 Genistein</td>
<td>15.305</td>
<td>269</td>
<td>1.23%</td>
</tr>
</tbody>
</table>

Table 2: Bone mineral density (BMD) and bone turnover biomarkers before and after soy intake.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before soy</th>
<th>After soy</th>
</tr>
</thead>
<tbody>
<tr>
<td>T score of hip</td>
<td>-1.97 ± 0.13</td>
<td>-1.76* ± 0.12</td>
</tr>
<tr>
<td>T score of lumbar spine</td>
<td>-1.47 ± 0.13</td>
<td>-1.47 ± 0.14</td>
</tr>
<tr>
<td>Osteocalcin (ng/ml)</td>
<td>22.44 ± 0.60</td>
<td>30.93* ± 0.57</td>
</tr>
<tr>
<td>CTX (ng/ml)</td>
<td>2.22 ± 0.10</td>
<td>1.48* ± 0.08</td>
</tr>
</tbody>
</table>

Values significantly differ according to T-test: *: significant <0.05, CTX: telopeptides of collagen type I

Table 3: Clinical and biochemical parameters before and after soy (Mean ± SE).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before soy</th>
<th>After soy</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>31.70 ± 0.79</td>
<td>31.39 ± 0.83</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>123.26 ± 2.37</td>
<td>123.26 ± 2.16</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>76.63 ± 1.62</td>
<td>77.33 ± 1.50</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>11.35 ± 0.71</td>
<td>9.02* ± 0.35</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>8.49 ± 0.65</td>
<td>6.44* ± 0.41</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.82 ± 0.02</td>
<td>0.77* ± 0.02</td>
</tr>
<tr>
<td>F Glucose (mg/dl)</td>
<td>98.85 ± 6.02</td>
<td>94.73 ± 5.54</td>
</tr>
<tr>
<td>F Insulin (uU/ml)</td>
<td>5.40 ± 0.62</td>
<td>4.15* ± 0.45</td>
</tr>
<tr>
<td>HOMA</td>
<td>1.54 ± 0.30</td>
<td>1.30 ± 0.26</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>188.86 ± 7.23</td>
<td>159.60* ± 4.72</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>97.09 ± 5.23</td>
<td>83.56* ± 4.27</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>53.09 ± 0.88</td>
<td>65.81* ± 0.80</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>75.60 ± 3.06</td>
<td>63.95* ± 1.86</td>
</tr>
<tr>
<td>TSH (uIU/ml)</td>
<td>1.97 ± 0.13</td>
<td>1.40* ± 0.08</td>
</tr>
<tr>
<td>Hb (gm/dl)</td>
<td>13.01 ± 0.15</td>
<td>12.83 ± 0.16</td>
</tr>
<tr>
<td>TLC (10³/cmm)</td>
<td>5.98 ± 0.29</td>
<td>6.11 ± 0.28</td>
</tr>
<tr>
<td>PLT (10³/cmm)</td>
<td>264.49 ± 7.91</td>
<td>263.37 ± 8.64</td>
</tr>
</tbody>
</table>

Values significantly differ according to T-test: *: significant <0.05, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, ALT: alanine transaminase, AST: aspartate transaminase, F: fasting, HDL: high density lipoprotein, LDL: low density lipoprotein, TSH: thyroid stimulating hormone, Hb: hemoglobin, TLC: total leucocytic count, PLT: platelets

**DISCUSSION**

Soy foods have long been a subject of scientific investigation due to the medical advantages related with their utilization as they have protective impact against osteoporosis and cardiovascular diseases 23. In the present study, the effect of soy on bone health and some metabolic parameters was investigated in apparently healthy Egyptian postmenopausal women. Studies assessing the effects of soy isoflavones on bone health showed a lot of discrepancy. In the current study, we observed a significant increase in mean osteocalcin levels along with a significant decrease in mean CTX levels after soy isoflavones ingestion. Additionally, there was an increase in BMD at proximal femur after ingestion of soy capsules with no change in BMD at lumbar spine.

Lee and his colleagues reported increase in bone formation markers: Bone alkaline phosphatase and osteocalcin in postmenopausal women after ingestion of 70mg isoflavones daily for 12 weeks 24. A significant increase in BMD at lumbar spine and to lesser extent at proximal femur after consumption of isoflavones was observed by a meta-analysis study 25. Recently, Zhang and colleagues reported decline in the loss of BMD in perimenopausal women after administration of soy isoflavones 26. On the other hand, several studies reported no beneficial effect of soy isoflavones on BMD of the spine, the total hip, or the femoral neck neither on bone turnover markers in postmenopausal women 27.

The mechanisms of action of isoflavones on bone are not fully understood. Several mechanisms have been postulated. Genistein...
isoflavone stimulates osteoblasts through binding to ERs which leads to increase bone formation. Moreover, genistein inhibits osteoclasts by promoting the expression of osteoprotegerin, which is an osteoclastogenic inhibitor responsible for neutralizing the effect of RANKL (receptor activator of nuclear factor-κB ligand) and daidzein induces apoptosis of osteoclasts 29.

Furthermore, isoflavones increase the synthesis of Insulin-like growth factor 1 (IGF-1) at the bone level and it has been known that IGF-1 increases activity of osteoblasts 29. There is some incongruity in the literature about the hypcholesterolemic effect of soy isoflavones. In the current study, we reported a beneficial effect of soy isoflavones on lipid profile in the form of a significant decrease in total cholesterol, triglycerides and LDL-c, accompanied by a significant increase in HDL-c. Similar results were reported in a previous meta-analysis study which documented the beneficial effects of soy which were more prominent in hypercholesterolemic, obese and diabetic individuals 30. In the same context, isoflavones prevented dyslipidemia in rats fed high cholesterol diet 31. Other studies deduced that soy isoflavones lowered TC and LDL-c but had no effect on TG and HDL-c 32.

The hypcholesterolemic effect of isoflavones may be exerted through decreasing lipid reabsorption, bile acid synthesis and hepatic lipid synthesis. The underlying mechanism is probably through its action as ligands for peroxisome proliferator activated receptors (PPARs), liver X receptor, and farnesoid X receptor 31. The activation of peroxisome proliferator-activated receptors (PPAR) is also responsible for the effect of soy on glucose metabolism.

A notable decrease is reported currently in fasting insulin after soy ingestion whereas there were no significant changes in fasting glucose and HOMA I-R test. Charles and colleagues observed in their study a remarkable decrease in serum creatinine in our patients after soy ingestion.

Clinical evidence for hypotensive effect of soy is still controversial. A recent meta-analysis study reviewing clinical studies involving non-hypertensives and hypertensive patients revealed that phytoestrogen/soy derivatives caused insignificant reduction of SBP and DBP 33 which commensurate with the results of our study where there was no effect of soy ingestion on blood pressure.

On the contrary, a previous meta-analysis of 14 randomized controlled trials revealed that isoflavones ingestion significantly decreases systolic blood pressure but not diastolic blood pressure in normotensive adults. The mechanisms underlying the effect of soy on BP are vasodilatation through interaction with the estrogen-response element of genes related to endothelial nitric oxide (NO) synthase that increases endogenous production of NO 34. In addition, animal study reported that soy isoflavones increase renal blood flow and sodium excretion 35. This mechanism may explain the significant decrease in serum creatinine in our patients after soy ingestion.

There are some concerns about the use of soy in patients with hypothyroidism as it interferes with the absorption of synthetic thyroid hormone. Isoflavones were reported to inhibit the activity of thyroid peroxidase (TPO), an enzyme involved in the synthesis of triiodothyronine (T3) and thyroxine (T4) 36. A recent meta-analysis study reported soy protein and/or isoflavones supplementation caused a remarkable decrease in TSH with no change in FT3 or FT4 suggesting that the adverse effect of soy, it is not clinically significant 37. Surprisingly, in the current study, we reported a significant decrease in TSH after soy consumption, unfortunately we did not assess FT3 or FT4. None of the participants developed symptoms suggestive of hyperthyroidism. Previous study in pre-ovariectomized monkeys revealed that dietary soy increased triiodothyronine and prevented decline in thyroxine, which suggested that soy protein and isoflavones consumption did not adversely affect or might even preserve thyroid function in postmenopausal women 38. In addition, the European Food Safety Authority (EFSA) risk assessment on 2015 reported that food supplements containing isolated isoflavones did not cause significant effects on thyroid function in peri- or post-menopausal women 39. The discrepancy in the results between different studies assessing the effect of soy may be due to differences in the constituents of the soy preparations, doses, durations and the populations chosen for the studies. The study faced some limitations as the decline in the number of the participants due to the prolonged duration of the study as well as the absence of a placebo group where each participant served as her own control.

CONCLUSION

The study suggests the ingestion of soy bioactive fraction exerted a beneficial effect on bone health in postmenopausal women. It induced a prominent decrease in bone resorption marker along with the increase in bone formation marker. It caused intensification in bone density at proximal femur. Moreover, soy bioactive fraction had a hypcholesterolemic effect with no adverse actions on thyroid and kidney functions. Consequently, soy bioactive fraction can be safely used as a complementary alternative for HRT in postmenopausal women to improve bone health and decrease cardiovascular risk.

REFERENCES


Salam, et al.: Effect of Soybean on Bone Health and Some Metabolic Parameters in Postmenopausal Egyptian Women


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**GRAPHICAL ABSTRACT**

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