The Possible Improving Effects of γ-Irradiated and/or Extruded Soy Flour on Hypercholesterolemic Rats

Refaat G. HAMZA1, Safaa AFIFI1,*, Abdel-Rahman B. ABDEL-GHAFFAR2, Ibrahim H. BORAI2

1 Department of Food Irradiation Research, National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority, 3 Ahmed El Zamor St., El Zohour District, 8th District, Nasr City, Cairo, Egypt; refaat.galal2009@yahoo.com (*corresponding author)
2 Department of Biochemistry, Faculty of Science, Ain Shams University Cairo, Egypt

Abstract

Hypercholesterolemia is serious conditions that can cause fatal complications without careful management. Among the dietary supplementation with functional food, soybeans possess variety of antioxidant compounds that may lower incidence of hypercholesterolemia and degenerative cardiovascular disease. Thus, the purpose of this study is to determine the effect of gamma-irradiated and/or extruded soy flour on hypercholesterolemic rats. Processing of soy flour by γ-irradiation and/or extrusion reduced the amount of antinutritional factors such as tannin and trypsin inhibitor and resulted in different changes in the total amino acids and fatty acid contents. The animals maintained on the HCD showed remarkable decrease in the level of HDL-C associated with significant increase in the values of serum total lipid, total cholesterol, triglyceride, LDL-C, vLDL-C and the risk ratio in addition to serum concentration of urea, creatinine and uric acid in comparison with those of the control group. However, dietary supplementation of raw and treated soy flour resulted in reduction in the bad changes induced by HCD in the above mentioned parameters. In conclusion, treated soy flour supplementation in diet of rats pointed out to its hypocholesterolemic effect and its ability to improve lipid profile and kidney function of hypercholesterolemic rats.

Keywords: extrusion, gamma-irradiation, hypercholesterolemia, soy flour

Introduction

Hypercholesterolemia is the presence of a high level of total cholesterol in the bloodstream (Barbana et al., 2010) and it is closely associated with obesity, diabetes mellitus, and several other metabolic syndromes (Aleisa et al., 2013). Also, it seems to be an important risk factor accounting for severe atherosclerotic diseases (Augusti et al., 2012).

Several studies indicated that soybean (Glycine max (L.) Merrill) is an effective dietary component and contains many bioactive components such as soy protein and soy isoflavones that can improve lipid profiles and reduce risk factors for chronic diseases like hypercholesterolemia and cardiovascular disease (Llaneza et al., 2011).

Moreover, soybean contains several antinutritional factors, which could limit their consumption such as trypsin inhibitors (TI) and tannin content (Gu et al., 2010) and removal of undesirable components is essential to improve the nutritional quality of soy. Various conventional and simple processing methods have been used such as gamma-irradiation (Dixit et al., 2011) and extrusion (Frias et al., 2011) in order to inactivate or reduce the antinutritional substances.

Therefore, the objective of the present study was oriented to the use of γ-irradiation and/or extrusion for activation or removal of certain antinutritional factors as well as to study the biochemical effect of γ-irradiated and/or extruded soy flour on lipid profile in hypercholesterolemic rats.

Materials and methods

Material

Freshly dried soybean seeds were obtained from the Agriculture Research Institute, Ministry of Agriculture and Land Reclamation, Giza, Egypt. Seeds were sorted by discarding damaged and immature ones. They were stored in air-tight containers at room temperature (25±1 °C) prior to further use.

Processing of soy bean seeds

α-Gamma irradiation treatment

Soybean seeds were packed in polyethylene bags, and sealed by heat. Each bag contained about 500 g for gamma irradiation treatment. They were subjected at ambient temperature to gamma irradiation from 60Co source at the National Center for Radiation Research and Technology (NCRRT) - Nasr City, Cairo, Egypt. The facility used was the Indian Gamma Chamber 400 A, 60 Co facility. The doses applied were 5, 10 kGy, delivered at a dose rate...
Animals

The experiments were conducted on male albino senile rats (130-150 g). The animals were housed under conditions of controlled temperature (30±2 °C) with natural light.

The animals were randomly divided into 8 groups, each consisted of 10 rats.

• Group I: Fed on the basal diet for 10 weeks and considered as control group.
• Group II: Fed on hypercholesterolemic diet for 10 weeks and considered as a positive control group.
• Group III: Fed on hypercholesterolemic diet containing raw soybean flour for 10 weeks and considered as a treated control group.
• Group IV: Fed on hypercholesterolemic diet with γ-irradiated soybean flour (5 kGy) for 10 weeks.
• Group V: Fed on hypercholesterolemic diet with γ-irradiated soybean flour (10 kGy) for 10 weeks.
• Group VI: Fed on hypercholesterolemic diet with extruded soybean flour for 10 weeks.
• Group VII: Fed on hypercholesterolemia diet with γ-irradiated (5 kGy) and extruded soybean flour for 10 weeks.
• Group VIII: Fed on hypercholesterolemic diet with γ-irradiated (10 kGy) and extruded soy flour for 10 weeks.

At the end of the experimental period, the rats in each group were fasted overnight, anaesthetized with diethyl ether and sacrificed. Blood samples were collected by heart puncture, allowed to coagulate and centrifuged to obtain serum for biochemical analysis.

Biochemical Analysis

Total lipids (TL), Total cholesterol (TC), triglycerides (TG) and high-density lipoprotein-cholesterol (HDL-C) were determined according to the procedure described by Zollner and Krisch (1962), Richmond (1973), Fassati and Prencipe (1982) and Lopez-Virella et al. (1977).

Low-density lipoprotein-cholesterol, very Low-density lipoprotein-cholesterol and risk ratio were evaluated according to Friedewald et al. (1972) formulas, respectively by the following equations:

\[
LDL-C (mg/dl) = TC - (TG/5 + HDL-C),
\]

\[
vLDL (mg/dl) = TG/5
\]

\[
\text{Risk ratio} = TC/HDL.\]

Serum uric acid determination was carried out according to Young (1990), serum creatinine determination was carried out according to Giorgio (1974) and urea was determined by Crouch and Batton (1977).

Statistical analysis

Statistical analyses were performed using computer program Statistical Packages for Social Science (SPSS, 1998), and values compared with each other using suitable tests.
Results and discussion

A significant reduction was noticed in the values of tannin content and trypsin inhibitor (TI) by processing of raw flour and the highest reduction was observed in irradiated (10 kGy) + extruded soy flour (Fig. 1). The animals maintained on the HCD showed significant high values of some lipid contents associated with remarkable decrease in the level of HDL-C in comparison with those of the control group. However, dietary supplementation of raw and treated soy flour resulted in reduction in the bad changes induced by HCD in the above parameters. Derangements in cholesterol metabolism have been associated with the etiology of most human diseases. It is widely reported that high dietary cholesterol intake occasioned by a defect in cholesterol transportation, biosynthesis or catabolism is a risk factor in coronary heart disease (CHD) and atherosclerosis (30). Hence, prevention of hypercholesterolemia will be a positive step in the right direction for the management and treatment of cardiovascular diseases. The results of the present investigation show that rats fed with cholesterol rich diet developed hypercholesterolemia with a significant elevation in serum total lipid, total cholesterol (TC), triglycerides and low density lipoprotein (LDL-C) while there was a significant decrease in the HDL-C compared to control (Tab. 2). These results are in agreement with earlier reports on dietary hyperlipidemia (Abdulazeez, 2011) and hypercholesterolemia (Olorunnisola et al., 2012). Moreover, prolonged administration of a high-cholesterol diet...
Tab. 2. Effects of dietary supplementation by raw and treated soy flour to HC rats on lipid profile

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>TL (mg/dl)</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>Risk ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>499.634±12.774ab</td>
<td>138.352±5.079b</td>
<td>105.469±1.976c</td>
<td>48.878±0.582a</td>
<td>68.238±0.398a</td>
<td>21.094±0.396a</td>
<td>2.845±0.023h</td>
</tr>
<tr>
<td>HCD</td>
<td>1310.75±5.689a</td>
<td>295.779±1.867a</td>
<td>202.128±2.056a</td>
<td>27.74±1.004a</td>
<td>227.614±1.512a</td>
<td>40.426±0.411a</td>
<td>10.724±0.356g</td>
</tr>
<tr>
<td>Raw</td>
<td>993.590±2.493ac</td>
<td>237.508±1.849c</td>
<td>162.810±1.750c</td>
<td>32.781±0.673a</td>
<td>172.165±1.111a</td>
<td>32.561±0.348b</td>
<td>7.253±0.111b</td>
</tr>
<tr>
<td>R1</td>
<td>772.121±3.775bd</td>
<td>200.662±2.738c</td>
<td>139.291±2.211c</td>
<td>35.488±0.885a</td>
<td>137.317±2.295a</td>
<td>27.858±0.443a</td>
<td>5.662±0.123b</td>
</tr>
<tr>
<td>R2</td>
<td>754.782±3.024be</td>
<td>192.552±1.689c</td>
<td>134.191±1.649c</td>
<td>38.567±0.959a</td>
<td>127.147±0.356a</td>
<td>26.838±0.09a</td>
<td>5.001±0.09</td>
</tr>
<tr>
<td>H</td>
<td>840.857±1.510e</td>
<td>210.840±3.617f</td>
<td>146.615±2.019d</td>
<td>34.467±1.054b</td>
<td>147.920±2.708a</td>
<td>29.323±0.403b</td>
<td>5.907±0.141c</td>
</tr>
<tr>
<td>HR1</td>
<td>614.518±2.827ef</td>
<td>179.385±1.558g</td>
<td>130.615±1.038e</td>
<td>40.508±1.079d</td>
<td>112.754±0.713e</td>
<td>26.123±0.207g</td>
<td>4.440±0.087f</td>
</tr>
<tr>
<td>HR2</td>
<td>563.367±2.253ef</td>
<td>166.256±1.693g</td>
<td>121.65±1.108d</td>
<td>42.374±0.819d</td>
<td>99.545±1.429g</td>
<td>24.337±0.221f</td>
<td>3.926±0.072f</td>
</tr>
</tbody>
</table>

Tab. 3. Effects of dietary supplementation by raw and treated soy flour to hypercholesterolemic rats on kidney function

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Urea (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>41.479±0.289g</td>
<td>5.355±0.082b</td>
<td>1.186±0.032f</td>
</tr>
<tr>
<td>HCD</td>
<td>59.633±0.311a</td>
<td>8.936±0.082a</td>
<td>2.069±0.062a</td>
</tr>
<tr>
<td>Raw</td>
<td>50.668±0.471b</td>
<td>7.078±0.047b</td>
<td>1.529±0.028b</td>
</tr>
<tr>
<td>R1</td>
<td>45.810±0.337d</td>
<td>6.491±0.023d</td>
<td>1.331±0.021cd</td>
</tr>
<tr>
<td>R2</td>
<td>45.115±0.194b</td>
<td>6.22±0.016d</td>
<td>1.301±0.018de</td>
</tr>
<tr>
<td>H</td>
<td>47.904±0.319f</td>
<td>6.629±0.021d</td>
<td>1.40±0.018f</td>
</tr>
<tr>
<td>HR1</td>
<td>44.672±0.383f</td>
<td>5.720±0.022f</td>
<td>1.268±0.015ef</td>
</tr>
<tr>
<td>HR2</td>
<td>43.218±0.305f</td>
<td>5.533±0.019f</td>
<td>1.202±0.016f</td>
</tr>
</tbody>
</table>

abMean within same column followed by different letters are significantly different at [P<0.05]. Values are means of three replicates (± SE). Raw = raw soy flour; R1=irradiated at dose 5kGy; R2= irradiated at dose 10kGy; H= extruded; HR1 = irradiated (5kGy) + Extruded; HR2 = irradiated (10kGy) + Extruded.
to animals will accelerate the synthesis of TG, inhibit the metabolism of fatty acids and diminish the secretion of TG from the liver to blood by decreasing the β-oxidation of fatty acids. It leads to the accumulation of excess TG in the liver and the content of TC in mouse serum increased significantly, compared with the normal control group rats (Feng et al., 2011).

On the other hand, the present study showed that consumption of HCD containing either raw or processed soy flour have benefit effects on lipid profile which lowered the levels of total lipids, cholesterol, triglycerides and low density lipoprotein (LDL), and significantly increased HDL-C concentration compared to HCD fed rats. Moreover, results of rats fed HCD with γ-irradiated (10kGy) +extruded soy flour provided marked effect on serum lipid and lipoprotein concentrations than the other groups. That refers to the both effect of gamma irradiation and extrusion which cause increase in the contents of essential amino acids and phenolic compound as well as removal of large portion of the antinutritional contents.

The mechanism for the cholesterol lowering effects of soy protein is that peptides resulting from the digestion of soy protein up regulate hepatic LDL receptors (Manzoni et al., 2003). Isoflavones may work by making the liver more efficient to remove the bad cholesterol from the blood by increasing LDL-receptor densities in the liver (Baum et al., 1998). In addition, Lee et al. (2012) reported that soy flour decreased low density lipoprotein–cholesterol and increased high density lipoprotein–cholesterol. High density lipoprotein (HDL) may enhance the removal of cholesterol from peripheral tissue to the liver for catabolism and excretion.

As a result of HCD intake in this study, urea, creatinine and uric acid concentrations were increased in the serum of hypercholesterolemic rats (Tab. 3). The elevated urea level in hypercholesterolemic rats is likely due to increased amino acid catabolism, impaired kidney function or liver damage (Pedraza et al., 2004). Yang et al. (2012) observed an increased serum creatinine levels and induced severe renal tubular necrosis in rats fed the high-cholesterol diet for 8 weeks but not in rats fed the normal diet or high-cholesterol diet for 2 weeks. The authors concluded that long-term hypercholesterolemia appeared to be a risk factor for contrast-induced acute kidney injury (CI-AKI), which might be associated with disorders in intrarenal prostaglandins and abnormalities in renal nitric oxide system induced by lipid peroxidation.

However, supplementation of soy flour (raw or processed) to the HCD induced reduction of urea, creatinine and uric acid levels and that could be attributed to phenolics and isoflavones scavenging properties and prevention of renal tissue damages via protecting lipids structures in renal cells from the peroxidation by ROS induced by hypercholesterolemic diet. Similarly, Khan and Sultana (2004) observed a marked reduction in the levels of blood urea nitrogen and serum creatinine, marker parameters of kidney damage in soy isoflavones pretreatment groups shows that antioxidants of soy is effective in improving kidney function.

Conclusions

The results of our study showed that the soy flour can play a role in the prevention of risk of developing high blood cholesterol and a significant decrease in the concentration of total lipid, triglyceride, total cholesterol, low-density lipoprotein, risk factor. Along with a marked increase in the concentration of high-density lipoprotein, in addition to a marked improvement in kidney function of rats fed a high cholesterol diet supplemented with raw and processed soybean flour compared to rats fed on HCD only.

References


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