Hepatoprotective and Hypolipidemic Effects of Red Ginseng Crude Extract in Obese Rats

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Abstract
The effect of red ginseng extract (RGE) on serum liver enzymes, lipids and some metabolic hormones was studied in obese rats. Sixty male rats were distributed into 6 equal groups. Group (1) was fed on basal diet (negative control), while the other groups were fed on high fat diet for 8 weeks to induce obesity and hyperlipidemia. Thereafter, Group (2) was kept as positive (obese) control, while rats of groups (3), (4), (5) and (6) were orally given RGE at 50, 100, 150 and 200 mg/kg\(^{-1}\)/day, respectively for 4 weeks. At the end of experiment (4 weeks), blood was collected from the rats for estimating serum biochemical constituents. Histological examination of the liver was carried out. The results showed that RGE significantly decreased serum levels of AST, ALT and ALP enzymes; TC, TG and LDL and insulin but decreased leptin hormone in obese-treated rats. The large dose (200 mg/kg\(^{-1}\)) of RGE ameliorated the degenerative changes which seen in liver of obese rats. This study recommends that intake of red ginseng roots may be useful for obese patients who suffer from liver diseases associated with hyperlipidemia.

Key words: Red ginseng; Hepatoprotective; Hypolipidemic; Histopathology; Obese rat.

Introduction

Obesity is defined as an excessive fat accumulation in the body that represents a risk factor to health. It results from an imbalance between energy intake and energy expenditure associated with genetic, metabolic, and behavioral components. The rapid development of the obesity might reflect substantial changes in other factors such as diet\(^1\). Obesity is one of the leading causes of death in humans and represents a risk factor for many chronic diseases such as heart and liver diseases\(^2\).

Red ginseng (Family Araliaceae) is one of slow growing perennial plants with fleshy roots which belong to the Panax genus. Its roots are rich in glycosylated saponins named ginsenosides which have been reported to produce various biological properties. The crude extract of red ginseng roots and its ginsenosides were found to produce hypoglycemic and antidiabetic activities\(^3, 4, 5, 6\); anticarcinogenic effect\(^7\); hepatoprotective action\(^8\), hypocholesterolemic and hypolipidemic effects\(^9\) and antigastic ulcer activity\(^10\) in humans and
The present work aimed to study the hepatoprotective and hypolipidemic effects of the crude extract of red ginseng roots and its effect on some metabolic hormones in obese rat model.

Material and Methods

1. Plant: Dried red ginseng roots (Family Araliaceae) were purchased from a local market of Agricultural Herbs and medicinal plant, Cairo, Egypt. The roots were authenticated by a taxonomist at the Botany Department, Faculty of Science, Cairo University. Red ginseng roots were finely grinded into a fine powder which subjected thereafter to the alcohol extraction.

2. Preparation of plant extract: The crude extract of red ginseng roots was prepared according to Shalaby and Hamowieh\textsuperscript{11}. Two hundred grams of dried fine powder of red ginseng roots were soaked in one liter of 70% methyl alcohol and kept in a refrigerator with daily shaking for 5 days. The methanol was then evaporated using a vacuum rotatory evaporator (Model No. 570, made in Germany). Twenty grams of the semisolid extract were suspended in 2 ml of Tween 80 (suspending agent) and then distilled water was added till 100 ml to obtain 20% liquid extract.

3. Rats: Sixty sexually mature male Sprague Dawley rats weighing 140 -150 g body weight and 10-12 weeks old were used. Animals were obtained from the Laboratory Animal Colony, Helwan, Egypt. Rats were housed in a well ventilated animal room under standard conditions of 24 ± 3°C temperature, relative humidity 50 ± 5% and 12 hr light/12 hr dark cycle at the Animal House of Pharmacology Department, Faculty of Veterinary Medicine, Cairo University. Feed and water were provided \textit{ad libitum} and rats were acclimatized to the environment for 7 days before start of the experiment.

4. Preparation of basal diet: Basal diet was prepared according to the method of Reeves et al.,\textsuperscript{12} It is consisted of 20 % protein (casein), 10 % carbohydrate (sucrose), 4.7% fat (corn oil), 2% choline chloride, 1% vitamin mixture, 3.5 % salt mixture and 5% fibers (cellulose). The remainder was corn starch up to 100 %.

5. Induction of obesity: Experimental obesity and hyperlipidemia model was induced by feeding of rats for 8 weeks on a high fat - diet which supplies 59% calories from fat + 21% calories from carbohydrate + 20% calories from protein. This model in rats closely resembles the reality of obesity in humans as mentioned by Bhatt et al.,\textsuperscript{13}

6. Experiment and grouping of rats: The experiment was carried out on 60 mature male Sprague Dawley rats randomly distributed into 6 equal groups. Group (1) was fed on basal diet and kept as a negative control, while the other five groups were fed on a high fat - diet for 8 weeks for induction of obesity and hyperlipidemia. After 8 weeks feeding on experimental diet, Group (2) was left obese as a positive control, while groups (3), (4), (5) and (6) were orally given, using stomach tube of rats, the red ginseng extract at 50, 100, 150, and 200 mg/kg\textsuperscript{1}, respectively, once daily for 4 weeks. The rats were euthanized using ether anesthetic and blood samples were collected from the orbital plexuses of veins. Blood was left to clot and centrifuged at 3000 rpm for 15 minutes for separation of the serum which was kept in a refrigerator at –20°C pending to the biochemical analyses. Livers of the rats were
preserved in 10% neutral formalin solution till processed for histological examination. All procedures involving animals were performed in accordance with the guidelines for the use and care of laboratory animals which approved by the Departmental Committee on the use and care of laboratory animals, National Research Center, Dokki, Egypt.

7. **Biochemical analyses:** Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were chemically determined according to the method of Bergmeyer et al.,\(^\text{14}\) using kits (purchased from Alkan Company for Biodiagnostic Reagents, Dokki, Cairo, Egypt) and alkaline phosphatase (ALP) was estimated according to the method of Roy\(^\text{15}\) using kits. Serum levels of total cholesterol (TC) were calorimetrically determined using kits according to the method of Richmond\(^\text{16}\); and triglycerides (TG) according to the method of Wahlefeld\(^\text{17}\). High density lipoprotein (HDL) was determined using kits according to the method of Wahlefeld\(^\text{17}\). Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) were calculated mathematically. Concentrations of free thyroxine (T4) hormone were determined using radioimmunoassay kits according to the method described by Patrono and Peskar\(^\text{18}\) and insulin was estimated by hormone specific antibody radioimmunoassay (RIA) kits according to the method of Eskander and Jun\(^\text{19}\). Leptin hormone was measured using enzyme-linked immunosorbent (ELISA) assay as described by Xiong et al.,\(^\text{20}\).

8. **Histological procedure:** Livers of the rats were taken and fixed in 10% neutral formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. These specimens were then cleared in xylene, embedded in paraffin boxes, sectioned at 4-6 microns thickness and stained with Hematoxylin and Eosin (H&E) then microscopically examined using light microscope as described by Carleton\(^\text{21}\).

9. **Statistical analysis:** The data were expressed as mean ± Standard errors (S.E.). Differences between means in different groups were tested for significance using a one-way Analysis of Variance (ANOVA) followed by Duncan’s multiple range test according to Snedecor and Cochran\(^\text{22}\). Differences were considered significant at level P<0.05 using computerized SPSS program version 15.

**Results**

1. **Biochemical analyses:** The results showed that feeding rats on HFD for 8 weeks caused significant (P < 0.05) increases in serum levels of AST, ALT and ALP enzymes as compared to the normal control rats. Oral administration of red ginseng crude extract to obese rats for 4 weeks produced significant reductions in the elevated liver enzymes, in a dose dependent manner as recorded in Table (1).

2. Feeding of the rats on HFD for 8 weeks significantly (P <0.05) increased the serum levels of total cholesterol and triglycerides. Oral administration of red ginseng crude extract to obese rats for 4 weeks significantly (P < 0.05) decreased serum levels of total cholesterol and triglycerides, in a dose dependent fashion, compared to the positive control (obese) group as recorded in Table (2).

3. Data in Table (3) show that rats fed on HFD for 8 weeks had a significant increase in serum level of LDL compared to the negative control group. The crude extract of red ginseng when orally given to experimentally obese rats for 4 weeks significantly decreased the elevated LDL level, but non significant changes in HDL and VLDL in the serum were observed.
Table 1. Effect of red ginseng extract (RGE) on serum levels of aspartate amino transferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) enzymes in obese rats.

<table>
<thead>
<tr>
<th>Groups and Treatments</th>
<th>Parameters</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (Normal rats)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>65.6 ± 1.8</td>
<td>37.5 ± 1.6</td>
<td>86.5 ± 1.9</td>
</tr>
<tr>
<td>Group 2 (Obese rats)</td>
<td></td>
<td>120.6 ± 2.1</td>
<td>60.5 ± 2.4</td>
<td>110.4 ± 2.7</td>
</tr>
<tr>
<td>Group 3 RGE (50 mg/kg)</td>
<td></td>
<td>117.6 ± 2.3</td>
<td>55.5 ± 2.8</td>
<td>108.7 ± 2.5</td>
</tr>
<tr>
<td>Group 4 RGE (100 mg/kg)</td>
<td></td>
<td>112.3 ± 2.4</td>
<td>53.7 ± 2.2</td>
<td>103.3 ± 2.8</td>
</tr>
<tr>
<td>Group 5 RGE (150 mg/kg)</td>
<td></td>
<td>108.8 ± 2.1</td>
<td>49.5 ± 2.6</td>
<td>100.5 ± 2.2</td>
</tr>
<tr>
<td>Group 6 RGE (200 mg/kg)</td>
<td></td>
<td>94.5 ± 1.6</td>
<td>45.5 ± 1.9</td>
<td>98.4 ± 1.2</td>
</tr>
</tbody>
</table>

Means ± SE with different superscript letters in the same column differ significantly at P < 0.05 using one way ANOVA test. n=10 rats.

Table 2. Effect of red ginseng extract (RGE) on serum levels of total cholesterol (TC) and triglycerides (TG) in obese rats.

<table>
<thead>
<tr>
<th>Groups and Treatments</th>
<th>Parameters</th>
<th>TC (mg/dL)</th>
<th>TG (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (Normal rats)</td>
<td></td>
<td>96.98 ± 1.4</td>
<td>55.43 ± 1.5</td>
</tr>
<tr>
<td>Group 2 (Obese rats)</td>
<td></td>
<td>122.95 ± 2.6</td>
<td>70.32 ± 1.9</td>
</tr>
<tr>
<td>Group 3 RGE (50 mg/kg)</td>
<td></td>
<td>108.97 ± 2.8</td>
<td>65.60 ± 1.4</td>
</tr>
<tr>
<td>Group 4 RGE (100 mg/kg)</td>
<td></td>
<td>104.90 ± 2.2</td>
<td>63.50 ± 1.1</td>
</tr>
<tr>
<td>Group 5 RGE (150 mg/kg)</td>
<td></td>
<td>99.90 ± 3.5</td>
<td>60.50 ± 1.2</td>
</tr>
<tr>
<td>Group 6 RGE (200 mg/kg)</td>
<td></td>
<td>93.45 ± 4.2</td>
<td>57.50 ± 1.4</td>
</tr>
</tbody>
</table>

Means ± SE with different superscript letters in the same column differ significantly at P < 0.05 using one way ANOVA test. n=10 rats.

Rats fed on HFD for 8 weeks had significant decreases in serum levels of insulin and an
increase in leptin hormone. There were non-significant changes in thyroxine levels between obese and control normal rats. Oral administration of red ginseng crude extract to obese rats for 4 weeks significantly ($P < 0.05$) increased serum levels of insulin and decreased leptin hormone, compared to the positive control (obese) group as recorded in Table (4).

2. Histopathological examination: Examination of liver sections of normal rats fed on basal diet showed normal histological structure of hepatic lobule (Fig.1). Livers of obese rats that fed on HFD for 8 weeks revealed marked congestion of hepatic central vein and degeneration of hepatic sinusoids (Fig.2) associated with hyperplasia of bile duct (Fig. 3). Oral administration of red ginseng crude extract at 50 or 100 mg kg$^{-1}$ for 4 weeks to obese rats showed moderate congestion of hepatic central vein and degeneration of hepatic sinusoids (Fig.4). The dose 150 mg kg$^{-1}$ of red ginseng extract showed mild congestion of hepatic central vein and hepatic sinusoids (Fig.5). In obese rats given 200 mg kg$^{-1}$ of ginseng crude extract for 4 weeks, the examination of liver sections showed almost normal histological structure of hepatic lobule (Fig.6)

Table 3. Effect of red ginseng extract (RGE) on serum levels of lipoprotein fractions (HDL, LDL and VLDL) in obese rats.

<table>
<thead>
<tr>
<th>Groups and Treatments</th>
<th>Parameters (mg/dL)</th>
<th>HDL</th>
<th>LDL</th>
<th>VLDL</th>
<th>Treatments</th>
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</thead>
<tbody>
<tr>
<td>Normal rats</td>
<td></td>
<td>53.82 ± 1.8$^a$</td>
<td>26.50 ± 1.6$^c$</td>
<td>11.06 ± 1.9$^a$</td>
<td></td>
</tr>
<tr>
<td>Obese rats</td>
<td></td>
<td>49.73 ± 2.1$^b$</td>
<td>59.50 ± 1.4$^a$</td>
<td>14.72 ± 3.7$^a$</td>
<td></td>
</tr>
<tr>
<td>RGE (50 mg/kg)</td>
<td></td>
<td>48.95 ± 1.3$^b$</td>
<td>48.50 ± 1.8$^b$</td>
<td>12.52 ± 1.5$^a$</td>
<td></td>
</tr>
<tr>
<td>RGE (100 mg/kg)</td>
<td></td>
<td>48.90 ± 2.4$^b$</td>
<td>44.70 ± 1.2$^b$</td>
<td>12.10 ± 2.8$^a$</td>
<td></td>
</tr>
<tr>
<td>RGE (150 mg/kg)</td>
<td></td>
<td>47.10 ± 2.1$^b$</td>
<td>40.50 ± 1.6$^b$</td>
<td>12.50 ± 1.2$^a$</td>
<td></td>
</tr>
<tr>
<td>RGE (200 mg/kg)</td>
<td></td>
<td>47.45 ± 1.6$^b$</td>
<td>35.50 ± 1.9$^b$</td>
<td>11.30 ± 1.3$^a$</td>
<td></td>
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</tbody>
</table>

Means ± SE with different superscript letters in the same column differ significantly at $P < 0.05$ using one way ANOVA test. n=10 rats.

Discussion

In the present study, the obesity model was induced by feeding rats on a high fat-diet for 8 weeks. This model closely resembles the reality of obesity in humans as mentioned by Bhatt et al., 13. However, experimental obesity could be induced in rat by other methods such as feeding a high carbohydrate - diet, anterior hypothalamus - lesion and genetically induced obesity as mentioned by Pierpaoli and Lesnikov 23.
Table 4. Effect of red ginseng extract (RGE) on serum levels of free thyroxine (T4), insulin and leptin hormones in obese rats.

<table>
<thead>
<tr>
<th>Groups and Treatments</th>
<th>Thyroxine (µg/dL)</th>
<th>Insulin (µU/ml)</th>
<th>Leptin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (Normal rats)</td>
<td>4.50 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.49 ± 0.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.0 ± 1.2&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group 2 (Obese rats)</td>
<td>4.15 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.58 ± 1.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>31.7 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>RGE (50 mg/kg)</td>
<td>4.35 ± 1.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.15 ± 2.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.6 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>RGE (100 mg/kg)</td>
<td>4.50 ± 1.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.56 ± 1.78&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.9 ± 1.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>RGE (150 mg/kg)</td>
<td>4.15 ± 1.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.15 ± 1.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.4 ± 1.9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>RGE (200 mg/kg)</td>
<td>4.50 ± 0.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>59.50 ± 0.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.4 ± 1.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means± SE with different superscript letters in the same column differ significantly at P < 0.05 using one way ANOVA test. n=10 rats.

Fig. 1. Liver of a control rat showing normal histological structure of hepatic lobules.
Fig. 2. Liver of an obese (non treated) rat showing marked congestion of hepatic central veins (Arrow) and degeneration of hepatic sinusoids (Arrow).
Fig. 3. Liver of an obese (non treated) rat showing hyperplasia of bile duct (Arrow).
Fig. 4. Liver of an obese rat given ginseng extract at 100 mg/kg for 4 weeks showing moderate congestion of hepatic central vein (Arrow) and degeneration of hepatic sinusoids (Arrow).
Fig. 5. Liver of an obese given ginseng extract at 150 mg/kg for 4 weeks showing mild congestions of hepatic central vein (Arrow) and hepatic sinusoids (Arrow).
Fig. 6. Liver of an obese rat ginseng extract at 200 mg/kg for 4 weeks showing nearly normal histological structure of hepatic lobules. (H & E X400)
The hepatoprotective effect of red ginseng extract was evident in this study from decreases of the elevated liver enzyme (AST, ALT and ALP) and amelioration of the degenerative changes which seen in liver of the treated obese rats. This effect was in accordance with that reported by previous authors who concluded that the isolated saponins of Korean red ginseng caused a hepatoprotective effect and induced restoration of hepatic enzymes (AST, ALT and ALP) in CCl4 - intoxicated rats. Moreover, Korean red ginseng accelerated the liver regeneration and ameliorated liver injury after 70 % hepatectomy in rats. The mechanism(s) of hepatoprotection of red ginseng could be explained through an inhibition of activity of cytochrome P450 enzymes in rat liver microsomes as mentioned by Kim et al., Other mechanism may be due to the antioxidant activity of red ginseng because of its high total phenolic compound content as reported by Kim et al.,

The hypolipidemic effect of red ginseng was similar to that previously reported by authors who concluded that red ginseng lowered the elevated levels of total cholesterol, triglycerides and LDL in man, rats and rabbits. They attributed the effect of red ginseng on serum lipids due to its content of saponins. The hypolipidemic of red ginseng could be possibly explained by preventing intestinal absorption of fat in obese rats (fed on high fat- diet) and / or decreasing synthesis of cholesterol in the liver via inhibition of enzymes responsible for its novo synthesis.

Rats fed on a high fat - diet for 8 weeks had significantly lower level of insulin than that of normal rats fed on basal diet. This finding agreed with that previously reported by Huang et al., who concluded that high fat-diet resulted in impaired pancreatic function of insulin secretion in rats. Red ginseng significantly increased serum insulin level, in a dose dependant manner, in obese rats. This result was similar to that previously reported by Ng and Yeung in diabetic mice and by Takaku et al., in obese rats. It has been concluded that hyperinsulinemia and insulin resistance are common features of obesity in humans and experimental animals.

Feeding high fat – diet to rats for 8 weeks increased the level of leptin hormone as compared to the normal control rats. This finding agreed with the previously reported by Inoue who concluded that high fat - diet increased serum leptin level in rats. Red ginseng significantly decreased serum leptin level in obese rats. This result agreed with those previously reported that saponins of red ginseng reduced body weight, decreased serum leptin level and depressed appetite in obese rats.

Concerning the effect of red ginseng extract on liver histology of obese rats, the results showed that the large dose of this extract ameliorated the degenerative changes which seen in liver sections. These findings were similar to those previously reported Kwon and Jang who found that Korean red ginseng accelerated liver regeneration and ameliorated liver injury after 70% hepatectomy in rats. In addition, Lee et al. and Jeong et al., concluded that red ginseng saponins had a hepatoprotective activity against CCL4 and tert-butyl hydroperoxide induced - hepatotoxicity in rats respectively.

Conclusions: Oral administration of red ginseng crude extract to obese rats caused marked hepatoprotective and hypolipidemic effects. These effects were proved using biochemical blood parameters and histopathological examination of the liver. This study suggests that intake of red ginseng roots may be beneficial for patients who suffer from liver diseases associated with hyperlipidemia.
EFFECTS OF RED GINSENG CRUDE EXTRACT IN OBESE RATS

References


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