Effects of dietary Yucca schidigera supplementation on plasma leptin, insulin, iodated thyroid hormones and some biochemical parameters in rats

I. KUCUKKURT1*, Y. DUNDAR1

1Department of Biochemistry, Faculty of Veterinary Medicine, University of Afyon Kocatepe, 03030, Afyonkarahisar, TURKEY
*Corresponding author: kurt@aku.edu.tr

SUMMARY
The aim of this study was carried out to investigate the effects of Yucca schidigera (Ys) which contains steroid saponins, on hormonal regulations of the energy metabolism in rats. Animals were allotted into 3 equal groups of 15 rats each according to the addition of the Ys powder (0, 100 and 200 ppm) to standard diets for 4 weeks and plasma concentrations of hormones (leptin, insulin and iodated thyroid hormones), some analytes (glucose, BUN, total protein, vitamin A and β-carotene) and lipid profiles (cholesterol, triglycerides, LDL and HDL) were monitored at the end of the experimental period. Significant increases in leptin and insulin concentrations were recorded in Ys supplemented rats and were associated with significant decreases in the total concentrations of the thyroid hormones as well as in the free fractions in rodents supplemented with 200 ppm. In parallel, marked decreases in glycemia and BUN concentrations were observed in all supplemented rats and were significantly and inversely correlated to the peptide hormones and positively to the thyroid hormones whereas the concentrations of cholesterol, triglycerides and LDL were dramatically depressed in rats receiving 200 ppm Ys. These results illustrate that the dietary Ys supplementation interferes with the energy metabolism by limiting distribution of energy compounds in the organism and by modulating hormone secretions.

Keywords: Yucca schidigera, rat, steroidal saponin, leptin, insulin, iodated thyroid hormones, blood biochemistry, lipid profile, energy metabolism

RESUME
Le but de cette étude a été de rechercher les effets de Yucca schidigera (Ys) qui contient des saponines stéroïdiennes sur les régulations hormonales du métabolisme énergétique chez le rat. Pour cela, les animaux ont été répartis en 3 groupes égaux de 15 rats chacun en fonction de la supplémentation réalisée en Ys (0, 100 et 200 ppm) d'un régime alimentaire standard pendant 4 semaines et les concentrations plasmatiques hormonales (leptine, insuline et hormones thyroïdiennes iodées), celles de différents analytes (glucose, BUN et protéines totales) ainsi que les profils lipidiques ont été déterminés à la fin de la période expérimentale. Des augmentations significatives des concentrations en leptine et en insuline ont été observées chez les rats supplémentés et ont été associées à des diminutions significatives des concentrations totales des hormones thyroïdiennes iodées ainsi que des fractions libres chez les rongeurs supplémentés par 200 ppm. En parallèle, de nettes diminutions de la glycémie et des concentrations de BUN ont été observées chez tous les animaux supplémentés et elles ont été corrélées significativement et inversement aux concentrations des hormones peptidiques et positivement à celles des hormones thyroidiennes iodées. Les concentrations plasmatiques de cholestérol, de triglycérides et de LDL se sont avérées remarquablement diminuées chez les rats recevant 200 ppm. Ces résultats montrent qu’une supplémentation alimentaire en Ys interfère avec le métabolisme énergétique en limitant la distribution dans l’organisme des composés énergétiques et en modulant les sécrétions hormonales.

Mots-clés : Yucca schidigera, rat, saponines stéroïdiennes, leptine, insuline, hormones thyroïdiennes iodées, biochimie sanguine, profil lipidique, métabolisme énergétique.

Introduction

Yucca schidigera (Ys), a worldwide known plant contains herbal chemicals such as steroidal saponins, phenolic substances, fiber, resveratrol and stilbenes. It is widely used in industry and animal breeding since it contains steroidal saponins [5, 19, 24, 30]. According to the studies, saponin rich plants have been proved to decrease the absorption of nutrients generally in the digestive tract and change the metabolism in this way [5, 19]. The Ys is a saponin rich plant and hypoglycaemic, hypcholesterolemic, antioxidiant, anti-inflammatory, antimicrobial, anti-protozoan and anti hypertensive effects were reported with low Ys contents in diets [2, 8, 18, 22, 25, 33].

Leptin hormone that is secreted from fatty tissue regulates carbohydrate and lipid metabolism and plays an important role in cardiovascular functions, reproduction, lactation and controlling immune system [4, 9, 23]. On the other hand, leptin has effects on regulation of appetite and energy expenditure [3, 6]. In parallel with these literatures, it is thought that the plants exhibiting some effects on the lipid metabolism also have effects on the energy metabolism and consequently on leptin secretion.
Although the literature related to the hypocholesterolemic, hypoglycaemic, anti-inflammatory and anti/protozoan effects of saponin rich plants are common, there aren't any studies dealing with the interactions with hormones such as leptin and thyroid that take part in energy metabolism. This study has been conducted for the purpose of searching the effects of dietary Ys addition at low doses on the secretion of leptin, insulin, and thyroid hormones, and on some biochemical parameters.

Materials and Methods

ANIMALS AND STUDY DESIGN

The Ys standard powder (Sarsaponin 30° contains more than 8% steroidal saponin) was provided by Desert King International (San Diego, CA, USA) and prepared for administration according to the producer's instructions.

Forty-five male Sprague-Dawley rats (3 months old, 180-250 g weight) were used in this study. The study protocol was also approved by the Ethical Committee of the Afyon Kocatepe University (B.30.2.AKÜ.0.8.Z.00/092). They were housed under standard conditions of temperature (23 ± 2°C), humidity, and dark–light cycle (12 hours light/12 hours dark). The animals were fed with standard rat feed (SRF) supplied by Bil-Yem Ltd. (Turkey). Tap water was available ad libitum. All the animals were carefully monitored and maintained. They were randomly allotted into 3 equal groups: rats of the group C were fed ad libitum with standard rat feed (SRF) whereas in the 2 other groups, animals received ad libitum standard rat feed (SRF) supplemented with Ys standard powder (Sarsaponin 30°) at the doses of 100 and 200 ppm, respectively for 4 weeks.

BIOCHEMICAL AND ENDOCRINE ANALYSES

At the end of the experiment, the blood samples were taken from all rats by cardiac puncture into sterile microtubes containing lithium heparinate as anticoagulant after one-night fasting and the animals were sacrificed by deep anaesthesia with a combination of ketamine (80 mg/kg i.p.) and xylasine HCl (Rompun® Bayer Ilac Sanayii, Istanbul; 10 mg/kg i.p.). Plasmas were prepared by centrifugation (1509 g, 10 minutes, 4°C) to measure biochemical parameters.

Plasma leptin (EZRL-83K) and insulin (EZRMI-13K) concentrations were determined using specific rat ELISA kits (Linco Research, Inc, St. Charles, USA) according to the manufacturer's instructions. The concentrations of the Total tri-iodothyronine (T₃) (DSL-10-3100S), total tetra-iodothyronine (T₄) (DSL-10-3200), and free fractions (Free tri-iodothyronine (FT₃) (DSL-10-41100) and Free tetra-iodothyronine (FT₄) (DSL-10-40100), respectively) were determined by specific ELISA-tests (DSL diagnostic systems laboratories, Inc, Texas, USA).

Plasma glucose, total cholesterol and total protein concentrations were measured with commercially available assay kits (Chema Diagnostica, Italy). Urea-N or BUN (blood urea nitrogen) (BioSystems, Spain), triglyceride (Globe Diagnostic), high density lipoprotein (HDL), high density lipoprotein (LDL) concentrations were measured with commercially available assay kits (Human, Germany). The plasma vitamin A and β-carotene concentrations were estimated by the method of SUZIKI and KATOH [31] using a spectrophotometer (Shimadzu UV-1601 visible spectrophotometer, Kyoto, Japan).

STATISTICAL ANALYSES

Statistical analyses of data were analyzed with SPSS statistical software (SPSS for Windows; Release 10.0.1 Standard Version). Comparisons between different groups were performed by one-way ANOVA; if ANOVA revealed significant differences, the post-hoc comparisons were performed by Duncan multiple range tests. Differences between means of p < 0.001, p < 0.01 and p < 0.05 were considered as significant. The results are expressed as means ± standard error.

Results

The variations of the hormonal status in rats according to the dietary Ys supplementation for 4 weeks were reported in the Table I. Plasma leptin concentrations were markedly elevated in the 2 Ys supplemented groups compared to the control group (p < 0.01), and this variation was maximal in animals receiving 100 ppm Ys (100 ppm vs. 200 ppm: p < 0.01). In the same way, insulin concentrations were also significantly increased in both supplemented groups (p < 0.01) independently of the added doses. By contrast, the plasma concentrations of thyroid hormones appeared decreased in rats receiving Ys: total and free fractions of tri-iodothyronine (T₃) and tetra-iodothyronine (T₄) concentrations were dramatically depressed in rats supplemented with 200 ppm Ys compared to the controls (p < 0.05) but only TT₃ and TT₄ concentrations were significantly lowered in rats supplemented with 100 ppm Ys (p < 0.05), the FT₃ and FT₄ concentrations showing intermediate values. As shown in Table II, positive significant associations between insulin and leptin concentrations were evidenced in all rats (r = 0.416; p < 0.05), whereas total concentrations of the iodated thyroid hormones significantly and inversely correlated with leptin (r = -0.335, p < 0.05 with TT₃ and r = -0.408, p < 0.05 with TT₄) or insulin concentrations (r = -0.363, p < 0.05 with TT₃ and r = -0.310, p < 0.05 with TT₄).

As shown in Table III, plasma glucose concentrations showed a statistically significant decrease in both supplemented groups (p < 0.05) and BUN concentrations significantly declined in supplemented rats according to the dose of Ys added to the diets compared to the control rats (p < 0.01). Plasma total protein, vitamin A and β-carotene concentrations also tended to be depressed in supplemented rodents but not significantly compared to the controls. No significant associations between glycaemia and
Table I: Concentrations of some hormones involved in the energetic metabolism (thyroid hormones, leptin and insulin) in rats supplemented with 100 and 200 ppm Ys (Yucca schidigera) powder for 4 weeks compared to the not supplemented controls (15 animals in each group). Results are expressed as means ± standard errors.

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Control</th>
<th>Ys supplementation</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 ppm</td>
<td>200 ppm</td>
<td></td>
</tr>
<tr>
<td>Leptin (µg/L)</td>
<td>0.36 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.43 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Insulin (µg/L)</td>
<td>0.16 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.21 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>TT&lt;sub&gt;3&lt;/sub&gt; (µg/L)</td>
<td>8.5 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.4 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>FT&lt;sub&gt;3&lt;/sub&gt; (ng/L)</td>
<td>1.58 ± 0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.03 ± 0.21&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>TT&lt;sub&gt;4&lt;/sub&gt; (µg/L)</td>
<td>75.9 ± 5.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.1 ± 3.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>FT&lt;sub&gt;4&lt;/sub&gt; (ng/L)</td>
<td>2.83 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.40 ± 0.15&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

TT<sub>3</sub>: Total tri-iodothyronine; FT<sub>3</sub>: Free tri-iodothyronine; TT<sub>4</sub>: Total tetra-iodothyronine; FT<sub>4</sub>: Free tetra-iodothyronine

Different superscripts a,b,c in the same row indicate significant differences (p < 0.05 or more).

Table II: Correlations between endocrine and biochemical parameters in rats dietary supplemented with Ys (100 and 200 ppm) or not. Significant correlations were indicated in bold.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Ys supplementation</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 ppm</td>
<td>200 ppm</td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>0.416</td>
<td>-0.335</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>BUN (mmol/L)</td>
<td>0.188</td>
<td>-0.408</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>0.368</td>
<td>0.405</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Vitamin A (µg/L)</td>
<td>0.202</td>
<td>0.145</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>β-carotene (µg/L)</td>
<td>0.232</td>
<td>0.257</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

BUN: blood urea nitrogen

Different superscripts a,b,c in the same row indicate significant differences (p < 0.05 or more).
BUN concentrations were evidenced \((r = 0.117; \ p > 0.05)\), otherwise, negative significant associations between BUN and leptin concentrations were evidenced in all rats \((r = -0.361; \ p < 0.05)\). In addition, positive significant associations between BUN and \(FT_1\) concentrations were evidenced in all rats \((r = 0.500; \ p < 0.05)\).

The plasma cholesterol, triglyceride and LDL concentrations significantly decreased in Ys supplemented rats compared to the controls \((p < 0.05)\). On the other hand, Ys supplementation has induced significant increases in HDL concentrations compared to the control group \((p < 0.05)\) independently of the added dose (figure 1). As shown in Table II, positive significant associations between cholesterol and triglyceride concentrations were evidenced \((r = 0.368; \ p < 0.05)\), as well as between these 2 biochemical parameters and LDL concentrations in one hand \((r = 0.405 \ p = 0.420\), respectively; \(p < 0.05)\) and total and free fractions of iodated thyroid hormones on the other hand \((r = 0.320 \ p = 0.323\), respectively with \(TT_3\), \(r = 0.377\) and \(r = 0.300\) between cholesterol and \(FT_1\) and between cholesterol and \(FT_4\) \(p < 0.05\). Additionally, LDL concentrations were also positively correlated with \(FT_1\) \(r = 0.334, p < 0.05\) and \(TT_3\) \(r = 0.407, p < 0.05\) while HDL concentrations were negatively associated with LDL \(r = -0.400, p < 0.05\), \(TT_3\) \(r = -0.309, p < 0.05\) and \(TT_4\) \(r = -0.439, p < 0.05\). On the other hand, the plasma lipid profiles concentrations were inversely correlated with leptin \(r = -0.414\) for cholesterol and \(r = -0.484\) for LDL, \(p < 0.05\) and insulin plasma concentrations at a lesser extend \(r = -0.335\) for LDL, \(p < 0.05\) whereas HDL concentrations were positively correlated \(r = 0.366\) with leptin and \(r = 0.351\) with insulin, \(p < 0.05\).

The dietary Ys supplementation has also induced significant increases in plasma insulin concentrations in the present study. Our findings are in line with the studies that DUFFY et al. [7] conducted on rats. DUFFY et al. [7] added Ys to rats' diet with butanol extract, without butanol extract and as Ys only in a dose of 200 mg/kg and stated that in the groups which were applied Ys only and Ys without butanol extract, serum insulin concentrations increased significantly; in the group with butanol extract no change was observed. In diabetic rats, PATEL et al. [26] reported decreases in the plasma leptin concentrations and suggested that insulin may be partly involved in the regulation of the leptin secretion.

Our findings demonstrate that a dietary Ys addition has efficiently altered the thyroid status in rats by decreasing the secretion of iodated thyroid hormones accordingly to the administered doses. In agreement, although a study specially focusing on saponins and thyroid hormones was not available, there are some evidences that natural and synthetic flavanoids might lead to a decrease in \(T_3\) and \(T_4\) concentrations in the short and long terms [32]. On the other hand, it was observed in the present study that the thyroid status was inversely correlated to the plasma leptin and insulin concentrations in rats. In the same way, FAIN et al. [10] demonstrated that leptin mRNA synthesis decreased at a rate of 40% when \(T_3\) was applied to rats with hyperthyroidism, and ZABROCKA et al. [34] have reported marked decreases in serum leptin concentrations and mRNA synthesis as well as the amount of white fat tissue, accepted as an indicator of leptin synthesis, in rats treated with various doses of \(T_3\). However, some studies failed to evidence correlations between thyroid hormones and leptin [12].

As plants including saponin are known to decrease plasma glucose concentrations, the decline in glycaemia observed in the present study in rats dietary supplemented with Ys powder was expected as previously demonstrated [1, 11, 21]. ASLAN et al. [2] also stated that addition of 100, 150 and 200 ppm Ys to the ration for 8 weeks significantly decreased plasma glucose concentrations and that saponins might be effective by preventing glucose absorption in intestines. According to DUFFY et al. [7], glucose concentrations were not significantly coupled to variations of the insulinaemia...
induced by Ys supplementation in presence or not of butanol extract. However, in the present study, decline in glycaemia may be related to a speeding insulin dependent glucose entrance into the cell.

Nevertheless, the same authors found significant decreases in BUN concentrations in rats supplemented with Ys (200 mg/kg) with or without butanol whereas KILLEEN et al. [16] reported that serum urea and ammonia concentrations decreased and naturally urine urea concentrations naturally increased in rats supplemented with Ys alone (without butanol) but not in rats receiving butanol extract. One hypothesis is that plants involving saponin decrease the digestibility of proteins in monogastric species by forming unabsorbed saponin-protein complexes [29]. However, plasma total protein concentrations in the present study were included in the usual range and poorly fluctuated among control and Ys supplemented groups. Another explanation would be related to the insulin effects which may limit protein and amino-acid utilization leading to reduction in the N turn over.

It is stated that saponins from plants taken in low quantity may limit lipid and glucose absorption [2] leading to a decrease in lipid and carbohydrate metabolisms [13, 28]. HAN et al. [14] observed that Platycodi radix saponins prevented fat absorption in the intestines from rats fed with a fatty diet by inhibiting pancreatic lipase, leading to significantly low triglyceride accumulation in liver and low cholesteroledema. Similarly, ZHAO et al. [35] orally administered 35 to 70 mg/kg/day Platycodi radix saponins in rats fed with a fatty diet for 4 weeks and reported significant decreases in serum cholesterol, triglyceride and LDL concentrations dependent to the saponin dose. In agreement, plasma total cholesterol, triglyceride and LDL concentrations were dramatically depressed in Ys supplemented rats contrary to the plasma HDL concentrations which have increased. In a study, in which effects of Panax ginseng extract on lipid metabolism was investigated, it was reported that an eight-week application significantly decreased serum total cholesterol, triglyceride and LDL concentrations and raised HDL concentrations which have increased. Changes in lipid metabolism may be explained by saponins in Panax ginseng extract according to KIM et al. [17].

As far as the blood concentrations of antioxidants (vitamin A and β-carotene) are concerned, no significant differences were evidenced among the control and Ys supplemented group. JENKINS and ATWAL [15] stated that fodder and 0.9% triterpenoid saponin given to chickens negatively affected body weight, fodder consumption, but that steroid saponins had no effect. It was seen here that Ys addition had no effect on vitamin A and β-carotene concentrations.

As a conclusion, Ys supplementation has induced increases in plasma leptin and insulin concentrations as well as in HDL concentrations and decreases in circulating concentrations of thyroid hormones, glucose, BUN, cholesterol, triglyceride, and LDL lipoproteins in rats, suggesting that plant saponins may interfere with energy metabolism and inherent hormonal regulations. However, further studies are needed for identifying all efficient plant substances and for determining with accuracy how they interact with hormone secretion.

Acknowledgement

This study was supported by the Afyon Kocatepe University Scientific Research Projects Commission under Project number 06.VF.08.

References

10. FAIN J.N., CORONEL E.C., BEAUCHAMP M.J.B.S.: Expression of leptin and β2-adrenergic receptors in rat
Dietary Yucca Schidigera on Metabolism in Rats


