Original Article

Effect of *Quercus infectoria* and *Zataria multiflora* extracts on the expression of Apo-B100 and PPAR-α in liver and adipose tissues in insulin resistant rats

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Abstract

Background: Insulin resistance can increase the risk of metabolic syndrome. Studies have shown that expression of PPAR alpha improved insulin function in patients with insulin resistance. Also ApoB100 is an essential ligand for the receptors of low-density lipoproteins (LDL). Increased plasma level of apoB100 is a risk factor for cardiovascular disease (CVD) and its increased production leads to insulin resistance. The aim of this study was to evaluate the effects of Q. Infectoria and *Z. multiflora* extracts on the expression of PPARα gene in adipose tissue and Apo-B100 genes in hepatic tissues of insulin-resistant rats. **Materials and Methods:** Forty Wistar rats were divided into 1- healthy control, 2- high fat control, 3- fenofibrate,4- Q. Infectoria and 5- *Z. multiflora* groups. All groups were fed with high fat diet for 6 weeks expect for the healthy control. Glucose tolerance test was performed to confirm insulin resistance in rats. Then groups 3, 4, and 5 were treated by fenofibrate, Q. Infectoria and *Z. multiflora* extracts respectively. After sacrificing the rats, their liver and fat tissues were removed. Real-time PCR was used to assess PPARα and ApoB100 gene expressions. **Results:** All groups had significant weight gain after 8 weeks. Expression of PPAR-α and ApoB100 genes were the same in Q. Infectoria, *Z. multiflora*, fenofibrate and healthy control groups.**Conclusion:** In conclusion, Q. Infectoria and *Z. multiflora* extracts decreased ApoB100 and increased PPARα gene expressions but these changes were not statistically significant.

Keywords: Quercus infectoria, Zataria multiflora, insulin resistance, ApoB100, PPARα

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Introduction

Metabolic syndrome (MetS) significantly increases the risk of cardiovascular diseases (CVD). The risk of CVD in patients with metabolic syndrome is twice as much as the healthy people (1). According to the American Heart Association, 35% of the adult American population is affected by the metabolic syndrome (2).

There is a resistance to glucose uptake in the presence of insulin in the liver of patients with metabolic syndrome, which leads to hyperinsulinemia. Insulin resistance can lead to hypertriglyceridemia and increased levels of very low density lipoprotein (VLDL) (3).

According to World Health Organization (2) MetS is defined by the coexistence of 3 or more of the following characteristics: 1) body mass index (BMI) ≥ 30kg/m2; 2) HDL < 35 mg/dl in men and 39 mg/dl in women; 3) triglycerides ≥150 mg/dl; 4) Blood

pressure >140/90 mmHg; and 5) hyperglycemia (fasting glucose ≥110 mg/dl).

Adiponectins activate adenosine 5' AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptors (PPARs) –α, which improve insulin function in patients with insulin resistance (4). Hyperinsulinemia leads to more expression of microsomal triglyceride transfer protein (MTP); hence stabilizing apolipoprotein B100 (apoB100) which leads to increased serum level of VLDL(4, 5) .

ApoB100 is an essential ligand for the receptors of low-density lipoproteins (LDL) ApoB100 is made in the liver and is part of VLDL. Increased plasma level of apoB100 is a risk factor for CVD and its increased production leads to insulin resistance (6, 7). Also PPAR-α is a transcriptional factor that regulates those genes that are involved in fatty acid oxidation PPAR-α are expressed in adipose tissue and contribute to the growth and differentiation of adipocyte and lipid metabolism.

In spite of some advancement in introducing new drugs to control metabolic syndrome, 22-44% of the adult American population are still affected by the disease(9). It is therefore necessary to find a useful treatment for patients affected with metabolic syndrome.

Using herbal products has gained an increasing attention because of being inexpensive, easily accessible, and having lower side effects and high capacity to control the disease(10). Extracts of Quercus infectoria (Q. Infectoria) and *Z. multiflora* (*Z. multiflora*) can inhibit intestinal α-glucosidase and decrease post prandial blood glucose level (11). Extracts of Q. Infectoria has anti-inflammatory effects(12, 13) and can probably increase insulin signaling through decreasing inflammatory factors(14). In the previous study, Q. Infectoria and *Z. multiflora* extracts significantly decreased blood glucose and triglycerides in insulin resistant rats(15).

So the aim of this study was to evaluate the effects of Q. Infectoria and *Z. multiflora* extracts on the expression of PPARα and Apo-B100 genes in hepatic and fat tissues of insulin-resistant rats.

Methods

This was an experimental study to evaluate the effects of Q. Infectoria and Z. multiflora extracts on the expression of genes related to insulin resistance compared with high fat, as control in insulin-resistant rats. The study population was Wistar rats. The sample size was calculated as 40 Wistar rats.

Aerial parts of Q. Infectoria and Z. multiflora were used to prepare the extracts. After collection, the herbs were identified and confirmed by a botanist, and then they were washed and dried at room temperature in a dark place. The dried herbs were then well grounded and transformed to powder. One hundred grams of the powder was then transferred to an Erlenmeyer flask and 1000 ml solvent was added and the mixture was shaken for 1 hour at 37ºC and was then left in the dark for 48 hours. After 48 hours, the extract was filtered using Whitman no.44 filter paper and was dried at room temperature. The dried powder was kept at -20ºC (3).

To prepare a high fat diet, a mixture of 30% sugar, 35% sheep fat, and 35% powdered milk were weighted and water was added to transform it to a paste (15). The paste was made in the form of a standard pellet and dried in the vicinity of the air. Then, it was placed in the refrigerator for daily consumption. The food was prepared for 24 hours. It was to be rebuilt the next day.

After 6 weeks of feeding with the high fat diet in high fat control group, glucose tolerance test was performed to confirm insulin resistance in rats. For this purpose, after 12 hours of food deprivation, 2 g/kg body weight of glucose was injected intrastomach to the animal and one hour later blood glucose was measured.

Wistar rats(n=40) were divided into 5 groups including: 1) healthy control group that was fed with normal diet and received no drug; 2) high fat control group that was fed with high fat diet and received no drug; 3) positive control that was fed with high fat diet and received fenofibrate (125 mg/kg/day); 4) Q. Infectoria group that was fed with high fat diet and received Q. Infectoria extract (1000 mg/kg/day); 5) *Z. multiflora* group that was fed with high fat diet and received *Z. multiflora* extract (1000 mg/kg/day).

After sacrificing the rats, ApoB100 and

PPARα gene expressions were assessed in liver and fat tissues real-time PCR RNA extraction was done by using AccuzolTM total RNA Extraction Reagent (Bioneer Company, Korea) according to the kit instructions.

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) software version 18.0 for Windows and Tukey Post Hoc test. P values of <0.05 were considered statistically significant.

Results

Insulin resistance was observed between the HFD and healthy control rats after 6 weeks of feeding with the high fat diet. Blood glucose in the healthy control group was less than 110 mg/dl and in the high fat control group was more than 150 mg/dl. Insulin levels in the healthy control group were less than 50 pmol/l and in the high fat control group was more than 101 pmol/l. These results show that in rats fed the HFD up to 6 weeks, insulin resistance was developed.

We measured the body weight of rats before and after the feeding programs. As depicted in figure 1, 6-week feeding of rats with the HFD resulted in significant increases in body weight as compared to the normal-fed rat. The highest weight gain was in the high-fat control and the groups treated with Q. Infectoria and *Z. multiflora* had the least weight gain.

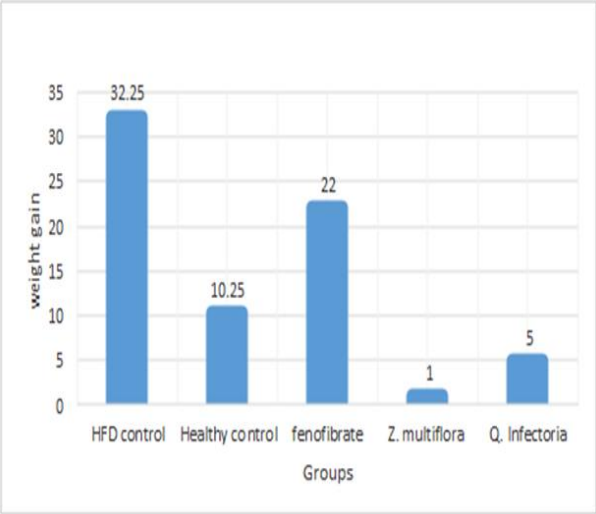

Figure 1. Weight gain in different groups of rats. Data are shown as mean ± SD

The highest food intake was in the healthy control group (21.63 ± 1.92 gr/day). Food intake in other groups were significantly lower than the healthy control group (p<0.001) and had almost equal rates (Table 1).

Table 1. Food intake in rats treated with different drugs compared to controls. Values are shown as mean ± SD

Groups	Mean food intake (g/day)	Standard deviation
Healthy control	21.63	1.92
High fat control	13.50	2.67
Z. multiflora	12.50	2.67
Q. Infectoria	11.50	1.64
Fenofibrate	13.50	2.67

Results indicated that Q. Infectoria, and *Z. multiflora* groups had the highest adipose tissues PPAR-a mRNA expressions between all groups. However, Comparison showed that there was not significant difference between high fat control with the healthy control, fenofibrate, Q. Infectoria, and *Z. multiflora* groups (Table 2).

Table 2. PPAR gene expression compared to high fat control group

Groups	Mean difference	P. value
Healthy control	0.74	0.95
Z. multiflora	0.46	0.99
Q. Infectoria	0.49	0.99
Fenofibrate	o.4o	0.99

The ApoB100 gene expression of liver tissues were compared between the groups. Q. Infectoria had maximum reduction effect on the ApoB100 gene expression among all groups. Also *Z. multiflora* group was shown lower gene expression than high fat control group. But none of these groups showed significant differences (Table 3).

Table 3. ApoB100 gene expression in groups compared to high fat control group

groups	Mean difference	P. value
Healthy control	0.19	1.00
Z. multiflora	0.43	0.99
Q. Infectoria	1.86	0.08
Fenofibrate	1.43	0.31

Discussion

Polyphagia causes obesity, which in turn leads to oxidative stress and finally to metabolic syndrome. In this study, food ingestion and weight change in rats was evaluated. We found that the healthy control group had the highest food ingestion. Other groups had almost equal rates of food ingestion. After the healthy control group, the highest food ingestion was in the high fat control, followed by fenofibrate, Z. multiflora, and Q. Infectoria groups.

Evaluation of the weight change in the groups showed that all the groups had weight gain, with the highest value in the high fat control group followed by fenofibrate, healthy control, Q. Infectoria, and Z. multiflora groups. Our results showed that the groups treated with Q. Infectoria and Z. multiflora had the least weight gain. As weight gain has a direct correlation with insulin resistance and metabolic syndrome, these herbs can be useful in preventing weight gain and decreasing insulin resistance. The reason for this effect might be attributed to increased basal metabolic rate, decreased intestinal absorption, or decreased lipid storage in the rats.

It is generally accepted that PPAR-α regulated genes that are important in fatty acid oxidation. PPAR-α is mainly expressed in fat tissues and is activated by fatty acids and fibrates. PPAR-α plays a role in growth and differentiation of adipocytes, lipid metabolism, lipoprotein synthesis, and tissue inflammatory responses (15). Animal studies have shown that fatty acids regulation is done by PPAR-α(16).

In our study PPAR-α gene expression at the mRNA level in Q. Infectoria, and Z. multiflora groups was increased but this increase was not statistically different from the high fat control group. Li and colleagues showed that green tea leaf could increase glucose and lipid metabolism in insulin resistant hamsters. Increased expression of PPAR-α was also shown in other investigation(17).

In another study to evaluate the effect of garden sage on PPAR-α gene expression, the expression of PPAR-α was increased in the group that had received garden sage, which led to decreased serum level of lipids(18). Other studies have also shown that phenolic polymethoxylate compounds, which are a subgroup of flavonoids, could increase PPAR-α in the liver of the hamsters fed with high fructose diet. The compound also changed adipocytokine secretion, which could lead to decreased insulin resistance in the animals(19).

In a study to assess the effect of Punica granatum on PPAR activation, Haung found that the plant could not change the animals' blood glucose level but it could increase mRNA of PPAR genes. The researcher noted that Punica granatum could affect tissue insulin sensitivity (20).

Apolipoproteins carry lipids in the body. ApoB100 is specifically involved in the synthesis of VLDL and is an essential ligand for LDL receptors. Increased level of such apolipoproteins has a direct correlation with CVD and insulin resistance. Of various modalities to treat insulin resistance those that can inhibit ApoB100 gene expression are among the successful treatments. In our study, we found that ApoB100 gene expression in Q. Infectoria, and Z. multiflora groups decreased, however, it was not statistically different from high fat control group. In another study, 8-week consumption of green tea could significantly affect ApoB100 expression and decreased risk of CVDs (21).

Insulin resistance is accompanied by decreased tissue sensitivity to insulin, and increased serum levels of insulin, glucose, and lipid. In our study, Q. Infectoria, and Z. multiflora did prevent severe weight gain in the rats, which can prevent obesity and in turn insulin resistance. On the other hand, ApoB100 gene expression in Q. Infectoria, and Z. multiflora groups decreased and PPAR-α gene expression in Q. Infectoria, and Z. multiflora groups increased, however, ApoB100 gene expressions in the liver and PPARα gene expression in fat tissues of the rats in Q. Infectoria, and Z. multiflora groups were not

significantly different from the high fat control group.

Conflicts of Interest

The authors have no conflict of interest to declare.

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