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Effects of *Piper sarmentosum* (Kaduk) Water Extract on Adiponectin and Blood Glucose Levels in Ovariectomy-Induced Obese Rats

[1]A. Aida Azlina, [2]S. Farihah, [1]M.S. Qodriyah and [1]M.F. Nur Azlina
[1]Department of Pharmacology,
[2]Department of Anatomy, Faculty of Medicine, Universiti Kebangsaan Malaysia,
Jalan Raja Muda Aziz, 50300 Kuala Lumpur, Malaysia

Abstract: This study was conducted to evaluate the effects of *Piper sarmentosum* (PS) extract and glycyrrhizic acid (GCA) on plasma adiponectin and blood glucose in ovariectomy-induced obese rats. Twenty eight female Sprague-Dawley rats were randomly divided into four groups. Three groups were ovariectomized (OVX), while the remaining group underwent sham operation. The OVX groups were given PS water extract (0.125 g kg^{-1}), GCA (0.120 g kg^{-1}) and water (CTRL), respectively, while the Sham-Operated (SHM) group received only water. Plasma adiponectin and blood glucose were measured at zero, three and five months of treatment, while body weight was measured weekly. All the OVX groups had a significant reduction ($p<0.05$) in the plasma adiponectin compared to the SHM group. After three and five months of treatment, both PS and GCA treated group showed a significant increment ($p<0.05$) in the plasma adiponectin level compared to CTRL group. While, the blood glucose level, only PS treated group showed significant reduction ($p<0.05$) after three and five months of treatment compared to CTRL group but no significant difference ($p<0.05$) occurred in body weight compared to CTRL group. Our finding suggests that water extract of *Piper sarmentosum* may have the ability to reduce the amount of visceral fat in the body as shown by the increment of plasma adiponectin and improve blood glucose levels in obese rats.

Key words: *Piper sarmentosum*, obesity, adiponectin, ovariectomy, blood glucose

INTRODUCTION

Adipose tissue is an endocrine organ that plays a role in energy homeostasis. Adipocytes does not only secrete Free Fatty Acid (FFA) but also releases a large variety of different protein factors, including leptin, plasminogen inhibitor 1 (PAI-1), angiotensin II, Acylating Stimulating Protein (ASP), tumor necrosis factor-alpha (TNF-α), resistin, interleukin-6 (IL-6), adiposin and also adiponectin. All this protein factors are collectively termed as adipocytokines (Greenberg and McDaniel, 2002; Tsao *et al*., 2002; Steppan and Lazar, 2002; Havel, 2002; Berg *et al*., 2002). Dysregulated secretion of adipocytokines has been shown to results in metabolic pertubations associated with pathophysiology of obesity and type-2 diabetes (Berg *et al*., 2002).

Adiponectin is a protein hormone that modulates a number of metabolic processes including glucose regulation and fatty acid catabolism, which is exclusively expressed and

Corresponding Author: Nur Azlina Mohd. Fahami, Department of Pharmacology,
Faculty of Medicine, Universiti Kebangsaan Malaysia,
Jalan Raja Muda Aziz, 50300 Kuala Lumpur, Malaysia
Tel: 603-92897507 Fax: 603-26938205

secreted from visceral, subcutananeous and bone marrow fat depots (Weyer *et al*., 2001; Arita *et al*., 1999) into the bloodstream and abundant in plasma. It plays a role in the suppression of the metabolic derangements that may result in the development of type-2 diabetes, obesity, atherosclerosis and non-alcoholic fatty liver disease. Previous studies have demonstrated that adiponectin levels are inversely related to visceral area and low levels have been associated with obesity, type-2 diabetes and cardiovascular diseases (Arita *et al*., 1999; Hotta *et al*., 2001).

In Malay traditional society, water decoction of the *Piper sarmentosum* leaves are being used for the treatment of cough, headache, waist pain and arthritis, while the water decoction of the roots are used in treatment of menstrual pain and to improve urination. Moreover, the pounded leaves are useful in the treatment of eczema. Most parts of the plant have potential benefits. The water extract of the whole plant had been shown to induce hypoglycemic effect in streptozocin-diabetic rats (Peungvicha *et al*., 1998). While the methanolic extract of the leaves was found to possess a marked neuromuscular blocking activity in the rat phrenic nerve-hemidiaphragm preparation (Ridtitid *et al*., 1998). Furthermore, the chloroform and methanol extracts of the leaves have the ability to act as an antiplasmodial agent against *Plasmodium falciparum* and *Plasmodium berghei* parasites (Najib Nik *et al*., 1999). In addition, the methanolic extracts of the leaves was found to have higher level of antioxidant activity compared to other traditional medicine plants (Chanwitheesuk *et al*., 2005). Therefore, the present study investigates the effects of water extracts of *Piper sarmentosum* on the adiponectin and blood glucose levels in ovariectomy-induced obese rats.

MATERIALS AND METHODS

Preparation of *Piper sarmentosum* Water Extract

Fresh leaves (3 kg) of *Piper sarmentosum* were collected from the Ethnobotanic Garden, Forest Research Institute Malaysia (FRIM) after being identified and confirmed by a plant taxonomist from the Medicinal Plant Division (voucher specimen, FRI 45870) FRIM. All the extraction procedures were performed at the FRIM laboratory. Fresh leaves of the plants were cleaned with tap water and dried at room temperature before been chopped into small pieces. The leaves were then boiled with distilled water (90%,v/v) at 80°C for 3 h. The water extract was then concentrated and followed by freeze-drying to form powder. The powdered extract was stored at 4°C until further use.

Animals Preparation

All procedures were carried out in accordance with the institutional guidelines for animal research of the Universiti Kebangsaan Malaysia (FAR/2006/AZLINA/19-APRIL/168-MARCH-2007). Twenty-eight female Sprague-Dawley rats weighing 180-200 g were maintained in normal room temperature with adequate ventilation and illuminated for 12 h daily (lights on from 0700 to 1900). Two rats were kept in each cage and allowed free access to rat chow (Gold Coin, Malaysia) and water. The rats were randomly divided into four experimental groups. Three groups underwent an experimental ovariectomy and one group underwent a sham operation. The ovariectomized groups were given *Piper sarmentosum* water extract (PS), glycyrrhizic acid (GCA) and water (CTRL) respectively, while the sham-operated (SHM) group received only water.

Experimental Protocol

The procedures were carried out in accordance with the institutional guidelines for animal surgery of the Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC).

The rats were anaesthetized with intramuscular injection of ketamine (Troy Laboratories, Australia) and xylazine (Troy Laboratories, Australia) cocktail (1:1). The hairs on the both sides of the body were shaved off from the lowest rib to the hip region. For the rats which underwent a sham operation, only skin and muscles were cut but the ovaries were spared. Meanwhile for rats in the ovariectomized groups, bilateral ovariectomy was performed using an incision 1.5 cm inferior to the palpated rib cage. The ovaries and surrounding tissue were removed; the incision was closed by suturing the muscles and stapling the skin. Antibiotic cream was applied on the wound and the animal was placed on paper towels in a cage until awake. *Piper sarmentosum* and glycyrrhizic acid were administered by oral gavage at a dose of 1.25 mg kg^{-1} (Peungvicha *et al*., 1998) and 1.20 mg kg^{-1} (Al-Wahaibi, 2007) respectively for five months. The control groups were sham administered water by oral gavage. Blood sampling were collected from orbital sinus on 0, 3 and 5 months of treatments.

Plasma Adiponectin and Blood Glucose Measurement

Blood glucose levels were monitored using the Glucometer (Optium). Plasma adiponectin levels were determined using the rat adiponectin enzyme-linked immunoabsorbent assay (ELISA) kit (Linco Research, USA).

Body Weight Measurement

Body weight was taken every week by using the electronic weighing scale (Tanita, Japan) in order to obtain the weight measurement.

Statistical Analysis

All data were tested for normal distribution and presented as Mean±SD. Differences in the body weights and blood pressure were analyzed by a one-way Analysis of Variance (ANOVA) followed by Tukey test for multiple group comparisons. A value of $p<0.05$ was taken as significant. All statistical analysis was conducted using the SPSS version 11.5 software.

RESULTS AND DISCUSSION

All the ovariectomized groups had a significant reduction ($p<0.05$) in the plasma adiponectin compared to the sham-operated group. However, after 3 and 5 months of treatment, both *Piper sarmentosum* water extract (PS) and glycyrrhizic acid (GCA) treated group showed a significant increment ($p<0.05$) in the plasma adiponectin level compared to CTRL group (Fig. 1).

Meanwhile, only the blood glucose level in *Piper sarmentosum* water extract (PS) treated group showed significant reduction ($p<0.05$) after 3 and 5 months of treatment compared to CTRL group (Fig. 2).

The results also showed that ovariectomized rats had a significant increased in body weight after three months compared to the sham-operated rats. However, there was no significant increased between all the treated groups in body weight after three months of treatment (Fig. 3).

Visceral obesity and features of the metabolic syndrome are caused by highly production of glucocorticoid exclusively within visceral adipose tissues (Masuzaki *et al*., 2001). Glucocorticoid potentiates the adipogenic process and anabolic lipid metabolism in adipocytes (Berger *et al*., 2001). During differentiation and maturation process, adipocytes

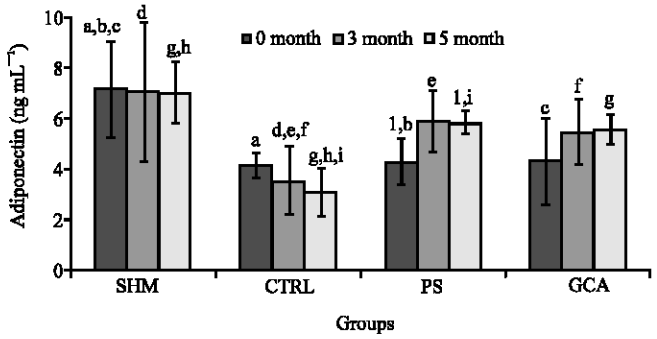

Fig. 1: Plasma adiponectin levels on 0 month, 3 months and 5 months of treatment (N = 7). Values are Mean±SD. The ovariectomized groups are CTRL, PS and GCA, while the sham-operated group is SHM. The data are the Mean±SD. Same letters shows statistically significant between groups ($p<0.05$). Same numbers are statistically significant within group ($p<0.05$)

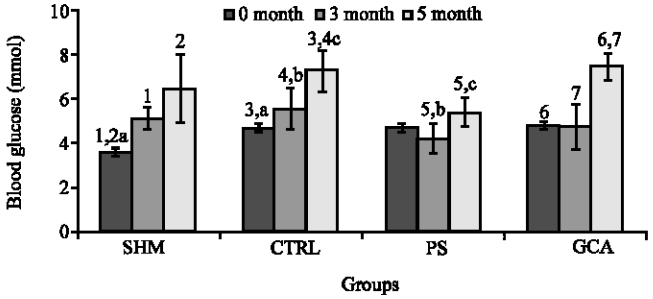

Fig. 2: Blood glucose levels on 3 months and 5 months of treatment (N = 7). Values are Mean±SD. The ovariectomized groups are CTRL, PS and GCA, while the sham-operated group is SHM. The data are the Mean±SD. Same letters shows statistically significant between groups ($p<0.05$). Same numbers are statistically significant within group ($p<0.05$)

release a variety of adipocytokines (Greenberg and McDaniel, 2002; Tsao *et al*., 2002; Steppan and Lazar, 2002; Havel, 2002; Berg *et al*., 2002).

Adiponectin is an adipocyte-derived protein that is abundantly expressed and secreted from adipocytes. Recent preclinical and clinical studies have implicated adiponectin as an important mediator of insulin sensitivity in peripheral tissue (Tsao *et al*., 2002; Steppan and Lazar, 2002; Havel, 2002; Berg *et al*., 2002). Plasma adiponectin concentrations are markedly reduced with increasing insulin resistance in obese patients with type-2 diabetes (Weyer *et al*., 2001; Arita *et al*., 1999; Hotta *et al*., 2001; Nadler *et al*., 2000; Hara *et al*., 2002; Comuzzie *et al*., 2001; Stefan *et al*., 2002; Lindsay *et al*., 2002; Rolland *et al*., 2007).

Present findings showed that plasma adiponectin levels (Fig. 1) were reduced and the blood glucose levels (Fig. 2) were increased in all ovariectomized groups compared to the sham-operated group. The reduction of the plasma adiponectin levels and the increment of the blood glucose levels are caused by obesity due to ovariectomy procedure. These results were comparable with Almonte *et al*. (2003) finding, which reported that adiponectin expression was significantly reduced in Zucker diabetic fatty (ZDR) rats. They also reported

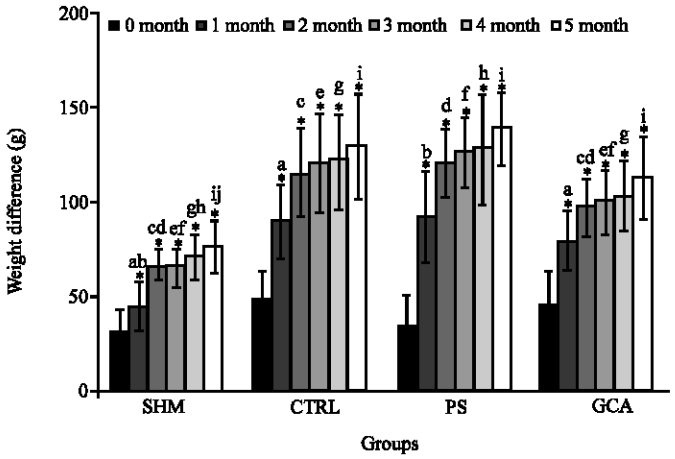

Fig. 3: Weight difference between group over time. Same letters are statistically significant ($p<0.05$) between groups. *Shows significant difference between 0 month (*$p<0.05$). N = 7 in each group. The data are the Mean±SD

that plasma adiponectin levels correlated positively with insulin sensitivity and negatively with the degree of hyperglycemia, hyperinsulinemia, hyperlipidemia and percentage of visceral fat pad mass (Almonte *et al*., 2003).

However, after 3 and 5 months of treatment, both PS and GCA treated group showed a significant ($p<0.05$) increment in plasma adiponectin levels compared to CTRL group (Fig. 1). This increment led to the idea that the adipocytes had been reduced. In support of this idea, our previous studies provides additional data showing that both PS and GCA treated group showed an improvement in lipid profile compared to CTRL group in ovariectomized induce obese rats.

Meanwhile, only the blood glucose levels in the PS treated group showed a significant reduction ($p<0.05$) compared to the CTRL group (Fig. 2). This reduction was predictable since Peungvicha *et al.* (1998) reported that *Piper sarmentosum* water extract shown hypoglycemic effects in diabetic induced rats (Peungvicha *et al*., 1998). Even though the GCA treated group showed an increment plasma adiponectin levels, but it did not show any reduction in blood glucose levels (Fig. 2). This can probably be explained by the fact that glycyrrhizic acid is known to be derived from licorice which is commercially used as artificial food sweetener in food industry. Thus, prolonged consumption could possibly lead to the increased in blood glucose levels.

Ovariectomy is known to cause the increase in body weight which results in obesity (Chu *et al*., 1999). Present finding showed that the body weight was increase in the ovariectomized rats by up to 20% as compared to the SHM group after one month of surgery. This finding was comparable with a study by Kano and Doi (2006). The weight difference for ovariectomized rats increased almost double between weeks 4 to weeks 8 compared with the sham-operated rats.

After five months of treatment, significant ($p<0.05$) reduction in body weight only occurred in GCA treated group. There was no dissimilarity for the PS treated group, as compared to CTRL group. However this result was not correlated with the adiponectin plasma level. A probable explanation could be that the body weight that we measured was the total body weight of the rats which included the subcutaneous fat as well as the visceral fats and it is known that adiponectin plasma level is more correlated with the visceral fat.

In conclusion, we showed that both *Piper sarmentosum* water extract and glycyrrhizic acid increased the plasma adiponectin levels but only *Piper sarmentosum* water extracts improved blood glucose levels. This finding suggests that *Piper sarmentosum* water extract may have the ability to reduce the amount of visceral fat as well as the ability to improve blood glucose levels in ovariectomy-induced obese rats.

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