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Research Article

Effect of Sukun Leaf Extract [*Artocarpus altilis* (Park.) Fosberg] on Insulin Resistance in Obese Rats (*Rattus norvegicus*): A Study of Free Fatty Acid (FFA) Levels

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Abstract

Background and Objective: The state of obesity is closely related to insulin resistance, which leads to type 2 diabetes. Free Fatty Acids (FFAs) play a role in the development of insulin resistance. *Artocarpus altilis* plants have traditionally been used by the people of Indonesia for the treatment of diabetes mellitus. This study aimed to determine the concentrations of *A. altilis* leaf extract that can improve insulin resistance in obese rats (*Rattus norvegicus*) through identification of the concentrations that can decrease the levels of FFA. **Methodology:** This study is a Randomized Controlled Trial (RCT). The rats were divided into five groups, each consisting of five rats. The rats were administered a high-fat diet (45%) (open source) to induce fattening and their weights were measured for assessing obesity. The fasting blood sugar (GDP) was monitored through gluco DR tests to ensure that the mice exhibited an increased glucose concentration. Group I was designated the negative control group, the rats in group II were administered metformin HCl and formed the positive control group and groups III, IV and V were denoted the 5, 10 and 15% *A. altilis* test groups. The groups underwent treatment for 14 days and ELISA was performed for the assessment of the FFA levels in each group after testing. A data analysis using nonparametric tests was performed to assess the significance of the differences among the groups at a 95% confidence level. All of the data were analyzed with SPSS version 21.0 (SPSS, Inc., Chicago, IL, USA). **Results:** The FFA levels in the negative control and the10% *A. altilis* ($p = 0.000$) and 15% *A. altilis* ($p = 0.016$) extracts did not show any significant differences, differences in the FFA content were found between the positive control and the 5% *A. altilis* (0.034) and 10% *A. altilis* ($p = 0.020$) test groups. **Conclusion:** The 10% *A. altilis* extract can lower the FFA levels in obese mice. Therefore, *A. altilis* should be considered for use in the prevention of insulin resistance.

Key words: *A. altilis*, obesity, FFA, insulin resistance

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Obesity is associated with insulin resistance and an increase in the incidence of obesity is followed by an increase in the incidence of type 2 diabetes. Insulin resistance is a condition defined by a reduced response to normal circulating insulin and plays an important role in the development of type 2 diabetes mellitus[1]. Obesity is a risk factor for type 2 diabetes, dyslipidemia and cardiovascular disease. In obese individuals, adipose tissue releases a number of non-esterified fatty acids, glycerol, hormones, cytokines, pro-inflammatory and other factors involved in the development of insulin resistance[2]. Several studies have demonstrated the role of insulin in the glucose homeostasis pathway and in controlling blood pressure and vascular reactivity, which are factors that contribute to the definition of metabolic syndrome[3]. Free Fatty Acids (FFAs) also play a role in the development of insulin resistance through the accumulation of lipids, the activation of several protein kinase C isoforms and a reduction in the tyrosine phosphorylation of IRS 1 and 2[4]. The FFAs are important mediators secreted from adipocytes and serve as a link between obesity and insulin-resistance conditions. At present, FFAs have been considered pathogenic factors for the development of type 2 diabetes mellitus[5].

The plasma FFA concentrations in obese individuals are generally higher than those in non-obese individuals. A high concentration of FFAs in plasma contributes to the development of insulin resistance in the muscles, liver and pancreas[6]. Improvements in insulin resistance can be obtained by an increase in the number of adipocytes, a decreased clearance of FFA and increased lipolysis (caused by an increase in the rate of lipolysis in adipocyte cells)[7]. The available evidence strongly suggests that FFAs can inhibit the action of insulin in multiple target tissues, such as skeletal muscle, liver and vascular endothelial cells. Long-term increases in the concentrations of FFAs (chronic condition) will induce insulin resistance in some of these target tissues and trigger inflammation and atherosclerosis[8].

A qualitative analysis of the active substances in the *Artocarpus altilis* leaf extract revealed that the active substances include phenols, flavonoids, alkaloids and saponins concluded that methanol extracts of dried leaves of *A. altilis* contain alkaloids, flavonoids, tannins, phenols and saponins[9,10]. The role of polyphenol as an antioxidant is thought to protect pancreatic β cells from the toxic effects of free radicals that are produced under conditions of chronic hyperglycemia. According to Kaneto *et al.*[11], the provision of antioxidants can improve the mass of pancreatic β cells and maintain their insulin content. These cells (muscle cells, adipose cells and liver cells) contain insulin receptors and the binding of free radicals to these cells increases insulin signaling to induce intracellular GLUT4 translocation to the cell membrane, which ensures that the cells can take up glucose from the blood. This study aimed to determine the concentrations of *A. altilis* leaf extract that can improve insulin resistance through identification of the concentrations that can cause decreases in the FFA levels in obese rats (*Rattus norvegicus*).

MATERIALS AND METHODS

The leaf materials used in this study were in the form of leaves from *Artocarpus altilis* plants (Park) or Fosberg leaf powder with 90% ethanol. The ethanol extract was obtained and then concentrated using a rotary evaporator (BuchiLabortechnik AG, Switzerland) to obtain a liquid extract. The liquid extract was then evaporated until it thickened. Subsequently, the ethanol extract of *A. altilis* was prepared into suspensions with concentrations of 5, 10 and 15% w/v. The test animals used were healthy, 4-month-old, Wistar male rats weighing 150-200 g. The rats were divided into five groups and fed a high-fat diet of 45% fat rodent diet (open source) until their weight reached 300 g. Increases in the blood glucose level were assessed by monitoring the fasting blood sugar (GDP) of the rats using the GlucoDR tool (HimedicalCo., LTD, South Korea). Group I was the negative control group, the rats in group II formed the positive control group and were administered metformin HCl and groups III, IV and V were denoted the 5, 10 and15% *A. altilis* test groups, respectively. After 14 days of treatment, the FFA levels in each group were assessed through a quantitative sandwich enzyme immune assay (ELISA). The significance of the differences among the groups was assessed through nonparametric tests with a 95% confidence level. All statistical analyses were performed using the Statistical Package for Social Science (SPSS) version 21.0 for windows (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

In the present study, the effects of the *A. altilis* extracts on the FFA levels were analyzed statistically using nonparametric tests and are presented in Table 1.

Table 1 shows , the average levels of FFAs presented the most significant differences between the negative control and the 10% *A. altilis* extract test group, which presented FFA levels as 234.51±4.32 and 141.61±27.02 μmol L^{-1}. The mean FAA levels of the 15 and 5% *A. altilis* extract groups, were 168.13±30.71 and 229.67±37.10 μmol L^{-1}, respectively,

Table 1: Average FFA levels in each group after treatment

Groups	n	Mean	SD
Negative control	5	234.51	4.32
Positive control	5	188.06	33.59
A. altilis extract (5%)	5	229.67	37.10
A. altilis extract (10%)	5	141.61	27.02
A. altilis extract (15%)	5	168.13	30.71

Table 2: Inter-group differences in the post-treatment FFA levels

Groups	Variance level	p
Negative vs. positive control	46.45	0.020
Negative control vs. 5% *A. altilis*	4.84	0.794
Negative control vs. 10% *A. altilis*	92.90	0.000
Negative control vs. 15% *A. altilis*	48.36	0.016
Positive control vs. 5% *A. altilis*	-41.61	0.034
Positive control vs. 10% *A. altilis*	46.45	0.020
Positive control vs. 15% *A. altilis*	1.93	0.917
5% *A. altilis* vs. 10% *A. altilis*	88.06	0.000
5% *A. altilis* vs.15% *A. altilis*	43.54	0.028
10% *A. altilis* vs. 15% *A. altilis*	-44.51	0.025

Source: Primary data 2016

which were also significantly different from the levels detected in the negative control.

Table 2 shows the 15% *A. altilis* extract significantly decreased the FAA levels to levels that were significantly greater than those detected in the negative control but these levels were not significantly different from those of the positive control. In addition, the 10% *A. altilis* extract also decreased the FFA levels to levels that were significantly higher than those found in both the negative and the positive control groups. Furthermore, even though the 5% *A. altilis* extracts lightly decreased the FFA level, the resulting level was not significantly different from the levels detected in the negative and positive control groups. In the fasting state, insulin suppression and stimulation of glucagon production control the blood glucose concentrations. These processes allow the heart to mobilize glucose from stored glycogen and synthesize glucose from amino acids and pyruvate (gluconeogenesis). Moreover, under conditions of low insulin levels, the absorption of glucose by muscles is minimized and adipocyte tissue releases FFAs[12]. Insulin not only reacts with glucose but also increases the esterification of fatty acids to triglycerides and inhibits the hydrolysis of triglycerides and the release of FFAs into the circulation (lipolysis). Thus, an increase in the level of insulin resistance in adipose tissue increases the release of FFAs into circulation. Several studies have shown that high concentrations of FFAs are associated with the onset of peripheral insulin resistance and liver disease. Increased levels of FFAs and intracellular lipids block insulin signaling, leading to a decrease in the insulin-stimulated conductivity of glucose into muscles, a process that might be mediated by a decrease in GLUT-4 translocation. The suppression of glucose transport to muscles causes decreases in glycogen synthesis and glycolysis in muscles. In the liver, high FFA levels might contribute to hyperglycemia due to the antagonistic effect of insulin on endogenous glucose production[4].

Glucose is stored as fat in fat tissues. In addition to encouraging fat synthesis, insulin also inhibits the breakdown of fat in adipocytes. Similarly, insulin encourages the absorption of amino acids and protein synthesis and inhibits the breakdown of proteins. The anabolic effect of insulin causes an increase in glycogen synthesis and a decrease in the decomposition of fat and protein[13,14]. The roles of the active compounds in *A. altilis* extracts in the homeostasis of blood glucose are unclear. However, it is suspected that the acetone-water-soluble compounds comprise a class of polyphenols of unknown type. The active compounds of this class of polyphenols act as antioxidants that can prevent and reduce free radicals by reacting directly with them[15]. The active compounds of the *A. altilis* extracts encourage communication within cells to induce the activation and increase the insulin sensitivity of receptors that can function as GLUT4 to transport glucose from the blood circulation into the cells and decrease the blood glucose levels. Blood glucose is transported in the form of glycogen in the liver and muscle cells through a process called glycogenesis and is stored as fat in adipose cells through the process of lipogenesis. The addition of these energy reserves will result in weight gain[16].

Polyphenols can act as insulin secretagogues or insulin memetics and thereby reduce the complications of diabetes. Polyphenols stimulate the synthesis of glycogen in the muscles through the mechanism of insulin transduction signaling and play their role by stimulating glucose uptake into peripheral tissues as well as regulating the activity and improving the function of enzymes to control the metabolic pathway. As a result, polyphenols can reduce the blood glucose levels[17].

Artocarpus altilis extracts contain polyphenols that are thought to synergize and enhance antioxidant activity by increasing the levels of cellular antioxidant enzymes, such as superoxide dismutase (SOD), catalase and glutathione peroxidase[18]. Polyphenols are capable of stimulating a 16% increase in insulin secretion from pancreatic β cells by affecting Peroxisome Proliferator Activated Receptors (PPARs)[19]. Polyphenols suppress the absorption of glucose or improve glucose tolerance. Moreover, polyphenols stimulate glucose uptake in peripheral tissues and thereby regulate the activity and expression of enzymes involved in carbohydrate metabolism pathways, which can be considered insulin mimetic due to their influence on insulin signaling[20,21].

Most obese individuals have high levels of plasma FFAs and it is known that high insulin levels lead to peripheral (muscular) resistance due to the inhibition of both insulin-stimulated glucose uptake and glycogen synthesis.

This mechanism involves the accumulation of intramyocellular diacylglycerol and the activation of protein kinase C. The FFAs also cause liver insulin resistance by inhibiting the ability of insulin to suppress glycogenolysis. In contrast, 30-50% FFA levels support basal insulin secretion and potentiate insulin secretion stimulated by glucose. The ability of FFAs to stimulate insulin explains why the majority (~80%) of obese individuals do not develop type 2 diabetes. These individuals are able to compensate for FAA-induced insulin resistance by enhancing insulin secretion in a FAA-mediated manner. Individuals who are unable to achieve this compensation (perhaps due to genetic reasons) eventually develop type 2 diabetes. The FFAs have the ability to anticipate and track IKB/NFkB, which is involved in many inflammatory processes. Thus, increased FFA levels in plasma not only cause insulin resistance in skeletal muscle and the liver but also might play a role in the pathogenesis of coronary artery disease. In cells with insulin receptors (muscle cells, adipose cells and liver cells), the binding of free radicals will increase insulin signaling and intracellular GLUT4 translocation to the cell membrane, which would allow the cell to uptake glucose from the blood[22,23]. In general, a decrease in oxidative stress can reduce insulin resistance and inhibit pancreatic β cell damage.

CONCLUSION

It is concluded that, the 10% *A. altilis* extract can lower the FFA levels in obese mice and should thus be considered for use in the prevention of insulin resistance.

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