The Efficacy of Combination Extract Andrographis

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The Efficacy of Combination Extract *Andrographis peniculata* and *Syzygium polyanthum* on Glucose Uptake in Skletal Muscle in Diabetic Rats

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Abstract

Background
Insulin resistance is impaired insulin signaling cascade in target cells to respond normal or elevated circulating insulin to the final cellular effect, such as translocation of vesicles containing GLUT4 glucose transporters, which is the major mediator of glucose removal from the circulation and a key regulator of whole-body glucose homeostasis. Among the several plants, *Andrographis peniculata* (sambiloto) and *Syzygium polyanthum* (Daun salam).

Methods
In the present study, a combination was made, *Andrographis peniculata* (sambiloto) and *Syzygium polyanthum* (Daun salam), and to determine the effects of combination on tretament insulin resistance. Male Wistar rats (weight, 200-300 g) were randomized into five groups (6 rats/group). Group 1: negative group. Group 2: positive group (metformin 63 mg/kgBW). Group 3,4 and 5: treatment with extract combination, each group 250 mg/kgBW, 500 mg/kgBW and 1000 mg/kgBW. Rats were induced by high fat diet-glucocorticoid for insulin resistance. Insulin and GLUT-4 were assayed by ELISA.

Results
Treatment with extract combination (250, 500 and 1000 mg/kgBW) and metformin for 2 weeks showed a sigificant decrease in fasting plasma insulin compare with the negative control rats with a reduction of 11,2%, 33,6%, 20% and 19,4%, respectively.

Conclusions
Two weeks treatment either extract combination or metformin in diabetic rats, significantly increased GLUT 4 level ($p < 0,05$) with a percentage increase of 6,68%, 15,21%, 12,76% and 1,77%.

Keyword : Andrographis-Syzygium-GLUT4-Diabetics Rat

Background

World Health Organization (WHO) has published that Diabetes mellitus (DM) is one of the leading causes of death worldwide. Type 2 diabetes is the most common form of which is 90-95% of all cases of diabetes. Epidemiological studies have shown an increasing trend of the incidence and the prevalence of type 2 DM in many countries in the world; including Indonesia is ranked number 7 of the 10 largest countries of diabetics worldwide. This disease is characterized by elevated blood sugar levels, which is preceded by insulin resistance or abnormal insulin secretion plays an important role in the onset and progression of disease. Insulin resistance is impaired insulin signaling cascade in target cells to respond normal or elevated circulating insulin to the final cellular effect, such as translocation of vesicles containing GLUT4 glucose transporters, which is the major mediator of glucose removal from the circulation and a key regulator of whole-body glucose homeostasis. GLUT4 is particularly expressed in adipose tissue and skeletal muscle. Insulin resistance in Human type 2 DM has been proposed to connect with functional defect in the muscle glucose transport system. An important mechanism in the treatment of DM is to stimulate plasma glucose uptake into peripheral tissues including the skeletal muscle and adipose tissue. Skeletal muscle is the primary tissue responsible for glucose use in the postprandial state. In animal models of diabetes, a decrease in muscle GLUT4 is caused by to provide to the insulin resistance and participates the hyperglycemic state.[1-3]

In the recent years, the various pharmacology activities of *Andrographis paniculata* either alone or in combination with other medicinal plants have been examined preclinically, and the results supported the clinical trial in human. Many bioactive compounds of *Andrographis paniculata* that have been isolated and identified indicated beneficial health effect for complex disease such as diabetes. Its major constituents are lactones, diterpenoids, diterpene glycosides, flavonoid, and flavonoid glycosides. Among those, diterpene lactone andographolide was reported to posseess antidiabetic activity and showed α-glucosidase inhibitory effects in a concentration manner . Beside lowering blood glucose, *Andrographis paniculata* canpreserve pancreatic beta cells at the same time. While antidiabetic potential of *Andrographis paniculata* has been investigated widely in streptozocin- or alloxan-induced diabetic animals, the antidiabetic potential of *Syzygium polyanthum* (Sp) or Indonesian bay leaf has not been studied intensively .[4-6]

Syzygium polyanthum herb empirically was said to have cleanse heat, to detoxify toxins, to dry moist, especially in fever therapy, headache, cough due to hot lungs, inflammation / pain of throat, dysentry, pain or heat sensation when urinating, eczema, etc . In Indonesia, *Syzygium polyanthum* is used as antiinflammatory medicine, antipiretic, and to detoxify toxins. Meanwhile, root and leaves are used to cure the bite of snake and insects in India . *Syzygium polyanthum* consists of essential oil (citral, eugenol), tannin and flavonoid. It is proven to cure diarrhea in mice, which was observed in amount, consistency of the feces, and the duration of the diarrhea. The water extract also lowers cholesterol in rats heart cell culture .[7,8]

Realizing the beneficial mechanisms of action and effects, this opens up the chance to combine both of the plants to have a synergistic effect. The main objectives of the present investigation were to evaluate the antidiabetic effect of mixture of aqueous extract of *Andrographis paniculata* and *Syzygium polyanthum* leaves on glucose uptake and on high fat diet-glucocorticoid (HFD/Gc) induced diabetic rats.

Methods

The leaves of *Andrographis paniculata* and *Syzygium polyanthum* were collected from Gaharu Plantation in Gandus District, Palembang, South Sumatera Province, Indonesia, in the month of August-September, identified and authenticated by the Indonesia Science Institute (LIPI). The collected plant material was made free from foreign organic matter.   Metformin obtained from Dexa Medica PT in Palembang, South Sumatera Province. Random Access Analyzer Bio System® and Bio System reagent for measuring blood glucose levels in diabetic rats. ELISA reader Bio Rad® was used in ELISA analysis for GLUT4 and insulin, using the GLUT4 *for* Rat ELISA kit from Qayee-Bio and Insulin for Rat ELISA kit from Sun long Biotech. Dexamethasone sodium phosphates were produced by Indofarma®. High fat diet consists of margarine and coconut oil was purchased from the local market, Palembang, Indonesia. All the other chemical used for the experiments were of analytical grade. All the other chemical used for the experiments were of analytical grade.

The collected *Andrographis paniculata* and *Syzygium polyanthum* leaf were washed, rinsed, blotted, sliced and ground. The extraction process was carried out at 90°C for 15 minutes in ratio of plant to water 1:10. The extract was filtered, concentrated, and evaporated in rotary evaporator.

Male Wistar rats (170-230 grams) purchased with animal health certificate from the veterinarian in the Department of Agriculture, Bandung, and West Java. They were 10 weeks old and have the lowest fasting blood glucose level of 5.6 mmol/L. All of them maintained in an air conditioned room (25+1 ºC), with a 12 h light - 12 h dark cycle and fed with standard diet and water ad libitum. Those were housed in the Animal House Faculty of Medicine, Sriwijaya University (Palembang, Indonesia) for 7 days before starting the experiment. The study approved by Health Research Review Committee of Mohammad Hoesin Central General Hospital and Faculty of Medicine Sriwijaya.

Rat diabetes induced by HFD 5 mL and Gc 250 ug/kg b.w./day using modification the method as describe by Sivabalan et al [15] for 2 weeks, and then fasting blood glucose level, collected from the orbital sinus puncture were checked. Rats with fasting blood glucose level over 11.1mmol/L were used. After the experiment, the animals terminated by intraperitoneal injection of ketamin 70 mg/kg b.w. HFD/Gc rats divided into five groups with 6 rats in each group as follows: Group I: diabetic control negative rats orally administered with tween 80 0.5%. Group II : Diabetic control positive rats orally administered with metformin 63 mg/kg b.w./day.Group III-V: diabetic rats orally administered extract combination with 250, 500 and 1000 mg/kg b.w./day respectively. [16] All treatment dissolved in tween 80 0.5% ad 2 mL, for 2 weeks. Fasting blood glucose and body weight measured before and after treatment. On the 15th day of administration, blood sample collected for fasting blood glucose and insulin measurement. During fasting, rats were deprived of food overnight for 12 h but had free access to water. Then, animals terminated. Ractus abdominis muscle (50 mg) collected from all groups for GLUT4 level measurement.

This assay used to measure level of fasting insulin from plasma preparation (whole blood collected into tube with anticoagulant-EDTA, incubated at room temperature for 20 minutes, and then centrifugated for 20 minutes at 3.000 rpm, supernatant were collected as plasma samples) and GLUT4 from tissue sample (adipose tissues were placed on a separate micro tube, washed 3 times with PBS 1%, homogenize by hand, add PBS, centrifuge for 20 minutes at the speed of 3000 rpm, supernatant collected) as described in manufacturer's instructions of ELISA kit. Fasting blood glucose level estimated by kits as mentioned by the manufacturer's instructions. Homeostasis model assessment of insulin resistance (HOMA-IR) calculated by the formula: $HOMA-IR = FPG(mmol/L) \times FPI(\mu IUmL)22.5$ FPG for fasting plasma glucose and FPI for fasting plasma insulin

Statistical analysis was performed using SPSS software package version 18. The values were analysed by paired t test, unpaired t test, one-way analysis of variance (ANOVA)

followed by boferroni pos-hoc test. All results were expressed as mean + SD. A vallue of p<0.05 was considered statistically significant.

Results

The extract combination at doses 250, 500, 1000 mg/kgBW and metformin for 2 weeks revealed a significant increase in body weight compare negative control with increase 22,5%; 6,1%; 20,1% and 40,4%. The fasting plasma glucose level of HFD-Gc for 2 weeks induce diabetic rats were significant increase (p<0.001) more than 11,1 mmol/L, and then treatment with extract combination (250, 500 and 1000 mg/kgBW) and metformin for 2 weeks showed a sigifican decrease in fasting plasma glucose compare with the negative control rats with a reduction of 35,6%, 48,3%, 37,6% and 47,3%, respectively. Similarly, the fasting plasma insulin levels were also increased significantly in the negative control rats induced HFD and Gc (p<0.001) compared with the previous condition. Treatment with extract combination (250, 500 and 1000 mg/kgBW) and metformin for 2 weeks showed a sigifican decrease in fasting plasma insulin compare with the negative control rats with a reduction of 11,2%, 33,6%, 20% and 19,4%, respectively. The insulin resistance (HOMA-IR) was inclined 6-7 fold (p<0.001) in the HFD-Gc induced diabetic control negative group compared they had been before. Administration of extract combination (250, 500 and 1000 mg/kgBW) and metformin for 2 weeks showed a sigifican decrease in HOMA-IR compare with the negative control rats with a reduction of 53,68%, 60,03%, 58,21% and 56,33%, respectively, compared with the diabetic negative control rats.

Table 1. The Efficacy of Extract Combination on body weight, fasting plasma glucose, fasting plasma insulin and HOMA-IR in Diabetic Rats

Group (n=6 each group)	Body weight (g)		Fasting Plasma Glucose (mmol/L)		Fasting Plasma Insulin (µU/mL)		HOMA-IR	
	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14
Negative control	207±5.71	177±17.01	11.87±0.88	13.47±0.95 a	10.35±0.81	10.53±0.82 a	5.46±0.84	6.30±1.98 a,c
DM+EC 250 mg/kgBW	204±11.11	250±17.19 a,c	11.55±0.44	7.21±1.67 a,c	10.76±0.76	9.75±0.87 a,c	5.52±0.56	3.12±0.87 a,c
DM+EC500 mg/kgBW	198±14.91	210±8.33 b,c	11.99±0.84	7.74±1.19 a,c	11.98±0.56	7.40±0.76 a,c	6.38±0.87	2.55±0.98 a,c
DM+EC1000 mg/kgBW	209±9.49	251±18.06 a,c	15.21±0.95	7.86±1.83 a,c	10.45±0.34	8.45±0.54 a,c	7.06±0.67	2.95±0.76 a,c
Metformin	192±6.78	269±12.29 a,c	12.12±0.84	6.39±1.27 a,c	11.15±0.74	9.21±1.67 a,c	6.00±0.89	2.62±0.98 a,c

DM= diabetes melitus, EC= extract combination; Paired t test, a $p<0.05$; Unpaired t test, b $p<0.05$ VS metformin; c $p<0.05$ VS negative control; Significance level was determined by one way ANOVA followed by bonferroni pos-hoc test

Table 2 shows GLUT4 level in diabetic rats induced HFD-Gc as negative control and subjected to extract combination (250,500 and 1000 mg/kgBW) and metformin treatment for 2 weeks. Two weeks treatment either extract combination or metformin in diabetic rats, significantly increased GLUT 4 level ($p<0,05$) with a percentage increase of 6,68%, 15,21%, 12,76% and 1,77%.

Table 2. The Efficacy of Extract Combination on GLUT 4 in Diabetic Rats

Group (n=6 each group)	GLUT 4 Skletal Muscle (pg/mL)
Negative control	395.56±28.98
DM+EC 250 mg/kgBW	421.59±41.87
DM+EC500mg/kgBW	455.34±34.98
DM+EC1000mg/kgBW	445.95±43.76
Metformin	402.56±32.98

DM= diabetes melitus, EC= extract combination; Paired t test, a $p<0.05$; Unpaired t test, b $p<0.05$ VS metformin; c $p<0.05$ VS negative control; Significance level was determined by one way ANOVA followed by bonferroni pos-hoc test

Discussion

In this study, the HFD administration in combination with Gc (250 ug/kg/day) for 2 weeks to rats resulted in pathogenesis of T2DM such as hyperglycemia, hyperinsulinemia and increased HOMA-IR index, similarity Diabetic rats model from Sivabalan *et al* (2008) that had shown in negative control. HFD induce insulin resistance in muscle (Wilkes et al.,1998) by forming fatty acid intermediates that activate PKC and inhibits the activation of Akt2 thereby preventing translocation GSV,] which in turn decreased GLUT4 expression in skeletal muscle.

At the same time, glucocorticoids interfere with lipid metabolism leading to the accumulation of lipid outside of adipose tissue such as muscle that to an able the photogenes is of insulin resistance as mentioned before, and increased liver gluconeogenesis.[9,10]

The results from this study showed that extract combination of *Andrographis peniculata* and *Syzygium polyanthum* had significant activity as an antihyperglycemic, improved insulin resistance in skeletal muscle of rat model induced by HFD-Gc, and increased GLUT4. It is very interesting that 500 mg/kg b.w./day of extract combination exhibited increased GLUT4 level more than metformin.

GLUT4 has an important role in the homeostasis of glucose contained in the adipose tissue, muscles and heart. The sensitivity of tissues to insulin can be significantly improved through the expression of GLUT4.[11-13] How does this fraction increase GLUT4 level in skeletal muscle will require further research.

Conclusion

The extract combination of *Andrographis peniculata* and *Syzygium polyanthum* showed the ability to improve glucose uptake by elevated levels of GLUT 4 in skletal muscle.

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