

Original Article

ANTIDIABETIC STUDY OF COMBINATION OF *ANDROGRAPHIS PANICULATA* (BURM. F.) WALLICH. EX NEES. HERBS EXTRACT AND *GUAZUMA ULMIFOLIA* LAMK. LEAVES EXTRACT IN OBESE DIABETIC MICE MODEL

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Received: 08 Oct 2015 Revised and Accepted: 25 Nov 2015

ABSTRACT

Objective: This research was to investigate the activity of *Andrographis paniculata* extract (APE) and *Guazuma ulmifolia* extract (GUE) in treating obesity integrated diabetic conditions.

Methods: There were two stages of inductions: the first stage was a 42 d of high carbohydrate intake (obese with insulin resistance mice model), followed by second stage induction by giving of alloxan 50 mg/kg bw intravenously (obese diabetic mice model). Animals with glucose level >200 mg/dl were then treated with metformin 195 mg/kg bw, glibenclamide 0.65 mg/kg bw, APE 2 g/kg bw, GUE 0.5 g/kg bw, combination of APE 1 g/kg bw and GUE 0.25 g/kg bw, or combination of APE 2 g/kg bw and GUE 0.5 g/kg bw for 14 d.

Results: The results showed that APE alone, GUE alone, and the combination of APE 2 g/kg bw and GUE 0.5 g/kg bw could improve insulin sensitivity in obese with insulin resistance mice model. Whereas the combination of APE 2 g/kg bw and GUE 0.5 g/kg bw could significantly decrease blood glucose level and body weight ($p < 0.05$) in obese diabetic mice model compare to APE alone, GUE alone, or a combination of APE 1 g/kg bw and GUE 0.25 g/kg bw.

Conclusion: It is concluded that APE 2 g/kg bw, GUE 0.5 g/kg bw, and combination of APE 2 g/kg bw and GUE 0.5 g/kg bw can improve insulin resistance conditions caused by obesity, while combination of APE 2 g/kg bw and GUE 0.5 g/kg bw has the best activity in treating obese diabetic conditions.

Keywords: Diabetes mellitus, Obesity, *Andrographis paniculata*, *Guazuma ulmifolia*, Mice.

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INTRODUCTION

Diabetes mellitus is a metabolic disorder that affects the body's ability to produce or use insulin. Insulin is a hormone produced in the pancreas that responsible for transport glucose (blood sugar) from the bloodstream into the cells so they can break it down and use it for immediate fuel. People cannot live without insulin [1]. Diabetes mellitus is indicated by the blood glucose level elevation [2]. The frequency of type 2 diabetes mellitus is 90% of cases globally [3]. Type 2 diabetes mellitus is a metabolism disorder with multifactorial cause, including lack of insulin secretion and peripheral insulin resistance. Pancreatic β cell dis-function is a major cause of this disorder, but the factor that trigger this β cell dis-function is still unclear [4].

Obesity is the main cause in type 2 diabetes mellitus case. About 80% of type 2 diabetes patients are overweight [3, 5]. Normal glucose regulation is controlled by balance act between pancreatic β cell insulin secretion and insulin sensitivity in peripheral tissue (muscle, liver, and adipose tissue). Insulin resistance is a major symptom in a metabolic syndrome and frequently develop into type 2 diabetes mellitus. Decreasing insulin sensitivity and damaged pancreatic β cell are the major pathogenesis in the type 2 diabetes mellitus [6].

Clinically, obesity is defined as a condition where individual's body mass index is more than or equal to 30 kg/m². Obesity is able to affect an individual in any age, but the exact etiology of this disease is still unclear. But it is already known that obesity is a result from chronic imbalance between energy intake and energy spending [5]. Obesity and insulin resistance usually initiate impaired glucose condition. So the link between obesity and type 2 diabetes mellitus is insulin resistance, which is marked by lower decreasing in glucose level upon glucose administration. It is known that severity and duration of obesity is the main risk factor in type 2 diabetes mellitus. In simple term, elevated body weight can cause hyperinsulinemia and insulin resistance [5].

Andrographis paniculata (Burm. F.) Wallich. ex. Nees., belongs to Acanthaceae family [7], known in Indonesia as sambiloto, traditionally

has been used as a hypoglycemic agents [8]. Previous research shows that *Andrographis paniculata* has the ability to lower blood glucose level in diabetic animal [9-11]. Major chemical compounds in *A. paniculata* are diterpenoid, flavonoid, and polyphenol. Main chemical constituent and active compound is andrographolide, belongs to diterpenoid group, a crystalline compound with bitter taste, mostly contain in leaves (>2%) [12, 13]. *A. paniculata*'s mechanism in decreasing blood glucose level is by increasing insulin secretion from undamaged pancreatic β cell, improving insulin sensitivity, and slowing down the insulin resistance development [14, 15].

Guazuma ulmifolia Lamk., belongs to Sterculiaceae family [16], known in Indonesia as jati belanda, traditionally has been used as a body weight reducing agents. A chemical constituent in *G. ulmifolia* is an alkaloid, flavonoid, saponin group, and tannin as main constituent [8]. Tiliroside is the marker compound in *G. ulmifolia* [17]. The previous study showed that *G. ulmifolia* had the ability to inhibit cholesterol and LDL level elevation [18]. Moreover, *G. ulmifolia* shows antidiabetic activity; the mechanism was thought to increase insulin secretion [19].

The epidemiology study suggested that increasing prevalent of diabetic was parallel to obesity. Body mass index and waist circumference played an important role in type 2 diabetes mellitus development [6]. So one of the aim of this study was to develop an obese diabetic mice model. Based on the previous study and traditional use, a combination of these two plants was thought to be effective in treating the obese diabetic condition. The combination on these two plant extracts in treating obese diabetic condition in mice has never been investigated before.

MATERIALS AND METHODS

Identification and authentication of plant material

Identified *Andrographis paniculata* (Burm. f.) Wallich. ex. Nees. and *Guazuma ulmifolia* Lamk. plants were obtained from Bumi Herbal, Bandung, West Java, Indonesia. Fresh plants were dried at 60-70 °C and then grinded into small pieces.

Preparation of ethanolic extract of *Andrographis paniculata*

1 kg dried herbs of *Andrographis paniculata* was macerated with 10 L ethanol, twice. The mixture was filtered using a filter paper, and the filtrate was concentrated using rotary vacuum evaporator at 60 °C [14].

Preparation of water extract of *Guazuma ulmifolia*

1 kg dried leaves of *Guazuma ulmifolia* was mixed with 15 L water and boiled for 30 min. The mixture was filtered and dried using freeze dryer [18].

Animals

Male Swiss-Webster mice 2-3 m old weighing 20-35 g from Pharmacology Laboratory, Bandung Institute of Technology. The animals were kept at standard laboratory conditions at 24-26 °C, humidity 70-75%, and 12 h light/dark cycle. Animals were fed with standard chow and water ad libitum. The methods in this study were performed in accordance with ethics and guide for animals care and used.

Oral glucose tolerance test

Before the commencement of the experiment and after first stage induction using high carbohydrate diet, an oral glucose tolerance test (OGTT) using glucose 3 g/kg bw was performed after a 12-hour fasting period. Blood glucose concentration from the tail vein was measured using the Easy Touch® blood glucose meter at 0 and 30, 60, 90 and 120 min after glucose administration [14].

Insulin tolerance test

After first stage induction using high carbohydrate diet, an insulin tolerance test (ITT) using insulin 0.75 U/kg bw, ip, was performed. Blood glucose concentration from the tail vein was measured using the Easy Touch® blood glucose meter at 0, 15, 30, 45, and 60 min [20].

Induction of obese diabetic mice

There were two stages of induction. The objective of the first stage was to develop obese with insulin resistance mice model. Forty two days of high carbohydrate diet intake [21] was performed, then insulin tolerance test was done. Then to develop obese diabetic mice,

the second stage induction was performed by administering aloxan 50 mg/kg bw intravenously [22].

Antidiabetic assessment of extracts on induced animals

After aloxan administration and hyperglycemic condition developed (blood glucose > 200 mg/dl), animals divided into 7 groups (positive control, metformin 195 mg/kg bw, glibenclamide 0.65 mg/kg bw, APE 2 g/kg bw, GUE 0.5 g/kg bw, combination of APE 1 g/kg bw and GUE 0.25 g/kg bw, combination of APE 2 g/kg bw and GUE 0.5 g/kg bw) and 1 negative control group. The dose was selected based on previous researches or based on traditional use. [8, 14, 23, 24]. All groups were being treated with standard drugs or extracts for 14 d. Body weight and blood glucose level were measured as a parameter during the experiment.

RESULTS AND DISCUSSION

Effect of single and combination extracts in reducing blood glucose upon glucose administration

Oral glucose tolerance test was performed to the healthy animal as a preliminary study. Blood glucose level profile was shown in fig. 1.

Thirty minutes after glucose administration, blood glucose level in all groups increased as a response to oral glucose administration. Blood glucose increasing at minutes 30 in the control group was significantly higher among the other groups.

At minutes 30 after glucose administration, a combination of *Andrographis paniculata* extract 2 g/kg bw and *Guazuma ulmifolia* extract 0.5 g/kg bw showed the lowest blood glucose level. From this study, it is known that both *Andrographis paniculata* extract and *Guazuma ulmifolia* extract, in single or combination administration, have the ability to suppress the increase of blood glucose upon glucose administration.

Increase of body weight can reduce insulin sensitivity

Body weight increasing profile during high carbohydrate diet was shown in fig. 2.

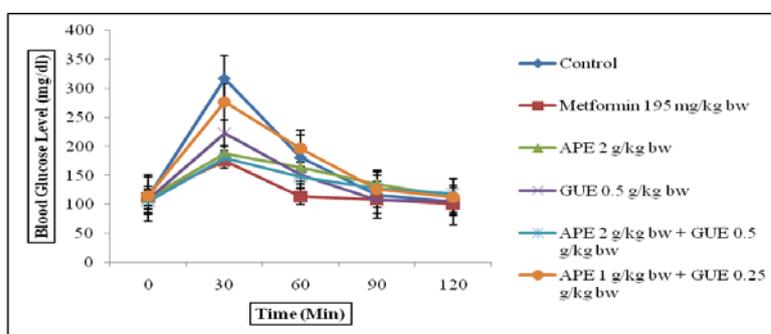


Fig. 1: Blood glucose level profile in oral glucose tolerance test

Note: APE = *Andrographis paniculata* extract, GUE = *Guazuma ulmifolia* extract. $p < 0.05$, $n \geq 4$ mice/group

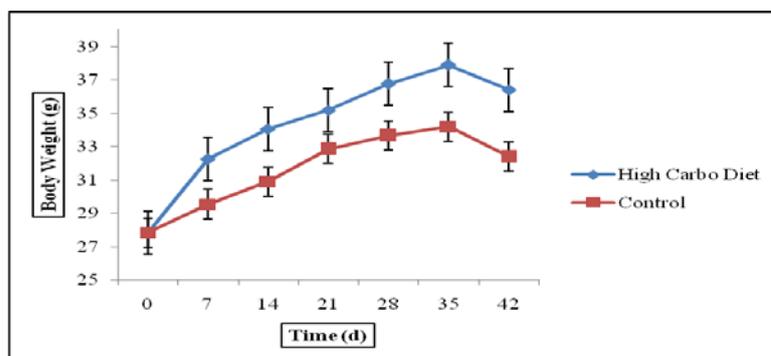


Fig. 2: Body weight profile during high carbohydrate diet intake

Note: $p < 0.05$, $n \geq 4$ mice/group

After 42 d administration of high carbohydrate diet, body weight increasing percentage in the treatment group was significantly higher ($p < 0.05$) compared to the control group. It suggests that high carbohydrate diet intake can induce obesity in mice compared to control group. This result collaborated with a previous study that done by Setiawan F, et al. [21].

Insulin tolerance test was performed after 42 d of high carbohydrate diet induction. Insulin sensitivity was stated with K_{ITT} . Lower K_{ITT} indicates lower insulin sensitivity.

According to table 1, the high carbo diet group had lower K_{ITT} value compared to the control group, although it was statistically

unsignificant ($p = 0.183$). However, it suggested that body weight elevation could cause insulin sensitivity decreasing. This data is collaborate with a study done by Ble-Castillo et al. that showed a high carbohydrate diet induce a higher insulin resistance and hepatic cholesterol content compared to a high-fat diet [25].

Effect of single and combination extracts in reducing blood glucose upon glucose administration in obese mice model

Oral glucose tolerance test was performed to obese with insulin resistance mice model in order to compare the blood glucose lowering activity between single and combination extracts. Blood glucose level profile showed in fig. 3.

Table 1: K_{ITT} value

Group	K_{ITT}
High carbo diet	0.316±0.785
Control diet	0.565±0.478

Note: Data are presented as mean±SD, $p < 0.05$, $n = \geq 4$ mice/group.

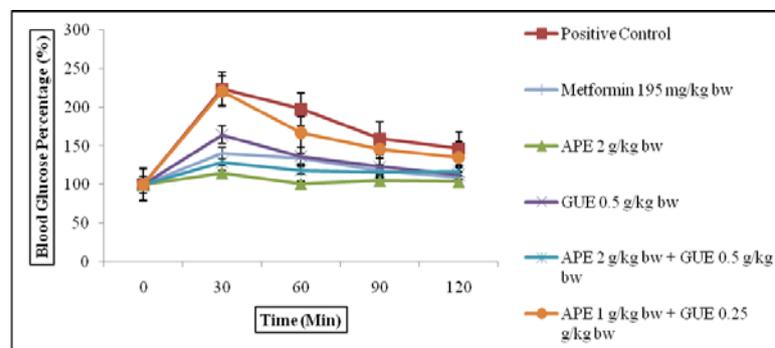


Fig. 3: Blood glucose percentage profile in oral glucose tolerance test to obese mice

Note: APE = *Andrographis paniculata* extract, GUE = *Guazuma ulmifolia* extract. $p < 0.05$, $n \geq 4$ mice/group

On this study, oral glucose tolerance test was performed to obese mice model with insulin resistance. The aim of this test was to investigate the activity of both single and combination extracts in improving insulin sensitivity. Metformin, one of oral antidiabetic drugs and belongs to biguanide group, is used as standard drugs. It has the ability to increase insulin sensitivity in hepatic and peripheral tissue (muscle), that improve glucose uptake to insulin-sensitive tissue. Detail mechanism of how biguanide group increase sensulin sensitivity is still under observation, though it is thought that activation of adenosine-5-monophosphate, increasing tyrosin kinase activity, and activation of glucose transporter-4 take an important place. Metformin do not have any activity to pancreatic β cell [26].

At minutes 30 after glucose administration, metformin as a standard drug group attenuated the increase of blood glucose level significantly compared to positive control group. The similar result was observed in *Andrographis paniculata* extract 2 g/kg bw and the

combination of *Andrographis paniculata* extract 2 g/kg bw and *Guazuma ulmifolia* extract 0.5 g/kg bw. This suggests that *Andrographis paniculata* extract 2 g/kg bw and the combination of *Andrographis paniculata* extract 2 g/kg bw and *Guazuma ulmifolia* extract 0.5 g/kg bw can improve insulin sensitivity in obese with insulin resistance mice model.

Effect of single and combination extracts in obese diabetic mice model

Developed obese diabetic mice model was divided into 7 groups (positive control, metformin 195 mg/kg bw, glibenclamide 0.65 mg/kg bw, APE 2 g/kg bw, GUE 0.5 g/kg bw, combination of APE 1 g/kg bw and GUE 0.25 g/kg bw, and combination of APE 2 g/kg bw and GUE 0.5 g/kg bw) and negative control group. Standard drugs and extracts were given for 14 d. The pparameters' were blood glucose level and body weight measurement. Blood glucose level profile during the treatment period was shown on table 2.

Table 2: Blood glucose level percentage profile

Group	Percentage of blood glucose level increasing profile (d)			
	3	7	10	14
Negative control	100.30±7.64	124.89±32.15	114.31±31.82	93.53±19.79
Positive control	95.49±13.34	106.57±34.88	118.09±38.00	117.13±25.22
Metformin 195 mg/kg bw	89.65±21.15	98.01±42.00	114.44±21.33	106.21±48.29
Glibenclamide 0.65 mg/kg bw	108.32±32.86	106.38±18.40	114.41±32.82	88.33±17.00
APE 2 g/kg bw	81.65±27.31	85.26±35.65	97.06±33.46	82.15±22.92
GUE 0.5 g/kg bw	92.35±15.78	124.67±22.48	132.05±32.75	117.80±36.27
APE 2 g/kg bw & GUE 0.5 g/kg bw	71.02±13.03	81.21±36.50	83.80±37.69	66.01±40.91*
APE 1 g/kg bw & GUE 0.25 g/kg bw	78.50±11.39	89.61±24.09	99.86±13.12	98.50±51.13

Note: Data are presented as mean±SD, $n \geq 4$ mice/group, * means significantly different compared to positive control, $p < 0.05$

According to table 2, glibenclamide, *Andrographis paniculata* extract 2 g/kg bw, combination of *Andrographis paniculata* extract 2 g/kg bw and *Guazuma ulmifolia* extract 0.5 g/kg bw, and combination of *Andrographis paniculata* extract 1 g/kg bw and *Guazuma ulmifolia* extract 0.25 g/kg bw group showed blood glucose decreasing after 14 d of treatment. But significant blood glucose level decreasing percentage ($p < 0.05$) was shown only by a combination of *Andrographis paniculata* extract 2 g/kg bw and *Guazuma ulmifolia* extract 0.5 g/kg bw group.

Body weight, another parameter in this study, was also measured. According to table 3, all groups had body weight decreasing

compared to before treatment's value. After 14 d treatment, glibenclamide and combination of *Andrographis paniculata* extract 2 g/kg bw and *Guazuma ulmifolia* extract 0.5 g/kg bw group showed significant decrease in animal's body weight ($p < 0.05$) compared to control group.

The result showed that after 14 d treatment, combination of *Andrographis paniculata* extract 2 g/kg bw and *Guazuma ulmifolia* extract 0.5 g/kg bw could significantly decrease both blood glucose level and body weight compared to positive control group. The result from this study showed comparable data with the hypothesis.

Table 3: Body weight percentage profile

Group	Percentage of body weight profile (d)			
	3	7	10	14
Negative control	91.54±2.14	97.06±3.20	92.64±1.53	92.93±0.78
Positive control	87.59±2.60	85.59±3.79	85.00±4.19	85.47±4.67
Metformin 195 mg/kg bw	85.92±3.39	84.81±2.50	84.35±3.60	81.88±4.70
Glibenclamide 0.65 mg/kg bw	88.42±2.68	91.49±15.86	82.86±2.87	79.20±4.11*
APE 2 g/kg bb	86.82±3.19	85.08±3.26	84.29±2.85	85.71±3.08
GUE 0.5 g/kg bb	87.54±0.96	86.59±2.07	85.51±2.58	85.38±3.85
APE 2 g/kg bb & GUE 0.5 g/kg bb	85.73±2.94	82.32±3.59	79.79±3.41	78.94±2.91*
APE 1 g/kg bb & GUE 0.25 g/kg bb	87.95±1.31	82.69±5.60	81.42±4.48	81.61±4.75

Note: Data are presented as mean±SD, n≥4 mice/group, * means significantly different compared to positive control, $p < 0.05$

Andrographis paniculata's mechanism in decreasing blood glucose level is by increasing insulin secretion from undamaged pancreatic β cell, improving insulin sensitivity, and slowing down the insulin resistance development [13, 14]. The mechanism of *Guazuma ulmifolia* in reducing body weight is still unclear. In this study, a combination of *Andrographis paniculata* 2 g/kg bw and *Guazuma ulmifolia* extract 0.5 g/kg bw showed the best activity in reducing blood glucose and body weight of obese diabetic mice model (table 3 & 4). This result suggests that one of the probable mechanism of this extract combination is by improving insulin sensitivity and decreasing body weight.

CONCLUSION

From this study, it is concluded that *Andrographis paniculata* extract 2 g/kg bw, GUE 0.5 g/kg bw, and combination of *Andrographis paniculata* extract 2 g/kg bw and *Guazuma ulmifolia* extract 0.5 g/kg bw can improve insulin resistance conditions caused by obesity while combination of *Andrographis paniculata* extract 2 g/kg bw and *Guazuma ulmifolia* extract 0.5 g/kg bw give the best antidiabetic activity to treat obese diabetic conditions.

CONFLICT OF INTERESTS

Declared none

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