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A combination of bitter gourd ethanolic extract with ant lion larvae aqueous extract for a blood glucose-lowering agent

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Abstract

Many herbs have been developed as functional foods, drugs and supplements for lowering blood glucose in diabetic patients. Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia and distinctive complications. Traditionally, both bitter gourd (*Momordica charantia* L.) and ant lion (*Myrmeleon* sp.) larvae were consumed by some people for treating DM. In the study, a combination of bitter gourd and ant lion larvae was evaluated for its antidiabetic effect in insulin resistance rats, a model of type 2 DM. In the study, insulin resistance condition in rat was induced by intraperitonial injections of human long acting insulin 0,5 IU/kgBW three times daily for 15 days. A single treatment was per orally administered in the rats at the day 16. Blood glucose levels before and one hour after drug administration were determined and compared using pair-sample t test. The combination of bitter gourd and ant lion larvae (75:25 w/w percentage) exhibited hypoglycaemic effect by 32.20±2.57%. Metformin, an antidiabetic agent with extrapancreatic action, decreased the blood glucose level by 39.29±2.96%. The result indicated that the combination of bitter gourd and ant lion larvae is potential to be developed as a blood glucose-lowering agent for diabetic patients.

Introduction

Diabetes mellitus (DM) is a group of diverse metabolic disorders characterized by hyperglycemia and distinctive complications (Leu and Zonszein, 2010). The disease is also characterized by absolute insulin deficiency or decreased insulin sensitivity. Subsequently, it results in a glucose uptake deficiency by insulin-sensitive tissue (Neal, 2002). Other symptomps that may appear are premature atherosclerotic cardiovascular disease and small vessel disease manifested such as : retinopathy with potential loss of vision; nephropathy leading to renal failure; and peripheral neuropathy with a high risk of foot ulcers and amputations (Leu and Zonszein, 2010). Diabetes mellitus is spread widely, not only in developed country, but also in developing country (Hossain *et al*., 2007). Modern lifestyle with high-fat diet increases risk factor for type-2 DM (Koh-Banerjee *et al*., 1997; Rang *et al*., 2003).

In developing countries, increasing number of diabetic patients related to insulin resistance (type 2 DM) is very high. Developing countries are undergoing rapid shift in nutrition consumption along with increasing obesity, metabolic syndrome and type 2 diabetes mellitus. Nutrient consumption patterns have been shifted from a healthy traditional high-fiber, low-fat, low-calorie diet toward increasing consumption of calorie-dense foods containing refined carbohydrates, fats, red meats, and low fiber (Misra *et al*., 2010). Insulin resistance is clinical condition of insulin-decreasing potency to increase glucose uptake into body cells. It is related to obesity and decreased physical activity, and long term consumption of high calorie (Rang *et al*., 2003; Meier, 2004). Insulin resistance is also defined as a subnormal biologic response to a given concentration of insulin (Fonseca and John-Kalarickal, 2010). It is the early and main character of type-2 diabetes (Sear *et al*., 2009).

Bitter gourd (*Momordica charantia* L.) is slim vines and cultured in tropical region for its edible fruit. Its leaf decoction is used for constipation, treating liver degeneration, and expel intestine worms in Indonesia. The plant was also resported to possess antioxidant activity (Aminah and Anna, 2011). In addition, the fruit flesh is used for treating diabetes in Malaysia (Islam *et al*., 2011). The main constituents of bitter gourd possessing hypoglycemic effect are charantin, p-insulin (insulin-like polypeptide), cucurbutanoid, momordicin, and oleanolic acid

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Harinantenaina *et al*., 2006; Bano *et al*., 2011). The pharmacological properties of p-insulin are similar to these of insulin (Singh *et al*., 2011).

Ant lion (*Myrmeleon* sp.) larvae contains a substance exhibiting an inhibitory property in α-glucosidase activity. The substance also exibited an inhibitory effect on carbohydrate catabolism for its antidiabetic activity (Sutanto, 2008). In the previous study, ant lion larvae juice decreased the glucose level in rats significantly (Kurniasih *et al*., 2006).

Based on the facts, both extracts were already reported for their hypoglycemic activity both scientifically and empirically. Action mechanism of hypoglycemic activity of both extracts is suggested to be different. The novelty of the study was to study the hypoglycemic activity of combination of bitter gourd and ants lion larvae in insulin-induced type 2 DM, a model of type 2 DM rats with insulin resistance.

Materials and Methods

Materials

Bitter gourds were collected from the area around Sleman, Jogjakarta Indonesia, and identified by a botanist at Laboratory of Plant Taxonomy, Faculty of Biology Universitas Gadjah Mada Indonesia. The fruit flesh was chopped, dried, and ground. The fruit flesh powder then macerated in ethanol 96% (one liter per day) for three days extraction. Subsequently, it was filtered and concentrated to yield a etanolic extract, and then diluted in distilled water for being administered in the rats. Ant lion larvae was collected from the area around Bantul, Jogjakarta Indonesia, and identified in at Laboratory of Animal Taxonomy, Faculty of Biology Universitas Gadjah Mada Indonesia. It was ground and suspended in in distilled water for being administered in the rats. Basic doses of bitter gourd and ant lion larvae were 60 mg/200 g BW and 50 mg/200 g BW, respectively. In the study, they were combined with ratio of 50:50; 75:25 and 25:75. For comparison, glibenclamide (PT. Indofarma Tbk. Indonesia) at 0,091 mg/kgBW and metformin (PT. Bernofarm Indonesia) at 9,05 mg/kgBW was used. Other materials were human long acting insulin (Monotard® HM), GOD-PAP Reagent Kit (DiaSys).

Animals

Wistar rats weighing 100-150 g were obtained from Laboratory of Pharmacology and Toxicology, Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia. The animal handling protocols of this study were in accordance with the guidelines of the animal care of the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia.

Experimental design

Type 2 diabetic condition in the rat was induced by an intraperitoneal injection of human long acting insulin (Monotard ® HM) at 0,5 IU/kgBW three times daily for 15 days (Chi *et al*., 2000). After fasting for 8 h, at day 16 the blood glucose level was measured by GOD-PAP Reagent Kit. The diabetic rats were divided into six groups consisting of three-five rats each as follows:

Group 1 – the rats received oral saline 10 ml/kg BW (control group).
Group 2 – the rats received a single dose of the combination of bitter gourd (60 mg/200 g BW) and ant lion larvae (50 mg/200 g BW) with a ratio of 50:50 w/w percentage, orally.
Group 3 – the rats received a single dose of the combination of bitter gourd (60 mg/200 g BW) and ant lion larvae (50 mg/200 g BW) with a ratio of 75:25 w/w percentage, orally.
Group 4 – the rats received a single dose of the combination of bitter gourd (60 mg/200 g BW) and ant lion larvae (50 mg/200 g BW) with a ratio of 25:75 w/w percentage, orally.
Group 5 – the rats received metformin dose 9,05 mg/kgBW, orally.

The combination of bitter gourd and ant lion larvae, metformin or glibenclamide were per orally administered at the day 16. One hour after administration, the the blood samples were collected from retro-orbital plexus, and centrifuged at 1,000 rpm for 10 min. Serum was removed for determination of glucose by GOD-PAP Reagent Kit (DiaSys).

Statistical analysis

The results were expressed as mean±SEM. The data were subjected to the one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test to compare more than two groups. While the unpaired or paired t test was used to compare the mean of two groups. P-values of less than 0.05 were considered significant.

Results and Discussion

To induce type 2 DM in rats, insulin was administered for 15 days. Reportedly, long term administration of insulin in normal rats induced hyperinsulinemia, and then induced insulin resistance DM (a type 2 DM). At the day 16, the blood of normal rats and diabetic rats were collected, and the blood glucose levels were measured. The blood glucose

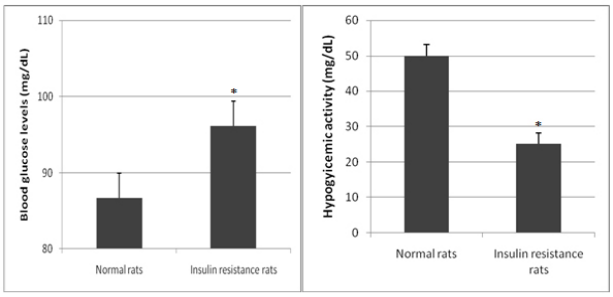

Figure 1. Blood glucose levels in normal rats and insulin-induced diabetic rats (a); and hypoglycemic action of glibenclamide (0,091 mg/kgBW) in normal rats and insulin-induced diabetic rats. Data represent mean ± SEM, and are three-four independent experiments. *P < 0.05 compared to the control value (normal rats).

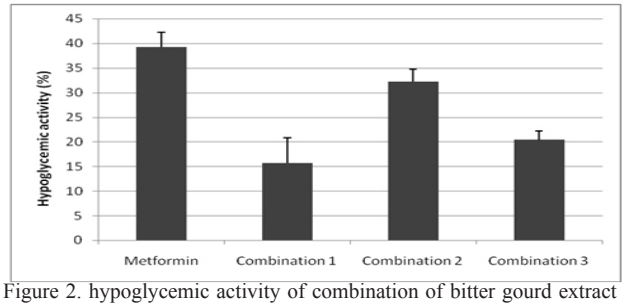

Figure 2. hypoglycemic activity of combination of bitter gourd extract and lion ant extract; and metformin (positive control) in insulin resistance rats. Data represent mean ± SEM, and are three-four independent experiments. *P < 0.05 compared to the control value (normal rats). Combination 1 : combination of bitter gourd (60 mg/200 g BW) and lion ant (50 mg/200 g BW) with a ratio of 50:50; combination 2 : ratio of 75:25; and combination 3 : ratio of 25:75.

levels of normal rats and diabetic rats were shown in Figure 1a. The blood glucose levels of insulin-induced type 2 diabetic rats (96.17±3.19 mg/dL) were higher than these of normal rats (86.68±3.29 mg/dl) significantly (P<0,05). In the study, glibenclamide, an sulfonylurea antidiabetic drug, was used to confirm an insulin resistance condition in the rats. In Figure 1b, glibenclamide (0,091 mg/kgBW) markedly suppressed the blood glucose levels with hypogycemic activity of 49.86±3.75%. In contrast, hypoglycemic activity of glibenclamide in insulin resistance rats was only 25.05±5.89%. It facts indicate that hypoglycaemic activity of glibenclamide has been reduced when administered in insulin resistance rats. The result showed that intraperitoneal injection of human long acting insulin three times daily for 15 day induces insulin resistance in rats.

In the study, all treatment could decrease the blood glucose levels in diabetic rats in comparison to this of the control group value. The combination of bitter gourd (60 mg/200 g BW) and ant lion larvae (50 mg/200 g BW) with a ratio of at the ratio of 50:50; 75:25 and 25:75 w/w percentages decreased the blood glucose levels significantly. Metformin, an biguanid antidiabetic drug, suppress the blood glucose levels by 39.29±2.96%. The hypoglycemic activity of combination of bitter gourd extract and lion ant extract with a ratio of at the ratio of 50:50; 75:25 and 25:75 w/w percentages were 15.69±5.51%; 32.20±2.57% and 20.38±1.87%. The results were shown in Figure 2.

Insulin resistance is a condition in which the tissues exhibit lower normal response for same level of blood insulin concentration (Fonseca and John-Kalarickal, 2010). For the therapy of insulin resistance, metformin is used to increase insulin sensitivity (Herzlinger and Abrahamson, 2010). However, glibenclamide is less suitable for treating

Table 1. Some informations of hypoglycemic activity of bitter gourd (*Momordica charantia* L.) in diabetic subjects

No.	Part of the plant	Extract	Hypoglycemic activity	Reference
1.	fruit pulp.	saponin-free methanol extract of pulp juice.	hypoglycaemic effect in type 2 diabetic rats.	Ali *et al.*, 1993
	seed and whole plant.	methanol extract of seed, and of whole plant. saponin-free methanol extract of whole plant.	no hypoglycaemic effect in type 1 diabetic rats.	
2.	fruit	water extract	decreasing effect on blood glucose and increasing effect on insulin in type 2 diabetic rats with hyperinsulinemia. increasing effect on GLUT4 protein.	Miura *et al.*, 2001
3.	whole-plant	aqueous extract	hypoglycaemia in normal rats and STZ-treated diabetic rats	Ojewole *et al.*, 2006
4.	fruit pulp	ethanolic extract	Improvement of fasting blood glucose, serum insulin and β-cell function in neonatally streptozotocin-induced type 2 diabetic rats	Hafizur *et al.*, 2011
5.	fruit	aqueous extract	alleviate pancreatic damage and increase the number of ß-cells in neonatal STZ-induced type-II diabetic rats	Abdollahi *et al.*, 2011
6.	whole fruit	acetone extract	Regeneration of beta cells in islets of Langerhans of pancreas of alloxan diabetic rats	Singh and Gupta, 2007

insulin resistance than metformin due to its pancreatic action of glibenclamide. In the study, metformin is used in control positive group. The administration of metformin succeded to decrease the blood glucose levels in type 2 DM rats (insulin resistance). However, administration of glibenclamide is less success than this of metformin.

To date, ant lion (*Myrmeleon* sp.) larvae is still being further investigated associated with its antidiabetic ativity. In Indonesia, ant lion larvae is empirically reported to possess antidiabetic activity, and widely used as a traditional medicine. Its scientific data associated with its antidiabetic ativity is very limited. Inline with empirical data, ant lion larvae juice was reported to decrease the glucose level in rats potently (Kurniasih *et al*., 2006). lion ants (*Myrmeleon* sp.) contains a substance exhibiting an inhibitory property in α-glucosidase activity. The substance also exibited an inhibitory effect on carbohydrate catabolism for its antidiabetic activity (Sutanto, 2008). In the other side, Bitter gourd contains several hypoglycaemic compounds such as charantin, cucurbutanoid, momordicin, and oleanolic acid (Harinantenaina *et al*., 2006). Reportedly, they showed potent hypoglycaemic effects in type 2 DM rats with different actions (Table 1). However, Ali *et al*. (1993) reported that the pulp juice of bitter gourd did not influence the blood glucose level in type 1 DM rats. In therapy of diabetes mellitus, drug combination therapy is sometimes needed in order to decrease the side effects or synergistic effects. In the study, bitter gourd was combined with ant lion larvae to provide a potent natural product combination. The combination at at the ratio of 75:25 w/w percentage exhibited most potent hypoglycaemic activity among the others in type 2 DM rats with insulin resistance. This combination is potential to develop as a blood glucose lowering agent in diabetic subjects associated with insulin resistance.

Conclusion

We concluded that the combination of bitter gourd (60 mg/200 g BW) and lion ants (60 mg/200 g BW) at the ratio of 75:20 w/w percentage decreased potently the blood glucose levels in insulin-induced type 2 diabetic rats. The hypoglycemic activity of this combination was 32.20±2.57%.

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