

Antidiabetic and Antioxidant Activities of 70% Ethanol-Diluted Extract of Piper Crocatum Leaves in Streptozotocin Induced Diabetic Rats

Aktifitas Antidiabetik dan Antioksidan Extract Etanol 70% Daun Sirih Merah (*piper crocatum*) pada Tikus Wistar Model Diabetes Mellitus

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ABSTRACT

Red betel plant (*Piper crocatum*) has been empirically used to cure diabetes mellitus. However, data regarding this plant are still limited. The aim of this study was to find antidiabetic and antioxidant activities of 70% ethanol-diluted extract of *Piper crocatum* leaves. Thirty Wistar rats aged 4 months were divided into 6 groups (I-VI) randomly. The groups of I-IV were injected with 40 mg i.p streptozotocin to induce DM. All groups were treated for 21 days. Group I (normal control group) and group II (diabetic control group) were given normal saline, while group III, IV, and V were treated with various doses of 70% ethanol-diluted extract of *Piper crocatum* leaves (50, 100, 200 mg/kg.b.w/day). Group VI (positive control group) was given glibenclamide 0,9 mg/kg.b.w/day. On day 21, the body weight, fasting blood glucose, plasma MDA, and insulin levels of all rats were examined. The data were processed using statistics to determine the effectiveness of antidiabetic and antioxidant activities of the extract. A significant decrease in fasting blood glucose and plasma MDA levels were observed in group III, IV, and V ($p=0,00$), when compared to group II (diabetic control group). A significant increase in body weight ($p=0,04$) and plasma insulin levels ($p=0,008$) occurred in group V only. In conclusion, seventy percent ethanol-diluted extract of *Piper crocatum* leaves has potential antidiabetic and antioxidant activities.

Keywords: Diabetes mellitus, insulin hormon, MDA, *Piper crocatum* leaves

ABSTRAK

Daun sirih merah (*Piper crocatum*) secara empiris telah digunakan dalam pengobatan diabetes melitus. Namun, data tentang hal ini masih terbatas. Tujuan penelitian ini adalah mengetahui aktifitas andiabetik dan antioksidan ekstrak etanol 70% daun *Piper crocatum*. Tiga puluh ekor wistar rat umur 4 bulan, dibagi menjadi 6 kelompok secara random. Kelompok II, III, IV, V, dan VI diinjeksi streptozotocin 40 mg i.p. untuk membuat tikus model diabetes melitus. Kemudian hari IV semua kelompok diperiksa kadar glukosa darah. Setelah itu diberi perlakuan selama 21 hari. Kelompok I (kontrol normal) dan kelompok II (kontrol DM) normal saline. Sementara kelompok III, IV dan V diberi ekstrak etanol 70% daun sirih merah dengan bervariasi dosis (50 mg/kg/BB/hari, 100 mg/kg/BB/hari, 200 mg/kg/BB/hari). Kelompok VI (kontrol positif) diberi glibenklamid 0,9 mg/kgBB/hari. Pada hari ke-21 semua anggota sampel diperiksa berat badan, kadar glukosa darah puasa, kadar hormon insulin dan kadar MDA plasma. Data diolah secara statistik untuk mengetahui efektifitas daya antidiabetik dan antioksidan ekstrak ini. Penurunan kadar glukosa darah puasa dan kadar MDA plasma yang signifikan ($p=0,00$), terjadi kelompok III, IV, dan V, dibanding kelompok II (kontrol DM). Sedangkan peningkatan signifikan hormon insulin ($p=0,008$) dan berat badan ($p=0,04$) hanya terjadi pada kelompok V. Kesimpulan, ekstrak etanol 70% daun sirih merah (*Piper crocatum*) mempunyai aktifitas anti diabetik dan anti oksidan yang cukup potensial.

Kata Kunci: Daun *Piper crocatum* , diabetes melitus, hormon insulin, MDA

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INTRODUCTION

Diabetes mellitus (DM) is a syndrome characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolisms associated with absolute or relative deficiency in insulin secretion and/or action (1). The prevalence of this disease is increasing, which is estimated at 10% among people over 60 years of age, and rises to 16-20% among those over 80 years of age. The overall prevalence among adults was 7,4% in 1995 and is predicted to be 9% in 2025 (2).

Management of DM without side effects is still a challenge in the medical community. DM therapy with insulin or oral hypoglycemic agents have failed to prevent complications in some patients (3,4). In addition, the drug ingredient has been being an imported commodity in Indonesia, so the drug prices are relatively expensive. Consequently, ongoing research is needed to find an alternative herbal medicine for DM treatment. Traditional medicinal plants contain mixtures of compounds which may possess efficacious antidiabetic activity and work synergistically. Besides, the price will be less expensive and profitable, especially for people living in developing countries (5,6).

Although the traditional treatment of DM in complementary medicine has been recommended, the mechanism of some herbs has been unknown (7). Traditional antidiabetic medicinal plants were to be studied to uncover their abilities to improve hyperglycemia in DM (4). WHO has strongly emphasized the rational use of traditional and indigenous natural medicines for treating DM (8).

Indonesia, as a tropical country, is rich in medicinal plants potentially efficacious as antidiabetic and antioxidant agents. One of the traditional medicinal plants that has long been believed to be efficacious against DM is leaves of *Piper crocatum*. Seventy percent ethanol-diluted extract of *Piper crocatum* leaves contains phytochemical compounds, including some classes of fatty acids, flavonoids, alkaloids, terpenoids, steroids, pyrimidine, essential oils, polyphenols, and vitamin E. The compound thought to have antidiabetic and antioxidant activities are polyphenols, alkaloids, flavonoids, terpenoids, and vitamin E (9,10).

Ethiopathogenesis of DM closely associated with oxidative stress, so the antioxidant agents is necessary in its treatment (7). This study is aimed to examine the antidiabetic and antioxidant activities of 70% ethanol-diluted extract of *Piper crocatum* leaves. The antidiabetic activity was observed by identifying its effects toward body weight, antihyperglycemia, and stimulation of insulin secretion, while the antioxidant effect was examined by measuring the decrease in plasma MDA (malondialdehyde) levels.

METHODS

Extraction of *Piper crocatum* Leaves

Piper crocatum was extracted in 70% ethanol through a percolation method performed at the Laboratory of Pharmaceutical Mathematics and Science of Sebelas Maret University.

Animals Tested

Thirty Wistar male rats (body weight of 150-200 grams) were maintained at a temperature of 25-30°C and 45-55%

humidity with 12 hours of light-dark cycle. All animals received standard laboratory food and water ad libitum. The study protocol has been reviewed and approved by the Ethical Review Committee, Faculty of Medicine Sebelas Maret University, Surakarta, Indonesia by decree No. 02/UN27.06/ERC/2012.

Induction of Diabetes and Animal Treatment

Intraperitoneal injection of 40mg/kg.bw streptozotocin (STZ) was given to rats in order to make animal models with DM (11-13). STZ was freshly dissolved in 0,1M citrate buffer (pH 4,5). The STZ was purchased from Sigma chemical company, St Louis, Missouri, USA. STZ caused degeneration of pancreatic β cells, the clinical symptoms of DM will appear on two to four days after injection. The animals with blood glucose levels exceeding 200 mg/dl were considered as diabetic (14).

Animals studied were separated into six groups and given treatment for 21 days after the STZ injection, as below:

- Group I : (Normal Control Group): The rats were given normal saline.
- Group II : (Diabetic Control Group): DM + normal saline
- Group III : DM + 70% ethanol-diluted extract of *Piper crocatum* leaves 50mg/kg.b.w/day.
- Group IV : DM + 70% ethanol-diluted extract of *Piper crocatum* leaves 100mg/kg.b.w/day
- Group V : DM + 70% ethanol-diluted extract of *Piper crocatum* leaves 200mg/kg.b.w/day.
- Group VI : (Positive Control Group): DM + glibenclamide 0,9mg/kg.b.w/day

Sample Collection

After 21 days of treatment, rats were fasted for 12 hours, then blood samples were collected. Retro-orbital blood samples were collected from the inner canthus of the eye under ether anesthesia using capillary tubes containing sodium fluoride. Plasmas were separated by centrifugation at 3000rpm for 10 minutes. Plasma samples were used for the measurement of glucose, insulin, and TBARS in different studied groups.

Biochemical Measurements

Fasting blood glucose levels were measured using the enzymatic colorimetric test "GOD PAP". Lipid peroxide levels were examined by measuring the plasma MDA levels using the spectrophotometric method of TBARS (units in mmol/l). While measurement of plasma insulin levels was performed with Rat/Mouse Insulin ELISA Kit. 96-well Plate (Cat. # EZRMI-13K).

Statistical Analysis

Data for various biochemical parameters were expressed as mean \pm SD and compared using one way analysis of variance (ANOVA) test. Values were considered statistically significant when $p < 0,05$. Statistical analysis was conducted using SPSS for Windows version 17.

RESULTS

A significant body weight loss and increase in fasting blood glucose and plasma MDA were observed in diabetic control group when compared to normal control group (Table I). Changes in these parameters indicate the clinical and laboratory DM, so that the animal can be used as a rat model of DM. Administration of 70% ethanol-diluted

Table 1. Fasting blood glucose, plasma insulin, and plasma MDA levels on day 21

Group of treatment		Body Weight (gram) ± SD	Blood Glucose (mg/dl) ± SD	Plasma MDA (mmol/L) ± SD	Plasma Insulin (ng/ml) ± SD
I.	Normal control	236,40 ± 5,03	73,87 ± 0,88	1,25 ± 0,15	1,41 ± 1,03
II.	Diabetic control	188,40 ± 32,49 ^(a)	205,55 ± 1,47 ^(a)	8,36 ± 0,20 ^(a)	1,05 ± 0,24
III.	Diabetic + 50mg/kgbw/day	216,20 ± 9,93	168,64 ± 1,19 ^(b)	6,44 ± 0,17 ^(b)	0,73 ± 0,16
IV.	Diabetic + 100 mg/kgbw/day	209,60 ± 29,05	157,68 ± 3,57 ^(b)	4,33 ± 0,11 ^(b)	1,56 ± 0,51
V.	Diabetic + 200 mg/kgbw/day	220,00 ± 14,00 ^(b)	130,19 ± 3,16 ^(b)	2,27 ± 0,12 ^(b)	2,19 ± 0,15 ^(b)
VI.	Positive control	249,40 ± 33,13 ^(b)	137,48 ± 3,13 ^(b)	2,58 ± 0,10 ^(b)	1,07 ± 0,96

Keterangan:

(a) Statistically significant when compared with Normal Controls

(b) Statistically significant when compared with Diabetic Controls

extract of *Piper crocatum* leaves to diabetic rats significantly increased the body weight of rats in group V ($p=0,04$). Significant decrease in fasting blood glucose and plasma MDA were also observed in group III, IV, and V ($p=0,00$). However, a significant increase in serum insulin only occurred in group V ($p=0,008$) (dose 200mg/kg.b.w/day).

DISCUSSION

Streptozotocin (STZ) causes degeneration of the β cells of pancreatic Langerhans island, consequently inducing impaired insulin secretion (15). Low dose STZ-induced diabetic rat would represent a model for good non-insulin-dependent diabetes mellitus (NIDDM) experimental diabetic state and provide a relevant example of endogenous chronic oxidative stress due to hyperglycemia (4). Induction of diabetic animal models was confirmed by the appearance of DM signs, including body weight loss and increased fasting blood glucose level. Fasting blood glucose levels were examined on day four after injection of 40 mg/kg.b.w STZ. At this dose, STZ has caused hyperglycemia (blood glucose >200mg/dl) at groups II to VI.

Injection of STZ led to increased plasma MDA levels (7). There was an increase in plasma MDA levels in diabetic control group (group II) when compared to the normal group (group I) ($p=0,00$), suggesting that the rats experienced oxidative stress (Table I). Administration of 70% ethanol-diluted extract of *Piper crocatum* leaves in group III, IV, and V increased body weight (especially in group V) and decreased blood glucose levels. The administration of the extract decreased the plasma MDA levels significantly compared to group II ($p=0,00$). The greatest decrease occurs in group V (200mg/kg.b.w/day), which is even greater than group VI (positive control group). These results indicate that 70% ethanol-diluted extract of *Piper crocatum* leaves is able to act as a potential antioxidant material that can reverse oxidative stress conditions. It increased insulin secretion significantly at dose 200mg/kg.b.w/day. It also poses the greatest hyperglycemia reduction among the groups studied.

The generation of reactive oxygen species (ROS) has an important role in the development of atherosclerosis and complications of DM. The exposure of hyperglycemia condition to endothelial cells leads to an increase in production of superoxide anion, which potentially reduces nitric oxide levels, a potent endothelium derived vasodilator (16). There are two mechanisms that explain the rise of oxidative stress in DM: 1) the rise of free radicals during auto-oxidation of glucose, 2) reduction of

endogenous antioxidant activity due to hyperglycemia (17).

Piper crocatum leaves contain a number of compounds possessing antioxidant activities, such as polyphenols, alkaloids, flavonoids, terpenoids, and vitamin E. This study estimated that the antioxidant activity is an important efficacious factor toward diabetes (3). Polyphenol is a group of chemical substances in plants. These substances have a lot of phenol groups in their molecules. Some studies suggest that the group has a role as an antioxidant which is good for health. These polyphenols protect body cells from free radical damage by binding to free radicals and preventing inflammation processes in the cell body (18). Provision of antioxidant-rich polyphenol affects to up-regulate antioxidant activity by increasing the concentration of Total Glutathione (GSH), Antioxidant Capacity (AOC), and plasma HDL, decreasing plasma MDA, LDL and hydrogen peroxide (H_2O_2) levels (19). Some flavonoids have also been known to be able to cope with some diseases caused by free radicals, as DM (4).

Vitamin E also acts as an antioxidant that is quite effective against hyperglycemia. Vitamin E has a role in strengthening insulin activity and glucose metabolism (17,20). Vitamin E can increase the activity of glutathione peroxidase (GPX) and Superoxide Dismutase (SOD), and decrease plasma MDA level (21-23). Natural antioxidants from plants have an important role in maintaining cell membrane integrity by preventing peroxidation of polyunsaturated fatty acid (18).

This complete extract contains a mixture of active compounds. It is difficult to determine the best component of the extract, in which some of these components possibly affect the glucose metabolism synergistically through several mechanisms, such as through inhibition of carbohydrate digestion and glucose absorption in the intestine, stimulation of insulin secretion from the pancreatic β -cells, modulation of glucose release from liver, activation of insulin receptors and glucose uptake in the insulin-sensitive tissues, and modulation of hepatic glucose output (24,25).

This study suggests that 70% ethanol-diluted extract of *Piper crocatum* leaves has potential antidiabetic and antioxidant activities, especially at dose 200 mg/kg.b.w/day. The antidiabetic activity is able to improve weight loss, reduce hyperglycemia levels, and stimulate the secretion of insulin hormone. Besides, the antioxidant activity of this extract also plays an important role in the mechanism of against DM. This study still requires some advanced research stage to be applied as an anti-diabetes drug in humans.

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