HAYATI Journal of Biosciences, March 2008, p 45-48 Vol. 15, No. 1
ISSN: 1978-3019

SHORT COMMUNICATION

Potency of *Piper crocatum* Decoction as an Antihiperglycemia in Rat Strain *Sprague dawley*

MEGA SAFITHRI[1]*, FARAH FAHMA[2]

[1]Department of Biochemistry, Faculty of Mathematics and Natural Sciences, [2]Department of Agroindustrial Technology, Faculty of Agricultural Technology, Bogor Agriculture University, Darmaga Campus, Bogor 16680, Indonesia

Received July 21, 2006/Accepted November 13, 2007

Current researches for appropriate hypoglycemic agents focused on plants for traditional medicine. Traditionally in diabetic treatment, people used decoctions of *Piper crocatum* (Piperaceae). However, there is no phytochemical data of decoctions extract of *P. crocatum*. Hence, the aims of this study were to explore the phytochemical of *P. crocatum* decoctions and its antihyperglicemic activity. Fresh leaves of *P. crocatum* were boiled in water to obtain decoction and were examined phytochemical compounds by using Harbone assay. Antihyperglicemic activity of *P. crocatum* decoction extract was orally fed to alloxan induced diabetic rats. Results showed that *P. crocatum* decoction extract contained flavonoids, tanins, and alkaloids. Ten days of daily treatment of various doses decoction extract of *P. crocatum* led to reduce blood sugar level by 10-38% and prevent fall in body weight level by 5-52%. This result showed the same activity as Daonil treatment, which was the diabetic drug. Hence, this extract showed antihyperglicemic activity in alloxan-induced diabetic rats and increasing of their body weight.

Key words: *Piper crocatum*, antihyperglicemic, phytochemical

INTRODUCTION

Diabetes mellitus is a chronic disease caused by inherited and or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such a deficiency results the increasing of blood glucose concentrations, which in turn will damage many body systems, particularly the blood vessel and nerves (Long 1989). Statistical projections in Indonesia mentioned that number of diabetics will increase from 5.6 million in the year 2001 up to 8.2 million in the year 2020; hence, it will become the fourth in the highest number of diabetics in the world after India, China, and USA (King *et al.* 1998; Boyle *et al.* 2001).

Researches in traditional medicine for hypoglycemic agents have been focused on plants due to traditional medicine gives better treatments than drugs (Rates 2001). There are 46 kinds of plants that have been used for diabetes treatment, i.e. Myrtaceae, Achantacheae, and Labiatae. Those plants have been experimentally evaluated and the active compounds were isolated (Widowati *et al.* 1997). Petroleum ether, methanol, and aqueous extracts from tropical almond (*Terminalia catappa*) are rich in tannins that had antidiabetic activity (Nagappa *et al.* 2003). Ethanol extract of *Piper crocatum* contained alkaloids, steroids, and tannins (Sugiharti 2007), which are phytochemical compounds for of diabetes mellitus. Traditionally in diabetic treatment, people used decoctions of *P. crocatum*; however, there is no phytochemicals data of *P. crocatum* decoctions. Hence, the aims of this study were to explore the phytochemicals data of *P. crocatum* decoctions and its antihyperglicemic activity.

*Corresponding author. Phone/Fax: +62-251-623247,
E-mail: safithri@ipb.ac.id

MATERIALS AND METHODS

Plant Material. *Piper crocatum* were freshly collected from Bogor Agricultural University herbal garden. The material was identified in the Herbarium Bogoriense, Indonesian Institute of Sciences (LIPI), Bogor. Fresh leaves of *P. crocatum* (200 g) were boiled in one liter of water and terminated when the volume reached up to 100 ml. The decoction was filtered through Whatman no. 1 filter paper, and the resulting extract was used in biological assays.

Chemicals. Alloxan tetrahedrat was obtained from Sigma (A6316-10 G) and Daonil from Aventis Pharmacy (A:21.2).

Phytochemical Test (Harbone 1984)

Alkaloid Test. Two grams of sample was added with ten ml chloroform and 0.25 ml ammonia. Fraction of chloroform was dissociated and acidified with 0.5 ml of 2M H_2SO_4. Each reagent (Dragendorf, Meyer, and Wagner) was added to the acid fraction separately. The existence of alkaloid was marked with white, red, brown sediment after adding the Meyer, Dragendorf, Wagner reactant, respectively.

Saponin Test. One gram of sample was added with ten ml water and boiled up to 100 ^oC for 5 minute, and subsequently shake in 100 rpm up to 25 ^oC. The existence of saponin was marked with foam that lasted for 10 minutes.

Flavonoid and Fenolic Hidroquinon Test. One gram sample was added with 10 ml of 30% methanol and then was heated in 100 ^oC for 2 minutes. The filtrate was pipette into

spot plate and divided into two parts each was added with 10% (b/v) NaOH and 10% (b/v) H_2SO_4. The existence of fenolic hydroquinone and flavonoid were marked with red colour by adding 10% (b/v) NaOH and red colour by adding strong H_2SO_4, respectively.

Triterpenoid and Steroid Test. Two grams of sample was added with 25 ml of 30% ethanol and was heated and filtered, subsequently. The evaporation filtrate was added with one ml of ether. The ether layer was pipette into spot plate and was added with Liebermen Buchard reactant (0.15 ml acetic acid anhydride and 0.05 ml concentrated H_2SO_4. The existence of triterpenoid was marked as red or purple colour, while green colour for steroid.

Tannin Test. Ten grams of sample was added with 20 ml water and was boiled for 2 minutes. The filtrate was added with 5 ml 1% (b/v) $FeCl_3$ and the existence of tannin was marked with dark blue or dark green.

Experimental Animals. *Sprague dawley* albino male rats (200-350 g) were obtained from the experimental animal facility of The National Agency of Drugs and Food Control (BPOM) Republic of Indonesia. Two weeks prior and during the experiment, rats were fed with standard diet (14.74% carbohydrate, 17.31% protein, 10.63% fat, 5.88% water, 31.63% ashes). Randomized rats were acclimatized for 7 days in 25 $\pm$ 2 ^oC, 50-60% relative humidity, and artificial light from 6.00 am to 6.00 pm. Fasting animals were carried out for 18 h ad labium (Ozbek *et al.* 2004).

Experimental Design. All rat was randomly divided into six groups with four rats in each group. Group A was normal control rats, group B was diabetic control rats, and group C was glibenclamide control rats. Group D, E, and F composed of rats having decoction extract treatment with different dose (Table 1). Treatment with decoction extract was started 48 h after alloxan injection. Alloxan was used to increase the blood glucose. In this experiments, Daonil was used to reduce the decrease the blood glucose.

Blood samples were obtained from tail vein in fasting rats for 18 hours and blood glucose levels were measured using electronic glucometer (Miles Inc, USA). Fasting blood glucose and body weight were measured on day 1, 3, and 13 after injection of 0.9% NaCl and 150 mg/kg BB alloxan.

Statistical Analysis. All body weight and fasting blood sugar values were measured as mean $\pm$ standard error of mean (SEM) and analyzed with ANOVA and Duncan test. Differences among groups were considered significant at $P < 0.05$ levels (Nagappa *et al.* 2003).

RESULTS

Phytochemicals assay of *P. crocatum* decoction contained alkaloid, flavonoid, and tannin (Table 2). The effect of *P. crocatum* docoction extract treatment on rat body weight on day 1, showed that rats body weight in all groups did not differ significantly ($P < 0.05$) (Table 3). Rats body weight decreased on day 3 after NaCl (Group A) and alloxan (B, C, D, E, F group) induction; the highest degradation occurred at group D (17.28% from body weight of day 1). However, body weight degradation on day 3 in all groups did not differ significantly ($P < 0.05$).

On day 13, we measured rats body weight that orally given *P. crocatum* dose 0.322 g/kg BB, 3.22 g/kg, and 20 g/kg BB to rats (D, E, and F group). During 10 days treatment, *P. crocatum* extract reduced rats body weight as well as B and C group (Table 3). *P. crocatum* dose 3.22 and 20 g/kg BB (E and F group) reduced 17.05 and 5.43% of body weight, respectively. D, E, and F groups did not differ with C group ($P < 0.05$). The lowest degradation (51.94%) of body weight was shown from D group. E and F groups did not differ with B group, however D group did ($P < 0.05$). It means that the *P. crocatum* in 0.322 g/kg can suppress the reduction of rats body weight.

Measurement of blood glucose level was carried out on day 1, 3, and 13, in order to observe the effect of aquades, Daonil and *P. crocatum* extract orally and NaCl or alloxan. The induction influences blood glucose rats during experiment. Results indicated that on day 1 (before treatment), rats blood glucose in all groups did not differ significantly ($P < 0.05$) (Table 4) and performed at normal range (60-110 mg/dl). However, after NaCl and alloxan induction (on day 3), rats

Table 1. Six group of *P. crocatum* treatment rats

Group	Treatment
A	Vehicle control (Injection of 0.9% NaCl by intraperitoneal and orally aquades 1 ml per day in 10 days)
B	Diabetic control (Injection of 150 mg/kg alloxan by intraperitoneal and orally aquades 1 ml per day in 10 days)
C	Glibenclamide control (Injection of 150 mg/kg alloxan by intraperitoneal and orally 3.22 mg/kg Daonil per day for 10 days)
D	Decoction extract (Injection of 150 mg/kg alloxan by intraperitoneal and 0.322 g/kg by orally per day in 10 days)
E	Decoction extract (Injection of 150 mg/kg alloxan by intraperitoneal and 3.22 g/kg orally per day in 10 days)
F	Decoction extract (Injection of 150 mg/kg alloxan by intraperitoneal and 20 g/kg by orally per day in 10 days)

Tabel 2. Result of phytochemical assay of *P. crocatum*

Test	Result
Alkaloid	+
Flavonoid	+
Saponin	-
Triterpenoid	-
Steroid	-
Tanin	+

+: contain compounds test, -: do not contain compound test.

Table 3. The effect of 10 days treatment with docoction extract of *P. crocatum* on rat body weight

Group	Average body weight (g)		
	Day 1	Day 3	Day 13
A	321.50 $\pm$ 2.65a	284.50 $\pm$ 2.65a	291.50 $\pm$ 7.05a
B	300.75 $\pm$ 47.59a	253.75 $\pm$ 33.90a	221.50 $\pm$ 21.42c
C	313.25 $\pm$ 45.89a	264.75 $\pm$ 34.46a	236.00 $\pm$ 29.47bc
D	332.75 $\pm$ 11.61a	275.25 $\pm$ 14.61a	259.75 $\pm$ 10.27b
E	318.00 $\pm$ 22.45a	278.75 $\pm$ 25.20a	252.00 $\pm$ 27.87bc
F	311.25 $\pm$ 20.16a	270.25 $\pm$ 21.41a	239.75 $\pm$ 20.53bc

Different letter(s) in each column indicated significant difference on $P < 0.05$. The groups refer to Table 1.

Table 4. The effect of 10 days treatment with decoction extract of *P. crocatum* on blood glucose level

Group	Average blood glucose level (mg/dl)		
	Day 1	Day 3	Day 13
A	73.75 ± 2.5a	91.00 ± 13.14a	81.25 ± 5.06a
B	70.00 ± 8.48a	340.50 ± 47.90b	311.50 ± 123.35b
C	67.50 ± 3.32a	295.25 ± 88.97b	237.00 ± 106.99ab
D	69.75 ± 9.32a	360.75 ± 93.35b	323.00 ± 155.08b
E	82.25 ± 5.74a	279.50 ± 93.29b	213.50 ± 109.01ab
F	75.00 ± 5.72a	302.75 ± 37.70b	189.50 ± 121.98ab

Different letter(s) in each column indicated significant difference on $P < 0.05$. The groups refer to Table 1.

blood glucose increased. Induction of alloxan (150 mg/kg) (B, C, D, E, and F group) increased blood glucose up to 4-5 folds. The highest (41.72%) increasing occurred D group. Increase of blood glucose rats on day 3 after induction of alloxan, showed different significantly ($P < 0.05$) with A group (Table 4).

Rats blood sugar reduced up to 10-38% in ten days after treated with various doses of decoction extract of *P. crocatum* (D, E, and F group) such as 10.46% in D group. which was not differ significantly with B and C group blood glucose. However, D group was differ significantly with A group. It was indicated that decoction of *P. crocatum* dose 0.322 g/kg cannot reduce their blood glucose. Rats in E and F groups were able to reduce their blood glucose up to 23.61 and 37.41%, respectively, which were not differ significantly with A, B, and C (Daonil treatment) groups. It was indicated that decoction of *P. crocatum* dose 3.22 and 20 g/kg gives blood glucose rats towards to normal level.

DISCUSSION

This study was conducted to evaluate the antihyperglicemic activity of *P. crocatum* as a new herbal drug. We were the first team that identified *P. crocatum* in antidiabetic herbal drugs group. Phytochemical analysis of *P. crocatum* decoction extract showed the presence of alkaloids, flavonoids, and tannins. Phytochemical compounds such as, flavonoids, sterols/triterpenoids, alkaloids, and phenolics are known to be bioactive antidiabetic principles (Atta-Ur-Rahman 1989; Ivora *et al.* 1989; Kameswara *et al.* 1997).

P. crocatum improves the condition of diabetic rats as indicated by body weight (Table 3: D, E, F group) and blood glucose level (Table 4: D, E, F groups) parameters. Ten days treatment of various doses decoction extract of *P. crocatum* prevents rats body weight reduction up to 5-52%. Body weight reduction was also indicated by using aqueous extract of *Terminalia catappa* of 42 mg/kg for 12 days treatment in alloxan induced diabetes up to 63.09% (Nagappa *et al.* 2003).

The decoction extract of *P. crocatum* at 3.22 and 20 g/kg in alloxan-induced diabetic rats showed that it possessed an antihyperglicemic activity to reduce blood glucose up to 23.61 and 37.41%. This reduction was the same activity as Daonil treatment. Antihyperclicemic activity from alcoholic extract of Chinese squash (*Benincasa hispida*) at 200 mg/kg was significantly reduced blood glucose level up to 3.68% after 24 hours given to diabetic mice. The extract contained alkaloids, flavonoids, saponins, and steroids (Battu *et al.* 2007). Blood glucose reduction up to 21.54% occurred from alcohol extract treatment of gopher plant (*Euphorbia leucophyllum*) at 500 mg/kg as well. It was observed after 24 hours the extract was given to diabetic mice (Satyanarayana *et al.* 2006).

Flavonoids compound are known to regenerate damaged â-cells in the alloxan diabetic rats (Chakkravarthy *et al*. 1980). In our study, active compound in decoction of *P. crocatum* was derived from group of flavonoids, tannins, and alkaloids. Alloxan was used to induce diabetes by destroying the â-cells of pancreas (Szkdelski 2001). There is possibility that flavonoids in *P. crocatum* have the same mechanism to regenerate the damaged â-cells in the alloxan diabetic rats. Another bioactive compound in *P.crocatum* was tannins that posses multiple biological activities including the anticancer (Okuda *et al.* 1995), antioxidant (Hagerman *et al.* 1998), and antimicrobial (Cowan 1999). Banaba (*Lagerstroemia speciosa*) extract possessed activities that both stimulated glucose transport and inhibited adipocyte differentiation in 3T3-L1 cells (Liu *et al*. 2001). Some of the glucose transport-stimulatory compounds in banaba extract were ellagitannins (Hayashi *et al*. 2002). Further research need to explore tannin compound of *P. crocatum* for glucose transport-stimulatory mechanism. Different mechanisms to reduce blood glucose levels by using plant extract also shown in some plants exhibited properties similar to the well-known sulfonylurea drugs like Tolbutamide (Ivora *et al.* 1988).

ACKNOWLEDGEMENT

The authors wish to thanks the Institute of Research and Community Empowerment (LPPM) Bogor Agricultural University for funding this research through Basic Research Project in the year 2005.

REFERENCES

Atta-Ur-Rahman ZK. 1989. *Medicinal plants with hypoglycemic activity. J Ethnopharmacol* 26:1-5.

Battu GR *et al*. 2007. Hypoglycemic and anti-hyperglycemic effect of alcoholic extract of *Benincasa hispida* in normal and in alloxan induced diabetic rats. *Phcog Mag* 3:101-105.

Boyle JP *et al*. 2001. Projection of diabetes burden through 2050: Impact of changing demography and disease prevalence in the U.S. *Diabetes Care* 24:1936-1940.

Chakkravarthy BK, Gupta S, Gambir SS, Gode KD. 1980. Pancreatic Beta Cell Regeneration: A novel antidiabetics mechanism of *Pterocarpus marsupium* Roxb. *Indian J Pharmacol* 12:123-127.

Cowan M. 1999. Plant products as antimicrobial agents. *Clin Microbial Rev* 12:564-582.

Hagerman AE *et al*. 1998. High molecular weight plant poliphenolics (tannins) as biological antioxidant. *J Agric Food Chem* 46:1887-1892.

Hayashi T *et al*. 2002. Ellagitannins from Lagerstroemia speciosa as activator of glucose transport in fat cells. *Planta Med* 68:173-175.

Ivora MD, Paya M, Villar A. 1988. Hypoglicemic and insulin release effects of tormentic acid, a new hypoglycemic natural product. *Planta Med* 54:282-286.

Ivora MD, Paya M, Villar A. 1989. A review of natural products and plants as potent ant diabetic drugs. *J Ethnopharmacol* 27:243-275.

Kameswara RB, Giri R, Kesavulu MM, Apparao CH. 1997. Herbal medicine in the management of diabetes mellitus. *Manphar Vaidhya Patrika* 1:33-35.

King H, Aubert RE, Herman WH. 1998. Global burden of diabetes 1995-2025: Prevalence, numerical estimates and projections. *Diabetes Care* 21:1414-1431.

Liu F *et al*. 2001. An extract of lagerstroemia speciosa L. has insulin-like glucose uptake-stimulatory and adipocyte differentiation-inhibitory activities in 3T3-L1 cells. *J Nutr* 131:2242-2247.

Long BC. 1989. *Essential of Medical Surgical Nursing*. New York: Mosby pr.

Nagappa AN, Thakurdesai PA, Venkat Rao N, Jiwan S. 2003. Antidiabetic activity of *Terminalia catappa* Linn fruits. *J Ethnopharmacol* 88:45-50.

Okuda T, Yoshida T, Hatano T. 1995. Hydrolysable tannins and related polyphenols. *Fortschr Chem Org Naturst* 66:1-17.

Ozbek H, Ceylan E, Kara M, Ozgokce F, Koyuncu M. 2004. Hypoglicemic effect of *Rheum ribes* roots in alloxan induced diabetic an normal mice. *Scand J Lab Anim Sci* 31:113-115.

Rates SM. 2001. Plants as a source of drugs. *Toxicon* 39:603-613.

Satyanarayana T, Katyayani BM, Hema LE, Anjana AM, Chinna EM. 2006. Hypoglycemic and anti-hyperglycemic effect of alcoholic extract of *Euphorbia leucophylla* in normal and in alloxan induced diabetic rats. *Pharmacoognosy Magazine* 2:244-255.

Sugiharti NP. 2007. Aktivitas Antibakteri Ekstrak Daun Sirih Merah (*Piper crocatum*) [Thesis]. Bogor: Bogor Agricultural University.

Szkdelski T. 2001. The mechanism of alloxan and streptozotocin action in â cells of the rat pancreas. *J Physiol Res* 50:536-546.

Widowati L, Dzulkarnain, Sa'roni. 1997. Tanaman obat untuk diabetes melitus. *Cermin Dunia Kedokteran* 116:53-60.