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Research Article

ANTIOXIDANT ASSAY OF *PHASEOLUS VULGARIS* L BEANS ETHANOLIC EXTRACT *IN VITRO* AND *IN VIVO*

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ABSTRACT

Objectives: Antioxidant is a substance such as Vitamin C that able to remove potentially damaging oxidizing agents. Malondialdehyde (MDA) is an important marker of oxidative stress that have correlation with Type 2 diabetes mellitus (DM) progression. *Phaseolus vulgaris* L (PV) (beans) is one of the alternative medicines to treat DM in Indonesia. We investigated the antioxidant activity of ethanolic extract on PV (EEPV) beans using *in vitro* and *in vivo* model.

Methods: Antioxidant assay *in vitro* was conducted using 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. Alloxan (120 mg/kg, intraperitoneally)-induced diabetic rats were divided into 5 groups (n=5), i.e., NC: Normal control, P1: Diabetic-control, P2, P3, and P4 (200 mg/kg, 400 mg/kg, and 600 mg/kg of EEPV, orally) for 28 days. MDA serum level was evaluated using spectrophotometer. The data were analyzed by one-way ANOVA test.

Results: The antioxidant assay *in vitro* showed that EEPV has very weak (IC50=641.87 µg/ml, whereas Vitamin C as standard showed very strong activity (IC50=2.95 µg/ml). MDA level were 2.80±0.09 nmol/ml (NC), 3.30±0.42 nmol/ml (P1), 3.27±4.23 nmol/ml (P2), 2.93±0.05 nmol/ml (P3), and 3.17±0.25 nmol/ml (P4). There was no significant differences among normal control, diabetic-control, and EEPV-treated groups (p>0.05).

Conclusion: EEPV showed a very weak antioxidant activity *in vitro*.

Keywords: Antioxidant, *Phaseoleus vulgaris L*, Beans, Ethanolic extract, *In vitro*, *In vivo*.

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INTRODUCTION

Free radicals, highly reactive, able to damage molecules, are known increasingly as an underlying mechanism of cells injury that affecting human health and disease, associated with diabetes mellitus (DM) Type 2 [1].

In diabetes, impaired glucose metabolism may lead to an increase in hydroxyl radical production. Free radicals may also be formed through the autoxidation of unsaturated lipids in plasma and membrane lipids. The free radicals product may react with polyunsaturated fatty acids in cell membranes leading to lipid peroxidation [2]. Thus, it will damage the membrane lipid bilayer structure that causes membrane-bound receptors and enzymes inactivation, so that the tissue permeability is increased. The end products of lipid peroxidation are propanal, hexanal,4-hydroxynonenal, and malondialdehyde (MDA) [3]. MDA is a highly mutagenic compound that represents the activity of free radicals in the cells so can be used as a biomarker of the lipid peroxidation [4-6].

Beans (*Phaseolus vulgaris* L. [PV]) are grain legumes, commonly used as cuisine, that have been consumed among people worldwide [7]. Conventionally, people were also use it as a herb to get its weight loss activity, antidiabetes, and antioxidant [8].

The previous study showed that oral administration of aqueous extract of PV (AEPV) (200 mg/kg body weight [BW]) for 45 days to diabetic rats significantly decreased the plasma thiobarbituric acid (TBA)-reactive substances and hydroperoxides. AEPV at 400 mg/kg BW was also significantly reduce (p<0.05) the values of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase [8].

Many studies have shown that oxidative stress/free radical is strongly associated with diabetes and particularly with the complications of diabetes [9]. Our previous study showed the antihyperglycemic activity of ethanolic extract on PV (EEPV). Therefore, the current study was conducted to evaluate its antioxidant activity.

METHODS

Chemical and reagents

Alloxan, formalin buffer 10%, heparin sodium, sodium chloride, cell lysis buffer, paraffin wax, TBA reagent, aquabidest, 70% and 80% aqueous alcohol and 96% absolute alcohol, xylitol, glycerin, Mayer's hematoxylin, eosin, Canada balsam. All other chemicals were of analytic grade.

Animals

Male healthy Wistar rats (*Rattus norvegicus*) were obtained from animal house of Universitas Sumatera Utara. The rats with following inclusion criteria were included in the study, i.e., age 2–3 months, 150–200 g BW. The study was conducted after approved by Animal Research Ethics Committees, Faculty of Mathematics and Natural Sciences (FMIPA), and Universitas Sumatera Utara (No. EC: 115/KEPH-FMIPA/2017).

Plant material and preparation of EEPV

The PV beans (Fig. 1) were collected at the Berastagi city, on of district in Medan (Sumatera Utara) (Fig. 2), in February 2017 and was authenticated by the Department of Botany, Universitas Sumatra Utrara. EEPV was done with maceration method that used ethanol solvent with ratio 1:10 (w/v). The dried PV was dissolved by 10 parts after that poured with 75 parts of ethanol 96% as the essence. Closed and left

for 5 days and shielded from the light while stirring occasionally. After 5 days, the solution was filtered, the pulp was squeezed, and washed with enough liquid essence to obtain 100 parts. The extract was obtained evaporated at a temperature of 50°C.

Induction of diabetes

Alloxan solution in 0.1 M citric buffer was administered at single dose of 120 mg/kg BW intraperitoneally. Diabetes was confirmed by determining the blood glucose concentration using glucometer (®glucoDrTM model AGM-2100), after 72 h of alloxan injection. The rats with blood glucose level >250 mg/dl were selected for the study [10,11].

Experimental design

The animals were divided randomly into five groups of five rats each and treated as follows:

1. Group I (NC): Normal control rats (standard pellets and water *ad-libitum*) for 28 days.
2. Group II (P1): Diabetic control rats were administered with 120 mg/kg of alloxan, standard pellets, and water *ad libitum* for 28 days.
3. Group III (P2): Diabetic rats+EEPV at dose of 200 mg/kg BW/day for 28 days.
4. Group IV (P3): Diabetic rats+EEPV at dose of 400 mg/kg BW/day for 28 days.
5. Group V (P4): Diabetic rats+EEPV at dose of 600 mg/kg BW/day for 28 days.

Fig. 1: *Phaseolus vulgaris L*

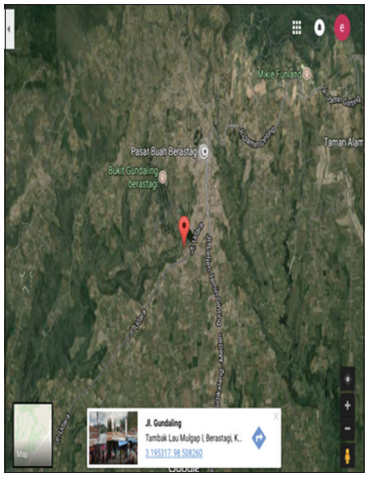

Fig. 2: Location of Berastagi

Antioxidant assay: 1,1 Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH radical-scavenging activities of experimental autoimmune encephalomyelitis were determined according to Molyneux [12].

DPPH preparation

20 mg of DPPH was dissolved in methanol until 50 ml to obtain 400 mcg/ml of DPPH solution.

Determination of maximum wavelength

As much as 5 ml of DPPH solution was put in a 50 ml of mixed flask, and the volume was adjusted with methanol to the mark line, to obtain a solution with a concentration of 40 mcg/ml. The absorbance was measured at 400–800 nm wavelength. The spectrum obtained then was observed.

Measurement of DPPH absorbance after addition of EEPV

As much as EEPV 100 mg was dissolved in 50 ml of methanol to obtain 2000 µg/ml (LIB). Different sample volumes of LIB (10, 12.5, 15, 17.5, 20 ml) were put in mixed flask (50 ml)+5 ml DPPH (400 µg/ml) to obtain different extract concentration, i.e., 400, 500, 600, 700, 800, and DPPH 40 mcg/ml. After 10–30 min, the absorbance was measured at maximum wavelength 516.5 nm.

Measurement of DPPH absorbance after addition of Vitamin C

Vitamin C as much as 25 mg crystal was dissolved in methanol until 100 ml, to obtain 250 µg/ml (LIB). Different volume of LIB (0.2, 0.4, 0.6, 0.8, 1 ml) were put 50 ml mixed flask of each. 5 ml DPPH (400 µg/ml) was added to obtain different Vitamin C concentrations, i.e., 1, 2, 3, 4, 5, and DPPH 40 µg/ml). The absorbance was measured at wavelength 516.5 nm after 10–30 min.

The assay was performed in triplicate. The percentage of DPPH radical scavenging activity was calculated as follows

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{ADPPH} - \text{A sample}}{\text{A DPPH}} \times 100$$

A DPPH = Absorbance of DPPH without sample
A sample = Absorbance of DPPH with sample
IC_{50} was determined using regression linear equation.

Measurement of MDA level

MDA level from the blood sample is determined by TBA method that measured using spectrophotometer at wavelength 532 nm. The presence of MDA is identified by the pink color formation after it reacted with TBA in acidic conditions. Measurement of MDA conducted at the Laboratory of Biochemistry Faculty of Medicine, Universitas Sumatera Utara. Data were analyzed using one-way ANOVA, $p<0.05$ considered as statistically significant.

RESULTS

DPPH radical scavenging assay

Tables 1-3 show the activity of EEPV and Vitamin C as radical scavengers.

The antioxidant assay *in vitro* showed that EEPV has very weak (IC50=641.87 µg/ml, whereas Vitamin C as standard showed very strong activity (IC50=2.95 µg/ml).

MDA level

MDA level of this study is shown in Table 4.

There was no significant differences among normal-control, diabetic-control, and EEPV-treated groups ($p>0.05$).

DISCUSSION

Diabetes can be produced in animals by alloxan induced, result in the production of active oxygen species from an imbalance between radical-generating and radical-scavenging systems, i.e., increased free

radical production or reduced activity of antioxidant defenses or both. Several studies have shown that DM (Types 1 and 2) is accompanied by the increased formation of free radicals and decreased antioxidant capacity, leading to oxidative damage of cell components [13]. Scavengers of free radicals are effective in preventing diabetes in these animal models.

PV has a notable place in the folklore throughout the world and in the traditional usage of many cultures such as antidiabetic [14,15]. In Indonesia, this plant commonly grows in Karo lands, especially Berastagi, one of the districts in North Sumatera, Indonesia. Local residents also often use it to alleviate the rise of blood glucose levels.

The present study continued with antioxidant activity test using DPPH radical-scavenging method. This assay was used to evaluate the strength of antioxidant properties of EEPV compare to Vitamin C. Vitamin C, is a water-soluble free radical scavenger that can change to the ascorbate radical by donating an electron to the lipid radical to terminate the lipid peroxidation chain reaction [16]. The roles of many dietary polyphenolic constituents derived from plants are more effective antioxidants *in vitro* than Vitamins E or C, and thus might contribute significantly to the protective effects [17]. The more polar ones (ethyl acetate and n-butanol) are those that generally have higher antioxidant activity [18].

Table 1: DPPH radical scavenging activity of EEPV

EEPV (µg/ml)	Absorbance	DPPH radical scavenging activity (%)
0	0.8080	0
400	0.5893	27.0627
500	0.5053	37.4587
600	0.4227	47.6898
700	0.3777	53.2591
800	0.2780	65.5940

DPPH: 1,1-Diphenyl-2-picrylhydrazyl, EEPV: Ethanolic extract on *Phaseolus vulgaris* L

Table 2: DPPH radical scavenging activity of Vitamin C

Vitamin C (µg/ml)	Absorbance	DPPH radical scavenging activity (%)
0	0.8497	0
1	0.7407	12.8286
2	0.5773	32.0518
3	0.4350	48.8034
4	0.2650	68.8113
5	0.0953	88.7799

DPPH: 1,1-Diphenyl-2-picrylhydrazyl

Table 3: IC_{50} of EEPV and Vitamin C on DPPH free radical

Sample	IC_{50} (mcg/ml)
EEPV	641.87
Vitamin C	2.95

DPPH: 1,1-Diphenyl-2-picrylhydrazyl, EEPV: Ethanolic extract on *Phaseolus vulgaris* L

Table 4: The MDA level of rats on day 28

Group	MDA (nmol/ml)	p
K	2.80±0.09	0.147
P1	3.30±0.42	
P2	3.27±4.23	
P3	2.93±0.05	
P4	3.17±0.25	

MDA: Malondialdehyde

Tables 1-3 show the activity of EEPV and Vitamin C as radical scavengers. The higher of EEPV lowered the absorbance which means the scavenging activity of EEPV increased following the higher concentration of EEPV. The IC50 values showed the inhibition concentration of DPPH at 50%. The results showed that IC50 of EEPV was 641.87 µg/ml whereas Vitamin C as standard was at 2.79 µg/ml. A compound claimed to have antioxidant activity when the IC50 as the following categories: <50 µg/ml (very strong), 50–100 µg/ml (strong), 101–150 µg/ml (moderate), and 151–200 µg/ml (weak). Therefore, the present study concluded that EEPV has very weak antioxidant properties while Vitamin C showed a very strong antioxidant activity.

In the present study, the increased levels of MDA clearly show that diabetic group (P1) was exposed to an increased oxidative stress through lipid peroxidation [19]. Decreased levels of MDA showed in P3 (EEPV at dose of 400 mg/kg BW).

The reports about the status of antioxidants and antioxidant enzymes in diabetic patients are very contradictory, both increase and decrease of antioxidant activity have been reported [19]. Products of membrane lipid peroxidation and other oxidants like H2O2 may react with superoxide dismutase resulting in oxidative modification thereby causing loss of enzyme activity [20]. Furthermore, diabetic hyperglycemia leads to glycation and inactivation of superoxide dismutase thus attributing to its decrease.

CONCLUSION

EEPV showed a very weak antioxidant activity *in vitro.*

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CONFLICTS OF INTERESTS

The author declares no conflicts of interest.

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