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Hypoglycemic Activity of Nano Particles from Temuru Leaves and Temuru(*Murraya Koenigii* (L.) Spreng) Leaf Extract on Alloxan Induced rats and Antioxidant Activity

Rini Handayani*, Urip Harahap, Karsono

Department of Pharmacology, Faculty of Pharmacy, University of Sumatra Utara, Indonesia

Abstract : The purpose of this study was to examine the hypoglycemic activity of nanoparticles and ethanol extract oftemuru leaves(*Murraya koenigii* (L.) Spreng) and measure the antioxidant activity of nano-particles and ethanol extract of temuru leaves. Nano particles oftemuru leaves were obtained by using the milling method.The characteristics of nano particles were analyzed using SEM (Scanning Electron Microscope) and PSA (Particle Size Analyzer), while the ethanol extract of temuru leaves obtained by maceration. The hypoglycemic test utilized mice that induced by alloxan 150 mg/kg intraperitoneally. The measurement of blood glucose levels (BGL) of mice was using the glucotesttools and determination of total blood cholesterol levels of mice was using enzymatic colorimetric method. Determination of antioxidant activity was using DPPH (2,2-diphenyl-1-picrylhidrazyl). Paired samples statistic analysis have shown that the mean of reduction of fasting bloodgluoselevel ingroups of mice that were given nano-particles and ethanol extract of temuru leaves significantly different from the diabetic control groups successively with significant value 0.003; 0.002, P <5%. Analysis of the antioxidant activity of ethanol extract of leaves temuru showed the $IC_{50}$of 34.16 ppm and $IC5_0$of nano particles of 328.42 ppm. The results showed that the nano particles and ethanol extract of the leaves can reduce the blood glucose level of mice. Antioxidant activity of ethanol extract stronger than nano particles of temuru leaves.

Keywords : *Murraya koenigii*,hypoglycemic, nano particles, blood glucose level, antioxidant.

Introduction

Diabetes Mellitus (DM) is now a major health problem in the world[1]. WHO estimated that number of diabetes sufferers continuously to rise in the world[2]. The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030[3].

Indonesia is amongcountries that rich in biodiversity, ranked four after Brazil, Columbia and China[4]. Temuruleaves used as raw materials in almost all traditional Indian medicine, efficacious to cure various diseases including dizziness, stomachache, itchy skin, insect bites, nausea, diarrhea and dysentery[5], antibacterial[2] and fungisid[6]. In Indonesia, the utilization of temuru leaves as a medication not yet widely known, especially in the public areas of Sumatra who often use the temuru leaves as a spice.

Some phytochemical compounds have been shown to inhibit the α-glucosidase enzymes, such as alkaloid[7], polyphenol[8], flavonoid, and triterpen[9]. Ten plants of Rubiaceae family and five plants from Apocynaceae family have antidiabetic activity as well by inhibiting the α-glucosidase enzymes. The contents of chemical compounds that are found in 80% ethanol extract of five simplicias family of Apocynaceae are tannin, glycoside, saponin, and anthraquinone. Ten plants of family ofRubiaceae contain terpenoid, tannin, glycoside, and anthraquinone[10]. Phytochemical compounds of alkaloid, terpene, saponin, tannin, glycoside, flavonoid, and quinone inhibit the α-glucosidase enzymes[11,12].Temuru leaves contain alkaloid[13,14]. Water extractand ethanol extract showed that temuru leaves contain phenol, sterol and steroid, saponin, quinone, alkaloid, flavonoid, tannin, and essential oil[15.16].

Several studies have shown that temuru leaves (*Murraya koenigii* (L.) Spreng) have many benefits for the treatment of diabetes[17,18], cholesterol[19] and for antioxidant[18,20].

The particle size of a drug clinically could affect the release of active substances[21]. The reduced particle size will improve drug solubility[22], this is because the surface area of the particles become larger and the properties of particles changed, thus increasing the bioavailibility of drugs in the body[23]. The shape and size of the particles affect the solubility, absorption and distribution of drugs, and therefore the shape and size of particles is one of the factors that affect effectiveness of the drugs[24].

Nano technology in the processing of natural materials simplicia can provide many advantages such as increased drug absorption and bioavailibility, side effects from the use of solvents can be avoided[25], the process of providing products became faster, more economical, and nature-friendly. Therefore, through this research activity would develop an innovation of temuru leaves nanoparticles preparation technology with hypoglycemic activity.

Antioxidants help reduce the risk of complications from type 2 diabetes and reduce insulin resistance[26]. Various studies have shown that phenolic and flavonoid compounds found in plants could counteract free radicals[27]. Temuru leaves contain phenolic and flavonoid compounds. Several studies have shown temuru leaves have antioxidant activity[28,29].

This study aimed to show nanoparticles hypoglycemic activity and ethanol extract of temuru leaves and its antioxidant activity.

Material and Methods

Materials

Materials of plants were obtained from Banda Aceh, Aceh Province, Indonesia.

Methods

Preparation the ethanolic extract

The extraction process was done by maceration method to dried temuru leaves that have been smoothed by using ethanol 96%.

Preparation of the nano particles

The preparationof the nanoparticles of temuru leaves was using milling method performed at the Research Center for Biology of LIPI Bogor. Examination of the characteristics of nano particles of temuru leaves was using SEM (Scanning Electron Microscope) and PSA (Particle Size Analyzer).

Testing of hypoglycemic activity

Hypoglycemic activity testing of the testing animals include laboratory animals acclimation, measurement of fasting blood glucose level fasting, a preliminary test with glucose tolerance methods, inducing alloxan, and the provision of treatment.

Methodology of this study was an experimental method with the design used was a completely randomized design (CRD). The observation duration of hypoglycemic activity of male Wistarmice was 12 days. Wistar male mice as many as 54 were divided into nine (9) groups as follows:

a. Group 1 was a group consistedof mice induced by alloxan without treatment as a diabetic control group.
b. Group 2 was a group consistedof mice induced by alloxan and given Na.CMC 0.5% as a solvent control group.
c. Group 3 was a group consistedof mice induced by alloxan and was given suspension of ethanol extract of leaves temuruwith dose of 50 mg / kg body weight.
d. Group 4 is a group consistedof mice induced by alloxan and was given suspension of ethanol extract of leaves temuru dose of 100 mg / kg body weight.
e. Group 5 was a group consistedof miceinduced by alloxan andwas given ethanol extract suspension of leaves temuruwith a dose of 150 mg / kg body weight.
f. Group6 is a group consistedof mice induced by alloxan and was given suspension ofnano particles oftemuru leaves with a dose of 50 mg / kg body weight.
g. Group 7was a groupconsistedof mice induced by alloxan and wasgiven suspension ofnano particles oftemuru leaves with a dose of 100 mg / kg body weight.
h. Group 8 was a group consisted of mice induced by alloxan and wasgiven suspension oftemuru leaves nano particleswith a dose of 150 mg / kg body weight.
i. Group 9 was a group consisted of mice induced by alloxan andwas given Acarbose 50 mg / kg body weightas a control of positive.

Analysis of antioxidant activity with DPPH method

The calculation used in the determination of free radical trapping activity was IC_{50} (Inhibitory Concentration), these values describe the concentration of testing compounds that can trap free radicals by 50%.

Data Analysis

Data were analyzed with Paired Samples Test Statistic to observe a significant difference between control groups and treatment groups, one way ANOVA test and Tukey test HSD (Honestly Significant Difference) to observe a significant difference in reduction of blood glucose level of mice induced byalloxanin treatment groups each time of observation.

Results and Discussion

The Characterization of NanoParticles and Ethanol Extract of Temuru Leaves

The characterization of nano particles and ethanol extract of temuru leaves include determination of level of water content, determination of levels of water-soluble extract and ethanol-soluble extract, the determination of total levels of ash and acid insoluble. Characterization was carried out to ensure the quality of simplicia and extract.

The hypoglycemic activity of nano particles and ethanol extract of temuru leaves in male mice which induced by alloxan can be observed from the miceblood glucose level that was measured every 3 days for 12 days long. Fasting blood glucose level measurement of mice was using enzymatic method. This method was chosen because it is very sensitive in measuring blood glucose level. Blood glucose levelmeasurementwas performed everyday at 09.00 PM. Hypoglycemic activity of each treatment groupof male mice after alloxan inducementcan be viewed in Table 1.

Table 1.The results of measurement of blood glucose level (mean ± SD, n = 5) on male micein each treatment group after inducing alloxan

Treatment Groups	Blood glucose level (mg/dL) on the day (mean ±SD, n=5)				
	3	6	9	12	15
CN	287,00±43,53	315,80±37,98	205,20±22,53	160,00±28,38	101,40±9,28
CD	279.2±25,05	324.6±36,34	214±73,71	105.4±6,54	91.8±9,96
N-1	336,50±85,04	220±69,64	155,83±53,24	99,17±9,43	95,76±8,71
N-2	367,33±130,13	247,83±71,18	176,33±58,01	113,16±12,66	99,50±2,81
N-3	273,80±40,04	153,80±32,37	111,90±9,21	96,40±18,22	94,00±12,06
E-1	304,40±55,07	183,80±36,36	120,40±20,99	104,60±15,27	97,40±10,24
E-2	356,00±105,84	190,75±53,13	125,25±26,96	98,50±12,87	91,25±5,32
E-3	366,50±109,98	242,17±90,36	113,17±5,19	95,17±3,66	93,50±7,01
Acarbose	280,00±39,67	113,00±24,32	103,60±6,43	95,60±4,93	105,60±7,37

Information: CN: control of negative ; CD: control of diabetic ; N-1: nano particles of temuru leaveswith dose of 50mg / kgbw; N-2: nano particles of temuru leaves with dose of 100mg / kgbw; N-3: nano particles of temuru leaves with dose of 150mg / kgbw; E-1: ethanol extract of temuru leaves with dose of 50mg / kgbw; E-2: ethanol extract of temuru leaves with dose of 100mg / kgbw; E-3: nano particles of temuru leaves with dose of 150mg / kgbw ; kgbw: kilogram of body weight.

The mean of measurement results ofblood glucose level in male mice after alloxan induction showed a reductionofblood glucose level in each oftreatment groups of nano particles and ethanol extractof temuru leaves of doses of 50 mg / kg bw; 100 mg / kgbw and 150 mg / kgbw. This provednano particles and ethanol extract of temuru leaves have hypoglycemic activity in male mice that induced by alloxan.

The results of paired samples statistic analysis, the mean of fasting blood glucose level reduction each time of observation, male mice groupswhich were given nano particles of temuru leaves, significantly different from the diabetic control group (significant value is 0.003; P <5%). Diabetic control group was a group of male mice induced by alloxan but were not given any treatment. The results of paired samples statistic analysis have beenproved that nano particles of temuru leaves have hypoglycemic activity. Nano particles of temuru leaves known based on statisticallyANOVA analysis can lowering fasting blood glucose level in male mice that induced by alloxan significantly in the days of hypoglycemic activity observation.

ANOVA statistical analysis results have been showed that ethanol extract of temuru leaves can lowering fasting blood glucoselevel of male mice induced by alloxan significantly in the observation days of hypoglycemic activity. The results of paired samples statistic analysis, the average reduction in fasting blood glucoselevel on each time observation of male mice groups which were given ethanol extract of temuru leaves, significantly different from the diabetic control group (significant value is 0.002; P <5%).

The comparison of hypoglycemic activity of nano particles and ethanol extract of temuru leaves

The comparison of hypoglycemic activity of nano particles and ethanol extract of temuru leaves has been viewedfrom the mean ofblood glucoselevel decrease in male mice induced by alloxan. The mean amounts of reduction in blood glucoselevel in each test group are shown in Table 2.

Table 2.The mean ofreductionofblood glucoselevel in the test groups each timeof observation

Groups	Blood glucoselevel on day 3	Blood glucose level on day 6	Reductionof blood glucose level (mg/dL)	Blood glucose level on day 9	Reduction of blood glucose level (mg/dL)	The meanofBGL reduction (mg/dL)
C(-)	287	316	-28.8	205	111	41
CD	279	325	-45.4	214	111	33
N-1	337	220	116.5	156	64	90
N-2	367	248	119.5	176	72	96
N-3	274	154	120	112	42	81
E-1	304	184	120.6	120	63	92
E-2	356	191	165.3	125	66	115
E-3	367	242	124.3	113	129	127
Acb	280	113	167	104	9	88

Information:
C(-): control of negative; CD: control of diabetic; N-1: nano particles of temuru leaves with a dose of 50 mg / kgbw; N-2: nano particles of temuru leaves with a dose of 100 mg / kgbw; N-3: nano particles of temuru leaves with a dose of 150 mg / kgbw; E-1: ethanol extract oftemuruleaves with a dose of 50mg / kg; E-2: ethanol extract of temuruleaves with a dose of 100 mg / kg; E-3: ethanol extract of temuruleaves with a dose of 150mg / kg; Akb: Acarbose; Kgbw: Kilogram of bodyweight

Antioxidant Activity

The analysis results of IC_{50} valuescan be viewed in Table 3.

Table 3. The IC_{50} values of nano particles of temuru leaves and ethanol extract oftemuru leaves

Testing solution	IC_{50}(ppm)
Nano particles of temuru leaves	328,42
Ethanol extract of temuru leaves	34,16

Based on category of the IC_{50} value as antioxidant then antioxidant activity of nano particlesof temuru leaves categorized as weak and ethanol extracts of leaves temuru had a very strong antioxidant activity.

Temuru leaves had the effect of lowering blood glucose levels of diabetic mice and had the potential to reduce the level of TBARS (Thiobarbituric Acid Reactive Substances) by inhibiting the formation of lipid peroxidation, thus helping to prevent complications of diabetes associated with the level of oxidative stress[30].

The antioxidant activity of ethanol extract of temuru leaves in this study was greater than the antioxidant activity of nano particles oftemuru leaves. This was presumably because the amounts of nano-particles andethanol extract of temuru leaves used in the study were similar. The nano particles of temuru leaves in this study were crude drug powders that sized <50 nm and ethanol extract of temuru leaveswas crude extract that obtained by using maceration method with 96% ethanol. Therefore it was necessary to doa conversion of the amount of nano-particlestemuru leaves used against ethanol extract of temuru leaves so it could be compared its antioxidant activity.

Antioxidants can bind free radicals that can reduce the risk of type 2 diabetes and beneficial in reducing insulin resistance[31]. Antioxidants play an important role in preventing complications of DM[32].

Conclusion

The hypoglycemic activitiy of nano particles of temuru leaves was not significantly different with hypoglycemic activity of ethanol extract of temuru leaves and the antioxidant activity of ethanol extract of temuru leaves greater than the nano particles oftemuru leaves.

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