Pharmacology and Clinical Pharmacy Research
Volume 4 No. 2 August 2019
ISSN:2527-7322 | e-ISSN: 2614-0020
doi: 10.15416/pcpr.v4i2.24524

Anti-Hyperglycemic Activity of *Phyllanthus emblica* Leaves and Bark in Alloxan-Induced Diabetic Rats

Maria Ulfah, Novi I. Fauzi, Nela Simanjuntak, Anastasia Sinuraya
Indonesian School of Pharmacy, Bandung, West Java, Indonesia

Abstract

Phyllanthus emblica is empirically used to treat various diseases. Chemical compounds in this plant includes benzene derivatives, diterpen and monoterpen, furanolacton, flavonoids and sterols. The purpose of this research is to investigate anti-hyperglycemic activity of *P. emblica.* The diabetic animal model was obtained by administration of alloxan 120 mg/kg BW intraperitonial. The rats were divided into 9 groups, *i.e.,* normal group, negative control (1% CMC), positive control (glibenclamide 0.5 mg/kg BW) and *P. emblica* leaves and bark ethanol extract at the dose of 500, 750, and 1000 mg/kg BW. Determination of flavonoid content was performed through colorimetric method using UV-Vis spectrophotometer at 425 nm. After 7 days of induction, the entire group was treated for 21 days, fasting blood glucose was performed on days 0, 1, 8, 15 and 22. Then the data of fasting blood glucose level in mice was treated with one way ANOVA analysis and advanced test with post hoc Least Significant Differences (LSD) method. The percentage of the blood glucose decrease from the animal treated with leaves extract at doses of 500, 750 and 1000 mg/kg BW, respectively, were 22.47%; 21.03%; and 24.52%, while those of bark extract were 32.19%; 31.61%; and 37.24%, respectively. Determination of total flavonoid level showed that the highest amount of flavonoids was observed in leaves (35.838 mg/g Quercetin). In conclusion, *P. emblica* bark and leaves showed anti-hyperglycemic activity.

Key words: anti-hyperglycemic, alloxan, *Phyllanthus emblica,* Wistar

Introduction

One of the traditional remedies used for diabetes mellitus is *P. emblica*. It is a native Indian plant. In Indonesia this plant is called *Malaka*. All parts of the plant have potential to be used for medicinal purpose. This potential is influenced by the biodiversity of the secondary metabolites in the plant. The secondary metabolites compounds contained in this plant include benzene and monoterpenes, furanolactone, flavonoids and sterols.[1] The presence of certain secondar metabolite is usually similar in the same plant, nevertheless the level varies between one part of the plant with others.[2]

Flavonoid compounds have recently attracted researchers to examine its biological activities, including anti-hyperglycemic activity. The potential effect of flavonoid as anti-diabetes is mainly through the modulation effect

Corresponding author: Maria Ulfah. Indonesian School of Pharmacy, Bandung, West Java, Indonesia.
Email: maria.ulfah@stfi.ac.id.
Received: 24 June 2019. **Revised**: 15 July 2019 **Published:** 30 August 2019.

of glucose transporter by increasing GLUT-2 expression in pancreatic β cells and increasing expression and promoting GLUT-4 translocation through PI3K/AKT,CAP/Cb1/TC10 and AMPK pathways. Assessment of recent findings on the beneficial effects of flavonoids on the management of diabetes focus in exploring the role of flavanoid compounds in modulating glucose transporter proteins at the cellular and molecular levels.[3,4]

The hyperglycemic activity of *P. emblica* has been reported by Shamim *et al* where *P. emblica* fruit extract at a dose of 200 mg/kg BW was reported to significantly decrease blood sugar levels in alloxan-induced mice. A decrease in blood glucose level similar to that of chlorpamamide at doses of 84 mg/kg BW.[5-8] Nevertheless, there is limited information on anti-hyperglycemic activity of other part of this plant, such as seeds, leaves, bark, and roots. Flavonoids content in the part of the plant may also influence its biological activies. Therefore, this study was conducted to investigate the effect of flavonoids in leaves and bark ethanolic extract of *P. emblica* on anti-hyperglycemic activity in alloxan-induced diabetic rats.

Methods

Materials

The materials used in this study included leaves and bark of *P. emblica* obtained from Jelekong Village, Bandung, glibenclamide (Sigma-Adlich), alloxan (Sigma-Aldrich), quercetin (Sigma Aldrich), methanol, carboxyl methyl cellulose (CMC), 96% ethanol, aluminum chloride ($AlCl_3$), sodium acetate, destilled water, and materials for testing simplicia parameters

Plant determination

Plant determination was conducted at Herbarium Jatinangor Laboratory of Plant Taxonomy Department of Biological Science of Universitas Padjadjaran and School of Life Science Technology (SITH) Institute Technologi Bandung.

Animal Model

Male Wistar rats (2-3 months old, 150-250 g) were used in this study. Rats were provided by Animal Laboratory of The Wistar, Bandung and kept under usual management condition in this institution. All conducted methods have followed the standard of management care for laboratory animals.

Extraction

Leaves and bark of *P. emblica* were dried and ground. *P. emblica* leaf extract was made by maceration method. Three hundred and fifty grams of leaves powder was macerated in 9600 ml of ethanol 96% for 18 hours, with occasional stirring, The extract was evaporated with a low pressure vaporizer to obtain a thickened extract. *P. emblica* bark extract was made through soxhlet method. The powder was wrapped with filter paper and heated with 1800 ml ethanol. The extract was thickened using rotary evaporator.

Table 1. Extraction of *P. emblica* Leaves and Bark

Part	Amount	Thickened Extract	Yield Extract
Leaves	700 g	288.36 g	41.19%
Bark	940 g	200 g	21.27%

Table 2. Phytochemical Screening of *P. emblica*

Secondary metabolites	Leaves		Bark	
	Raw	Ethanol Extract	Raw	Ethanol Extract
Alkaloid	+	+	+	+
Flavonoid	+	+	+	+
Saponin	+	+	+	+
Tannin	+	+	+	+
Quinone	+	+	-	-
Monoterpenoid and Sesquiterpenoids	+	+	-	-
Steroid	-	-	-	-
Triterpenoid	-	-	-	-

+ = presence, - = absence

Determination of flavonoids

Phytochemical screening of leaves and bark extract were performed to examine the presence of alkaloids, steroids/triterpenoids, saponins, tannins, flavonoids, quinones, monoterpenoid, and sesquiterpenoids. Determination of flavonoid content was performed through colorimetric method using UV-Vis spectrophotometer at 425 nm.

Quercetin spectrum with methanol yielded a wavelength of band I of 371 nm, with the band II not being apparent. In addition of $AlCl_3$ and HCl shift reagents there was a batochromic shift in band I of 53.5 nm with a wavelength of 424.5 nm which states that the compound has 5-OH, C-flavonoid ring. In the addition of the NaOH reagent there was a shift in band I of 65.5 nm with a wavelength of 436.5 nm which states that the compound has 4'-OH in the flavonoid B ring which is similar to flavonol/quercetin. The complex between quercetin with $AlCl_3$ and sodium acetate 15 ppm gives an absorbance of 1.682 and the maximum wavelength (λ) at 425 nm. The absorbance of the extract was measured using three repetitions.

Antihyperglycemic assay

Rats were acclimatized to the environment. All groups, except normal group was induced with alloxan intraperitonial with dose 120 mg/kg BW, alloxan was dissolved in physiological NaCl. Fasting blood glucose levels and body weight of rats were examined on days 0, 3, 6 and 7. Fasting blood glucose levels were examined using glucosemeter. Blood sample was collected from tail. Rats are classified as diabetes if fasting blood glucose level > 140 mg/dl.

Table 3. Total Flavonoid in *P. emblica* Extract

Ethanol Extract	Levels of Flavonoids (mg/g Quercetin)
Leaf 1000 mg	35.838
Leaf 750 mg	26.878
Leaf 500 mg	17.919
Bark 1000 mg	28.992
Bark 750 mg	20.294
Bark 500 mg	14.496

ISSN:2527-7322 | e-ISSN: 2614-0020

Diabetic rats were divided into 9 groups and each group consisted of 5 rats. The treatment for 9 groups included normal group, negative control (1% CMC), positive control (glibenclamide 0.5 mg/kg BW) and *P. emblica* leaves and bark ethanol extract at the dose of 500, 750, and 1000 mg/kg BW. Fasting defined as the rats were left 18 hours without food, only drink provided. Fasting blood glucose and body weight of rats were examined on days 0, 1, 8, 15, and 22. One way ANOVA analysis and advanced test with post hoc Least Sinificant Differences (LSD) method were perffromed.

Results and Discussion

Plant determination results showed that the plant used in this study was *P. emblica.* The extract obtained were 288.36 g and 200 g for leaves and bark, respectively. The results of phytochemical screening showed that bark and leaves of *P. emblica* contained flavonoids, saponins, and tannins. This flavonoid compound is considered responsible in antihyperglyccemic activity of *P. emblica.* Determination of total flavonoid level showed that the highest amount of flavonoids was observed in leaves (35.838 mg/g Quercetin).

In this study, alloxan was used to induce diabetes. Alloxan is a pyrimidine derivative, with another name 2,4,5,6-tetraoxypirimidine; 2,4,5,6-pyrimonyron and 1,3-diazinan-2,4,5,6-tetron; mesoxalylurea 5-oxobarbiturate acid. Pure alloxan is obtained from the oxidation of uric acid by nitric acid. Alloxan is an unstable, hydrophilic compound. The activity of this free radical compound increase the concentration of calcium cytosol that causes rapid destruction of pancreatic β cells. Alloxan compounds also have specific cytotoxic properties in Langerhans β cells and evoke radical groups that damage Langerhans β cells due to increased free radicals in the body.[9,10]

Bark ethanol extract exhibited better activity compared to leaves extract. The percentage of the blood glucose decrease from the animal treated with leaves extract at doses of 500, 750 and 1000 mg/kg BW, respectively, were 22.47%; 21.03%; and 24.52%, while those of bark extract were 32,19%; 31.61%; and 37.24%, respectively.

Based on one way ANOVA analysis, leaves and bark ethanol extract of *P. emblica* at doses of 500, 750 and 1000 mg / kg BW exhib-

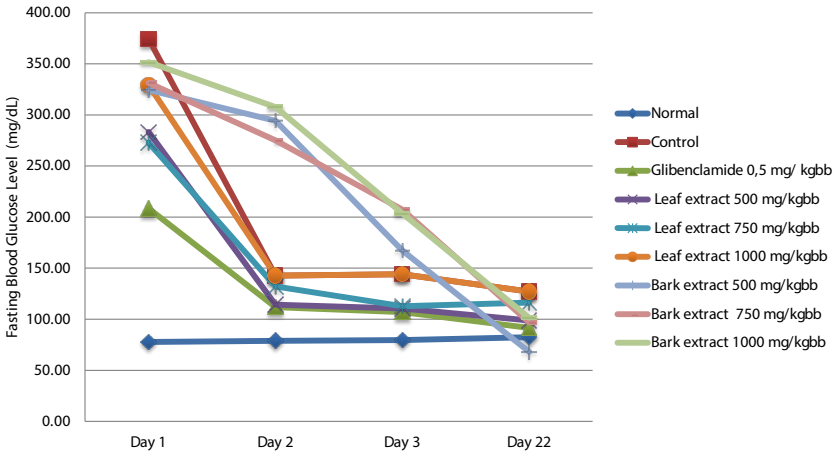

Figure 1. Fasting Bloof Glucose After Treatment

ISSN:2527-7322 | e-ISSN: 2614-0020

ited antihyperglycemic effect with P <0.05 at week 2-3.

Our study found that the greater level of flavonoids showed the greater anti-hyperglycemic activity. Flavonoids regulate glucose transporter protein in cells by increasing GLUT-2 expression in pancreatic β cells. Beside, presence of C-2-C-3 double bond and C-4 ketonic group can improve α-glucosidase activities that are are related with antidiabetic property.[11-14]

Conclusion

P. emblica bark and leaves showed antihyperglycemic activity.

Acknowledgement

The authors would like to thank Indonesian School of Pharmacy (STFI) and the Hazanah foundation for their support during the research.

Funding

None.

Conflict of Interest

None declared.

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