Antidiabetic Activity of the Leaf Extract of *Eurycoma Longifolia* Jack. in Streptozotocin-Nicotinamide Induced Diabetic Model

Ruqiah Ganda Putri Panjaitan*, Agus Astuti

**ABSTRACT**

Background: One of the medication attempts in diabetes mellitus is by utilising plants that are potent as an antioxidant. *Eurycoma longifolia* Jack, known as “Longjack,” in English, is a medicinal plant and reportedly effective as an antioxidant. Objectives: This study was aimed to examine the antidiabetic effectiveness of ethanol extract of longjack leaf in diabetes mellitus rats. Methods: This study used the total of 24 male white rats which were grouped into four. The normal and the negative control groups were administrated with CMC/Na 0.5% dose 2 ml/200 g body weight; one group was administrated with ethanol extract of longjack leaf dose 176.4 mg/200 g body weight; and the positive control group was administrated with glibenclamide dose 0.09 mg/200 g body weight. Before the extract administration, all of the experimental animals were prior induced into diabetic condition with streptozotocin-nicotinamide. Results: The levels of blood glucose and malondialdehyde in rats after the 14-day extract treatments of the experimental animals were prior induced into diabetic condition with streptozotocin-nicotinamide.

**INTRODUCTION**

Diabetes mellitus is a syndrome of metabolic disorder of carbohydrates, lipids, and proteins occurring as a result of insulin limitation and the decline of tissue sensitivity against insulin. Diabetes mellitus causes hyperglycaemia, lipid abnormality, and other metabolic disorders. Diabetes is classified into two types, i.e. diabetes type 1 and type 2. Diabetes type 1 is noticeable with the destruction of autoimmune of pancreatic β cells, which are an insulin producer, whereas diabetes type 2 is manifested due to the occurrence of hyperglycaemia which is caused by the reduction of insulin secretion triggered by the resistance of insulin.

Diabetes mellitus type 2 is a health problem faced by the worldwide community and closely related to obesity, hypertension, and other health diseases; this condition may even become worse resulting in serious complication on other organs in the body, such as heart attack, heart failure, kidney failure, dementia, blindness, and lower limb amputations.

Previous studies revealed that one of the triggered factors in hyperglycaemia is the upsurge of free radical production or reactive oxygen species (ROS); the intensification of ROS activity can injure various tissues in the body. Moreover, the hyperglycaemia condition also promotes the increase of ROS production in all tissues through the process of glucose auto-oxidation and protein glycosylation. Meanwhile, in the oxidative stress condition, the levels of antioxidant enzymes extensively affect the susceptibility of various tissues associated to complications in patients with diabetes. The main target of ROS is lipid, and one of the decomposition products from lipid oxidation is malondialdehyde, which is formed from prostaglandin biosynthesis, such as endoperoxides from polyunsaturated fatty acids. Previously, it has been reported that malondialdehyde is systematically linked to metabolic parameters in patients with diabetes type 1 and 2, and is stated that the patients with diabetes in severe metabolic control will show the high plasma levels of malondialdehyde and are significantly different with diabetic patients in better metabolic control. In the hyperglycaemia condition, the rise of ROS production exceeding the antioxidant capacity of cells will lead to the escalation of oxidative stress accompanied by the lipid peroxidation in cell membranes so that it will increase malondialdehyde as a result of lipid peroxidation.

Medicinal plants are reported to containing various secondary metabolites that play a role as antioxidant. Antioxidant is a vital substance that protects our body from damages due to oxidative stress by free radicals. Several studies showed that some plants are widely contributed in diabetes management because the phytochemical compounds have potency as an antioxidant, anti-inflammation, and the decline of blood glucose. There are plants available in diabetes medication, such as ladies’ fingers seed (*Abelmoschus esculentus*) and longjack root (*Eurycoma longifolia* Jack.).

Indonesia is one of the countries that has massive biodiversity and local knowledge correlated to the utilisation of medicinal plants. *Eurycoma longifolia Jack.* which is classified into Simaroubaceae

Cite this article: Panjaitan RGP, Astuti A. Antidiabetic Activity of the Leaf Extract of *Eurycoma Longifolia* Jack. in Streptozotocin-Nicotinamide Induced Diabetic Model. Pharmacogn J. 2021;13(6)Suppl: 1582-1588.
Longjack is also one of the Indonesia’s tropical plants and well known as a raw material in the manufacture of both modern and traditional drugs. Previous studies demonstrated various medicinal activities from the longjack root, such as anti-hyperlipidaemia, anti-inflammation, and analgesic; anti-obesity; hepatoprotector, and were safely proven in blood profile of lactating mothers. Not only that, the longjack root is also a plant used in traditional medicine to treat diabetes mellitus. In accordance with the diverse activities owned by the longjack root, the study results of qualitative phytochemicals exhibited that methanol extract of the longjack root contained phenol, flavonoid, terpenoid, triterpenoid, glycoside, and steroid.

The findings revealed that any phytochemical compounds in a plant are not only concentrated in certain parts of the organs, but also in specific areas. If a plant has compounds in certain parts, it may be considered that other parts have also similar compounds even in different levels. Currently, the longjack’s organ often used is the root. However, if this part is excessively used in future, it may have an adverse impact on the survival of this plant species. Therefore, it is necessarily conducted a research to investigate another part of the plant’s organ, which is the leaf. Several studies related to the longjack plant demonstrated that the longjack leaf can be used to treat itching; the stem is efficacious for dysentery; the stem bark is useful for worms; the petiole has the activity of healing the cut wounds in mice. Furthermore, it is stated that this healing power is correlated to the content of compounds in the longjack leaf. In connection with this, the aim of this study was to determine the antidiabetic activity of ethanol extract of longjack leaf in streptozotocin-nicotinamide induced rats.

**MATERIALS AND METHODS**

**Experimental Animals**

All experimental animals used in this study were the male white Wistar rats aged 2 months with the body weights ranging from 148-209 g. Those animals were collected from the laboratory of The Centre for Food and Nutrition Studies, University of Gadjah Mada, Yogyakarta, Indonesia. Before treatments began, all rats were acclimatised for 7 days. During this acclimisation period, they were fed with standard diet of ayam Bangkok AD II and drunk ad libitum. Overall procedures related to the management of animal model in this study was ethically approved in the letter of 1.253/XI/HREC/2019 from the Ethic Commission of Research and Health Research of Public Regional Hospital (RSUD) Dr. Moewardi, Surakarta, Indonesia.

**Plant Extraction**

The fresh longjack leaves were collected from Peramas Mount, Mount Palung National Park, North Kayong Regency, West Kalimantan, Indonesia. The result of plant determination was stated in a letter numbered 123/A/LB/FMIPA/UNTAN/2018. The leaves were then separated from the stalks, cleansed, and weighted for the wet weight of 4.2 kg. After that, they were dried and collected the dry weight of 1.55 kg. Next, the leaves were extracted using 96% distilled ethanol solvent. The yielding of samples was conducted in room temperature for 24h with threefold repetitions. The filtrates of the sample yields were then evaporated using vacuum rotary evaporator. The extract total obtained was 62.2 g with the yield of 4.02%. The extraction process was conducted in this study following to Harborne (1987).

**Production of CMC-Na 0.5% Solution**

All procedures of the production of CMC-Na 0.5% solution referred to Salma et al. (2013). A total of 0.5 g CMC-Na (Sigma-Aldrich) was placed into a beaker glass and dissolved in ±30 ml warm aquades until homogenised. The homogenous CMC-Na solution was then moved to a 100 ml volumetric flask, added by aquades till 100 ml, and stirred up until well-mixed. The dosage used in this trial followed Saputri & Zahara (2016) at 2 ml/200 g body weight.

**Production of Streptozotocin Solution**

Streptozotocin (STZ) solution and dosages were correspondingly made following Ghasemi et al. A total of 216 mg streptozotocin (Cayman Chemical) was diffused in 72 ml buffer citrate with pH 4.5, which was initially prepared before inoculation. Then, the homogenisation was done by homogeniser. The dosage of streptozotocin solution used in the injection was 9 mg/200 g body weight.

**Production of Nicotinamide Solution**

Dosages and NA (Nicotinamide) solution were prepared according to the method of Ghasemi et al.(2014). A total of 528 mg nicotinamide (Sigma-Aldrich) was suspended in 72 ml sodium chloride 0.9% (PT Widrata Bhakti) until homogenous using homogeniser. Nicotinamide dose injected to the rats was 22 mg/kg body weight.

**Production of Glibenclamide Suspension**

Glibenclamide suspension and dosage administration were implemented using the method of Salma et al. (2013). The dose administration of glibenclamide (PT Indofarma) for adults is 5 mg. Thus, the rat dosage conversion is 0.09 mg/200 g body weight. A glibenclamide tablet (PT Indofarma) of 27 mg was ground using mortar, then was diffused in 15 ml of CMC-Na 0.5%.

**Antidiabetic Activity Test**

Rats were divided into 4 groups consisting of 6. The first group was the normal control; the second one was negative control with CMC-Na 5% dose 2 ml/200 g body weight; the third one was the group with the ethanol extract administration of longjack leaf dose 176.4 mg/200 g body weight; and the fourth one was the positive control with the glibenclamide administration dose 0.09 mg/200 g body weight. After acclimatisation, on the 0 day the initial blood draw was done when all experimental animals were yet to receive treatments. Then, they were induced with STZ-NA. The induction of STZ-NA was completed by injecting streptozotocin dose 9 mg/200 g body weight and nicotinamide dose 22 mg/200 g body weight which was early administered 15 mins before streptozotocin.

**Rat Models with Diabetes Mellitus type 2**

Induction procedure of type-2 diabetes mellitus in rats referred to Ghasemi et al. (2014). Rats were injected via intraperitoneal with the combination of streptozotocin dose 9 mg/200 g body weight and nicotinamide dose 22 mg/200 g body weight which was early administered 15 mins before streptozotocin.
Measurement of Blood glucose Levels

Blood sample in rats were obtained from sinus orbitalis using microhematocrit. The collected blood samples were placed into eppendorf tubes and centrifuged at 4000 rpm for 15 mins. After the sera were parted, the serum separation was done by adding glcuses GOD FS DiaSys into test tubes. The samples in the test tubes were then homogenised using vortex. The absorbance measurement was completed by using spectrophotometer at 500 nm.

Measurement of Malondialdehyde Levels

Measurement of malondialdehyde levels in blood serum was finalised using the method of Thiobarbituric Acid Reactive Substance. A total of 0.5 ml blood serum was added with 0.5 ml trichloroacetic acid 30% and centrifuged at 3000 rpm for 5 mins. Supernatant was then collected. A total of 0.5 TBA 10% was added into 0.5 supernatant and boiled at 100°C for 30 mins, then was cooled at room temperature. The absorbance was then read at 532 nm. Malondialdehyde concentration is µl/1ppm.

Data Analysis

This study was conducted using completely randomised design. Overall, the data was statistically analysed using SPSS 24 for Windows and continued with the Duncan test at a confidence rate of 5%.

RESULTS AND DISCUSSION

The treatment of diabetes mellitus can be done using medicinal plants. Some plants having the antioxidant content of flavonoid, tannin, alkaloid, and terpenoid are reportedly efficacious in decreasing blood glucose level.

Diabetes mellitus is marked with hyperglycaemia condition which causes oxidative stress. This condition will encourage the formation of ROS in various tissues through the auto-oxidation process. Reactive oxygen species will straightforwardly oxidase and damage DNA, protein, lipid which ended up with complications due to diabetes. In hyperglycaemia condition, the increase of ROS production exceeding the antioxidant capacity of cells will initiate oxidative stress accompanied by the occurrence of lipid peroxidation in cell membranes so that it will increase malondialdehyde as a result of lipid peroxidation and injuries in tissue cells. Other study results also proved that species oxygen reactive plays a main role in the pathogenesis of type-2 diabetes mellitus.

According to Hidayaturrahmah et al. (2020) blood glucose levels in normal rats ranged from 50-135 mg/dl. In Table 1, it can be seen that the average levels of blood glucose on the day 0 for all treatments were in normal rates, 66.29-68.40 mg/dl. Similarly, in the same table, it was also shown that blood glucose levels in the experimental rats in the normal control on the measurement day 0, 3, and 18 were in normal rates; these groups shown to imply that all animal models were not diabetic without STZ-NA induction. Thereafter, on the day 3 another blood draw was carried out to investigate the escalation of blood glucose levels due to STZ-NA induction. The results demonstrated that the average levels of blood glucose in animal models on the groups with the administration of CMC-Na, ethanol extract of longjack, and glibenclamide were 277.19-281.04 mg/dl, implying that all experimental animals had type-2 diabetes mellitus. In accordance with Ghasemi et al. (2014) it stated that if blood glucose levels in rats >250 mg/dl after STZ-NA injection indicating all rats had type 2 diabetes mellitus. This occurred because of streptozotocin injection will expand the capacity of free radicals due to the release of oxide nitrogen radicals so that it leads damages in pancreatic β cells. In contrast, nicotinamide is an antioxidant of vitamin B3 derivate (Niasin) which acts to protect pancreatic β cells from the adverse effects of streptozotocin cytotoxic. In connection with the nicotinamide role, the aim of nicotinamide administration in this study was to prevent streptozotocin from damaging DNA that might lead to massive destruction in pancreatic β cells. Moreover, on...
the day 18 the measurement of blood glucose and malondialdehyde levels was recorded to determine the antidiabetic activity of ethanol extracts. The findings revealed that the average levels of blood glucose on the day 18 ranged from 148.63-285.84 mg/dl, suggesting the decline of blood glucose levels on the groups with the administration of glibenclamide and longjack ethanol extract. Conversely, the elevation of blood glucose levels occurred in the group of CMC-Na injection.

Table 2 presented the average levels of malondialdehyde from all treated groups ranging from 1.13-10.03 nmol/ml, implying that malondialdehyde levels in the experimental rats on the normal control were at normal rates. In contrast, the groups with the injections of CMC-Na, longjack ethanol extract, and glibenclamide experienced declines. Responding to Sutaryono et al. (2016) the normal levels of malondialdehyde are 1.09-1.64 nmol/ml. Malondialdehyde is a product of lipid peroxidation generally used as an indicator of the occurrence of oxidative stress. Normal group showed the levels of blood glucose in normal rates and had the lowest level of malondialdehyde compared to other groups (1.13 nmol/ml); this group was not exposed by streptozotocin. Fitriana et al. (2017) disclosed that streptozotocin can advance lipid peroxidation and malondialdehyde level. More than that, the balance between free radicals and antioxidant can be found in the normal body. Nevertheless, the balance can change when the production of free radicals rose; stress antioxidant can be found in the normal body.

Inflammation, and oxidative stress in muscles and lipids, as well as glucose metabolism in hepatocytes, diminishing insulin resistance, insulin secretion and the proliferation of pancreatic β cells, lessening body weight. In addition, the potential properties are confirmed by the antioxidant contents found in the ethanol extract of longjack so that it may repair damages due to free radical attacks.

CONCLUSION

Ethanol extract of the longjack leaf dose 176.4 mg/200 g body weight has an antidiabetic property in terms of reducing blood glucose and malondialdehyde levels in streptozotocin induced rats, which is equivalent to the administration of glibenclamide dose 0.09 mg/200 g body weight.

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Pharmacognosy Journal, Vol 13, Issue 6 (Suppl), Nov-Dec, 2021

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**GRAPHICAL ABSTRACT**

*Longjack (Eurycoma longifolia Jack.)*

![Extracted](image)

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**Table 1. Average levels of blood glucose in rats on each treatment group on the day 0, 3, and 18**

| Treatment Group | Average Levels of Blood Glucose (mg/dl) |
|-----------------|-----------------------------------------|
|                 | D-0             | D-3             | D-18            |
| Normal Control  | 66.36±1.42      | 67.54±1.23      | 78.73±7.20      |
| CMC-Na 5% dose  | 66.29±1.87      | 277.19±3.01     | 285.84±17.56    |
| Ethanol Extract | 68.40±1.71      | 281.04±1.79     | 156.77±8.49     |
| Glibenclamide   | 67.16±2.27      | 279.28±3.30     | 148.63±23.81    |

Information: * Same letters shown not significantly different on the Duncan test at 5% Numbers shown after ± demonstrated SD (Standard Deviation)

**Table 2. Average levels of malondialdehyde on each treatment group on the day 18**

| Treatment Group | Malondialdehyde Level (nmol/ml) |
|-----------------|---------------------------------|
| Normal Control  | 1.13±0.22                       |
| CMC-Na 5% dose  | 10.03±0.34                      |
| Ethanol Extract | 3.86±0.71                       |
| Glibenclamide   | 3.64±1.77                       |

Information: * Same letters shown not significantly different on the Duncan test at 5% Numbers shown after ± demonstrated SD (Standard Deviation)
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Cite this article: Panjaitan RGP, Astuti A. Antidiabetic Activity of the Leaf Extract of *Eurycoma Longifolia* Jack. in Streptozotocin-Nicotinamide Induced Diabetic Model. Pharmacogn J. 2021;13(6)Suppl: 1582-1588.