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Original Article

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

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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 were 78.73 mg/dl and 1.13 nmol/ml, respectively in the normal control group; 285.84 mg/dl and 10.03 nmol/ml were in the negative control group; 156.77 mg/dl and 3.86 nmol/ml in the group with the administration of ethanol extract of longjack leaf; and 148.63 mg/dl dan 3.64 nmol/ml in the group of glibenclamide administration ($p<0.05$). The reduction of blood glucose and malondialdehyde levels in the groups of ethanol extract administration of longjack leaf dose 176.4 mg/200 g body weight was similar to the glibenclamide administration dose 0.09 mg/200 g body weight. **Conclusion:** The ethanol extract of longjack leaf was effective as an antidiabetic.

Key words: Blood glucose level, Diabetes mellitus, *Eurycoma longifolia* Jack. leaf.

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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.[1-3] 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,[4] 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.[5,6] 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.[7]

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.[6,8-10] 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.[6,8-10] 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.[6,9,11] 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.[12] 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 peroxidase in cell membranes so that it will increase malondialdehyde as a result of lipid peroxidation.[13]

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.[14-16] 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.[17,18] There are plants available in diabetes medication, such as ladies' fingers seed (*Abelmoschus esculentus*)[16] and longjack root (*Eurycoma longifolia* Jack.).[19]

Indonesia is one of the countries that has massive biodiversity and local knowledge correlated to the utilisation of medicinal plants.[20,21] *Eurycoma longifolia* Jack. which is classified into Simaroubaceae

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family, is one of the famous medicinal plants in the Southeast Asia.[22,23] 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[24]; anti-obesity[25]; hepatoprotector[14], and were safely proven in blood profile of lactating mothers.[26] Not only that, the longjack root is also a plant used in traditional medicine to treat diabetes mellitus.[23] 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, glycoside, cardiac glycosides, and protein.[27] On the other side, Kaimal *et al.* (2010)[28] stated that the responsible compounds for decreasing blood glucose level are flavonoid, phenol, diterpenoid, 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.[29] Currently, the longjack's organ often used is the root.[14,26,30,31,32] 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 fruit is efficacious for dysentery; the stem bark is useful for worms[23]; the petiole has the activity of healing the cut wounds in mice.[33] Furthermore, it is stated that this healing power is correlated to the content of compounds in the longjack leaf.[33] 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 acclimatisation 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 Health Research of Regional Public 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 *vacum 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).[34]

Production of CMC-Na 0,5% Solution

All procedures of the production of CMC-Na 0,5% solution referred to Salma *et al.* (2013).[35] 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)[36] at 2 ml/200 g body weight.

Production of Streptozotocin Solution

Streptozotocin (STZ) solution and dosages were correspondingly made following Ghasemi *et al.*.[37] 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).[37] 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).[35] 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%.

Rat Models with Diabetes Mellitus type 2

Induction procedure of type-2 diabetes mellitus in rats referred to Ghasemi *et al.* (2014).[37] 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.

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 via intraperitoneal. Nicotinamide was given 15 mins early before streptozotocin. Next, on the day 3 the second blood draw was performed to investigate blood glucose levels after injections. According to Ghasemi *et al.*(2014).[37] rats considered to have type 2 diabetic condition are when the blood glucose levels > 250 mg/dl. In this study, all of blood glucose levels in the rats after injections were above the normal rate of 200 mg/dl. Hence, this study was then continued to the next steps of the extraction administration for 14 days consecutively from the day 4 to 17. On the day 18, the last blood draw was performed to determine blood glucose and malondialdehyde levels after the treatments. All rats were prior fasted for ±12-15h when each blood draw implemented.

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 serums were parted, the serum separation was done by adding *glucoses* 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 Subtance. 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.[38,39] Longjack or pasak bumi is a medicinal plant used in herbal medicine because of the potential properties, such as antidiabetic.[23] Methanol extract of the longjack root was reported to have compounds of phenol, flavonoid, heart glycosides, protein, and terpenoid.[27] The use of longjack is more concentrated on the root, while the leaf is still rare to be used.[14,26,27] Therefore, this study was focused to evaluate the antidiabetic activity of ethanol extract of longjack leaf in streptozotocin-nicotinamide induced rats. According to Mahajan *et al.* (2020)[10]; Szkudelski (2012)[40] the streptozotocin administration was reported to damage pancreatic β cells by forming free radicals, similarly nature impairments occur in diabetes. Moreover, the decline of blood glucose levels made malondialdehyde levels also decreased.[12] Table 1 and 2 displayed the test results of antidiabetic activity of ethanol extract of longjack in STZ-NA induced rats.

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.[41] 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[13] and injuries in tissue cells.[42] Other study results also proved that species oxyegn reactive plays a main role in the pathogenesis of type-2 diabetes mellitus.[10,40] On the other side, the escalation of malondialdehyde levels is a result of the rise of ROS production so that malondialdehyde becomes one of the probes to identify the oxidative stress in cells. The measurement of malondialdehyde is not only through plasma and serum, but also from various tissues in kidney tissues in patients with diabetes.[9]

According to Hidayaturrahmah *et al.* (2020)[43] blood glucose levels in normal rats ranged from 50-135 mg/dl.[43] 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)[37] it stated that if blood glucose levels in rats >250 mg/dl after STZ-NA injection indicating all rats had type 2 diabetes mellitus.[37] 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.[37] 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.[37] 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

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	$66.36^{a} \pm 1.42$	$67.54^{a} \pm 1.23$	$78.73^{a} \pm 7.20$
CMC-Na 5% dose 2 ml/200 g body weight	$66.29^{a} \pm 1.87$	$277.19^{b} \pm 3.01$	$285.84^{c} \pm 17.56$
Ethanol Extract of Longjack Leaf dose 176.4 mg/200 g body weight	$68.40^{a} \pm 1.71$	$281.04^{c} \pm 1.79$	$156.77^{b} \pm 8.49$
Glibenclamide dose 0.09 mg/200 g body weight	$67.16^{a} \pm 2.27$	$279.28^{b,c} \pm 3.30$	$148.63^{b} \pm 23.81$

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	$1.13^{a} \pm 0.22$
CMC-Na 5% dose 2 ml/200 g body weight	$10.03^{c} \pm 0.34$
Ethanol Extract of Longjack Leaf dose 176.4 mg/200 g body weight	$3.86^{b} \pm 0.71$
Glibenclamide dose 0.09 mg/200 g body weight	$3.64^{b} \pm 1.77$

Information: * Same letters shown not significantly different on the *Duncan* test at 5%

Numbers shown after ± demonstrated SD (Standard Deviation)

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)[44] the normal levels of malondialdehyde are 1.09-1.64 nmol/ml.[44] Malondialdehyde is a product of lipid peroxidation generally used as an indicator of the occurrence of oxidative stress.[45] 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)[46] disclosed that streptozotocin can advance lipid peroxidation and malondialdehyde level.[46] 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 oxidative is a product of the imbalance. Stress oxidative can occur due to free radical upsurge or the decline of antioxidant activity, nor can be both.[8] On the group with CMC-Na administration, the blood glucose levels elevated joining with the highest level of malondialdehyde at 10.03 nmol/ml. Unfortunately, CMC-Na does not have any potential compounds that can reduce both blood glucose and malondialdehyde levels. Fitriana *et al.* (2017)[46] stated that streptozotocin will improve lipid peroxidation and malondialdehyde level, while Dewi *et al.* (2016)[47] confirmed that CMC-Na has also no beneficial substances that are capable of overcoming streptozotocin effects. On the groups with the administration of glibenclamide experienced the reduction of blood glucose and malondialdehyde levels. Glibenclamide is reported to have stimulus effects on pancreatic β cells to secrete insulin so that it can downgrade blood glucose rates.[48] Furthermore, on the group with the administration of longjack ethanol extract had an activity of protection for the body from free radical attacks caused by streptozotocin. This was noticeable of the decrease of blood glucose and malondialdehyde levels. Khanam *et al.* (2014)[27] revealed that the longjack root has the contents of phenolate, flavonoid, and terpenoid. Moreover, Panjaitan *et al.* (2020)[33] also disclosed that the ethanol extract of longjack stalks has the contents of alkaloid, terpenoid, saponin, tannin, and phenol. All metabolite secondary contents in various organs of the plant are reported to have antioxidant properties. Flavonoid is reported to have an ideal structure as an antioxidant to block free radicals. The structure is combined with the group of phenol which is composed by more than one aromatic and hydroxyl groups as well as conjugated double bonds where the structure is required in capturing free radicals.[49] Flavonoid can also provide beneficial effects for diabetes by motivating insulin secretion and the proliferation of pancreatic β cells, lessening apoptosis, treating hyperglycaemia through the management of glucose metabolism in hepatocytes, diminishing insulin resistance, inflammation, and oxidative stress in muscles and lipids, as well as increasing the glucose absorption in skeletal muscles and white adipose tissues.[41] Flavonoid also has a potential property in stabilising the products of the regeneration process of peroxyl and hydroxyl radicals. Stability of phenoxy radicals is believed to slow the speed of propagation reaction on the process of lipid auto-oxidation.[44] Meanwhile, alkaloid is believed to reduce blood glucose level.[50,51,52] Therefore, the results of this study suggested the antidiabetic effect of ethanol extract of longjack assessed by the levels of blood glucose and malondialdehyde. 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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GRAPHICAL ABSTRACT

Longjack (*Eurycoma longifolia* Jack.)

Extracted

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	$66.36^{a} \pm 1.42$	$67.54^{a} \pm 1.23$	$78.73^{a} \pm 7.20$
CMC-Na 5% dose 2 ml/200 g body weight	$66.29^{a} \pm 1.87$	$277.19^{b} \pm 3.01$	$285.84^{c} \pm 17.56$
Ethanol Extract of Longjack Leaf dose 176.4 mg/200 g body weight	$68.40^{a} \pm 1.71$	$281.04^{c} \pm 1.79$	$156.77^{b} \pm 8.49$
Glibenclamide dose 0.09 mg/200 g body weight	$67.16^{a} \pm 2.27$	$279.28^{bc} \pm 3.30$	$148.63^{b} \pm 23.81$

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	$1.13^{a} \pm 0.22$
CMC-Na 5% dose 2 ml/200 g body weight	$10.03^{c} \pm 0.34$
Ethanol Extract of Longjack Leaf dose 176.4 mg/200 g body weight	$3.86^{b} \pm 0.71$
Glibenclamide dose 0.09 mg/200 g body weight	$3.64^{b} \pm 1.77$

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

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