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Research Article
Effects of *Tectona grandis* L. Extract in Diabetic Rats on Nitric Oxide and Malondialdehyde Levels

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Abstract

Background and Objective: Diabetes mellitus is characterized by hyperglycemia caused by disturbances in pancreatic β cells resulting in the formation of reactive oxygen species which in this study was characterized by increased levels of nitric oxide and malondialdehyde in male Wistar rats with diabetes mellitus models. **Materials and Methods:** Animal modeling of diabetes mellitus using 40 mg kg^{-1} b.wt. streptozotocin intraperitoneally. The antioxidant activity is based on the ferric reducing antioxidant power (FRAP) method. Determination of nitric oxide and MDA levels using the Griess method and Thiobarbituric Acid Reactive Substances (TBARS) Assay, respectively. The animal models were divided into six treatment groups, normal control (KN), positive control (K^+) (glibenclamide), negative control (K^-) (Na-CMC 0.5%), teak leaf ethanol extract group at a dose of D_1 (100 mg kg^{-1} b.wt.), D_2 (200 mg kg^{-1} b.wt.) and D_3 (300 mg kg^{-1} b.wt.). **Results:** The antioxidant activity showed that the IC_{50} values of vitamin C and the ethanol extract of *Tectona grandis* leaves were 18.208 and 62.236 µg mL^{-1}, respectively. The NO levels in KN = 83.133 µmol L^{-1}, K^+ = 118.300 µmol L^{-1}, K^- = 317.467 µmol L^{-1}, D_1 = 210.133 µmol L^{-1}, D_2 = 184.467 µmol L^{-1} and D_3 = 129.300 µmol L^{-1}. The MDA levels at KN = 3.767 µmol L^{-1}, K^+ = 8.854 µmol L^{-1}, K^- = 31.032 µmol L^{-1}, D_1 = 27.010 µmol L^{-1}, D_2 = 20.166 µmol L^{-1} and D_3 = 15.512 µmol L^{-1}. **Conclusion:** The ethanol extract of teak leaves *Tectona grandis* L. at a dose of 300 mg kg^{-1} reduces nitric oxide levels and plasma malondialdehyde levels in rats through the activity of antioxidant compounds.

Key words: Antioxidant, blood glucose, nitric oxide, malondialdehyde, *Tectona grandis* L., diabetes mellitus (DM)

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder that causes hyperglycemia due to insulin deficiency, insulin resistance or both[1]. According to the International Diabetes Federation (IDF), the global number of people with diabetes mellitus will be around 578 million in 2030 and 700 million in 2045[2], with the number of DM sufferers in Southeast Asia expected to rise to 88 million in 2019, 115 million in 2030 and 153 million in 2045[2] while, the number of DM sufferers in Southeast Asia is estimated to continue to increase to 88 million people in 2019, 115 million people in 2030 and 153 million people in 2045. Diabetes mellitus type II shows the highest prevalence in the world[3]. The enhancement of DM cases also occurs in Indonesia, where Indonesia is the 7th country with the high prevalence of diabetes mellitus in the world, with 10 million people. Cases of diabetes mellitus rank 4th out of 10 non-communicable diseases (PTM) in Indonesia with a prevalence of 1.5%[2]. Data from the Central Statistics Agency show that DM in Sulawesi is in 5th place out of the ten most common diseases, with 2436 cases[4].

Uncontrolled hyperglycemia conditions lead to insulin resistance and impaired secretion in pancreatic β cells, resulting in the formation of reactive oxygen species (ROS), which can lead to enhance the oxidative stress inside the body. Oxidative stress refers to the imbalance between free radicals and antioxidants that leads cellular injury to the body's organs[5]. This condition can be caused by the formation of ROS exceeding its metabolic capacity[6]. The enhancement of reactive oxygen species (ROS) production also causes endothelial damage, thereby inhibiting the Endothelial Nitric Oxide Synthase (eNOS) process and decreasing nitric oxide (NO) production. This is the cause of microvascular complications that often occur in type II DM, including retinopathy, nephropathy and neuropathy, which are the main causes of blindness, end-stage renal disease[7]. Nitric oxide (NO) is formed by endothelial nitric oxide synthase (eNOS), which plays a crucial role in endothelial homeostasis as a vasodilator. This endothelial damage can be seen from the decreased nitric oxide (NO) levels in the endothelial tissue[8]. In addition, oxidative stress can affect the peroxidation of fatty acids or lipids, which causes the formation and increased levels of the compound malondialdehyde (MDA). The MDA originates from two different sources: The food intake and lipid peroxidation in the tissues. The generation of MDA on a scale and the rate of lipid oxidation in tissues are affected by two factors, endogenous and exogenous influences[9,10].

Antioxidants are chemical components that can neutralize free radicals. Antioxidants can protect the body against damage caused by reactive oxygen, inhibit degenerative diseases such as DM and prevent complications. There are two kinds of antioxidants: Endogenous antioxidants produced by the body and exogenous antioxidants from outside the body. If used for a long time, synthetic endogenous antioxidants such as vitamins A, D and E are reported to cause side effects[11]. Therefore, exploring natural sources of antioxidants in plants continues to be carried out. One of the powerful antioxidant compounds is flavonoids[12,13].

Tectona grandis is a plant that has the potential to be developed as an antidiabetic drug and free radical prevention. This plant contains saponins, carbohydrates, cardiac glycosides, steroids, triterpenes, alkaloids, tannins, phenolics, flavonoids, proteins and amino acids. Traditionally *Tectona grandis* has been used to treat diabetes, lipid disorders, inflammation, ulcers and bronchitis. In a different study, the ethanol extract of the bark and the methanol extract of the leaves of *Tectona grandis* showed antidiabetic and antioxidant activity[14-17]. Exploratory research on the pharmacological activity of *Tectona grandis* is still limited. In a previous study, we showed that the ethanol extract of *Tectona grandis* leaves had antidiabetic activity at an effective dose of 200 mg kg^{-1} b.wt.[18]. Therefore, in this study, an *in vitro* antioxidant follow-up test was carried out using the FRAP method, determination of NO and MDA levels from blood samples of tested animals with diabetes.

MATERIALS AND METHODS

Study area: This research was carried out in 2021 in the laboratories of the Faculty of Pharmacy, Haluoleo University and the Faculty of Medicine, Haluoleo University.

Materials: The materials used in this study was the teak leaves *Tectona grandis* L., 1, 1, 3, 3-tetramethoxypropane (TMP) (sigma®), distilled water, 70% alcohol (Bratachem®), 1 N HCl (Merck®), 96% ethanol (Bratachem®), 1% $FeCl_3$ (Merck®), glibenclamide 5 mg, chloroform (Merck®), Na-CMC 0.5% (Bratachem®), rat feed, dragendorff reagent (Bratachem®), liebermann-burchard reagent (Bratachem®), magnesium powder (Bratachem®), streptozotocin (STZ) (sigma®), thiobarbituric acid (TBA) 1% (sigma®), white rat (Rattus norvegicus), trichloroacetic acid (TCA) 10% (sigma®), glacial acetic acid (Bratachem®), concentrated H_2SO_4 (Merck®), NaOH (Merck®), 0.1 M sodium citrate buffer (Merck®), N-(1-naphthyl) ethylenediamine dihydrochloride (Bratachem®), sulfanilamide (Merck®), orthophosphoric acid (Merck®).

Methods

Extraction: The leaves of *Tectona grandis* L. were taken from Danagoa Village, Tongkuno District, Muna Regency, Southeast Sulawesi. Samples were prepared into dry simplicia and ground into powder. Simplicia powder (4 kg) was macerated using ethanol as a solvent. Macerate was concentrated using a Rotary Vacuum Evaporator (Stuart RE300P®, United Kingdom) at a temperature of 40°C until a thick extract was obtained and the yield value was calculated.

Antioxidant assay: The antioxidant activity of the ethanol extract of *Tectona grandis* was carried out using the ferric reducing antioxidant power (FRAP) method with slight modification[19]. The standard solution used is ascorbic acid. The ethanol extract (25 mg) was dissolved in 25 mL ethanol and then homogenized to make a stock solution of 1000 ppm. Sample solutions were taken, respectively, 3, 2, 1, 0.5 and 0.1 mL from a 1000 ppm stock solution, placed in a test tube and the volume was made up to 10 mL using ethanol, homogenized. The extract and standard were made into a 1000 ppm stock solution with five concentration variants, namely 10, 50, 100, 200 and 300 ppm. Pipette (Eppendorf Research®, Germany) 1 mL each, add 1 mL of 0.2 m phosphate buffer (pH 6.6) and After that, 1 mL of 1% potassium ferricyanide was incubated for 20 min at 50°C. After incubation, 1 mL of 10% TCA was added and centrifuged at 3000 rpm for 10 min. After centrifugation, 1 mL of the supernatants were injected into a test tube, followed by 1 mL of sterile water and 0.5 mL of 0.1% ferric chloride. The solution stand for 10 min, then the absorbance of the solution was measured using an ultraviolet-visible (UV-Vis) spectrophotometer (Techcomp UV2500 UV-Vis Spectrophotometer®, Thailand) at 720 nm. The antioxidant activity of the extract was expressed in terms of its inhibition percentage and the IC_{50} value.

Animal modelling of diabetes mellitus: Diabetic animal modelling follows the method described by Nuralifah *et al*.[18]. The animal used is the *Rattus norvegicus* rat. Animals were acclimatized and given standard pellets and drinks. The ethical clearance for animal subject use was approved by the committee on health research ethics of the institution of research and community service, Universitas Haluoleo, Kendari (ethical clearance certificate number: 1298/UN29.20/PPM/2022). Briefly, the animal model was fasted for 24 hrs and then given an injection of 40 mg kg^{-1} b.wt., streptozotocin in 0.1 M sodium citrate buffer pH 4.0 once and given 10% sucrose solution[20]. Blood glucose levels were measured before treatment, after induction and after treatment. Animals with blood glucose levels >150 mg dL^{-1} were used as diabetic animals for further determination. Animals were divided into 6 groups, with four animals in each group. The animal group consisted of the normal control group, the positive control group received 5 mg of glibenclamide, the negative control (NaCMC 0.5%) and the three dose groups of teak leaf ethanol extract (*Tectona grandis* L.), D_1 (dose of 100 mg kg^{-1} b.wt.), D_2 (200 mg kg^{-1} b.wt.) and D_3 (300 mg kg^{-1} b .wt.). The animal treatment was carried out for seven days and on the 8th day, euthanasia was carried out to take the animal's blood through the heart vein and it was accommodated in an EDTA tube. Blood was centrifuged for 15 min at 3000 rpm. The supernatant was taken and put into an Eppendorf tube and frozen at -20°C for further testing of NO and MDA levels.

Determination of nitric oxide (NO) and malondialdehyde (MDA) levels of diabetic animal models: Plasma nitric oxide (NO) levels were measured using the Griess method with modifications[21]. A total of 1 mL of plasma sample was pipetted and put into a 50 mL measuring flask, then 2.5 mL of griess A solution was added and shaken. After 5 min, another 2.5 mL of griess B solution was added, shaken and sufficient with distilled water on the measuring flask, then homogenized. Nitric oxide levels were measured using a UV-vis spectrophotometer at 540 nm.

The MDA levels were measured using the Thiobarbituric Acid Reactive Substances (TBARS) Assay method with modifications[22]. A total of 1.0 mL of plasma sample was pipetted and put into an Eppendorf tube, added 1.0 mL of TCA (trichloroacetic acid) solution and 1.0 mL of TBA (thioBarbituric acid) solution, then homogenized. The homogeneous mixture was heated at 95°C for 15 min using a water bath and then cooled to room temperature. After that, the solution was centrifuged at 3000 rpm for 10 min. The absorbance of the pink supernatant was measured using a UV-Vis spectrophotometer at a wavelength of 532 nm.

Statistical analysis: Data on levels of nitric oxide (NO) and malondialdehyde (MDA) were first tested for normality and homogeneity, then statistically analyzed using one way ANOVA IBM SPSS Statistics 24 application which had a significant difference ($p<0.05$) so that further tests were carried out using the *post hoc* test least significant difference (LSD).

RESULTS

Testing the antioxidant activity of the ethanol extract of teak leaves was carried out using the ferric reducing antioxidant power (FRAP) method. The antioxidant activity of the extract was expressed in terms of its inhibition percentage in inhibiting free radicals. This inhibition percentage was obtained from the difference in absorbance absorption in the sample as measured by a UV-Vis spectrophotometer at the maximum wavelength of 720 nm. The results of the percent inhibition of teak leaf ethanol extract can be seen in Fig. 1.

Modeling of test animals with induced intraperitoneal Streptozotocin at a dose of 40 mg kg^{-1} b.wt. Increased blood glucose levels were observed 2-4 days after STZ induction. It is expected that the blood glucose level of rat test animals is 150 mg dL^{-1} or more so they are said to have DM. Increased blood sugar levels can be observed in the average blood glucose levels before and after STZ induction and the decrease in glucose levels after treatment was shown in Fig. 2.

Type 2 diabetes mellitus is characterized by increased blood glucose levels or hyperglycemia. Hyperglycemic conditions cause insulin resistance and impaired secretion in pancreatic β cells so that they can increase the production of free radicals, such as reactive oxygen species (ROS). The ROS will exacerbate oxidative stress and result in insulin resistance. Increased activity of free radicals in the body, characterized by increased levels of MDA and NO. The MDA and NO levels in rat test animals after administration of teak leaf ethanol extract can be seen in Table 1.

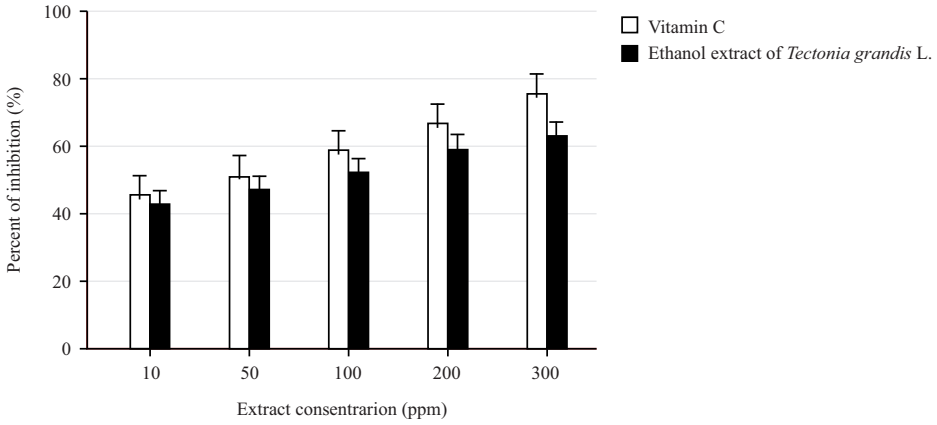

Fig. 1: Percent inhibition (%) of vitamin C and ethanol extract of *Tectona grandis* using the FRAP method

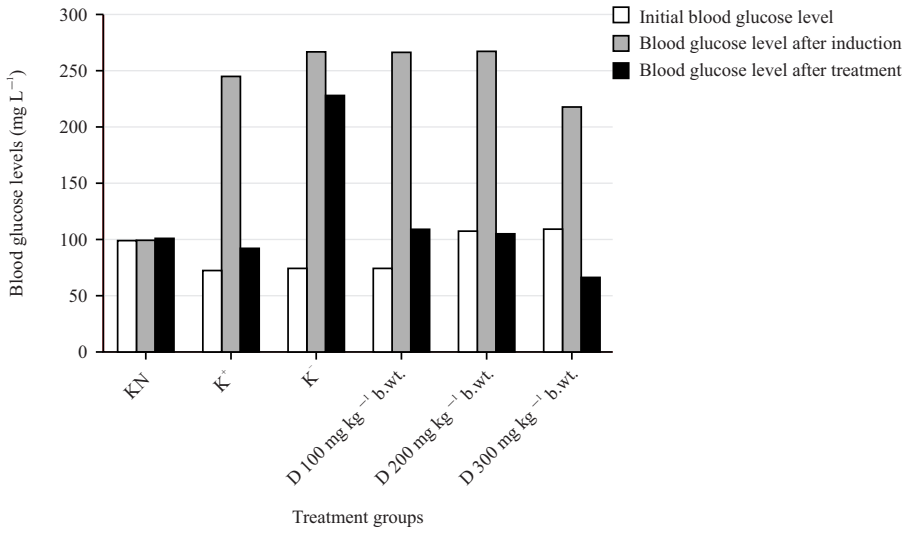

Fig. 2: Mean of blood glucose levels in the treatment group

Table 1: Average levels of nitric oxide (NO) and malondialdehyde (MO)

Group of test animals	Mean±SD NO levels (μmol L^{-1})	Mean±SD MDA levels (μmol L^{-1})
Normal control	83.133±0.710	3.767±0.130
Positive control	118.300±0.513	8.854±0.313
Negative control	317.467±0.852	31.032±0.016
Extract dosage 100 mg kg^{-1} BB	210.133±0.351	27.010±0.850
Extract dosage 200 mg kg^{-1} BB	184.467±0.483	20.166±0.798
Extract dosage 300 mg kg^{-1} BB	129.300±0.686	15.512±0.596

Significance was measured at ($p<0.05$)

DISCUSSION

Tectona grandis L. is a potential plant to be further developed as an herb with antidiabetic activity. Previous research has shown the effectiveness of this plant in reducing blood glucose levels in test animals. However, no studies have conducted the determination of NO and MDA levels. Therefore, in this study, NO and MDA levels were determined in diabetic animals that had been given extract therapy. This research began with preparing *Tectona grandis* L. ethanolic extract using the maceration method to obtain the crude extract. The percent yield of crude extract was 6.17%. The extract was then used for in vitro antioxidant assay using the FRAP technique. The FRAP was chosen because the process is easy, inexpensive and quick and the reagents are simple, do not need specific equipment and are highly reproducible[23,24]. The results of the antioxidant activity test showed that the IC50 values of vitamin C and the ethanol extract of *Tectona grandis* leaves were 18.208 and 62.236 μg mL^{-1}, respectively. The ability to neutralize oxidants from the ethanol extract of *Tectona grandis* is included in the strong antioxidant[25], with percent inhibition concentrations of 10, 50, 100, 200 and 300 ppm, are 44.549, 49.121, 54.396, 61.430 and 65.299%, respectively. The antioxidant activity of the ethanol extract of *Tectona grandis* in Fig. 1 shows an increment percentage of inhibition as the concentration rises. The greatest oxidant inhibition occurs at a concentration of 300 ppm.

Diabetic animal modelling was carried out using streptozotocin (STZ) induction and blood glucose levels measurements before, after and after treatment. The results showed the induction was successful. All groups had blood glucose levels >150 mg dL^{-1} (Fig. 2). The animals were then given treatment according to the group and blood glucose levels were measured again. The results show that the extract can lower blood glucose levels depending on the dose. The largest reduction in blood glucose levels was in the 300 mg kg^{-1} b.wt., group and the decrease was greater than that of the Glibenclamide positive control (Fig. 2).

The mean plasma NO levels in the control group (Table 1) were within the range of normal plasma NO levels of about 25-45 μmol L^{-1} [26]. This indicates that the plasma NO level formed is still physiological (has not been influenced by anything) and is still balanced (the body's normal condition). Likewise, in the positive control group, the mean plasma NO levels were also in the range of normal plasma NO levels. It was shown that glibenclamide plays a role in influencing plasma NO levels by reducing the amount of ROS (reactive oxygen species) production[27]. Meanwhile, the mean plasma NO levels in the negative control group exceeded the range of normal NO levels. This indicates that STZ increases the NO levels, which can damage pancreatic β cells by producing ROS[28]. The mean of plasma NO levels in each group showed that between the negative control group and three extracts, the 100, 200 and 300 mg kg^{-1} b.wt., doses had significant differences ($p<0.05$), this indicated that the three extract dose groups had different effectiveness from the negative control group on plasma NO levels. The 300 mg kg^{-1} b.wt., group showed the same effectiveness ($p>0.05$) as the positive control group.

The effectiveness of the extract in reducing the level of NO and MDA levels is caused by its chemical compound. Chemical compounds that are suspected as antidiabetic are flavonoids. Flavonoids are polyphenol compounds that can prevent and protect against damage to pancreatic β cells due to free radicals. These flavonoids can stimulate insulin secretion, thereby increasing the repair of pancreatic β cells so that complications can be prevented in patients with degenerative diseases such as diabetes mellitus associated with oxidative stress. There is an antioxidant mechanism in the ethanol extract of teak leaves which fights oxidative stress, which will result in a decrease in ROS or free radical levels[29,30].

The average plasma MDA level in the normal control group (Table 1) was included in the normal MDA level, which was less than 4 μmol L^{-1}. The normal control group had lower plasma MDA levels than the treatment control group because the plasma MDA levels formed were still physiologically unaffected and antioxidants were still able to neutralize them

under normal body conditions. The mean plasma MDA levels of the negative control group had a significant difference from the normal control group because after induction STZ was able to increase lipid peroxidation and also can increase the MDA levels. This high MDA level indicates a high amount of free radicals in the body. Meanwhile, the positive control group showed the mean plasma MDA levels from the negative control group. This indicated that glibenclamide might reduce the plasma MDA levels[31,32]. The mean plasma MDA levels in the ethanol extract group showed a significant difference ($p<0.05$) between the positive and negative control groups. The 300 mg kg^{-1} b.wt., extract showed lower MDA levels than the 100 and 200 mg kg^{-1} b.wt., extracts. However, it was not as effective as the positive control in reducing MDA levels. Based on this, MDA and diabetes mellitus are interrelated. Hyperglycemia conditions that occur in DM sufferers will result in increased production of free radicals, which will cause oxidative stress. Oxidative stress can cause lipid peroxidation, resulting in damage to cell membranes, which can be indicated by increased plasma levels and tissue malondialdehyde (MDA). Malondialdehyde is formed from lipid peroxidation in cell membranes, namely the reaction of free radicals (hydroxyl radicals) with Poly Unsaturated Fatty Acids (PUFA). Therefore, an increase in MDA indicates a fat peroxidation process which has a high potential to cause microvascular and macrovascular complications in DM patients[33,34].

Blood glucose that increases acutely or chronically will cause oxidative stress. Oxidative stress causes an increase in malondialdehyde (MDA) levels and decreases blood levels of nitrate and nitrite (NOx) as an intermediate metabolite of nitric oxide (NO). The results of this study were in line with the 2020 research by Serang and Hammi[29] that giving antioxidants is proven to reduce MDA levels. Antioxidants can also reduce MDA in a direct way, by capturing ROS directly and indirectly by inducing antioxidant enzymes, inhibiting pro-oxidant enzymes and producing phase II detoxification enzymes and antioxidant enzymes[29,34,35]. Nunes *et al.*[36] investigated glibenclamide increases nitric oxide levels and decreases oxidative stress in an *in vitro* model of preeclampsia. Glibenclamide administration had a positive effect on production in reducing oxidative stress and increasing antioxidant capacity[36].

The implication of current research results was to provide scientific information to the public regarding the efficacy of *Tectona grandis* L., leaves, which can be applied as an herbal medicinal plant. The limitation of this research is that only 1 type of animal test and modeling is used so that this research can be developed using several fractions with other modeling test animals such as hepatoprotectors.

CONCLUSION

The content of secondary metabolites contained in the leaves of *Tectona grandis* L. are alkaloids, flavonoids, tannins, terpenoids and saponins. The ethanol extract of *Tectona grandis* leaves has strong antioxidant activity which is effective in reducing plasma levels of NO and MDA in male Wistar rats with diabetes mellitus type II. Therefore, this plant is expected to be a candidate plant that can be used by people with diabetes mellitus and can prevent vascular complications related to NO and MDA levels.

SIGNIFICANCE STATEMENT

This study found that the ethanol extract Teak leaves (*Tectona grandis* L.) have strong antioxidant activity and are able to reduce blood glucose levels with parameters of nitric oxide (NO) and malondialdehyde (MDA) levels. This research will be a reference for further development to make teak leaves a traditional medicine in the treatment of type 2 diabetes mellitus.

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