



The Effect of Liposomes Ethanol Extract of Tekelan Leaves (*Chromolaena odorata* L.) on the SOD and MDA Level in Streptozotocin-Nicotinamide-Induced Diabetic Rats

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Abstract

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BACKGROUND: Oxidative stress occurs when the quantity of free radical molecules in the body exceeds the number of antioxidant molecules. Tekelan leaf (*Chromolaena odorata* L.) is an *Asteraceae* plant with antioxidant activity that inhibits an oxidative stress. According to the chemical component contained in Tekelan leaves, components such as flavones, flavonols, flavonones, chalcones, and hydroxy benzoic acid, are responsible for the antioxidant properties. Tekelan leaf ethanol extract liposomes are one of the preparations that can improve the level antioxidant activity of the product, in this study, the activity of liposomes ethanol extract of Tekelan leaves (*Chromolaena odorata* L.) is tested to male rats which induced by streptozotocin-nicotinamide and calculate the level of SOD and MDA.

AIM: The purpose of the study was to determine the activity of liposomes preparation of Tekelan leaf ethanol extract in male rats induced by STZ-nicotinamide by calculating MDA and SOD levels.

METHODS: Rats were given nicotinamide at a dose of 24 mg/200 g BW and streptozotocin at a dose of 12 mg/200 g BW at day 1, then continue to liposome and extract administration at doses of 20 mg/200 g BW, 40 mg/200 g BW, and 80 mg/200 g BW, respectively, for 15 days. At day 16, the blood were drawn and SOD and MDA levels were analyzed using a spectrophotometer.

RESULTS: The MDA level for liposome preparation at a dose of 80 mg/200 g BW was substantially lower (9.81 ± 0.08) than negative control group (15.94 ± 0.45). Similar result also reported in SOD level that liposome preparation at a dose of 80 mg/200 g BW (89.33 ± 0.30) is higher than negative control group (65.55 ± 0.30).

CONCLUSION: It concluded that the liposomes preparation of the ethanol extract of Tekelan leaves showed higher antioxidant activity than extract preparation, it showed a dose-dependent manner.

Introduction

Oxidative stress occurs when the quantity of free radical molecules in the body is not balanced with the number of antioxidant molecules in the body [1]. Body cells such as cells in the liver and kidneys can be damaged by free radicals [2]. Furthermore, free radicals may injure endothelial cells in the body including the pancreatic injury [3]. In rodent model, the pancreas will necrotize because of streptozotocin-induced diabetes. Streptozotocin enters pancreatic cells through the glucose transporter-2 (GLUT-2) enzyme and only damaging these cells [4].

Poly-ADP-ribosylase is activated when a cell's deoxyribose nucleic acid (DNA) is damaged, and free radicals are created, which can injure pancreatic cells. Nicotinamide, a B3 vitamin that acts as a precursor to the coenzyme nicotinamide adenosine dinucleotide (NAD), has been shown to reduce apoptosis [5]. When streptozotocin and nicotinamide are combined,

they can produce an insulin insufficiency model rather than an insulin resistance model. Antioxidants are substances that have the ability to delay or stop the oxidation of other molecules. Antioxidants prevent chain reactions by compensating for the electron shortage of free radicals and suppressing further oxidation reactions by being oxidized [6]. One of the plants that potential as antioxidant agent is Tekelan leaf (*Chromolaena odorata* L.).

Tekelan leaf (*Chromolaena odorata* L.) is an *Asteraceae* plant with antioxidant qualities that aid in the prevention of oxidation [7]. Antioxidants contained in Tekelan leaves can stabilize free radicals by transferring one or more electrons to them, effectively stopping the oxidation process [8]. The chemical components contained in Tekelan leaves, such as flavones, flavonols, flavonones, chalcones, and hydroxy benzoic acid, are responsible for the antioxidant benefits. In addition to flavonoids (aurone, chalcone, flavones, and flavonols), alkaloids, tannins, saponins, and other phenolic compounds act as antioxidants [9].

One of the preparation techniques to increase the activity of the substance is liposome preparation. Liposomes are a terrific technique to delivering more soluble and penetrate the substrate [10]. Liposomes have both hydrophilic and lipophilic features, which allow hydrophilic molecules to be trapped in the water core and lipophilic molecules in the lipid bilayer because it was easy to manufacture and have a high solubilization capacity [11]. Because of their phospholipid makeup, liposomes are biocompatible, biodegradable, and non-immunogenic (which is similar to cell membranes). Liposomes contain pharmaceuticals (active molecules) with the purpose to boosting solubility, reducing side effects, extending release, protecting medications, targeting drugs, and increasing efficacy [12]. In this research, the Tekelan leaf ethanol extract was designed into liposome preparation and determine the MDA and SOD levels in male rats caused by STZ-nicotinamide. Superoxide dismutase is an endogenous antioxidant that protects against free radicals by speeding up O₂- dismutation and limiting the amount of O₂- produced [1]. Malondialdehyde, or MDA, is a chemical that indicates oxidative stress in the body. MDA is the result of radical chemicals attacking polyunsaturated fatty acids with at least three double bonds in lipid membranes, causing lipid peroxidation [13].

Materials and Methods

Materials

The materials used in this study were Tekelan leaf that collected from Deli Tua Village (Deli Serdang Regency of Indonesia), ethanol 96% (Sigma), lipoid S75 (Sigma), lipoid DMPG-Na (Sigma), cholesterol (Sigma), Span 60, methanol p.a (Sigma), chloroform p.a (Sigma), trichloro acid (Supelco), heparin sodium (Inviclot), glibenclamide (Pratapa Nirmala), liposomes ethanol extract of Tekelan leaves, formalin (Segera Husada Medica), phosphate buffer saline (Max Lab), BTA (Ecosol), and TEP (Soho).

Extraction and phytochemical contents identification tekelan leaf ethanol extract

Phytochemical screening carried out on ethanol extract beetroot included examining the secondary chemical metabolites of alkaloids, flavonoids, glycosides, tannins, saponins, terpenoids, and steroids. A total of 2 kg of Tekelan leaf powder was extracted using 15,000 ml of 96% ethanol as the solvent by maceration method. Allow to sit for 5 days in a dark place, stirring occasionally. The pulp is pressed with a flannel cloth and then filtered using filter paper after 5 days of screening. The dregs were rinsed with

the remaining 5000 ml of ethanol solvent before being refiltered. A rotary evaporator was used to evaporate the solvent at a temperature of 50°C until a thick extract was obtained [14].

Preparation of liposomes by thin-layer hydration method

The preparations are divided into two mixtures in the formulation: A mixture (consisting of lipoid S75, lipoid DMPG-Na, cholesterol, and Span 60) and B mixture (consisting of methanol, chloroform, and ethanol extract of Tekelan leaves). Each material's specific gravity is used to compute its density. The entire amount of substance is 50 mmol. The extract was dissolved in methanol and chloroform in a concentration of 100 mg (5:5) concentration after being dissolved and homogenized in mixture. A rotary evaporator was used to evaporate the contents of a round tool flask, then heated to a temperature of 60°C at a pressure of 200 mBar for 1 hour, repeated it until a thin film formed on the round bottom flask's walls, and then added PBS to the flask. Extracted liposomes were purified using a PD 10 column, placed in a container, and shrunk using ultrasonic with a 30 min sonication duration [15].

Experimental animals

Twenty-seven healthy male white rats, aged 2–3 months and weighing 150–200 g, were used in the study. Rats were maintained for a week before treatment to allow them to acclimate the condition. Rats were treated after a week of adaptation. Rats were injected with 120 mg/kgBW of nicotinamide diluted in 0.9% NaCl after 1 week of adaptation and then continue to STZ induction at a dose of 60 mg/kgBW dissolved in citrate buffer pH 4.5 in 15 min later.

Treatment regime of liposomes preparation and ethanol extract of tekelan leaves

Animals were separated into nine groups after being stimulated by nicotinamide and STZ, then continue to liposome and extract administration at doses of 20 mg/200 g BW, 40 mg/200 g BW, and 80 mg/200 g BW, respectively, for 15 days. At day 16, the blood were drawn and SOD and MDA levels were analyzed using a spectrophotometer. Treatment groups were consisted of:

- Group 1 was normal group
- Group 2 CMC-Na 1%BB (Negative control group)
- Group 3 Extract at dose of 20 mg/200 g BW/day
- Group 4 Extract at dose of 40 mg/200 g BW/day
- Group 5 Extract at dose of 80 mg/200 g BW/day
- Group 6 Liposome at dose of 20 mg/200 g BW/day
- Group 7 Liposome at dose of 40 mg/200 g BW/day

- Group 8 Liposome at dose of 80 mg/200 g BW/day
- Group 9 Glibenclamide at dose of 0.09 mg/200 g BW/day.

MDA level measurement

A thiobarbituric acid reactive substance (TBARS) assay was used to determine MDA levels in this study. MDA generates a TBA-MDA bond complex based on the interaction between MDA and TBA, which gives a pink to reddish color, then determined using a spectrophotometer. The standard for testing MDA was tetraethoxypropane (TEP). The maximum absorption wavelength was obtained by diluting tetraethoxypropane (TEP) in 100 mL of distilled water and shaking (primary standard), primary standard solution was taken back with 100 mL of distilled water. Add 0.5 mL TCA and 1 mL TBA and read at a wavelength of 400–800 nm to produce a wavelength of 532 nm [16].

Standard curve preparation

Standard curves were created using a tetraethoxypropane standard solution (1/80,000 times dilution). A 30 L, 50 L, 70 L, 90 L, and 110 L were taken from these solutions. Fill each test tube halfway. A 0.5 mL of 20% TCA solution, 1 mL of 0.67% TBA solution were added to each tube and agitated homogeneously after adding up to 1 mL of distilled water. A 1 ml distilled water, 0.5 ml 20% TCA solution, and 1 ml 0.67% TBA solution were added to the blank standard solution and agitated until homogenous. A replica of the blank standard solution was created. All tubes were placed in a water bath at 95–100°C for 10 min before being cooled with running water. At a wavelength of 532 nm, the absorbance was measured. A calibration curve was created by linking the absorption value as coordinates (Y) and the concentration of the standard solution (nmol/ml) as abscissa (X) from the measurement data [16], [17].

Sample rate measurement

(a) A 1 ml of sample was placed in a test tube, along with 0.5 ml of 20% TCA, and vortexed until homogenous. It was then centrifuged at 3000 rpm for 10 min, and the supernatant was collected. (b) A 1 mL of 0.67 percent TBA was placed in the tube, which was then placed in a water bath at 95–100°C for 10 min before being cooled with running water. A spectrophotometer was used to detect the absorbance at a wavelength of 531.5 nm [16].

Calculation of MDA levels:

$$Y = a + bX$$

Information:

$$X = \text{MDA level}$$

$$Y = \text{sample absorption}$$

SOD level measurement

The ability of SOD to convert superoxide anion (O_2^-) into molecular hydrogen peroxide and oxygen is the basis for measuring its antioxidant activity. The rate of autoxidation in the presence and absence of samples containing McCord Fridovich “cytochrome c” units was used to determine SOD activity. The results were then read using an ELISA kit at a wavelength of 450 nm.

Data analysis

SPSS version 21.0 was used to analyze the research data. Homogeneity, normality, one-way ANOVA, and the Tukey test were used to examine the data.

Results and Discussion

Identification of phytochemical content of tekelan leaf ethanol extract

Results showed that phytochemical shown in Table 1.

Table 1: Identification of phytochemical content

Chemical compound identification results		
Compound	Powder	Extract
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Saponins	+	+
Steroids/triterpenoid	+	+
Glycoside	+	+

–: Negative, +: Positive.

MDA level

In this study, MDA levels were measured using the thiobarbituric acid reactive substance (TBARS) method, which can be quantified spectrophotometrically. A 532 nm was the wavelength employed in this experiment. The results of the MDA level measurements are shown in Table 2.

Table 2: MDA levels measurement results

Group	Mean MDA level (nmol/mL) ± SD	(p-value)
Normal	11.74 ± 1.99	0.000 0.104 ^a
CMC-Na	15.94 ± 0.45	0.000 1.000 ^{ab}
Glibenclamide 0.09 mg/200 g BB	11.34 ± 0.22	0.000 0.082 ^a 0.104 ^a
Extract 20 mg/200 g BB	13.12 ± 0.22	0.000 0.076 ^{ad}
Extract 40 mg/200 g BB	12.09 ± 0.35	0.000 0.076 ^{ad}
Extract 80 mg/200 g BB	11.19 ± 0.42	0.000 0.104 ^{ad}
Liposome 20 mg/200 g BB	10.99 ± 0.08	0.000 0.082 ^{ad}
Liposome 40 mg/200 g BB	10.39 ± 0.12	0.000 0.082 ^{ad}
Liposome 80 mg/200 g BB	9.81 ± 0.08	0.000 0.082 ^{ad}

^aSignificantly different to CMC-Na group, ^bsignificantly different to glibenclamide group, ^dsignificantly different to normal group.

MDA value for liposome preparation at a dose of 80 mg/200 g BW was substantially lower (9.81 ± 0.08) than the MDA value of negative control group (15.94 ± 0.45). The similar results are also reported in extract preparation. Malondialdehyde (MDA) is the end product of radical molecules attacking lipid membranes containing polyunsaturated fatty acids with at least three double bonds, resulting in lipid peroxidation [13], [18]. The high level of MDA is influenced by lipid peroxide, which indirectly influences the high number of free radicals. The ability of liposomes and extracts to scavenge free radicals is due to phytochemical content of flavonoids that found in Tekelan leaves, which play a role in preventing damage and repairing pancreatic cells in diabetes, it is also play role in reducing lipid peroxidation by slowing the onset of necrosis in cells and increasing vascularization so that cell damage and regeneration can be avoided [19], [20]. Liposome preparation showed greater activity compared to the extract preparation, it proved that liposome increases the activity of Tekelan leaves extract.

Superoxide dismutase (SOD) activity

The activity of SOD was measured in % suppression of the auto-oxidation processes of nicotinamide and streptozotocin. Because of dismutation, the presence of SOD inhibits the autoxidation reaction. The results of the percentage of SOD inhibition measurement are shown in Table 3.

Table 3: Percentage of SOD inhibition measurement results

Group	Inhibition \pm SD (%)	Significance (p)
Normal	92.07 ± 0.81	-
CMC-Na	65.55 ± 0.30	1.000 0.000 1.000 ^{bc}
Glibenclamide 0.09 mg/200 g BB	75.00 ± 0.30	0.000 0.106 ^{ac}
Extract 20 mg/200 g BB	70.73 ± 0.30	0.000 1.000 ^{ac}
Extract 40 mg/200 g BB	73.37 ± 0.47	0.000 0.106 ^c
Extract 80 mg/200 g BB	77.24 ± 0.47	0.000 1.000 ^{ac}
Liposome 20 mg/200 g BB	82.32 ± 0.30	0.000 0.934 ^{ac}
Liposome 40 mg/200 g BB	83.03 ± 0.47	0.000 0.934 ^{ac}
Liposome 80 mg/200 g BB	89.33 ± 0.30	0.000

^aSignificantly different to CMC-Na group; ^bsignificantly different to glibenclamide group; ^csignificantly different to normal group.

Liposome preparation at a dose of 80 mg/200 g BW had a substantially higher SOD value (89.33 ± 0.30) than negative control group (65.55 ± 0.30), the presence of flavonoid components in the form of quercetin, an endogenous antioxidant that fights free radicals, is most likely to blame free by accelerating O₂- dismutation and maintaining a healthy equilibrium of O₂- and formation. As demonstrated by a decrease in membrane lipid peroxidation activity and an increase in endogenous antioxidant activity, particularly the SOD enzyme, which absorbs superoxide anions and transforms them to hydrogen peroxide, liposomes and a Tekelan leaf ethanol extract has antioxidant activity [21], [22]. The

ability of an ethanol extract of Tekelan leaves to bind to liposomes results in the formation of a liposome cross-linker. Because its value can indicate the ability of the cross-linker to convey the active ingredient to the therapeutic target, liposome adsorption efficiency has an impact on the drug delivery system. The solubility of the medication in the matrix or polymer and the amount of polymer utilized determine the entrapment efficiency of a particle. The medicine is strongly adsorbed in the polymer/matrix since the ethanol extract of is freely soluble in it [23], [24].

Conclusion

Liposomes preparation of the ethanol extract of Tekelan leaves showed higher activity in MDA and SOD level compare than extract preparation, it showed a dose-dependent manner.

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