

Utilization of tekelan leaf extract (*Chromolaena odorata* L.) based on nanoliposome technology as treatment of antihyperglycemia

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Abstract. Metabolic disease that occurs due to abnormalities in insulin secretion and insulin action is said to be diabetes or hyperglycemia. One alternative that can be used for hyperglycemia management is to use tekelan leaves (*C. odorata* L.). The leaves of this tekelan plant have low water solubility and high permeability. A liposome is a good carrier to increase the solubility and penetration of the compounds it carries. This study aimed to develop a nanotechnological liposomal preparation from the ethanol extract of tekelan leaves as a hypoglycemic agent in diabetic male rats induced by nicotinamide 120 mg/kg BW and streptozotocin 60 mg/kg BW. Antihyperglycemia treatment was applied to nanoliposomes at a dose of 25 mg / kg BW, 125 mg / kg BW, 250 mg / kg BW, CMC-Na and 0.45 mg / kg BW of glibenclamide. The results obtained were at 12 'sonication time, the resulting particle size is 145.5 ± 2.8 and PI 0.307 ± 0.03 . The results of antihyperglycemia testing were obtained at a nanoliposome dose of 250 mg/kg BW produced the lowest blood glucose levels, namely, 86 ± 4.30 mg / dL.

1 Introduction

Metabolic disease that occurs due to abnormalities in insulin secretion and insulin action is said to be diabetes or hyperglycemia. In the world, about 425 million people have diabetes. The number will increase to 600 million people estimated by 2040 [1].

Hyperglycemia medication consumption often causes side effects. Research is often conducted to find drugs that are more effective and safer [2]. A traditional plant that can be used as alternative medicine is the leaves of tekelan (*C. odorata* L.). There are active compounds in tekelan leaves, namely flavonol 3'4'-dihydroxiflavone (quercetin), which has antioxidant, antiproliferative, anti-inflammatory, anti-diabetic, anti-carcinogenic, antihypertensive, antidiabetic, and protects against various types of diseases such as osteoporosis, certain forms of cancer, lungs, and heart, also against aging [3].

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The body can be protected from several types of degenerative diseases by preventing the occurrence of lipid peroxidation in the presence of quercetin [4]. However, quercetin has a weakness in terms of water solubility and high permeability. For that, we need technology to increase bioavailability so that the maximum therapeutic effect is obtained.

There is one type of drug delivery that is currently being developed. These types are liposomes. With the presence of liposomes, solubility can be increased, and the penetration of compounds in the patient's body can grow. Liposomes are formed from the interaction between hydrophilic-hydrophobic compounds or between phospholipids and water molecules [5].

The novelty of this research is the development of a liposome nanotechnology preparation of tekelan leaf ethanol extract as an antidiabetic which was tested on white male rats.

2 Research method

2.1 Research tools

The tools used in this study were glass tools (stirring rod, watch glass, glass beaker, measuring cup, turtle spoon), 500-1000 μ L micropipette, analytical scale (Metler Toledo), rotary evaporator (Heidolph), Transmission Electron Microscopy (JEM 1400, JEOL), Particle Size and Zeta potential (Horiba SZ 100), pH meter (Horiba), cabinet dryer, furnace (thermolyne, thermos scientific), pycnometer (pyrex), atomic absorption spectrophotometry (PerkinElmer, PnAAcle 900T), oven (Memmert), glucose stick (Aqu-Check), animal scales.

2.2 Research materials

The materials used in this study were tekelan leaves obtained from Deli Tua (North Sumatra), aquabidestilata (Ikapharmindo), nicotinamide (Sigma Aldrich), streptozotocin (Sigma Aldrich), Lipoid S75, Lipoid DMPG-Na, cholesterol (Sigma Aldrich), span-60, blue tip, microtubes, glucose (Sigma Aldrich), glibenclamide, NaCl (Sigma Aldrich), methanol (Merck), chloroform (Merck), and PBS (Phosphate Buffer Saline) (Merck).

2.3 Research stage

2.3.1 Preparation of tekelan leaf ethanol extract

Tekelan leaf powder obtained from refining tekelan leaves from the Deli Tua region, North Sumatra, was extracted using 1.5 L ethanol 96% by maceration method. After obtaining the liquid extract, it is concentrated with a rotary evaporator at a temperature of 50°C until a thick extract is obtained [6].

2.3.2 Phytochemical screening

Phytochemical screening was carried out to identify what metabolites are present in tekelan leaves. The phytochemical screening identified alkaloid compounds, flavonoids, tannins, steroids / triterpenoids, and glycosides [3].

2.4 Preparation of Liposome Extract Nanotechnology for Tekelan Leaves

The liposomes of the tekelan leaf ethanol extract were prepared using a thin layer hydration method. The liposome material was made in two mixtures, namely Mix A (consisting of S75 lipoids, DMPG-Na lipoids, cholesterol, and span 60). Mixture B (consisting of methanol, chloroform, ethanol extract of tekelan leaves). The two ingredients are put into a round appliance flask, then evaporated by a rotary evaporator and heated to a temperature of 60°C with a pressure of 200 mBar for 1 hour. This was done until a thin layer was formed on the walls of the round bottom flask, then PBS was added into the flask. The niosome results were taken. Furthermore, it was purified with a PD 10 column, collected into a container; then downsizing was carried out using ultrasonic with sonication times of 2 seconds, 4 seconds, 6 seconds, 8 seconds, 10 seconds, and 12 seconds. Analysis with a particle size analyzer (PSA). Liposome formulations can be seen in Table 1 below.

Table 1. Formulation of Tekelan leaf liposomes

| Ingredient Name | Formulations |
|-----------------------------------|--------------|
| Ethanol extract of tekelan leaves | 100 mg |
| Lipoid S75 | 95% |
| Lipoid DMPG-Na | 5% |
| Cholesterol | 5% |
| Methanol | 5 mL |
| Chloroform | 5 mL |
| PBS saline | 10 mL |

2.4.1 Liposome morphology test

The morphological characteristics and size of the liposomes were evaluated by TEM (*Transmission Electron Microscopy*). Liposome samples were diluted with 20x and 50x dilutions, then one drop was dropped on a formvar coated cumprum grid and dried at room temperature; after drying, samples were analyzed by TEM. The samples were put in a container and analyzed at 25°C [7].

2.4.2 Particle Size observation and polydispersion index

The particle size measurement and polydispersion index was carried out using a particle size analyzer (Horiba SZ 100), the sample was inserted into the cuvette after 100x dilution. The stability of the liposome preparations was evaluated organoleptically in the form of color changes and the formation of particulates that were dispersed into the PBS carrier liquid [8].

2.5 Production of hyperglycemia in rats

The mice were adapted for one week before being treated. After one week of adaptation, the mice were induced using nicotinamide 120 mg/kg BW. 15 minutes later, followed by streptozotocin induction at a dose of 60 mg /kg BW. The inducer is given intraperitoneal to cause type-2 diabetes mellitus. If there is an increase in rat blood glucose levels to ± 200 mg /dL, the rats are considered to have hyperglycemia [9].

2.5.1 Antihyperglycemia Test in Male White Rats

Tekelan leaf niosome antihyperglycemia test was given with a variety of doses, namely 25 mg/kg BW, 125 mg/kg BW, and 250 mg/kg BW, which was given orally once per day CMC-Na was used as a negative control, and glibenclamide 0.45 mg /kg BW was used as a positive control. The five groups were measured for their blood glucose levels on day 0, 3, 6, 9, 12, and 15. Normal glycemia levels are 75-150 mg/dL, mild glycemia 150-200 mg/dL moderate 200-400 mg/dL and severe glycemia conditions above 400 mg/dL [10].

3 Results and discussion

3.1 Results of phytochemical screening

The results of the phytochemical screening of tekelan leaves can be seen in Table 2 below.

Table 2. Phytochemical screening results

| Results of Tekelan Leaf Phytochemical Screening | | |
|---|--------|---------|
| Compound | Powder | Extract |
| Alkaloids | + | + |
| Flavonoids | + | + |
| Tannins | + | + |
| Saponins | + | + |
| Steroids/triterpenoids | + | + |
| Glycosides | + | + |

Based on Table 2, the results of phytochemical screening of powders and extracts produced secondary metabolites in the form of alkaloids, flavonoids, tannins, saponins, steroids/triterpenoids, and glycosides. This is in accordance with Marianne's research [6] which states that the ethanolic extract of tekelan leaves contains secondary metabolites of alkaloids, flavonoids, tannins, saponins, steroids/triterpenoids, and glycosides.

3.2 Results of liposome nanotechnology formulation, characterization, and physical stability

3.2.1 Organoleptic results

The organoleptic results observed were the distinctive odor of tekelan leaves, brownish-green color, and a cloudy consistency such as suspension.

3.2.2 TEM (Transmission Electron Microscopy) observation

Liposome nanotechnology TEM observations can be seen in Fig. 1 below.

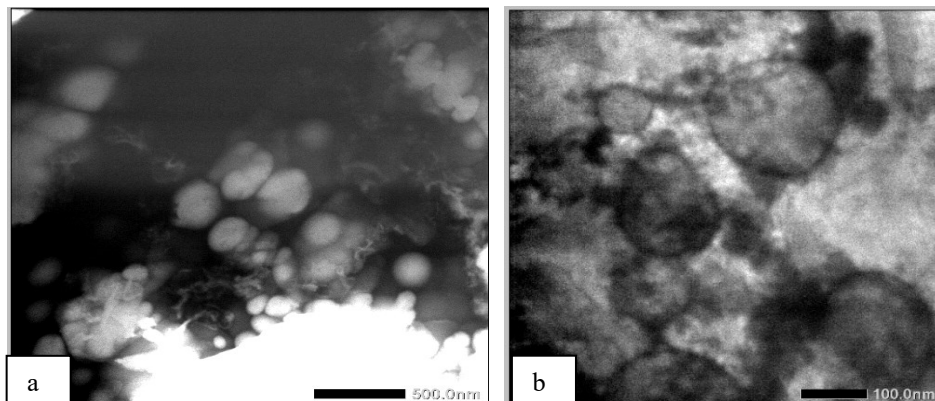


Fig. 1. TEM Results at 10,000 Times Magnification of Niosome Ethanol Extract Leaf Tekelan (*C. odorata* L.) Formulation-3 with Sonication Time 30 minutes. (a) before purification, image (b) after purification

The TEM (*Transmission Electron Microscope*) results from the liposomes of the tekelan leaf extract were somewhat rounded with different particle sizes before and after purification. Before undergoing purification, the TEM size of the liposomes of the tekelan leaf extract was 500 nm, and after undergoing purification, the TEM size was 100 nm.

Abinav et al. [10] stated that the size of the vesicle diameter is influenced by the amount of cholesterol, the solvent, and the surfactants that form it. [11] stated that the particle size after purification showed smaller results compared to that before purification. This indicates that purification has resulted in pure nanoparticles. The purification process was carried out to determine the percentage of the ethanol extract of tekelan leaves that could be absorbed in the resulting niosome vesicles. The absorption process can form better bilayer vesicles [12].

3.2.3 Results of observation of particle size and polydispersion index

The results of observing the particle size and polydispersion index can be observed in Table 3 below.

Table 3. Observation results of particle size and Liposome Polydispersion Index Tekelan leaf ethanol extract

| Sonication Time (minutes) | Particle Size (nm±SD) | PI (D±SD) |
|---------------------------|-----------------------|------------|
| 2 | 286,5±8,3 | 0,419±0,06 |
| 4 | 194,7±1,6 | 0,530±0,04 |
| 6 | 186,5±2,8 | 0,376±0,09 |
| 8 | 175,9±3,9 | 0,359±0,07 |
| 10 | 162,6±5,5 | 0,345±0,04 |
| 12 | 145,5±2,8 | 0,307±0,03 |

The ethanol extract of the tekelan leaves used for the manufacture of liposomes was 10%. A good liposome particle size is 100 nm - 150 nm, while a good PI (Polidispersity Index) is <0.7 Đ, PI is tested to determine the homogeneity of the solution, the smaller the PI, the better the homogeneity.

The sonication time of 12 minutes had the smallest particle size, namely 145.5 ± 2.8 and PI 0.307 ± 0.03 . The longer the sonication time will cause the liposome temperature to increase. This is because the energy used to reduce the size of the vesicles changes shape to heat. At one time, the vesicle size will reach the smallest (optimum) size, in this condition, the vesicle is relatively more stable [13].

Particle distribution is one of the important factors that need to be considered in the characteristics of liposomes. The good particle distribution indicates the ability of the preparation to get better at loading and releasing the drug to achieve the expected therapeutic effect. The longer the sonication time is carried out in making liposomes, the better the evenness of the particle distribution in terms of particle size, particle distribution graphs [14].

3.3 Results of Measurement of Average Blood Glucose Levels Before and After Induction of

3.3.1 Nicotinamide and Streptozotocin

Measurement of average blood glucose levels before and after nicotinamide and streptozotocin induction can be seen in Table 4 below.

Table 4. Average KGD measurement results before and after Induction Nicotinamide and Streptozotocin (mg / dL)

| Treatment | Mean fasting KGD \pm SD (mg/dL) | Mean KGD After Induction of Streptozotocin and Nicotinamide \pm SD (mg/dL) |
|---------------------------------|-----------------------------------|--|
| Na-CMC | 83,2 \pm 2,95 | 234,6 \pm 8,85 |
| Glibenklamide 0,45 mg/kg BW | 83,6 \pm 2,30 | 370,6 \pm 18,68 |
| Liposome extract 25 mg / kg BW | 83 \pm 2,24 | 286,6 \pm 10,85 |
| Liposome extract 125 mg / kg BW | 83,2 \pm 2,17 | 372,8 \pm 12,70 |
| Liposome extract 250 mg / kg BW | 87,4 \pm 1,14 | 367,6 \pm 13,90 |

Measurement of blood glucose levels for each treatment day 0, 3, 6, 9, 12, and 15 can be seen in Table 5 below.

Table 5. Measurement of blood glucose levels for each treatment (mg / dL)

| Treatment | Day-0 | Day -3 | Day -6 | Day -9 | Day -12 | Day 15 |
|------------------------------------|---------------------|---------------------|---------------------|----------------------------------|----------------------------------|----------------------------------|
| CMC-Na | 234,6 ± 8,85 | 234,6 ± 8,85 | 232,8 ± 8,75 | 231 ^b ± 9,43 | 229 ± 9,43 | 226,2 ^b ± 8,98 |
| Glibenklamide 0,45 mg/kg BW | 370,6 ± 18,68 | 344,2 ± 6,38 | 324,4 ± 16,64 | 220 ^a ± 31,18 | 166,6 ^b ± 15,47 | 110 ^a ± 9,30 |
| Liposome extract 25 mg / kg BW | 375,4 ± 10,14 | 325,4 ± 9,18 | 274 ± 8,72 | 230,6 ^a ± 5,50 | 171,2 ± 8,47 | 126,4 ^b ± 8,73 |
| Liposome extract 125 mg / kg BW | 372,8 ± 12,70 | 280,4 ± 15,47 | 271,8 ± 11,78 | 230,6 ^a ± 11,41 | 168 ^b ± 15,48 | 116,2 ^a ± 12,46 |
| Liposome extract 250 mg / kg BW | 286,6 ± 10,85 | 235 ± 5,96 | 160,2 ± 4,38 | 121,8 ^a ± 1,79 | 112,4 ^b ± 5,64 | 86 ^a ± 4,30 |

Information :

a = not significantly different from glibenclamide

b = not significantly different from CMC-Na

The results of measuring blood glucose levels showed that the liposome nanotechnology extract of ethanol leaves of tekelan leaves with a dose of 250 mg/kg BW had the lowest value in reducing glucose levels in mice, namely 86 ± 4.30 mg/kg BW. And based on the results of statistical observations, it can be seen that the liposomes of ethanol extract of leaves of tekelan at doses of 25 mg/kg BW, 125 mg/kg BW, and 250 mg/kg BW of body weight are not significantly different from glibenclamide doses of 0.45 mg/kg BW in terms of reducing blood glucose levels in rats—white male. However, the three liposome nanotechnology have significant differences with CMC-Na in terms of lowering blood glucose levels.

4 Conclusions

This study concluded that the liposome nanotechnology formulation obtained the smallest particle size in the 12th minute with a particle size of 145.5 ± 2.8 nm and a polydispersion index of 0.307 ± 0.03 . Based on the smallest particle size formulation, when tested as an antihyperglycemia with various doses, a nanoliposome dose of 250 mg/kg BW resulted in the lowest blood glucose levels of 86 ± 4.30 mg / dL.

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