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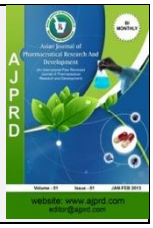

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Research Article

Antidiabetic Activity of Ethanol Extract of *Tetracera scandens* (L.) Merr. Stems

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A B S T R A C T

Diabetes mellitus is a metabolic disorder which had a high prevalence in Indonesia. Traditional medicinal plants have been developed to treat diabetes mellitus. One of them is *Tetracera scandens* (L.) Merr. This study aimed to determine the antidiabetic activity of the ethanol extract of *T.scandens* stems on blood glucose levels, insulin levels, and homeostatic model assessment for insulin resistance (HOMA-IR) in mice induced by alloxan. Animals were grouped into five groups: normal group, negative control, and extract doses of 175, 350, and 700 mg/kg BW. Animals were induced with an alloxandose of 175 mg/kg BW. The extract was given orally for 21 days. The results showed that the administration of ethanol extract of *T.scandens* stems could significantly reduce blood glucose levels, increase insulin levels and reduce insulin resistance ($p<0.05$).It can be concluded that the ethanol extract of *T.scandens* potential to develop as herbal medicine in diabetes mellitus therapy. Further study is needed to confirm the antidiabetic activity of *T.scandens*.

Keywords: alloxan, diabetes mellitus, blood glucose, insulin, insulin resistance

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INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia due to impaired insulin secretion, insulin action, and progressive changes in the structure of pancreatic β-cells[1]. Diabetes mellitus is prevalent in Indonesia. However, prevalence varies by province and ethnic group[2]. Numerous kinds of synthetic medications are used to treat diabetes, including metformin, sodium-glucose transport protein-2 (SGLT-2) inhibitors, glucagon-like peptide-1 receptor agonist (GLP-1RA), dipeptidyl-peptidase-4 (DPP-4) inhibitors, thiazolidinediones, and sulfonyl ureas [3]. However, because of the adverse effects of some medications, many people treat diabetes using herbal medicine, which is safer, more effective, and more economical[4].

Tetracera scandens (L.) Merr. (Fig.1) is one of the traditional medicinal plants used bythe community to treat sore throat, fever, flu, tuberculosis, postnatal tonic, snakebite, hepatitis, rheumatism, gout, inflammation, diarrhea, ulcers, burns, coughs, canker sores, diabetes mellitus, hypertension, urinary tract disorders, internal pain, and edema[5]. The stem of *T.scandens* has idioblast cells and unicellular glandular trichomes as secretory structures. The phytochemical reaction showed that the substances secreted by cat sandpaper idioblast cells mainly contain alkaloids, terpenoids, and phenols. *T.scandens* gland trichomescontain only flavonoids[6]. Flavonoid contentis known to regenerate damaged β-cells and is consideredan antihyperglycemic agent [7,8].

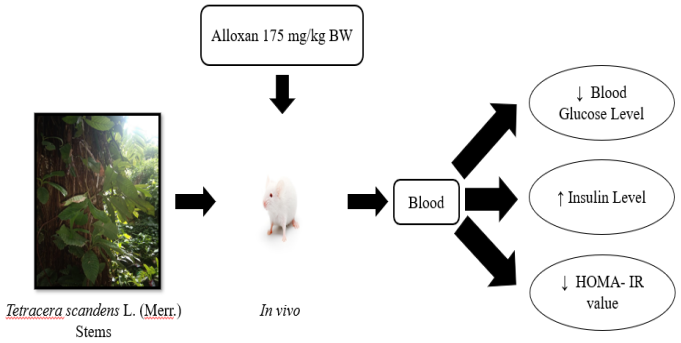

Figure 1: Antidiabetic activity of *Tetracera scandens* L. (Merr.)

Several studies have explored the activity of *T.scandens* as a traditional medicinal plant with the potential as antidiabetic. The in-vitro study showed that genistein and its derivatives 3,5-diprenyl genistein, 6,8-diprenyl genistein, derrone, and alpinumis of lavone isolated from the leaves of *T.scanden s* showed a significant effect on glucose uptake, adenosine-5'-monophosphate-activated protein kinase (AMPK) phosphorylation, Glucose Transporter-4 (GLUT4) and GLUT1 messenger RNA (mRNA) expression, andprotein-tyrosine phosphatase 1B (PTP1B) inhibition on L6 myotube [9]. Aqueous and methanol extract of *T.scandens leaves* showed a significant antihyperglycemic effect in alloxan-induced diabetic rats without hypoglycemia[10].

Activity hypoletin from the methanol extract of *T.scandens* leaves also showed anti-diabetic activity in vitro, increasing glucose absorption in adipocytes like insulin action. The leaves of *T.scandens* were discovered to have anti-diabetic and anti-obesity properties by enhancing blood glucose absorption and decreasing adipogenesis[11]. *T.scandens* leaves can also inhibit the α-glucosidase enzyme[5]. Based on this description, a study was conducted to determine the effect of the ethanol extract of *T.scandens stems* on glucose levels, insulin levels, and insulin resistance in white mice with diabetes mellitus.

MATERIALS AND METHODS

Material

The materials used were *T.scandens* (L.) Merr., 96% v/v ethanol (PT. Brataco), Hi-Pro-Vite 511 pellet food (PT. Charoen Indonesia), Sodium Carboxy Methyl Cellulose (Na CMC) (PT. Brataco), glucose(PT. Brataco), alloxan monohydrate (Aldrich),blood glucose test reagent (PT. Rajawali Nusindo), and insulin ELISA kit reagent (PT. Gamma Scientific).

Sample Preparation

The sample used was *T.scandens* stems as much as 1 kg obtained from Kanagarian Sitanang, Nagari District, Agam Regency, West Sumatra Province, Indonesia. Plant identification was carried out at the Andalas University Herbarium (ANDA), Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, West Sumatera, Indonesia. Wet sorting, washing, and chopping samples were transformed into simplicia.

Extraction

The ethanol extract of *T.scandens*stems was prepared by maceration in 96% v/v ethanol.1 kg of sticks was placed into the maceratorat a ratio of 1:10;add 10 liters of submersible solvent for the first 6 hours, stirring occasionally, and leave at room temperature for 18 hours. Filter the macerate using a flannel to separate it. Repeat the filtration procedure twice more using the same solvent type and concentration. Collect and concentrate all of the macerates using a rotary evaporator(Heidolph) until a thick extract is achieved.

Secondary Metabolite Screening

The secondary metabolite contents of *T.scandens* extract were determined. The assay was conducted to determine the alkaloids, flavonoids, phenolics, terpenoids, and steroid compounds [12].

Animals

Twentymale white mice aged 2-3 months and weighing 20-30 grams. Before being treated, the mice were acclimatized for seven days with adequate food and water. The mice used are healthy male mice with normal behavior, not showing significant abnormalities, and weight deviation during maintenance is not more than 10% of average body weight. Animals were randomly grouped into five groups consisting of 4 mice. Pharmacological activity assay on experimental animals has passed the ethical review of the Research Ethics Commission of the Faculty of Medicine, Andalas University No. 59/UN.16.2/KEP-FK/2020.

Dosage

The dosage of *T.scandens* stem ethanol extract given to experimental animals was 175, 350, and 700 mg/kg BW given orally.Inmice, hyperglycemia was induced with an alloxandose of 175 mg/kg BW.

Pharmacological Activity Test

In this study, mice were grouped into five treatment groups. Four groups (negative control group, groups given extract dose 175, 350, and 700 mg/kg BW) were induced with an alloxan dose of 175 mg/kg BW subcutaneously and given glucose of 10% for two days. The average group was only assigned Na CMC. The extracts were given for 21 days. After 21 days, the blood was taken from the animal. The animal fasted for 8 hours before taking the blood. 1 mL blood was taken from the sinus orbital of mice, put in the vacuum tube, and centrifuged for 20 minutes at 3000 RPM. The serum was separated. Blood glucose level was analyzed with a blood glucose test reagent (PT. Rajawali Nusindo) and measured using the clinical photometer 5010 v5+ (Riele). Insulin level was diagnosed with an insulin

ELISA kit reagent (PT. Gamma Scientific) and measured using an ELISA reader (RayBio)at 450 nm.

Calculation of HOMA-IR Value

Insulin resistance was assessed based on the homeostasis model assessment of insulin resistance (HOMA-IR). This method calculates insulin resistance in humans and animals using the formula: HOMA-IR =fasting insulin x fasting blood glucose / 405[13].

Statistical Analysis

The data were analyzed statistically using SPSS 24 parametric (ANOVA 1 way) and non-parametric (Kruskal-Wallis) analysis tests.

RESULTS

The sample used is a brownish stem and slightly green. It was identified at Andalas University Herbarium (ANDA), Andalas University, Padang, West Sumatera, Indonesia. The identification results showed that the plants used were *T.scandens* (L.) Merr. And the family Dilleniaceae.

The extraction of *T.scandens*stems was carried out by the maceration method. This method is simpleand doesnot require special equipment and heating, so it can overcome the possibility of compounds decomposing or evaporating due to heating. Ethanol was used because ethanol is a universal solvent that can dissolve polar, semi-polar, and non-polar compounds.

T.scandens macerated about 1 kg. The sample immersion was carried out for 24 hours, with the first 6 hours being occasionally stirred and repeated three times. It aims to attract the active compounds that are efficaciously contained in it. Furthermore, the collected macerate was concentrated with a rotary evaporator to evaporate the remaining solvent and water so that a thick extract weighing 77.4856 grams was obtained.

After obtaining a thick extract, the extract was characterized to see the quality of the quotereceived. Organoleptic determination showed that the sectionhad a reddish-black color, tasteless, odorless, andthick consistency. This organoleptic determination is one of the specific parameters determined by the five senses and aims for a simple and subjective initial recognition. Furthermore, a phytochemical screening test was carried out to determine the components of the compounds contained in the extract. The phytochemical screening test of *T. scandens* stem ethanol extract obtained the content of alkaloids, phenolics, terpenoids, and flavonoids in the section.

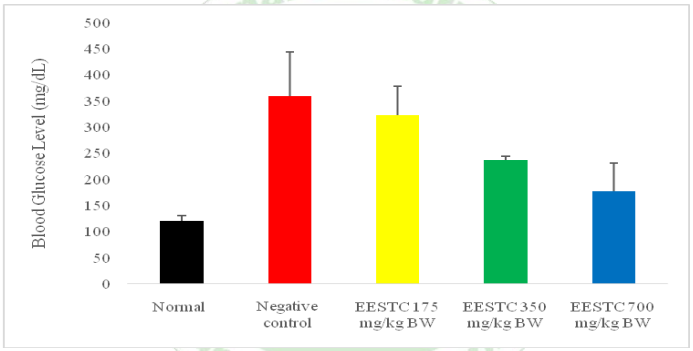

Figure 2: Blood Glucose Level

Furthermore, the antidiabetic pharmacological activity was tested on mice as experimental animals. Based on the results of the study in Fig.2, it was found that the three doses given (175, 350, and 700 mg/kg BW) significantly reduced blood glucose levels in mice induced by alloxan ($p < 0.05$), which doses 700 mg/kg BW had the most significant effect on lowering blood glucose levels. Likewise, the extract administration increases insulin levels significantly ($p < 0.05$) (Fig. 3).

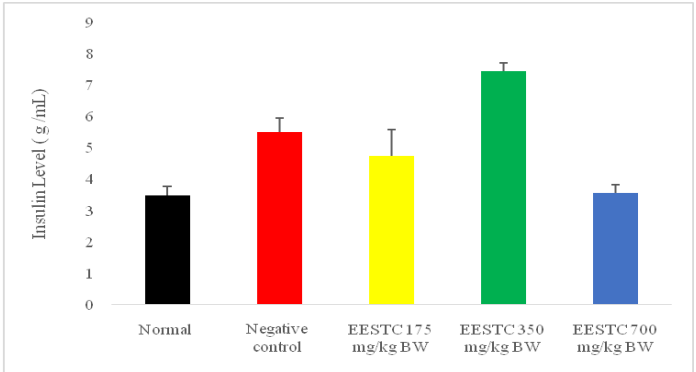

Figure 3: Insulin Level

After measuring blood glucose levels and insulin levels, the homeostatic model assessment for insulin resistance (HOMA-IR) value can be calculated. The HOMA-IR measurement is a practical and easy-to-use method. Based on the study results, it was found that the administration of the extract could significantly reduce the HOMA-IR value. It means there wasan increase in insulin sensitivity or a decrease in insulin resistance after administration of *T.scandens stem* ethanol extract($p < 0.05$), in which a dose of 700 mg/kg BW showed the most significant effect on reducing insulin resistance (Fig. 4).

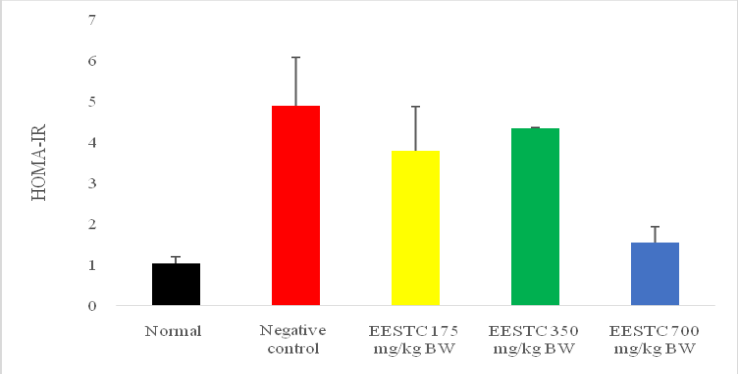

Figure 4: HOMA-IR value

DISCUSSION

Insulin resistance impairs the utilization of glucose in peripheral tissues by insulin. It can be because of failed phosphorylation of the insulin receptor substrate (IRS) complex, a decrease in GLUT-2 translocation, and glucose oxidation, preventing glucose from entering cells; it leads to hyperglycemia. The higher the HOMA-IR value, the more glucose is absorbed and used by body cells; as a result, blood sugar levels rise [14].

The researchers hypothesized that the decrease in blood glucose levels in mice was due to the presence of secondary metabolites like flavonoids, which have been shown to have an anti-hyperglycemic effect. Flavonoids were previously thought to be an effective antihyperglycemic agent due to restoring damaged cells in alloxan-induced diabetes[15]. Flavonoids are anti-inflammatory and can improve insulin sensitivity in pancreatic cells that produce insulin. Additionally, flavonoids can boost the capacity of cells to secrete insulin. Flavonoids protect β-cells against cytokines, glucotoxicity, and lipotoxicity by various mechanisms, including suppression of nuclear factor kappa B (NF-κB) signaling, activating the phosphatidylinositol-3-kinase/ protein kinase B (PI3K/Akt) pathway, inhibiting nitric oxide generation, and decrease of reactive oxygen species (ROS). Increased insulin secretory capability improves mitochondrial activity and enhances insulin secretion via phospholipase C and phospholipase C/ protein kinase C (PLC/PKC) or cyclic adenosine monophosphatase/ protein kinase A (cAMP/PKA) signaling [8].

CONCLUSION

In alloxan-induced rats, given an ethanol extract of *T.scandens* (L.) Merr. stems reduced glucose levels, increased insulin levels, and decreased insulin resistance or increased insulin sensitivity.

ETHICAL APPROVAL

The Research Ethics Commission approved the protocol study of the Faculty of Medicine, Andalas University No. 59/UN.16.2/KEP-FK/2020.

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CONFLICT OF INTERESTS

There is no conflict of interest.

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