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Effect of *Trichosanthes cucumerina* Methanol Extract on CRP and Fibrinogen Levels in Diabetic Ulcer Rat Models

Nazula Rahma Shafriani*, Tri Dyah Astuti, Shofy Farha Aulia Amri, Riski Aditya Permana, Zuvi Fridhoriansyah, Intan Maharani
Department of Medical Laboratory Technology, Faculty of Health Sciences, Universitas 'Aisyiyah Yogyakarta, Jl. Siliwangi No.63, Nogotirto, Gamping, Sleman, Yogyakarta Postcode, Indonesia.

Corresponding author*
nazula.rahma@unisayogya.ac.id

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Abstract

Diabetic ulcers can be fatal for people with long-term diabetes. This is caused by the spread of diabetic ulcers and inadequate care for the wound, which can induce an infection and even death. This study aims to determine inflammatory markers, specifically fibrinogen and C-Reactive Protein (CRP), as indicators of healing, particularly in a rat model of diabetic ulcers treated with *Trichosanthes cucumerina* methanol extract. The methods used were making extracts, measuring blood glucose levels, measuring CRP, and measuring fibrinogen levels in 25 male Wistar rats. Based on the results of glucose and fibrinogen examinations in all groups before treatment and after treatment, it showed a decrease in glucose and fibrinogen levels. Meanwhile, the results of CRP examinations, before and after treatment did not show any differences. The results of statistical analysis showed that there was a significant difference of $p<0.05$ in pre and post glucose, as well as pre and post fibrinogen.The extract that reduced fibrinogen levels the most was group 3 with 500 mg/KgBW. Meanwhile, CRP levels before and after treatment did not show a decrease.

Keywords: *Trichosanthes cucumerina*; *Ulcus diabetic*; CRP; Fibrinogen.

Abbreviations: CRP = C-Reactive Protein, DM = Diabetes mellitus, CuB = Cucurbitacin B, POCT = Point of care Testing, carbon tetrachloride (CCl4).

INTRODUCTION

Type 2 diabetes is a condition where blood sugar levels increase which can increase the risk of macro vascular and micro vascular damage thereby reducing the sufferer's quality of life. One of the complications of type 2 diabetes is neuropathy in the form of reduced sensation in the feet and is often associated with foot ulcers and even amputation (Fitria et al., 2017). According to Roza et al. (2015) chronic DM sufferers are increasingly likely to suffer from hyperglycemia which will ultimately cause retinopathy, nephropathy and diabetic ulcers. Blood sugar levels can be managed, but type 2 diabetes cannot be cured. Patients with type 2 diabetes have elevated C-Reactive Protein (CRP) levels as a result of an inflammatory response brought on by problems. CRP is an inflammatory marker for diagnosing infection (Hadavand et al., 2019). The plasma protein known as CRP is derived from the liver. Plasma CRP levels can increase rapidly to more than 1000 times above average values due to tissue damage or infection. Consequently, CRP is a monitoring marker in the acute phase, responding to tissue damage, infection, and inflammation. Research by Li et al. (2016) has shown that increased CRP levels are associated with diabetes.

Another marker of inflammatory reactions besides CRP is fibrinogen. Blood coagulation, fibrinolysis, cellular and matrix interactions, inflammation, wound healing, and neoplasia are all significantly impacted by fibrinogen. It was reported that serum fibrinogen levels increased due to an increase in acute phase proteins in type 2 diabetes sufferers (Korkmaz et al., 2018). Age, gender, smoking, body mass index (BMI), alcoholism, hypertension, glycemic management, lipid profile, and uric excretion rate are modifiable and non-modifiable factors affecting fibrinogen (Kattula et al., 2017).

There have been reports of the anti-inflammatory properties of *Trichosantes cucumerina* extract. Methanol and water extract at a 75mg/kg concentration demonstrated significant inhibition of carrageenan-induced edema in the hind leg. The anti-inflammatory effect induced by the methanol extract was comparable to that of the drug tested, indomethacin, and 750mg/kg of the extract at four and five hours. According to Ahuja et al. (2019), the entire fruit and seeds of *Trichosantes cucumerina* exhibited anti-inflammatory effect against

mice's acetic acid-induced vascular permeability, carrageenin-induced edema and cotton pellet-induced granuloma formation.

Cucurbitacin B (CuB) is a component of *Trichosantes cucumerina* extract. CuB exhibits pharmacological effects on various illnesses, inflammatory, antioxidant, antiviral, hypoglycemic, hepatoprotective and anti-cancer activities (Dai et al., 2023). The hepatoprotective effect of CuB was reported more than 20 years ago when its preventive and therapeutic effects against carbon tetrachloride (CCl4)-induced hepatotoxicity were confirmed. Later, Research showed that pre-treatment with CuB could significantly reduce liver lesions in a mouse model of CCl4-induced hepatotoxicity, reaffirming its protective effect against acute liver injury (Hunsakunachai et al., 2019). Research by Yang et al. (2020) showed that CuB can effectively inhibit liver fibrosis and suppress the release of cytokines including TGF beta 1, IL-5, IL-13, IL-6, and TNF alpha.

This study measured CRP and Fibrinogen levels in mice with a diabetic ulcer model. So far, the research that has been carried out is to determine the presence of CuB in inflammatory effects and supporting wound healing and no research has been carried out on its effect on rat therapy with diabetic ulcer models. As a result, it is critical to understand the presence of an inflammatory marker in diabetic ulcers, as the provides insight into how the ulcers heal.

MATERIALS AND METHODS

Materials

Trichosantes cucumerina methanol extract, whole blood, glucose trip (EasyTouch), serum, CRP latex reagent (Glory diagnostic), gel separator vacuum tube, citrate vacuum tube, Fibrinogen reference plasma reagent (MD pacific), distilled water, FIB reagent (MD pacific)

Instruments

Oven, rotary evaporator, POCT (EasyTouch) tool, test slide centrifuge, stir bar, micropipette, yellow tip, blue tip, TS6000 semi-automatic Coagulation Analyzer.

Procedure

Making Methanol Extract of Trichosanthes cucumerina.

50 kg of *Trichosanthes cucumerina fruit* was separated from the seeds, cut into thin slices with a thickness of ±5 mm, then dried in an oven at 50°C for ±72 hours. Next, the dried flesh of the *Trichosanthes cucumerina* is ground into powder with a size of 40 mesh, and simplicia powder is obtained. 1 kg of *Trichosanthes cucumerina* fruit simplicia powder was extracted using the maceration method using methanol solvent. The first maceration was carried out for 48 hours and the volume of methanol added was 2,200 ml and 1 kg of *Trichosanthes cucumerina* simplicia powder. The second maceration was carried out for 24 hours and the volume of methanol added was 850 mL. The third maceration was carried out for 24 hours and the volume of methanol added was 750 mL. The filtrate obtained was evaporated using a rotary evaporator at a temperature of 40°C and a rotation speed of 4 rpm (Duengo el al., 2016)

Treatment of Experimental Animals.

Male rats (*Rattus norvegicus*) from the same colony were 8-9 weeks old and weighed 180-200 grams. Group division was carried out by sampling. Samples were taken from the population and the size was determined based on the Federe formula. For five treatments, at least 5 repetitions are required for each treatment so the total mouse sample required for this research is 25 mice. Mice were obtained from the Biomedical Laboratory of Muhammadiyah University, Yogyakarta. The treatment began with adaptation and acclimatization of five groups of mice including negative control, positive control (Metformin and Amoxicilin), as well as three treatment groups with doses of methanol extract of eel bitter melon 125 mg/KgBW, 250 mg/KgBW, and 500 mg/KgBW. Before induction, blood was taken from the rat's tail to measure blood glucose levels. All mice were then induced with Alloxan 150 mg/KgBW for 96 hours until they developed diabetes mellitus. After being hyperglycemic, a 2 cm incision was made in the thigh and inoculated with Staphylococcus aureus to form diabetic ulcers within two days. The methanol extract of eel bitter melon was administered for 21 days at a dose of 1 ml per day according to each treatment group.

Blood Glucose Examination

Blood glucose levels were checked before treatment and after treatment (negative control, positive control, and therapy with *Trichosanthes cucumerina* Methanol Extract at a dose of 125 mg/KgBW, 250 mg/KgBW, 500 mg/KgBW), by taking samples. blood from the tail vein of mice in all groups. The first drop of blood is discarded, and the subsequent drop of blood is examined using a point of care test (EasyTouch). The reagent strip is inserted into the device, then blood is dripped onto the reagent strip. The results are read on the screen in less than 30 seconds. The number on the screen shows the blood sugar concentration in mg/dL (Masdar et al., 2021).

CRP examination

The CRP examination method is carried out qualitatively with CRP latex reagent. A total of 50 µL of serum samples from the treatment group, positive control (CP), and negative control (CN) were pipetted onto the slide circle. Next, 1 drop of latex reagent (CRP antigen) was added to each circle. This mixture was stirred with a stir stick for 2 minutes to homogenize it. The examination results are read under bright light and validated by observation under a microscope. If agglutination occurs,

it indicates a positive CRP result, whereas if there is no agglutination, the result is negative (Kalma, 2018).

Fibrinogen examination

Fibrinogen examination was carried out with the TS6000 semi-automatic Coagulation Analyzer, namely diluting the fibrinogen reference plasma reagent using 1 mL of distilled water, and diluting the "FIB" reagent with 2 mL of distilled water. Then, place the cuvette in the pre-warming sample position, add 20 µL of sample or control and 180 µL of Imidazole Buffered Saline (IBS) reagent into the cuvette, then incubate for 3 minutes in the prewarming sample position by pressing the clock icon on the screen. After that, move the cuvette to the test channel and add one magnetic ball into the cuvette, then add 100 microliters of FIB reagent using a micropipette connected to the tool. Press until you hear a "beep" sound, and wait until the reading process is complete to get the results.

RESULTS AND DISCUSSION

Based on the results of glucose and fibrinogen examinations in all groups before treatment and after treatment, it showed a decrease in glucose and fibrinogen levels which are presented in table 1. Meanwhile, for the results of CRP examinations, before and after treatment did not show any differences. The results of statistical analysis showed that there was a significant difference of $p<0.05$ in pre and post glucose, as well as pre and post fibrinogen.

Table 1. Results of glucose, fibrinogen and CRP examinations.

Parameter		Control (+)	Control (-)	Group I (125 mg/KgBW)	Group II (250 mg/KgBW)	Group III (500 mg/KgBW)	P Value
Fibrinogen (mg/dL)	**Pre**	526±87,77	436,2±88,56	472,6±102,57	543,2±37,29	508,6±56,14	
	Post	769,2±148,52	210±64,16	311±54,85	280,4±69,3	221±78,29	0,000
Glucose (mg/dL)	**Pre**	336,4±54,57	422,2±130,21	362±118,98	365,8±154,45	350,2±125,47	
	Post	264,8±40,81	178,2±13,97	223,6±52,1	228,2±53,76	190,8±39,92	0,035
CRP (mg/L)	≤ 15 mg/L						-

Discussion

Based on the results of examining blood glucose and fibrinogen levels, a strong relationship was found between these two variables. Fibrinogen levels rise in response to elevated blood glucose levels. This is consistent with studies by Rosyadi et al. (2018) that fibrinogen levels correlate with the process of diabetes in mice induced by streptozotocin. In addition, elevated blood glucose levels exacerbate the infection state (Fitria et.al., 2017).C-reactive protein (CRP) is an acute phase protein produced by the liver. CRP is frequently used as an indication of inflammation since a rise in CRP levels signals inflammation in the body. In chronic inflammatory diseases like type 2 diabetes, CRP levels often rise somewhat. Nevertheless, in this study, no relationship was found between glucose levels and CRP levels. These results are in line with (Faddah et al., 2012) which stated that CRP in rats was not the principal significant acute phase protein and that, in comparison to human CRP, its level below basal conditions was higher. Effect of *Trichosanthes cucumerina* methanol extract on fibrinogen shown that it has an impact on fibrinogen and glucose levels. The results of this research are in line with research conducted by Benny (2014) on the effect of *Trichosanthes cucumerina* in lowering blood sugar in experimental animals. Aside from that, *Trichosanthes cucumerina* has components that resemble sulfonylurea (an anti diabetic drug and is widely used). The phytochemicals such as, carbohydrates, alkaloids, steroids, saponins, flavonoids and tannin were detected from the medicinal plant, *T. cucumerina*. Methanol extract showed larger numbers of phytochemicals than ethyl acetate, acetone and water extract. Secondary metabolites are useful in cell growth, body building and replacement. They also function as immunological stimulants, antibacterial, anti-inflammatory agents, antiviral and detoxification activities. Plant derived tannin shown a range of antibacterial properties (Seshadri et.al., 2020). A decrease in blood glucose levels was followed by a significant decrease in fibrinogen levels in the 3 treatment groups. The concentrations used, namely concentrations of 125mg/kgBW (Body weight), 250mg/kgBW and 500mg/kgBW, have the ability to reduce blood glucose levels. In diabetic rats, administration of methanol extract from eel bitter melon with a concentration of 500mg/kgBW showed a more optimal effect in reducing blood glucose and fibrinogen levels. According to research by Afifah, et al (2021), extract of *Momordica charantia* can also reduce blood glucose levels in diabetic mice (Mus musculus) with concentrations of 100 mg/kgBW, 250 mg/kgBW and 400mg/kgBW.

CONCLUSIONS

Based on the results of research on the effect of methanol extract of *Trichosanthes cucumerina* on CRP and

fibrinogen levels in diabetic ulcer models, a significant reduction in fibrinogen levels was obtained before and after treatment. The extract that reduced fibrinogen levels the most was group 3 with 500 mg/KgBW of extract. Meanwhile, CRP levels before and after treatment did not show a decrease.

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