EFFECT OF CIPLUKAN (*Physalis angulata L.*) EXTRACT ON ANGIOTENSIN II AND BLOOD GLUCOSE LEVELS OF DIABETIC RATS MODEL

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Abstract. Diabetes mellitus (DM) is a metabolic disease that can cause various complications, including liver and kidney fibrosis. Angiotensin II is a substance that plays a role in the development of fibrosis through the mechanism of insulin resistance. Ciplukan (*Physalis angulata L.*) contains various active compounds, including physalin B, D, and F, with physalin B and D known to have antifibrotic effects. This study aims to determine the effect of different doses of Ciplukan on Angiotensin II levels and blood glucose in a diabetic rat model. The study design used a pre and post-test with a control group design. A total of 25 male Wistar rats (Rattus norvegicus) were divided into five groups: healthy control group [1], disease control group [2], administration of Ciplukan extract at a dose of 75 mg/kgBW [3], 150 mg/kgBW [4], and 300 mg/kgBW [5]. The results of the paired t-test showed significant differences in Angiotensin II levels in groups 3, 4, and 5, with p-values of 0.001, 0.004, and 0.05, respectively, and significant differences in blood glucose levels in groups 4 and 5 (p=0.001 and 0.035). The ANOVA test showed that Ciplukan did not affect Angiotensin II levels but affected blood glucose levels, with an effective 150 mg/kg BW dose.

Keywords: diabetes mellitus, angiotensin II, blood glucose, *Physalis angulata L.*

A. Introduction

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia resulting from abnormalities in insulin secretion, impaired insulin function, or both (1,2). The prevalence of DM is quite high globally, in Southeast Asia and Indonesia, with 424.9 million, 151.4 million, and 10.3 million people affected, respectively (1).

Diabetes mellitus can affect various organs, including retinopathy, neuropathy, coronary artery disease, nephropathy (1,2), and liver abnormalities, including liver fibrosis (3). The prevalence of diabetic kidney disease (DKD) is approximately 40% in type 2 DM cases and 30% in type 1 DM cases (4). A study by Siswandari et al. (2023) found that 32.78% of DM

patients experience liver fibrosis based on a FIB-4 score ≥1.43 (5). Fibrosis can progress to cirrhosis and liver carcinoma, where the incidence of liver carcinoma is higher in patients with fibrosis and cirrhosis, and 80-90% of liver carcinoma cases are associated with fibrosis (6–8).

Angiotensin II is a substance that influences the occurrence of fibrosis in DM through the mechanisms of insulin resistance and liver fibrogenesis (9,10). Angiotensin can also increase blood glucose levels through its effects on insulin sensitivity (11).

Various therapies have been developed both for treating diabetes and for preventing and managing liver complications, including the use of natural substances. One natural substance studied is Sun Chlorella (*Chlorella pyrenoidosa*), which has hepatoprotective effects (12). Another natural substance being developed is Ciplukan (*Physalis angulata L.*). Ciplukan is a member of the Solanaceae family and contains various active compounds, including saponins, flavonoids, polyphenols, alkaloids, protein, chlorogenic acid, citric acid, angulatin A, palmitic acid, acetic acid, protein, vitamin C, tannin, malic acid, physalin B, physalin D, and physalin F (13). Physalin B and D are known to have antifibrotic effects (14,15).

B. Methods

This study used a pre-test and post-test with a control group design, and the experiment was conducted using a completely randomized design. Twenty-five male Wistar rats (Rattus norvegicus), aged 2-3 months, weighing 150-200 grams, were used. The rats were divided into five groups: group 1 (healthy control), group 2 (disease control), group 3 (Ciplukan dose 75 mg/kgBW), group 4 (Ciplukan dose 150 mg/kgBW), and group 5 (Ciplukan dose 300 mg/kgBW) (16). Groups 2, 3, 4, and 5 were induced to become diabetic by injecting STZ-NA, i.e., streptozotocin 65 mg/kgBW and nicotinamide 230 mg/kgBW intraperitoneally, followed by 30% sucrose administration for 28 days. Ciplukan extract was then administered to groups 3, 4, and 5 for 28 days.

The ethanol extract of Ciplukan (*Physalis angulata L.*) was prepared by harvesting the plant at 1-1.5 months of age, washing it with clean water, draining it, cutting it into small pieces, and drying it by air-drying (without direct sunlight) until fully dried. The dried plant material was then blended into powder. The simplisia (dried plant material) was placed in a maceration container and kept away from sunlight.

Angiotensin II levels were measured using the ELISA method, while blood glucose levels were measured using the enzymatic method. The results were tested pre- and post-test using paired t-tests, and the effects of Ciplukan extract were evaluated using ANOVA.

C. Results and Discussion

The results of the study showed that the STZ-NA induction caused an increase in blood glucose levels, as seen in Table 1. There was a significant increase in the mean levels of Angiotensin II before and after treatment in groups 3, 4, and 5. The mean blood glucose levels significantly decreased in groups 4 and 5. This study did not show a significant correlation between Angiotensin II and blood glucose levels ($r=0.145$, $p=0.589$).

The results of the ANOVA test in Table 2 show that there was a significant difference in Angiotensin II levels between the disease control group [2] and the treatment groups [3, 4, 5] compared to the healthy control group [1], but no significant differences were found between the treatment groups themselves. A significant difference in blood glucose levels was found between the disease control group [2] and the treatment group [3] compared to the healthy control group [1], as well as between the treatment groups [3] and [4].

The administration of Streptozotocin-nicotinamide (STZ-NA) has been proven to cause hyperglycemia in experimental animals. Hyperglycemia, which triggers diabetes, occurs due to the destruction of pancreatic beta cells through mechanisms of DNA damage, oxidative stress, and beta cell apoptosis. Streptozotocin enters pancreatic beta cells via GLUT2 transporters.

Once inside the beta cells, STZ binds to methyl groups on DNA, particularly guanine, causing structural DNA damage and inhibiting DNA replication and gene transcription. Streptozotocin also triggers oxidative stress by producing reactive oxygen species (ROS), such as superoxide (O2-), hydrogen peroxide (H2O2), and hydroxyl radicals (•OH). These ROS cause damage to lipid membranes, proteins, and DNA. DNA damage and oxidative stress activate apoptosis in pancreatic beta cells through p53 and other transcription factors. Apoptosis leads to the death of pancreatic beta cells, resulting in decreased insulin production. The reduction in insulin production causes glucose in the blood to be unable to enter body cells, leading to the accumulation of glucose in the blood or hyperglycemia (17,18).

Table 1. Research results

Biomarkers	Groups	Mean Pre-test	Mean Post-test	Paired t-test
Angiotensin II (pg/mL)	1	47.6	47.4	0.706
	2	58.9	97.8	0.027*
	3	55.7	103.0	0.001*
	4	62.2	115.3	0.004*
	5	70.1	99.5	0.005*
Blood Glucose (mg/dL)	1	87.6	80.0	0.180
	2	535.0	603.6	0.061
	3	453.6	353.4	0.120
	4	447.8	136.0	0.001*
	5	368.4	234.8	0.035*

*Note: Significance p < 0.05

Table 2. Results of the ANOVA test

Groups	Angiotensin II					Blood glucose				
	1	2	3	4	5	1	2	3	4	5
1		0.002	0.001	0.000	0.001		0.000	0.001	0.858	0.085
2	0.002		0.989	0.520	1.000	0.000		0.002	0.000	0.000
3	0.001	0.989		0.797	0.997	0.001	0.002		0.008	0.262
4	0.000	0.520	0.797		0.613	0.858	0.000	0.008		0.432
5	0.001	1.000	0.997	0.613		0.085	0.000	0.262	0. 432	

This study shows a significant difference in Angiotensin II levels between the disease and healthy control groups. The difference in Angiotensin II levels may be due to fibrosis occurring in the liver, which aligns with previous research showing higher Angiotensin II levels in the fibrosis group compared to the healthy control group (19,20). There has been limited research on the effects of Ciplukan on Angiotensin II levels. Ciplukan has various therapeutic effects, including hypoglycemic, microbicidal, antiviral, immune system modulation, anti-inflammatory, and antioxidant properties. The active components of Ciplukan include saponins, flavonoids, polyphenols, alkaloids, protein, chlorogenic acid, citric acid with angulation A (WA), palmitic acid, acetic acid, protein, vitamin C, tannins, malic acid, physalin B, physalin D, and physalin F (13). The antifibrotic effects of Ciplukan are mainly attributed to physalin B and D.

In vivo studies have shown that administering physalin B reduces histopathological liver injury and collagen accumulation and decreases the expression of fibrogenic genes. Physalin B suppresses the expression of fibrotic markers in LX-2 cells and rat pHSC (primary hepatic stellate cells). The mechanism occurs through inhibition of GLI activity via non-canonical Hedgehog signaling. Physalin B blocks the formation of the lamina-associated polypeptide 2α (LAP2α)/histone deacetylase 1 (HDAC1) complex, thus inhibiting HDAC1-mediated deacetylation of GLI1. The acetylation of GLI1 regulated by physalin B and the decreased expression of GLI1 inhibit HSC activation (21).

The results of this study show that Ciplukan extract can decrease blood glucose levels, consistent with previous research (16,22). The active components of Ciplukan, such as flavonoids and alkaloids, may influence glucose metabolism. This effect occurs through mechanisms that stimulate insulin synthesis and secretion, restore damaged pancreatic beta cells, increase insulin sensitivity, enhance glucose uptake by fat and muscle cells, and affect enzymes involved in glucose metabolism (22,23).

This study did not show a relationship between Angiotensin II and blood glucose levels. Angiotensin II affects insulin sensitivity by decreasing insulin-stimulated tyrosine phosphorylation. This reduction disrupts the interaction between phosphatidylinositol-3-kinase and insulin receptor substrate (IRS-1), leading to downregulation of insulin receptor signaling. This will result in increased hepatic glycogenolysis and gluconeogenesis, which are processes of glucose production in the liver and contribute to elevated blood glucose levels (11,24).

D. Conclusion

The results of this study indicate that Ciplukan (*Physalis angulata L.*) extract has an effect in decreasing blood glucose levels with an effective dose of 150 mg/kgBW. However, it does not affect Angiotensin II levels.

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