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Innovative Therapy for Diabetes Mellitus Based on Tithonia Diversifolia Through Pancreatic β-Cell Viability

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ABSTRACT

Diabetes mellitus (DM) is a chronic metabolic disease with increasing global prevalence including in Indonesia. A key factor in the pathogenesis of DM is damage to pancreatic β cells which are essential for insulin production. This study aims to determine the effect of ethanol extract of Tithonia diversifolia leaves on blood glucose levels and pancreatic β cells in DM model rats. Tithonia diversifolia contains flavonoids and polyphenols which possess antioxidant properties. These compounds may reduce the production of reactive oxygen species (ROS) and offer cell protection The study used a laboratory-based experimental post-test only control group design. Four groups were used: a negative control group (K); three treatment groups (P1, P2, and P3) receiving *T. diversifolia* ethanol extract at doses of 50, 150, and 500 mg/kg body weight/day. Each group consisted of 9 males diabetic Wistar rats induced by streptozotocin. The results showed that the administration of T. diversifolia ethanol extract at a dose of 150 mg/kgBW/day resulted in a significantly higher average pancreatic β-cell count compared to the control group and the 50 and 500 mg/kg body weight (BW)/day treatment groups.

Keywords: Ethanol extract, pancreatic beta cells, tithonia diversifolia leaves.

1. Introduction

Diabetes mellitus (DM) is a crucial issue in Indonesia. The prevalence of DM in Indonesia is increasing, from 5.7% in 2007 to 6.9% in 2013 (Ministry of Health, 2014). The International Diabetes Federation (IDF) reported in 2014 that more than 387 million people were diagnosed with DM, while an estimated 175 million people were undiagnosed. The IDF also estimates that the number of sufferers will reach 592 million by 2035. DM causes 4.9 million deaths per year and accounts for 11% of total healthcare expenditures in the adult population (IDF, 2014). These data provide an overview of the development of DM, its public health impact, and the importance of greater attention to the disease.

DM can be classified based on its cause into type 1 and type 2 DM. The cause of type 1 DM is a deficiency of the hormone insulin due to damage to pancreatic β cells. Meanwhile, type 2 diabetes mellitus (DM) is caused by insulin resistance. In the early stages of type 2 diabetes, compensatory increases in insulin production are observed, which then progresses to damage pancreatic β-cells (Suyono, 2013; Setiati, 2017, and Solis-Herera *et al*., 2021). Because pancreatic β-cells play a crucial role in the progression of both types of diabetes, further research on this tissue is needed.

Currently, drug options for diabetes are limited. DM medications such as sulfonylureas and meglitinides present significant problems due to side effects like hypoglycemia, dizziness, and gastrointestinal issues. Additional risks include weight gain, lactic acidosis, or potentially serious conditions like diabetic ketoacidosis (Sarah *et al*., 2013; Chaudhury, *et al*. 2017). On the other hand, people have used certain types of plant leaves to lower blood glucose levels, one of which is the Tithonia diversifolia leaf. Several studies have shown that the leaf extract can lower blood glucose levels (Lin, 2012; Wahyuningsih *et al*., 2013; Juang, 2013; Eddouks *et al*., 2014; Barboza *et.al*, 2018).

Tithonia diversifolia is found in several places in the world, including Southeast Asia, Africa, and South America. This plant thrives at an altitude of 200-2,300 m above sea level, with a temperature of 15-31°C, and rainfall of 1,000-2,000 mm/year. The biological classification of this plant is as follows: kingdom plantae (plants), super division spermatophyta (seed-producing plants), division magnoliophyta (flowering plants), class magnoliopsida (dicotyledons), subclass asteridae, order asterales, family asteraceae, genus tithonia and species Tithonia diversifolia. Parts of the plant contain several compounds including alkaloids, anthracene, balsam, cardiac glycosides, saponins, saponin glycosides, tannins, and also essential oils (Ajao, A.A., and Moteetee, A., N., 2017; Barboza *et.al*, 2018).

However, the extent to which the leaf extract of this plant has an effect on pancreatic β cells has not been widely studied. This research is needed to prove the activity of the active ingredients contained in the plant so that it can be used as a drug innovation in the future. The results of this study are expected to initiate further research on plant-based drug innovations for diabetes mellitus that maintain pancreatic β-cell function, using plants that are widely available in Indonesia.

2. Methods

This was an experimental study using male Wistar rats as subjects. The study was conducted in the research laboratory of the Faculty of Medicine, Islamic University of Indonesia. The subjects were male Wistar rats, weighing 150-250 grams, healthy, and approximately two months old. The rats were acclimatized for seven days, provided with standard feed and water *ad libitum*. Their body weight and lengths were weighed and measured as baseline data. The standard feed composition used included: maximum water 12%, minimum crude protein 19%, minimum crude fat 5%, maximum crude fiber 4.5%, maximum ash 6.5%, calcium 0.9-1.2%, phosphorus 0.7-0.9%, and coccidiostat (+).

The welfare of the Wistar rats was strictly maintained throughout the study in accordance with ethical guidelines, including housing them in individual cages to prevent social stress and bullying. The animals were kept on a standard *ad libitum* diet and maintained under controlled light-dark cycles. Pancreatic tissue sampling was performed under local anesthesia to ensure analgesia. Following the procedure, the carcasses were ethically handled by securing them in a special biological waste container before burial. Ethical approval for this research was obtained from the Ethics Committee of the Faculty of Medicine, Universitas Islam Indonesia no. 1/Ka.Kom.Et/70/KE/II/2020.

The number of research subjects was calculated using the Charan method using the following formula: n = r + 1, where "n" is the number of samples and "r" is the number of groups studied.
So that the number of research subjects in each group is at least 5 rats. But in this study, the number was 9 rats for each group (Charan and Biswas, 2013; Murti, B., 2024).
After acclimatization, the rats were randomly divided into four groups and housed in separate experimental cages.

Diabetes was induced in the rats by intraperitoneal injection of streptozotocin at a low dose (40 mg/kg body weight) followed by low-dose streptozotocin (STZ) injection to cause mild dysfunction in β-cells without completely compromising insulin secretion. The rats were fasted for six hours before the injection (Guo *et. al.*, 2018). After 24 hours, blood glucose levels were measured. If hyperglycemia did not occur, the induction would be repeated at the same dose. Rats with blood glucose levels exceeding 200 mg/dL were randomly distributed into five groups, each containing six rats.

The names and treatments for each sample group were as follows:

1. Group K consisted of diabetic rats given drinking water *ad libitum*. Belum sesuai APA
2. Group P1 consisted of rats given Tithonia diversifolia extract at a dose of 50 mg/kg body weight/day.
3. Group P2 consisted of rats given Tithonia diversifolia extract at a dose of 150 mg/kg body weight/day.
4. Group P3 consisted of rats given Tithonia diversifolia extract at a dose of 500 mg/kg body weight/day.

Pancreatic tissue was harvested surgically under general anesthesia with a ketamine injection at a dose of 3 mg/kg body weight. The pancreatic tissue was stored in 70% alcohol and then sent for histopathological preparation and stain with Victorian blue. Primary data collected included blood glucose levels and pancreatic β-cell counts.

3. Results and Discussion

Microscopic slides to identify pancreatic β-cells were prepared using a microtome and Victorian blue staining. One specific histochemical technique for microscopic preparations is the Victoria Blue stain, which is often used to differentiate endocrine cells in the pancreatic islets of Langerhans, particularly α and β-cells. Common stains, such as Hematoxylin-Eosin (HE), are generally inadequate for this identification (Scott, C. R., *et al*., 2015). The preparation was observed using a light microscope at 400x magnification, after which pancreatic β-cells were identified and counted. Cell counting is performed using the "cell counter" or "point picker" tools to manually or semi-automatically mark and count objects on microscopic images displayed on a computer screen. Examples of microscopic images at 400x magnification are shown in Figure 1.

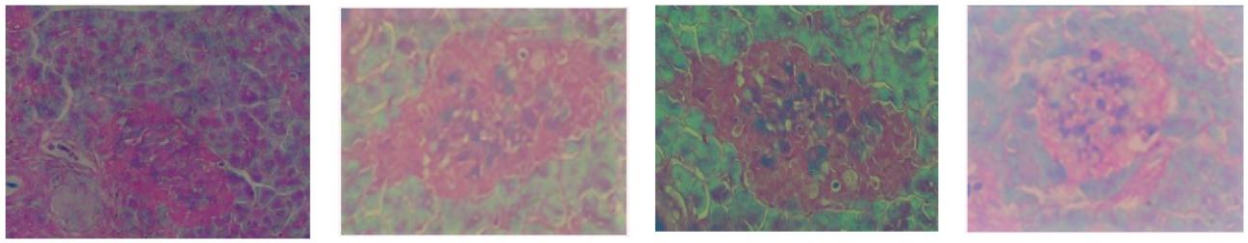

Figure 1. The microscopic photo of pancreatic tissue with 400X magnification, Victorian blue staining. K Group is a negative control; Treatment Group P1 receiving *T. diversifolia* ethanol extract at doses of 50 mg/kg body weight/day, P2 receiving *T. diversifolia* ethanol extract at doses of 150 mg/kg body weight/day, and P3 receiving *T. diversifolia* ethanol extract at doses of 500 mg/kg body weight/day.

Streptozotocin (STZ) injection selectively damages pancreatic β-cells. STZ enters the cells via the GLUT2 transporter. Once inside, STZ releases free radicals, including nitric oxide (NO), which causes DNA damage and severe oxidative stress. This damage triggers cell death through necrosis and apoptosis, ultimately resulting in insulin deficiency and hyperglycemia (Iwasaki, M., *et al*., 2016; Wu & Huan, 2018; García-Aguilar and Carlos Guillén, 2022).). In a study of normal rats, the average number of pancreatic beta cells in one islet was 84.56 ± 9.40 cells (per islet, calculated from 10 islets per preparation at 400x magnification) (Suarsana, I. N., *et. al*, 2010).

The variation in the average number of pancreatic beta cells in the islets of Langerhans may be due to several factors. Research highlights the central role of oxidative stress in the pathophysiology of pancreatic β-cell dysfunction and death in diabetes (Tanti, J. F., et al., 2019). Furthermore, pancreatic β-cell viability is not only affected by free radicals from agents like STZ but is also significantly influenced by internal and external antioxidant agents (Zhang, Y., *et al*., 2020). Another study on external antioxidants demonstrated the protective effect of administering quercetin (a flavonoid antioxidant) to diabetic rats, significantly shielding pancreatic β-cells from the damaging oxidative stress associated with diabetic conditions (Burlaka, 2021). In research on diabetic mice, the administration of ethanol extract proved effective in reducing blood glucose levels (Fitriyanto, 2020). Research on various parts of the plant also demonstrates benefits in reducing blood glucose levels in mice (Sintowati *et. al*, 2021). The benefits of the extract may be due to mechanisms that enhance pancreatic β-cell viability and thus maintain insulin production. Supporting this, the pancreatic β-cells in this study remained visible and quantifiable under the microscope, suggesting protection from necrosis or severe STZ-induced damage. as shown in Table 1**.** This protective action is strongly attributed to the antioxidant role of the *Tithonia diversifolia* extract.

Table 1. Pancreatic beta cells number of Langerhans islet.

Group	The number of pancreatic beta cells in each randomly selected islet of Langerhans in a group of rats.									Mean
KN	6	6	4	1	7	2	2	2	3	3.67±2.179
P1	5	12	15	17	13	1	5	2	2	8.00±6.225
P2	15	31	10	10	12	9	22	10	5	13.78±7.996
P3	14	2	1	5	5	1	1	1	1	3.44±4.304

Table note:
K Group is a negative control. The treatment groups received Tithonia diversifolia ethanol extract at the following doses: P1 Group: 50 mg/kg body weight/day, P2 Group: 150 mg/kg body weight/day, and P3 Group: 500 mg/kg body weight/day.

Analysis between groups using Statistical Package for the Social Sciences (IBM SPSS) version 23 software. Normality and homogeneity tests were carried out as a prerequisite for determining the type of parametric or non-parametric test to be applied. as presented in Table 2. The Shapiro-Wilk test was used because the sample size was relatively small. A p-value greater than the specified significance level of 0.05 indicates that the data are normally distributed. The p-value found in group P3 was less than 0.05, so the parametric test could not be used for the whole group. The Levene's test for homogeneity of variance yielded a p-value of 0.021, indicating that the variances between groups were unequal (heteroscedasticity). Consequently, a non-parametric test was chosen, utilizing the Kruskal-Wallis test to compare significant differences among three or more groups. Post-hoc analysis was then performed using the Mann-Whitney U test to compare specific pairs of groups.

Table 2. Mean, normality, and homogeneity test, number of pancreatic beta cells in each group.

Group	n	Mean±SD	Saphiro-Wilk test (p)	Homogeneity of variance (p)
K	9	3.67±2.179	0.195	0.021
P1	9	8.00±6.225	0.143	
P2	9	13.78±7.996	0.055	
P3	9	3.44±4.304	0.000	

The Kruskal-Wallis test is a nonparametric statistical test used to compare three or more independent groups to determine whether there are significant differences in the dependent variable. This test is a nonparametric alternative to one-way analysis of variance (ANOVA) and is often used when data are not normally distributed. The results of the Kruskal-Wallis test are presented in Table 3.

Table 3. Result of Kruskal Wallis

Statistic Test	Value
Chi-Square	10.628
df	3
Asymp. Sig.	0.014

The significance value of 0.014 indicates that there is a significant difference between the groups. This indicates a significant difference between two or more groups. To identify the differences between the groups, a Mann-Whitney test was performed. This test is a non-parametric alternative to the

independent t-test and assesses the difference in medians between two groups. The results shown in Table 4.

Table 4. Result of Mann Whitney Test (p-value).

	KN	P1	P2	P3
KN		0.245	0.001	0.189
P1			0.198	0.205
P2				0.018

Table 4 shows that the number of pancreatic beta cells in the P2 group was significantly higher than in the KN (negative control) and P3. However, this number was not significantly different from the number of pancreatic beta cells in P1.

The number of pancreatic beta cells in the group administered *Tithonia diversifolia* leaf ethanol extract at a dose of 150 mg/kgBW/day was significantly higher (or greater) than the control group and the 500 mg/kgBW/day dose group, but was not significantly different from the 50 mg/kgBW/day dose group. The Tithonia diversifolia extract effectively maintained pancreatic beta cell viability at a dose of 150 mg/kgBW, but failed to do so at the higher dose of 500 mg/kgBW.

The increased survival of pancreatic beta cells in this study may be due to several factors, including:

a. Antioxidant Activity.

Tithonia diversifolia extract is rich in phenolic and flavonoid compounds, such as quercetin, chlorogenic acid, caffeic acid, saponins, and tannins. These compounds act as antioxidants, protecting β cells from oxidative damage caused by oxidative stress, which is common in type 2 diabetes (Rahman, *et al*., 2021). Tithonia diversifolia contains flavonoids/polyphenols that have antioxidant properties. Examination results of total phenolic compounds and total antioxidant capacity of the aqueous extracts of insulin leaves were 55.92 ± 4.45 gallic acid equivalents GAE mg/g dry weight (93.09 ± 37.91 uM Trolox equivalents antioxidant capacity TEAC / mg dry weight). This function is due to various mechanisms, including reducing the production of reactive oxygen species (ROS), scavenging mechanisms, and protection by stabilizing free radicals. The process of reducing ROS production occurs due to suppression of glycogenolysis and gluconeogenesis, which can also be achieved by inhibiting enzymes in the ROS formation process, such as NADH oxidase (Fitriyanto *et al*., 2020).

b. Anti-inflammatory Effects

Chronic inflammation in the pancreas can lead to apoptosis (death) of β cells. Tithonia diversifolia has anti-inflammatory effects through inhibition of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6). It also regulates signaling pathways such as nuclear factor kappa B (NF-κB), which are involved in the inflammatory response (Oliveira *et al*., 2015). Tithonia diversifolia extract has been shown to inhibit liver injury and reduce Langerhans cell damage by downregulating IL-1β expression in alloxan-induced mice. Interleukin-1(IL-1β) is a pro-inflammatory cytokine whose upregulation is triggered by various signals, such as infection and injury. Primarily secreted by macrophages, IL-1β is processed by the inflammasome to its active form, which subsequently causes inflammation and contributes to the pathogenesis of various disorders, including cancer and autoimmune diseases (Solfaine, 2022).

c. β Cell Regeneration and Protection

Several in vitro and in vivo studies have shown that Tithonia extract can stimulate the regeneration of damaged pancreatic β cells. Reduces apoptosis (programmed death) through regulation of the Bcl-2 and Bax genes, proteins that regulate the life-death balance of cells (Sintowati *et al*., 2021).

4. Conclusion

Administration of Tithonia diversifolia leaf ethanol extract at a dose of 150 mg/kgBW/day can maintain the number of pancreatic β cells histopathologically.

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