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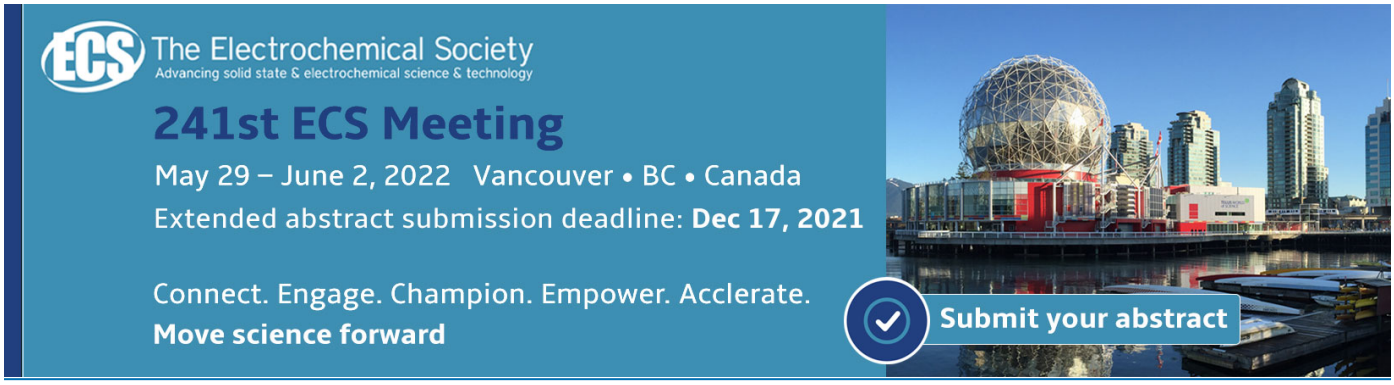

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Antioxidative Activity of *Tithonia Diversifolia* Extract in Streptozotocin-Induced Diabetic Rats.

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Abstract. Diabetes mellitus (DM) is metabolic disease characterized by high blood glucose. *Tithonia diversifolia* (TD) is a traditional herbal plant that contains anti-oxidative substances to reduce toxicity by free radical molecules. This study is aimed to analyzed effect of *Tithonia diversifolia* extract on diabetic rats. Dried leaves of *Tithonia diversifolia* plant (Balittro,Bogor) were sieved and macerated using 96% ethanol. TD leaves extract was dissolved in 0.1% sodium carboxymethyl cellulose (CMC-Na). Twenty-four male Wistar rats (*Rattus norvegicus*) were allocated into four groups; The control received normal saline (P0). The positive control received 0.1% CMC-Na (P1), the treatment received 100 mg/kg bw of TD extracts (P2) and cathecin 10 mg /kg bw (P3) respectively for 7 days. Bloods were collected for analysis of blood glucose (BG) and alkaline phospathase (ALP). The levels of MDA and SOD concentrations were conducted by Sandwich-ELISA. Based on result showed that feeding TD extract significantly could decrease the level of BG and ALP concentration compared to the positive control group ($p<0.05$). Level of MDA was decreased meanwhile level of SOD concentration significantly ($p<0.05$) on treatment group. It was concluded that administration of TD extract could restore normal blood glucose by antioxidant effect on diabetic rats.

1. Introduction

Diabetes mellitus is a metabolic syndrome consists of a group of cardio metabolic risk factors, with insulin resistance and adiposity as its main characteristics. Insulin is one of the main regulators of adipose tissue function. In someone with diabetes mellitus, endogenous insulin secretion is replaced by an exogenous supply, which is not regulated naturally [1-3].

Streptozotocin (Stz) is a natural nitrosourea product from Streptomyces achromogenes. Usually, single dose intraperitoneal injections (60 mg/kg bw) contain direct toxicity to β cells that produce necrosis within 48-72 hours and cause hyperglycemias[4,5].

Streptozotocin cytotoxic work is mediated by reactive oxygen species (ROS), where Streptozotocin and its reduction products enter the redox cycle, and form superoxide radical by-products. This radical is dismutase to become H2O2 to be a highly reactive hydroxyl radical that is formed by Fenton's reaction. Simultaneous ROS work with increased levels of cytosolic calcium which causes rapid destruction of pancreatic β cells [6].

Consumption of hypoglycaemic drugs in long term used can cause a number of side effects.[7]. Therefore, traditional medicine using medicinal plants is an alternative choice for prevention and treatment. Currently more people are returning to nature, including the use of ant diabetic drugs. One of the plants that have the potential of medicine is *Tithonia diversifolia*, that it has traditionally been used as a medicine for abdominal pain, bloating, diarrheal, and anti-inflammatory. The parts of *Tithonia diversifolia* plants that are used as a source of chemicals are leaves, roots, stems, fruit, and seeds. *Tithonia diversifolia* leaves contain active substances (phytochemicals) of alkaloids, saponins, saponin glycosides, tannins, volatile oil and antidiabetic [8,9].This study is aimed to analyzed effect of *Tithonia diversifolia* extract on diabetic rats.

2. Method

2.1. Design of research

This research was an experimental design with Randomized Post-test Control Group Design and was approved by Animal Care and Use Committee Faculty of Veterinary Medicine Universitas Airlangga Surabaya (No.2.KE.091052018). Dried leaves of *Tithonia diversifolia* plant was purchased from Balai Penelitian Tanaman Rempah dan Obat (Balittro) Bogor, West Java. Identification and extraction of *Tithonia diversifolia* leaves were conducted at the Department of Phytopharmacy, Faculty of Pharmacy, Universitas Gadjah MadaYogyakarta. The dried leaves were sieved and macerated using 96% ethanol as a solvent. The *Tithonia diversifolia* leaves extract was dissolved in a suspension containing 0.1% sodium carboxymethyl cellulose(CMC-Na).The CMC-Na suspension was used for treatment inthe positive control group for 7 days.

Preparation of a solution of *Tithonia diversifolia* leaves extract by weighing the extract as much as 40 mg in 10 ml of methanol, then 4 l of the solution was spotted on a silica gel plate F254, the ethanol extract of *Tithonia difersifolia* leaves was spotted on a TLC plate (Thin Layer Chromatography) with a distance of 1.5 cm from the bottom edge and 1 cm from the top edge of the plate, then allowed to dry. Then it was eluted with the selected mobile phase, namely toluene: ethyl acetate (7: 3), because the toluene: ethyl acetate mobile phase had good separation, then continued with UV light observations at wavelengths of 254 nm and 366 nm, then continued with spraying aimed to see the content of plant compounds on the TLC plate, namely with several visible spots such as AlCl3 (for flavonoids). Furthermore, the results of spraying with the spot viewer are measured and the Rf value of each spot is calculated from the spotting point, the Rf (Retardation Factor) value is between 0-1 which indicates the elution speed of a compound in the spot stain.

This study used twenty-four animals Wistar rats (*Rattus norvegicus*), male, age between 2-3 months, weight between 150-200 grams and physically fit. All rats were obtained from the Animal Laboratory Unit Faculty of Medicine,Universitas Airlangga Surabaya Indonesia. Rats were feed pellet and drinking *ad libitum*. All animals were adapted for 1 week. Diabetic rats were made by administering a single dose 60 mg/kg bw of Streptozotocin (Stz) and feed a high-fat diet for 2 weeks. Each group of Wistar rats consist 6 animals were allocated into:

1) Control group: normal saline; aquades (P0)
2) Positive control: Streptozotocin (Stz) + CMC-Na 0.01% (P1)
3) Treatment group: Streptozotocin (Stz) induced+ *Tithonia diversifolia* leaves extract 100 mg/kg bw (P2)
4) Drug Comparative group: Streptozotocin induced + catechin at a dose 10 mg/kg bw (P3)

The treatment group was given TD extract of 100 mg/kg bw, and the comparison drug group was given catechins 10 mg/kg bw (P3) for 7 days. All rats were sacrificed for blood samples on the 8th day. Blood glucose and alkaline phosphatase were measured by calorimetry. Level of malondialdehyde (MDA) and super oxide dismutase (SOD) concentrations were measured using the Avidin-Horseradish Peroxidise (HRP) Sandwich-Enzyme-Linked Immunosorbent Assay (ELISA) technique. Parametric data between the treatment and control groups were analyzed using the one-way Anova and the 95% confidence level significant difference test ($\alpha = 0.05$) with Duncan's post hoct test.

3. Results and Discussions

Identification of the active compound of *Tithonia diversifolia* (TD) leaves extract was conducted by Thin Layer Chromatography (TLC). The results of the TLC of TD leaves extract were positive for the flavonoid as active compounds (Figure 1.).

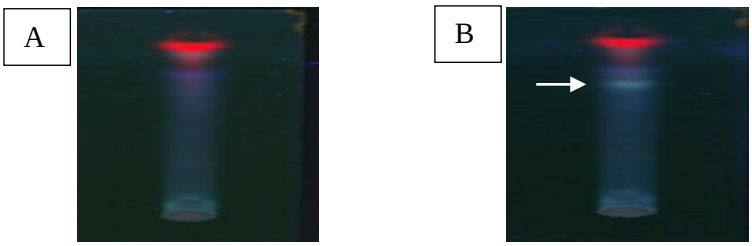


Figure 1. **A.** Before TLC with $AlCl_3$, **B.** Active compound of *Tithonia diversifolia* extract as flavonoid by TLC.

The chemical composition of the leaves, bark and roots of *T. diversifolia* contains saponins, polyphenols and flavonoids. Based on ethnomedicinal reviews, the chemical constituents and in vitro pharmacological properties of this plant have been identified, and it has been reported to have anti-malarial, anti-diabetic and anti-microbial properties. In addition, ethanol and methanol extracts from this plant have been found to have in vivo anti-inflammatory and anti-oxidative properties [8].

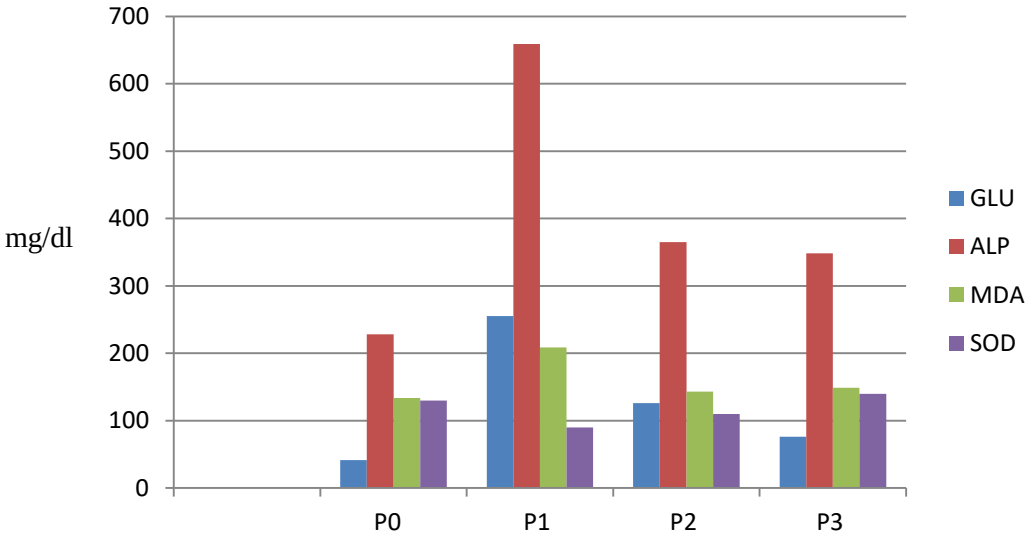
Diabetes mellitus (DM) is characterized by increased levels of glucose in the blood and often appears without symptoms. In type 2 diabetes mellitus, genetic and environmental factors have a considerable influence on the occurrence diabetes, including obesity, a high-fat and low-fibber diet, and lack of exercise [10,11]. Based on the examination of blood serum the following results were obtained in Table 1.

Table 1. TheAverage of Blood glucose (BG), ALP, MDA and SOD concentrations in the various groups.

Groups	BG (mg / dl),	ALP(mg / dl)	MDA(mg / dl),	SOD(mg / dl),
P0 (n=6)	41.3^{a}±09.3	228.3^{a}±084.2	133.5^{a}±07.9	0.13^{a}±0.1
P1 (n=6)	255.1^{b}±14.9	659.1^{b}±254.7	208.6^{b}±46.9	0.09^{b}±0.04
P2 (n=6)	126.1^{c}±14.3	364.8^{c}±93.1	143.9^{c}±06.1	0.11^{c}±0,04
P3 (n=6)	76.3^{d}±74.8	348.3^{c}±67.9	149.5^{c}±52.7	0.14^{a}±0.06

Superscript indicates a significant difference of $p \leq 0.05$, blood glucose (BG), alkaline phospatase (ALP), Malondialdehyde (MDA) and superoxide dismutase (SOD) n=number of rats; P0=control group, P1=positive group, P2=treatment group, P3= catechin group

The results of the study showed that the administration of ethanol extract of *Tithonia diversifolia* has effect of reducing blood glucose and alkaline phospatase concentrations in the treatment group. Feeding of per oral *Tithonia diversifolia* leaf extract dose of 100 mg/kg bw were decrease blood glucose levels at 126.1 mg/dl (group P2) significantly different with positive control group (P1).



P0=control group, P1=positive group, P2=treatment group, P3= catechin group

Figure 2. Effects of oral administration of *Tithoniadiversifolia* extracts on blood glucose, Alkaline phospatase, MDA and SOD in various groups.

Tithonia diversifolia leaves extract has been shown to reduce blood glucose and alkaline phospatase levels in Wistar rats in this study indicating that *Tithonia diversifolia* extract is proven in preventing diabetic rat by streptozotcin (STz) and high fat diets. Hypoglycaemic activity of *Tithonia diversifolia* leaves extract occurs after the metabolic process during the administration of a high fat diet and extracts treatment for 7 days. Blood glucose and alkaline phosphatase levels were decrease significantly in the treatment group *Tithonia diversifolia* extract (P2) compare with in the control positive group (P1) and the lowest blood glucose concentration in the group with cathecin (P3).The level of MDA concentration in the positive control was highest than treatment and the control group. Feeding by per oral *Tithonia diversifolia* leaves extract dose of 100 mg/kg bw can decrease MDA levels at 143 mg/dl (group P2) compared with positive control (P1) at 208 mg/dL but it's not significantly different with P3 group at 149 mg/dl. The results showed a decrease in blood glucose levels followed by increasing of superoxide dismutase (SOD) activity in the treatment group. In the group feeding with *Tithonia diversifolia* extract (P2), level of SOD concentration was increased significantly at 0.11 mg/dL compared to the positive control group (P1) at 0.09 mg/dL, and catechin group (P3) at 0.14 mg/dL. This indicates that the administration of *Tithonia diversifolia* leaves extract can stimulate anti-free radical activity to inhibit oxidative stress produced by diabetic rat.

3.1. Malondialdehyde (MDA)

Increased oxidative stress occurs in all treatment groups with the induction of streptozotocin and a high-fat diet. In the positive control group with extracts had the highest MDA levels and significant differences with MDA levels in the treatment group and the catechin group. Malondialdehyde (MDA) is a stable end product of fat peroxidation produced from interactions with free radicals in the phospholipid membrane in hopes. Malondialdehyde is found in the red blood cell membrane. As previously known, lipids / fat are the initial targets that will be damaged by free radicals. Therefore, MDA can also be used as a parameter to determine free radicals that try to damage body tissues [12].
Increasing of MDA level in diabetic rats were indicates that the more severe the level of diabetes mellitus, the more free radicals that attack the cell, so the higher the serum MDA content in the blood. Conversely, an increase in the amount of serum MDA also plays a role in the development of diabetes mellitus which leads to other micro vascular complications such as: nephropathy, retinopathy, and neuropathy [13]. Previously study [14] shows that serum MDA was found to be higher in people with diabetes mellitus who used insulin than diabetics without insulin therapy

High MDA levels indicate the presence of free radicals that occur by the process of metabolic disease in diabetes by means of fatty acid peroxidation. Increased MDA also means an increase in lipid peroxidation which can be an indication of a decrease in the amount of antioxidants in the body, both enzymatic antioxidants and non-enzymatic antioxidants [6].
Oxidized lipids can produce MDA as decomposition products. The mechanism for the formation of MDA involves the formation of prostaglandins, such as endoperoxide [15]. Increased MDA in serum, plasma, and various tissues occurs in people with diabetes mellitus [16]. In patients with diabetes mellitus complicated by myocardial infarction, MDA serum is found to be higher than that of people without diabetes mellitus without complications patients with type 2 diabetes with complications of myocardial infarction, there are more free radicals that attack the heart cells or pancreatic cells. Whereas, in patients with type 2 diabetes without complications, free radicals only attack pancreatic cells [12].

3.2. Superoxide dismutase (SOD)

Superoxide dismutase (SOD) is an antioxidant enzyme that acts as a catalyst in superoxide anion dismutase which is radical into hydrogen peroxide and oxygen molecules. SOD has a role in protecting cell and tissue damage caused by Reactive Oxygen Species (ROS). SOD is an antioxidant that acts against superoxide, both in the kidneys which are at risk of developing diabetes nephropathy or in eye tissue which is at risk of developing diabetes retinopathy. In excessive amounts, SOD and other antioxidants, will fight oxidative stress, reduce ROS levels, and increase the amount of antioxidant enzymes, thus preventing the occurrence of diabetes mellitus. Increased levels of SOD have been shown to reduce oxidative stress and neuronal apoptosis in mice, thus preventing diabetes. Research in diabetic induced mice found that there was a decrease in SOD and other antioxidant enzymes in liver tissue [17].
Decreasing the activity of SOD and other antioxidant enzymes is associated with an increase in free radicals in other organs, especially the pancreas, so that SOD and other enzymes focus on protecting pancreatic cells from damage. Another reason for the decline in this activity is that SOD and other antioxidant enzymes protect other tissues which are the effects of the development of diabetes mellitus, such as: kidney and retinal tissue; resulting in a decrease in SOD activity in the liver [16].
The administration of *Tithonia diversifolia* extract were showed to reduce glucose levels and stimulated anti-free radical activity to inhibit oxidative stress. This indicates that *Tithonia diversifolia* extract is beneficial in preventing streptozotcin-induced diabetes on rats.

4. Conclusions

Based on the research can be concluded that administration of oral TD extract could inhibit diabetic rat by decreasing in blood glucose and alkaline phosphatase concentrations. Antidiabetic of TD extract is increased SOD and lowering MDA concentration in rats.

Acknowledgments

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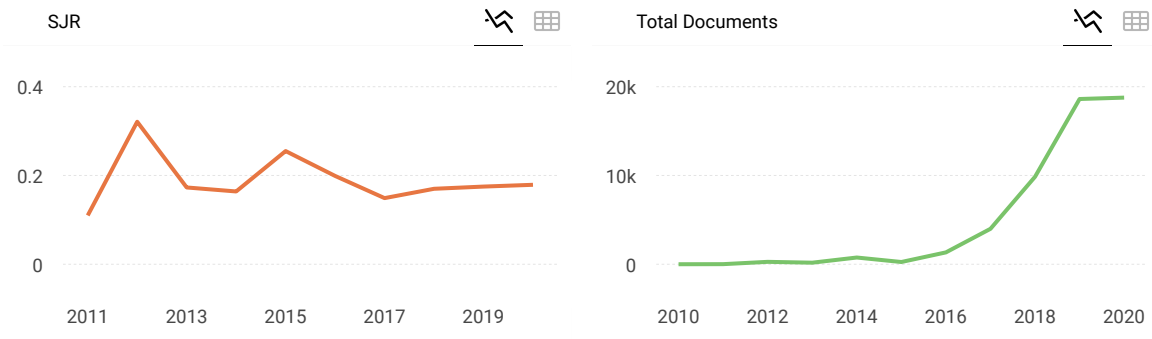

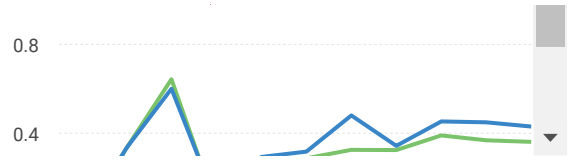

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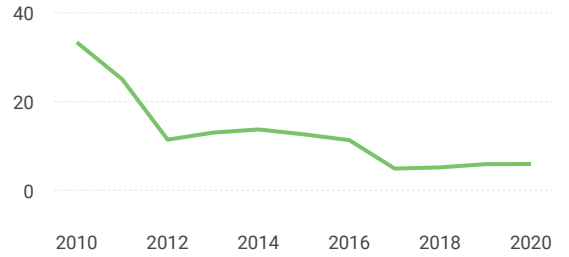

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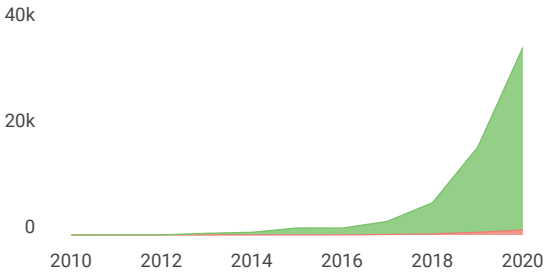

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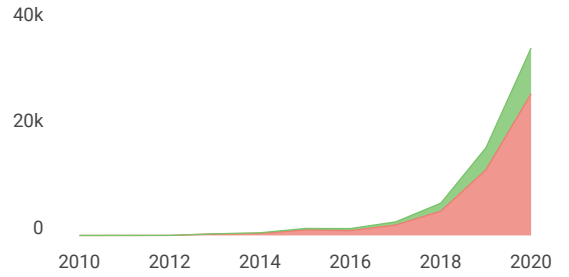

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