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The Influence of Ethanol Extract and Variety of Fractions from *Tithonia Diversifolia* on Blood Glucose Level of Male White Mice

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ABSTRACT

Tithonia diversifolia have properties of insulin to lower blood glucose levels, such as phenolic compounds, terpenoids and flavonoids. This study aimed to anti-hyperglycemic activity of ethanol extracts and n-hexane fraction, acetate-ethyl fraction, and the remaining fraction of leaves from *Tithonia diversifolia* on male white mice. This study use five treatment groups: control group is negative, extract, n-hexane fraction, acetate-ethyl fraction and the remaining fraction. Each group is given treatment by name the group, except for the control group. The dose used for each group is the same, ie 10.78 mg / kg. Treatment was given for 21 days, followed by taking the measurements of blood glucose levels on days 0, 7th, 14th and 21st. Calculation of blood glucose levels is done by cutting the tip of the mice's tail to get the blood was measured using glucometer. The results showed all dose treatment were not significantly affect blood glucose levels of male white mice decreased ($P > 0.05$). The research showed that ethanol extract, hexane fraction, ethyl acetate fraction, the remaining fraction leaves of *Tithonia diversifolia* have no ability to lower blood glucose levels male white mice.

Keywords: *Tithonia diversifolia*, diabetes melitus, glucose

INTRODUCTION

Kidney is the organ which has the primary function to eliminate product discharge body [4]. Diabetes included in the world's top ten causes of their decline in productivity and growth in humans. This makes diabetes a significant threat to the world in the 21st century. Globally, there have been 4.6 million people every year who have died from diabetes. In some countries, even found the children and young persons belonging to have died from lack of insulin without any prior diagnosis. [5].

One of the Indonesian plants is a plant *Tithonia diversifolia*. *Tithonia diversifolia* plant is commonly used as a cure wounds or bruises, and as a remedy against flatulence. Many also used as a cure leprosy, liver disease, diabetes drugs and anticancer properties. From research conducted by Asri Sulistijowati S and Educate Gunawan that *Tithonia diversifolia*, these plants contain active substances that belonged to the essential oils, alkaloids, flavonoids, saponins, triterpenoids and polyphenols. *Tithonia diversifolia* leaves 12 compounds contain at least 14 compounds terpenoids and flavonoids [6].

Tithonia diversifolia plant is one of the herbs used in curing people with diabetes mellitus. decoction of leaves of *Tithonia diversifolia* believed efficacious in treating diabetes mellitus[3]. Research has been done on this plant, one of which is a decrease in blood sugar levels in diabetic mice induced by alloxan. It is also useful as anti-inflammatory and analgesic activity, as well as antimalarial *Tithonia diversifolia* leaf extract in mice in vivo. [9].

The based on the above, then further research is to see the effect of some factions and leaf extract *Tithonia diversifolia* (*Tithonia diversifolia*) on blood glucose levels of male white mice.

MATERIALS AND METHODS

Tools and materials

The tools used are analytical balance (Satorius), microscopy (Olympus), micro pipette (Socorex), pipette, oral needles, animal cages, a pipette, needle oral, beakers, test tubes, beaker glass, bottles, vials, Glucometer (NESCO), rotary evaporator, separating funnel, tube racks, cage rat, a rat drinking, and knives.

Materials used are thick extract of *Tithonia diversifolia* [11], 70% ethanol (Brataco), aquadest (Brataco), Na CMC (Merck), n-hexane solution (Merck), and acetate ethyl solution (Merck).

Animals used are male white mice weight 20-25 g [1], and the age were 2 months before use acclimatization for one week at the Pharmacology Laboratory of the Faculty of Pharmacy, Andalas University.

Plant material

The aerial parts of *T. diversifolia* (Hemsl.) A. Gray were collected in Bukittinggi, Sumatera Barat in Mei 2016 and identified by Biology Department of Andalas University.

Extraction and Fractination

The dried aerial parts of *T. diversifolia* (1,5 kg) were macerated with 70% EtOH at room temperature [7]. The EtOH extract was concentrated to an aqueous residue and suspended with water. The concentrated extract was fractination by n-hexane and acetate-ethyl solution [2].

Dosage Planning

- Negative control group (K1) was given physiological solution (0.9%).
- Test group (K2), was given the suspension of ethanol extract at a dosage of 10, 78 mg/ kgbw for 21 days.
- Test group (K3), was given the suspension of n-hexane fraction at a dosage of 10, 78 mg/ kgbw for 21 days.
- Test group, (K4), was given the suspension ethyl- acetate fraction at a dosage of 10, 78 mg/ kgbw for 21 days.
- Test group (K4), was given the suspension of remaining fraction at a dosage of 10, 78 mg/ kgbw for 21 days.

RESULTS AND DISCUSSION

Identification of *Tithonia diversifolia* has been done in the herbarium ANDA, Universitas Andalas Padang. The results of the identification of states that samples be employed in this study are *Tithonia divrsifolia* (Hemsl) A. Gray



Picture 1. Photo of *Tithonia divrsifolia* (Hemsl) A. Gray

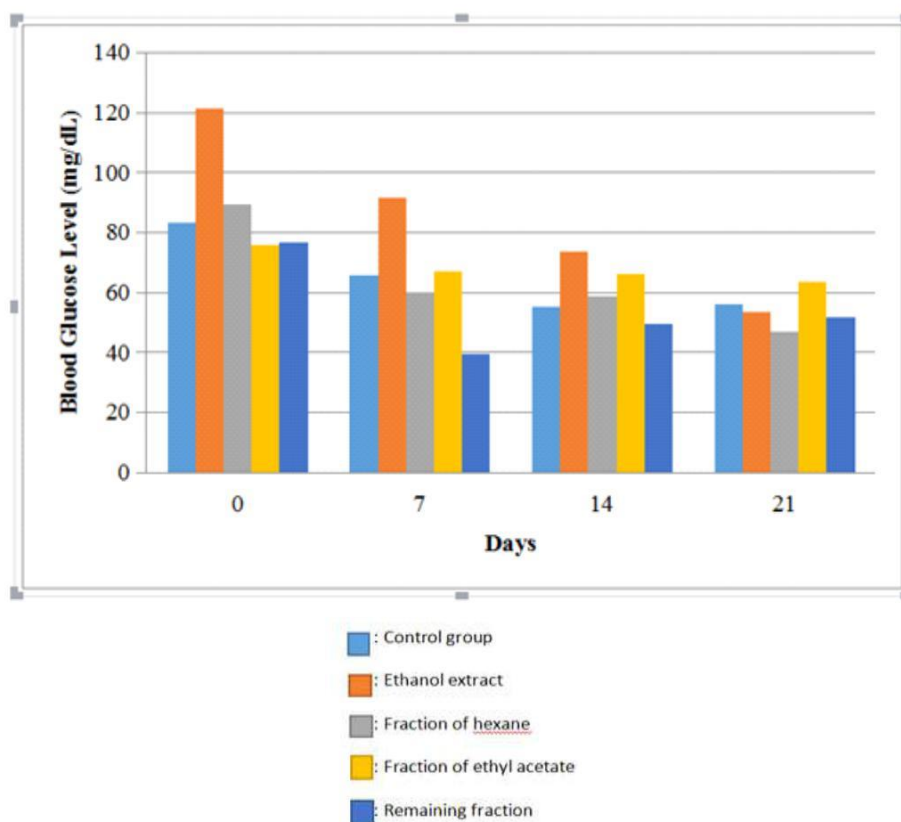
The preparation used in the form of *Tithonia diversifolia* (Hemsl) A. Gray extract, which in previous studies has been proven as an anti-diabetic agent [10] and decrease the blood glucose level [11].

In this study the effect of extract ethanol and variety of fractionation with same dosage to blood glucose level. Observation do on days 0, to see normal value before the given induction for 21 days, then checks to day 7,14, 21 used glucometer [8] .

In this experiment the animals were divided into 5 groups of the first group which is negative control only given suspension of NaCMC the second, third , fourth, fifth is a group that was given treatment in induction with extract dan variety of fractionation for 21 days at a dosage 10,78 mg/kgbw. From the results, the data is quite diverse with a standard deviation in each group were not big enough. Differences arising is a reasonable due to differences physiological condition of each individual such as weight, age, the body's metabolic processes of each animal during treatment will affect blood sugar measured. Measurement of blood sugar levels done on day 8, -15 and -22. The parameters measured were the blood sugar levels are influenced by the type of treatment and time. This type of treatment or the group observed that the test group which gives effect more effective in decreasing blood sugar levels, then for a time duration of breast be seen with the expected effect of the drop in their blood sugar levels better.

When viewed as a percentage compared with the control group, the group extracts and fractions residual value losses against blood glucose. While groups of hexane and ethyl acetate group experienced no significant decrease in blood sugar levels compared with controls. Further followed by statistical analysis. The statistical analysis used was ANOVA (analysis of variants). One of the requirements to perform statistical analysis of test data obtained are to be normally distributed. Therefore, the processing of the blood glucose levels were obtained beginning with test normalias according to Shapiro-Wilk. The normality of the test shows that the blood glucose level data is normally distributed with significant values > 0.05, the data processing can be followed by analysis of variance.

Table 1. The bar chart showed comparison of the blood glucose levels of the male white mice between the duration and treatment of *Tithonia diversifolia*



ANOVA test used is a two-way ANOVA for factors that affect the observed there are two types of treatment or group and duration of administration. Blood sugar levels are calculated on a day-to-8, -15, and -22 with different variations of the test group and the type fraction ketal leaf extract *Tithonia diversifolia* showed mixed results. For this type of treatment yielded significant results or meaningful 0.000. This effect is due to figures that were significantly ($p < 0.05$), which means the type of treatment or a group giving a real difference to the levels of blood sugar levels. But for a time also results in a significant or meaningful to the value of 0,000. which means $p < 0.05$ so time is significantly different on blood sugar levels. Likewise for the correlation between the type of treatment or group with the time showed no significant or meaningful results, namely 0.031 ($p > 0.05$). This illustrates that the overall group and time was not significantly different from the type of treatment.

Table 2. Effect of dose and duration of administration of ethanol extract and fraction of *Tithonia diversifolia* leaves on average blood glucose levels white male mice in each treatment group

| Group | Day 0 | Day 28 | Blood Glucose Level (%) |
|-----------------------|-------------|-------------|-------------------------|
| Ethanol Extract | 121,5±17,25 | 53,75±11,00 | 55,76 |
| N-hexana fraction | 89,43±27,20 | 47,14±6,09 | 47,28 |
| Acetate-etyl fraction | 75,83±31,42 | 63,50±20,52 | 16,26 |
| Residual fraction | 76,57±38,40 | 52,00±18,38 | 32,08 |

For this type of treatment followed by a further test of Duncan's multiple range which generates 3 subset. From 3 subset formed seen that there are two groups of tests are on differences compared with the control group. However, due to the significance value greater than 0.05, the type of treatment does not give a real difference to the decrease in blood sugar levels (Appendix 3, Table 14). It concluded that the ethanol extract of leaves of *Tithonia diversifolia* (Hemsl.) A. Gray did not give a significant effect on blood sugar levels.

Table 3 Further test results Duncan's multiple range against darahh glucose levels of factor in the type of treatment / group (SPSS 17.0)

| Level | | Duncan ^{a,b,c} | | |
|------------------------|----|-------------------------|-------|-------|
| Group | N | Subset | | |
| | | 1 | 2 | 3 |
| Residual fraction | 28 | 54.46 | | |
| control | 32 | 59.31 | 59.31 | |
| n-hexane fraction | 28 | 63.93 | 63.93 | |
| Acetate-ethyl fraction | 24 | | 68.13 | |
| extract | 16 | | | 85.12 |
| Sig. | | .112 | .140 | 1.000 |

CONCLUSION

Based on research that was done the induction extract ethanol and variety of fractination of *Tithonia diversifolia* to male white mice, it can make conclusion :

Data research provides results that variations fraction and leaf extracts *Tithonia diversifolia* not leave blood sugar levels decrease with time male white mice significantly, because based on data obtained Annova bidirectional significance value > 0.05

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