Molekul, Vol. 13. No. 1, Mei 2018 : 72 – 79 doi: 10.20884/1.jm.2018.13.1.417

Ethanol Extract of *Tithonia diversifolia* (Hemsley) A Gray Standardized Ameliorates Hyperglycemia, Polyphagia, and Weight Loss in Diabetic Rats

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Received February 7, 2018; **Accepted** April 3, 2018; **Available online** May 31, 2018

ABSTRACT

Diabetes mellitus is a state of chronic hyperglycemia which causes various complications. Traditionally, The *Tithonia diversifolia* (Hemsley) A Gray leaf has long been used for the treatment of diabetes. The aim of this study is to investigate the effect of the *T. diversifolia* leaf on blood glucose, polyphagia, and weight loss in a diabetic rat model. Rats were made diabetic with intraperitonial injection of Nicotinamide and Streptozotocin and divided into 5 groups. Group 1 were healthy rats, group 2 were diabetic rats, while groups 3, 4, and 5 were diabetic rats treated with 25, 50, and 100 mg/kg body weight of 70% ethanol extract of *Tithonia diversifolia* leaf respectively for 28 days. Blood was taken after treatment for measuring glucose. The ethanol extract of *T. diversifolia* leaf decreased blood glucose in diabetic rats ($P<0.05$). The ethanol extract of *T. diversifolia* leaf significantly suppresses polyphagia and improves diabetic rat weight ($P <0.05$). In conclusion, *Tithonia diversifolia* ethanolic extract has anti-hyperglycemic effect and ameliorated the effect of diabetes mellitus symptoms, namely polyphagia and weight loss.

Keywords: Anti-hyperglycemia, Diabetes Mellitus, Polyphagia, *Tithonia diversifolia*

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder which prevalence continues to rise. Prevalence of DM in 2010 reached 2.3% and estimated in 2030 reached 7.7% (Shaw, Sicree, & Zimmet, 2010). Furthermore, the prevalence of DM is also higher in developing countries compared with developed countries. In Indonesia, the prevalence of DM continues to increase, in 2007 by 2.3% and in 2013 reached 5.3% (Kementerian Kesehatan Republik Indonesia, 2013). Diabetes mellitus is usually in the elderly, but it has begun to shift to young adults, teenagers, and even children due to increased sedentary life, poor diet, and obesity (International Diabetes Federation, 2017).

Diabetes mellitus is characterized by chronic hyperglycemia due to insufficient insulin production, insulin action failure, or both (American Diabetes Association, 2008; Ozougwu, Obimba, Belonwu, & Unakalamba, 2013). Insulin plays a role in moving glucose into cells to be converted into energy. Insulin deficiency causes glucose to not move into the cells that affect glucose accumulated in the blood (Mane, Chaluvaraju, Niranjan, Zaranappa, & Manjuthej, 2012). Chronic hyperglycemia causes various complications in DM such as retinopathy, neuropathy, nephropathy, cardiovascular complications and ulceration (Bastaki, 2005).

Polyphagia and weight loss are typical symptoms of DM (Olokoba, Obateru, & Olokoba, 2012). Glucostat Theory of Feeding Regulation describes the phenomenon of polyphagia in DM. In the hypothalamus, there is a center of satiety and starvation that regulates food intake. The adequacy of glucose in cells activates the satiety center in the hypothalamus. In contrast, glucose-deficient cells activate the starvation center that triggers a continuous desire to eat (Ganong, 2006; Guyton & Hall, 2006). Furthermore, lipolysis is responsible for weight loss in DM (Mohamed, Mohamed, & Rashid, 2004).

The use of conventional medicines as anti-hyperglycemia has been reported to have some side effects (Defronzo, Mehta, & Schnure, 2015). Based on this, it needs a breakthrough in the treatment of DM through the discovery of new drugs by exploring the medicinal plants that have traditionally been used to treat anti-diabetes. Currently, there are many studies related to the anti-hyperglycemic

activity of herbs. Medicinal plants with anti-hyperglycemic activity can be used as an alternative to treat DM. *Tithonia diversifolia* (Hemsl.) A. Gray, known as Mexican sunflower, is found in America, Asia, and Africa. In Indonesia, *Tithonia diversifolia* plants have different names i.e. "Kembang Bulan", "Rondo Noleh" or "Rondo Semoyo", "Kirinyu" (Sunda), and "Kayu Paik" (Minang). Traditionally, *Tithonia diversifolia* leaves have been widely used as anti-hyperglycemic. Furthermore, *Tithonia diversifolia* contains flavonoids, diterpenes, and sesquiterpene lactones which are known to have antioxidant effects (Sharma, Singh, & Singh, 2013).

Medicinal plant with high antioxidant levels are very important to fight oxidative stress in DM (Nasri, Shirzad, Baradaran, & Ra, 2015).Various studies have shown that *Tithonia diversifolia* also has anti-cancer agents and anti-inflammatory activity (Owoyele, Wuraola, Soladoye, & Olaleye, 2004; Wahyuningsih, Wirohadidjojo, Hidayat, & Sadid, 2015). However, there are very few studies related to the influence of *Tithonia diversifolia* leaves on DM. This study aimed to determine the effect of *Tithonia diversifolia* on blood glucose, polyphagia, and weight loss in diabetic rats.

EXPERIMENTAL SECTION

Chemicals

Streptozotocin (Nacalai), nicotinamide (Sigma), Glucose oxidase/peroxidase reagent (sigma), Pulvis Gummi Arabicum (PGA), pure water, vitamin free casein, DL-methionine, whole wheat flour, vitamin mix, AIN 76 mineral mix, choline chloride, alphacelnon nutritive bulk, corn oil, normal saline, buffer citrate.

Extraction

We use 70% ethanol extract of *Tithonia diversifolia* leaves from the Herbal Laboratory of Pharmacology and Therapeutic Department, Medical Faculty, Universitas Gadjah Mada. One kg of *T. diversifolia* leaves dried powder was macerated by 2 liters of ethanol (70%) for 72 hours. The filtrate was separated by filtration (Buchner funnel) and maceration was repeated 3 times. The second and third maceration was macerated for 24 hours. The filtrates obtained were combined and evaporated in vacuo to dryness by rotary evaporator. The ethanol extract has been standardized with the Tagitinin C marker by using TLC chromatography and TLC densitometer and has been reported. Tagitinin C is an active compound found in *Tithonia diversifolia* plant. TLC chromatography shows the position of Tagitinin C marker is at Rf value of 0.32. Based on TLC densitometer, Tagitinin C concentration at 5000 µg of ethanol extract 70% *Tithonia diversifolia* is equal to 2.87% (Wahyuningsih, Wijayanti, Budiyanto, & Hanafi, 2015; Wahyuningsih et al., 2015).

Animal and care

Sprague Dawley adult rats (10 weeks, 175-250 g) were used for this study. The rats were acclimatized for 7 days in an air-conditioned room at 25^{o} C with 12h light and 12 dark cycles with free access to food (semipurified diet for rats and mice) and water. The research proposal was approved by the Ethical Clearance Committee of Integrated Research and Testing Laboratory, Universitas Gadjah Mada, certificate number 00059/04/LPPTNIII/2016.

Experimental design

Twenty rats were divided into 5 groups. Group 1 consisted of healthy control rats, group 2 served as diabetic control rats, group 3 as diabetic rats treated with ethanol extract of *Tithonia diversifolia* leaf standardized dose of 25 mg/kg BW, group 4 as diabetic rats treated with ethanol extract of *Tithonia diversifolia* leaf standardized dose of 50 mg/kg BW, and group 5 as diabetic rat treated with ethanol extract of *Tithonia diversifolia* leaf standardized dose of 100 mg/kg BW. Administration of ethanol extract of *Tithonia diversifolia* leaf for 28 days.

Induction of diabetes and measurement of blood glucose

Diabetes was induced by intraperitoneal (i.p) injection of nicotinamide (NA) in normal saline at a dosage of 230 mg/kg body weight and streptozotocin (STZ) in buffer solution (0.1 mol/l of citrate, pH 4.5) at a dosage of 65 mg/kg body weight. Injection of NA 15 min was before injection of STZ. At this dose, STZ induced hyperglycemia (blood glucose > 150 mg/dl) in rats was measured at 120 hours post-injection by GOD PAP method (Ghasemi, Khalifi, & Jedi, 2014). After the treatment is finished, blood glucose levels are checked to determine the effectiveness of the treatment.

Body weight and food intake

The weight of rats weighed every week using a digital scales "Mettler Toledo". Feeding intake is calculated based on feeding as much as 15 g/day minus the residual feed. The residue of the feed is separated from rats stool and weighed daily by using digital scales.

Statistical analysis

Statistical analyses were performed using One-Way ANOVA followed by post hoc (LSD) tests. The paired-t-test was performed to determine differences before and after treatment. Differences were considered to be significant at $p< 0.05$ compared to the control group.

RESULTS AND DISCUSSION

In this study, we studied the activity of *Tithonia diversifolia* leaves against hyperglycemia, polyphagia, and weight loss. *Tithonia diversifolia* leaf-related studies as anti-hyperglycemia have been done before, but studies examining the effects of *Tithonia diversifolia* leaves in suppressing polyphagia and improving weight in diabetic rats have never been done. We managed to prove that *Tithonia diversifolia* leaves have the ability to lower blood glucose levels, suppress polyphagia, and improve weight in diabetic rats.

We used a combination of nicotinamide and streptozotocin to make DM type 2. Streptozotocin is a diabetogenic agent that destroys pancreatic beta cells through its bonding with GLUT 2 glucose transporter. Streptozotocin causes DNA damage resulting in increased activity of poly (ADP-ribose) polymerase (PARP-1) and impacts on the reduction of NAD + and ATP resulting in loss of pancreatic beta energy and apoptosis (Ghasemi et al., 2014; Goud, Dwarakanath.V, & Swamy, 2015). Streptozotocin has a long half-life so it is more stable in making hyperglycemia and the damage caused more specifically that in beta cells (Islam, Rupeshkumar, & Reddy, 2017). Nicotinamide inhibits the activity of the PARP-1 enzyme thus preventing the depletion of NAD + and ATP in STZ-exposed cells (Szkudelski, 2012). The use of nicotinamide is intended to prevent severe pancreatic damage due to the direct use of STZ can result in the destruction of pancreatic beta cells and induce an autoimmune process against pancreatic beta cells characteristic of DM type1. The combination of NA-STZ has been widely used to make animal models of DM type 2.NA-STZ induction has managed to make rats blood glucose levels higher than normal rats.

Tithonia diversifolia also proved to be antihyperglycemic in KK-Ay strains (Miura, Nosaka, Ishii, & Ishida, 2005). In this study, *Tithonia diversifolia* had a smaller effective dose than previous studies. This finding is an important concern because high dosages are associated with the toxicity of natural ingredients. The smaller doses in the herbs can avoid the risk of toxicity of the plant and the use of natural materials can be as little as possible so as to be more visible in the application.

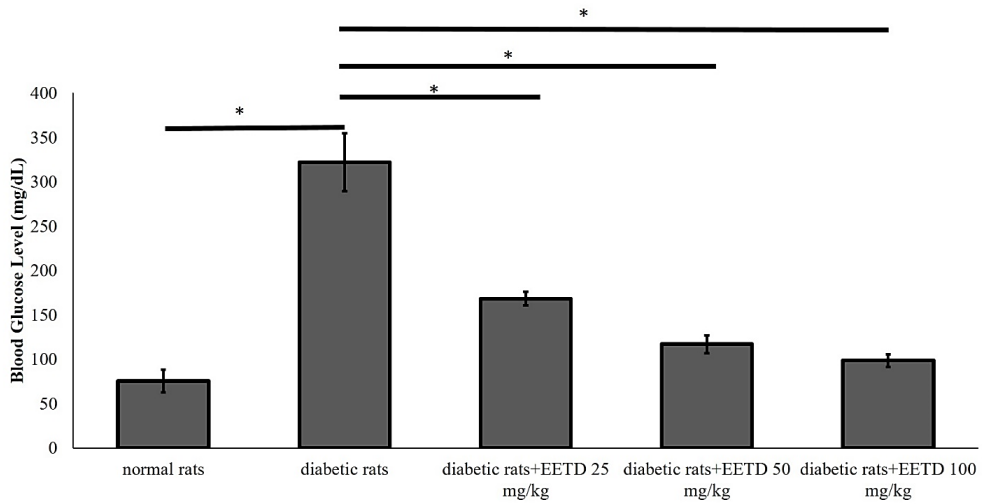
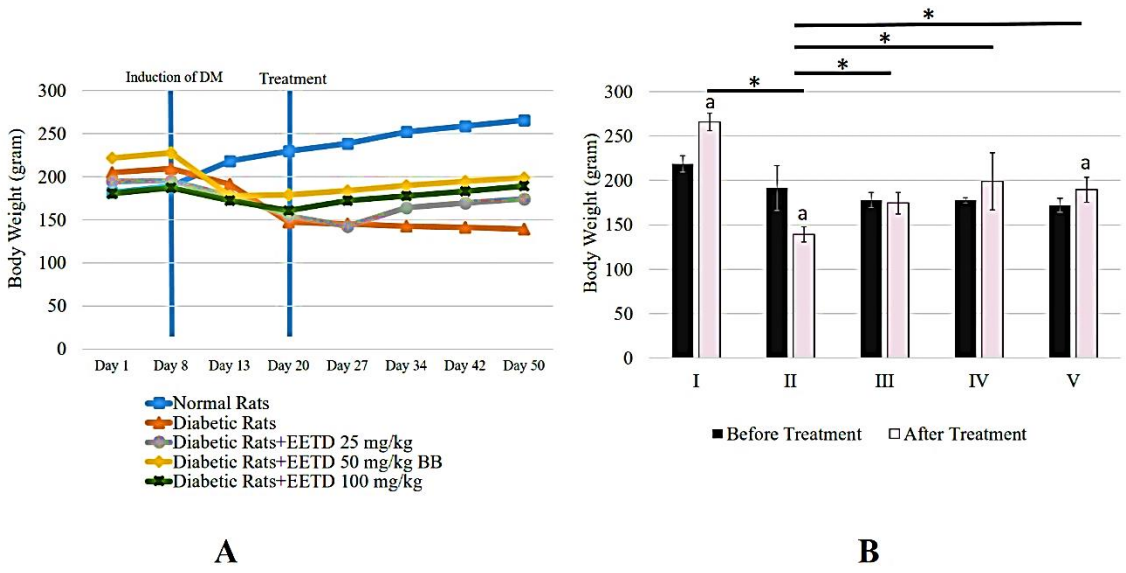


Figure 1. Effect of Extract Ethanol of *Tithonia diversifolia* leaf on blood glucose level (mg/dL) in diabetic rats. *P<0.05 significant difference compared with the diabetic control group



A **B**

Figure 2. A. Body weight changes in Rats. Day 1= early weight of rats, day 8=weight of rats before induction of DM, day 13= weight of rats five days after induction of DM, day 20 = weight of rats after chronic diabetes/before administration of extract, days 27 to 50= weight of rats during the administration of extract. **B.** Effect of Extract Ethanol of *Tithonia diversifolia* leaf on body weight (g) in diabetic rats. I= normal rats, II= diabetic rats, III= diabetic rats+ethanol extract of *Tithonia diversifolia* at dose 25 mg/kg, IV= diabetic rats+ethanol extract of *Tithonia diversifolia* at dose 50 mg/kg, V= diabetic rats+ethanol extract of *Tithonia diversifolia* at dose 100 mg/kg. $^{a}P<0.05$ significant difference compared with before treatment. *P<0.05 significant difference compared with diabetic control group.

The previous study examined the toxicity of *Tithonia diversifolia* leaf and obtained the result that *Tithonia diversifolia* leaf water extract had LD_{50} (lethal dose) of 120 mg/kg BW and the maximum tolerated dose was 100 mg/kg BW (Oyewole, Magaji, &Awoyinka, 2007). Furthermore, toxicity test of 70 % ethanol extract of *Tithonia diversifolia* leaves proved that 70% ethanol extract of *Tithonia diversifolia* leaves a single dose of 400-1600 mg/kg BW was a toxic dose due to damage to kidney and liver, while repeated dose of 400 mg/kg BW caused damage irreversible in the kidneys and liver (Elufioye, Alatise, Fakoya, Agbedahunsi, & Houghton, 2009). Other studies confirmed that a safe dose of *Tithonia diversifolia* water extract was at a dose below 100 mg/kg BW (Passoni, Oliveira, Chagas-Paula, Gobbo-Neto, & Da Costa, 2013).

Figure 2 presents data on the effect of ethanol extract of *Tithonia diversifolia* leaf to diabetic rat weight. The results showed DM causes significant weight loss. In normal rats, weight gain occurred until the end of the study. Whereas in diabetic rats, weight loss occurred until the end of the study. In groups 3, 4 and 5, weight loss was before ethanol extract of *Tithonia diversifolia* leaves, but after administration of ethanol extract of *Tithonia diversifolia* leaves, the rat weight increased until the end of the study (**Figure 2.A**). We performed paired t-test analysis to determine differences before and after treatment. The statistic test stated that there was a difference of body weight between before and after treatment in normal rats group, diabetic rats without treatment, and diabetic rats treated with ethanol extract of *Tithonia diversifolia* leaves dose of 100 mg/kg BW. In the normal group of rats and diabetic rats treated with ethanol extract of *Tithonia diversifolia* leaves dose of 100 mg/kg BW occurred a significant weight gain, otherwise in the group of diabetic rats without treatment there was a significant weight loss. The ANOVA-one way test proved that there was a difference in body weight in the normal group of rats and the ethanolic extract treatment of *Tithonia diversifolia* leaves of all doses compared with diabetic rats (**Figure 2.B**). Weight loss is caused by insulin resistance that affects glucose failure in cells. Failure of insulin action causes the occurrence of lipolysis. This leads to fat disassembly and lowering the fat period (Adiwijono & Ahmad

H., 1993; Ashaeryanto, Tiara & Diana, 2011; Krauss, 2004).

Weight loss in diabetic rats without treatment was not in line with dietary intake. The data of feeding intake was obtained from the average intake of food each day before and after treatment. **Figure 3** showed the food intake of diabetic rats without treatment has increased significantly, although the weight of diabetic rats continues to decline. In contrast to other groups that were more stable in food intake. Feeding intake in the group of normal rats and diabetic rats treated with of ethanol extract of *Tithonia diversifolia* leaves at all doses did not differ significantly between before and after treatment. These results indicate that an increase in dietary intake in the diabetic rats' group as one of the symptoms of hyperglycemia is polyphagia. In other studies, it has been shown that the induction of streptozotocin increases feed intake in mice. Chronic hyperglycemia has a positive association with polyphagia but is negatively correlated with weight (Tian et al., 2010).

Tithonia diversifolia has been shown to improve insulin resistance (Miura et al., 2005). Furthermore, through in vitro studies using HepG2 cells, it is known that the compounds of the sesquiterpene lactone, thyrotundin and tagitinin A, can increase the activity of peroxisome proliferator-activated receptors (PPARs) γ which is to increase insulin sensitivity (Lin, 2012). Sesquiterpene lactones from *Tithonia diversifolia* plant also proved to increase glucose uptake in 3T3-L1 adipocyte cell cultures with glucose exposure (Zhao, Li, Chen, Xi, & Sun, 2012). The glucose that moves in the cell prevents lipolysis on adipose tissue, so there is no breaking of the fatty period, and the weight of the diabetic rat can gradually improve (Krauss, 2004).

Furthermore, satiety centers in the hypothalamus will also be activated due to the adequacy of glucose in the cell and reduce polyphagia in diabetes. Increased cell glucose uptake, reduced lipolysis, and gluconeogenesis result in reduced blood glucose concentrations (Janani & Ranjitha Kumari, 2015). *Tithonia diversifolia*'s ability to increase insulin sensitivity is responsible for reducing hyperglycemia, improving feed intake, and increasing weight in diabetic rats.

The ability of *Tithonia diversifolia* leaf extract activates PPARs γ as well as the conventional drug work mechanism of DM i.e. thiazolidinediones in controlling blood sugar levels. Activation of PPARs γ can increase the expression of a number of genes related to lipid and glucose metabolism and regulate insulin transcription in pancreas β cells (Grygiel-Górniak, 2014; Leonardini, Laviola, Perrini, Natalicchio, & Giorgino, 2009). However, in its development thiazolidine-diones have a pleiotropic effect that increases the risk of miocard infarction as reported in previous studies (Defronzo et al., 2015), so that *Tithonia diversifolia* leaf extract can be a complementary or alternative therapy in the treatment of DM.

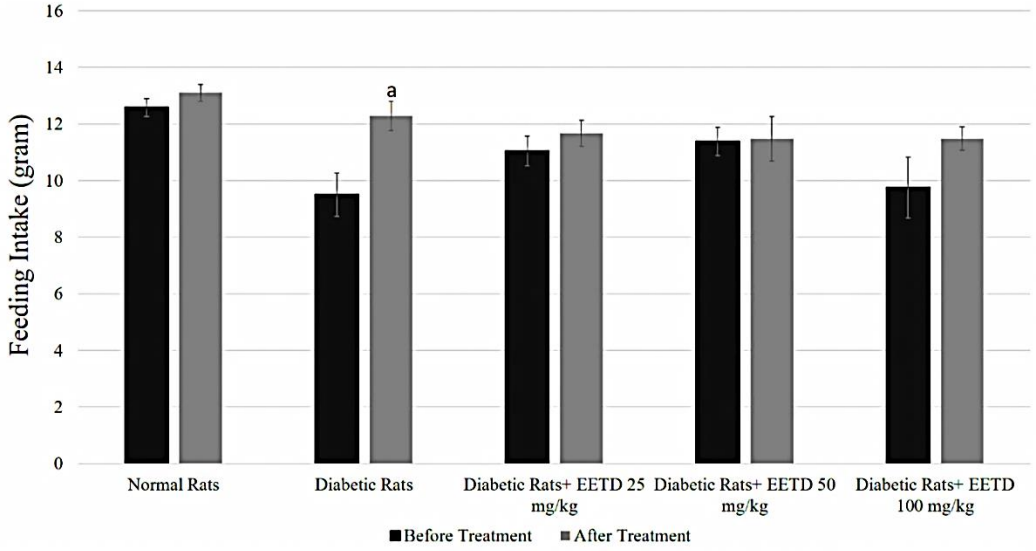


Figure 3. Effect of Extract Ethanol of *Tithonia diversifolia* leaf on feeding intake (gram) in diabetic rats. [a]P<0.05 significant difference compared to before treatment.

The ameliorative effect of *Tithonia diversifolia* leaf also due to its antioxidant effect which can decrease oxidative stress caused by diabetes. Diabetes mellitus leads to increased production of reactive oxygen species (ROS) and decreased antioxidants that play an important role in the complications of DM (Rochette, Zeller, Cottin, & Vergely, 2014). The antioxidant effect of *Tithonia diversifolia* extract has been reported to reduce free radicals by its free radical scavenger capacity (Di Giacomo et al., 2015; Tania P et al., 2016). Thongsom et al. demonstrated that total antioxidant content of *Tithonia diversifolia* was 93.09±37.91 µMTEAC/mg dry weight. *Tithonia diversifolia* also has the ability to decrease lipid peroxidation levels in mice with induced diabetes (Thongsom et al., 2013). The antioxidant activity is strongly related to flavonoids, saponin, phenol, terpenoid, and alkaloids which can prevent the formation of ROS due to diabetes and consequently inhibit lipid peroxidation (Tania P et al., 2016; Thongsom et al., 2013).

CONCLUSION

NA-STZ induction can increase blood glucose levels along with typical symptoms of diabetes mellitus i.e. polyphagia and weight loss. Oral administration of ethanol extract of *Tithonia diversifolia* leaf at a dose of 100 mg/kg BW was proven to increase weight, suppress polyphagia, and decreased blood glucose in a diabetic rat model.

ACKNOWLEDGEMENT

The authors would like to thank Mr. Yuli, animal house laboratory of PAU, Universitas Gadjah Mada, Mr. Ngakirno, pharmacology and therapy department, medical, faculty, Universitas Gadjah Mada, Mr. Parno and Mrs. Nia, physiology department, medical faculty, Universitas Gadjah Mada for their help and use of laboratory facilities. This publication is a part of the Fauziyah's dissertation.

REFERENCES

ADA. (2008). Diagnosis and classification of diabetes mellitus. Diabetes Care, 31(SUPPL. 1), 55–60.

Adiwijono & Ahmad H. (1993). Dislipidemia pada Diabetes Mellitus Tipe II: Patofisiologi dan Pendekatan Terapi. Berkala Ilmu Kedokteran.

Ashaeryanto, Tiara, I. ., & Diana, K. (2011). Berat Badan Menurun (Modul Tuto). FK Universitas Haluoelo Kendari.

Bastaki, S. (2005). Review Diabetes mellitus and its treatment. Int. J. Diabetes Metabol, 13, 111–134.

Defronzo, R. A., Mehta, R. J., & Schnure, J. J. (2015). Pleiotropic Effects of Thiazolidinediones : Implications for the Treatment of Patients With Type 2 Diabetes Mellitus. Hospital Practice, 41(2), 132–147.

Di Giacomo, C., Vanella, L., Sorrenti, V., Santangelo, R., Barbagallo, I., Calabrese, G., … Acquaviva, R. (2015). Effects of *Tithonia diversifolia* (Hemsl.) A. Gray Extract on Adipocyte Differentiation of Human Mesenchymal Stem Cells. Plos One, 10(4), e0122320.

Elufioye, T. O., Alatise, O. I., Fakoya, F. a., Agbedahunsi, J. M., & Houghton, P. J. (2009). Toxicity studies of *Tithonia diversifolia* A. Gray (Asteraceae) in rats. Journal of Ethnopharmacology, 122(2), 410–415.

Ganong, W. . (2006). Review of Medical Physiology, (21st ed.). California: Lange Medical Publications.

Ghasemi, A., Khalifi, S., & Jedi, S. (2014). Streptozotocin-nicotinamide-induced rat model of type 2 diabetes (review). Acta Physiologica Hungarica, 101(4), 408–420.

Goud, B. J., Dwarakanath.V, & Swamy, B. K. C. (2015). Streptozotocin - A Diabetogenic Agent in Animal Models. IJPPR. Human, 3(1), 253–269.

Grygiel-Górniak, B. (2014). Peroxisome proliferator-activated receptors and their ligands: nutritional and clinical implications – a review. Nutrition Journal, 13, 1–10.

Guyton, A. C., & Hall, J. E. (2006). Textbook of Medical Physiology. Philadelphia: W.B. Saunders.

International Diabetes Federation. (2017). IDF Diabetes Atlas 8th Edition.

Islam, M., Rupeshkumar, M., & Reddy, K. B. (2017). Streptozotocin is more convenient than Alloxan for the induction of Type 2 diabetes. International Journal of Pharmacological Research, 7(1), 6–11.

Janani, C., & Ranjitha Kumari, B. D. (2015). PPAR gamma gene - A review. Diabetes and Metabolic Syndrome: Clinical Research and Reviews, 9(1), 46–50.

Kementerian Kesehatan Republik Indonesia. (2013). Riset Kesehatan Dasar.

Krauss, R. M. (2004). Lipids and Lipoproteins in Patients With Type 2 Diabetes. Diabetes Care, 27, 1496–1504.

Leonardini, A., Laviola, L., Perrini, S., Natalicchio, A., & Giorgino, F. (2009). Cross-Talk between PPARγ and Insulin Signaling and Modulation of Insulin Sensitivity. Hindawi, 1–12.

Lin, H. R. (2012). Sesquiterpene lactones from *Tithonia diversifolia* act as peroxisome proliferator-activated receptor agonists. Bioorganic and Medicinal Chemistry Letters, 22(8), 2954–2958.

Mane, K., Chaluvaraju, K., Niranjan, M., Zaranappa, T., & Manjuthej, T. (2012). Review of insulin and its analogues in diabetes mellitus. Journal of Basic and Clinical Pharmacy, 3(2), 283–93.

Miura, T., Nosaka, K., Ishii, H., & Ishida, T. (2005). Antidiabetic effect of Nitobegiku, the herb *Tithonia diversifolia*, in KK-Ay diabetic mice. Biological & Pharmaceutical Bulletin, 28(11), 2152–2154.

Mohamed, E., Mohamed, M., & Rashid, F. A. (2004). Dyslipidaemic pattern of patients with type 2 diabetes mellitus. Malaysian Journal of Medical Sciences, 11(1).

Nasri, H., Shirzad, H., Baradaran, A., & Ra, M. (2015). Antioxidant plants and diabetes mellitus. J Res Med Sci, 20, 491–502.

Olokoba, A. B., Obateru, O. A., & Olokoba, L. B. (2012). Type 2 diabetes mellitus: A review of current trends. Oman Medical Journal, 27(4), 269–273.

Owoyele, V. B., Wuraola, C. O., Soladoye, A. O., & Olaleye, S. B. (2004). Studies on the anti-inflammatory and analgesic properties of *Tithonia diversifolia* leaf extract. Journal of Ethnopharmacology, 90(2–3), 317–321.

Oyewole, I. O., Magaji, Z. J., & Awoyinka, O. A. (2007). Biochemical and toxicological studies of aqueous extract of *Tithonia diversifolia* (Hemsl.) leaves in wister albino rats. Journal of Medicinal Plants, 1(September), 030–033.

Ozougwu, J. C., Obimba, K. C., Belonwu, C. D., & Unakalamba, C. B. (2013). The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. Journal of Physiology and Pathophysiology, 4(4), 46–57.

Passoni, F. D., Oliveira, R. B., Chagas-Paula, D. A., Gobbo-Neto, L., & Da Costa, F. B. (2013). Repeated-dose toxicological studies of *Tithonia diversifolia* (Hemsl.) A. gray and identification of the toxic compounds. Journal of Ethnopharmacology, 147(2), 389–394.

Rochette, L., Zeller, M., Cottin, Y., & Vergely, C. (2014). Diabetes, oxidative stress and therapeutic strategies. Biochimica et Biophysica Acta - General Subjects, 1840(9), 2709–2729.

Sharma, S. K., Singh, L., & Singh, S. (2013). A review on medicinal plants having antioxidant potential. Indian Journal of Research in Pharmacy and Biotechnology, 1(3), 404–409.

Shaw, J. E., Sicree, R. a., & Zimmet, P. Z. (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Research and Clinical Practice, 87(1), 4–14.

Szkudelski, T. (2012). Streptozotocin-nicotinamide-induced diabetes in the rat. Characteristics of the experimental model. Experimental Biology and Medicine, 237, 481–490.

Tania P, M., Castilo B, D. Del, Serrão P, C. D., Lobato R, A. B., Silva R, R., Oliveira P, F. De, … Susan, S. (2016). Antioxidant effect of plant extracts of the leaves of *Tithonia diversifolia* (Hemsl.) A. Gray on the free radical DPPH. Journal of Chemical and Pharmaceutical Research, 8(8), 1182–1189.

Thongsom, M., Chunglok, W., Kuanchuea, R., Thongsom, M., Chunglok, W., & Kuanchuea, R. (2013). Antioxidant and Hypoglycemic Effects of *Tithonia diversifolia* Aqueous Leaves Extract. Advances in Environmental Biology, 7(9), 2116–2125.

Tian, H., Wei, L., Xu, Z., Zhao, R., Jin, D., & Gao, J. (2010). Correlations between blood glucose level and diabetes signs in streptozotocin induced diabetic mice. Global J Pharmacol.

Wahyuningsih, M. S. H., Wijayanti, M. A., Budiyanto, A., & Hanafi, M. (2015). Isolation and Identification of Potential Cytotoxic Compound From Kembang Bulan [*Tithonia diversifolia* (Hemsley) a Gray] Leaves. International Journal of Pharmacy and Pharmaceutical Sciences, 7(6), 298–301.

Wahyuningsih, M. S. H., Wirohadidjojo, Y. W., Hidayat, R., & Sadid, A. (2015). Antifibrotic Effect of Standardized Ethanol Extract of *Tithonia diversifolia* (Hemsley) A . Gray on Keloid Fibroblasts. International Journal of Pharmacognosy and Phytochemical Research, 7(4), 642–647.

Zhao, G., Li, X., Chen, W., Xi, Z., & Sun, L. (2012). Three new sesquiterpenes from *Tithonia diversifolia* and their anti-hyperglycemic activity. Fitoterapia, 83(8), 1590–1597.