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by Cek Turnitin UWKS

Submission date: 17-Jan-2024 01:46PM (UTC+0700)

Submission ID: 2190953736

File name: 4.pdf (388.57K)

Word count: 3383

Character count: 17879

Tithonia Diversifolia vs Catechin: Role in Regulating Blood Glucose, Malondialdehyd, and Super Oxide Dismutase Level on Rat Induced Diabetes Mellitus and High-Fat Diet

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Abstract

Tithonia diversifolia (TD) is known as medicine plant which contains antioxidants and has been known as anti-diabetic medicine. Diabetic patient has increased blood glucose, reactive oxygen species (ROS), and malondialdehyd (MDA) level. In other side, the super oxide dismutase (SOD) level commonly attenuates. The aims of this study was to analyze the effect of Tithonia diversifolia and catechin in blood glucose, malondialdehyd (MDA), and Super Oxide Dismutase (SOD) level on diabetic and high fat induced rats.

The design of this study was Randomized Posttest Control Group Design using Wistar strain rats which were divided into 4 groups. After treatment, all groups of rats were sacrificed to take blood samples then measured blood glucose, MDA and SOD level. Statistical analysis of data to examine differences in treatment and control was conducted by one way Anova test with a 95% confidence level ($\alpha=0.05$). There was difference of glucose level ($p=0.020$) between control and treatment groups, but there were no differences in MDA level ($p=0.103$) and SOD level ($p=0.207$).

Keywords: *Tithonia diversifolia, catechin, malondialdehyd, super oxide dismutase, blood glucose level, diabetes.*

Introduction

Tithonia diversifolia is known as medicine plant. It has several functions relate with disease prevention and treatment. The habitats of Tithonia diversifolia is in tropical and subtropical area. In Indonesia, this plant is known as Kembang Bulan. Traditionally, all part of this plant, especially leaves, use to treat wounds, abdominal pain, abscesses, malaria, fever, hepatitis, and diabetes mellitus [1]. It contains active substances such as: alkaloids, saponins, saponin glycosides, tannins, balsam, and volatile oil [2].

Alkaloids play as antioxidant. It reduces ROS which causes oxidative damage by peroxidation, oxidation of cellular lipid, proteins, and DNA. Saponins can reduce blood cholesterol level, plays as anti-hepatic fibrosis agent, and acts as anti-inflammation [3-5]. Tannins are polyphenolic compound which in certain dose has role as anti-diabetic, anti-adipogenic, antipyretic, and antiseptic [2,6].

It is commonly found in diabetic patient that the number of ROS increase and leads to damage tissue, such as: damaging macro-vascular and micro-vascular which contributes to the other complication development like nephropathy and retinopathy diabetic [7]. ROS incline to attack phospholipid bilayer in cell membrane. The stabilized final substrate which produced by this reaction called malondialdehyde (MDA), which could be the marker of the amount of free radicals which damaging human tissues.

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MDA blood level in diabetic patient which has heart complication is higher than diabetic patient without complication [8]. The similar condition also found in diabetic patient with insulin therapy than without insulin therapy [9]. The increasing amount of MDA is marker of increasing lipid peroxidation, which imply the reduction amount of body's antioxidant [10]. Antioxidants in the body include enzymatic and non-enzymatic antioxidants.

One of enzymatic antioxidants is superoxide dismutase (SOD). It has role as a catalyst in the reaction of changing radical superoxide anion to hydrogen peroxide and oxygen molecule. It has important function in protecting cell from damaging. In type 2 diabetic patients, SOD inhibit the complication of nephropathy and retinopathy diabetes mellitus [11]. Extracellular SOD has big responsibility in protect pancreas beta cell from ROS's damaging.

According to the description above, researchers thought that it's necessary to conduct research about *Tithonia diversifolia* leaves extract and its effects to MDA, SOD, and blood glucose level in rats induced diabetes and high fat diets, comparing with catechin's action as comparative drugs.

Material and Method

The research's design was Randomized Posttest Control Group Design. Animals that used in this study were Wistar strain rats (*Rattus norvegicus*), male, age between 2-3 months, weight between 150-200 grams and physically fit obtained from the Animal Implementing Unit Faculty of Medicine, Universitas Airlangga Surabaya Indonesia. The sample size were 8 rats x 4 groups = 32 rats.

Rats were given food special pellet feed and drinking ad libitum. After being adapted for 1 week, rats were divided in 4 groups. Group of white rats contained 8 rats. There were 1 control group and 3 treatment groups. Control group was given aquades. Treatment groups were induced a dose 60 mg/kg bw of Streptozotocin (Stz) and giving a high-fat diet for 30 days. In 31st day, D2 group was given 100 mg/kg bw extract, and D3 group was given catechin as comparative drug at 10 mg/kg bw for 7 days. After that, all rats were taken for blood samples to measure the level of blood glucose, MDA and SOD level. Data were analyzed by SPSS 21 with ANOVA to measure the difference blood glucose, MDA, and SOD level among each group.

Finding:

Table 1. Average glucose levels (mg/dl) in the control and treatment groups

Groups	n	Mean±SD	Min	Max	p
D0	8	150,375±15,070	130,00	175,00	0,020*
D1	8	226,625±74,630	152,00	359,00	
D2	8	203,75±62,490	142,00	327,00	
D3	8	234,125±122,804	130,00	490,00	

*p<0.05 imply there is significant difference between groups

Table 2. Difference blood glucose level among each control group and treatment groups

Paired Groups	p
D0 – D1	0,002*
D0 – D2	0,007*
D0 – D3	0,105
D1 – D2	0,442
D1 – D3	0,505
D2 – D3	0,878

*p<0.05 imply there is significant difference between paired groups

Table 3. Average MDA levels (mg/dl) in the control and treatment groups

Groups	n	Mean±SD	Min	Max	p
D0	8	108,171±26,669	75,801	156,250	0,103
D1	8	176,404±60,078	108,897	284,040	
D2	8	209,237±129,145	106,246	503,730	
D3	8	196,647±87,953	95,298	389,162	

Table 4. Average SOD levels (mg/dl) in the control and treatment groups

Groups	n	Mean±SD	Min	Max	p
D0	8	0,138±0,059	0,052	0,257	0,207
D1	8	0,091±0,039	0,005	0,145	
D2	8	0,109±0,044	0,037	0,202	
D3	8	0,142±0,065	0,018	0,239	

Tithonia diversifolia leaves extract is more effective to reduce blood glucose level than catechin, the comparative drugs. It has been proven by the results which showed that D2 had lower level of blood glucose than D3 (Table 1). Tithonia diversifolia extract can also be a barrier to hyperinsulinemia in diabetes with streptozotocin induction and a high-fat diet. There was differences in the average glucose levels in the control and treatment groups.

The elevation of blood glucose happened in rat-induced streptozotocin. This is consistent with Samarghandian et al that blood glucose increases significantly after streptozotocin induction^[12]. Olukunle et al reported that Tithonia diversifolia leaves extract significantly decrease the plasma glucose of rats induced diabetes mellitus because it improved insulin action in the cellular level^[13]. Thongsom et al analyzed the total phenolic content of Tithonia diversifolia leaves is 27.2 ± 4.45 GAE mg/g and antioxidant capacity is 93.09 ± 37.91 uM TEAC/mg dry weight^[14]. This amount is higher than antioxidant capacity in dietary vitamin and mineral.

Sibul et al reported significant positive correlation between phenolic and total antioxidant activity which reduce the number of reactive oxygen species (ROS)^[15]. It has been known that increasing ROS also happen in diabetes patient. In diabetes mellitus patient, ROS attacks beta cell of pancreas and imply to inflammation and cell dysfunction^[16]. Tagne et al reported that Tithonia diversifolia also contained flavonoid which showed as anti-diabetic effect by reducing the oxidative stress^[1,17]. Flavonoid enhances GLUT-2 expression

in beta cell and GLUT-4 translocation^[18]. Therefore, it could be concluded that flavonoid improves glucose metabolism^[19].

Nagao et al reported that there was no significant difference in blood glucose level between control and catechin treatment group in diabetes mellitus patients^[20]. Other study showed that catechin injection with dosage more than 20 mg/kg BW among treatment group can reduce glucose level^[12]. It has been proven that catechin has anti-diabetic effect^[21]. In this research, the dosage of 10 mg/kg BW catechin which was given to rats induced diabetes. It can be concluded that the catechin dosage in this study was lower, therefore blood glucose level wasn't reduced significantly.

Streptozotocin also causes the elevation level of MDA, beside of blood glucose level^[12]. MDA can be used as the marker of oxidative stress level, especially in lipid peroxidation^[10]. In diabetes mellitus patient, oxidative stress alters macronutrients metabolism and increases risk of endothelial dysfunction and trigger the development of atherosclerosis^[12]. Oxidative stress increases the progression and complication development of diabetes mellitus. MDA level increased significantly in diabetic patient with complication^[22]. According to the result, MDA level of D2 treatment group was higher than other group. D2 was treated by Tithonia diversifolia leaves extract. D3, group with comparative drugs – catechin – had lower level of MDA than D2. Meanwhile, there was no significant different between each groups in this study ($p>0.05$).

Thongsom et al reported that injection of Tithonia

diversifolia in rats induced diabetes was significantly reduce the MDA level in liver and pancreas tissue [14]. It refers that Tithonia diversifolia treatment reduce the lipid peroxidation. Injection of catechin also related with increasing antioxidant enzyme and reducing lipid peroxidation [12]. Catechin enriched was observed decrease intra-abdominal fat [23] which is correlate with degenerative disease incidence, such as: diabetes mellitus. In this study, treatment groups were given high fat diet.

More amount of catechin in treatment group, the MDA level was lower [12]. According to the result (Table 3), in the dosage of 10 mg/kg BW; catechin was more effective to reduce MDA level than Tithonia diversifolia leaves extract in the dosage of 100 mg/kg BW. It also relates with catechin's role in preventing the onset of pancreatic islet cells from damaging of ROS [24,25].

Hyperglycemia also causes increased production of ROS because of auto-oxidation of glucose and protein glycolylation [14] [26]. Free radicals are molecules that are easy to diffuse and damage the biomolecules which cause short-lived cells [27,28]. In normal metabolism, ROS is produced from oxygen molecular [29]. During oxidative stress, the amount of antioxidant is not enough to handle free radicals or ROS which play an important role in several diseases' pathophysiology by means of redox reaction [30,31]. Higher redox reaction between ROS and antioxidant caused higher level of MDA level.

SOD 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 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 [7]. Research in diabetic induced mice found that there was a decrease in SOD and other antioxidant enzymes in liver tissue [32].

The result showed that there was no significant difference of SOD level between all groups, but catechin-treatment group had higher level of SOD than Tithonia diversifolia group (Table 4). SOD activity has increased in catechin-treat-diabetic rats [12]. The SOD level was higher in treatment group with highest dose of catechin [12]. When the SOD level is high, more amount of SOD prevents human body from ROS; therefore the level of MDA is lower. The other side, when the SOD

level is low; the body's antioxidant capacity is also lower and MDA is formed more because of ROS.

Conclusion

Streptozotocin induction causes changes in glucose levels in the treatment group. There were differences in glucose levels in the treatment and control groups. Both Tithonia diversifolia and catechin have antioxidant and anti-diabetic action which relate with blood glucose and oxidative stress. In this study, oxidative stress was determined by MDA and SOD level. There were no significant differences in MDA and SOD level, even the MDA level was higher in Tithonia diversifolia leaves extract treatment group and SOD level was higher in catechin treatment group.

Conflict of Interest: All authors who have participated in preparation of this manuscript declare that we have no conflict of interest.

Source of Funding: This study was supported by The Ministry of Research, Technology and Higher Education of Indonesia. The authors were grateful for Institution of Research and Innovation Universitas Airlangga for completion of research project (Grant No.200/UN3.14/LT/2018).

Ethical Clearance: The approval to conduct this research was obtained from The Animal Care and Use Committee (ACUC) Faculty of Veterinary Medicine, Universitas Airlangga Surabaya (No.2.KE.091.05.2018).

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