International Journal of Research and Review
Vol. 9; Issue: 6; June 2022
Website: www.ijrrjournal.com
Research Paper *E-ISSN: 2349-9788; P-ISSN: 2454-2237*

Antioxidant Potential of White Turi Stem (*Sesbania grandiflora*) in Reducing Malondialdehyde (MDA) and Blood Glucose Levels in Type 2 Diabetes Mellitus Model Mice

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DOI: https://doi.org/10.52403/ijrr.20220655

ABSTRACT

Type 2 Diabetes Mellitus triggers hyperglycaemia conditions resulting in oxidative stress which is characterized by increased production of Reactive Oxygen Species (ROS) inducing in cell damage, resulting in lipid peroxidation which decomposes in the blood into Malondialdehyde (MDA). The use of natural antioxidants is believed to be safer and more effective in the long term. One of the plants that has a strong antioxidant content and activity is the White Turi Plant (Sesbania grandiflora). This study aimed to examine the antioxidant activity of white turi (Sesbania grandiflora) stem extract in reducing MDA and blood sugar levels in Type 2 DM model mice. A quasi-experimental method with an in vivo design was used in this study. A sample of 30 hyperglycaemic mice was divided into 5 groups: group K- was given aquadest and standard feed, group K+ was given glibenclamide 0.013 mg/20gBW, group I was given extract at a dose of 2.8 mg/20gBW, group II was given extract at a dose of 5, 6 mg/20gBW and group III was given extract at a dose of 11.2 mg/20gBW. At the end of the study, blood glucose and MDA levels will be measured as a biomarker of oxidative stress. Repeated measurement Anova and One-way Anova test was used in this study, it was found that white turi (*Sesbania grandiflora*) stem extract at a dose of 2.8 mg/gBW had an effect on decreasing blood glucose levels in mice. Moreover, based on the results of the MDA level test, it showed that there was an effect of white turi stem extract on reducing MDA levels in the blood of mice. In conclusion, white turi stem (Sesbania grandiflora) has the potential to reduce MDA and blood sugar levels in Type-2 DM model mice.

Keywords: [White Turi Stem (*Sesbania grandiflora*), Malondialdehyde, Blood Glucose, Reactive Oxygen Species, Diabetes Mellitus Type2]

INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disease characterized by persistent hyperglycemia due to insulin production, secretion and function. In general, DM is classified into Type 1 DM, Type 2 DM, Gestational Diabetes Mellitus, and other types of DM. [1] Based on data from the International Diabetes Federation (IDF) in 2011 there were 336 million people with T2DM and 4.6 million deaths occur every year. Meanwhile, the World Health Organization (WHO) estimates that there will be an increase in the number of DM cases in Indonesia, to 21.3 million in 2030. As many as 90% of all DM cases are Type 2 DM.[2]

Lifestyle factors have a major contribution to the increased cases of T2DM such as obesity, poor diet, and lack of physical activity that induces hyperglycemia conditions resulting in oxidative stress which is characterized by increased production of Reactive Oxygen Species (ROS), impaired pancreatic β cell function and insulin resistance.[1] Oxidative stress arises when there is an imbalance between the number of antioxidants and oxidants in the body due to insulin resistance, thereby damaging the body's biomolecules such as lipids, proteins, and DNA which results in damage to pancreatic β cells, as well as increased blood glucose levels. [1,3,4] One of the oxidative stress markers is an increase in malondialdehyde (MDA) levels.[5]

The currently available pharmacotherapy treatment for T2DM is oral anti-diabetic medication, and insulin therapy. However, currently available treatment has not been able to control and reduce the risk of complications and side effects in Type 2 DM so that effective drugs are needed at a lower cost in the therapy of type 2 DM.[6] One alternative to DM treatment is to utilize the antioxidant content of natural ingredients. This treatment is believed to be safer for consumption in the long term, effective, and able to reduce the burden of treatment costs.[7] One of the natural ingredients that has strong antioxidant contents and activity is the White Turi Plant (*Sesbania grandiflora*). White Turi (*Sesbania Grandiflora*) is a plant that comes from the Fabaceae family and is easily found in various tropical areas, especially Indonesia. All parts of the white turi plant are known to have various benefits for humans.[3]

White turi stems are proven to contain antioxidant compounds of alkaloids, flavonoids, saponins, steroids, triterpenoids, phenolics, and tannins with strong antioxidant effects with IC_{50} values below 100 so that they have the potential to be developed as natural antioxidants.[8] Antioxidants in white turi stem, especially flavonoids, work as scavengers, hydrogen donors, electron donors, metal-chelating agents, and chain-breaking of free radicals. In addition, products from flavonoids such as tannins in white turi stems also have a structure that can donate their H atoms as a damper for the effects of free radicals. Tannins have good reducing and inhibitory properties of oxidation reactions.[8] The antioxidant content in white turi stems can reduce insulin resistance, improve pancreatic β-cell function, increase GLUT-4 expression, reduce mitochondrial damage, decrease inflammation, and inhibit α-glycosidase activity as well as reduce the production of ROS, thereby reducing the level of malondialdehyde in the blood which is a marker of oxidative stress. [9–11]

Seeing this potential, the authors are interested in carrying out a research entitled Antioxidant Potential of White Turi Stem (*Sesbania grandiflora*) in Reducing Malondialdehyde (MDA) and Blood Glucose Levels in Type 2 Diabetes Mellitus Model Mice.

MATERIALS & METHODS

This research is a quasi-experimental study with an in vivo design. The research was conducted at the Integrated Biomedical Laboratory of Udayana University, Sudirman Street, Denpasar, Bali for 4 months. This research has obtained an ethical statement from the Ethics Commission of Sanglah Hospital/Udayana University Medical Faculty.

The instruments used in this study include measuring cups, Erlenmeyer flask, scissors, blender, sieve, filter paper, analytical balance, sample bottles, animal cages, injection equipment, oven, Buchner funnel, rotary vacuum evaporator, photometry, incubator and Nesco Multicheck Glucose set, centrifuge, vortex mixer, water bath, spectrophotometer, micro pipette, test tube, microtube and gastric probe. The materials used in this study were young stems of white turi (*Sesbania grandiflora*) and alloxan monohydrate. The solvents used were ethanol, normal saline, 1N HCL, Sodium Thiosulfate, Thiobarbituric Acid,

and sterile aquabidest for injection. The research was started by collecting young stems of white turi plant (*Sesbania grandiflora*), then washed thoroughly. Turi stems that have been washed, then drained and cut into pieces to facilitate the drying process. The drying process was carried out under indirect sunlight until simplicia was formed.[8] Dried simplicia was then made dry selection and then mashed using a blender and filtered using a sieve. Turi stem simplicia that has been filtered is then weighed as much as 800 grams, and macerated into 1.600 ml of 70% ethanol solvent (1:2) for 24 hours at room temperature and stirred for several times. The maceration results obtained were then filtered using filter paper and a Buchner funnel, then the residue was re-macerated using the same steps for 3 times. Then the filtrate was evaporated using a rotary vacuum evaporator at a heating temperature of 55^{o}C to obtain a concentrated extract.

The experimental animals used were balb/c white mice with an average body weight of 15-30 grams and 2-3 months old and male. The mice used were 30 individuals and were given standard feed and drinking ad libitum. Mice were acclimatized for 7 days before being given treatment. After acclimatization, the mice were checked for initial blood glucose before being induced by alloxan. Before being induced by alloxan, mice were fasted for 12 hours while still being given water. Alloxan was injected intraperitoneally at a dose of 150 mg/kgBW. Then wait for 5 days until there was an increase in blood glucose levels in mice. After the mice experienced hyperglycemia, the mice were then divided into 5 groups, namely group K- (negative control) only given aquadest and standard feed, group K+ (positive control) given glibenclamide 0.013 mg/20gBW, treatment group I was given ethanol extract of white turi stem with dose of 2.8 mg/20gBW, treatment group II was given ethanol extract of white turi stem at a dose of 5.6 mg/20gBW and treatment group III was given ethanol extract of white turi stem at a dose of 11.2 mg/20gBW. All treatment and control groups (+) were also given aquadest and standard feed ad libitum. Drugs and extracts were administered orally using a gastric probe. Blood glucose measurements were carried out using a Nesco Multicheck glucometer. Blood samples were taken through the peripheral veins of mice and then the blood obtained was tested with a glucometer. Measurement of blood glucose levels in mice was carried out every 4 days on days 4, 8 and 12. Before measuring the mice were fasted for 8-12 hours.

Measurement of MDA levels in mice was carried out using the Thiobarbituric Acid Reactive Substance (TBARS) method. On the 12th days, the mice blood samples were taken again and allowed to stand until serum was formed. Next, 100 µL of serum was added with 1 ml of 0.9% Nacl, then centrifuged at 8,000 rpm for 20 minutes. Then, 550 µL of distilled water and 100 µL of TBA were added, then homogenized with a vortex. After that, it was homogenized again by adding 250µL of 1N HCL, then vortexed again. Then 100 µL of Na-Thio was added, and homogenized at 500 rpm for 15 minutes. Then the supernatant formed was transferred to a new microtube, then heated in a 100^{o}C water bath for 30 minutes. Then the absorbance was measured using a UV-1601 spectrophotometer with a wavelength of 535 nm.(12) Lastly, the experimental animals were terminated by being anesthetized until they died.

Statistical Analysis

Data obtained from the study of blood glucose and MDA levels in mice were analyzed using the SPSS 25.0 program through the Repeated Analysis of Variance (Repeated ANOVA) and One-way ANOVA. This method of analysis was carried out if the data met the assumption that the sample came from an independent group and the variance similarity was based on the homogeneity test and the data were normally distributed with the Shapiro-Wilk normality test with a significant p value of >0.05. Furthermore, the data obtained will

be presented in tabular form with a significant p-value in the analysis <0.05.

RESULT

The results of the mice blood sugar measurements were then collected and analyzed. The Sapiro-Wilk test showed that the data were normally distributed and homogeneous, so a repeated one-way ANOVA test was performed with the results in Table 1.

Table 1. Mean blood glucose levels of mice and the results of the repeated ANOVA test.

Treatment Groups	Pre-Alloxan	Day 0	Day 4	Day 8	Day 12	P Value
Controls (+)	97.3±13.8	115.1±17.9	131.6±18.1	123.1±17.2	129.6±21.7	0.126
Controls (-)	91.7±8.6	117.2±32.6	119.3±25.7	121.1±24.9	132.8±19.1	0.439
Treatments I	96.7±18.0	131.8±20.1	118.1±12.0	112.3±20.8	98.8±10.4	0.049*
Treatments II	90.8±3.9	113.4±15.4	117.8±38.2	126.2±11.3	114.6±17.5	0.768
Treatments III	88.1±21.1	113.2±20.3	116.1±26.3	141.7±31.6	112.0±18.5	0.180
*Statistically Significant (p<0.05)						

A significant decrease in blood glucose levels was found in the treatment group I with blood glucose levels on day 0 was 131.8±20.1 mg/dL and on day 12 there was a decrease in the average fasting blood glucose level of 98.8±10.4 mg/dL. So that the white turi stem extract at a dose of 2.8 mg/gBW had a significant effect on reducing blood glucose levels in mice (p<0.05). Meanwhile, in the treatment groups II and III, no significant decrease in blood glucose levels was found. The average blood glucose levels in the positive and negative control groups tended to increase on day 12 and the intervention given to the control group did not have a significant effect on reducing blood glucose levels in mice (p value>0.05). On the 12th day, mice blood samples were collected for further analysis of MDA levels using the TBARS (Thiobarbituric Acid Reactive Substance) method. Furthermore, MDA levels were examined, mice plasma were analyzed by one way ANOVA test with a significance value <0.05, all data were normally distributed and all data were homogeneous.

Table 3. MDA test results for mice

Treatment Groups	Average of MDA Level ±DS	P Value
Controls (+)	10.7± 1. 7	0.001*
Controls (-)	10.4± 0. 6	
Treatments I	15.9 ± 1.9	
Treatments II	12.9 ± 0.9	
Treatments III	11.4± 1.7	

***P value <0.05 statistically significant**

The results of the repeated one way ANOVA test analysis showed that there was a difference in the mean levels between the treatment groups which resulted in a significance value of 0.001, so that there was an effect of white turi stem on MDA levels with the largest decrease in MDA in group III. Thus, it can be concluded that white turi (*Sesbania grandiflora*) extract at a dose of 11.2 mg/20gBB had an effect on reducing MDA levels in alloxan-induced DMT2 mice.

DISCUSSION

Diabetes mellitus can be triggered by excess ROS production, this excess production triggers oxidative stress which at least causes insulin resistance through five molecular mechanisms, namely pancreatic β-cell dysfunction, decreased expression of Glucose Transporter Type 4 (GLUT-4), mitochondrial dysfunction, and insulin signaling pathway disturbances. An increase in the number of ROS interferes with the performance of pancreatic β-cell function through the mechanism of increasing apoptosis, disrupting K_{ATP} channels, inhibiting pancreatic duodenal transcription factors PDX-1 and MafA, decreasing pancreatic β-neogenesis, mitochondrial dysfunction, and activation of the Nf-kb, JNK/SAPK molecular pathway, p38 Mitogen-Activated Protein Kinase (MAPK), and the hexoamine pathway.[9] Administration of alloxan in mice caused damage to pancreatic β-cells by forming reactive oxygen compounds that form superoxide radicals. Alloxan also interferes with the process of cell oxidation, excreting calcium ions from the mitochondria, causing the death of pancreatic cells due to hemostatic disorders. As a result, there is damage to insulin receptors and β-cells of the islets of Langerhans of the pancreas. This causes clinical conditions in the form of increased blood glucose levels in mice.[13]

The administration of white turi stem extract in alloxan-induced diabetes mellitus mice was able to reduce blood glucose levels, this was due to the active ingredient in the form of antioxidants in it. Based on research conducted, turi plants contain strong antioxidants in the leaves, flowers, stems, and roots. In this study, phytochemical tests were found on acetone extracts of white turi leaves and stems containing flavonoids, phenolic compounds, saponins, tannins, triterpenoids, alkaloids, and steroids. with antioxidant activity in turi stems which is stronger against DPPH radicals with an IC50 value of 54.2608 compared to acetone extract of white turi leaves of 56.5707.[8] Another study showed that the ethanol extract of turi leaves 20% w/v gave significant results in lowering blood glucose levels and increasing insulin sensitivity.[14] Similar results were also obtained in the study by giving turi leaf decoction to mice.[15] Based on research on the antioxidant activity of white turi leaves and stems, the IC50 value was not significantly different, but the antioxidant activity was stronger in white turi stem extract. Therefore, white turi stem have the potential to have the same effectiveness in lowering blood glucose levels.[8]

White Turi contains secondary metabolites that work as antioxidants, namely flavonoids and tannins. Flavonoids are able to work through various mechanisms as antidiabetic with the activity of reducing oxidative stress (ROS). Flavonoids are antioxidants that work as scavengers, hydrogen donors, electron donors, metal-chelating agents, chain breaking of free radicals. Then the tannin content in white turi also has potential as an antioxidant with a structure that can donate its H atoms to reduce the effects of free radicals. Tannins are flavonoid derivatives with good reducing and inhibitory properties of oxidation reactions.[8] Turi stem flavonoids prevent DMT2 through various mechanisms, namely reducing the accumulation and breaking down of ROS so that insulin resistance decreases and improves pancreatic β-cell function, increases GLUT-4 expression, reduces mitochondrial damage, decreases inflammation, and inhibits α-glycosidase activity.[9–11]

The results of this study indicate that there is an increase in glucose levels due to the induction of alloxan in mice model of type 2 diabetes mellitus. Then the treatment was carried out by giving aquadest and standard feed to the K- group, the K+ group (positive control) was given glibenclamide 0.013 mg/20gBW, the first treatment group was given white turi stem ethanol extract at a dose of 2.8 mg/20gBW, the second treatment group was given White turi stem ethanol extract dose of 5.6 mg/20gBW and treatment group III was given white turi stem ethanol extract at 11.2 mg/20gBW ethanol extract. After examination on the 12th day, data analysis was carried out, it was found that the treatment group I with white turi stem extract at a dose of 2.8 mg/20gBW had a significant value ($p=0.049$). Meanwhile, in the control and other treatment groups, the results were not significant with $p>0.05$. In general, the larger the dose, the better the benefit. In addition, when viewed from previous studies that showed antioxidant activity that was not significantly different from turi leaves which had shown its effectiveness as an anti-diabetic, it is necessary to conduct further research with a longer period of time and a more uniform sample population related to white turi stem extract.

Free radicals and Reactive Oxygen Species that trigger the process of oxidative stress in diabetes mellitus are caused by an imbalance of pro-oxidants and antioxidants. Excessive levels of pro-oxidants are triggered by conditions of dyslipidemia and insulin resistance in DMT2 conditions. The content of flavonoid compounds and tannins in white turi stem extract acts as a scavenger in reducing and inhibiting the process of oxidative stress.[8] Malondialdehyde (MDA) is a secondary product of lipid peroxidation. Cellular damage due to free radicals can be identified through MDA levels.[15] Increased

levels of MDA in the blood is a marker of increased oxidative stress in the condition of type 2 diabetes mellitus.[5]

Based on the results of the study, the average level of MDA in each treatment group was obtained with a significance value of 0.001. This means that there is an effect of white turi stem extract in reducing MDA levels in alloxan-induced T2DM mice. The decrease in MDA levels in this study was in the treatment group I with the administration of white turi stem extract at a dose of 2.8 mg/20gBW having a mean MDA ±SD value of 15.9 ± 1.9 while the second treatment group with a dose of 5.6mg/20gBW had an MDA mean value ± SD of 12.9 ± 0.9. In the treatment group III who received the extract at a dose of 11.2/mg/kgBW, it was seen that the effect of white turi stem extract had the greatest decrease in MDA levels in DMT2 mice. So that in this study the administration of white turi stem extract which has an antioxidant role with a certain dose was able to reduce oxidative stress which was measured from a decrease in blood MDA levels in alloxan-induced T2DM mice.

CONCLUSION

It can be concluded that white turi (Sesbania grandiflora) extract at a dose of 2.8 mg/gBW had an effect on reducing blood glucose levels in mice and a dose of 11.2 mg/gBB for a decrease in blood MDA levels in T2DM mice. However, this research is certainly not without its shortcomings. There are limitations in the time of the study so that the results obtained are less than optimal. Therefore, suggestions for further research are necessary to conduct further research on the effect of white turi (Sesbania grandiflora) stem extract in reducing reactive oxygen species levels in type 2 diabetes mellitus mice with larger doses and longer durations in order to obtain better results.

Acknowledgement: None

Conflict of Interest: None

Source of Funding: None

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How to cite this article: Putu Novi Handayani, Ni Made Ayudia Shanty, Ni Kadek Saras Dwi Guna et.al. Antioxidant potential of white turi stem (sesbania grandiflora) in reducing malondialdehyde (MDA) and blood glucose levels in type 2 diabetes mellitus model mice. *International Journal of Research and Review*. 2022; 9(6): 520-526. DOI: *https://doi.org/10.52403/ijrr.20220655*

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