The Use of *Meemba* Leafe (*Azadirachta Indica*) for Recovery of Mouse β-Pancreas due to the Type-2 Diabetes Mellitus

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Abstract. One of the serious diseases that has been a concern in medicine is Diabetes Mellitus (DM). The rate of morbidity and mortality is significantly high. The medicine that comprehensively recovers the disease is necessary. This enables β pancreas recovery. The extract of *Meemba* Leaf is expected to fulfill this requirement. This research was carried out to analyze and prove the impact of *Meemba* leaf extract on reduction of blood glucose level of Mouse with type 2 Diabetes Mellitus (DMT2). The testing animals were modified to suffer from type 2 Diabetes Mellitus. *Meemba* leaf extract was given and the effect was observed until the blood glucose level reached the normal condition. The animal testing had been successfully modified to suffer from type 2 Diabetes Mellitus. The intervention by giving *Meemba* leaf extract of 250 mg/kg BW (bodyweight) had demonstrated gradual improvement of the disease indicated by reduction of blood glucose level. After 28 days, the disease seems to be recovered. This research findings are expected to contribute the essential treatment of the disease by supporting β pancreas cells improvemnt. The extract of *Meemba* leaf has shown the ability to do so. This material is extensively available, affordable with low price and, therefore, this will help people to recover from DM.

Keywords: Diabetes Mellitus; β pancreas cell, hyperglycemia; *Meemba* leaf extract; blood glucose level

1 Introduction

Medical science is currently developing rapidly. There are some diseases for which treatment has been found both in terms of the method and used substances. One of the diseases that is becoming the focus of treatment in the field of medicine is Diabetes Mellitus. Research in the field of medicine and pharmacy that has a focus on recovering the disease is carried out quite intensively. This is because the disease has extensive implications and is a health issue on a global scale [1]. It therefore needs good treatment that is empirically proven for better patient treatment [2]. Diabetes Mellitus (DM) is a serious disease that has long-term impact on personal, family and social life. The disease is in the top 10 causes of death that take the lives of millions of people. It is about

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80% of people suffering from DM are those living in low- and middle-income countries. The regions heavily affected by DM disease are China and India [3]

The prevalence of morbidity and mortality for diabetes mellitus is quite high and there is a significant cost for treatment of the disease. The International Diabetes Federation (IDF) has estimated that in 2017 there were 451 million people with diabetes in worldwide level (aged 18-99 years). These figures are predicted to rise reaching 693 million people by 2045. It is estimated that almost half of the people (49.7%) living with diabetes mellitus are undiagnosed. In 2017 it was estimated that around 5 million deaths worldwide were caused by diabetes in the age range of 20-99 years [4]. The global healthcare expenditure for diabetics was estimated at USD 850 billion in 2017. According to the trend, new estimates of diabetes prevalence, diabetes deaths and health care spending due to diabetes present a huge burden on social, financial and health systems over the world [5].

In 2005, it was estimated that more than 20 million people in the United States had diabetes. About 30% of these people have undiagnosed cases. The increased risk of diabetes is mainly related to age, ethnicity, family history of diabetes, smoking, obesity, and lack of physical activity. Diabetic-related complications including cardiovascular disease, kidney disease, neuropathy, blindness, and lower limb amputation are significant causes of increased morbidity and mortality among diabetics and result in a heavy economic burden on the U.S. health care system. By 2050, the number of people in the United States diagnosed with diabetes is estimated to increase to 48.3 million [6]

Diabetes Mellitus is a disease characterized by high glucose level in the blood. Diabetes and lack of control over glucose concentrations in the blood are increasing rapidly as one of the major chronic degenerative disorders [7]. This occurs due to the complex metabolic disorders associated with increased insulin resistance, insulin incapability and malfunction of β pancreas-cell, abnormal metabolism of glucose and lipids, subclinical inflammation and the increased of oxidative stress [8]. Oxidative stress is indicated to be a major cause in the pathogenesis of the disease. The pathogenesis of Diabetes Mellitus includes the process of oxidative stress in which there is a state of imbalance between free radicals and antioxidants. The production of free radicals in Diabetes Mellitus due to glucose auto-oxidation exceeds the ability of intracellular antioxidants to neutralize them, causing cell damage. Free radicals can increase the peroxidation of lipids that decompose into *Malondialdehyde* (MDA) in the blood and are reactive compounds as markers of oxidative stress. A persistent of high blood glucose levels may cause damage on blood vessels that affect heart, eye, kidney, and nerve function and are likely to result in various complications[8][9][10]. Type-2 Diabetes Mellitus contributes 90% of the Diabetes cases and its prevalence rate shows a consistent increment. Factors causing this increase include age, lifestyle and obesity [11]. The main factors of the Type-2 Diabetes Mellitus on a global scale are obesity, lack of physical activity, increased consumption of unhealthy foods such as processed meats, modified wheat and sugar-sweetened beverages [12].

Diabetes Mellitus affects the role of β pancreas cell. For the person who is not diabetic there is a grow in mass of β cell. However, the function β-cell is concurrent with individuals with normal body mass. Preliminary data suggest that weight loss can lead to some decrease in cell mass β and function in non-diabetic individuals. The average

sell β mass is reduced in Type-2 Diabetes Mellitus regardless of obesity, but the variation between individuals is quite diverse. The functioning of β cells, however, is severely impaired. Weight loss interventions and antihyperglycemic treatments, improving the function β pancreas cell, but without changes in cell mass [3]. A growing research suggests that herbs can effectively treat DMT2. The current research looks at the effect of herbal therapy on DMT2 through anti-inflammatory and anti-oxidation characteristics, regulation of blood lipid metabolism, anti-glycose effects and other mechanisms [13]. The effectiveness of herbal medicine in type-2 Diabetes Mellitus is given in Fig. 1.

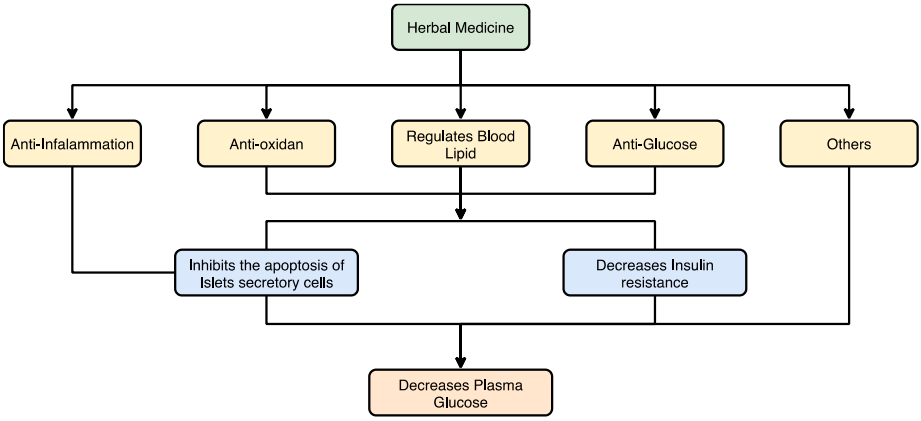

Fig. 1. The effect of herbal medicine in type 2 diabetes mellitus [13]

In this research the treatment and recovery of type-2 Diabetes Mellitus is carried by giving the extract of *Meemba* leaf. *Meemba* leaf contains flavonoid and alkaloids compounds that work by two main mechanisms, i.e. intra-pancreatic and extra-pancreatic. Flavonoid and alkaloids compounds in the intra-pancreatic mechanism work by repairing (regeneration) the damaged β pancreas cells and protecting cells from damage and stimulating the release of insulin. Alkaloids have been shown to have the ability to regenerate damaged β pancreatic. Alkaloids are also able to stimulate the sympathetic nerves (sympathomimetic) which has an effect on increasing insulin secretion[14].

Meemba leaf extract contains active compounds of flavanoid that have antioxidant properties. It can protect damage to pancreatic cells due to the free radicals. Alkaloids work to suppress blood glucose level in the extra-pancreatic mechanism by increasing blood glucose transport, inhibiting glucose absorption in the colon, stimulating glycogen synthesis and inhibiting glucose synthesis by inhibiting the enzyme glucose 6-phosphatase, fructose 1.6-biphosphatase which is an enzyme that plays a role in gluconeogenesis, as well as increasing glucose oxidation through glucose 6-phosphate dehydrogenase. Inhibition of the enzymes 6-phosphatase and fructose 1,6-biphosphatase will decrease glucose formation from substrates other than carbohydrates. Flavonoids absorbed in the blood will increase the solubility of blood glucose so that it is easy to secrete through urine [20]

Meemba leaf extract has shown to be an insect repellent, for lowering inflammation, treating diabetes, and for treating cancer. The benefits found in *Meemba* leaf compounds and its extracts for health are extensive, however if the processing and the use is not standardized, then it may result in side effects of damaging the liver and kidneys. The recent research on *Meemba* leaf extract is highlighted focusing on its main aspects, such as antioxidants, and their potential role in the treatment of diabetes. In this research, there is a section that examines how toxicity levels *Meemba* leaves to rat, thus encouraging further research for the development of better products for human use[16].

2 Method

2.1 Research Design

The type of this research is experimental. The study was carried out by giving treatment to the animals test that have been divided into control groups and treatment groups and then compared based on the effects [17]. In this research, a mouse that has been induced with streptozotocin suffering from Type-2 Diabetes Mellitus is employed. This is to observe the effectivity of *Meemba* leaf extract on the treatment of the disease.

The research design used is the pre- and post-test control group design. This design was chosen because it can produce data with high validity and treatment can be regulated by researchers. The use of this design can control the occurrence of testing bias and testing interactions[18].

2.2 Development Animals Test

For preparing the research object, a number of wistar male white rats aged 2-3 months, body weight 150-250 g, was treated by feeding them with standardized rat food. This was carried out in the Faculty of Pharmacy, Muhammadiyah University of Surakarta. The decision of using animals for the experiments is based on the consideration that wistar male white rats are most often used in biomedical research because they are genetically similar to humans and have adaptability in laboratory environments [15].

The minimum number of samples used in this study is calculated according to the formula in [19]. Based on the formula, the minimum number of samples for each group was 4 wistar rats. To anticipate the presence of animals excluded from the sample e.g. due to death during the study, the number of samples was increased. The amount of addition depends on the magnitude of the risk of an exclusion event. Considering this fact, in this study 5 Wistar male white rats were used for each group [19].

The preliminary research in this study was carried out by modelling the animals test to suffer from Type-2 Diabetes Mellitus. This was carried out by induction of streptozotocin (STZ). In this stage, obtaining the right dose of STZ that enables making the model without getting dead is challenging. In this study, the injection of STZ at the dose of 35-60 mg/kg Body Weight (BW) was used. This causes type-2 Diabetes Mellitus, damages Pancreatic β cells but enables pancreas to be reversible. The first group of rats was given treatment (STZ 50 mg/kg BW) and Nicotinamide-induced 110 mg/kg

BW intraperitonially, after 15 min induced STZ 50 mg/kg BW then was not fed during a night. Rat is successful to have type 2 DM if the blood glucose level is 200 mg/dL [17]. The rats were used in experiment is shown in Fig. 2.

Fig. 2. Wistar Rats used for experiment

2.3 *Meemba* Leaf Extraction and Application

Various studies on Meemba leaf are normally aimed at testing antioxidant effects and testing the body's natural defenses. One such study used Meemba leaf and methanol to extract the active compounds of Meemba leaf [21]. For making the extract of Meemba leaf, a total of 1 kg of the leaf powder was mixed with 1 liter of 96% ethanol and was then left for 2 days while stirred occasionally. The solution is then filtered with filter paper, macerated and evaporated to exclude the main solvent with a rotary evaporator. The solution was thickened with a water bath, Meemba leaf extract can be seen in Fig. 3.

Fig. 3. The Extract of *Meemba* Leaf

In this study, the rats were firstly adapted for one week to undergo a standardized diet before involved in the experiment. Before the treatment, the blood was taken from the rats to check their blood glucose levels. For the group of normal control, the rats

simply stay and were given a standardized diet. Another group was inducted with STZ 50 mg/kg BW followed by the injection of Nicotinamide after 15 minutes. After 7 days, the blood glucose levels, and the weight were checked for both groups. After this step, the treatment was carried out. After streptozotocin-induced 3 days later wistar rats start to be given Meemba leaf extract for 28 days by checking their blood glucose levels on the days of 4, 7, 14, 21, and 28. The application of neem leaf extract to wistar rats can be seen in Fig. 4.

Fig. 4. The Intervention with *Meemba* Leaf Extract

3 Result And Discussion.

Nowadays, various methods and pharmacotherapy are being developed using *Meemba* leaf extract, and therefore, *Meemba* leaf extract is increasingly interested because of its benefits[22]. This is due to *Meemba* leaf extract shows enormous potential as an alternative pharmacotherapy[23]. This study also shows very significant results, from the achieved results in the reduction of rat blood glucose levels and the overall restoration of β pancreas cells as mentioned in [24].

Table 1. Intervention of *Meemba* leaf extract on blood glucose level

No	Day	Blood glucose level (mg/dL)
1	0	88
2	4	254
3	7	266
4	14	304
5	21	217
6	28	142

Wistar rats, which had been induced with streptozotocin to suffer from type-2 diabetes mellitus, after three days were given *Meemba* leaf extract of 250 mg/kg-BW. The blood glucose level was tested at day of 7, 14, 21, and 28. It was recorded that at the beginning there was an increase in blood glucose levels. After some time, there was a decrease near normal and at the day of 28, the blood glucose levels were again tested.

It was measured that the blood glucose levels were normal, i.e. 142 mg / dL. The results of this study can be seen in Table 1 and Fig. 5.

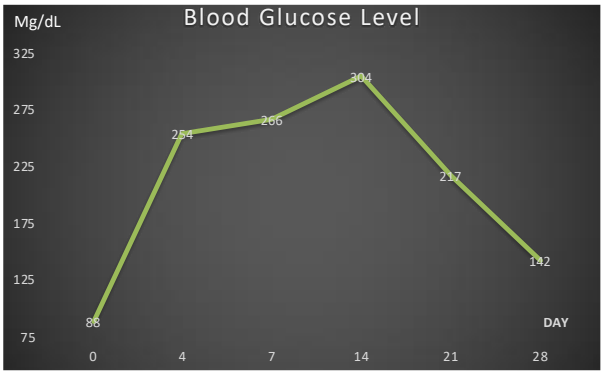

Fig. 5. The effect of giving *Meemba* leaf extract on bllod glucose level of Type-2 Wistar rat

4 Conclusion

Diabetes mellitus is one of global health problems resulting in physical and financial drawbacks for the patients suffering from its. Many herbal plants have been developed for the recovery of diabetes mellitus mainly through its anti-inflammatory, anti-oxidation, blood fat regulation and anti-glucose properties. In this case, it is presented the effect of *Meemba* leaf extract in lowering blood glucose levels in DMT2 wistar rats.

In this study, there is an effect of *Meemba* leaf extract in male wistar rats with a decrease in blood glucose levels gradually approaching normal and on day 28 when blood sugar returned tom 142 mg/dL. Biochemical analysis of *Meemba* leaf extract contains active compounds of alkaloids and flavonoids that have been shown to be useful in lowering blood glucose levels in male wistar rats for the treatment of type 2 diabetes mellitus.

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References

1. A. Ghasemi, S. Khalifi, and S. Jedi, “Streptozotocin Nicotinamide Induced Rat Model of Type 2 Diabetes (review),” *Acta Physiol. Hung.*, vol. 101, no. 4, pp. 408–420, Dec. 2014, doi: 10.1556/APHYSIOL.101.2014.4.2.
2. P. Saeedi *et al.*, “Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th

edition," *Diabetes Res. Clin. Pract.*, vol. 157, p. 107843, Nov. 2019, doi: 10.1016/j.diabres.2019.107843.
3. R. A. DeFronzo *et al.*, "Type 2 diabetes mellitus," *Nat. Rev. Dis. Prim. 2015 11*, vol. 1, no. 1, pp. 1–22, Jul. 2015, doi: 10.1038/nrdp.2015.19.
4. K. Ogurtsova *et al.*, "IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040," *Diabetes Res. Clin. Pract.*, vol. 128, pp. 40–50, Jun. 2017, doi: 10.1016/J.DIABRES.2017.03.024.
5. N. H. Cho *et al.*, "IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045," *Diabetes Res. Clin. Pract.*, vol. 138, pp. 271–281, Apr. 2018, doi: 10.1016/j.diabres.2018.02.023.
6. A. D. Deshpande, M. Harris-Hayes, and M. Schootman, "Epidemiology of Diabetes and Diabetes Related Complications," *Phys. Ther.*, vol. 88, no. 11, pp. 1254–1264, Nov. 2008, doi: 10.2522/ptj.20080020.
7. L. Hieronymus and S. Griffin, "Role of Amylin in Type 1 and Type 2 Diabetes," *Diabetes Educ.*, vol. 41, pp. 47S-56S, Sep. 2015, doi: 10.1177/0145721715607642/ASSET/IMAGES/LARGE/10.1177_0145721715607642-FIG2.JPEG.
8. O. Mendes, L. Koetzner, and J. Chen, *Nutraceutical Impact on the Pathophysiology of Diabetes Mellitus*, Second Edi. Elsevier Inc., 2018.
9. M. Koshizaka *et al.*, "Obesity, Diabetes, and Acute Coronary Syndrome: Differences Between Asians and Whites," *Am. J. Med.*, vol. 130, no. 10, pp. 1170–1176, Oct. 2017, doi: 10.1016/J.AMJMED.2017.03.030.
10. T. Issar *et al.*, "Altered peripheral nerve structure and function in latent autoimmune diabetes in adults," *Diabetes. Metab. Res. Rev.*, vol. 36, no. 3, p. e3260, 2020, doi: 10.1002/dmrr.3260.
11. A. P. Hills *et al.*, "Epidemiology and determinants of type 2 diabetes in south Asia," *Lancet Diabetes Endocrinol.*, vol. 6, no. 12, pp. 966–978, Dec. 2018, doi: 10.1016/S2213-8587(18)30204-3.
12. L. Luo *et al.*, "A new 7-gene survival score assay for pancreatic cancer patient prognosis prediction.," *Am. J. Cancer Res.*, vol. 11, no. 2, pp. 495–512, 2021, Accessed: Apr. 04, 2021. [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/33575083.
13. G. M. Pang *et al.*, "Herbal medicine in the treatment of patients with type 2 diabetes mellitus," *Chinese Medical Journal*, vol. 132, no. 1. Lippincott Williams and Wilkins, pp. 78–85, Jan. 05, 2019, doi: 10.1097/CM9.0000000000000006.
14. S. V. M. Larantukan, N. L. E. Setiasih, and S. K. Widyastuti, "Pemberian Ekstrak Etanol Kulit Batang Kelor Glukosa Darah Tikus Hiperglikemia," *Indones. Med. Veterinus*, vol. 3, no. 4, pp. 292–299, 2014.
15. S. O. Adewole, E. A. Caxton-Martins, and J. A. O. Ojewole, "Protective Effect of Quercetin on the Morphology of Pancreatic β-Cells of Streptozotocin-Treated Diabetic Rats," *African J. Tradit. Complement. Altern. Med.*, vol. 4, no. 1, p. 64, 2007, doi: 10.4314/AJTCAM.V4I1.31196.
16. J. F. Islas *et al.*, "An overview of Neem (Azadirachta indica) and its potential impact on health," *J. Funct. Foods*, vol. 74, p. 104171, Nov. 2020, doi: 10.1016/J.JFF.2020.104171.
17. B. Ichsan, "Pengantar Metodologi Penelitian Kedokteran Dan Kesehatan Masyaraka," *J. Metodol. Penelit. Kesehat.*, vol. 7, no. 5, pp. 57–60, 2016.
18. Kemenkes RI, "Rencana Aksi Kegiatan Direktorat Lesehatan Keluarga," 2020.
19. W. N. Arifin and W. M. Zahiruddin, "Sample Size Calculation in Animal Studies Using Resource Equation Approach," *Malays J Med Sci*, vol. 24, no. 5, pp. 101–105, 2017, doi: 10.21315/mjms2017.24.5.11.

20. C. Ogbonnaya Eleazu, K. Chinedum Eleazu, S. Chukwuma, and U. N. Essien, "Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk to humans," 2013.
21. M. Mirfeizi, Z. Mehdizadeh Tourzani, S. Z. Mirfeizi, M. Asghari Jafarabadi, H. R. Rezvani, and M. Afzali, "Controlling type 2 diabetes mellitus with herbal medicines: A triple-blind randomized clinical trial of efficacy and safety," *J. Diabetes*, vol. 8, no. 5, pp. 647–656, Sep. 2016, doi: 10.1111/1753-0407.12342.
22. R. Al Akeel, A. Mateen, K. Janardhan, and V. C. Gupta, "Analysis of anti-bacterial and anti oxidative activity of Azadirachta indica bark using various solvents extracts," *Saudi J. Biol. Sci.*, vol. 24, no. 1, pp. 11–14, Jan. 2017, doi: 10.1016/J.SJBS.2015.08.006.
23. S. Farhat Basir and S. Shailey, "Strengthening of antioxidant defense by Azadirachta indica in alloxan-diabetic rat tissues," *J. Ayurveda Integr. Med.*, vol. 3, no. 3, pp. 130–135, 2012, doi: 10.4103/0975-9476.100174.
24. G. Mccalla, O. Parshad, P. D. Brown, and M. T. Gardner, "Beta Cell Regenerating Potential of Azadirachta indica (Neem) Extract in Diabetic Rats," *West Indian Med J*, vol. 65, no. 1, p. 13, 2016, doi: 10.7727/wimj.2014.224.

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