THE EFFECT OF KERSEN (*Muntingia calabura*) EXTRACT ON THE NUMBER OF PANCREATIC BETA CELLS: EXPERIMENT STUDY IN RAT

Sri Mega Muthmainah S.P [1], Muhammad Fadhol Romdhoni [2*], Ira Citra Ningrom[3], Yunia Annisa[4]

[1]*Faculty of Medicine, University Muhammadiyah Purwokerto*
[2]*Pharmacology Department, Faculty of Medicine, University Muhammadiyah Purwokerto*
[3]*Pathology Anatomy Department, Faculty of Medicine, University Muhammadiyah Purwokerto*
[4]*Internal Medicine Department, Faculty of Medicine, University Muhammadiyah Purwokerto*

***) Correspondence Author**
Muhammad Fadhol Romdhoni
Pharmacology Department, Faculty of Medicine
University Muhammadiyah Purwokerto , Purwokerto 53113, Indonesia
Email: m.fadholromdhoni@ump.ac.id

Abstract

Type 2 diabetes mellitus is a common type of diabetes which can be caused by an increase in blood sugar levels resulting in a decrease in the number of pancreatic beta cells, dysfunction or apoptosis. To increase the number of pancreatic beta cells, antioxidants are needed. The antioxidant content of kersen leaves can improve pancreatic beta cell disorders due to high blood sugar levels by reducing ROS and RNF so that oxidative stress decreases and the number of pancreatic beta cells increases. Aim of this research, to determine the effect and the most effective dose of kersen leaves ethanol extract on the number of pancreatic beta cells of white rats wistar strain induced by streptozotocin-nicotinamide (STZ-NA). This study used a true experiment post-test only with control group design with STZ-NA-induced KI-KV group and normal control KVI group. KI-KIII was given kersen leaves extract at a dose of 0.3; 0.5 ;0.7 mg/gBW. KIV positive control. KV negative control. At the end of the study, the pancreas of rats was made preparations with immunohistochemical staining and the average pancreatic beta cells from the 5 islets of Langerhans were calculated. The average number of pancreatic beta cells that were most effective from the kersen leaves treatment group was KI (dose of 0.3 mg/gBW) with an average of 39.9. There is an effect of ethanol extract of kersen leaves on increasing the number of pancreatic beta cells in white rats of wistar strain induced by STZ-NA with an effective dose of 0.3 mg/gBW.

Keywords: Type-2 Diabetes Mellitus, The Number of beta cell, *Muntingia calabura*, Kersen

Abstrak

Diabetes melitus tipe 2 merupakan jenis diabetes yang umum terjadi yang dapat disebabkan oleh peningkatan kadar gula darah yang mengakibatkan penurunan jumlah sel beta pankreas, disfungsi atau apoptosis. Untuk meningkatkan jumlah sel beta pankreas, diperlukan antioksidan. Kandungan antioksidan daun kersen dapat memperbaiki gangguan sel beta pankreas akibat tingginya kadar gula darah dengan cara menurunkan ROS dan RNF sehingga stres oksidatif menurun dan jumlah sel beta pankreas meningkat. Penelitian ini bertujuan untuk mengetahui pengaruh dan dosis efektif ekstrak etanol daun kersen terhadap jumlah sel beta pankreas tikus putih galur wistar yang diinduksi *streptozotocin-nicotinamide* (STZ-NA). Penelitian ini menggunakan posttest true experiment only dengan desain kelompok kontrol dengan kelompok KI-KV yang diinduksi STZ-NA dan kelompok KVI kontrol normal. KI-KIII diberi ekstrak daun kersen dengan dosis 0,3; 0,5 ;0,7 mg/gBW. KIV kontrol positif. Kontrol negatif KV. Pada akhir penelitian, pankreas tikus dibuat preparat dengan pewarnaan imunohistokimia dan dihitung rata-rata sel beta pankreas dari 5 pulau Langerhans. Rata-rata jumlah sel beta pankreas yang paling efektif dari kelompok perlakuan daun kersen adalah KI (dosis 0,3 mg/gBB) dengan rata-rata 39,9. Ada pengaruh ekstrak etanol daun kersen terhadap peningkatan jumlah sel beta pankreas tikus putih galur wistar yang diinduksi STZ-NA dengan dosis efektif 0,3 mg/gBB

Kata Kunci: Diabetes melitus tipe 2, Jumlah sel beta pankreas, *Muntingia calabura*, Kersen

INTRODUCTION

A good diet can be seen from the amount and type of food. Excessive consumption of carbohydrates and sugar will cause health problems.[1] The condition of consuming excessive food and lack of physical activity makes a person susceptible to hyperglycaemia (high blood sugar levels) which is called type 2 diabetes mellitus.[2]

NIDDM (non-insulin dependent diabetes mellitus/type 2 diabetes mellitus) accounts for approximately 90% of diabetes worldwide. Indonesia is included in the 10 countries with the most diabetes cases at the age of 20-79 years.[3] NIDDM is associated with elevated blood sugar levels.[4] The body's inability to compensate for high blood sugar levels is caused by several factors such as genetics, environment, and lifestyle.[5] Increased blood sugar levels can result in a decrease in the number of pancreatic beta cells which will affect the reduction in insulin levels.[5]

The progressive decrease in pancreatic beta cells in NIDDM patients requires one of the therapeutic managements in the form of insulin injection.[6] Not all Indonesian people can reach the price of insulin pens, so many choose to consume herbal plants because besides being cheap, herbal plants are also easy to find and can be processed independently at home.[7]

Herbal plants found by Indonesians have many benefits, one of which is kersen leaves (*Muntingia calabura L. folium*) which is useful for lowering blood sugar levels.[8] *Muntingia calabura L. folium* contains flavonoids, phenolics, and saponins which are used as antioxidants.[9] The antioxidant content of kersen leaves can improve pancreatic beta cell disorders in the body due to high blood sugar levels.. [8]

MATERIALS AND METHODS

This study used 24 male white rats (Rattus norvegiccus) wistar strain which were divided into 6 groups, namely the control group (positive, negative, normal) and the treatment group given ethanol extract of kersen leaves KI: 0.3 mg/gBB, KII: 0.5 mg/gBW and KII: 0.7 mg/gBW. This research is experimental research with true experiment post test only with control group design with simple random sampling technique.

Preparation of ethanolic extract kersen leaves (*Muntingia calabura L. folium)*

Making the extract begins with picking leaves from trees around the Kembaran District, Banyumas Regency, then determination at the Pharmacy Biology Laboratory, University of Muhammadiyah Purwokerto. The leaves are washed and dried, then put into a maceration container and keep away from sunlight. [8,10,11] Kersen leaves powder was extracted by maceration method by soaking in solvent (96% ethanol).[11] One part of simplicia is added to 10 parts of solvent, soak for 2x24 hours and stir occasionally. [10,12] After that, filter with filter paper, and the macerate is evaporated using a rotary evaporator until the filtrate is obtained, followed by evaporation with a water bath so that the extract becomes thick.. [11,13]

Induction with streptozotocin and nicotinamide

After the 7-day acclimatization process, the rats were weighed and made sure the rats were moving swiftly and there were no signs of illness. Rats were injected with Nicotinamide at a dose of 230 mg/kgBW intraperitoneally, 20 minutes later induced STZ (Streptozotocin) at a dose of 65 mg/kgBW intraperitoneally and waited for 5 days.

Giving ethanol extract of kersen leaves (*Muntingia calabura L. folium)*

After the rats were declared to have NIDDM, give ethanol extract of kersen leaves (*Muntingia calabura L. folium*) with a dose of KI: 0.3 mg/gBW, KII: 0.5 mg/gBW, and KIII: 0.7 mg/gBW. The positive control group was given 500 mg of metformin which was converted to 9 mg of rats, and the negative control group was given aquadest. The extract was administered using a sonde for 14 days.

Preparation of microscopic preparations

After the treatment was completed, the animals were anesthetized and then terminated using the cervical dislocation method[14], then paraffin blocks were made and pancreatic tissue preparations were made with immunohistochemical staining, then the average number of pancreatic beta cells was calculated from 5 pancreatic islets per preparation with 400x magnification with a Leica attachment camera microscope. The calculation of pancreatic beta cells was carried out by researchers assisted by anatomical pathologists.[15]

RESULT

Normality test

The Shapiro-Wilk test shows p>0.05, meaning that the data is normally distributed.

Deskriptif test

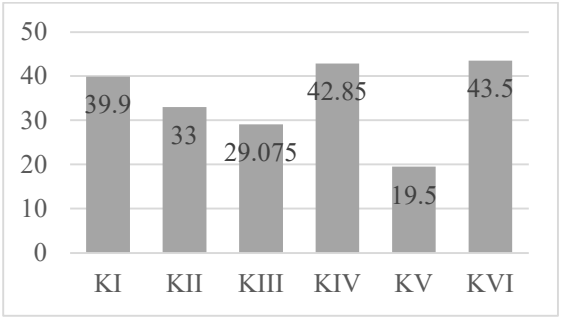

Figure 1 Histogram of Average Pancreatic Beta Cells

From Figure 1 we can see that the average number of pancreatic beta cells in the treatment group has increased compared to the negative group. The average number of beta cells given kersen leaves extract was not in line with the dose given. The smaller the dose, the more the average number of pancreatic beta cells increases, it can be seen in KI with a dose of 0.3 mg/gBW showing the highest average amount compared to KII (0.5 mg/gBW) and KIII (0.7 mg/gBW). At the end of the study, it turned out that the average number of beta cells in the treatment group had not yet reached the number of beta cells in the normal group, namely KVI, but what was close to normal was KI or a dose of 0.3 mg/gBB.

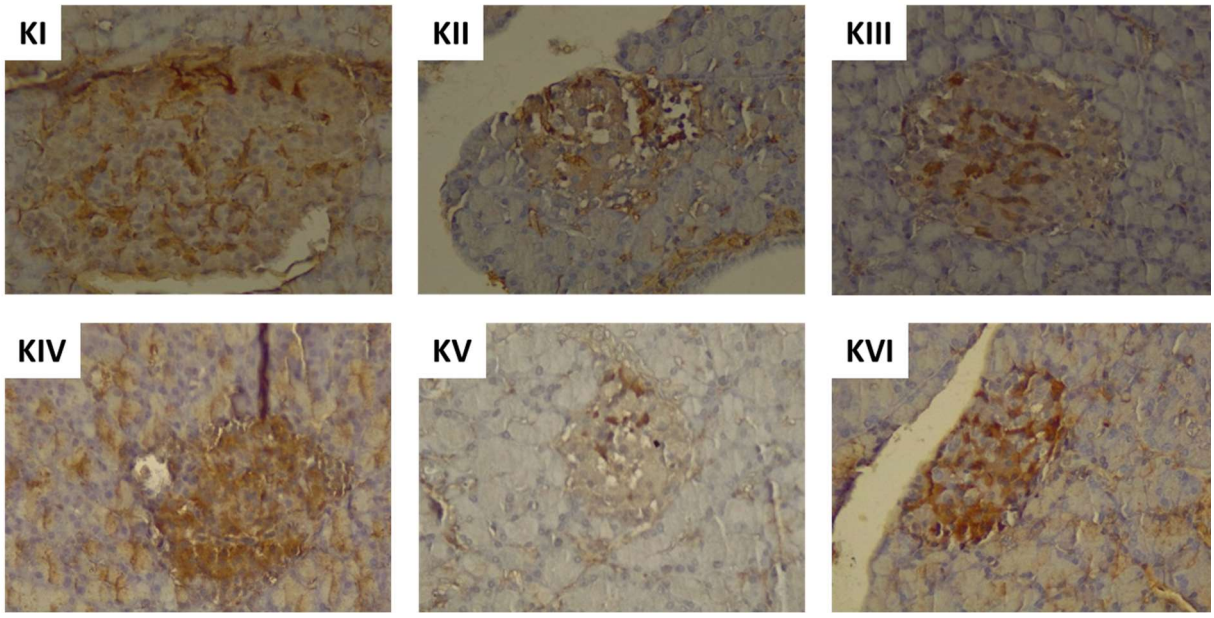

Figure 2 Pancreatic Beta Cells at 400x Magnification using IHC staining

In Figure 2, it can be seen that there is a significant difference between the number of beta cells in the positive control compared to the negative control, and there is a significant difference between KI (dose of kersen extract 0.3 mg/gBW) and KV (negative control group). It can be seen in the picture that dark brown cells in the islets of Langerhans in KIV are more than KV, and it can be seen that dark brown cells in KI are more than KV.

Homogeneity test

The significance value in this study is 0.146, meaning that the data is homogeneous because p>0.05

***One-Way ANOVA* test**

The One-Way ANOVA test in this study showed a significance result of 5.3x10-5, p<0.05, meaning that there was a significant difference.

***Post Hoc LSD* test**

From the results of the Post Hoc LSD test, it was found that between KI and KIII and KV, KII with KIV, KV, and KVI, KIII with KI, KIV, KV, and KVI, KIV with KII, KIII, and KV, KV with KI, KII, KIII, KIV, and KVI, KVI with KII, KIII, and KV have a significance of <0.05, which means that there is a significant difference between the groups. In contrast, KI with KII, KIV, and KVI, KII with KI and KIII, KIII with KII, KIV with KI and KVI, KVI with KI and KIV had no significant difference because the significance value was >0.05.

DISCUSSION

In this study, it was found that ethanol extract of kersen leaves (*Muntingia calabura L. folium*) could increase the number of pancreatic beta cells in white rats (*Rattus norvegicus*) wistar strain induced by streptozotocin-nicotinamide. The increase in the number of pancreatic beta cells can be seen from the results of the study which showed the average number of pancreatic beta cells in 5 pancreatic islets in KI, KII and KIII as the kersen leaves extract treatment group had an average number that increased from KV/negative control but not more increased from KIV/positive control seen from the mean KVI as normal control. Of the three treatment groups given kersen leaves extract, the results of KI showed that the average result of pancreatic beta cells increased the most from the negative control, KII increased secondly after KI, and the third increased KIII was not much different from KII.

The results of this study showed KII with a dose of 0.5 mg/gBW kersen leaves extract as a reference for effectiveness because in the study (Syahara, Harahap

& Widyawati, 2019) a dose of 500 mg/kgBW was the most effective dose to raise insulin levels and lower blood sugar levels, which means it also affects the increase in the number of pancreatic beta cells. The average result of the number of pancreatic beta cells in KII increased from the negative control which means the active substance content in kersen leaves works. Likewise, the dose of KI was reduced to 0.3 mg/gBW to determine whether the active substance in kersen leaves was still functioning or not and the results showed a more significant increase than KII. For KIII, the dose was increased to 0.7 mg/gBW and had the lowest average number of pancreatic beta cells compared to other treatment groups. From the results obtained, it was stated that as the dose of kersen leaves ethanol extract (*Muntingia calabura L. folium*) increased, the average number of pancreatic beta cells in rats did not increase because the dose-response relationship of an active substance was not always linear. also with the availability and characteristics of the drug receptor to cause an effect.[16,17] Drug response at low doses generally increases with dose, but with increasing dose the response may be less due to the possibility of reaching the dose with no further increase in response.[17] At higher doses, there may be a decrease in the effect, indicating that there is a saturation of the reaction between the drug molecules and the receptor which results in no increase in the effect.[16]

The increase in the number of pancreatic beta cells in KI, KII and KIII occurs because the administration of kersen leaves extract (*Muntingia calabura L. folium*) contains active substances in the form of flavonoids, saponins, phenolics which act as antioxidants.[9] The dominant compounds in kersen leaves are flavonoids, which are used to protect against free radicals.[18,19] Flavonoids can prevent oxidative stress, flavonoids can act as antioxidants directly or indirectly, for direct antioxidants flavonoids will donate hydrogen ions which will neutralize the toxic effects of free radicals, while indirect antioxidants flavonoids will increase endogenous gene expression.[20] Kersen leaves also have other antioxidants such as saponins and phenols. Saponins can regenerate cells in the pancreas so that the number of pancreatic beta cells will increase and insulin secretion will also increase.[21] Phenol can also donate hydrogen ions and can neutralize free radicals in the oxidation process.[20] Kersen leaves antioxidants can improve the sensitivity/response of pancreatic beta cells due to high blood sugar levels in rats that have been induced with *STZ-NA* to develop type 2 diabetes.[8,19,22] *STZ* is a compound that will increase *ROS* and *RNS* levels so that oxidative stress is high which makes pancreatic beta cells damaged, while *NA* plays a role here to help kersen leaves to inhibit apoptosis in the oxidative stress pathway and can reduce *ROS* and *RNF* which result in decreased oxidative stress so that it can reduce oxidative stress so that increase the number of beta cells[23,24] The antioxidants in kersen leaves will bind to free radicals so that it will reduce insulin resistance.[8] The antioxidants in kersen leaves, especially flavonoids, will inhibit pancreatic beta cell damage by way of beta cells in the islets of Langerhans to regenerate and re-issue insulin into the blood, making it suitable for type 2 DM patients. [25]

The average increase in the number of pancreatic beta cells in the 5 islets of Langerhans in KIV/positive controls who were given 9 mg metformin was much higher than that of KV/negative controls by looking at KVI/normal controls. The mean number of pancreatic beta cells in the metformin-treated group increased compared to the negative control group, possibly due to reduced beta cell apoptosis, increased beta cell formation or both. Metformin is an antihyperglycemic agent that can improve insulin sensitivity and does not cause hypoglycemia.[26] The mechanism of metformin which is a biguanide is not known with certainty, but its primary effect is to reduce hepatic glucose production by activating the enzyme AMP-activated protein kinase, slowing glucose absorption in the gastrointestinal tract and increasing glucose excretion from the blood.[17] Metformin is the first line in patients with type 2 diabetes because it is safe and does not pose a risk of myocardial infarction. [26]

The average number of pancreatic beta cells in KV/negative control has the smallest number compared to other groups, this can occur because the negative group is a group that was induced by *STZ-NA* to become a type 2 DM animal model by only being given aquadest and standard feed when the other group was given the intervention kersen leaves extract and metformin. Type 2 DM patients usually have resistance or a large beta cell deficiency, so the number of beta cells is insufficient to overcome insulin resistance or increased blood sugar, so that if it is not inhibited, the number of pancreatic beta cells can continue to decrease due to damage.[17] In type 2 DM patients, beta cells exposed to hyperglycemia will produce ROS, as ROS increase will cause pancreatic beta cell damage.[26] The quality and quantity of pancreatic beta cells are influenced by things such as the regeneration process, beta cell survival, cellular mechanisms, adaptability, cell apoptotic processes or failure of metabolic compensation.[26]

CONCLUSION

The ethanol extract of kersen (Muntingia calabura L. folium) can increase the number of pancreatic beta cells of white rats (Rattus norvegicus) wistar strain induced by STZ-NA with an effective dose of 0.3 mg/gBW.

ACKNOWLEDGEMENTS

The author would like to thank the Faculty of Medicine, University of Muhammadiyah Purwokerto for their support during the preparation of this manuscript.

DISCLAIMER

This article was presented at the 16th Indonesian Pharmacological Association national congress in 2022 and won 4th place, but has never been considered and published in any journal.

REFERENCES

1. Susanti S, Bistara DN. Hubungan Pola Makan Dengan Kadar Gula Darah Pada Penderita Diabetes Mellitus. *J Kesehat Vokasional*. 2018;3(1):29. doi:10.22146/jkesvo.34080
2. Masi GNM. Kadar Gula Darah Pada Pasien Diabetes Melitus Pancaran Kasih Gmim Manado. 2017;5.
3. Diabetes Federation International. *IDF Diabetes Atlas Ninth Edition 2019*.; 2019. http://www.idf.org/about-diabetes/facts-figures.
4. Soelistijo SA, Lindarto D, Decroli E, et al. Pedoman pengelolaan dan pencegahan diabetes melitus tipe 2 dewasa di Indonesia 2019. *Perkumpulan Endokrinol Indones*. 2019:1-117. https://pbperkeni.or.id/wp-content/uploads/2020/07/Pedoman-Pengelolaan-DM-Tipe-2-Dewasa-di-Indonesia-eBook-PDF-1.pdf.
5. Keresztes P, Peacock-Johnson A. CE: Type 2 diabetes: A pharmacologic update. *Am J Nurs*. 2019;119(3):32-40. doi:10.1097/01.NAJ.0000554008.77013.cf
6. Bahendeka S, Kaushik R, Swai AB, et al. EADSG Guidelines: Insulin Storage and Optimisation of Injection Technique in Diabetes Management. *Diabetes Ther*. 2019;10(2):341-366. doi:10.1007/s13300-019-0574-x
7. Helen Anjelina Simanjuntak. Utilization of Diabetes Mellitus Medicinal Plants In Simalungun Ethnic Society of Simalungun Regency (North Sumatera Province , Indonesia). *J Biol Lingkungan, Ind Kesehat*. 2018;5(1).
8. Aligita W, Susilawati E, Sukmawati IK, Holidayanti L, Riswanti J. Antidiabetic activities of Muntingia calabura L. leaves water extract in type 2 diabetes mellitus animal models. *Indones Biomed J*. 2018;10(2):165-170. doi:10.18585/inabj.v10i2.405
9. Minh NP, Nhan NPT, Khoa NNA, Thao NN, Tram NT, Sang VP. Different Parameters of Herbal Tea Production from Straw Berry (Muntingia calabura) Leaf. *J Pharm Sci Res*. 2019;11(4):1451-1454.
10. BPOMRI. *Acuan Sediaan Herbal*. Jakarta: Direktorat Obat Asli Indonesia Badan Pengawasan Obat dan Makanan RI; 2012.
11. Syahara S, Harahap U, Widyawati T. Activity of Muntingia calabura Leaves Ethanolic Extract on Glucose and Insulin Blood Levels in Streptozotocin-induced Rat. *Asian J Pharm Res Dev*. 2019;7(4):8-11. doi:10.22270/ajprd.v7i4.552
12. Herlina. Science & Technology Indonesia Antidiabetic Activity Test Of Ethanolic Seri Leave ' s (Muntingia Calabura L .) Extract In Male Rats Induced By Alloxan. *Sci Technol Indones*. 2018;3:7-13.
13. Sari DI, Triyasmono L. Rendemen dan Flavonoid Total Ekstrak Etanol Kulit Batang Bangkal (Nauclea subdita) dengan Metode Maserasi Ultrasonikasi. *J Pharmascience*. 2017;4(1). doi:10.20527/jps.v4i1.5755
14. Perdana AG, Soesilowati R, Romdhoni F, Adi R, Putra N. Pengaruh aspartam terhadap struktur histologi hepar tikus (rattus norvegicus) jantan galur wistar model diabetes melitus. *Ibnu Sina Biomedika*. 2018;2(1):1-7.
15. Ningrom, Ira Citra, Puspasari, Dik, Wijaya, Indra ,Karlowee,Vega MeiraDewi, Amarwati siti. Perbedaan antara ekspresi tumor activating macrophage (tam/cd68) dan tumor supressor gene (p16) pada karsinoma sel skuamosa serviks uteri yang residif dan tidak residif di rsup dr. Kariadi semarang. 2018;volume 3 n.
16. Jaya, Bhaktiar Mulya;Bahar, Yenni;Koosgiarto, Dharma;Karita D. Pengaruh Pemberian Ekstrak Etanol Daun Kemangi (Ocimum sanctum) Terhadap Kadar Ureum dan Kreatinin Tikus Putih Galur Wistar (Rattus norvegicus) yang Diinduksi Sisplatin. 2018:1-9.
17. Katzung BG, Masters SB, Trevor AJ. *Farmakologi Dasar & Klinik*. Vol 53.; 2018.
18. Andalia N, Safrida, Sabri M. Efektivitas pemberian ekstrak daun kersen (muntingia calabura l.) Terhadap struktur mikroskopis sel beta pankreas tikus hiperglikemik. *J EduBio Trop*. 2017;5(1).
19. Sasmita FW, Susetyarini E, Husamah H, Pantiwati Y. Efek Ekstrak Daun Kembang Bulan (Tithonia diversifolia) terhadap Kadar Glukosa Darah Tikus Wistar (Rattus norvegicus) yang Diinduksi Alloxan. *Biosfera*. 2017;34(1):22. doi:10.20884/1.mib.2017.34.1.412
20. Wayan Chitra Septiana MA. Journal of nutrition. *J Nutr Coll*. 2016;Volume 5,. doi:10.1038/184156a0
21. Parawansah, Giatna S, Yusuf MI. Uji Efek Antidiabetik Ekstrak Daun Andong (Cordyline fruticosa L . A . Cheval) Mus musculus yang Diinduksi Streptozotosin. *Medula*. 2015;2(2):156-160.
22. Prahartini A, Sahid N, Murbawani E. Pengaruh Bubuk Daun Kenikir (Cosmos Caudatus) Terhadap Kadar Glukosa Darah Tikus Diabetes Diinduksi Streptozotocin. 2016;5(2):51-57.

doi:10.14710/jnc.v5i2.16359
23. Maiese K. New Insights for nicotinamide: Metabolic disease, autophagy, and mTOR. *Front Biosci - Landmark*. 2020;25(11):1925-1973. doi:10.2741/4886
24. Raza H, John A. Streptozotocin-induced cytotoxicity, oxidative stress and mitochondrial dysfunction in human hepatoma HepG2 cells. *Int J Mol Sci*. 2012;13(5):5751-5767. doi:10.3390/ijms13055751
25. Kurnia DC. Pemanfaatan Daun Kersen (Muntingia calabura L.) dalam Penanganan Diabetes Mellitus. *Berk Ilm Mhs Farm Indones*. 2020;7(1):017-025. doi:10.48177/bimfi.v7i1.7
26. Eva D. *Diabetes Melitus Tipe 2*. padang: pusat penerbitan bagian ilmu penyakit dalam FK andalas; 2019.