

Activity of Ethanol Extract of *Gynura procumbens* (Lour.) Merr. Leaf to Decrease Blood Glucose Level and Recover Pancreatic Histopathology in White Male Mice Induced by Alloxan

Rahimatul Uthia^{*1}, Suci Fitrima¹, Rahmad Abdillah¹, Sri Oktavia¹

¹School of Pharmaceutical Science (STIFARM) Padang, Padang-25176, Indonesia

Abstract : Introduction : Diabetes mellitus is a chronic condition indicated by the increasing of blood glucose level which is caused by lack of insulin as consequence from disturbance in the insulin secretion. This research is aimed to see the influences of ethanol extract of *Gynura procumbens* (Lour.) Merr. toward the reducing of blood glucose level and to fix pancreatic histopathology.

Methods : methodology used in this research is methodology of experimental using animal testing. Mice divided into 5 groups consists of negative control, positive control, Group of 50 mg/kg BW dose, 150 mg/kg BW, 300 mg/kg BW and 200 mg/kg BW alloxan induced animal intraperitoneally. The Extract is orally given for 7 days. The research data is analyzed using one way ANOVA test and Followed by Duncan test.

Result : the result of research showed that giving ethanol extract of *Gynura procumbens* (Lour.) Merr. with 50 mg/kg BW doses, 150 mg/kg BW, 300 mg/kg BW was able to reduce the level of blood glucose significantly ($P < 0.05$) and pancreatic histopathology illustrate the perceptual structure of endocrine cubicles which homogenous spreading out on Langerhans Island and proportional looked of cytoplasm.

Conclusion : this discovery showed that giving ethanol extract of *Gynura procumbens* (Lour.) Merr. in those three doses above was able to reduce the level of blood glucose and recover pancreatic histopathology which has damaged.

Keywords : *Gynura procumbens* (Lour.) Merr., Diabetes Mellitus, pancreatic cell

Introduction

Lifestyle changes could trigger negative impact in form of the emerging of various degenerative diseases, one of them is diabetes mellitus. Based on WHO data in 2016 total population of Indonesia 258 million people Which 28.100 males and 28.200 females in 30-69 range of ages die caused by diabetes, in the other hand, up to 70 years old 16.300 males and 34.800 females die caused by diabetes. Prevalence of diabetic female is more susceptible to diabetes in 30-70 range of age.¹

Hyperglycemia is one among many indication of diabetes mellitus which indicated by the increasing of blood glucose levels and progressive change concerning on structure of pancreatic histopathology. Damaged

Rahimatul Uthia *et al* / International Journal of ChemTech Research, 2018,11(10): 142-148.

DOI= <http://dx.doi.org/10.20902/IJCTR.2018.111018>

pancreas could be triggered by many factors, those aregenetical, microbe infection, substancialdiabetogenic and free radical.²

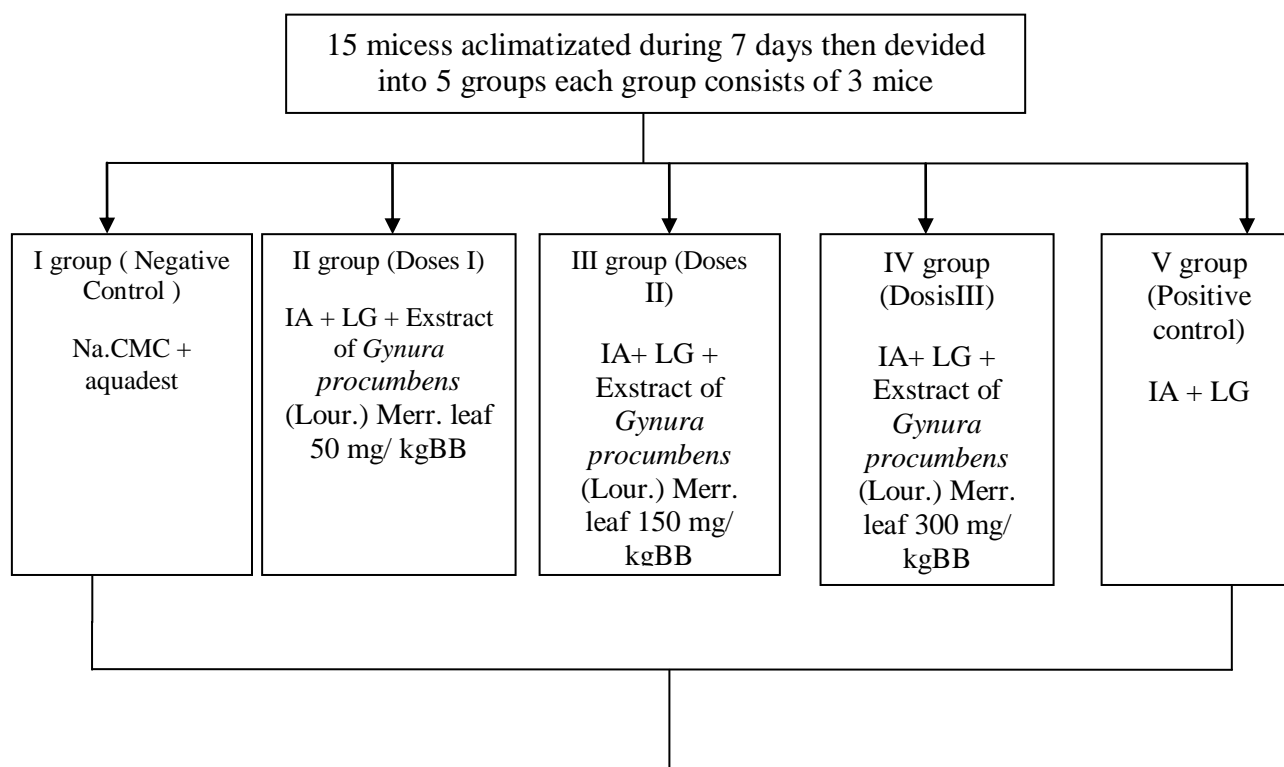
One way to cure diabetes mellitus bydoing a therapy called herbal therapy. Herbal therapy in question isprocess of healing diabetes mellitus byconsumingconcoction extracted from variousmedicinal plants.³One of medicinal plant used as a traditional medicineis*Gynura procumbens* (Lour.) Merr..Kaewseejan, *et al*had proventhatleaf of*Gynura procumbens* (Lour.) Merr. contains flavonoid particularly such as myricetin and kaempferol.⁴The main benefit of *Gynura procumbens* (Lour.) Merr.leafas anti-diabetes are flavonoid and polyphenol.⁵Lately,reportedthat ekstracted water of *Gynura procumbens* (Lour.) Merr.is not only able to reduceblood glucose levelsbut also increaselevel of glucose absorption by muscle to diabetic micewhichinduced by streptozotocin (STZ).⁶Regarding to previous researchabout thehistopathology illustration of beta cell of pancreas toginger juice, essential oil contentand flavonoid ingingeris able to recoverthe beta cell of pancreas induced by alloxan.⁷

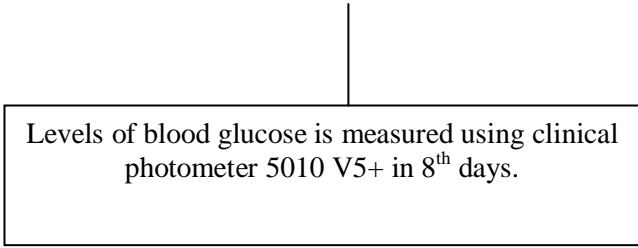
Methods

Materials

Substances used are *Gynura procumbens* (Lour.) Merr. leaf, ethanol 95 % (PT Bratacem), glucose powder (PT Bratacem), aquadest (PT Bratacem), alloxan monohydrat (Aldin), Sodium chloride (NaCl) 0,9 % (PT Widatra Bhakti), alcohol 70 % (PT Bratacem), acohol 96 % (PT Bratacem), formaline 37 % (PT Bratacem), dyeHaematoxyllin (SPI-Chem), Eosin (The SCIENCE Company), xylol (Merck), Mayer's albumin (DiaSys), paraffin (Merck), and adhesive ethelan (Merck), *Natrium Carboxy methyl cellulose* (NaCMC) (PT Bratacem), mercury chloride (Merck), potassium iodide (Merck), hydrochloric acid anhydrous (Merck), chloroform (PT Bratacem), concentrated sulfuric acid (Merck), reagen blood glucose checking (PT Rajawali Nusindo), kaempferol (Merck), magnesium sulfate powder (Merck), acetic acidanhydrous (Merck), boric acid (Merck), n-Hexan (PT Bratacem), ethyl acetate (PT Bratacem), formic acid (PT Bratacem), citric acid (Merck), Alumunium chloride (Merck), sodium acetate (Merck).

Research procedure





Levels of blood glucose is measured using clinical photometer 5010 V5+ in 8th days.

information:IA = Induction of Alloxan

LG= Liquid of Glucose

Figure 1: Research procedure

Histopathologyprepare

Making histopathologyprepareusing paraffin methodology.⁸

1. The dissected white male mice's Pancreas, washed first in to soluted NaCl (Sodium Chloride) 0,9 %.
2. Then Fixed by Soluted formaline 10 % for 3 hours.
3. Dehydrate sequentially using alcohol 70 %, 95 %, 100 % in an hour for each.
4. Moving on to clearing process using xylol for twice, in an hour for each.
5. In filtrated into liquid paraffin for two hours and cycled for three hours and thirty second in the incubator at a temperature 56-60 °C.
6. Commit to embedd ing process which is by planting tissues into a print using pure paraffin medium .
7. The planted plant being formed into a wood beam then cut using 5µm rotary microtom. Sticked on glass object which has applied mayer's albumin glue previously (egg whites and gliserine), and then drained it up .
8. Put the piece of bond-looked tissues in water bath filled of water at maximum temperature 40 °C.

3.3.17 Coloring Prepare using Haematoxylin-Eosin color⁸

1. The slice which has put on glass object then *deparafinisaze* with xylol for twice in 5 minutes.
2. Rehydrate using alcohol 100 %, 95 %, 70 % during 2 minutes for each.
3. Wash it in water flow.
4. Paint it using *Haematoxylin* for 2 minutes.
5. Wash again with water until clean and cristal clear.
6. Then dip it in to solution of HCl 0,4 N for twice till three times.
7. After that wash it again with water flow.
8. Paint it using *eosin* for 5 minutes.
9. Dehydrate with alcohol 70 %, 95 %, 100 % for each in 2 minutes.
10. *Clearing* it using *xylol* for twice, for each in 2 minutes after that drained it with wind blow.
11. Conducted to *mounting* which is by giving ethelan glue to prepare and close it off with cover glass.
12. Observe it under the microscope.

Data Analysis

Statistical analysis of results was done by ANOVA test followed by Duncan test for determination of variance.

Result and Discussion

Sample used in this research is *Gynura procumbens* (Lour.) Merr. leaf from Kampung Jua, West Sumatera. Identifying the plant was done in Herbarium Laboratory department of Biology MIPA Faculty, University of Andalas (UNAND) campus of Limau Manih Padang west Sumatera. The aim of identification is to find out sample identity which is used in. Based on the result of identification discovered that sample used in this research is leaf of *Gynura procumbens* (Lour.) Merr., Compositae family.

Table No. 1: The result of characterization from *Gynura procumbens* (Lour.) Merr. leaf:

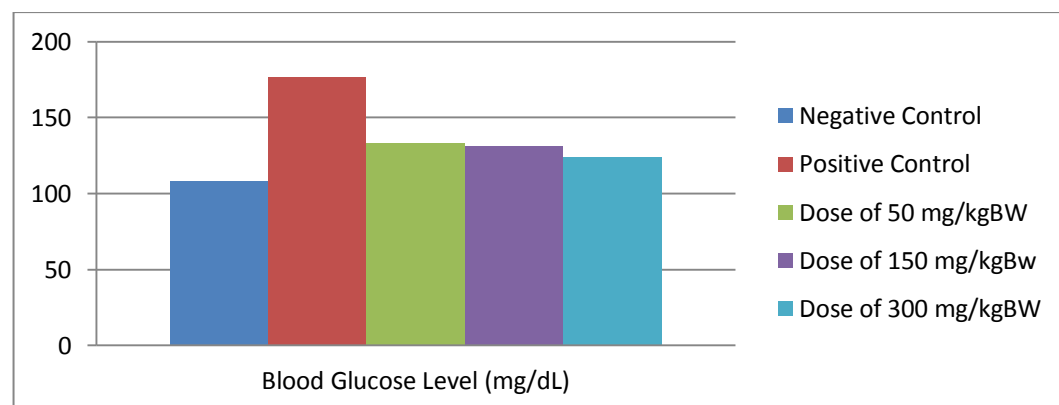
Characteristic ⁹	Percentage (%)
shrink dehydration of simplicia	7,58 ± 1,66
Ashes content of simplicia	6,49 ± 0,64
Unsoluble ashes content simplicia acid	0,98 ± 0,04
Soluble compound content in water simplicia	26,09 ± 1,96
Soluble compound content ethanol simplicia	6,18 ± 2,33
rendemen extract	12,06
Water content extract	9,42 ± 0,82
Ashes extract content	2,94 ± 0,06
Unsoluble ashes content extract acid	0,50 ± 0,08
flavonoid extract content	

The Effect of Giving Ethanol Extract from *Gynura procumbens* (Lour.) Merr. Leaf toward Blood Glucose Level of Male White Mice

The next step is in vivo test from ethanol extract of *Gynura procumbens* (Lour.) Merr. leaf for 7 days. In this step, mice which is induced by alloxan then ethanol extract of *Gynura procumbens* (Lour.) Merr. is given to animal group by 5 oral treatments everyday for a week. During a week treatment, ethanol extract of *Gynura procumbens* (Lour.) Merr. shows a good effect to the reducing of blood glucose level of mice. The data of mice glucose levels during a week treatment explained on Table No. 2 and picture No. 2.

Tabel No. 2: The result of blood glucose level of white male mice after being induced by alloxan doses 200 mg/kg BB intraperitoneally (i.p) and given 10 % of glucose as well as giving ethanol extract of *Gynura procumbens* (Lour.) Merr. in various of doses.

Group of Doses	Blood glucose level (mg/dL)			Average (\bar{X}) ± Standart of Deviation (SD)
	1	2	3	
Negative Control	82	100	143	108 ± 31.34
Control Positive	162	184	185	177 ± 13.00
Doses 50mg/kg BW	126	126	148	133 ± 12.70
Doses 150mg/kg BW	109	133	151	131 ± 21.07
Doses 300mg/kg BW	105	126	141	124 ± 18.08

**Picture 2. Diagram of Blood Glucose levels based on negative control, positive Control and various doses of giving ethanol extract of *Gynura procumbens* (Lour.) Merr. toward the reducing blood glucose levels of white male mice**

The picture above shows that the percentage of negative control and those three doses is almost similar, beside that doses group and positive control shows a significantly different range of score. Surprisingly, from three doses given, doses 50 mg/kg BW, doses 150 mg/kg BW dan doses 300 mg/kg BW can reduce blood glucose level of an alloxan-induced white male mice.

The result of statistical test of one way variant analysis from homogenous table data variable of blood glucose levels shows a significant result which is (sig. 0,430). So it could be followed by one way Anova test. Based on the result of the calculation shows that sig score of blood glucose levels with score of (sig. 0,021) which means giving ethanol extract of *Gynura procumbens* (Lour.) Merr. is influential to reduce blood glucose levels of alloxan-induced mice suffering diabetes mellitus. Then statistical analysis followed by Duncan test, which the result shows that various doses of ethanol extract of *Gynura procumbens* (Lour.) Merr. is able to give the same impact to decrease the blood glucose levels but from the research data we can see that the biggest percentage of decreasing blood glucose levels is on doses 300 mg/kg BW.

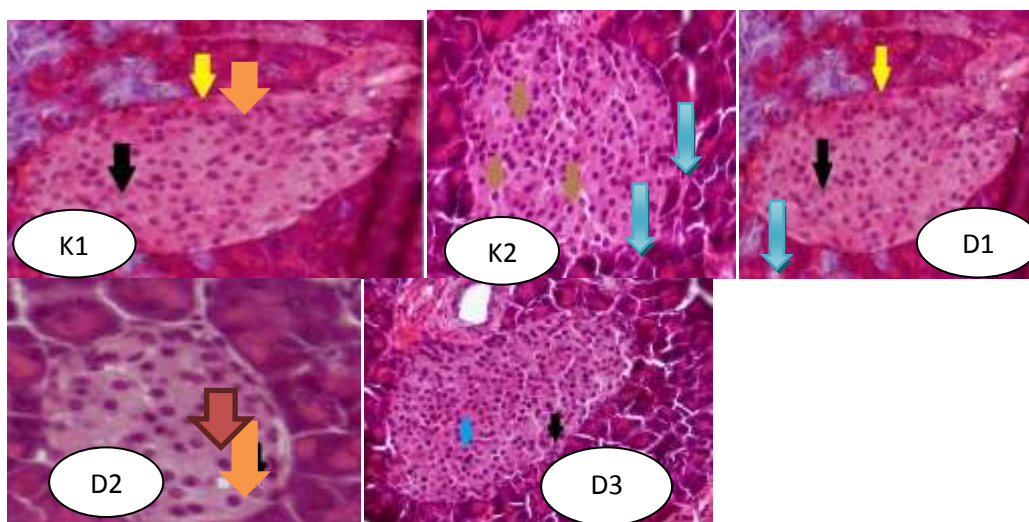
Pancreatic Histopathology of White Male Mice

After a week of treatment, the next stage is to take the mice's pancreas by doing surgery to be used as prepare and committed to painting process using *Haematoxyllin-Eosin* (HE) method. Illustration of pancreas histology gotten from the result of checking process and analyzing process microscopically with scale of 100x to prepare of mice's pancreas illustrated on picture 2.

K1 shows that there is a regularity of structure of endocrine cell in negative control which spreading out on *Langerhans land* with homogenous form of cell and proportional-looking size of cytoplasm to the large core, with HE painting technique we could see the purplish blue core of endocrine with more rounded shape and nucleus is clear, along with pink cytoplasm and proximate form of cell and full fill the whole space of *Langerhans island* so in the normal group shows that endocrine condition is intact and homogenous structure of cell.¹⁰

Morphology change seen on positive control which induced by alloxan that there is lesion on pancreas tissues in form of degeneration of endocrine cell headed to necrotic cell is illustrated on Picture No.3 which is K2. Degeneration of endocrine cell is seen in the core which change into polymorph (non-homogenous). The change is illustrated in form of size changing become smaller (picnosis) or even faded and more like an empty cytoplasm.¹¹ Beside that there is also clumping cytoplasm which shows that there is denaturation of protein which trigger leukocyte reaction. Then, necrotic tissue decreased by granulation tissues and finally formed "scarring".¹² This case explains that by giving *alloxan* it can damage endocrine cell of pancreas particularly beta cell and as a consequence insulin secretion into blood vessels decreases.¹³

Changes on cells caused by substances which have similar cytotoxic effect with alloxan which is depreciation of pancreas islands, reducing amount of beta cells, and demise of cells. This case happen caused by alloxan toxic on beta cells initiated by free radicals which is formed by redox reaction. Alloxan and its reduction product, creating redox cycle superoxide radicals formation. This radical is dismutated to hydrogen peroxide. Radical hydroxyl with high reactivity initiated by Fenton reaction. Free Radicals action with high stimulation increases cytosol calcium concentration which trigger fast destruction of pancreas beta cells. The increasing amount of concentration calcium in cytosol also caused by alloxan which induced calcium spending from mitochondria which trigger the disrupting process of pancreas beta cells oxidation. Because of disrupted pancreas beta cell so insulin is not perfectly formed with the result of that is the increasing of blood glucose levels.¹⁴



PictureNo. 3:Illustration of Langerhans islet cells Histopathology white male mice scale of 100x with HE painting., ➡ =normal cell, ➡ = necrosis, ➡ =disappearing of cell's core,

➡=endocrine started to regenerate becoming normal form of cell

Discussion

Based on observation using HE painting technique on doses 50 mg/kg BW can show that there is a change on its pancreas cells. That change includes endocrine cells which started to regenerate into normal form of cell, even though there are several degenerated endocrine cells and there is still inflamed cell but only on doses 50 mg/kg BW which endocrine cell is getting better. On doses 150 mg/kg BW shows that there is a regeneration process of endocrine cells but the amount of it is not more than doses 300 mg/kg BW can be seen on picture 2 which is D2. Majority of endocrine cells on doses 50 mg/kg BW are still degenerating which is almost likely identified to negative control but illustration of empty *Langerhans islet* is less finding but not on doses 150 mg/kg BW and doses 300 mg/kg BW, on doses 300 mg/kg BW is clearly seen that there is regenerating happened on *Langerhans islet* closed to negative control which necrotic relative endocrine cell is less (proven by reducing of an empty space caused by necrosis) and there is endocrine cell which stay in normal condition. Quantitatively, this case illustrates that there is the increasing of endocrine cells (picture n0.3 which is D3). If it being seen from the result of the research which has been done, based on research data, the tested group which has been given by ethanol extract of *Gynura procumbens* (Lour.) Merr. is able to reduce the level of blood glucose and shown the obvious betterment toward pancreatic organ.

This case happened because of the existence of bioactive compound contained in *Gynura procumbens* (Lour.) Merr. leaf which is flavonoid identified as a group of polyphenol compound which is assumed can reduce blood glucose levels by improving the taking of glucose from blood into cell.⁵ Flavonoid is also has antioxidant activity which is able to comprehend free radical as a cause of damage on pancreas beta cell and hamper the damage so beta cell which left is still functional.¹⁵

Antioxidant contained in *Gynura procumbens* (Lour.) Merr. leaf is also able to protect beta cell for being normal and enable the regeneration of existed beta cell through the process of mitosis or through establishment of new islet by committing to proliferation and differentiation of endocrine from ductal cells and ductular.¹⁵ The existence of recovery on beta cells the sources of insulin, so it triggers the increasing amount of insulin in Mice's body which is able to facilitate the entry of blood glucose into cell as the result, it can reduce the blood glucose level.

Conclusion

From the research which has been done we can conclude that giving ethanol extract of *Gynura procumbens* (Lour.) Merr. leaf has been proven can definitely reduce the levels of blood glucose on diabetic white male mice which induced by aloksan and histopathologically giving the extract can also recover the

illustration of Langerhans island of pancreas endocrine cell distribution on diabeticmice induced by aloksan which endocrine cell committed to regenerate into a normal form.

References

1. World Health Organization. Web World Health Organization – Diabetes country profiles, 2016, Accessed at December 10th 2016 from http://www.who.int/diabetes/country-profiles/idn_en.pdf?ua=1
2. Isselbacher K. J., Braunwald E., Martin J. B., Fauci A. S. and Kasper D. L.. Harrison prinsip-prinsipilmupenyakitdalam (13th edition). Translated by: A. H. Asdie., Penerbit BukuKedokteran EGC, Jakarta, 2000
3. Utami P., Tanaman obat untuk mengatasi diabetes mellitus, PT AgroMedia Pustaka, Jakarta, 2003
4. Kaewseejan N., Sutthikum V. andSiriamornpun S., Potential of *Gynuraprocumbens* leaves as source of flavonoid enriched fractions with enhanced antioxidant capacity, Journal of functional foods, 2015, 12, 120-128
5. Utami P. And Puspaningtyas D. E., The miracle of herbs, PT AgroMedia Pustaka, Jakarta, 2013
6. Zurina H., Yam M. F., Mariam A. and Ahmad Pauzi M. Y., Antidiabetic properties and mechanism of action of *Gynura procumbens* water extract in streptozotocin-induced diabetic rats, Molecules, 2010, 15, 9008-9023
7. Djama'an Q., Goenarwo E. and Mashoedi I, Pengaruh air perasan jaheterha dapka darglukosadara hdangambaran histopatologisel beta pancreas, Sains Medika Jurnal Kedokteran dan Kesehatan, 2012, 4, 115-226
8. Leeson C. J., Carneiro J. And Kelley R. O, Histologi dasar (8th edition). Translated by: J. Tambayong, Penerbit Buku Kedokteran EGC, Jakarta., 1995
9. Kementerian Kesehatan Republik Indonesia, Farmakope Herbal Indonesia (Edisi 1), Kementerian Kesehatan Republik Indonesia, Jakarta, 2010
10. Fiore M.S.H, Atlas histology manusia (6th edition). Penerjemah: M. Martoprawiro, S.K. Siswojo, I. Suryono, J. Tambajong, Penerbit Buku Kedokteran EGC, Jakarta, 1992
11. Diani A. R., Sawada G., Wyse B., Murray F. T. and Khan M., Pioglitazone preserves pancreatic islet structure and insulin secretory function in three murine models of type 2 diabetes. Am. J. Physiol. Endocrinol. Metab, 2004, 286, 116-122
12. Thomas, C. Histopatologi: bukuteks dan atlas untuk pelajaran patologi umum dan khusus (10th edition) translated by: H. Tonang, L. Widjajadan L. Libertus, Penerbit Buku Kedokteran EGC, Jakarta, 1988
13. Boudreau M. D., Taylor H. W., Baker D. G. and Means J. C, Dietary Exposure to 2-Aminoanthracene Induces Morphological and Immunocytochemical Changes in Pancreatic Tissues of Fisher-344 Rats. Toxicological sciences, 2006, 93, (1), 50-61
14. Rohilla A. and Ali S, Alloxan induced diabetes: mechanisms and effects. International Journal of Research in Pharmaceutical and Biomedical Sciences, 2012, 3, 819-823
15. Lukiati B., Maslikah S. I. and Nugrahaningsih, Potensi ekstrak etanol labu siam (*Sechium edule*) untuk perbaikan kerusakan sel beta pankreas dan kadar nitrogen oksida pada tikus yang mengalami diabetes melitus. Jurnal kedokteran hewan, 2016, 10(1), 24-27.
