Journal of Health Science
(Jurnal Ilmu Kesehatan)
https://www.ejournalwiraraja.com/index.php/JIK
2356-5284 (Print) |2356-5543 (online)

The Effect Of Water Concentration Of Red Belt Leaves (*Piper Crocatum*) On The Kidney And Pancreatic Histopathology Of Diabetes Mice (*Mus Musculus*)

Surati[1], Desi Armalia [2], Putri Kurniasiwi[3], Jon Farizal[4]
[1,3] Medical Laboratory Technology, Poltekkes of the Ministry of Health Semarang
[2] Anatomy-Histology Departemen, Faculty of Medicine, Diponegoro University Semarang
[3] Medical Laboratory Technology, Poltekkes of the Ministry of Health Bengkulu
Email: [1]analis_surati@yahoo.com,[2]desy.armalina@gmail.com, [3]putrikurniasiwi@poltekkes-smg.ac.id, [4]jonfarizal77@gmail.com
Correspondence Author: putrikurniasiwi@poltekkes-smg.ac.id

Article Information
Article History:
Received: 10 – 10 -2023
Revised: 23-10-2023
Accepted: 24-11-2023

Keywords :
Decoction of red betel leaves
Kidneys
Mus musculus
Diabetes Pancreas

ABSTRACT
Red betel leaves contain flavonoid compounds that can reduce blood sugar levels and minimize tissue damage by reducing lipid peroxide. This study aims to determine the effect of red betel leaf boiled water with a concentration of 25%, 50%, 75%, and 100% on the histopathology of the kidneys and pancreas of diabetic mice and to determine the most effective concentration. This experimental research was completely randomized with 2 control groups, K+ and K-, and 4 treatment groups, P1, P2, P3, and P4, with a sample population of 18 mice. Experimental animals were treated by giving red betel leaf boiled water with a concentration of 25%, 50%, 75%, and 100%, 50 mg and a sonde dose of 0.13 ml for 7 days. Then the kidneys and pancreas were taken, processed histologically, and observed with a 400x magnification microscope in 3 fields of view. The research results show sig. 0.015, it means that the histopathological appearance of the pancreas of mice P3, and P4 has a significant difference compared to mice in the K(+) group. Concentrations of 75% and 100% resulted in enlargement of kidney cell size approaching the size of healthy kidney cells and there was the best improvement in the pancreas organ. The conclusion is that concentrations of 25%, 50%, 75%, and 100% of red betel leaf boiled water affect the histopathology of the kidneys and pancreas of diabetic mice.

Introduction

Diabetes is a chronic disease characterized by increased blood glucose levels and can cause damage to the heart, blood vessels, kidneys, and nerves. The most common diabetes is type 2, which occurs because the body does not produce enough or a prevalence rate of 9.3% of the total population of the same age (Ministry of Health of the Republic of Indonesia, 2020).

Based on the health profile of Central Java province in 2019, the number of DM sufferers in Central Java was 652,822 people (Central Java Health Office, 2019).

The pancreas is an organ with a dual function, namely as an exocrine and endocrine gland. The pancreatic endocrine gland consists of islets, which are clusters scattered throughout the exocrine pancreas. insulin or becomes resistant to insulin (WHO, 2023).

According to the International Diabetes Federation (IDF) organization, in 2019, of the 10 countries with the highest number of diabetes cases, Indonesia was in 7th position, with a total of around 10.7 million cases. IDF estimates that at least 463 million people aged 20 – 79 years worldwide have diabetes, The islets of Langerhans consist of several different types of cells that produce certain hormones, namely beta (β) cells that produce the hormone insulin, alpha (α) cells that produce the hormone glucosinolate, delta (δ) cells that produce the hormone somatostatin and pancreatic polypeptide (PP) cells that produce pancreatic polypeptide hormone (Wilujeng & Pangestu, n.d.)

The kidney is the primary organ that often experiences problems from the effects of a compound. The kidneys have a high blood

volume flow, filtering toxic substances in the glomerular filtrate and carrying them through tubular cells (Putri, 2017). This is considered to make people living with diabetes miserable, so diabetes is believed to be a dangerous and frightening disease (Hartini, 2009).

Chronic hyperglycemia in diabetes mellitus sufferers can cause hypoxia in the interstitium of the kidneys, which will have an impact on the Glomerular filtration rate (GFR) (Dewi, 2021). Complications of diabetes mellitus in the kidneys that can result in kidney failure are called Diabetic Nephropathy. High glucose levels are the main cause of structural changes in the kidneys (Satria et al., 2018).

Insulin and oral hypoglycemic drugs are used as temporary treatments to control blood sugar levels in people living with diabetes, but there is another alternative, namely taking herbal medicines. Antidiabetic herbs are used in red betel leaves (*Piper crocatum*) because this plant contains secondary metabolic compounds that act as hypoglycemic agents (Handini & Rohmah, 2018). A herbal medicine that can empirically treat diabetes mellitus is red betel leaf (*Piper crocatum*) (Kendran et al., 2013).

The red betel plant has many benefits, such as flavonoid and polyphenol compounds, which can function as antioxidants, drugs to lower blood sugar levels (antidiabetic), anti-cancer, antiseptic, and anti-inflammatory. The content of other substances in the form of alkaloid compounds has anti-neoplastic properties, which are effective in inhibiting the growth of cancer cells (Reveny, 2011). Flavonoid and alkaloid compounds are active substances that have been studied to have hypoglycemic activity. Flavonoids can inhibit the action of the α-glucosidase enzyme in luteolin. The glucosidase enzyme is an enzyme that plays a role in determining the ability of a plant as an antidiabetic agent in vitro using inhibition techniques (Kendran et al., 2013).

This research uses mice because they are often used in laboratory research, especially those related to the field of histopathology. Mice have advantages such as a relatively short life cycle, easy to handle, reproductive characteristics similar to other mammals, anatomical structure, physiology day, alloxan was induced at a dose of 100mg/KbBB, which was converted from a human dose to mice. For example, the and genetics similar to humans (Mutiarahmi, C.N., 2021). Previous research by Shinta and Sudyanto (2016) concluded that boiled water from red betel leaves (*Piper crocatum*) reduced blood glucose levels in male white mice. A significance value of <0.05 indicates this, so it is concluded that boiled water from red betel leaves (*Piper crocatum*) can be used as an alternative herbal medicine to lower blood glucose (Shinta & Sudyanto, 2016). According to research by Nasi, Liestiono S et al. (2015), giving boiled water from red betel leaves (*Piper crocatum*) to animals tried to reduce blood sugar levels.

Histopathological changes in the islets of Langerhans in people living with diabetes have also been reported by several researchers, namely that there are changes quantitatively (reduction in number or size) and qualitatively (necrosis, degeneration and myloidosis, vacuolization) (Nubatonis et al., 2018). Meanwhile, another research concluded that the results of observations on kidney morphology showed that there was no change in color in the control and treatment groups. Still, the ethanol extract of peridot leaves at a dose of 300 affected reducing the diameter of the glomerulus (Putri, 2017).

The research was carried out with the aim of determining the effect of boiled water from red betel leaves (*Piper crocatum*) with varying concentrations of 25%, 50%, 75% and 100% on the histopathology of the kidneys and liver of mice (*Mus musculus*) by observing tubule cell size and diameter. Glomerulus and assess the quality of the cell nucleus and cytoplasm on pancreatic histopathology preparations.

METHOD

This research is a type of experimental research with a Completely Randomized Design (CRD) with two control groups, namely K+ and K-, and four treatment groups, namely P1, P2, P3, and P4, each given ARDSM concentrations of 25%, 50%, 75%, and 100%. Calculation of the sample population from the Gomez and Gomez formula resulted in 18 mice whose kidney and liver histopathological preparations were made and observed under a microscope. Statistical tests in this reasearch used Kruskal Wallis based on the results of pancreatic histopathological observations, and continued by using Mann Whitney test.

The research began with the adaptation of the mice for seven days; then, on the 8th weight of mice is 25 grams = 25g/1000Kg = 0.025Kg = 0.025Kg x 100mg = 2.5mg. Mice were induced with 0.0125 ml of alloxan

intraperitoneally which will have the effect of increasing blood glucose levels. Alloxan was induced once in groups K+, P1, P2, P3 and P4. On day 11, the mice's blood glucose levels were measured to ensure that the mice were suffering from diabetes with glucose levels > 200 mg/dl. Make red betel leaf boiled water with concentrations of 100%, 75%, 50% and 25%. The red betel leaves used are the half-old ones from segments 6 – 12. The sonde dose calculation is as follows: Weight of red betel leaf x conversion factor = 50 grams x 0.0026 = 0.13ml

After the sacrifice, give water from boiled red betel leaves for seven days. Then, the process of maceration and histotechnics of the kidney and liver organs was carried out, and observations were carried out at 400x magnification with repetition in 3 fields of view. Observations were made by measuring tubular narrowing and glomerular diameter.

The assessment criteria for tubular narrowing are that the tubule is narrowed decrease of 16.41%, P(2) of 20.4%, P(3) a decrease of 21%, P (4) amounting to 58.4%.

a. Kidney Observation

Based on the research carried out, the results of microscopic examination measurements of kidney tubule cells in the K+, K-, P1, P2, P3, and P4 groups can be seen in Figure 1 as follows:

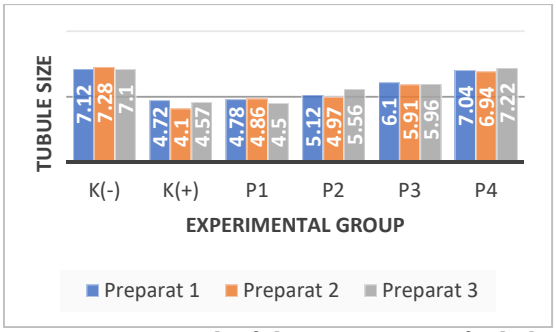


Figure 1. Graph of the average size of tubule constriction per preparation

Images of the results of histopathological observations measuring renal tubules can be seen in Figure 2 as follows:

and closed, the shape is irregular, and the brush border is no longer visible on the proximal tubule and the results of the observations are recorded. Meanwhile, the criterion for assessing glomerular diameter is by measuring the glomerular area using an audiovisual program on a computer.

RESULTS

The results of blood sugar measurements in K- or healthy mice that were not given any treatment showed that all three mice had normal blood sugar. Normal blood sugar levels in mice range from 62.8 mg/dl-176 mg/dl, and mice are declared hyperglycemic if their blood glucose levels are >200 mg/dl. The results of blood sugar measurements in the Positive Control (+) group who were given alloxan induction showed that blood sugar levels decreased after being given boiled red betel leaf water. However, this value was still at a level above normal (Hyperglycemia). The results of blood sugar measurements in treatment groups P1, P2, P3, P4 experienced a decrease in P(1) a

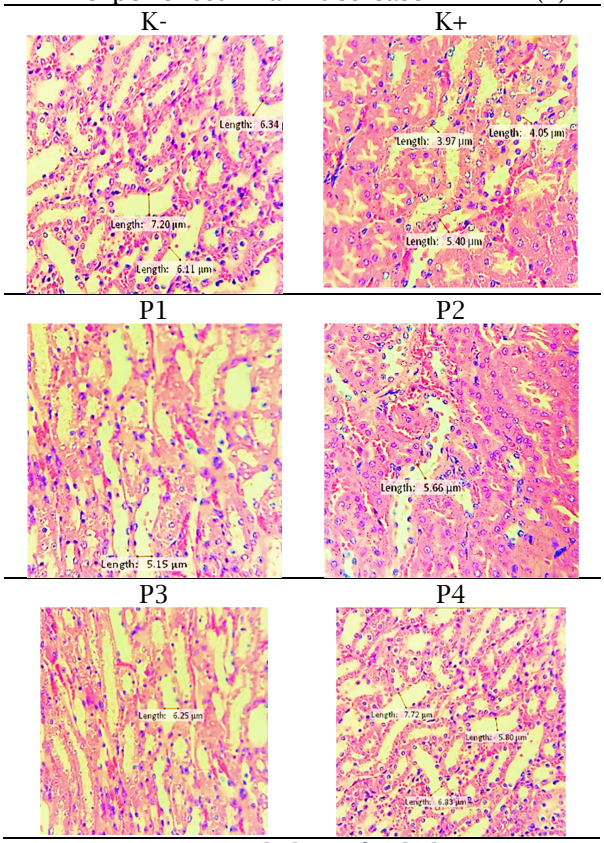


Figure 2. Histopathology of Tubules K (-), K (+), P1, P2, P3, and P4

The results of microscopic examination observing kidney diameter in groups K+, K-, P1,

P2, P3 and P4 can be seen in Figure 3 as follows:

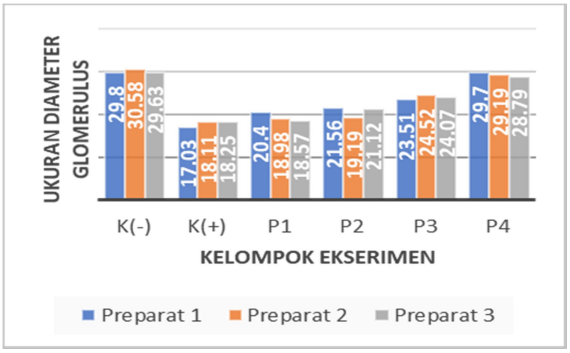


Figure 3. Graph of Average Glomerular Diameter Size Per Preparation

Images of histopathological observations of kidney diameter can be seen in Figure 4 as follows:

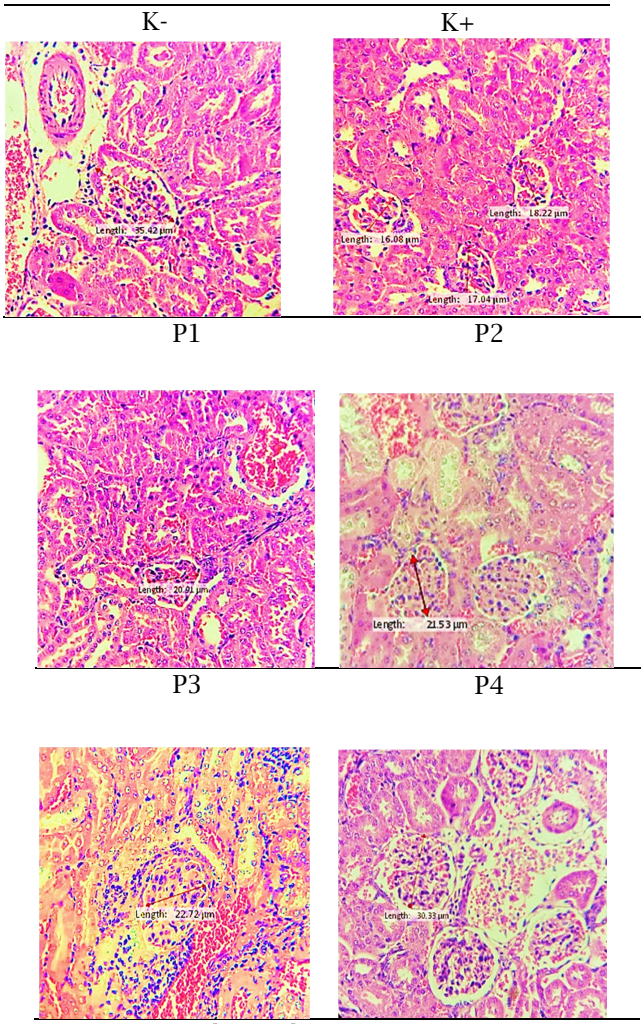


Figure 4 Glomerular Diameter Sizes K(-), K(+), P1, P2, P3, and P4

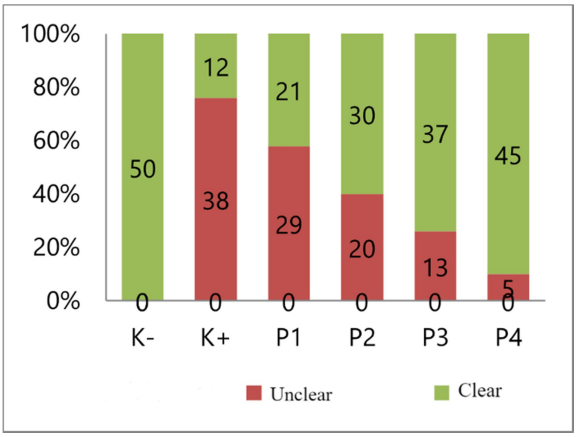
The data from reading the sizes of tubule and glomerular cells in Figure 1 and Figure 3 were then subjected to statistical tests using SPSS. The normality test in this study used the Shapiro-Wilk test because the sample size was <50. The normality test results obtained a significant value of > 0.05 so that the data is normally distributed. Then, a homogeneity test was carried out with a significant result of >0.05, meaning the data is homogeneous.

The multivariate ANOVA (MANOVA) test showed that the average measurements in each research group showed different results and significant ranges. The average results of negative controls or healthy mice are considered a reference for normal values for the size of changes in kidney tissue. The MANOVA statistical test on the Pairwise Comparison table states that the significance between one experimental group and another is >0.05, so the data is considered to have no significant differences.

b. Pancreas Observation

The research continued with histopathological observations of the cell nuclei and cytoplasm of the islets of the Langerhans pancreas of mice to determine the cell necrosis experienced in each treatment.

Graph 4. Cell nuclei and cytoplasm of islets of Langerhans pancreas of mice (Mus Musculus)



Source: Primary Data, 2022

Based on the observation graph of cell nuclei and cytoplasm in the islets of Langerhans of the mouse pancreas, the clear category of K (-) was the most frequently found and the percentage of histopathological reduction in the clear category of each treatment on K (-) was calculated, namely the decrease that occurred in K (+) on K (-) amounted to 76%, the decrease that

occurred in P(1) to K(-) was 58%, the decrease that occurred in P(2) to K(-) was 40%, the decrease that occurred in P(3) to K(-) of 26%, the decrease that occurred in P(4) against K(-) was 10%. The description of the results of histopathological observations of the cell nuclei and cytoplasm of the islets of Langerhans of the mouse pancreas can be seen as follows:

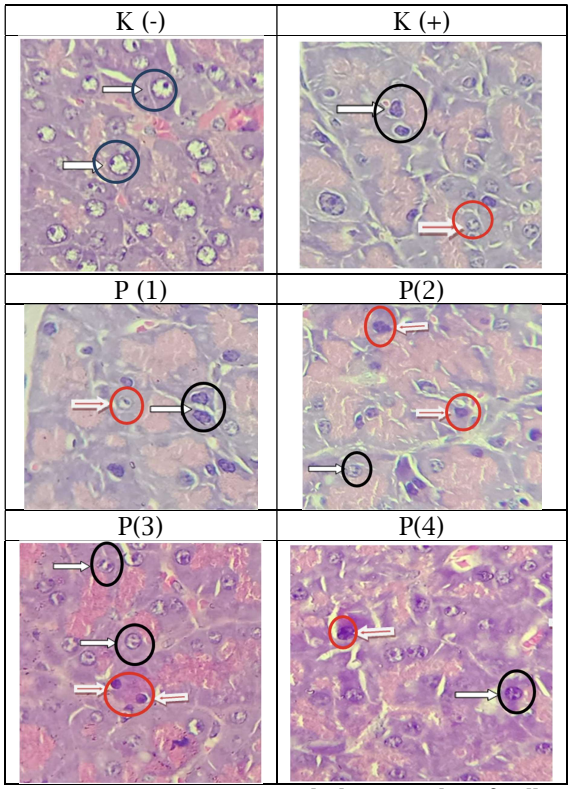


Figure 5 Histopathology results of cell nuclei and cytoplasm of islets of Langerhans pancreas of mice *(Mus Musculus)*

Details:
The cell nucleus and cytoplasm are clear.
The cell nucleus and cytoplasm are less clear.

substances that can make cells fragile and can repair damaged cells. Antioxidants are essential substances that function as an antidote to free radicals. For people with diabetes mellitus (DM), antioxidants can minimize tissue damage by reducing lipid peroxide. Diabetes caused by alloxan can damage the cells that makeup kidney tissue, such as the tubules and glomerulus. Degeneration is often found in glomerular cells, while necrosis is more often in tubular cells. (Yulinta et al., 2013)

The microscopic observations of the kidneys showed that renal tubule and glomerular cells were experiencing

In Figure 5. K- treatment is negative control or healthy mice without treatment, K+ is positive control mice, which only induces alloxan, P1 in mice treated with 25% red betel leaf boiled water, and P2 is mice given concentration of red betel leaf boiled water. 50%, P3 mice were given red betel leaf boiled water with a concentration of 75%, and P4 mice were given red betel leaf boiled water with a concentration of 100%.

Statistical tests use Non Parametrics by applying several unrelated samples, namely the Kruskal Wallis test. The significance value obtained was 0.015, which means the value is smaller than 0.05 (<0.05), so it was concluded that the null hypothesis was rejected. The alternative hypothesis was accepted, meaning that the research results showed an effect of ARDSM administration on the histopathology of the mouse pancreas. Then, the Mann-Whitney test was carried out to determine the significant difference in the number of cell nuclei and cytoplasm of the mouse pancreas (*Mus Musculus*). The results were that in the P3 and P4 groups, there was a significant difference in K+, and there was no significant difference in K-

DISCUSSION

Red betel leaves contain good ingredients for lowering blood sugar levels by drinking boiled water (Hertarinda, 2013). The best content of red betel leaves in reducing blood glucose levels is flavonoid compounds. Alkaloids and flavonoids are active compounds with hypoglycemic activity (lowering blood glucose levels). Flavonoids work to inhibit the glucosidase enzyme in luteolin. The glucosidase enzyme is an enzyme that functions to determine a plant's ability as an antidiabetic in vitro using inhibition techniques. (Direktorat P2PTM, 2021).

Flavonoid compounds are antioxidants. Antioxidants are substances that can kill other degeneration and necrosis. This damage occurred in the K (+) group, mice induced by alloxan without treatment, and all treatment groups, including P1, P2, P3, and P4; this shows that administration of alloxan can affect cell damage in the kidney. The lightest cell degeneration and necrosis were found in groups P3 and P4 with a concentration of boiled red betel leaf water of 75% and 100% with a sonde dose of 0.13 ml; tissue enlargement occurred with microscopic observation of cell size, which was almost the same as group K (-) or healthy mice. This shows that boiled red betel leaf water with 75% and 100% concentrations contains more active compounds than concentrations of

25% and 50%. This is because if the extract concentration is high, the active substance content will also be higher so that the treatment potential increases.

The results of microscopic observations of the pancreas of mice in (K-) without treatment showed that the pancreas was in a normal condition, and the cell nuclei and cytoplasm were visible, characterized by purplish blue cell nuclei and quite large amounts of pink cytoplasm. In the group (K+) induced by alloxan, cell changes occurred characterized by cell nuclei and cytoplasm that were less clear with faded color. Meanwhile, the treatment group treated with boiled water from red betel leaves (*Piper crocatum*) P1, P2, P3, and P4 showed an increasingly better condition; the cells began to look normal, which can be seen from the round cell nuclei, and the regular arrangement of the cell shapes can be seen as seen in the observation K-.

CONCLUSION

Based on the results of research observations and discussions, it can be concluded that the histopathology of the kidneys of diabetic mice that were given water from boiled red betel leaves resulted in an increase in tubular cell size by 34.3% and glomerular diameter by 35% at a concentration of 75% and there was an improvement in tubular cell size by 34.3%. 58.2% and glomerular diameter of 64.2% at 100% concentration compared to diabetic mice. Therefore, it can be concluded that the best concentration is boiled red betel leaf water with a concentration of 75% and 100%.

REFERENCES

Dewi, M. P. S. (2021). *Kadar Glukosa Darah Sewaktu pada Pasien PROLANIS Trimester I dan Trimester II*. *03*(01), 56–60.

Dinkes Jateng, (Dinas Kesehatan Provinsi Jawa Tengah). (2019). Profil Kesehatan Provinsi Jateng Tahun 2019. *Dinas Kesehatan Provinsi Jawa Tengah*, *3511351*(24), 61.

Direktorat P2PTM, (Direktorat P2PTM). (2021). *Penyakit Diabetes Melitus*.

Handini, H. D., & Rohmah, J. (2018). Original Research Articles Efektivitas Formulasi Ekstrak Daun Sirih Merah (Piper crocatum) Dengan Ekstrak Daun Pandan Wangi (Pandanus amaryllifolius Roxb .) Terhadap Hiperglikemia Serta Histopatologi Pankreas Mencit D-IVTeknologi Laboratorium Medis , F. *Medicra (Journal of Medical Laboratory Science/ Technology)*, *1*(2), 54–67.

Harahap, R. S. (2020). *Studi Literatur Perbandingan Efektifitas Streptozotocin dan Aloksan sebagai Agen Diabetagonik pada Uji In Vivo*.

Hertarinda. (2013). *Uji Toksisitas Subkronis Daun Sirih Merah (Piper crocatum Ruiz & Pav.) pada Tikus Terhadap Gambaran Mikroskopis Ginjal dan Kadar Kreatinin*.

Kamaliani, B. R., Setiasih, N. L. E., & Winaya, I. B. O. (2019). *Gambaran Histopatologi Ginjal Tikus Wistar Diabetes Melitus Eksperimental yang Diberikan Ekstrak Etanol Daun Kelor*. *21*.

Kemenkes RI, (Kementerian Kesehatan Republik Indonesia). (2020). Tetap Produktif, Cegah Dan Atasi Diabetes Mellitus. In *pusat data dan informasi kementrian kesehatan RI*.

Kendran, A. A. S., Gelgel, K. T. P., Pertiwi, N. W. L., Anthara, M. S., & Dharmayuda, A. A. G. O. (2013). Toksisitas Ekstrak Daun Sirih Merah pada Tikus Putih Penderita Diabetes Melitus. *Jurnal Veteriner*, *14*(4), 527–533.

Khoirin, W. A., & Hartono, R. (2021). Kadar Hemoglobin pada Pasien Diabetes Mellitus Tipe 2. *Jaringan Laboratorium Medis*, *03*(01), 24–29.

Mutiarahmi, C. N., Hartady, T., & Lesmana, R. (2021). Use of Mice As Experimental Animals in Laboratories That Refer To the Principles of Animal Welfare: a Literature Review. *Indonesia Medicus Veterinus*, *10*(1), 134–145. https://doi.org/10.19087/imv.2020.10.1.134

Nubatonis, D. C., Ndaong, N. anggadewi, & Selan, Y. N. (2018). *Pengaruh Pemberian Ekstrak Etanol Daun Sambiloto(Andrographis paniculata Nees) Terhadap Histopatologi Pankreas Mencit (Mus musculus) Diabetes Melitus (DM) Tipe I*. *3*(14), 63–65. http://eprints.mums.ac.ir/236/%0Ahttps://ijogi.mums.ac.ir/article_8979.html

Putri, M. S. (2017). Pengaruh Ekstrak Etanol Daun Pirdot (Saurauia vulcani Korth.) Terhadap Gambaran Histologi Ginjal Mencit Jantan(Mus musculus L.) yang Diinduksi Diabetes dengan Aloksan. In *Analisis Kesadahan Total dan Alkalinitas pada Air Bersih Sumur Bor dengan Metode Titrimetri di PT Sucofindo Daerah Provinsi Sumatera Utara*.

Refdanita, Veryanti, P. R., Wulandari, A., Muti, A. F., & Sianturi, S. (2019). *Petunjuk dan Paket Materi Praktikum Farmakologi*. Institut Sains dan Teknologi Nasional Jakarta.

Safira, K. (2018). *Buku Pintar Diabetes: Kenali, Cegah, dan Obati!* HEALTHY.

Satria, H., Decroli, E., & Afriwardi, A. (2018). Faktor Risiko Pasien Nefropati Diabetik Yang Dirawat Di Bagian Penyakit Dalam Rsup Dr. M. Djamil Padang. *Jurnal Kesehatan Andalas*, *7*(2), 149.

Shinta, D. Y., & Sudyanto, S. (2016). Pemberian Air Rebusan Daun Sirih Merah (Piper crocatum Ruiz &Pav) Terhadap Kadar Glukosa dan Kolesterol Darah Mencit Jantan. *Sainstek : Jurnal Sains Dan Teknologi*, *8*(2), 180.

Wilujeng, & Pangestu, W. (n.d.). *Pengaruh Pemberian Infusa Albedo Semangka Terhadap Diameter Pulau Langerhans Pankreas Tikus Jantan (Rattus norvegicus) Sebagai Sumber Belajar Biologi*. Retrieved November 10, 2021, from https://eprints.umm.ac.id/69800/

World Health Organization. 2023. *Diabetes.*

Yulinta, N. M. R., Gelgel, K. T. P., & Kardena, I. M. (2013). Efek Toksisitas Ekstrak Daun Sirih Merah Terhadap Gambaran Mikroskopis Ginjal Tikus Putih Diabetik yang Diinduksi Aloksan. *Buletin Veteriner Udayana*, *5*(8 (27)).