Veterinary Biomedical & Clinical Journal

ISSN 2622-1012
Vet Bio Clin J. Vol. 6 No. 1, January 2024 pp: 8-15
DOI: 10.21776/ub.VetBioClinJ.2024.006.01.2

Research Article

Effect of *Piper crocatum* Extract on Kidney Histopathology in Alloxan-Induced Wistar White Rats (*Rattus norvegicus*)

Rondius Solfaine[1*], Diyana Rahmadhani[2]

[1] Pathology Laboratory, Faculty of Veterinary Medicine, University of Wijaya Kusuma Surabaya, Surabaya, Indonesia
[2] Veterinary Professional Program, Faculty of Veterinary Medicine, University of Wijaya Kusuma Surabaya, Surabaya, Indonesia
[*] *Email*: rondius@uwks.ac.id

Citation :
Solfiane, R. & Rahmadhani, D. (2024). Effect of *Piper crocatum* Extract on Kidney Histopathology in Alloxan-Induced Wistar White Rats (*Rattus norvegicus*). *Veterinary Biomedical and Clinical Journal*, 6(1), 8–15. https://doi.org/10.21776/ub.VetBioClinJ.2024.006.01.2

Article history :
Received : 20 October 2023
Accepted : 22 June 2024

Abstract: The prevalence of diabetes mellitus is steadily increasing. According to the International Diabetes Federation in 2021, the number of diabetes sufferers in Indonesia has rapidly increased in the last ten years. This study aims to determine the effect of red betel leaf extract (*Piper crocatum*) on kidney histopathology of Wistar white rats (*Rattus norvegicus*) induced by alloxan. A total of 25 white male *Wistar rats* aged ± 3 months were used in this study. All samples were divided randomly into five treatment groups, namely: (K-) healthy rats that were only given distilled water; (K+) given a single dose of alloxan 120 mg/kg bw/intraperitoneal; (P1) rats given alloxan 120 mg/kg bw/intraperitoneally and red betel leaf extract 100 mg/kg bw/orally; (P2) rats given alloxan 120 mg/kg bw/intraperitoneally and red betel leaf extract 150 mg/kg bw/orally; (P3) rats were given alloxan 120 mg/kg bw/intraperitoneally and red betel leaf extract 200 mg/kg bw/orally. Treatment was given for 14 days. Blood glucose levels were measured using the Glucometer (EZ Smart) colorimeter method. On the day 15th, all rat were euthanized and necropsied to remove the kidneys and then made into preparations with HE staining. The histopathological lesions observed were necrosis, degeneration, and inflammatory cell infiltration. Histopathological observations were carried out using a microscope with 40x magnification. Data resulting from observations were analyzed using SPSS 20 with Kruskal-Wallis and Mann-Whitney ($P \leq 0.05$). It revealed that red betel leaf extract has a potential ability to reduce the effects of hyperglycemia, and by inducing 2% of red betel leaf extract at dose of 100 mg/kg bw, 150 mg/kg bw, and 200 mg/kg bw could reduce necrosis, inflammatory cell infiltration, and degeneration significantly in the kidneys of white rats.

Keywords: histopathology, *Piper crocatum, Rattus norvegicus*

INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by hyperglycemia caused by insulin deficiency (type 1), insulin resistance, or both (type 2). Apart from type 1 and type 2, there is also gestational diabetes, which appears during pregnancy, and diabetes that related to certain conditions or certain diseases. The effective treatment is very important to prevent serious long-term complications (Dewi et al., 2014). Statistical reports from the International Diabetes Federation (IDF) showed that approximately 230 million people have diabetes in the world, and the number of people living with diabetes is expected to reach 350 million in 2025, with high prevalence in Indonesia, India, and Pakistan (Yulinta et al., 2013). Meanwhile, in animals, diabetes cases most often occur in dogs over five years of age, with an incidence rate of around 13 cases per 10,000 dogs each year (Fall et al., 2007).

Chronic hyperglycemia is a major cause of diabetes complications, including diabetic nephropathy, diabetic neuropathy, and diabetic retinopathy. Diabetic nephropathy, which causes end-stage renal disease, involves several pathogenic mechanisms such as aldose reductase, increased

hexosamine, activation of protein kinase C, impaired insulin action, and the formation of advanced glycation end products. In addition, diabetic nephropathy is characterized by various disorders such as nephrotic syndrome, glomerulosclerosis, persistent albuminuria, decreased glomerular filtration rate, increased arterial blood pressure, and obstruction of fluid excretion.

The use of chemical drugs to treat diabetic nephropathy can cause side effects such as gastrointestinal disorders, hypoglycemia, kidney damage, liver damage, cardiovascular effects, allergic reactions, electrolyte disturbances, and risk of infection. Patients need to monitor their condition regularly and communicate with their doctor to manage possible side effects, perhaps by adjusting the dose or type of medication.

The use of herbal medicines so far has only been empirical, namely based on doses and effects obtained from the experiences of variations in each person (Yulinta et al., 2013). Indonesia has enormous potential to provide natural medicinal ingredients used to maintain health. More than 25,000-30,000 plant species exist in Indonesia, and around 6,000 types of plants can be used as traditional herbal medicines. One of the plants commonly used by Indonesian people as an ingredient in traditional medicine is red betel leaf (*Piper crocatum*). The part of the red betel plant widely used as medicine is the leaves. Red betel plants are used by extracting them first to take the active ingredients in red betel leaves (*Piper crocatum*) (Yulinta et al., 2013).

The red betel plant (*Piper crocatum*) is an ornamental plant belonging to the *Piperaceae family*, growing on vines with heart-shaped leaves and stems, which grow alternately from the stem with the appearance of the leaves being silvery red and also shiny. The red betel plant (*Piper scrotum*) has several benefits, including flavonoid and polyphenol compounds, which can function as antioxidants, antidiabetics, anticancers, antiseptics, and anti-inflammatories. Another substance contained in red betel (*Piper scrotum*) has antineoplastic properties, which are effective in inhibiting the growth of cancer cells (Dewi et al., 2014). This study aims to determine the histopathological features of the kidneys by administering red betel extract (*Piper crocatum*) to the kidneys of Wistar rats induced by alloxan.

MATERIALS AND METHODS

The method for making red betel leaf extract was carried out at the Pharmacy Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga, using a blender to make red betel leaves become powder form. Then, the powder was extracted with 70% ethanol solvent using a vacuum rotary evaporator, followed by a freeze-dryer drying process. Induction of diabetes in mice was carried out by administering alloxan 120 mg/kg bw intraperitoneally. The research involved 25 male white *Wistar rats* which were divided into five treatment groups: (K-) healthy rats given distilled water; (K+) alloxan-induced rats; (P1) to (P3) rats were induced by alloxan and given red betel leaf extract at doses of 100 mg/kg bw, 150 mg/kg bw, and 200 mg/kg bw orally for 14 days, respectively. Blood glucose levels were measured with a Glucometer (EZ Smart). On the day 15th, all mice were euthanized and necropsied to remove the kidneys, which were then prepared using HE staining for histopathological analysis.

The level of damage on kidney was analyzed using a scoring method which categorized the extend of lessions as follows: 0 (no change); 2 (lesions < 25%); 4 (26-50% of lesions); 6 (51-75% of lesion); and 8 (lesions > 76%). This analysis was carried out on five (5) fields for each histopathology slide using a light microscope with 40x magnification.

Data Analysis

Data obtained for all parameters were analyzed using Statistics Packages for Social Science (SPSS) 20 with Kruskal-Wallis ($P \leq 0.05$). When a significant different is observed, the Mann-Whitney test will be conducted to evaluate the differences between groups.

RESULT AND DISCUSSION

Blood Sugar Levels and Alloxan Induction

Rats were categorized have diabetes when blood sugar levels are ≥126 mg/dl (Rashmi et al., 2023). Average of blood sugar levels in white rats (*Rattus norvegicus*) after alloxan induction on this study can be seen on **Table 1**.

Table 1 . Average of blood sugar levels in white rats (*Rattus norvegicus*)

Group	Blood Sugar Levels *(Mean ± Std. deviation)*		
	Pre induction	**Post induction**	**Post-treatment**
K-	81.80 ^{a} ± 17.10	84.80 ^{a} ± 8.79	85.00 ^{a} ± 4.36
K+	89.60 ^{a} ± 17.70	128.20 ^{b} ± 30.37	130.00 ^{b} ± 69.67
P1	87.00 ^{a} ± 11.49	151.00 ^{c} ± 41.07	146.00 ^{c} ± 78.17
P2	83.80 ^{a} ± 8.82	171.40 ^{d} ± 110.08	144.40 ^{c} ± 74.74
P3	82.60 ^{a} ± 9.10	226.80 ^{e} ± 199.87	81.40 ^{a} ± 18.53

Note: (K-) control negative; (K+) control positive (single dose of alloxan 120 mg/kg); (P1) 120 mg/kg of alloxan & 100 mg/kg of red betel leaf extract; (P2) 120 mg/kg alloxan & 150 mg/kg red betel leaf extract; (P3) 120 mg/kg alloxan & 200 mg/kg red betel leaf extract. Pre-induction: average blood sugar levels of white rats before alloxan induction; Post-induction: average blood sugar levels after alloxan induction; Post-treatment: average blood sugar levels after being given red betel leaf extract. Different superscripts in rows and columns indicate significant differences ($p \leq 0.05$).

On the day 6th after alloxan induction, K+, P1, P2, and P3 group experienced an increase of blood sugar levels (with 128.20 ± 30.37, 151.00 ± 41.07, 171.40 ± 110.08, 226.80 ± 199.87; mean ± SD, respectively) and were categorized as diabetes. This proves that alloxan induction in this study was successful in causing diabetes mellitus in white rats (*Rattus norvegicus*).

Histopathological Scoring

The data in this study were obtained based on histopathological analysis of the kidneys in white rats (*Rattus norvegicus*) using this scoring method. The Kruskal-Wallis test in the negative control (K-), positive control (K+), first treatment (P1), second treatment (P2) and third treatment (P3) groups for necrosis cell lesions in the kidneys of white rats (*Rattus norvegicus*) showed the value probability (Sig.) = <.001 so <.001 < 0.05, stating that there is a real difference in the data (**Table 2**).

Table 2. Mean scoring of necrosis, degeneration, and inflammatory cell infiltration in glomeruli and interstitial tubules

Group	Lesion Form *(Mean ± Std. deviation)*		
	Necrosis	**Degeneration**	**Inflammatory Cell Infiltration**
K-	2.00 ^{a} ± 0.00	1.60 ^{a} ± 0.55	1.00 ^{a} ± 0.00
K+	4.80 ^{c} ± 1.10	2.40 ^{b} ± 0.55	2.00 ^{b} ± 0.00
P1	6.00 ^{d} ± 0.00	3.00 ^{c} ± 0.00	2.00 ^{b} ± 0.00
P2	3.20 ^{b} ± 1.10	2.40 ^{b} ± 0.55	1.60 ^{c} ± 0.55
P3	2.40 ^{ab} ± 0.89	2.00 ^{ab} ± 0.00	1.80 ^{c} ± 0.45

Note: Different superscripts indicate a significant difference ($P<0.05$). Meanwhile, the same superscript states no significant difference ($P>0.05$).

The highest necrosis histopathological lesion was in the first treatment group (P1) at 6.00, while the lowest was in the negative control treatment (K-) at 2.00. The results of analysis using the Mann-Whitney method used to determine differences between treatments and doses of red betel leaf extract (*Piper crocatum*) given to necrotic lesions gave results for the negative control group (K-), positive control (K+), first treatment (P1), the second treatment (P2) and the third treatment (P3) have values that indicate whether there is a significant difference or no significant difference, namely P1 with P2, P1 with P3, P3 with K+, P1 with K-, K+ with K -, shows the Asymp probability value. Sig. (2-tailed) < 0.05, then Ho is rejected, meaning there is a significant difference in effectiveness. Meanwhile, in groups P2 with P3, P1 with K+, P2 with K+, P2 with K-, and P3 with K-, the Asymp probability value is shown. Sig. (2-tailed) > 0.05, then Ho is accepted, meaning there is no significant difference in effectiveness.

The highest degenerating histopathological lesion was in the first treatment group (P1) at 3.00, while the lowest was in the negative control group (K-) at 1.60. The results of analysis using the Mann-Whitney method, which was used to determine differences between treatments and doses of red betel leaf extract (*Piper crocatum*) given to degenerative lesions, gave results for the negative control group (K-), positive control (K+), first treatment (P1), the second treatment (P2) and the third treatment (P3) have values that indicate whether there is a significant difference or no significant difference, namely P1 and P3, P1 and K-.

The highest degenerating histopathological lesions were in the positive control group (K+) and the first treatment (P1), which had the same highest value of 2.00, while the lowest was in the negative control group (K-) of 1.60. In the treatment group given red betel leaf extract (*Piper crocatum*) at a dose of 150 mg/kg bw (P2) and a dose of 200 mg/kg bw (P3), kidney histology changes occurred in the form of necrosis with almost the same degree of severity but not lower when compared with a group (K+). Comparison between groups (K-), (K+), (P1), (P2), and (P3) showed significant differences in the degree of necrosis. The necrosis that occurred in group (P1) was higher when compared with groups (P2) and (P3). This shows the possibility that red betel leaf extract at a dose of 150 mg/kg bw and 200 mg/kg bw is still better in providing the effect of regenerating kidney cell necrosis than the relatively lower dose of 100 mg/kg bw so that the regeneration process of necrosis cells due to alloxan administration is not maximum.

Based on histopathological changes in inflammatory cell infiltration lesions in the kidneys of rats (*Rattus norvegicus*) induced by alloxan and given 2% red betel leaf extract (*Piper crocatum*) at a dose of 100 mg/kg bw (P1), 150 mg/kg bw (P2), 200 mg/kg bw (P3) and two control treatments, namely negative when given distilled water and positive when induced by alloxan. Comprehensive histopathological structure observation in the kidneys of white rats (*Rattus norvegicus*) in the negative control (K-), positive control (K+), first treatment (P1), second treatment (P2), and third treatment (P3) groups. Infiltration of inflammatory cells in the glomerular area and renal tubules is characterized by the accumulation of purplish neutrophil cells with hematoxylin-eosin (HE) staining. Also, necrotic changes appear, characterized by the cell nucleus shrinking until the cell undergoes lysis. The results of histopathological examination of the kidneys of white rats (*Rattus norvegicus*) in various treatments can be seen in **Figure 1.**

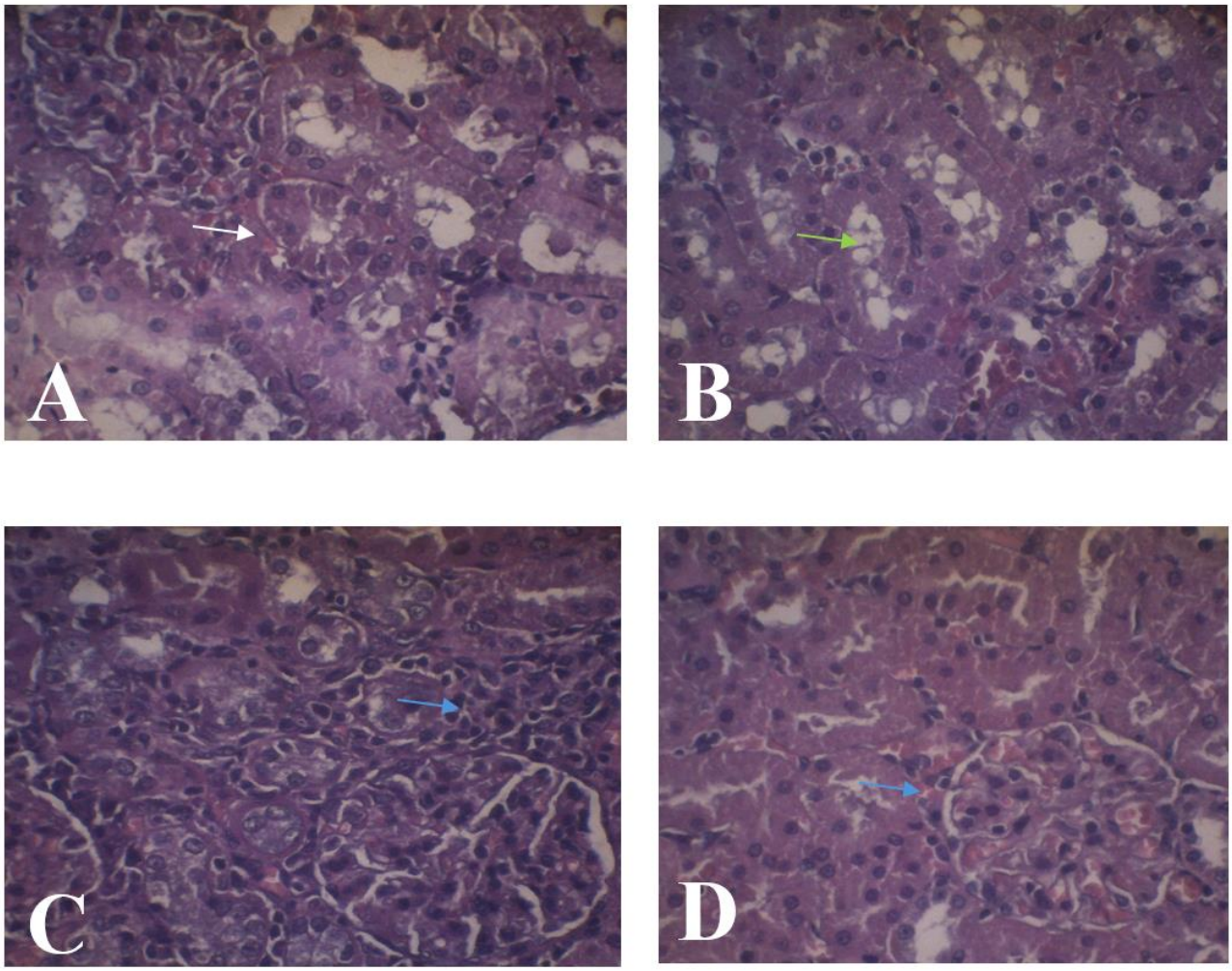

Figure 1. Histopathological analysis of kidney tissue shows various pathological changes: A) In group P1, lesions of inflammatory cell infiltration and fatty degeneration were seen in the glomerular area (white arrow); B) The K+ group shows necrosis and inflammatory cell infiltration in the tubules and interstitium (green arrow); C & D) In group P2, signs of congestion are visible (blue arrows). All these changes were observed using Hematoxylin-Eosin (HE) staining at 40x magnification.

According to research by Puspitasari & Choerunisa (2021), alloxan can cause permanent hyperglycemia within two to three days by damaging pancreatic beta cells, which can cause insulin production to decrease. After 14 days of administering red betel leaf extract, the blood sugar conditions of white rats *(Rattus norvegicus*) in the treatment groups (P1, P2, and P3) experienced a decrease in blood sugar levels. Red betel leaf extract (*Piper crocatum*) is suspected to contain chemical compounds in the leaves. According to Wati et al. (2020), betel leaf extract contains chemical compounds in the leaves, including flavonoids, alkaloids, and amino acids. The flavonoid content in red betel leaves (*Piper crocatum*) can regenerate cells in the islets of Langerhans so that they can increase the production of the hormone insulin.

Hyperglycemia in diabetes mellitus can cause damage to the kidney filter area or glomerulus. Therefore, an abnormal amount of blood protein is excreted into the urine. In normal kidneys, large molecular proteins cannot pass through the glomerulus, but these proteins can pass through in pathological conditions. Rats are said to have diabetes if blood sugar levels are ≥126 mg/dl. The

results of the induction of test animals into diabetes mellitus after administration of alloxan. The condition of sugar levels showed a significant decrease in the treatment group (P1, P2, P3) compared to the positive control group after induction with alloxan.

According to research by Dharmayudha et al. (2014), treatment with 2% red betel leaf extract (*Piper crocatum*) at a dose of 50 mg/kg BW and a dose of 100 mg/kg BW can suppress the increase in blood glucose levels in white rats (*Rattus norvegicus*) by activating cells. Pancreatic beta to produce insulin. This study used higher doses of red betel leaf extract (*Piper crocatum*) (100 mg/kg bw, 150 mg/kg bw and 200 mg/kg bw) to more clearly determine the effectiveness of red betel leaf extract in treating diabetes mellitus. The results showed that administering a higher dose of red betel leaf extract (*Piper crocatum*) than previous research on white rats (*Rattus norvegicus*) could be more effective in suppressing an increase in blood glucose levels by activating pancreatic beta cells to increase insulin production, compared with a dose of the lower one.

Red betel leaf extract (*Piper crocatum*) was given to experimental animals affected by diabetes mellitus to reduce blood glucose and prevent the intestinal organs from absorbing the glucose they eat. Red betel leaf extract (*Piper crocatum*) can stimulate the body's pancreatic beta cells to produce more insulin. Red betel leaves contain momorcidin, a type of glucoside that can reduce blood glucose levels and help the pancreas produce insulin.

Diabetes mellitus can result in the release of calcium ions from mitochondria, which disrupts homeostasis and becomes an initial trigger for necrosis or cell death. The necrosis process begins with changes in the morphology of the cell nucleus, where pyknosis occurs, followed by karyorrhexis (rupture of the cell nucleus), and finally, karyolysis (loss of the cell nucleus). Based on this research, histopathological changes in necrotic lesions in the kidneys of white rats (*Rattus norvegicus*) induced by alloxan and given 2% red betel leaf extract (*Piper crocatum*) at a dose of 100 mg/kg bw (P1), 150 mg/kg bw (P2), 200 mg/kg bw (P3) and two control treatments, namely negative by giving distilled water and positive induced by alloxan.

Degeneration in the epithelium of the renal tubules is caused by enlargement of the epithelial cells, which causes the lumen of the tubules to appear narrowed; this shows that the administration of alloxan can cause damage to the cells that make up the kidney tissue which occurs in the positive control (K+) group as shown in **Figure 1**. Degeneration is found in cells in the glomerular area and renal tubular epithelium, and high-scoring results indicate that alloxan administration causes damage to the cells that make up the kidney. Metabolic processes disrupted due to minor injury and affecting the structures within cells are also called cell degeneration. Cell degeneration consists of 2 types, namely cell swelling and fatty changes; the swelling that occurs in cells is caused by cells that cannot regulate the balance of ions and fluids, causing hydration in the cells, while fatty changes manifest as fat vacuoles in the cytoplasm caused by hypoxia or toxic materials (Jannah & Budijastuti, 2022).

Giving red betel leaf extract (*Piper crocatum*) at 200 mg/kg bw to the samples showed a milder microscopic picture of degeneration than mice that were only given alloxan. Giving red betel leaf extract can influence the acceleration of repair or durability of the cells that make up the kidney even though the dose given is low. Red betel leaf extract at 200 mg/kg BW doses contains active flavonoids and alkaloids. Which is greater than that of red betel leaf extract at a dose of 100 mg/kg bw and a dose of 150 mg/kg bw, so that white rats given red betel leaf extract at a dose of 200 mg/kg bw were able and effective in improving the microscopic appearance of the cells that make up the kidney. Red betel leaf extract (*Piper crocatum*) contains secondary metabolite compounds: alkaloids, flavonoids,

saponins, and tannins (Assiam et al., 2014). Inflammatory cell infiltration found in kidney tissue is thought to be triggered by alloxan-induced inflammation. Treatment with red betel leaf extract (*Piper crocatum*) reduced cell infiltration compared to the positive control group.

The active compounds in red betel leaf extract (*Piper crocatum*) reduced histological damage to the kidneys in the treatment group at a dose of 100 mg/kg bw, even though inflammatory cell infiltration occurred. However, the treatment groups with 150 mg/kg bw and 200 mg/kg bw showed lower inflammatory cell infiltration. Research also notes that administration of alloxan induces inflammation in the kidneys, a response to damage and vulnerability of endothelial cells to toxins circulating in the blood circulation (Taek et al., 2020). Therefore, tissue damage caused by alloxan in diabetes mellitus involves inflammatory mechanisms triggered by toxic compounds, free radicals, and other harmful compounds, ultimately resulting in inflammatory cell infiltration that can be observed microscopically (Cahyani et al., 2022).

CONCLUSION

Giving red betel leaf extract (*Piper crocatum*) can reduce the effects of hyperglycemia. In addition, administering 2% red betel leaf extract at a dose of 100 mg/kg bw, 150 mg/kg bw, and 200 mg/kg bw can reduce necrosis, inflammatory cell infiltration, and degeneration. in the kidneys of white rats (*Rattus norvegicus*).

REFERENCES

Assiam, N., Setyawati, I., & Sudirga, S. K. (2014). Effect of Dosage and Duration of Treatment of Red Calliandra Leaf Extract (Calliandra calothyrsus Meissn.) on the Histological Structure of Mouse Kidneys (Mus musculus L.). *Symbiosis II Journal*, *2*, 236–246.

Cahyani, D., Maliza, R., & Setiawan, H. (2022). The Effect of Arabica Coffee Fruit Skin Extract (Coffea arabica L.) on the Histopathology of Mice (Mus musculus L.) Liver Induced by Ethanol. *Journal of Biotechnology and Natural Science*, *1*(1), 12–20. https://doi.org/10.12928/jbns.v1i1.4734

Dewi, Y. F., Anthara, M. S., & Dharmayudha, A. A. G. O. (2014). Effectiveness of Red Betel Leaf Extract (Piper crocatum) on Blood Glucose Level Decreased White Male Rats (Rattus norvegicus) Induced Alloxan. *Buletin Veteriner Udayana*, *6*(1), 73–79.

Dharmayudha, A. A. G. O., Anthara, M. S., Wiranata, I. M. A., & Sudimartini, L. M. (2014). Effectiveness of Red Betel Leaf Extract (Piper crocatum) on Improvement Body weight of male white rats (Rattus novergicus) with diabetes Alloxan induction. *Buletin Veteriner Udayana*, *6*(2), 175–179.

Fall, T., Hamlin, H. H., Hedhammar, Å., Kämpe, O., & Egenvall, A. (2007). Diabetes Mellitus in a Population of 180,000 Insured Dogs: Incidence, Survival, and Breed Distribution. *Journal of Veterinary Internal Medicine*, *21*(6), 1209. https://doi.org/10.1892/07-021.1

Jannah, D. R., & Budijastuti, W. (2022). Histopatological Overview Kidneys Toxicity of A Male Rat (Rattus norvegicus) Being Given Yakon Tuber (Smallanthis sonchifolius). *LenteraBio : Berkala Ilmiah Biologi*, *11*(2), 238–246. https://doi.org/10.26740/lenterabio.v11n2.p238-246

Puspitasari, V., & Choerunisa, N. (2021). Systematic Review: Antidiabetic Effect of Bitter Melon Fruit (Momordica charantia Linn.) on Blood Glucose Levels in Alloxan-Induced Rats. *Generics: Journal of Research in Pharmacy*, *1*(2), 18–27. https://doi.org/10.14710/genres.v1i2.11052

Rashmi, P., Urmila, A., Likhit, A., Subhash, B., & Shailendra, G. (2023). Rodent models for diabetes. *3 Biotech*, *13*(3), 80. https://doi.org/10.1007/s13205-023-03488-0

Wati, Y. S., Zukhra, R. M., & Permanasari, I. (2020). Konsumsi Rebusan Daun Sirih Merah Efektif Terhadap Perubahan Kadar Gula Darah Penderita Diabetes Mellitus. *Al-Insyirah Midwifery: Jurnal Ilmu Kebidanan (Journal of Midwifery Sciences)*, *9*(2), 91–99. https://doi.org/10.35328/kebidanan.v9i2.729

Yulinta, N. M. R., Gelgel, K. T. P., & Kardena, I. M. (2013). The Toxic Effect of Red Betel Leaf Extract on Microscopic Kidney in Alloxan Induced Diabetic Rats. *Buletin Veteriner Udayana*, *5*(2), 114–121.