http://ejournal.seaninstitute.or.id/index.php/healt
Jurnal Eduhealt, Volume 14, No. 04 2023
ISSN. 2808-4608

Effectiveness Test of Ethanol Extract of Red Betel Leaves (Piper Crocatum Ruiz & Pav) Against Histopathological Features of The Pancreas and Blood Sugar Levels of Alloxan-Induced Male Mice (Mus Musculus L)

Mahral Effendi Sembiring[1], Ali Napiah Nasution[2*], Linda Chiuman [3]
[1,2,3]Master Study Program in Biomedical Sciences, Faculty of Medicine, Dentistry and Health Sciences, Prima Indonesia University, Medan

ARTICLE INFO

Keywords:
Red Betel Leaves, Diabetes Mellitus, Pancreas, Blood Sugar

ABSTRACT

Diabetes Mellitus induces hyperglycemia. Low insulin production causes insulin resistance and altered protein, fat, and carbohydrate metabolism. Much research has shown that traditional herbal treatments can treat diabetes. Diabetes increases free radical production. Secondary metabolic plants include antioxidants that treat extreme illnesses like diabetes. This study examines the efficacy of ethanol extract from red betel leaves (EERBL) in lowering blood glucose levels in vivo, the optimal dose, secondary metabolite content based on phytochemical screening, and alloxan-induced pancreatic hyperglycemia histopathology. Mouse. Six 20-30 gram groups of mice were treated. Group III got 0.65 mg/kgBW glibenclamide, and Groups IV, V, and VI received oral gavage EERBL 100, 300, and 500 mg/kgBW. Group I was in standard control without alloxan, and Group II was in alloxan control. Hyperglycemic mice received 150 mg/kg BW intraperitoneal alloxan. Red betel is 96% ethanol-macerated. Oral red betel leaf extract was given for 21 days to monitor blood glucose and pancreatic function. Splitting red betel leaves to male mice significantly altered blood glucose and pancreatic histology. Red betel goes low cuts close and pancreatic histology by 58.4%. Blood glucose readings were Kruskal-Wallis significant at 0.004 or 0.05. Red betel leaf extract reduced blood glucose and enhanced pancreatic histology in male mice.

Email :
mahralsembiring123@gmail.com

1. INTRODUCTION

Diabetes mellitus causes hyperglycemia due to insulin action anomalies, activity, or both. Diabetes mellitus is caused by many anatomical and chemical issues. Diabetes mellitus also causes insulin deficiency and reduced insulin activity (Sugiarta & Darmita, 2020). Diabetes mellitus has three forms: insulin-dependent type (type 1), insulin-deficient type (type 2), and chemical- and drug-induced and gestational types. Insulin secretion and action anomalies induce hyperglycemia in type 2 diabetes. (2019, Decroli E). DM prevalence has increased over the previous 20 years. Diabetes will affect 300 million people by 2025, according to WHO predictions. The World Health Organization estimates 21.3 million Indonesians will have diabetes by 2030, up from 8.4 million in 2000 (IDF Committee, 2019).

The CDC reports 21 million Americans with DM. This represents 7% of Americans. DM across all age groups, maybe 4.4%, or 366 million cases, by 2030. Diabetes is frequent in adults over 65, and men are more likely than women (Lestari, 2018). Over 90% of people with diabetes have type-2 diabetes. About 3 to 6 percent of white adults have type 2 diabetes. The International Diabetes Federation (IDF) says that there were 336 million type-2 diabetics in the world in 2012 and that the disease killed 4.6 million people each year, or one person every seven seconds. More people worldwide, including in Indonesia, get diabetes mellitus every year. Based on information about people with diabetes mellitus in Indonesia in 2012, the International Diabetes Federation (IDF) said

that 4.8% of the population had diabetes mellitus and that about 59% of those people had not been tested. In 2013, there were 33 million more people with DM than the previous year, two times as many. The International Diabetes Federation (IDF) responded 2014 that Indonesia has the fifth-highest number of people with diabetes (Decroli, Kam, & Dillasamola, 2019).

The International Diabetes Federation (IDF) estimates that 463 million diabetics aged 20–79 exist worldwide in 2019. At that age, 9.3% of the world's population has it. Diabetes will affect 9.65% of men and 9% of women in 2019, according to IDF. Diabetes is expected to reach 19.9%, or 111.2 million people aged 65 to 79, as the population matures. RI Ministry of Health (2020) forecasts show the population would climb to 578 million in 2030 and 700 million in 2045. Indonesia is anticipated to have over 600 million DM sufferers by 2035, up from 300 million in 2013 (Novalinda, Priastomo, & Rijai, 2021).

According to 2019 Riskesdas, 2% of Indonesians are diagnosed with diabetes by age 15. Compared to the 2013 Riskesdas data, which had a patient rate of 1.5%, this result implies an increase in the 15-year-old diabetes rate. However, blood sugar tests showed that diabetes mellitus prevalence increased from 6.9% in 2013 to 8.5% in 2018. Treatment residents show that just 25% of people with diabetes realize they have the disease. Diabetes can be treated with pharmacological and non-pharmacological treatments. Currently, pharmaceutical diabetes treatment uses syntheti. Therefore, herbal remedies are merely an alternative While. While synthetic anti-diabetes medicines are helpful, they can cause hypoglycemia, dizziness, nausea, vomiting, constipation, and liver issues (Chaudhury et al., 2017). Thus, new anti-diabetic medication discoveries are hoped for. The WHO recommends using plants as a source of diabetic treatment components (Modak, Dixit, Londhe, Ghaskadbi, & Devasagayam, 2007; Vieira et al., 2019).

Medical research shows that developed and developing nations use herbal treatments more. Even the therapy employing these plants is receiving global interest. According to WHO data, 65% of community treatments in developed and developing countries employ traditional medicines made from natural substances (Ekor, 2014). Plant medicine advantages are still based on empirical facts and unproven claims. Traditional communal use for treatment yields this. Herbal medicine is being examined for its health advantages and ability to treat disease as pharmaceutical and medical technology advances and natural constituent chemistry is understood. Indonesia has many medicinal plants that help treat diabetes. Red betel (Piper crocatum Ruiz & Pav) is an Indonesian plant info frequently utilized by the people (Gholam & Firdausy, 2022). Must decorate with this crimson betel plant. People don't realize red betel's cancer, antibacterial, anti-diabetic, hypertension, and anti-hypercholesterolemia properties (Fadlilah Muhammad, 2015).

According to Evidence-Based Medicine, red betel leaves are rarely used as a medical treatment. This may be because red betel has not been well-known for a long time. Therefore, there is little scientific knowledge about its advantages and few research articles in scientific journals at home and abroad. Leaves, stems, fruits, flowers, and roots are medicinal plant parts. Public knowledge and treatment information sources have made people more comfortable using natural pharmaceutical treatments and trusting their health benefits. Communities that employ raw materials are encouraged to use plants to treat and prevent disease. Red betel (Piper crocatum Ruiz & Pav.) is a rare medicinal plant. Because of its antibacterial properties, some red betel treats canker sores and toothache (Puspita, Safithri, & Sugiharti, 2019). Red betel leaf also treats diabetes, inflammation, hypertension, hepatitis, nosebleeds, antiseptics, hemorrhoids, and cancer. A betel leaf herbal tea decoction can treat gout, gastritis, and tiredness, and it is anti-infective and anti-fungal (Jovanović et al., 2018).

Red betel leaves (Piper crocatum Ruiz & Pav) include polyphenols, essential oils, saponins, alkaloids, tannins, and flavonoids (Lister et al., 2019). Active secondary metabolites such as flavonoids, alkaloids, polyphenolics, tannins, saponins, and essential oils provide red betel leaf (Piper crocatum Ruiz & Pav.) Its medicinal properties Flavonoids Polyphenol Solic have antioxidant, anti-diabetic, anti-cancer, antibacterial, and anti-inflammatory properties. Alkaloid chemicals may decrease cancer cell proliferation, making them anti-neoplastic. Saponins in red betel leaves increase collagen production, which can cure wounds (Januarti, Wijayanti, Wahyuningsih, & Nisa, 2019). Phenolic molecules have varied biological activities. Bioactive chemicals from the phenolic group can

act as antioxidants, prevent degenerative diseases, including cancer and premature aging, and boost the immune system.

Flavonoids are polyphenolic chemicals, with C15 having two phenolic nuclei and three carbon units. Flavonoids are C6–C3–C6 molecules. Structure-wise, all flavonoids are derivatives of the major flavones found in plant white flour. All flavonoid derivatives share some features. Flavonoids include anthocyanins, proanthocyanidins, flavonols, flavones, glycoflavones, biflavonyls, chalcones and aurons, flavanones, and isoflavones, which are abundant in plants. All the d betel leaves' secondary metabolites make them medicinal (Nuralifah, Muhammad Fitrawan, Parawansah, & Trisetya, 2022). A major energy source for the body is glucose. Daily eating provides glucose as fat, protein, and carbs. The body uses glucose for energy. The body's sugar level is called blood glucose. Blood glucose levels over normal indicate diabetes (Putra, Aulia, & Wahyuni, 2017).

This research used alloxan-induced mice (Mus musculus L.). Experimental animals are given alloxan to cause diabetes. A deadly glucose analog in pancreatic beta cells, alloxan produces superoxide, H2O2, and hydroxyl radicals. Super hydroxide radicals boost hydrogen peroxide and hydroxyl radicals, which damage pancreatic beta cells and limit insulin synthesis and secretion, causing hyperglycemia. This study employed alloxan to induce diabetes because it forms hydroxy radicals in pancreatic beta cells, causing pancreatic damage. The researcher wants to study "Test of the Effectiveness of Red Betel Leaf Ethanol Extract (Piper crocatum Ruiz & Pav) on Pancreatic Histopathology and Blood Glucose Levels of Alloxan-Induced Male Mice (Mus Musculus L)."

2. METHOD

An experimental research design is a collection of standard operating procedures and protocols for doing research with a controlled experimental design and at least two independent variables. Here, one uses the first set of variables as a constant against which one can evaluate the second set's variability (Notoatmodjo, 2018). This study used glassware, 40 mesh sieves, maceration vessels, blenders, mouse cages, husks, blenders, oral probes, analytical balance scales, ovens, filter paper, evaporating cups, drying cabinets, stir bars, glucose tests, test strips, microscopes, dropper pipettes, scalpels, Rotary Evaporators, injection and oral syringes, test tubes, analytical balances, stampers and mortars, gram scales, and water baths. Consuming and drinking mice, cages, and husks were consistent for all test animals. All test animals were acclimatized to the same conditions a week before the trial. Before, test animals undergo a fast of 18-22 hours without food but are given water. Red betel leaves (Piper Crocatum Ruiz & Pav) from plantations in Berastagi, Karo Regency, were utilized to make Simplicia poSimpliciaethanol extract. Aquadest, hydrochloric acid, 1% Na-CMC, 0.65% glibenclamide, alloxan, 96% ethanol, magnesium powder, sodium hydroxide, sodium chloride, coloring solution, hematoxylin hematoxylin-eosinaffin, acetone, alcohol, Mayer reagenMayeragendrof Ldrag-drop bouchWagner aBouchardan-bouchLieberman-Bouchardle mice were induced by alloxan on days 0, 3, 6, 9, 12, 15, 18, and 20 using a glucometer, blood glucose readings were again taken to assess their diabetic status. Mice were diagnosed with diabetes if their blood glucose levels were ≥ 200 mg/dL. Diabetic mice were separated into six groups of 5 mice each and treated for 21 days with the following:

a. Group I (Normal): Male mice (Mus musculus L) were fed and drank 0.5% Na-CMC suspension without induction.
b. Group II (negative control): Male mice (Mus musculus L) administered Alloxan 150 mg/kg bb, i.e., drinking 0.5% Na-CMC suspension.
c. Group III (positive control): Male mice (Mus musculus L) stimulated by alloxan, i.e., received 0.65 mg/kg bb glipalamide orally daily.
d. Group IV, male mice (Mus musculus L) induced with alloxan received 100 mg/kg bb EEDSM orally daily.
e. Group V, male mice (Mus musculus L) induced by alloxan received 300 mg/kg EEDSM orally daily, once a day.
f. Group VI, male mice (Mus musculus L) stimulated by alloxan received 500 mg/kg bb EEDSM orally once daily.

After 21 days of treatment, mice in 6 groups were fasted for at least 10 hours before KGD measurement and pancreatic tissue removal (2021, Pertiwi MB et al.). Size of KGD before and after alloxan induction at 150 mg/kg BW. If there is no increase in KGD after three days there, it is measured again three days following installation. If two measures show no KGD > 200 mg/DL, the animals are re-induced with the same dose. Three days after installation, KGD was measured again. Repeat KGD measurements three days later until KGD is > 200 mg/DL. Once KGD > 200 mg/DL, mice will be treated according to their three-up. Every three days to 21 days, KGD would be measured throughout treatment (Fadah & Nugrahaningsih, 2020).

Research data was analyzed using SPSS 22. For data normality, the Shapiro-Wilk method was used. If data are normally distributed (P > 0.05), use One-Way ANOVA to calculate group mean differences. The Post Hockey test (HSD) determines if treatments differ significantly (P 0.05). If data are irregular, the Kruskal-Walli's test is used.

3. RESULTS AND DISCUSSION

The study found that ethanol extract of red betel leaves (EEDSM) lowers mice's blood sugar after alloxan induction. Based on mice's blood glucose levels before and after alloxan induction. The graph above shows mice getting medicine has much lower blood sugar than those without therapy. The standard role group received no alloxan induction or red betel leaf ethanol extractant, with an average blood sugar of 121.6 g/dL.As a negative control, alloxan caused the highest mean blood sugar concentration of 409.6 mg/dL. The positive control test showed 174.6 mg/dL blood sugar after glibenclamide.

Tests of blood sugar levels as treatments 1, 2, and 3 showed that 100 mg EEDSM had an average KGD of 168.6 mg/dL, 300 mg had 169.5 mg, and 500 mg had 169.5 mg. The study above showed that the standard control test differed from the negative control group, treatment groups 1, 2, and 3. Based on SPSS Kruskal-Wallis test findings for mouse blood sugar, Asymp was obtained. The p-value value is 0.0 4 or p<p-valueed betel leaf extract of 100 mg reduced blood glucose from 409 mg/dL to 168.6 mg/dL, which was very specific and influential. EEDSM extract a00 mg and 500 mg also lowered blood sugar to 169.5 mg/dL. Normal blood sugar levels before and after alloxan induction and red betel leaf ethanol extract are shown below.

Table 1. Results of Measuring Blood Sugar Levels

Groups	Standard Deviation (STD)
Normal	25.1925915
Alloxan	171.6259499
Glibenclamide	46.0304247
EEDSM 100 mg	45.5060435
EEDSM 300 mg	48.5582124
EEDSM 500 mg	46.1378369

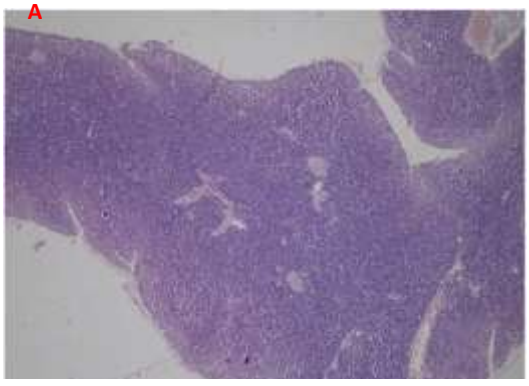

Figure A. Pancreatic tissue, HE, 40X

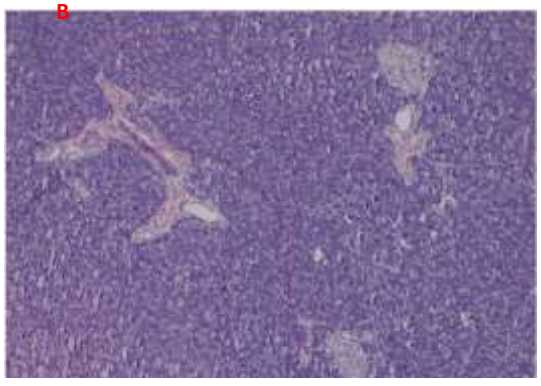

Figure B. Pancreatic tissue, HE, 100X

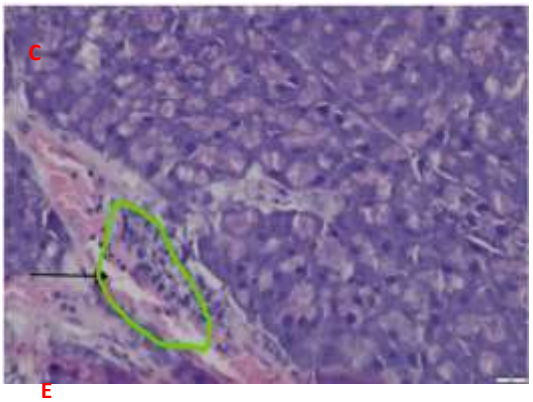

Figure C. Pancreatic tissue (K-2), HE, 400X, neutrophil 40X

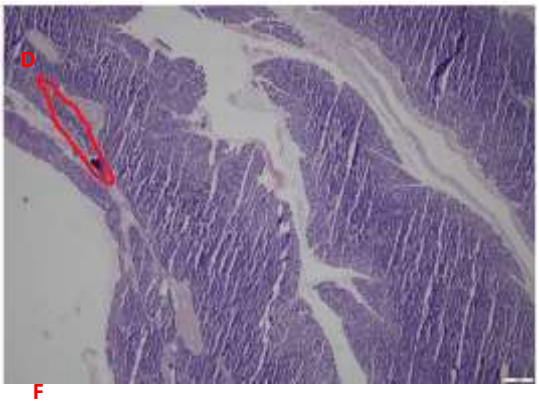

Figure D. Pancreatic tissue (K-1), HE, 40X, MN and PMN inflammatory cell groups, 100X 40X

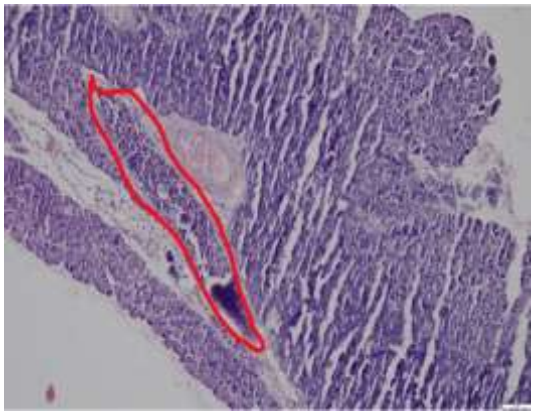

Figure E. Pancreatic tissue (K-1), HE, 100X, MN and PMN inflammatory cell groups

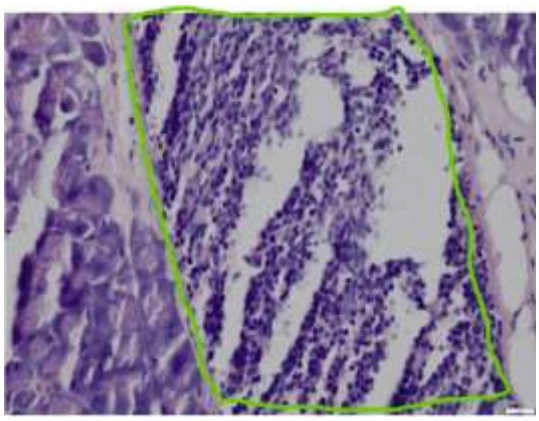

Figure F. Pancreatic tissue (K-1), HE, 400X, MN and PMN inflammatory cell groups

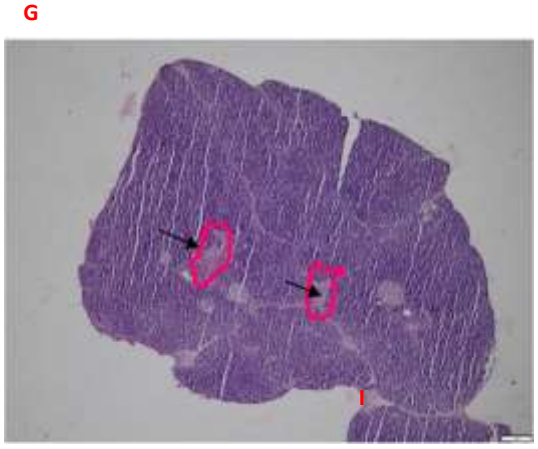

Figure G. Pancreatic tissue (KP) 5, HE, 40X

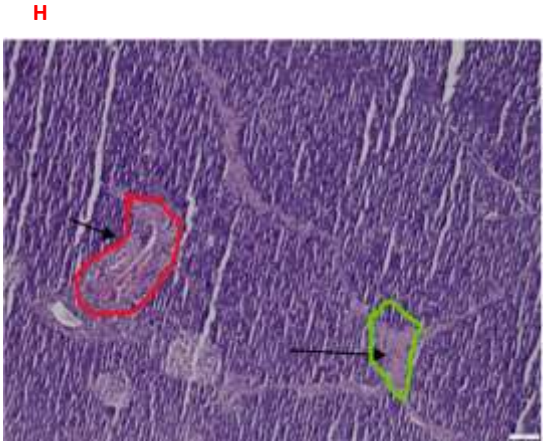

Figure H. Pancreatic tissue (KP 5), HE, 100X, interlobular connective tissue with infiltration

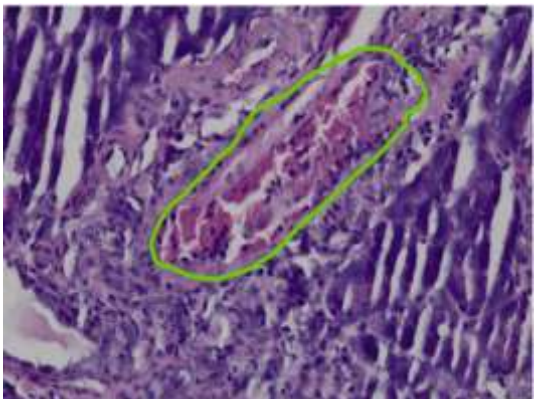

Figure I. Pancreatic tissue (KP 5), HE, 400X, interlobular connective tissue with inflammatory cell infiltration.

Islets of Langerhans comprise 65% pancreatic beta cells, and a significant number will impact their morphology. Damage to these cells will affect their quantity and size. Beta cells regulate blood glucose by secreting insulin. Hyperglycemia results from beta cell dysfunction or injury, which disrupts insulin synthesis. Hyperglycemia activates metabolic pathways to maintain glucose levels but also increases oxidative stress (ROS), which can damage or disable pancreatic beta cells. Thus, antioxidant chemicals are needed to protect pancreatic beta cells from oxidative processes (Coskun, Kanter, Korkmaz, & Oter, 2005; Newsholme, Keane, Carlessi, & Cruzat, 2019).

This study showed that red betel leaf extract lowers blood sugar in diabetic mice. The secondary metabolites in red betel leaves affect pancreatic beta cells in the islets of Langerhans to regulate blood glucose levels through several pharmacological mechanisms. According to phytochemical screening, red betel leaves contain flavonoids, phenols, alkaloids, steroids, terpenoids, tannins, and glycosides. Antioxidants include flavonoids, phenolic derivatives, alkaloids, and tannins. Phenolic flavonoids stimulate insulin secretion. Flavonoids and tannins can inhibit α-glucosidase, reducing glucose absorption and increase in the digestive tract (Ahmed et al., 2019).

Alkaloid group chemicals can repair injured pancreatic β-cells, increasing insulin production and controlling blood glucose levels. Saponins shrink the stomach mucous membrane and reduce glucose absorption with alkaloids and flavonoids. Tannins boost glucose and fat metabolism, reducing blood calorie accumulation, and they act as chelators to shorten the small intestine epithelial barrier, decreasing meal absorption (Serang & Febrianto, 2018).

In diabetic mice, blood vessels dilate, congest, and fill with erythrocyte cells and lymphocytes after incision, and polymorphonuclear (PMN) and mononuclear (MN) inflammation and neutrophils in the connective tissue, interstitial bleeding, and erythrocytes in the lumen of the blood vessel after alloxan administration. Despite alloxan induction and red betel leaf extract treatment at numerous doses, no cells or tissues were necrosed. Due to a lack of alloxan doses and high doses of red betel leaf extract with enough flavonoids, phenols, alkaloids, and tannins to repair tissue in the pancreas islets of Langerhans, the researchers found no necrotic cells or tissues. Researchers believe that increased connective tissue is caused by the activation of Interleukin-1β, a mitogen in fibroblast activity, leading to increased inflammatory cytokines in the body due to lipid deposits and alloxan as free radicals. This occurs without administering compounds that counteract free radicals, such as flavonoids, phenols, alkaloids, and tannins. Alloxan can harm pancreatic endocrine cells, particularly β cells, reducing insulin production into blood vessels. This insulin secretion decreases and raises blood glucose (Setiadi, 2020).

The researchers hypothesized that human error, such as starting an alloxan dose too small, cutting action, preservation to staining with hematoxylin and eosin, or concentration of red betel leaf extract that is still very thick and large, could cause an excessive concentration of bioactive

compounds. Red betel leaf ethanol extract reduces free radicals and inflammatory mediators that injure pancreatic cells.

Some cells are repaired and restored, lymphocyte numbers alter, and inflammatory cells infiltrate during cell repair. Tissue healing boosts lymphocytes. In the subsequent trial, it should supply the proper alloxan and red betel leaf extract dose. Thus, it will reduce, improve, or avoid inflammation, bleeding, connective tissue proliferation and thickening, and blood vessel dilatation and congestion (Serang & Febrianto, 2018). Flavonoids, polyphenols, alkaloids, and tannins in red betel leaves act as antioxidants to decrease macrophages and cell damage by suppressing inflammatory reactions. Plants with higher bioactive chemicals produce antioxidant capacity and enhance inflammatory repair (Villarreal-Soto et al., 2019).

Hematoxylin and Eosin staining showed that all pancreatic tissue was expected. Hence, the score remained normal. No necrotic pancreatic tissue was found. Compared to pancreatic tissue before alloxan induction, the histological picture of the tissue is disturbed by an inflammatory reaction characterized by PMN (polymorphonuclear) and MN (mononuclear), minimal or extensive interstitial bleeding, and increasing or thickened connective tissue, the lumen of blood vessels filled with erythrocyte cells, and inflammation. Only inflammation was reduced by 100 mg of red betel leaf extract compared to alloxan induction. This shows that the diabetic pancreas may be repaired to produce insulin and manage blood sugar. Oxygen-free radical molecules cause toxicity and oxidative stress, which damages pancreatic beta. Alloxan, utilized in this investigation, causes oxidative stress. Cells necrose and shrink due to alloxan toxicity. Alloxan also causes chronic hyperglycemia, which impairs the levels of Superoxide Dismutase (SOD), which converts superoxide into hydrogen peroxide and oxygen, and Glutathione Peroxidase (GPx), an antioxidant that catabolizes hydrogen peroxide. This defensive mechanism uses H2O2.

Increased ROS production causes excess ROS levels, suppressing pancreatic duodenal homeobox factor-1 (PDx-1) status, a gene that maintains pancreatic islet cell function and inhibits proliferation. Normal or injured by inflammation, hemorrhage, blood vessel dilatation, or congestion. Numerous antioxidant substances must be consumed to protect pancreatic tissue from free radical damage (Serang & Febrianto, 2018). Flavonoids and phenols are antioxidants that increase glucose-stimulated insulin release and inhibit cytokine-induced beta-cell dysfunction. These benefits result from modifying insulin secretion's triggering and amplification pathways.

The antioxidant activity of flavonoids maintains the signaling mechanism that induces beta cell insulin release, as ROS impede the mitochondrial electron transport chain and change the K-ATP channel. To show that red betel leaf can be used to treat diabetes mellitus, one of which lowers blood sugar in mice and is almost as effective as glibenclamide. In addition to flavonoids, tannins can enhance glucose transport and block differentiation in 3T3-L1 adipocytes by reducing α-amylase and α-glucosidase activity (Tan, Chang, & Zhang, 2017). Alkaloid chemicals in red betel leaves promote β-cell regeneration in injured islets of Langerhans, such as alloxan (Latuhihin, Watuguly, Kakisina, & Kustarini Samsuria, 2020).

Glibenclamide, an oral hypoglycemic sulfonyl urea derivative, decreases blood sugar by increasing insulin production and pancreatic cell repair. Blocking ATP-sensitive potassium channels depolarizes the cell membrane and opens voltage-dependent calcium channels, letting Ca2+ into the cytosol. Until pancreatic cells' intracellular calcium levels rise and drive insulin secretion and repair (Scarl et al., 2020). Red betel leaves contain saponins that block alpha-glucosidase, lowering blood glucose. This enzyme breaks down carbohydrate molecules into glucose (Lister E I N, 2020). In addition to saponins, red betel leaves contain flavonoids that boost insulin production and beta-cell regeneration. By reducing glucose absorption and regulating carbohydrate metabolism enzymes such as alpha-glucosidase, flavonoids can also lower blood sugar. Flavonoids minimize blood sugar, like oral antihyperglycemics. Oral antihyperglycemics increase insulin secretion and receptor sensitivity, glucose absorption in peripheral tissues and muscle, fat and liver tissue insulin sensitivity, and polysaccharide breakdown. Flavonoids reduce blood sugar, like oral hypoglycemics, by stimulating insulin production. Red betel leaf extract lowers blood sugar like glipalamide, the gold standard.

4. CONCLUSION

The study found that red betel leaf extract can lower blood glucose levels and repair damage to pancreatic tissue and β cells in diabetic mice. This damage is caused by inflammation, interstitial bleeding, blood vessel dilatation, and connective tissue proliferation. Red betel leaf extract reduced blood glucose levels most at 100 mg with a mean post-induction value of 406 mg/dL to 168.6 mg/dL, followed by 300 mg and 500 mg. According to the researchers, red betel leaf extract at 100 mg, 300 mg, and 500 mg improved the histopathological picture of pancreatic tissue and decreased inflammation even after induction. No necrosis was identified by pancreatic alloxan. Due to the apparent absence of necrosis, the alloxan dose may have been insufficient, the red betel leaf extract dose large and dense, and the interval from induction to pancreatic injury may have been too short. These positive alterations imply pancreatic healing, allowing insulin production and regular blood sugar levels. Red betel leaves' flavonoids, phenols, alkaloids, and tannins lower blood glucose and heal pancreatic tissue. Thus, red betel leaf ethanol extract lowers blood sugar and repairs diabetes-damaged pancreatic tissue.

REFERENCES

Ahmed, M., Ji, M., Qin, P., Gu, Z., Liu, Y., Sikandar, A., … Javeed, A. (2019). Phytochemical screening, total phenolic and flavonoid contents and antioxidant activities of Citrullus colocynthis L. and Cannabis Sativa L. *Applied Ecology and Environmental Research*, *17*(3), 6961–6979. https://doi.org/10.15666/aeer/1703_69616979

Chaudhury, A., Duvoor, C., Reddy Dendi, V. S., Kraleti, S., Chada, A., Ravilla, R., … Mirza, W. (2017). Clinical Review of Antidiabetic Drugs: Implications for Type 2 Diabetes Mellitus Management. *Frontiers in Endocrinology*, *8*(January). https://doi.org/10.3389/fendo.2017.00006

Coskun, O., Kanter, M., Korkmaz, A., & Oter, S. (2005). Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and β-cell damage in rat pancreas. *Pharmacological Research*, *51*(2), 117–123. https://doi.org/10.1016/j.phrs.2004.06.002

Decroli, E., Kam, A., & Dillasamola, D. (2019). The percentage of depressive symptoms in patients with type 2 diabetes mellitus in M Djamil General Hospital Padang, Indonesia. *Marmara Pharmaceutical Journal*, *23*(2), 292–297. https://doi.org/10.12991/jrp.2019.136

Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Neurology*, *4 JAN*(January), 1–10. https://doi.org/10.3389/fphar.2013.00177

Fadah, I., & Nugrahaningsih, W. (2020). Efek Pemberian Ekstrak Kulit Buah Jeruk Bali (Citrus maxima) terhadap Kadar Glukosa Darah dan Kadar MDA Tikus Hiperglikemia. *Life Science*, *9*(1), 62–71. Retrieved from http://journal.unnes.ac.id/sju/index.php/LifeSci%0AEfek

Fadlilah Muhammad. (2015). Benefit of Red Betel (Piper Crocatum Ruiz & Pav.)As Antibiotics. *J Majority*, *4*(3), 71–74.

Gholam, G. M., & Firdausy, I. A. (2022). Molecular docking study of natural compounds from red betel (Piper crocatum Ruiz & Pav) as inhibitor of secreted aspartic proteinase 5 (Sap 5) in Candida albicans. *Sasambo Journal of Pharmacy*, *3*(2), 97–104. https://doi.org/10.29303/sjp.v3i2.145

IDF Committee. (2019). International Diabetes Federation Atlas. In *Pfizer - MSD - Alliance - Lilly - Novo Nordisk* (9th ed.). Online Edition: Pfizer - MSD - Alliance - Lilly - Novo Nordisk. https://doi.org/10.1016/S0140-6736(55)92135-8

Januarti, I. B., Wijayanti, R., Wahyuningsih, S., & Nisa, Z. (2019). Potensi Ekstrak Terpurifikasi Daun Sirih Merah (Piper crocatum Ruiz &Pav) Sebagai Antioksidan Dan Antibakteri. *JPSCR : Journal of Pharmaceutical Science and Clinical Research*, *4*(2), 60. https://doi.org/10.20961/jpscr.v4i2.27206

Jovanović, M., Milutinović, M., Kostić, M., Miladinović, B., Kitić, N., Branković, S., & Kitić, D. (2018). Antioxidant capacity of pineapple (Ananas comosus (L.) Merr.) extracts and juice. *Lekovite Sirovine*, *38*(38), 27–30. https://doi.org/10.5937/leksir1838027j

Kemenkes RI. (2019). Laporan Nasional Riset Kesehatan Dasar - 2018. In *Kementerian Kesehatan RI*

(Vol. 1). Retrieved from https://www.kemkes.go.id/article/view/19093000001/penyakit-jantung-penyebab-kematian-terbanyak-ke-2-di-indonesia.html

Latuhihin, Y. G., Watuguly, T., Kakisina, P., & Kustarini Samsuria, I. (2020). Potential of Binahong (<i>Anredera cordifolia</i> [Tenore] Steen) in Reducing TNF-<i>α</i> Expression on Regeneration of Pancreas <i>β</i> Cells on White Rats (<i>Rattus norvegicus</i>) Diabetes Mellitus Models. *Journal of Biosciences and Medicines*, *08*(06), 37–49. https://doi.org/10.4236/jbm.2020.86004

Lestari, I. C. (2018). Antidiabetic Effects Of Mahkota Dewa (Phaleria Macrocarpa) Leaf Ethanol Extract In Diabetic Rat With Streptozotosin Induction. *Biomedika*, *10*(2), 94–101. https://doi.org/10.23917/biomedika.v10i2.7019

Lister, I. N. E., Ginting, C. N., Girsang, E., Armansyah, A., Marpaung, H. H., Sinaga, A. P. F., … Rizal, R. (2019). Antioxidant properties of red betel (Piper crocatum) leaf extract and its compounds. *Journal of Natural Remedies*, *19*(4), 198–205. https://doi.org/10.18311/jnr/2019/23633

Modak, M., Dixit, P., Londhe, J., Ghaskadbi, S., & Devasagayam, T. P. A. (2007). Indian herbs and herbal drugs used for the treatment of diabetes. *Journal of Clinical Biochemistry and Nutrition*, *40*(3), 163–173. https://doi.org/10.3164/jcbn.40.163

Newsholme, P., Keane, K. N., Carlessi, R., & Cruzat, V. (2019). Oxidative stress pathways in pancreatic β-cells and insulin-sensitive cells and tissues: Importance to cell metabolism, function, and dysfunction. *American Journal of Physiology - Cell Physiology*, *317*(3), C420–C433. https://doi.org/10.1152/ajpcell.00141.2019

Notoatmodjo, S. (2018). *Metodologi Penelitian Kesehatan* (3rd ed.). Jakarta: Rineka Cipta.

Novalinda, N., Priastomo, M., & Rijai, L. (2021). Literature Review: Bahan Alam yang Berpotensi sebagai Antidiabetes. *Proceeding of Mulawarman Pharmaceuticals Conferences*, *14*, 389–397. https://doi.org/10.25026/mpc.v14i1.595

Nuralifah, N., Muhammad Fitrawan, L. O., Parawansah, P., & Trisetya, M. (2022). Histopatologi Organ Pankreas Tikus DM tipe 2 yang diberi Ekstrak Etanol Daun Gedi Merah (Abelmoscus manihot L. Medik). *Journal Syifa Sciences and Clinical Research*, *4*(1), 141–151. https://doi.org/10.37311/jsscr.v4i1.13566

Puspita, P. J., Safithri, M., & Sugiharti, N. P. (2019). Antibacterial Activities of Sirih Merah (Piper crocatum) Leaf Extracts. *Current Biochemistry*, *5*(3), 1–10. https://doi.org/10.29244/cb.5.3.1-10

Putra, A. M. P., Aulia, D., & Wahyuni, A. (2017). Uji Aktivitas Ekstrak Etanol Daun Belimbing Wuluh (Averrhoa bilimbi L.) Terhadap Penurunan Kadar Glukosa Darah Mencit Putih Jantan yang Diinduksi Aloksan. *Jurnal Ilmiah Ibnu Sina*, *2*(2), 263–269.

Scarl, R. T., Corbin, K. L., Vann, N. W., Smith, H. M., Leslie, S., Sherman, A., … Arbor, A. (2020). Intact pancreatic islets and dispersed beta-cells both generate intracellular calcium oscillations but differ in their responsiveness to glucose. *Cell Calcium*, 1–28. https://doi.org/10.1016/j.ceca.2019.102081.Intact

Serang, Y., & Febrianto, Y. (2018). Pengaruh Pemberian Ekstrak Etanol Daun Jeruk Nipis (Citrus aurantifolia) Pada Protekso Pankreas Tikus Diabetes yang Diinduksi Aloksan. *Jurnal Farmasi & Sains Indonesia*, *1*(2), 56–62.

Setiadi. (2020). Pengaruh Ekstrak Kulit Lidah Buaya Terhadap Kadar Gula Darah Dan Gambaran Histopatologi Pankreas Tikus Yang Diinduksi Aloksan. *Life Science 9*, *9 (2)*(2), 171–185.

Sugiarta, I. G. R. M., & Darmita, I. G. K. (2020). Profil penderita Diabetes Mellitus Tipe-2 (DM-2) dengan komplikasi yang menjalani rawat inap di Rumah Sakit Umum Daerah (RSUD) Klungkung, Bali tahun 2018. *Intisari Sains Medis*, *11*(1), 7–12. https://doi.org/10.15562/ism.v11i1.515

Tan, Y., Chang, S. K. C., & Zhang, Y. (2017). Comparison of α-amylase, α-glucosidase and lipase inhibitory activity of the phenolic substances in two black legumes of different genera. *Food Chemistry*, *214*, 259–268. https://doi.org/10.1016/j.foodchem.2016.06.100

Vieira, R., Souto, S. B., Sánchez-López, E., Machado, A. L., Severino, P., Jose, S., … Souto, E. B. (2019). Sugar-lowering drugs for type 2 diabetes mellitus and metabolic syndrome—review of

classical and new compounds: Part-i. *Pharmaceuticals*, *12*(4). https://doi.org/10.3390/ph12040152

Villarreal-Soto, S. A., Beaufort, S., Bouajila, J., Souchard, J. P., Renard, T., Rollan, S., & Taillandier, P. (2019). Impact of fermentation conditions on the production of bioactive compounds with anticancer, anti-inflammatory and antioxidant properties in kombucha tea extracts. *Process Biochemistry*, *83*(April), 44–54. https://doi.org/10.1016/j.procbio.2019.05.004