

HEPATOPROTECTIVE AND HISTOLOGICAL PANCREAS EFFECTS OF *Sarang Banua* (*Clerodenrum fragrans* Vent Willd) LEAF EXTRACT IN ALLOXAN-INDUCED DIABETIC RATS

Murniaty Simorangkir^{1,✉}, Erlintan Sinaga² and Saronom Silaban³

¹Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Negeri Medan, Medan 20221, Indonesia

²Department of Biology, Faculty of Mathematics and Natural Science, Universitas Negeri Medan, Medan 20221, Indonesia

✉Corresponding Author: murniatysimorangkir@unimed.ac.id

ABSTRACT

Research on anti-diabetic herbal plants and their safety continues to grow by the increasing number of diabetics, especially in Indonesia, which is rich in plant biodiversity. This study investigated the effect of *Sarang banua* (*Clerodendrum fragrans* Vent Willd) leaf extract on the hepatoprotective effect, histology of the pancreas, and serum glucose levels of white male rats (*Rattus novergicus*) induced diabetes by alloxan. Diabetic rats were given ethanol extract of *C. fragrans* leaf (100, 200, and 300 mg/kg bw), ethyl acetate extract (200, 300 mg/kg bw), and metformin (125 mg/kg) orally for 14 days. Enzym activities and glucose levels in serum were determined at the end of treatments. The histology of rat pancreas and liver were observed under an electron microscope with 10x magnification. The data obtained were analyzed by ANOVA followed by the Least Significant Difference test. The results showed that the administration of ethanolic extract of *C. fragrans* leaves at 100mg/kg bw significantly decreased the serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), and serum glucose levels in the alloxan-induced diabetic rat ($p < 0.05$). *C. fragrans* leaf extract decreased the level of histological damage liver and pancreas of alloxan-induced diabetic rats. The *C. fragrans* improved the protective effects on the liver, and pancreas, and decreased serum glucose in alloxan-induced diabetic rats.

Keywords: *Clerodenrum fragrans* Vent Willd, Diabetic, Hepatoprotective, Histological Pancreas.

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INTRODUCTION

People with diabetes continue to experience an increase in Indonesia, It is predicted to reach 21.3 million people by 2030. Diabetes is a metabolic disorder disease characterized by blood glucose levels above normal (hyperglycemia). This data shows that diabetes is a non-communicable disease that requires efforts to prevent and treat it.¹ Research on traditional herbal plants with anti-diabetic properties continues to grow along with the increasing number of diabetics, especially in Indonesia rich in plant biodiversity. In addition to testing the efficacy of herbal plants, testing for toxic effects needs to be carried out to maintain the safety of herbal use.² In research on the anti-diabetic efficacy of herbs, alloxan can be used to make experimental animals with diabetes conditions.³ Giving alloxan can damage pancreatic cells resulting in impaired insulin production, so that blood glucose levels increase.^{4,5} The organ that functions in the metabolism of drugs and toxins in the liver. Hyperglycemia in diabetic conditions can lead to oxidative stress and liver damage, which causes an increase in blood Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) activity.⁶ Increased blood SGOT and SGPT activity is an indicator of alloxan hepatotoxicity. The *Sarang banua* plant (local name) is widely found in the Simalungun area, North Sumatra, Indonesia and is traditionally used as a diabetes medicine. The result of the determination by Herbarium Bogoriense, Research Center for Biology-LIPI Bogor, and the *Sarang banua* is *C. fragrans* Vent Willd, family Verbenaceae. The genus *Clerodenrum* consists of 500 species throughout the world. Still, only about 11 species have been studied for their chemical content and biological activity,⁷ and in Indonesia, there are 17 plants of the genus *Clerodenrum*.⁸ The *Sarang banua* plant contains flavonoids, triterpenoids, saponins, tannins, and quinones that have medicinal potential,⁹ and have antioxidant, and antibacterial

activity.¹⁰ The ethanol extract of the leaves of *C. fragrans* contains 13.47% flavonoids using standard quercetin.¹¹ Flavonoids have a hypoglycemic effect by suppressing the peroxidase action, thereby inhibiting ROS formation by neutrophils.¹² Several research results of plants belonging to the genus *Clerodendrum*, including *C. capitatum*, have hypoglycemic activity,¹³ *C. inerme*,¹⁴ and *C. serratum*¹⁵ showing the potential hepatoprotective of CCl₄-induced mice. This study investigated the effect of *Sarang banua* leaf extract on the hepatoprotective effect, histology of the pancreas, and serum glucose levels of white male rats (*Rattus novergicus*) induced diabetes by alloxan. Parameters observed were SGOT, SGPT, glucose levels in alloxan-induced diabetic rat serum, and histological damage of liver and pancreas tissue.

EXPERIMENTAL

Material

The materials used were vacuum rotary evaporator (Heidolph), analytical balance, micropipette, microtube, hematocrit microcapillary, centrifuge, plastic rat cage, syringe 5 mL, 3 mL, 1 mL (Terumo), Eppendorf tube, 3 mL vaculab, instrument oral sonde, surgical instrument, Naso Gastric Tube (NGT) size 5 mL (Terumo), glass vial, vortex (SBS), shaker incubator, microtome, glucometer, spectrophotometer micro lab 300 (Elitech), slide and coverslip, camera microscope, rotary evaporator. The materials used were distilled water, ethanol solvent (Merck), *n*-hexane (Merck), ethyl acetate (Merck), alloxan monohydrate, metformin, Bouin's solution, ethanol, xylol, paraffin, hematoxylin-eosin staining.

Preparation of *C. fragrans* Leaf Extract

Samples of fresh *Sarang banua* leaves (*C. fragrans* Vent. Willd) were taken from Simalungun, North Sumatra, Indonesia and have been determined by Herbarium Bogoriense (No. 1680/IPH.1.01/If.07/VI/2017). A total of 5.2 kg of fresh *Sarang banua* leaf were separated from the petioles, washed with clean water, and dried away from sunlight. The dried leaves were made into flour with 60 mesh (620 g). A total of 600 g of *Sarang banua* leaf flour was extracted by maceration. The extraction process uses organic solvents with stratified polarity, namely *n*-hexane, ethyl acetate, and ethanol. Each extract was filtered and concentrated using a vacuum rotary evaporator at a temperature of 50°C.¹⁶ The two thick extracts were then used in the hepatoprotective, histological examination of the pancreas, and serum glucose levels of alloxan-induced diabetic male rats.

Preparation of Test Animals

The animal test used were white male rats (*Rattus novergicus*) aged 6-8 weeks, body weight $\pm$ 150-200 g, and healthy (active and not disabled). During the study, rats were fed standard pellet feed and drinking water *ad libitum*. After acclimatization for two weeks, the test animals were given treatment. All aspects of veterinary care comply with the guidelines and technical requirements approved by the Animal Research Ethics Committees/AREC, USU FMIPA No. 0413/KEPH-FMIPA/2021, 8 July 2021.

Hepatoprotective, Histological Pancreas, and Anti-Diabetic Test of *C. fragrans* Extract

Male white rats (*R. novergicus*) were acclimatized for two weeks before being given treatment. The experiment consisted of 8 groups, and each group used three rats, so the total number of test animals was 24. Before being given alloxan, the glucose levels of the rats were checked with a glucometer (initial data). Seven groups of rats were induced by diabetes with alloxan at a dose of 125 mg/kg bw intraperitoneally (IP) (modified by Adeoye *et al.*)¹⁷ except for the normal group. After three days of alloxan injection, they were measured using a glucometer. Furthermore, the rats were given *C. fragrans* extract orally every day for 14 days and metformin for the positive control group. The treatments were: K0 (normal, without alloxan and extract), K1 (alloxan), K2 (alloxan and metformin), K3 (alloxan and ethanol extract of *C. fragrans* 100 mg/kg bw, K4 (alloxan and ethanol extract 200 mg/kg bw), K5 (alloxan and ethanol extract 300 mg/kg bw), K6 (alloxan and ethyl acetate extract 200 mg/kg bw), K7 (alloxan and ethyl acetate extract 300 mg/kg bw). After 14 days of treatment, rats' blood, liver, and pancreas were taken.

Measurement of SGPT, SGOT, and Serum Glucose Levels

The rats' blood was drawn through the orbital sinus using microcapillaries and a non-heparin tube.¹⁸ Serum was separated by centrifugation at 3000 rpm for 10 minutes. Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) were measured spectrophotometrically,

Austria: DIALAB® Produktion und Vertrieb von chemisch-technischen).¹⁹ The rat serum glucose level was measured by GOD-PAP.

Liver histopathology and Degree of Hepatocyte Damage

The rats were anesthetized with ether, the abdomen was opened, and the liver was taken. Cuts of rat liver were cleaned with 0.9% saline solution and fixed with 10% formalin solution. The preparations were made using the paraffin method and stained with hematoxylin-eosin.²⁰ Histopathological analysis was carried out using the Manja Roenigk Histopathology Scoring model using a microscope with a magnification of 10 x 40x, including normal hepatocytes and hepatocytes damaged by necrosis, parenchymatous degeneration, and hydropic degeneration. Then calculate the percentage of damage.²¹

Pancreatic Histopathology

The rats were anesthetized with ether, the abdomen was opened, and the pancreas was removed, and fixed with Bouin's solution. Then, preparations were made using the paraffin method, cut 5 m thick, and stained with hematoxylin-eosin staining.²²

Data Analysis

The research data was shown in the form of mean $\pm$ SD and analyzed by one-way analysis of variance (ANOVA), followed by the least significant difference test with a significant level of 5%, using the SPSS 24.0 application.

RESULTS AND DISCUSSION

Hepatoprotective Effect of *Sarang banua* Extract

The effect of ethanol extract and ethyl acetate extract of the leaves of the *C. fragrans* Vent Willd on the levels of SGOT, SGPT, and serum glucose of alloxan-induced diabetic male white rats (*R. norvegicus*) was shown in Table-1.

Table-1: Average SGOT, SGPT, and Serum Glucose Levels in Rats after Administration of Alloxan and *C. fragrans* Vent Willd Extract

Group	SGOT level (U/L)	SGPT level (U/L)	Serum Glucose Level (mg/dL)
K ₀ . Normal	52.67 $\pm$ 7.51 ^a	40.67 $\pm$ 6.11 ^a	85.33 $\pm$ 3.21 ^a
K ₁ . (Alloxan)	91.33 $\pm$ 20.50 ^b	60.33 $\pm$ 2.52 ^b	155.33 $\pm$ 6.42 ^b
K ₂ . (Alloxan + Metformin)	63.33 $\pm$ 7.09 ^a	41.67 $\pm$ 3.51 ^a	70.00 $\pm$ 4.35 ^c
K ₃ . (Alloxan+EECf100mg/kg bw)	54.00 $\pm$ 3.61 ^a	33.00 $\pm$ 3.00 ^a	75.66 $\pm$ 3.18 ^c
K ₄ . (Alloxan+EECf200mg/kg bw)	61.33 $\pm$ 5.51 ^a	40.33 $\pm$ 5.51 ^a	93.00 $\pm$ 8.88 ^a
K ₅ . (Alloxan+EECf300mg/kg bw)	68.67 $\pm$ 20.03 ^{ad}	43.66 $\pm$ 7.64 ^a	99.33 $\pm$ 1.82 ^a
K ₆ . (Alloxan+EAECf200mg/kg bw)	62.67 $\pm$ 1.53 ^{cd}	50.33 $\pm$ 6.60 ^c	125.66 $\pm$ 4.50 ^d
K ₇ . (Alloxan+EAECf300mg/kg bw)	71.00 $\pm$ 19.08 ^{cb}	56.33 $\pm$ 5.03 ^b	131.33 $\pm$ 2.41 ^d

Values are shown as mean $\pm$ SD, n=3. Different superscripts in the same vertical row showed significant differences (p<0.05).

Giving alloxan, metformin, ethanol extract of *C. fragrans* (EECf) 100 mg/kg bw, EECf 200 mg/kg bw, EECf 300 mg/kg bw, ethyl acetate extract *C. fragrans* (EAECf) 200 mg/kg bw, EAECf 300 mg/kg bw significantly (p<0.05) on serum glucose levels compared to normal group rats. Alloxan administration of 125 mg/kg bw to the alloxan-induced diabetic rat's group (K₁) showed higher serum glucose levels (155.33 $\pm$ 6.42 mg/dL) than the normal group (K₀) without alloxan (85.33 $\pm$ 3.21 mg/dL) (Table-1). Giving alloxan can damage pancreatic cells resulting in impaired insulin production, so blood glucose levels increase.^{4,5} However, after administration of ethanolic extract of *C. fragrans*, leaves orally for 14 days, there was a decrease in rat serum glucose levels. Administration of ethanol extract of *C. fragrans* at a dose of 100 mg/kg bw/day for 14 days orally in alloxan-induced rats (K₃), showed the lowest serum glucose levels (75.66 $\pm$ 3.18 mg/dL), significantly different from other treatments (p<0.05). This indicates that the administration of the ethanolic extract of the leaves of *C. fragrans* significantly reduced serum glucose. The average serum glucose level of rats given the ethanol extract of *C. fragrans* 100 mg/kg bw (75.66 $\pm$ 3.18 mg/dL) was not different (p>0.01) from the diabetes drug metformin (70.00 $\pm$ 4.35 mg/dL) (Table-1). This decrease in blood glucose levels indicates the recovery of pancreatic cell function after administration of *C. fragrans* extract. The leaf extract of *C. capitatum* (100, 400, and 800 mg/kg bw) also had hypoglycemic and hypolipidemic activity

significantly lowering blood glucose.¹³ Giving alloxan, metformin, ethanol extract of *C. fragrans* 100 mg/kg bw, 200 mg/kg bw, 300 mg/kg bw, ethyl acetate extract 200 mg/kg bw, 300 mg/kg bw significantly ($p < 0.05$) on the activity of the SGOT and SGPT enzymes in rat serum (Table-1 and Fig.-1).

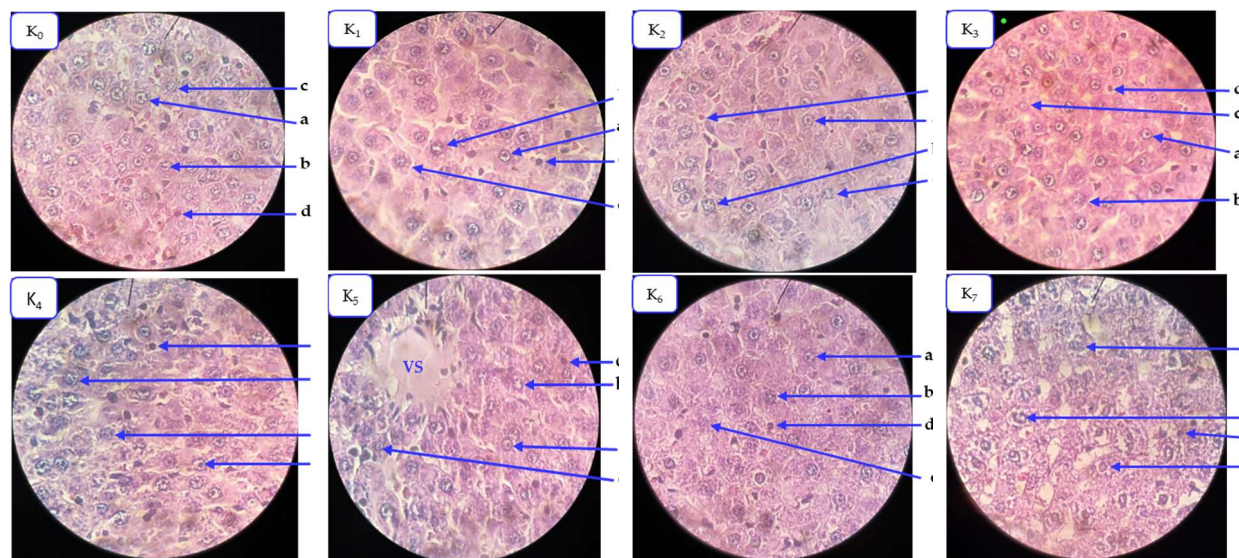


Fig.-1: Histological Pictures of White Rat Liver in Each Treatment with Alloxan and *Sarang banua* Leaf Extract (*C. fragrans*) (100 x magnification). VS: Central Vein, a. Normal, b. Parenchymatous degeneration, c. Hydropic degeneration, d. Necrosis. K0 (Normal), K1 (Alloxan), K2 (Metformin), K3 (*C. fragrans* Leaf Ethanol Extract 100mg/kg bw), K4 (*C. fragrans* Leaf Ethanol Extract 200mg/kg bw), K5 (*C. fragrans* Leaf Ethanol Extract 300mg/kg bw), K6 (*C. fragrans* Leaf Ethyl Acetate Extract 200mg/kg bw) and K7 (Ethyl Acetate Extract 300mg/kg bw)

Administration of alloxan 125 mg/kg bw showed higher serum SGOT and SGPT enzyme activities (91.33 ± 20.50 U/L and 60.33 ± 2.52 U/L) in alloxan groups rats than the normal groups (52.67 ± 7.51 U/L and 40.67 ± 6.11 U/L) (Table-1). SGOT and SGPT activities can be used to assess the extent of liver damage. Liver damage/necrosis in diabetic rats can cause an increase in SGOT enzyme activity, and serum SGPT due to leakage from the liver cytosol into the bloodstream,⁶ which is also an indicator of alloxan hepatotoxicity. The results of this study showed that the liver hepatocyte necrosis score of alloxan-induced rats (18.90%) was greater than the liver hepatocyte necrosis score of normal rats (8.10%) (Table-2). This indicates that alloxan-induced rat liver damage occurred. An increase in SGPT or SGOT can be used as a marker of impaired hepatocellular liver cell integrity.⁶ However, administration of *C. fragrans* leaf ethanol extract for 14 days in alloxan-induced rats resulted in a decrease in serum SGOT and SGPT. Administration of ethanolic extract of *C. fragrans* 100 mg/kg bw/day for 14 days orally in alloxan-induced rats resulted in lower serum SGOT and SGPT enzymes activities (54.00 ± 3.61 U/L and 33.00 ± 3.00 U/L) than the groups of alloxan-induced rats (91.33 ± 20.50 U/L and 60.33 ± 2.52 U/L) and did not differ significantly ($p > 0.01$) with the normal groups (52.67 ± 7.51 U/L and 40.67 ± 6.11 U/L), (Table-1).

A decrease in serum SGOT and SGPT to normal conditions indicates a recovery (hepatoprotective effect) of the liver after administration of ethanol extract of *C. fragrans* 100 mg/kg bw. This hepatoprotective effect is supported by data on liver tissue necrosis scores of rats treated with ethanolic extract of *C. fragrans* 100 mg/kg bw (9.10%) lower than alloxan-induced liver necrosis scores (18.90%) (Fig.-1 and Table-2). Secondary metabolites produced by plants are one of the current alternatives in the treatment of liver damage.²³

Administration of *C. fragrans* ethanol extract 100 mg/Kg bw/day for 14 days to alloxan-induced rats, showed the lowest serum SGOT and SGPT activities which were not significantly different ($p > 0.01$) with the normal group, the group metformin and ethanol extract group *C. fragrans* 200 mg/kg. This indicates that the administration of *C. fragrans* ethanol extract 100 mg/kg bw, 200 mg/kg bw, and metformin can reduce serum SGOT and SGPT to normal conditions. Changes in liver cell wall permeability or damage

result in an increase in SGPT or SGOT, so this can be used as a marker of impaired hepatocellular liver cell integrity.²⁴ Decreased SGPT activity in rat serum indicated a recovery of liver function (hepatoprotective) after administration of *C. fragrans* extract in alloxan-induced rats.

Table-2: The Level of Damage to Liver Hepatocytes of White Rats after Being Given Alloxan and *C. fragrans* Extract

Group	Normal (%)	Parenchymatous Degeneration (%)	Hydrographic Degeneration Degenerasi Hidrofik (%)	Necrosis (%)
K ₀ Normal	89.90%	1.50%	1.50%	8.10%
K ₁ (Alloxan)	56.80%	8.10%	16.20%	18.90%
K ₂ (Metformin)	44.40%	27.80%	11.10%	16.70%
K ₃ (EECf100mg/kgbw)	63.60%	15.90%	11.40%	9.10%
K ₄ (EECf200mg/kgbw)	51.40%	11.40%	8.60%	14.60%
K ₅ (EECf300mg/kgbw)	50.00%	15.60%	18.80%	15.60%
K ₆ (EAECf200mg/kgbw)	41.20%	29.40%	11.80%	17.60%
K ₇ (EAECf300mg/kgbw)	11.80%	23.50%	47.10%	17.60%

The administration of ethanol extract of *C. fragrans* leaves was better at reducing serum SGPT activity and restoring the liver function of alloxan-induced rats compared to *C. fragrans* leaf ethyl acetate extract significantly ($p < 0.01$). This hepatoprotective effect is supported by data on liver tissue necrosis scores of rats treated with ethanolic extract of *C. fragrans* 100 mg/kg bw (9.10%) lower than alloxan-induced liver necrosis scores (18.90%). The hepatoprotective effect of this *C. fragrans* leaf extract can be seen from the histological images of the rat liver in each treatment. The results of observations of the level of liver damage in the normal group showed a central vein with prominent small nuclei, and hepatocytes neatly arranged in sinusoids. While the liver tissue sections of the oxalan-induced diabetic rat cohort showed abnormal central veins with relatively large-sized nuclei, hepatocytes were not well-arranged in the sinusoids. The liver sections of rats treated with *C. fragrans* extract showed that the central veins were normal, the hepatocytes were well preserved, and the sinusoids were well demarcated. It turned out that the administration of ethanolic extract of *C. fragrans* leaves at a dose of 100 mg/kg bw to alloxan-induced rats showed the lowest level of damage (9.10% necrosis) close to the liver damage level of normal group rats (8.10%) compared to the 200 mg/kg dose ethanol extract, 300 mg/kg ethyl acetate extract and metformin. This indicates the recovery of liver tissue in the alloxan-induced rat group given the ethanolic extract of *C. fragrans* leaves at a dose of 100 mg/kg bw. This was also supported by the activity of the enzymes SGOT, SGPT and the lowest serum glucose levels (54.00 ± 3.61 U/L, 33.00 ± 3.00 U/L, and 75.66 ± 3.18 mg/dL) were found in the group of rats given the ethanol extract of *C. fragrans* leaves at a dose of 100 mg/kg compared to other treatments. Gopal and Sengottuvelu (2008),¹⁴ reported that 200 mg/kg bw ethanol extract of *C. inermis* leaves showed hepatoprotective activity in CCl₄-induced rat liver damage (0.5 ml/kg bw), significantly lowering serum alanine aminotransferase (ALT) enzyme, aspartate transaminase (AST), alkaline phosphatase (ALP) and increase glutathione. Agarwal et al. (2013),²⁵ found that the leaf alcohol extract of *C. serratum* (200 mg/kg bw) and aqueous extract (200 mg/kg bw) had a significant hepatoprotective effect by restoring the levels of AST, ALT, and ALP induced mice CCl₄. Patel et al.¹⁵ reported that the biomarker ursolic acid isolated from the alcoholic root extract of *C. serratum* had the effect of restoring AST, ALT, and ALP levels to normal to stabilize plasma membranes and repair liver tissue damage caused by CCl₄. In addition, ursolic acid normalizes antioxidant disorders by maintaining glutathione levels and inhibiting malondialdehyde production. Vidya et al.²⁶ reported that administration of an alcoholic root extract of *C. serratum* (20 mg/kg bw) for two weeks significantly decreased serum bilirubin levels and enzyme markers of CCl₄-induced rat liver function, suggesting the potential of a hepatoprotective agent possibly due to its inhibitory activity radicals from flavonoids present in medicinal ingredients.

Histology of the Pancreas

Histological images of the pancreas and the results of the histological scoring of the pancreas after being induced by alloxan and extracts of the *C. fragrans* leaf were shown in Fig.-2 and Table-3.

In normal rats, the islets of Langerhans were seen in a normal state, and the cell nucleus was clearly visible, surrounded by normal acinar cells. Normal pancreatic tissue consists of small glands lined with simple cuboidal epithelium with round nuclei, relatively uniform, arranged in microfollicular. Among them appear

paler islands of Langerhans, with relatively uniform rounded nuclei. The average score of the level of histological damage to the pancreas showed that the normal group of rats was 0.06 ± 0.139 . In the alloxan-induced diabetic rat group, there was a decrease (atrophy) in the size of the islets of Langerhans.

Table-3: The Results of Scoring the level of Histologic Damage to the Pancreas of White Rats (*R. norvegicus*) after being Induced By Alloxan and Leaf Extract of *C. fragrans* Vent Willd

Group	Pancreas Histological Scoring					Mean $\pm$ SD
	1	2	3	4	5	
K ₀ Normal	0.31	0.00	0.00	0.00	0.00	0.06 ± 0.139
K ₁ (Alloxan)	1.65	1.67	2.50	2.68	2.50	2.20 ± 0.498
K ₂ (Metformin)	0.40	1.30	0.50	0.8	1.67	0.97 ± 0.618
K ₃ (EECF100mg/kgbw)	0.50	1.41	0.6	0.20	1.52	0.91 ± 0.657
K ₄ (EECF200mg/kgbw)	0.40	1.40	0.40	0.32	1.30	0.76 ± 0.537
K ₅ (ECF300mg/kgbw)	0.30	1.60	0.36	0.30	1.41	0.79 ± 0.653
K ₆ (EAECF200mg/kgbw)	0.20	1.42	0.30	0.20	1.33	0.69 ± 0.627
K ₇ (EAECF300mg/kgbw)	0.20	1.35	0.22	0.20	1.1	0.49 ± 0.572

Acinar cells and islets of Langerhans were desquamated with an average score of 2.20 ± 0.498 of histological damage to the pancreatic rat pancreas (Fig.-2 and Table-3). Histological damage to the pancreas is characterized by changes in the shape of the pancreas in the form of shrinking the size of the islets of Langerhans,²⁷ and swelling of the cell nucleus in the islets of Langerhans.²⁸ Giving alloxan makes hyperglycemia in rats.²⁹ In this study, alloxan administration (125mg/kg bw, I.P) caused hyperglycemia in rats after 72 hours of alloxan induction. Alloxan is an important ingredient used to make diabetic experimental animals in rats, mice, rabbits, and dogs.³ The mechanism of alloxan-inducing diabetic rats is by damaging pancreatic cells. This mechanism begins with the entry of alloxan through the glucose transporter GLUT2 and then reacts with glutathione. This process will produce dialuric acid and other products in the form of Reactive Oxygen Species (ROS). These hydroxyl radicals cause pancreatic-cell death due to their very low antioxidant defense ability.^{30,31} This results in decreased insulin production so that glucose levels in the blood increase.^{4,5} In this study, the administration of alloxan (125mg/kg bw, I.P) showed that the serum glucose levels of rats (155.33 ± 6.42 mg/dL) were higher than the normal group (85.33 ± 3.21 mg/dL). In addition, metformin administration showed a decrease in the level of histological damage to the pancreas of rats compared to the alloxan group. Metformin is an oral hypoglycemic agent that works actively in lowering blood sugar levels.³² The administration of ethanol and ethyl acetate extracts of *C. fragrans* leaves showed a decrease in the level of histological damage to the pancreas of rats compared to the alloxan group. This indicates a repair of pancreatic cells. Administration of ethanol extract of *C. fragrans* at a dose of 100 mg/kg bw, 200 mg/kg bw, and ethyl acetate extract at a dose of 300mg/kg bw in hyperglycemic rats showed an improvement in pancreatic cells which was marked by a decrease in the level of histological damage to the pancreas of rats (0.91 ± 0.657 ; 0.76 ± 0.537 and 0.49 ± 0.572) (Fig.-2 and Table-3).

In this study, administration of ethanolic extract of *C. fragrans* at a dose of 100 mg/kg bw orally for 14 days in alloxan-induced diabetic rats showed the lowest glucose levels (75.66 ± 3.18 mg/dL) compared to ethanolic extract of *C. fragrans* 200 mg/kg bw (93.00 ± 8.88 mg/dL) and ethyl acetate extract 300 mg/kg bw (131.33 ± 2.41 mg/dL). This showed that the hepatoprotective effect of the ethanolic extract of *C. fragrans* leaves at a dose of 100 mg/kg bw on alloxan-induced diabetic rats was greater than that of the ethanol extract of 200mg/kg bw, 300mg/kg bw, and ethyl acetate extract 300 mg/kg bw. However, in terms of the level of histological damage to the pancreas of rats, the administration of ethyl acetate extract 300 mg/kg bw had lower damage. The results of the toxicity test on *Artemia salina* Leach, ethanol extract, ethyl acetate, and *n*-hexane of *C. fragrans* leaves, showed LC₅₀ values of 26.25; 37.50; 41.97 μ g/mL, which indicates the bioactive potential of *C. fragrans* extract.¹⁶ Relevant research results show that the polyphenol content of *S. cumini* plant extract can increase the natural antioxidant activity of superoxide dismutase, catalase, and glutathione peroxidase, thereby increasing insulin secretion.²⁸

The results of the docking molecular simulation test showed that polyphenols, tannin skeletons, and the flavonoid family had a high binding affinity for the four receptors in testing the anti-diabetic activity of the bioactive plant compound *Euphorbia thymifolia*.³³ The hepatoprotective, histological effects of the pancreas and alloxan-induced

decrease in rat serum glucose may be caused by the content of secondary flavonoid metabolites and antioxidant activity of the ethanol extract of the leaves of *C. fragrans*.^{11,34,35}

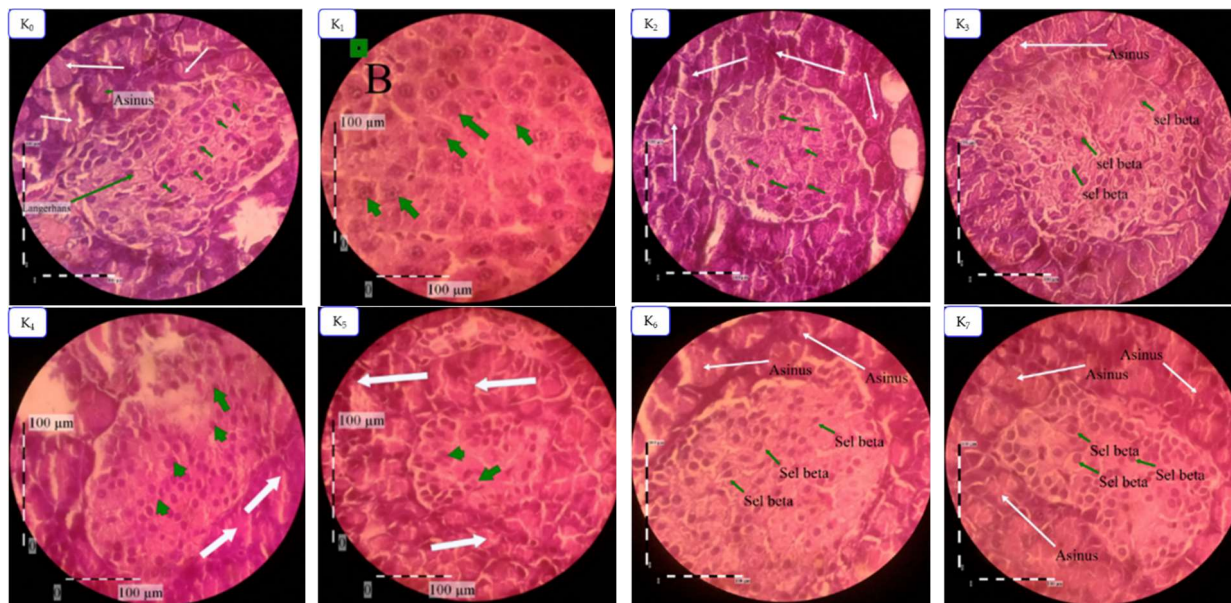


Fig.-2: Histology of the Pancreas of White Rats after Administration of Alloxan and *C. fragrans* Leaf Extract. K0 (Normal), K1 (Alloxan), K2 (Metformin), K3 (*C. fragrans* Leaf Ethanol Extract 100mg/kg bw), K4 (*C. fragrans* Leaf Ethanol Extract) 200mg/kg bw), K5 (*C. fragrans* Leaf Ethanol Extract 300mg/kg bw), K6 (*C. fragrans* Leaf Ethyl Acetate Extract 200mg/kg bw) and K7 (Ethyl Acetate Extract 300mg/kg bw)).

In this study, the ethanol extract of the leaves of *C. fragrans* had a significant positive effect on the hepatoprotective effect, histology of the pancreas, and serum glucose levels of alloxan-induced rats. This result may be caused by the content of secondary flavonoid metabolites and the antioxidant and immunostimulant activities of the leaf extract of *C. fragrans*.^{36,37,38,39} Administration of ethanolic extract of *C. fragrans* leaves 100 mg/kg bw resulted in the lowest levels of SGOT, SGPT and liver damage, pancreas and serum glucose levels compared to metformin, ethanol extract of *C. fragrans* at a dose of 200 mg/kg bw, 300 mg/kg, ethyl acetate extract 200 mg/ kg bw, 300 mg/kg bw. The administration of ethanolic extract of *C. fragrans* Vent Willd leaves 100 mg/kg bw significantly decreased the serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), and serum glucose levels in the alloxan-induced diabetic rat ($p < 0.05$). The *Sarang banua* (*C. fragrans*) leaf extract has a protective effect on the liver, and pancreas, and decreased serum glucose of white male rats (*Rattus novergicus*) with alloxan-induced diabetes. The results of this study can be used to develop the *C. fragrans* plant as an antidiabetic herbal ingredient and safe to use.

CONCLUSION

The administration of ethanolic extract of *C. fragrans* Vent Willd leaves 100 mg/kg bw significantly decreased the serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), and serum glucose levels in the alloxan-induced diabetic rat ($p < 0.05$). *C. fragrans* leaf extract decreased the level of histological damage liver and pancreas of alloxan-induced diabetic rats. The *C. fragrans* improved the protective effects on the liver, pancreas, and decreased serum glucose in alloxan-induced diabetic rats.

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