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**Original Research Article**

# Regenerative Effects of Bay Leaf (*Syzygium polyanthum* (Wight) Walp.) Extract on Renal and Pancreatic Histopathology Damage in Alloxan-Induced Hyperglycemia Mice

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**Abstract:** *Syzygium polyanthum (Wight) Walp,* known as Indonesian bay leaf, is a plant widely used by many ethnic groups in Southeast Asia as a traditional medicinal plant. This study aims to reveal the regenerative effects of bay leaf extract on histopathological damage to the kidneys and pancreas of hyperglycemic mice induced by alloxan. The test animals were divided into 5 groups, each consisting of 5 mice. The first group was only given food and water as a control. The second group was induced by alloxan as a negative control. Groups 3, 4, and 5 were induced by alloxan and bay leaf extract at doses of 150, 250, and 350 mg/kgBW, respectively. Alloxan induction was carried out once every two days for 6 days, while bay leaf extract was given after induction once daily for 14 days. Blood glucose levels and kidney and pancreas damage scores of mice were examined on days 1, 10, and 25. The results of the experiment showed that blood glucose levels, renal dan pancreatic histopathology damage in mice decreased after being given the extract for 14 days. Thus, it can be concluded that bay leaf extract has regenerative properties against renal dan pancreatic histopathological damage in alloxan-induced hyperglycemic mice.

**Keywords:** *Syzygium polyanthum*, Hyperglycemia, Alloxan-induced, Histopathology, Regenerative effects.

## INTRODUCTION

Bay leaf (*Syzygium polyanthum* (Wight) Walp.), Myrtaceae family, is a plant native to Southeast Asia, including Thailand, Malaysia, and Indonesia. Many ethnic groups in the region have used this plant as a nutritional food and traditional medicine. The nutritional aspects of bay leaves include being rich in vitamins such as Vitamin B2, B3, and C. Traditionally, the medicinal benefits of this plant are to treat asthma, cancer, diabetes, endometriosis, postpartum, hypertension, ulcer, fever, skin diseases (Ismail & Ahmad, 2019; Abdulrahman *et al*., 2020; Mahmoud Dogara, 2022).

The chemical compounds that have been reported to have been extracted and characterized from bay leaf plants are phenolics, flavonoids, monoterpenes, sesquiterpenes, oxygenated monoterpenes and oxygenated sesquiterpenes (Abdulrahman, 2022). The bio-activities that have been demonstrated by the plant extracts of this plant include antioxidant, antihypertensive, antihyperglycemic, antibacterial, antifungal, cytotoxic, and antidementia properties. Another positive aspect of this plant, which makes it worthy of being recommended as a medicinal plant, is its lack of signs of toxicity after long-term administration (Nurlely *et al*., 2024).

Experiments using rats as test animals, Simanjuntak *et al*., (2021) found that bay leaf extract for 21 days in benzene-induced rats every 3 and 6 days showed a protective effect against damage to pancreatic beta cells, and was also effective in reducing blood sugar levels (Simanjuntak *et al*., (2021). Next, also on rats, bay leaf extract also shown has a protective effect against renal damage. Butar *et al*., (2026) used bay leaf ethanol extract in ibuprofen-induced rats, revealing that bay leaf extract at a dose of 150 mg/kgBW improved histopathological damage to the renal epithelium, tubules,

glomeruli, and interstitial tissue (Butar *et al*., 2026). Next, Rizky *et al.,* (2026) used bay leaf nanoemulsion extract on Wistar rats and successfully revealed that at a concentration of 5% bay leaf extract could restore complications of kidney dysfunction in rats suffering from diabetes mellitus (Rizky *et al*., 2026).

This study aims to determine the effect of bay leaf extract on renal dan pancreatic histopathological damage in hyperglycemic mice after alloxan induction.

# MATERIAL AND METHODS

**Plant Samples and Extraction**

The fresh leaves of bay leaf plant (*Syzygium polyanthum*) were collective from Gang Dipangga of Jalan Pramuka in Bandar Lampung City, Indonesia. The plant leaves were washed and then air dried. Next, the air-dried bay leaves were ground in a blender and filtered to obtain a fine powder. The powder was then macerated using 96% ethanol in a beaker glass for 24 hours. This maceration was repeated three times until the solvent became clear. The ethanol extract was then filtered to obtain a filtrate which was then evaporated to form a paste which was used as a test material in this study.

**Test Animals and Experimental Design**

The test animals used were male Wistar mice (*Mus musculus* L.) aged 2–3 months and weighing 25–35 grams. The mice were fed standard pellet feed and water ad libitum daily. Prior to the experiment, the animals were acclimatized for 7 days to adapt to the laboratory environment. By using a completely randomized design, a total of 25 mice were divided into five groups (5 each) as follows:

1) Control group, given only food and water.
2) Negative control group, given only alloxan 150 mg/kgBW every two days for 6 days.
3) Treatment group 1 (BL-1): mice were given alloxan 150 mg/kgBW every two days for 6 days and bay leaf extract at a dose of 150 mg/kgBW once daily for 14 days.
4) Treatment group 2 (BL-2): Mice were given alloxan 150 mg/kgBW every two days for 6 days and bay leaf extract at a dose of 250 mg/kgBW once daily for 14 days.
5) Treatment group 3 (BL-3): mice induced with alloxan 150 mg/kgBW every 2 days for 6 days and bay leaf extract at a dose of 350 mg/kgBW once a day for 14 days.

**Alloxan Induction**

Alloxan induction was performed after the mice were fasted for 8-12 hours without food except for drinking water. Alloxan at a dose of 150 mg/g BW was administered intraperitoneally by injection, following Ighodaro, *et al*., (2017), every two days for six days. Two hours before alloxan induction, the mice were weighed and their blood sugar levels were checked (Ighodaro, *et al*., 2017).

**Administration of Extract**

After alloxan induction, mice were given bay leaf extract. The plant leaves extract in paste form was dissolved in water containing 1% CMC-Na (Sodium Carboxymethyl Cellulose). The extract was administered orally using an oral gavage needle once a day for 14 days.

**Blood Sugar Assessment**

The blood glucose levels of the experimental mice were examined on day 1 (before treatment), day 10 (after alloxan induction), and day 25 (after administration of bay leaf extract). Blood was drawn from the injured tail tip after the mice had been fasted for 8–12 hours. Blood glucose levels were checked with a glucose test meter using the Gluco Dr Biosensor (AGM-2100).

**Histopathological Examination**

After blood glucose levels were checked, histopathological examination of the kidneys and pancreas was performed. Mice were killed and dissected to remove the kidneys and pancreas. Histological slides were then prepared using the paraffin method with hematoxylin-eosin staining. Slides were examined under a microscope at 400x magnification. The level of renal histopathology damage is based on a glomerular and tubular damage score. The damage score ranges from 0 to 3, as described in Table 1.

**Table 1: Description of the damage score of renal histopathological damage of mice used in the study**

| Type of damage | | Score | Description |
|---|---|---|---|
| **Glomerular** | **Tubular** | | |
| Normal | Normal | 0 | No damage in glomerular dan tubular cells |
| Infiltration of inflammatory cells | Infiltration of inflammatory cells | 1 | There is an accumulation of lymphocyte cells in the glomerulus and tubules |

| Type of damage | | Score | Description |
|---|---|---|---|
| Glomerular | Tubular | | |
| Bowman spatial edema | Swelling of tubular epithelial cells | 2 | Widening of the Bowman's space. Swelling occurs in the tubular epithelial cells. |
| Necrosis | Necrosis | 3 | Glomerular and tubular cells are not intact, appear wrinkled and denser, are not vascular, have dark colored and small nuclei, and are broken into several clumps. |

Histopathological assessment of pancreatic damage is based on microscopic examination of the level of damage to the islets of Langerhans. The damage to the islets of Langerhans cells is determined using the scores (range from 0 to 4) described in Table 2.

**Table 2: Description of the damage score of pancreatic histopathological damage of mice used in the study**

| Score | Description |
|---|---|
| 0 | The cells and structures of the islets of Langerhans appear normal, with no evidence of damage |
| 1 | ¼ total pancreatic cell necrosis, there is cell degeneration in the form of cytoplasmic vacuoles |
| 2 | ½ pancreatic cell necrosis, with necrotic cells present throughout the entire field of view, and the cells exhibit karyorrhexis (nuclear fragmentation) |
| 3 | ¾ total pancreatic cell necrosis, there are inflammatory cells in the interstitial space of the pancreas |
| 4 | Necrosis of all pancreatic cells |

**Data Analysis**

Data on body weight and blood glucose levels of mice were analyzed using Repeated Measures ANOVA and continued with the Bonferroni post hoc test. Next, qualitative data of renal and pancreatic histopathological damage scores were analyzed using the Kruskal Wallis test and the Mann-Whitney post hoc test.

## RESULTS AND DISCUSSION

**Body Weight and Blood Glucose of Test Animals**

Data on the body weight of mice in normal conditions, after alloxan induction, and after being given bay leaf extract are presented in Table 3, while the blood glucose levels of mice before and after alloxan induction, and after being given bay leaf extract can be seen in Table 4.

**Tabel 3: Body weight of mice (g) before and after treatment (Mean ± SD)**

| Treatment group | Day-1 (before alloxan induction) | Day-10 (after alloxan induction) | Day-25 (after extract administration) |
|---|---|---|---|
| Control | 24.4 ± 1.14a | 27 ± 1.22a | 32.4 ± 3.29a |
| Negative Control | 31.6 ± 2.3a | 27.8 ± 3.42a | 31 ± 0.7a |
| BL-1 (150 mg/kg BW) | 33 ± 1.58ab | 32.8 ± 2.95ab | 29.8 ± 3.11ab |
| BL-2 (250 mg/kgBW) | 35.6 ± 1.81b | 35.4 ± 2.88b | 36.8 ± 5.71b |
| BL-3 (350 mg/kg BW) | 31.1 ± 4.36a | 28.4 ± 4.39a | 33 ± 2.0a |
| Control: normal mice; Negative control: Alloxan-induced mice; BL: alloxan induced and bay leaf extract; Values in the same column followed by the same superscript are not different statistically | | | |

**Table 4: Blood glucose level of mice (mg/dL) before and after treatment (Mean ± SD)**

| Treatment group | Day-1 (before alloxan induction) | Day-10 (after alloxan induction) | Day-25 (after extract administration) |
|---|---|---|---|
| Control | 95.4±8.9a | 115.2±9.6a | 96±3.5a |
| Negative Control | 117.6±13.2c | 166.6±36.4a | 120.6±29.1a |
| BL-1 (150 mg/kg BW) | 108.4±21.1b | 419.4±150b | 162.2±48.1b |
| BL-2 (250 mg/kgBW) | 99.8±11.6ab | 336±111.2ab | 109.8±19.2ab |
| BL-3 (350 mg/kg BW) | 106.4±7.7b | 454±111.3b | 111.4±36.5b |
| Control: normal mice; Negative control: Alloxan-induced mice; BL: alloxan induced and bay leaf extract; Values in the same column followed by the same superscript are not different statistically | | | |

Based on the data in Table 3, it is clear that alloxan induction effectively reduced the body weight of mice, but this body weight was successfully restored by administering bay leaf extract, especially at doses of 250 and 350 mg/kgBW. Furthermore, as shown in Table 4, alloxan induction was also shown to be effective in increasing blood glucose levels in

mice. The high glucose levels caused by alloxan induction were shown to be reduced by bay leaf extract from doses of 150 mg/kgBW to 350 mg/kgBW.

The positive effect of bay leaf extract on body weight as found in this study is understandable, as Ani et al.'s (2022) report found that bay leaf extract can indeed increase body weight in mice and maintain relatively normal physical performance (Ani *et al*., 2022). Several researchers have previously reported that lower blood glucose levels in experimental animals due to the administration of bay leaf extract. Wahjuni and Wita (2017) showed that bay leaf extract exhibited lower blood glucose level and lower level of 8-OHdG (Wahjuni & Wita, 2027). Furthermore, Wahjuni *et al.,* (2018) demonstrated that 5 mg/kg body weight of bay leaf extract exhibited antidiabetic effects by reducing AGE levels in alloxan-induced hyperglycemic rats (Wahjuni *et al.,* 2018). The antihyperglycemic properties of bay leaf extract are likely due to inhibition of glucose absorption from the intestine and increased glucose uptake by muscle tissue (Widyawati *et al.,* 2015). The high glucose uptake by muscle tissue is because bay leaf extract increases GLUT4 protein levels (Hayati & Hidaya, 2020).

**Renal and Pancreatic Histopathology of Test Animals**

Table 5 show renal dan pancreatic histopathological damage score of mice after treated by bay leaf extract for 14 days. Meanwhile, the visualization of renal and pancreatic histopathological damage are shown in Figures 1 and 2 respectively.

**Table 5: Renal dan pancreatic histopathological damage score of mice after treatment are shown in Table 5.**

| Mice group | Renal damage score | Pancreatic damage score |
|---|---|---|
| Control | $0.0 \pm 0.0^{a}$ | $0.0 \pm 0.00^{a}$ |
| Negative Control | $2.2 \pm 0.4^{b}$ | $2.0 \pm 0.00^{c}$ |
| BL-1 (150 mg/kg BW) | $2.2 \pm 1.0^{b}$ | $1.6 \pm 0.54^{c}$ |
| BL-2 (250 mg/kgBW) | $2.0 \pm 0.7^{b}$ | $1.2 \pm 0.44^{b}$ |
| BL-3 (350 mg/kg BW) | $1.0 \pm 0.0^{c}$ | $1.0 \pm 0.00^{b}$ |
| Control: normal mice; Negative control: Alloxan-induced mice; BL: alloxan induced and bay leaf extract; Values in the same column followed by the same superscript are not different statistically | | |

Photographic view renal and pancreatic histopathological damage shown by test animals of each treatment group are presented in Fig.1 and Fig. 2 respectively.

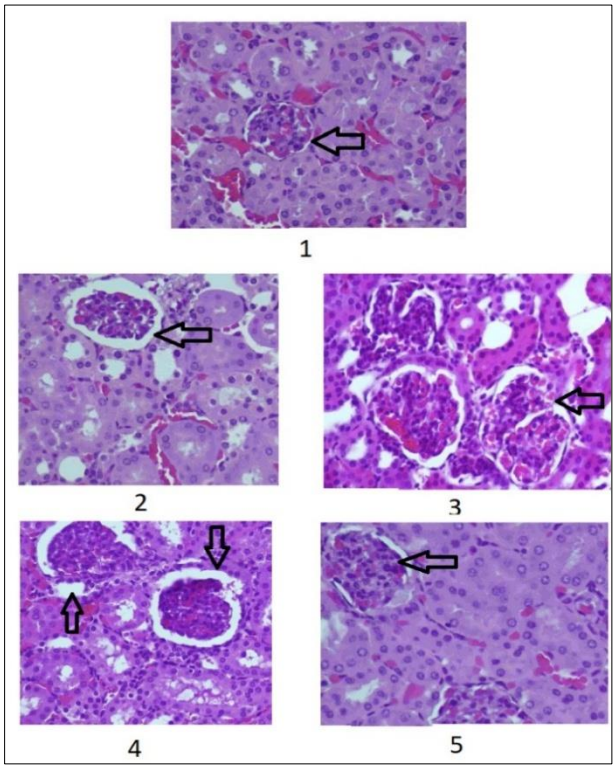


**Fig. 1: Photographic of renal histopathological damage to test animals after treatment. Arrows indicate renal glomerular abnormalities (1) Control: normal mice; (2) Negative control: Alloxan-induced mice; (3-5); BL: alloxan induced and bay leaf extract**

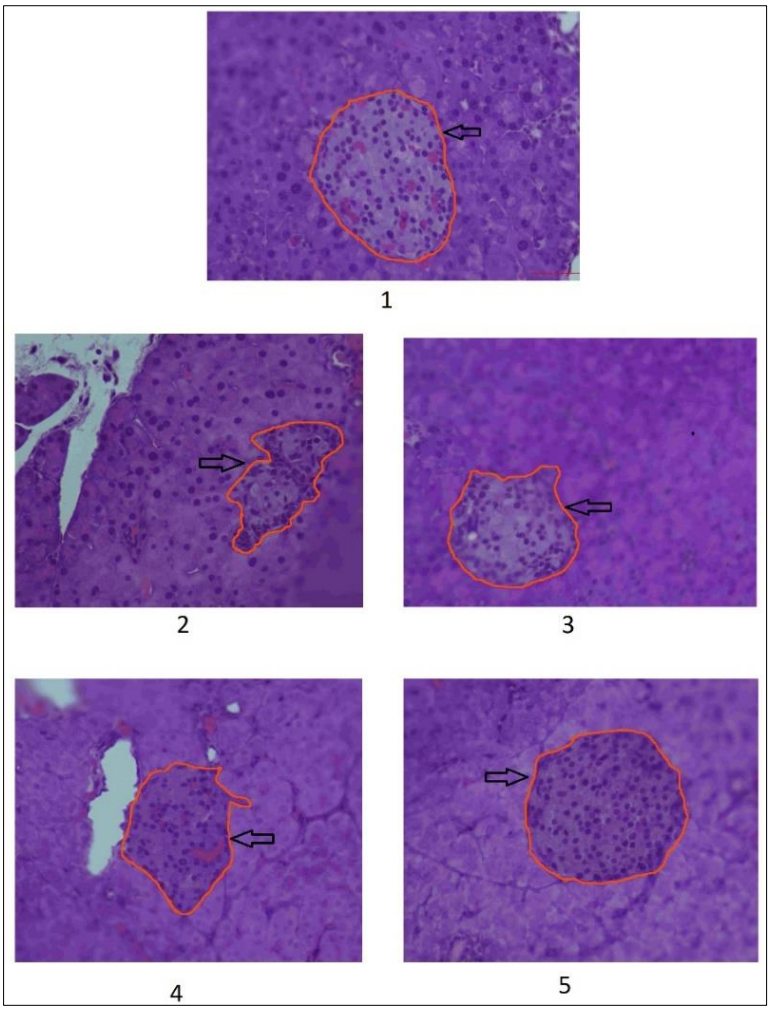


**Fig. 2: Photographic of pancreatic histopathological damage to test animals after treatment. Arrows indicate abnormalities of islet of Langerhans cells. (1) Control: normal mice; (2) Negative control: Alloxan-induced mice; (3-5); BL: alloxan induced and bay leaf extract**

Based on the data in Table 5 and Figures 1 and 2, it can be seen that alloxan induction effectively increased histopathological damage in the kidneys and pancreas of mice. This damage was proven to be reduced by bay leaf extract at all doses (150-350 mg/kg BW). The recovery of renal and pancreatic damage is very likely related to the anti-inflammatory and antioxidant properties of bay leaf extract. Clinical evidence suggests that antioxidant therapy in pre-dialysis patients with chronic kidney disease (CKD) can prevent progression to end-stage kidney disease (ESKD) and helpful in reducing the risk of cardiovascular events (Jun *et al.,* 2012; Tamadon *et al*., 2015). Antioxidants have also been shown to help treat pancreatitis (pancreas inflammation) (Swentek *et al*., 2021; Pădureanu *et al*., 2022). Apart from anti-oxidant ingredients, anti-inflammatory ingredients are also known to have anti-pancreatitis properties (Kňazovický *et al*., 2025)

The inflammatory and antioxidant properties of bay leaf extract have been demonstrated by numerous previous studies. Syabana *et al*., (2021), for example, found that the compounds trans-aconitic acid, gallic acid, and myricetin extracted from bay leaf plants showed antioxidant properties (Syabana *et al*., 2021). Meanwhile, Aditya *et al*., (2022) found that chemicals extracted from bay leaves such as deoxyphomalone and phloretin were proven to have anti-inflammatory, immunosuppressant and TNF expression inhibitor (Aditya *et al*., 2022).

## CONCLUSION

Bay leaf extract has been shown to be effective in lowering blood glucose levels and repairing histopathological damage to the kidneys and pancreas. Therefore, it can be concluded that Syzygium polyanthum (Wight) Walp leaf extract has a regenerative effect on kidney and pancreas damage in hyperglycemic mice.

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22. The results of the α-glucosidase activity test showed that the most active fractions were obtained from solvents with medium polarity: Fractions 9 and 10 (F9 and F10), obtained from gradient acetone-water 4:1 and 3:2, respectively. The IC50 values of F9 and F10 were 24.8 and 31.8 µg/mL, respectively. NMR data showed that F9 had more intense and

diverse signals in the aromatic region than F10. OPLS analysis results showed that some typical flavonoid signals abundant in F9 positively correlated with α-glucosidase activity. 2D NMR and UHPLC-HRMS analysis of F9 led to the conclusion that these signals could be attributed to myricetin-3-O-rhamnoside (myricitrin) and epigallocatechin-3-gallate (EGCG). In silico analysis confirmed these results, as myricitrin and EGCG had binding energies resembling acarbose as a positive control (-8.47, -8.19, and -10.13, respectively).

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