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Effect of Sapodilla (*Manilkara zapota L.*) Leaves Extract on Pancreas of Alloxan Induced Mice: A Histopathological Study

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Abstract

Diabetes mellitusis a metabolic disorder in the form of high levels of glucose in the blood. This study was conducted to determine the effect of *Manilkara zapota* leaf extract (MZLE) on alloxan-induced mice. 25 male mice ± 3 months, 25-35 g, treated with P1 (MZLE 140 mg/kg), P2 (MZLE 70mg/kg), K+ (Pioglitazone 20 mg/kg), P0 (normal control) and K- (diabetescontrol). Pancreas was taken on day 14 after being given therapy. Data analysis by Kruskal Wallis and MannWhitney. MZLE 140 mg/kg showed that the highest pancreatic protection compared to MZLE 70 mg/kg and pioglitazone 20 mg/kg.

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Key words: Diabetes, *Manilkara zapota*, Histopathology

Diabetes mellitus (DM) is a metabolic disorder in the form of high levels of glucose in the blood or insulin deficiency. The single diagnosis of diabetes was categorized on the criteria of hyperglycemia. Complications that occur in diabetes are very complex and multifactorial with environment and genetics (Cole and Florez, 2020).

Treatment of diabetes is carried out to maintain glycaemic control within the normal range and anticipate complications (Kuroda *et al.*, 2021). Pioglitazone is a standard drug in the treatment of diabetes but has side effects for sufferers such as oedema, digestive disorders, and even heart failure (Charbonneau and Capoccia, 2022). This gave rise to efforts to find other alternatives such as the use of herbal medicines with minimal side effects

(Khairullah *et al*., 2020, 2021, Safira *et al*., 2022).One of the medicinal plants that can be used is sapodilla manila (*Manilkarazapota* L.). The phytochemical identification of sapodilla leaf extract (*Manilkarazapota* L.) shows that it contains several phenolic compounds which play a role in reducing free radicals. Extracts of stem bark, leaves and seeds of *Manilkarazapota* L. have antioxidant activity that inhibits the alpha-glucosidase enzyme in the antidiabetic mechanism (Karle *et al*., 2022). Based on the description above, a study was conducted on the effect of administration of sapodilla leaf extract on the histopathological picture of the pancreas of mice induced by alloxan.

Materials and Methods

The population in this study were 25 malemice (*Mus musculus*) aged 3 months and 25-35 g. The treatment groups were divided as: P1 (MZLE 140 mg/kg), P2 (MZLE70 mg/kg), K+ (Pioglitazone 20 mg/kg), P0 (normal control) and K- (control diabetes, mice was given alloxan at a dose of 150 mg/kg dissolved in 1.5 mL of 0.9% NaCl solution).Ethicaltests were carried out at the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya with number 2.KEH.068.06.2022. Mice were placed in plastic cages at room temperature with a light-dark cycle of 12 hours. Mice were given food and water *ad libitum*. Mice were adapted for seven days (Solikhah *et al*., 2021).Induction type 1 DMwas carried out by injecting alloxan at a dose of 150 mg/kg dissolved in 0.9% NaCl. Checking the blood glucose levels of the mice was carried out five days after the injection. Mice with blood glucose levels ≥ 200 mg/Dl were categorized as DM mice and continued with extract and drug therapy (Solikhah *et al*.,2022). Euthanasia of mice was performed by injection of xylazine and ketamine. (Animal ethics committee reference letter No.2.KEH.068.06.2022). Postmortem examination of mice was conducted and pathology of pancreas was carried out on the 14th day after administration of drugs and extracts. After sampling the pancreas, the histopathological description is read for scoring in each treatment group with the following scoring parameters:

Establishment of Histopathology Preparate

In this study, the paraffin method was used to make microanatomical specimens. A 10% formalin buffer solution was used as the fixative solution. Thin tissue sections, approximately 4 mm thick were cut. Following the cutting process, tissue dehydration was carried out for 2 hours by immersing the tissue in alcohol with gradually increasing concentrations from low to high (70%, 80%, 95%, and 96%). The dehydration process was then followed by clearing using xylene, with the aim of making the tissue transparent. The result were placed in embedding cassettes, ranging from 1 to 5 pieces, adjusted according to the organ's size. Hematoxylin Eosin staining was used in this research (Solikhah *et al*., 2022). Microscopic observations of the pancreas were conducted using a Nikon Eclipse E200 light microscope at a magnification of 400x.

Results and Discussion

The results of the Kruskal Wallis test with the parameter of oedema between groups showed a value of p=0.001, inflammatory cell infiltration that occurred with a value of p=0.001 and necrosis revealeda value of p=0.012. Microscopic observations showed that there was an improvement in the histopathological picture in the P1

Table I. Average scoring of oedema, inflammatory cell infiltration and necrosis on the histopathological appearance of the pancreas of treated mice

Treatment	Oedema	Inflammatory cellinfiltration	Necrosis
P1	1.8± 0.28^{a}	1.64 ± 0.26^{a}	1.68 ± 0.22^{a}
P2	1.84 ± 0.26^{a}	1.72 ± 0.22^{a}	1.76 ± 0.26^{a}
K+	2.0± 0.26^{a}	1.72 ± 0.33^{a}	1.64± 0.26^{a}
K-	2.8 ± 0.2^{b}	2.7 ± 0.26^{b}	2.8 ± 0.24^{b}
P0	1.2 ± 0.2^{c}	1.12 ± 0.17^{c}	1.52 ± 0.38^{ac}

Note: Different superscripts (a, b,c) in the same column show a real (significant) difference (p□0.05).

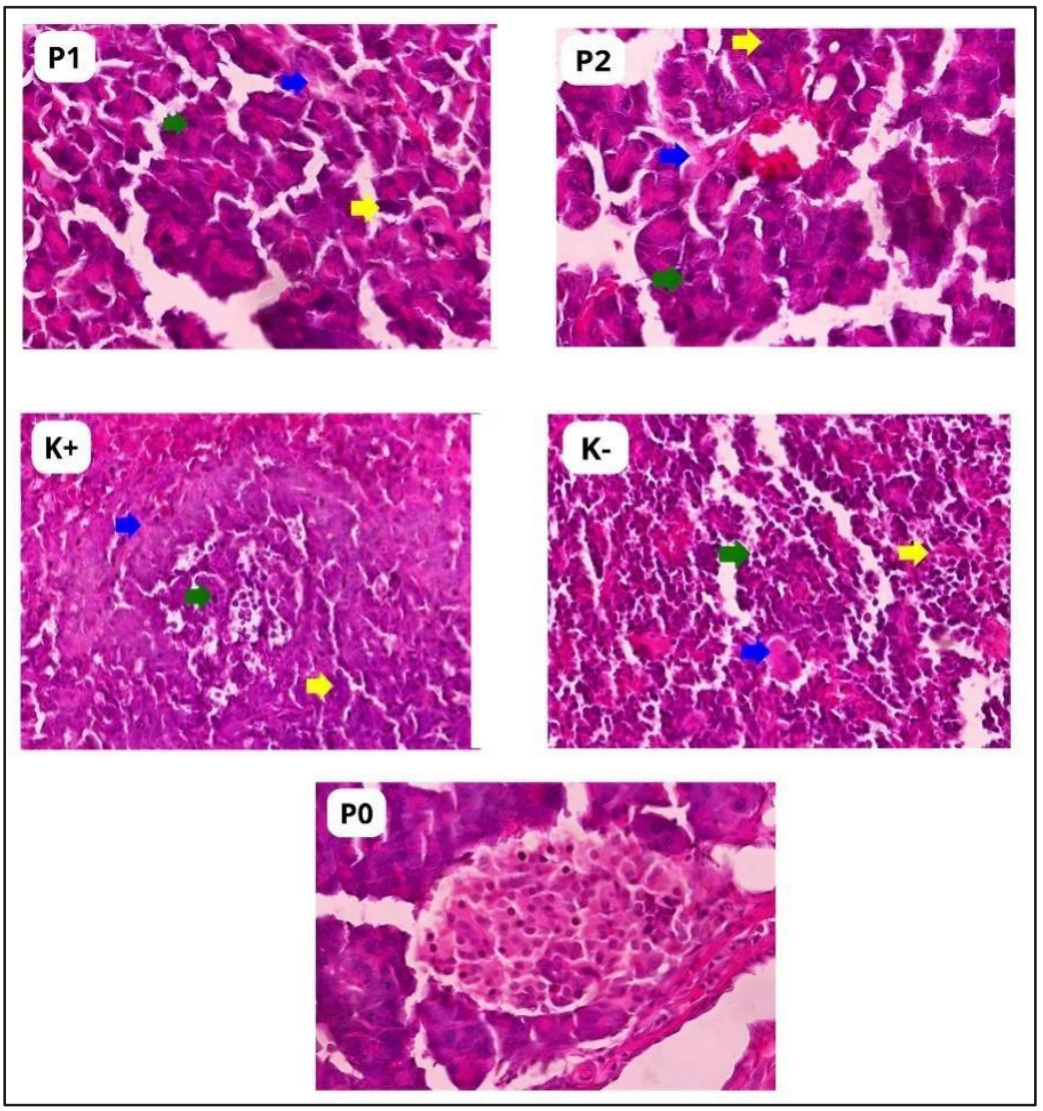

Fig 1. Microscopy of the pancreas with HE stains at 400x, oedema (blue), inflammatory cell infiltration (yellow), necrosis (green).

and P2 treatment groups where the oedema that occurred was slightly reduced and the acinar cell shape was still visible although in the K+ group the edema was still massive. The most severe inflammatory cell infiltration occurred in the diabetes control group. Necrosis that occurred in all extract and drug treatment groups had decreased compared to the K- group.

Histological picture of the P0 group showed that pancreatic cells were intact, clearly demarcated and acinar cells were still fused. Minimal inflammatory cell infiltration occurs. In contrast, in the K- group, pancreatic cells experienced necrosis. Alloxan causes pancreatic β cells to undergo necrosis in reactive oxygen species (ROS) (Solikhah, 2023). In general, necrosis begins with pyknotic nucleus then the cell loses the nucleus (karyolysis). This is an indication that mice experience destructive insulitis. Inflammatory cell infiltration occurs almost throughout the visual field. Pancreatic histopathology of alloxan-induced diabetic mice showed oedema of the white spaces between pancreatic lobules and acinar cells (Abdul and Moustafa, 2013). The diabetes control group was seen to have oedema with a score of 3 where the acini cells had separated from each other or were not intact.

The results of the data analysis showed that the P1 (MZLE 140 mg/kg) and P2 (MZLE 70 mg/kg) had significant differences from the K1group. Group treated with extract showed no significant difference to the normal group based on necrosis parameters. The two extract doses given did not show a significant difference in the three scoring parameters used. Increasing the drug dose should increase the response proportionately, however, the higher extract dose 140 mg/kg did not show a significant difference to the 70 mg/kg. This often occurs in drugs with natural ingredients because the compound consists various kinds of compounds that work together to cause effects, resulting in adverse interactions that cause effects that do not differ too much between doses. This may be related to the saturation of the associated receptors and the occurrence of interactions with chemical compounds in sapodilla leaves. Saturated receptors cause the dose to not reach the maximum effect.

Antioxidant compounds in sapodilla leaf extract capture free radicals which cause damage to pancreatic tissue so that infiltration of mononuclear cells into pancreatic tissue is also reduced (Gosh *et al*., 2015).

Data analysis showed that the P1, P2 and K- treatment did not show significant differences, but all three influenced repairing alloxan-induced diabetic mouse pancreatic damage. Pioglitazone has a diabetes therapy mechanism by inhibiting oxidative stress and ROS production through PPAR-γ-mediated mechanisms. An increase in total antioxidants can improve the histopathological structure of the affected organs caused by hyperglycaemia (Charbonneau and Capoccia, 2022).

Summary

Based on this study, administration of sapodilla leaves extract for 14 days on diabetic mice induced with alloxan could improve repair of pancreatic damage in diabetic mice. Administration of at a dose of 140 mg/kg BW showed the least damage compared to extract at a dose of 70 mg/kg and pioglitazone at a dose of 20 mg/kg.

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