ORIGINAL ARTICLE

The Effect of Ajwa Date (Phoenix dactylifera) Extract on The Histopathology of Pancreatic Islets in Mice with Diabetes Mellitus

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ABSTRACT

Introduction: It is necessary to develop alternative antidiabetic therapies that are safer and more affordable to overcome the high prevalence of diabetes mellitus in Indonesia. Ajwa dates (Phoenix dactylifera) have a high flavonoid content; hence, this study aimed to investigate their effect on streptozotocin-induced diabetes mellitus mice by examining the number of beta cells and the islets of Langerhans.
Methods: Twenty-five mice were divided into five groups: a negative control group (K1), a positive control group (K2), and three treatment groups (P1, P2, and P3). The K2, P1, P2, and P3 groups were induced by 100 mg/kg bw of streptozotocin. Additionally, the P1, P2, and P3 groups received oral treatment using ajwa date methanol extract at different doses of 3, 5, and 7 g/kg bw, respectively. The treatment was administered daily for four weeks. The initial analysis included the homogeneity test and the Shapiro-Wilk test. As the data were non-normally distributed, the analysis proceeded with the Kruskal-Wallis test ($p<0.05$).
Results: The comparative analysis revealed significant differences in the number of beta cells among the groups, with a notable decrease observed in the K2 group and an increase in each treatment group. The measurement of the islets of Langerhans exhibited significant differences among the groups, with $p=0.001$.
Conclusion: The administration of ajwa date methanol extract can affect the number of beta cells and the islets of Langerhans in mice with diabetes mellitus.

Keywords: Ajwa date extract; beta (β) cells; pancreatic islets; mice; diabetes mellitus

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Highlights:
1. This original study examined the antioxidant compounds derived from naturally sourced ajwa date (Phoenix dactylifera) extract.
2. Ajwa date extract has the potential to protect against histological damage, specifically to beta cells and pancreatic islets, in mice induced by streptozotocin.

Article history: •Received 5 January 2024 •Revised 9 March 2024 •Accepted 3 April 2024 •Published 31 August 2024

INTRODUCTION

Date palm trees are plants originating from the Middle East and South Africa. The plants have been widely cultivated in tropical and subtropical areas. Date palm trees offer numerous benefits, e.g., the trunk and frond of the plants can be used for construction materials and fuel. Palm fronds can be shaped for use as roofing or for the construction of huts. Additionally, dates have been known to be rich in sugar and vitamins (Tengberg, 2012). Another benefit of dates is that they can treat metabolic diseases because they contain antioxidant compounds (Dayang et al., 2014). One of the metabolic diseases is diabetes, which remains prevalent among many people who are unable to maintain a healthy diet and lifestyle.

The prevalence of diabetes around the world is remarkably high. According to data from the World Health Organization, the number of diabetic individuals increased from 108 million in 1980 to 422 million in 2014. This prevalence is increasing faster in low- and middle-income countries than in high-income countries. In 2021, the International Diabetes Federation (2013) reported that around 536.6 million people aged 20–79 years worldwide suffer from diabetes, resulting in a prevalence rate of 10.5%. In addition, the number of deaths caused by diabetes has reached 6.7 million.

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia (Baynest, 2015). Some types of diabetes mellitus are caused by a complex interaction between genetic and environmental factors. Factors contributing to hyperglycemia vary depending on

Available at https://e-journal.unair.ac.id/CIMRJ ; DOI: 10.20473/cimrj.v5i2.53610

the etiology of diabetes mellitus, including reduced insulin secretion, decreased glucose use, and increased glucose production (Loscalzo et al., 2022). In general, diabetes mellitus is divided into two different types, namely type 1 diabetes mellitus and type 2 diabetes mellitus. Type 1 diabetes mellitus is an autoimmune disease characterized by the progressive destruction of beta cells, leading to absolute insulin deficiency. On the other hand, type 2 diabetes mellitus is caused by insulin resistance and beta cell dysfunction, resulting in relative insulin deficiency (Kumar et al., 2014).

So far, definitive drug therapy options for diabetes mellitus are still limited to injectable and oral antihyperglycemic drugs, such as sulfonylureas, metformin, alpha-glucosidase inhibitors, dipeptidyl peptidase 4 (DPP-4) inhibitors, and insulin. However, it is important to note that each of these drugs carries its own side effects (Soelistijo et al., 2021). Ajwa dates (Phoenix dactylifera) have antihyperglycemic properties, meaning they can reduce high levels of glucose in the blood. Unlike existing oral and injectable antihyperglycemic drugs, ajwa dates do not have serious side effects (Maulana, 2020). In a prior study conducted by AlGeffari et al. (2016), a total of 19 patients were included as research participants. Among these participants, 10 patients received dates, while 9 patients were given 50 grams of glucose. Following the administration of the date palm diet, there was a decrease in hemoglobin A1c (HbA1c) levels along with improvements in glycemic indicators such as glycemic index and glycemic load. Furthermore, another study undertaken by Febrianti (2018) showed that administering ajwa date extract to pregnant mice for 30 days at varying doses of 3.12, 5.2, and 7.28 g/kg bw yielded significant differences. The administration of the largest dose, at 7.28 g/kg bw, demonstrated an effect on the blood glucose levels of the mice. These previous studies have suggested that dates have the ability to lower HbA1c and blood glucose levels, hence helping in the control of hyperglycemic diabetes mellitus. In light of the aforementioned data regarding the antioxidant properties of dates, this study aimed to investigate the potential effect of ajwa date methanol extract on pancreatic histopathology, particularly the number of beta cells and the islets of Langerhans, in mice with diabetes mellitus.

METHODS

This research was conducted at the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. Specifically, the sample preservation took place in the Experimental Animal Unit of the Department of Biochemistry, while the examination of the pancreatic islets was performed in the Histotechnique and Photomicroscopy Laboratory. The design used in this study was a randomized posttest-only control group design. The animal models of diabetes mellitus used in this study were Swiss Webster strain male white mice (Mus musculus). A total of 25 mice were divided by simple randomization into five groups: negative control group (K1), positive control group (K2), treatment group 1 (P1), treatment group 2 (P2), and treatment group 3 (P3). The K1 group, serving as the normal control, consisted of mice fed a standard diet. The K2, P1, P2, and P3 groups were once induced by 100 mg/kg bw (2.0 mL/kg bw) of streptozotocin, which caused diabetes mellitus in the mice. To determine the effect of the streptozotocin administration, the blood sugar levels of the mice were checked. A blood glucose concentration of >150 mg/dL (8.3 mmol/L) and/or a statistically higher concentration than that of the negative control group indicated adequate induction of diabetes mellitus by streptozotocin (Furman, 2021).

The K2 group, serving as the positive control, was not given any additional therapy. Meanwhile, the P1, P2, and P3 groups received a daily administration of ajwa date methanol extract at varying doses of 3, 5, and 7 g/kg bw, respectively. The oral administration of the ajwa date methanol extract was conducted daily for a period of 30 days. The doses of ajwa date extract were determined by referencing a prior study, where 3.12, 5.2, and 7.28 g/kg bw were used for different groups of mice (Agustina et al., 2019). On the 31st day, the mice were sacrificed. The preparations were made for the histopathological examination of the pancreas under a microscope.

The process of producing methanol extract from ajwa dates was conducted using the maceration method (cold extraction), with the intention of maintaining the integrity of the desired compound throughout the extraction process. This method provided advantages over other techniques because of its practicality, convenience, and the need for minimal solvents. The maceration method was selected because it could use different types of solvents according to their polarity properties for the targeted compound (Febrianti, 2018). The process of producing ajwa date methanol extract began by separating the fruit flesh from the seeds. The fruit flesh was thinly sliced and baked at 80 °C until it dried within 2 x 24 hours. The dates, which had turned into powder, were macerated using methanol solvent at a ratio of 1:2. The solution was allowed to stand for 2 x 24 hours, after which it was separated until the filtrate and residue were obtained. The filtrate obtained was then evaporated using a rotary evaporator to remove the solvent, resulting in a thick extract derived from the flesh of dates. This concentrated extract contained glucose, fructose, and sucrose compounds (Abdillah et al., 2017).

The pancreas of the mice was harvested after administering the ajwa date methanol extract for 30 days. The experimental animals were anesthetized by inhalation. Four experimental animals were placed within jars that had been infused with chloroform, inducing anesthesia until the mice lost consciousness. After around half a minute, the experimental animals were lifted from the anesthesia jars to undergo surgery (Agusfina & Julio, 2022). A surgical procedure known as necropsy was performed by making incisions in the skin and abdominal muscles of the mice until the abdominal cavity opened. The blood was removed until the heartbeat stopped, following which the pancreas organ was extracted. The pancreatic organs were collected and fixed in 10% formalin buffer, then preparations were made with hematoxylin and eosin staining (Akmal et al., 2023). The preparations were observed using an Olympus CX33 microscope at 400X magnification, and the cellSens and ImageJ imaging software were utilized for analysis. Observations of the islets of Langerhans were carried out on one preparation per sample, resulting in a total of five islets. The reading of the preparation was carried out by one of the authors (NA), which was then discussed and validated with the other authors (JS and THY). The average of the islets of Langerhans had an oval/elliptical shape, allowing the area to be calculated using the ImageJ software.

The data collected from the experiment were analyzed using IBM SPSS Statistics for Windows, version 27.0 (IBM Corp., Armonk, NY, USA). In the statistical analysis, the homogeneity test and the Saphiro-Wilk test were performed. The data were deemed evenly distributed if the Saphiro-Wilk test result exhibited a significance value of $p>0.05$. A homogeneity test was performed to determine whether

the data were homogeneous, with a significance value of p>0.05 (Garth, 2008). If the data were normally distributed and homogeneous, the analysis proceeded with a one-way analysis of variance (ANOVA) and a post-hoc test to assess if there was a significant difference. If the data did not follow a normal distribution, the Kruskal-Wallis test was used, followed by the Dunn-Bonferroni post-hoc test. A p-value below 0.05 for these two tests indicated a statistically significant result.

RESULTS

This study was experimental research that employed Swiss Webster strain male white mice (Mus musculus) as animal models of diabetes mellitus. As this study used a randomized posttest-only control group design, dependent variables were assessed at the end of the research. Following the administration of ajwa date methanol extract, the data on the beta cells were analyzed using the Kruskal-Wallis test. Table 1 shows the results from the analysis of the five groups.

Table 1. Results of post-treatment beta cell count analysis using the Kruskal-Wallis test

Groups	n	Mean±SD	p
K1	5	79.89±41.77	
K2	5	28.68±19.73	
P1	5	49.75±23.42	0.001
P2	5	44.77±27.71	
P3	5	32.01±27.18	

Note: K1 = negative control group (no treatment); K2 = positive control group (100 mg/kg bw of streptozotocin); P1 = treatment group 1 (100 mg/kg bw of streptozotocin and 3 g/kg bw of ajwa date extract); P2 = treatment group 2 (100 mg/kg bw of streptozotocin and 5 g/kg bw of ajwa date extract); P3 = treatment group 3 (100 mg/kg bw of streptozotocin and 7 g/kg bw of ajwa date extract); SD = standard deviation.

The data collected from the five groups were analyzed for normal distribution using the Saphiro-Wilk test, which resulted in a p-value of 0.001. Since the obtained p-value was below 0.05, it indicated that the data did not follow a normal distribution. Similarly, the homogeneity test resulted in a p-value of 0.001, which was substantially lower than the cut-off value of 0.05. This demonstrated that the variations

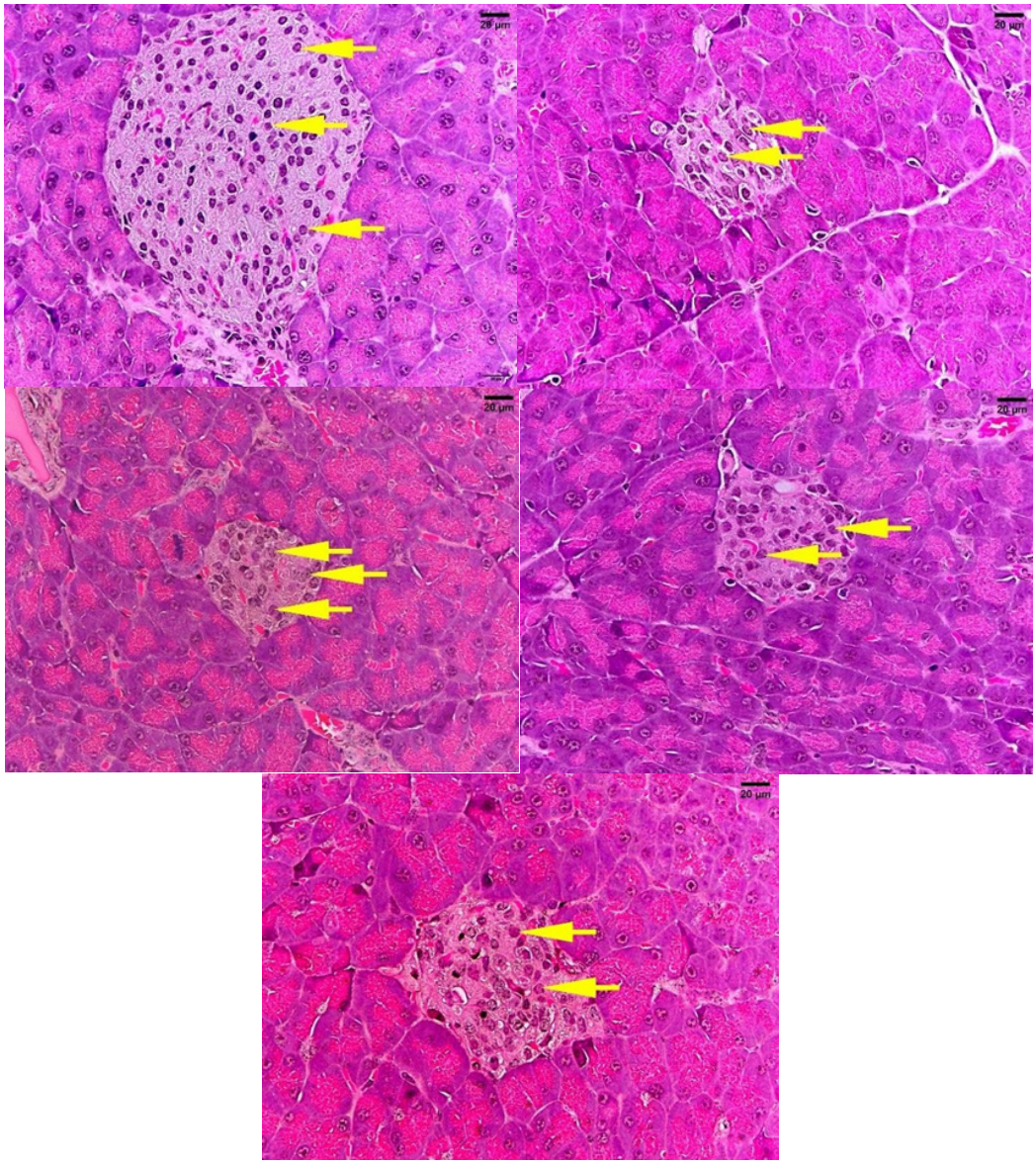

Figure 1. Appearance of beta cells in each group, as observed through hematoxylin and eosin staining at 400X magnification and 20 µm scale.

Note: K1 = negative control group (no treatment); K2 = positive control group (100 mg/kg bw of streptozotocin); P1 = treatment group 1 (100 mg/kg bw of streptozotocin and 3 g/kg bw of ajwa date extract); P2 = treatment group 2 (100 mg/kg bw of streptozotocin and 5 g/kg bw of ajwa date extract); P3 = treatment group 3 (100 mg/kg bw of streptozotocin and 7 g/kg bw of ajwa date extract).

in beta cell data among the groups were not homogeneous. Because the data were not homogeneous and non-normally distributed, the Kruskal-Wallis test was conducted. The test produced a significant p-value of 0.001. The p-value, which was below 0.05, indicated a statistically significant difference in the changes in the number of beta cells among the groups. The Kruskal-Wallis test was followed by a post-hoc Dunn-Bonferroni test. The Dunn-Bonferroni test yielded a p-value below 0.05, indicating a statistically significant difference in the changes in the number of beta cells of the mice. Specifically, there was a significant difference between the K1 group and the K2, P1, P2, and P3 groups, as well as between the K2 group and the P1, P2, and P3 groups.

Figure 1 displays the visual characteristics of beta cells in the five examined groups. The observation was performed using hematoxylin and eosin staining at 400X magnification and a 20 μm scale. The beta cells were painted using hematoxylin and eosin staining, resulting in fairly bright, round, and large nuclei, as shown by the yellow arrows in the figure. The P3 group exhibited a larger area of beta cells compared to the other treatment groups (P1 and P2). The results indicated a beneficial effect of the ajwa date methanol extract on the beta cells of diabetes mellitus mice.

The statistical analyses performed on the data of the islets of Langerhans were identical to those conducted for analyzing the number of beta cells. The Shapiro-Wilk test produced a p-value of 0.001 from the analysis of the data obtained from the five groups. The p-value, which was far below 0.05, indicated that the data were not normally distributed. A p-value of 0.001 was also yielded from the homogeneity test. This result demonstrated that the data were not homogeneous, as the p-value was considerably lower than 0.05. Due to the non-normal distribution and non-homogenous characteristics of the islets of Langerhans data, the analysis continued with the Kruskal-Wallis test (Table 2). The analysis revealed a statistically significant result, with a p-value of 0.001, which was lower than the threshold of 0.05. The p-value demonstrated a significant difference in the changes in the islets of Langerhans. The post-hoc Dunn-Bonferroni test revealed a p-value below 0.05, suggesting a statistically significant difference in the changes in the islets of Langerhans, particularly between the K2 group and the P1 and P2 groups.

Table 2. Results of pancreatic islet analysis using the Kruskal-Wallis test following the administration of ajwa date extract

Groups	n	Mean±SD (μm2)	p
K1	5	17503.48±10274.72	
K2	5	7103.79±5160.01	
P1	5	11953.47±5524.72	0.001
P2	5	12165.81±7039.49	
P3	5	10215.72±7756.73	

Note: K1 = negative control group (no treatment); K2 = positive control group (100 mg/kg bw of streptozotocin); P1 = treatment group 1 (100 mg/kg bw of streptozotocin and 3 g/kg bw of ajwa date extract); P2 = treatment group 2 (100 mg/kg bw of streptozotocin and 5 g/kg bw of ajwa date extract); P3 = treatment group 3 (100 mg/kg bw of streptozotocin and 7 g/kg bw of ajwa date extract); SD = standard deviation.

DISCUSSION

In this experimental study, animal models of diabetes mellitus were developed from Swiss Webster strain male white mice (Mus musculus). Male mice were chosen as research subjects because they have more stable hormonal conditions compared to female mice (Muhtadi et al., 2014). In addition, Swiss Webster strain mice (Mus musculus) have been frequently used as an experimental animal model for testing the effect of drugs on humans and the level of toxicity of toxins. Swiss Webster strain mice as animal models are easy to maintain in large quantities, only require minimal cost, possess diverse genetic variations, and have fairly good anatomical and physiological characteristics (Darmawan et al., 2014). The pancreatic histology observation was performed in this study to determine the condition of the mouse pancreas after being induced by streptozotocin and receiving ajwa date methanol extract for 30 days. The mice were dissected on the 31st day, and their pancreas was extracted to make preparations for hematoxylin and eosin staining. Afterwards, the beta cells and pancreatic islets of the mice were assessed to identify any changes in these markers across all groups.

Type 1 diabetes mellitus is characterized by the body's immune system that destroys beta cells in the pancreas, resulting in the inability of beta cells to produce insulin and regulate blood glucose levels. Consequently, the pancreas will try to produce more insulin (Ridwan et al., 2012). The mechanism of beta cell death in type 1 diabetes mellitus is the result of a combination of genetic and environmental factors. These factors lead to the accumulation of lymphocyte cells and macrophages in the islets of Langerhans, followed by the release of pro-inflammatory factors such as tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), and interferon alfa (IFN-α) (Pirot et al., 2008). These cytokines, along with the excessive formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) by immune cells, activate intracellular signaling pathways that promote autophagy, apoptosis, or necroptosis in pancreatic beta cells. Flavonoids have the ability to inhibit oxidative stress, which in turn triggers insulin secretion by pancreatic beta cells (Amani & Mustarichie, 2018).

In this study, the changes in the number of beta cells were quite significant in each control group and treatment group. The administration of different doses of ajwa date methanol extract to the treatment groups had an effect on the number of beta cells. The average number of intact beta cells did not differ much among the three treatment groups (P1, P2, and P3). However, there were cells in the islets of Langerhans that underwent vacuoles (necrosis), especially in the P1 group. A prior study conducted by El-Desouki et al. (2015) revealed that diabetes causes changes in mouse pancreatic tissue. These changes include vacuolization of islands, degeneration and necrosis of beta cells, dilation of intercalation channels, and infiltration of inflammatory cells. Meanwhile, the observation of the islets of Langerhans revealed different shapes and sizes within the five different groups. In the negative control group (K1), numerous islets of Langerhans retained their regular round or oval shape. It seemed that there was no damage to the islets of Langerhans, and the beta cells remained arranged and distributed evenly. There were no cells that experienced necrosis or inflammation. Meanwhile, in the positive control group (K2), the islets of Langerhans had small sizes. A significant number of beta cells had undergone necrosis. According to the statistical analysis, only the P1 and P2 groups exhibited significant results compared to the positive control group. It was inferred that administering doses of 3 and 5 g/kg bw of ajwa date methanol extract for 30 days was enough to affect the islets of Langerhans. However, the statistical data revealed a decreasing trend in the average number of cells in the islets of Langerhans, indicating that the lowest dose already had an effect on the islets of Langerhans.

Ajwa date extract contains a high concentration of

flavonoids. Research has demonstrated that flavonoids, such as quercetin, kaempferol, luteolin, and epicatechin, can increase the ability and insulin secretion capacity of pancreatic beta cells in rats (Ghorbani et al., 2019). Flavonoids have the ability to increase the capacity of beta cell secretion in animal models of diabetes. Prior research carried out by Masyita et al. (2018) provided evidence that jackfruit leaf ethanol extract contains beneficial flavonoid compounds. The administration of jackfruit leaf ethanol extract at a dose of 400 mg/kg bw effectively affected the regeneration of pancreatic beta cells after 21 days. Another study reported by Hafez et al. (2020) also showed that administering 5 mL/kg bw of date extract for eight weeks had an effect on diabetic rats. The date extract exerted a beneficial effect by restoring the function and structure of beta cells due to its flavonoid content, which possesses antioxidant and hypoglycemic properties.

Date extract has been proven to contain flavonoid compounds with reduction potentials, enabling them to reduce hydroxyl, alcosyl, peroskil, and superoxide radicals. The balance between antioxidants and oxidants can effectively reduce oxidative stress levels (Sayuti & Yenrina, 2015). Prior research has shown that hyperglycemia is associated with oxidative stress, elevated nitric oxide (NO) levels, and reactive oxygen species (ROS), all of which contribute to the development of insulin resistance (Chong et al., 2005). Prolonged or recurrent exposure of beta cells in the islets of Langerhans to hyperglycemia leads to progressive loss of beta cell phenotype, particularly a reduction in the expression of insulin-producing genes and major transcription factors. Increased blood sugar levels can also cause histopathological changes in the islets of Langerhans within the pancreatic tissue. This occurs through the direct glucotoxic effects on beta cells, which make up most of the endocrine cell mass in the islets of Langerhans. As a result, the cells will eventually experience apoptosis, leading to a decrease in the diameter and area of the islets of Langerhans (Farid et al., 2014). In research carried out by Wilujeng et al. (2023), male rats with diabetes mellitus were given a watermelon albedo infusion at different doses of 1,000, 1,500, and 2,000 mg/kg bw. Flavonoid compounds in the watermelon albedo infusion exhibited an effect on the diameter of the islets of Langerhans. Favonoids have demonstrated the ability to alter the secretory response of the islets of Langerhans, but with the presence of an additional 20 mmol of glucose/L (Castillo et al., 1989). In addition, a study conducted by Julianti et al. (2015) demonstrated that tapioca starch modified with 4% green tea extract, which contains flavonoids, had no effect on the islets of Langerhans. However, an effect was observed in terms of restraining the rate of pancreatic beta cell damage in diabetic rats.

This research raises awareness regarding the effect of ajwa date methanol extract on the number of beta cells and the islets of Langerhans in mice with diabetes mellitus. However, this study was only conducted within four weeks, which prevented an assessment of the long-term effect of administering ajwa date methanol extract. The experiment conducted in this study only involved three varying doses. Therefore, further research with more varied doses is necessary to determine the optimal dose and toxic dose of ajwa date methanol extract in regulating the blood sugar levels of diabetes mellitus mice.

The retrieval of variables from the preparations was insufficient, necessitating future research to make the preparations several times until the data are sufficient. This study was constrained by the lack of variation in data from the analysis of the number of beta cells and the islets of Langerhans in each group. Additionally, there were large gaps in the sample, resulting in non-normally distributed data. The possibility of sugar content in the ajwa date methanol extract might also affect the results of this study.

CONCLUSION

Administering ajwa date methanol extract can affect the number of pancreatic beta cells in mice with diabetes mellitus. Furthermore, the administration of ajwa date methanol extract at medium doses will potentially have an effect on the islets of Langerhans in mice with diabetes mellitus.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to the Experimental Animal Unit of the Department of Biochemistry and the Histotechnique and Photomicroscopy Laboratory, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, for providing access to their facilities.

CONFLICT OF INTEREST

None.

ETHICS CONSIDERATION

The Health Research Ethics Committee of the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, issued the ethical approval for this study, with registration No. 210/EC/KEPK/FKUA/2023 on July 31, 2023. Appropriate measures were implemented to ensure minimal discomfort for the mice involved in this study.

FUNDING DISCLOSURE

None.

AUTHOR CONTRIBUTION

NA contributed to the conceptualization as well as the analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, and collection and assembly of the data. IH, JS, and THY contributed to the analysis and interpretation of the data, critical revision of the article for important intellectual content, final approval of the article, and provision of study materials.

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