Baghdad Science Journal

Volume 22 | Issue 5 Article 10

5-17-2025

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How to Cite this Article

Syafitri, Anggun; Yuandani; Widiyawati, Tri; Anggraini, Dwi Rita; and Waruwu, Syukur Berkat (2025) "Antioxidant Capacity of Benalu Duku Leaves Alcoholic Extract on SOD Level and Pancreatic Cytology in Induced Diabetic Rats," *Baghdad Science Journal*: Vol. 22: Iss. 5, Article 10.
DOI: https://doi.org/10.21123/bsj.2024.11608

BAGHDAD SCIENCE JOURNAL 2025;22(5):1518–1525

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RESEARCH ARTICLE

Antioxidant Capacity of Benalu Duku Leaves Alcoholic Extract on SOD Level and Pancreatic Cytology in Induced Diabetic Rats

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ABSTRACT

Diabetes mellitus causes damage to pancreatic β cells and oxidative stress due to an imbalance of oxidants and antioxidants in the body. Controlling hyperglycemia by administering conventional drugs and with long-term use carries the risk of side effects, so traditional treatment is recommended. Benalu Duku (*Dendophthoe pentandra* (L.) Miq) is a plant considered a parasite. However, it has the potential to be developed as a diabetes drug because it contains metabolites that can be used as drugs that come from nature. This study aims to test phytochemicals and examine the effect of ethanol extract of Benalu Duku leaves (EEBD) on superoxide dismutase (SOD) levels in streptozotocin-nicotinamide-induced diabetic white Wistar rats, blood glucose levels were also examined, as well as conducting histological analysis of pancreatic β cells. The results of the phytochemical examination showed that it contained alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenoids. Research shows that giving EEBD for 28 days can significantly reduce blood glucose levels compared to the Na-CMC group. SOD levels also increased with respective values of 30.97 $\pm$ 0.84, 21.99 $\pm$ 0.61, 30.52 $\pm$ 1.30, 28.55 $\pm$ 1.30, 28.99 $\pm$ 0.95, and 29.00 $\pm$ 0.86 pg/mL. Pancreatic histology also showed differences between qualitative and quantitative, indicating pancreatic repair and increased surface area of the islets of Langerhans. This plant has the potential to be developed into a new medicinal ingredient that comes from nature.

Keywords: *Dendrophthoe pentandra*, Diabetic, Hematoxylin-eosin, Langerhans, Pancreatic, Superoxide dismutase

Introduction

Diabetes mellitus is a metabolic condition that can cause many long-term consequences, including nephropathy, neuropathy, retinopathy, and cardiomyopathy. Due to the accumulation of damage to several organs, this condition is sometimes known as a silent killer and is even a high-risk condition that can be fatal.[1,2] Diabetes causes chronic hyperglycemia due to impaired insulin secretion due to damage to pancreatic β-cells or impaired insulin receptor sensitivity.[3–5] Damage to pancreatic β-cells is often associated with oxidative stress due to an imbalance between oxidants and antioxidants in the body.[6–8] Persistent hyperglycemia causes increased production of free radicals, especially SOD. Increased glucose in diabetes results in the accumulation of ROS in pancreatic beta cells. This accumulation of ROS will damage the cells where they are located. In type 2 diabetes, ROS reduces insulin synthesis and activates the beta cell apoptosis pathway.[9,10] Controlling hyperglycemia in diabetes mellitus patients has been

Received 21 May 2024; revised 27 July 2024; accepted 29 July 2024.
Available online 17 May 2025

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https://doi.org/10.21123/bsj.2024.11608

controlled by administering oral antidiabetic agents. However, their use must be consumed for life, requiring high medical costs. Apart from that, long-term use poses a risk of severe side effects, so the use of traditional medicine is more recommended. [11–13]

Benalu Duku (*Dendrophthoe pentandra* (L.) Miq) is a parasitic plant included in 3000 other plant species with medicinal potential. Because it is considered a parasite, this plant is often thrown away because it is thought to interfere with the growth of Duku plants. However, leaves that have been considered parasitic benefit human health. [14,15] The compounds that have been isolated from Benalu Duku leaves are Quercetin 3-methyl ether-7-O-arabinoside, Quercetin 3- o-arabinoside-7-o-glucoside, and Quercetin 3-O-α-Rhamnoside, each of which has different activities. High as an antioxidant, antibacterial, and antidiabetic. [16–18] A flavonoid molecule called quercetin 3-methyl ether-7-O-arabinoside can be a free radical neutralizer, protecting pancreatic beta cells from harm and promoting insulin release. [19–21] This research was conducted to test the effect of ethanol extract of Benalu Duku leaves on SOD levels and the protective effect on pancreatic β cells in diabetic rats induced by streptozotocin-nicotinamide.

Materials and methods

Sample collection

Benalu Duku leaves were obtained from the Duku fields of Medan Johor Village, Medan, North Sumatra Province, Indonesia. Plant identification was carried out at the Medanense Herbarium, University of Sumatera Utara, Indonesia, with a number 136/MEDA/2022. Other materials used include nicotinamide (Brataco-Chem), streptozotocin (Brataco-Chem), natrium carboxymethyl cellulose 0.5% (Brataco-Chem), glibenclamide, ethanol 96%, distilled water, trichloroacetic acid 20%, thiobarbituric acid 0.67%, and enzyme-linked immunosorbent assay (ELISA) kit.

Extract preparation

Five hundred grams of Benalu Duku leaf simplicia powder was put into a vessel then added with ethanol solvent until it was soaked with ten parts of ethanol solvent, covered and soaked for the first 6 hours, then left for 18 hours. The macerate is separated by filtering. The filtering process was repeated three times, using half the solvent volume in the first filtering. The extract was concentrated using a rotary evaporator at a temperature of $\pm$ 40°C until a thick extract was obtained. Phytochemical screening tests examine flavonoid, alkaloid, saponin, tannin, glycoside, and steroid/triterpenoid compounds. [22,23]

Preparation of test animals

The test animals used were male white Wistar rats with a body weight of 180–200 grams, divided into six groups, each consisting of 4 animals. This research procedure was carried out based on guidelines and approval from the Animal Research Ethics Committees (AREC), Department of Biology, Faculty of Mathematics and Natural Sciences, University of Sumatera Utara, Indonesia, with approval number 0795/KEPH-FMIPA/2022. The rats were separated into six groups, each consisting of 4 rats, as follows:

- Group 1: Normal
- Group 2: Na-CMC (natrium carboxymethyl cellulose) 0.5%
- Group 3: Glibenclamide 0.45 mg/kg BW
- Group 4: EEBD (ethanol extract of benalu duku leaves) 100 mg/kg BW
- Group 5: EEBD 200 mg/kg BW
- Group 6: EEBD 400 mg/kg BW

Before the test, the rats were first acclimatized to the laboratory environment for a week, ensuring their comfort and reducing stress. They were handled carefully without causing fear. The night before treatment, the animals were fasted first. After fasting for 18 hours, rats were induced by administering 230 mg/kg BW nicotinamide solution intraperitoneally. After 15 minutes, continue with administration of 65 mg/kg BW streptozotocin solution intraperitoneally. Blood glucose levels were measured using a glucometer (Easy Touch®GCU) after 72 hours of induction. Rats with fasting blood glucose levels $>$200 mg/dL are said to be diabetic and can be used in testing. The extract was given orally every day for 28 days starting when the rats had diabetes. [24,25]

Measurement of SOD levels

On the last day, the rats were sacrificed using anesthesia. Blood was taken (3–3.5 mL) from the heart to collect plasma serum, which was used to measure SOD levels using the ELISA method. The absorbance was read with a microplate reader at a wavelength of 450 nm, and the levels were calculated. [26,27]

Histological observations

The rat pancreas was analyzed histologically using the hematoxylin-eosin staining method. [28] Then, observations can be made under a microscope with

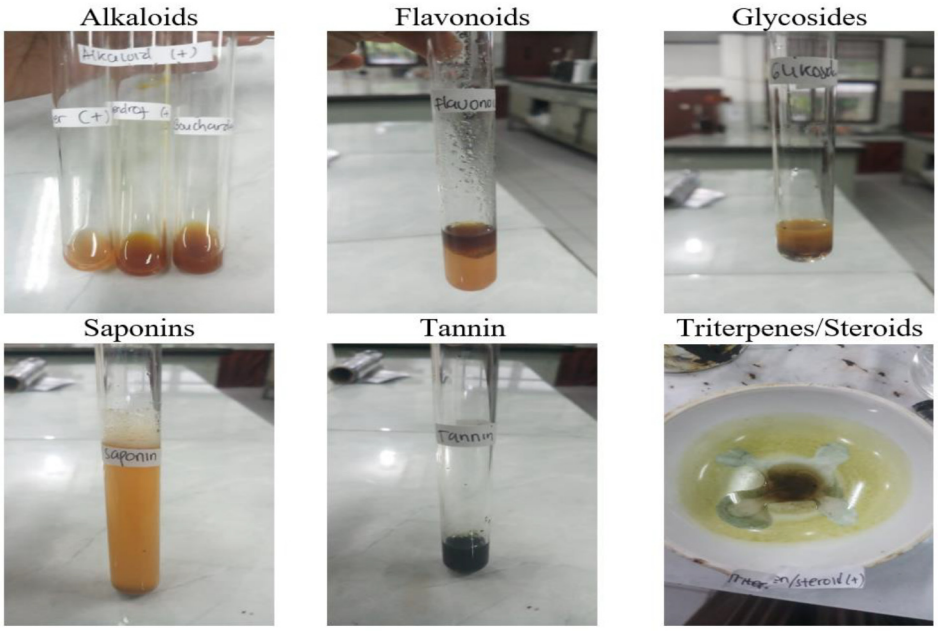

Fig. 1. Results of phytochemical screening of EEBD.

400x magnification.[29] The structure of the pancreatic islands of Langerhans is examined by observing the islands of cells buried in the exocrine tissue of the pancreas. Then, the examination continues by calculating the area of the pancreatic islands of Langerhans.[30]

Data analysis

Statistical analysis used Anova and the Post Hoc Tukey HSD test to determine whether there were fundamental differences between treatments.[31]

Results and discussion

Phytochemical screening

The results of phytochemical screening of the EEBD were obtained to obtain information on the classes of secondary metabolite compounds. Phytochemical examination showed the presence of alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenoid compounds. The results of phytochemical screening can be seen in Fig. 1. Phytochemical examination showed the presence of alkaloid compounds. When the Mayer reagent solution was added, it formed a white lumpy precipitate; with the Bouchardat reagent solution, a blackish brown precipitate was formed, and the Dragendorff reagent solution formed a red color. Examining the flavonoid compound group with magnesium powder and concentrated hydrochloric acid produces a red-colored solution. Examination of the group of glycoside compounds by adding Molisch's reagent and concentrated sulfuric acid forms purple rings. The sample was added with hot distilled water and shaken vigorously to produce stable foam, then 2 N HCl was added, indicating the presence of the saponin group of compounds. Adding $FeCl_3$ gives a blackish-green color, indicating the presence of tannin compounds. Examination of the triterpenoid/steroid compound group by adding a few drops of Liebermann-Burchard reagent produces a pink or purple color, which indicates the triterpenoid compound group.[32,33]

Blood glucose level examination results

Glucose was checked 72 hours after induction, and glucose was rechecked on the last day of treatment. The examination results are displayed in Table 1.

Blood glucose levels in induced rats were high on average except in the normal group (not induced). This shows that the rats used for the experiment were in a state of hyperglycemia. Streptozocin-nicotinamide induction can increase blood glucose levels.[34] On the last day, blood glucose levels decreased in all treatment groups. However, the Na-CMC group did not experience a significant decrease.

SOD level examination results

The results of examining SOD levels, as shown in Table 2.

Table 1. Results of blood glucose level analysis.

Groups	Blood Glucose Levels (mg/dL)	
	After Induction	Day 28
Normal	91.25 ± 8.17	93.75 ± 4.82
Na-CMC 0.5%	356.25 ± 23.05[b,c]	325.00 ± 16.67[b,c]
Glibenclamide 0.45 mg/kg BW	367.75 ± 16.19[a,c]	151.00 ± 23.08[a,c]
EEBD 100 mg/kg BW	365.50 ± 8.73[c]	206.00 ± 27.74[a]
EEBD 200 mg/kg BW	366.25 ± 11.05[c]	163.75 ± 46.60[a]
EEBD 400 mg/kg BW	367.75 ± 9.88[c]	153.00 ± 35.37[a]

Note: Data is presented in the form of average and standard errors. [a]: significantly different from the Na-CMC control group, [b]: significantly different from the glibenclamide group, [c]: significantly different from the normal group.

Table 2. Results of SOD level analysis.

Groups	SOD Levels (pg/mL)
Normal	30.97 ± 0.84[a]
Na-CMC 0.5%	21.99 ± 0.61[b,c]
Glibenclamide 0.45 mg/kg BW	30.52 ± 1.30[a]
EEBD 100 mg/kg BW	28.55 ± 1.30[a,c]
EEBD 200 mg/kg BW	28.99 ± 0.95[a]
EEBD 400 mg/kg BW	29.00 ± 0.86[a]

Note: Data is presented in the form of average and standard errors. [a]: significantly different from the Na-CMC control group, [b]: significantly different from the glibenclamide group, [c]: significantly different from the normal group.

The average SOD concentration value in the Na-CMC group had the lowest concentration. Administration of nicotinamide and streptozotocin as triggers of oxidative stress will reduce antioxidant resistance in the body by showing a decrease in SOD levels. Administration of nicotinamide may influence the SOD levels examined, where nicotinamide is a direct precursor of NAD^+ and an inhibitor of poly ADP ribose, which has the effect of increasing ATP levels in cells, thereby reducing cell damage. [35–37]

Administration of EEBD had a significant effect on increasing SOD concentrations. The three EEBD groups showed significant differences with the Na-CMC group. The chemical compounds such as flavonoids, tannins, and saponins from various types of plants are known to have antioxidant effects. [38,39] The low SOD levels in the Na-CMC group indicate the high oxidative stress that occurs in diabetic rats without therapy. Diabetes mellitus increases ROS, thereby exacerbating oxidative stress. Oxidative stress causes an imbalance in the system for the formation and capture of free radicals, thereby reducing antioxidant activity. [40–42]

Histological examination on pancreatic

Histological observations on pancreatic preparations from normal groups and treated groups showed different structures, as in Fig. 2. Based on the results of the observations, the normal group showed pancreatic features, such as pancreatic acinar cells and Langerhans cells. Langerhans cells appear with regular cell membranes; alpha cells are located at the edge, with small, dense, round nuclei and little cytoplasm (red stars), while beta cells are located in the center with larger, brighter nuclei, eosinophilic cytoplasm (yellow stars). In the positive control, Langerhans cells appeared with a large surface area with many cells as in the normal group, and the cell membrane was still regular (black arrow). Meanwhile, in the Na-CMC group, Langerhans cells appeared with a smaller surface area and fewer cells. The cells in the central (middle) section appear to be experiencing edema (hydrophilic degeneration) marked with a yellow star. In the 100 mg/Kg BW group, Langerhans cells showed a smaller surface area with fewer cells and irregular cell membranes (black arrows). The 200 mg/KgBW group showed Langerhans cells with a larger surface area than the normal group and a more significant number of cells. It can be seen that the cells in the central (middle) section are experiencing proliferation, marked by yellow stars and slightly irregular cell membranes (black arrows). Finally, in the 400 mg/Kg BW group, Langerhans cells appeared with a larger surface area and a more significant number of cells resembling the normal group. The cell membrane was slightly irregular (black arrow).

Induction of diabetes with streptozotocin and nicotinamide causes pancreatic beta-cell necrosis. [43] Beta cells show a significant decrease in moderate diabetes, whereas, in severe diabetes, beta cells are not even found. Langerhans cells with a smaller surface area and a smaller number of cells in the Na-CMC group indicate damage to the pancreatic tissue; the cells in the central part also experience hydrophic degeneration. Hydrophilic degeneration indicates that reversible changes have occurred in the cells, so if the toxic exposure is stopped, the damaged cells will return to normal. Continued degeneration will cause cell death. Liver cell death causes hepatocytes to be unable to return to their normal form

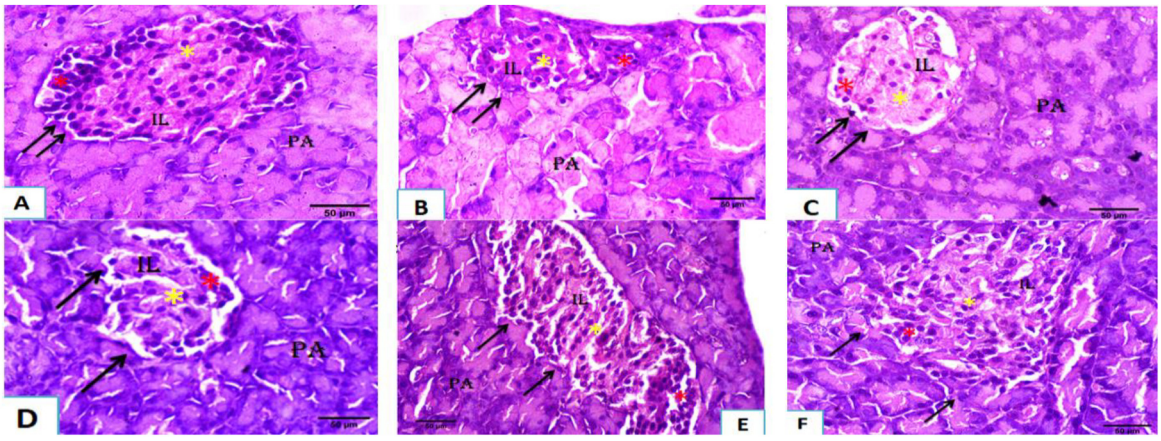

Fig. 2. Pancreatic histopathology (400x), A: Normal group; B: Glibenclamide; C: Na-CMC; D: EEBD 100 mg; E: EEBD 200 mg; F: EEBD 400 mg; IL: Islet of pancreas; PA: Panceatic acinar; *: alpha cells, *: beta cells;↑: cell membrane.

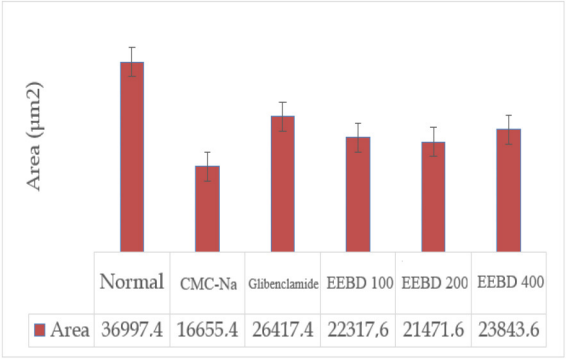

Fig. 3. Results of calculating the area of the pancreatic islets of Langerhans. Note: Data is presented in the form of average and standard errors.

(irreversible). Hydrophilic degeneration increases intracellular water content, which causes the cytoplasm and organelles to swell and form vacuoles. Damage to the cell membrane's permeability causes obstruction to sodium flow out of the cell, causing excessive ions and water to enter the cell. A lack of oxygen, calcium deficiency, severe shock, and diabetes mellitus can cause this hydrophilic degeneration.[44,45]

Quantitative calculations of Langerhans islands were conducted to assess improvements. The results are shown in Fig. 3. The area of the islets of Langerhans was highest in the normal group, significantly different from the Na-CMC group. The EEBD 200 mg/kg BW group had a lower mean area, while the highest was observed at the EEBD dose of 400 mg/kg BW. These findings suggest the potential for beneficial pharmacological effects of EEBD. In diabetes mellitus sufferers, Langerhans Islands undergo morphological changes in both number and size. The islets of Langerhans, a collection of endocrine glands spread across the pancreas, resemble islands with numerous blood capillaries passing through them.[46–48]

Conclusion

EEBD has potential sound pharmacological effects that can significantly increase the SOD levels of streptozotocin-nicotinamide-induced diabetic rats. On Langerhans Island, EEBD increased the Langerhans surface area of diabetic rats compared to Na-CMC group. The leaves of this plant have the potential to be developed in the future and analyzed regarding the active ingredients contained in this plant.

Acknowledgment

The authors would like to sincerely thank the Master of Pharmacy Study Program at the Faculty of Pharmacy, University of Sumatera Utara, Medan, Indonesia.

Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all the figures and tables in the manuscript are ours. Furthermore, figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- The author has signed an animal welfare statement.
- No human studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at Animal Research Ethics Committees (AREC), Department of

Biology, Faculty of Mathematics and Natural Sciences, University of Sumatera Utara, with approval number 0795/KEPH-FMIPA/2022.

Authors' contribution statement

This manuscript was created in collaboration with all authors: Y. conceptualized and designed the research, A.S. collected samples, performed analysis, and wrote the manuscript, D.R.A. performed histological analysis, T.W. the result interpretation, S.B.W. revisions, proofreading, edit the manuscript with revisions and now take care of publishing. All authors read and approved the final manuscript.

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القدرة المضادة للأكسدة للمستخلص الكحولي لأوراق بينالو دوكو على مستوى SOD وعلم خلايا البنكرياس في الجرذان المصابة بداء السكري

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الخلاصة

يتسبب داء السكري في تلف خلايا البنكرياس والإجهاد التأكسدي بسبب عدم توازن المواد المؤكسدة ومضادات الأكسدة في الجسم. إن السيطرة على ارتفاع السكر في الدم عن طريق إعطاء الأدوية التقليدية والاستخدام طويل الأمد ينطوي على مخاطر الآثار الجانبية، لذلك يوصى بالعلاج التقليدي. بينالو دوكو (*Dendophthoe pentandra* (L.) Miq) هو نبات يعتبر من النباتات الطبيعية. ومع ذلك، فمن الممكن تطويره كدواء لمرض السكري لأنه يحتوي على مستقلبات يمكن استخدامها كأدوية تأتي من الطبيعة. تهدف هذه الدراسة إلى اختبار المواد الكيميائية النباتية ودراسة تأثير المستخلص الإيثانولي لأوراق بينالو دوكو (EEBD) على مستويات فوق أكسيد ديسموتاز (SOD) في فئران ويستار البيضاء المصابة بداء السكري المستحث بالستربتوزوتوسين والنيكوتيناميد، كما تم فحص مستويات الجلوكوز في الدم، بالإضافة إلى إجراء التحليل النسيجي. تحليل خلايا البنكرياس. وأظهرت نتائج الفحص الكيميائي النباتي أنها تحتوي على قلويدات وفلافونيدات وجليكوسيدات وصابونين وعفص وترايتيربينويدات. تظهر الأبحاث أن إعطاء EEBD لمدة 28 يومًا يمكن أن يقلل بشكل كبير من مستويات الجلوكوز في الدم مقارنة بمجموعة Na-CMC. زادت مستويات SOD أيضًا بقيم 30.97 ± 0.84 و 21.99 ± 0.61 و 30.52 ± 1.30 و 28.55 ± 1.30 و 28.99 ± 0.95 و 29.00 ± 0.86 بيكوغرام / مل. وأظهرت أنسجة البنكرياس أيضًا اختلافات بين النوعية والكمية، مما يشير إلى إصلاح البنكرياس وزيادة مساحة سطح جزر لانجرهانس. يتمتع هذا النبات بإمكانية تطويره ليصبح مكونًا طبيًا جديدًا يأتي من الطبيعة.

الكلمات المفتاحية: *Dendrophthoe pentandra*، السكري، الهيماتوكسيلين أيوزين، لانجرهانز، البنكرياس، سوبر أكسيد ديسموتاز.