BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 14, Number 2, October 2025 | Pages: 1165-1169 | DOI: 10.14421/biomedich.2025.142.1165-1169 ISSN 2540-9328 (online)

Phytochemical Screening and Blood Glucose Response of Red Dragon Fruit Extract in Alloxan-Induced Mice

I Gusti Agung Istri Mas Dianti Pratiwi[1], Ni Putu Ayu Dian Maharani[1], Ni Made Asthi Pramesti Kirana[1], Putu Nadya Asti Utari[1], I Gede Ngurah Bagus Arthayasa[1], Luh Gde Evayanti[2], Made Dharmesti Wijaya[3]*

[1]Medical Study Program; [2]Department of Anatomy and Histology; [3]Department of Pharmacology,
Faculty of Medicine and Health Sciences, Warmadewa University,
Jl. Terompong No 24 Denpasar 80235, Tel. +62 361 240727, Indonesia.

Corresponding author*
dharmestiwijaya@warmadewa.ac.id

Manuscript received: 18 September, 2025. Revision accepted: 21 November, 2025. Published: 04 December, 2025.

Abstract

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by elevated blood glucose levels, which can lead to various complications. Natural products, including red dragon fruit (*Hylocereus polyrhizus*), have gained attention for their potential antidiabetic properties due to their phytochemical content, particularly flavonoids. This study aimed to evaluate the phytochemical composition of red dragon fruit extract and its effect on blood glucose levels in alloxan-induced diabetic mice. Phytochemical screening was conducted qualitatively to identify the presence of secondary metabolites. Male mice were divided into five groups: a negative control group (no treatment), a positive control group (metformin 10 mg/kg BW), and three treatment groups (T1, T2, T3) receiving red dragon fruit extract at doses of 18.2, 36.4, and 72.8 mg/kg BW, respectively. Diabetes was induced by a single intraperitoneal injection of alloxan monohydrate (140 mg/kg BW). Treatments were administered orally once daily for 6 days. Blood glucose levels were measured on days 0, 2, 4, and 6. Data were analyzed using the Friedmann test. Phytochemical screening confirmed the presence of flavonoids, tannins, alkaloids, and saponins in the extract. Statistical analysis showed no significant reduction in blood glucose levels after administration of red dragon fruit extract at any of the tested doses ($p>0.05$). Red dragon fruit extract did not exhibit a significant antihyperglycemic effect in alloxan-induced diabetic mice under the tested conditions. This may be attributed to suboptimal dosage, insufficient flavonoid concentration, or a less effective mechanism of action compared to standard therapy. Further studies are needed to optimize the formulation and evaluate its potential using different extraction methods or in combination with other bioactive compounds.

Keywords: Alloxan-induced mice; antihyperglycemic; *Hylocereus polyrhizus*.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. According to the International Diabetes Federation, approximately 589 million people aged 20-79 years worlwide were living with DM in 2024, and this number is projected to rise to 853 million by 2050. Indonesia ranks among the top ten countries with the highest number of DM cases, with and estimated 20.4 million people affected in 2024 (International Diabetes Foundation, 2025). Moreover, DM contributes significantly to morbidity and mortality, with complications such as cardiovascular disease, nephropathy, retinopathy, and neuropathy (Harreiter & Roden., 2023).

Diabetes mellitus management generally involves pharmacological and non-pharmacological approaches, including lifestyle modification and the use of antidiabetic medications (Harreiter & Roden., 2023). However, long-term use of synthetic drugs can lead to adverse effects and impose economic burdens on patients. This has encouraged the exploration of alternative therapies derived from natural products, particularly medicinal plants, which are believed to offer therapeutic benefits with minimal side effects (Ali et al. 2022; Pitaloka & Juwariyah, 2021).

One such plant is red dragon fruit (*Hylocereus polyrhizus*), which is widely cultivated and easily accessible in Indonesia. This fruit is known to contain various bioactive compounds, including phenolic acids (such as hydroxycinnamates), beta carotene, flavonoids, belatains, vitamin C, as well as linoleic and linolenic acids (Arivalagan et al. 2021). These phytochemicals possess antioxidant properties that may contribute in reducing oxidative stress and improving insulin sensitivity, which are important in the context of DM (Benson et al. 2023). In addition, the dietary fiber in red dragon fruit, particularly pectin, is suggested to help regulate blood glucose levels by slowing glucose absorption through increased intestinal viscosity and

decreased diffusion rates (Benson et al. 2023). The antioxidant and phytochemical compounds may also support vascular health and enhance cell membrane permeability, potentially increasing cellular responsiveness to insulin.

Considering the potential bioactive compounds present in red dragon fruit and the limited research on its overall phytochemical profile and antihyperglycemic effects, further investigation is warranted. Therefore, this study aimed to perform a qualitative phytochemical screening of red dragon fruit extract and to evaluate its effect on blood glucose levels in alloxan-induced diabetic mice.

MATERIALS AND METHODS

Materials

The materials used in this study included fresh red dragon fruits (*Hylocereus polyrhizus*) obtained from a traditional market in the Denpasar area, Bali; 25 male Swiss-Webster mice; standard mice feed; distilled water (5 L); 96% ethanol (OneMed); and alloxan monohydrate (Sigma-Aldrich). Rice husks were also used as bedding material for the mice. For the antioxidant activity assay, additional materials included 1% hydrochloric acid (HCl), ascorbic acid as a standard antioxidant, and DPPH (2,2-diphenyl-1-picrylhydrazyl) kit.

The tools utilized in this study included various standard glassware (beakers, glass funnels, measuring flasks, measuring cups, test tubes), filter papers, dropper pipettes, syringes (1 mL and 3 mL), glass stirrers, microscope slides, oral gavage (mice sonde), mice drinking bottles, mice cages, blood glucose strips, disposable gloves, face masks, hand sanitizers, tissues, glass jars, knives, cutting boards, filters, and a rotary evaporator. For the antioxidant activity test, additional tools used were a UV-Vis spectrophotometer, an analytical balance, aluminum foil, black glass bottles, dark vials, and other supporting laboratory equipment.

Preparation of red dragon fruit extract

Fresh red dragon fruits were thoroughly washed, peeled, and cut into small pieces. The fruit pieces were then dried in an oven at 40°C for seven days until completely dehydrated. The dried fruits were subsequently ground using a blender to obtain a fine powder. A total of 400 grams of the dried powder was weighed and subjected to maceration with 2.8 liters of 96% ethanol (sample-to-solvent ratio of 1:7). The maceration process was carried out for five days at room temperature, protected from light, with occasional stirring. After five days, the mixture was filtered, and the filtrate was concentrated using a rotary evaporator to obtain a thick extract.

Phytochemical screening

Phytochemical screening of the red dragon fruit extract was conducted qualitatively to detect the presence of flavonoids, alkaloids, saponins, and tannins. For flavonoid detection, the extract was placed into a test tube, then added with 1–2 grains of magnesium metal and 3 drops of concentrated hydrochloric acid, followed by the addition of amyl alcohol and vigorous shaking; the appearance of a reddish-yellow to red color indicated a positive result for flavonoids. Alkaloid detection involved mixing the extract with 1 mL of concentrated hydrochloric acid and 9 mL of distilled water, heating for 2 minutes, cooling, and filtering; the filtrate was divided into three test tubes, and the addition of Wagner's reagent to each was performed—precipitate formation in at least two tubes indicated a positive result for alkaloids. For saponins, the extract was added with 10 mL of hot water, cooled, shaken for 10 seconds, and then added with 2N hydrochloric acid; the formation of stable foam over 1 cm in height indicated the presence of saponins. Tannin detection was carried out by adding 1–2 drops of 1% ferric chloride ($FeCl_3$) solution to the extract; the appearance of a blackish-green coloration suggested a positive result for tannins. These tests were conducted following standard procedures for phytochemical analysis as described by Harborne (1988).

Animal preparation

The test animals used in this study were male Swiss-Webster mice, aged approximately 6 weeks and weighing around 25 grams. The mice, which were in healtaahy condition and exhibited normal activity, were obtained from the Animal Laboratory, Faculty of Medicine and Health Sciences, Warmadewa University. Prior to the experimental procedures, the mice were acclimatized in their new cages for 7 days at room temperature, with free access to standard feed and drinking water.

After the adaptation period, fasting blood glucose levels were measured to obtain baseline data. Diabetes mellitus was then induced by intraperitoneal injection of alloxan monohydrate at a dose of 140 mg/kg body weight. Alloxan induces diabetes by selectively damaging pancreatic β-cells, resulting in insulin deficiency and hyperglycemia (Woldekidan et al. 2021) Following induction, the mice continued to receive food and water ad libitum. On the seventh day after induction, following a fasting period of 12–18 hours, fasting blood glucose levels were measured again to confirm hyperglycemia. Mice with fasting blood glucose levels greater than 290 mg/dL were considered diabetic and included in the subsequent experimental groups (American Diabetes Association, 2024)

Treatment protocol

A total of 25 male Swiss-Webster mice were randomly divided into 5 groups, each consisting 5 mice. The groups received the following treatments: Group 1 (negative control) did not receive any treatment with red dragon fruit extract; Group 2 (positive control) was

administered metformin orally at a dose of 10 mg/kg body weight; meanwhile Group 3, 4, and 5 were treatment groups that received red dragon fruit extract at concentrations of 18.2 (T1), 36.4 (T2), and 72.8 (T3) mg/kg body weight, respectively.

The red dragon fruit extract was prepared by dissolving the thick extract in distilled water to obtain the desired concentrations. Each treatment, including the extract and metformin, was administered once daily via oral gavage for 14 consecutive days. During the treatment period, all mice provided standard feed and drinking water ad libitum. Blood glucose levels were measured on days 0, 2, 4, and 6 to monitor changes in response to the administered treatments.

Statistical analysis

The data obtained were analyzed using SPSS software. Prior to comparative testing, the data were assessed for normality and homogenity. Since the results indicated that the data were not normally distributed and variances were not homogeneous, a non-parametric approach was applied. The Friedman test was used to compare blood glucose levels among the five groups. A 95% confidence level ($p < 0.05$) was used to determine statistical significance.

Ethical approval

All animal procedures performed in this study were reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Udayana University (Approval No: 0324/UN 14.2.2.VII.14/LT/2025).

RESULTS AND DISCUSSION

A total of 10 kilograms of fresh red dragon fruit (*Hylocereus polyrhizus*) were oven-dried at 40°C for seven days, yielding approximately 1,000 grams of dried simplicia. The dried material was then subjected to extraction using the maceration method, with 400 grams of powdered simplicia immersed in 2,8 L of 96% ethanol. The maceration process was conducted at room temperature for five days with occasional stirring and protection from light. This procedure resulted in the production of 55 milligrams of thick extract. Maceration was selected as the extraction method in this study due to its simplicity, cost-effectiveness, and ability to preserve thermolabile phytochemicals through cold extraction (Rusip et al. 2022).

Phytochemical screening

Qualitative phytochemical screening tests were carried out to identify flavonoid, tannin, alkaloid and saponin in the red dragon fruit extract. The results confirmed that the extract contained all four compounds (Table 1).

Table 1. Phytochemistry of red dragon fruit extract.

Phytochemicals	Method	Results	Description
Flavonoids	Shinoda's test	+	Reddish-orange color formed
Tannins	$FeCl_3$	+	Blackish-blue color formed
Alkaloids	Wagner's test	+	Precipitate formed
Saponins	Froth test	+	Foam formed

Theoretically, the presence of these compounds is associated with the potential to lower blood glucose levels in alloxan-induced mice. Flavonoids are known to exert antidiabetic effects through multiple mechanisms, including enhancing insulin receptor sensitivity, stimulating insulin secretion, regenerating pancreatic β-cells, and inhibiting the digestive enzymes α-amylase and α-glucosidase (Liu et al. 2023). Similarly, tannins have been reported to inhibit glucosidase activity and improve insulin sensitivity, while alkaloids may stimulate insulin release or suppress hepatic gluconeogenesis (Ramadhan & Sujono, 2025). Moreover, saponins are recognized for their ability to inhibit intestinal glucose absorption and enhance insulin secretion (Idris *et al,* 2023). The synergistic presence of flavonoids, tannins, alkaloids, and saponins in the red dragon fruit extract therefore provides a theoretical basis for its potential antihyperglycemic effect. However, although the phytochemical profile of the extract suggested potential antihyperglycemic activity, further evaluation in this study showed that such effects were not observed under the tested conditions.

Blood Glucose Level

In vivo testing was conducted using 25 mice randomly divided into five groups. The mean blood glucose levels of each group are presented in Table 2, while the trends over time are visualized in Figure 1. Statistical analysis using the Friedman test showed no significant differences in blood glucose levels among the groups ($p = 0.537$, $p > 0.05$). These results indicate that red dragon fruit extract at doses of 18.2, 36.4, and 72.8 mg/kg BW did not significantly reduce blood glucose levels in alloxan-induced diabetic mice compared with control groups.

Table 2. Blood glucose levels (mg/dL) of alloxan-induced diabetic mice during 6 days of treatment.

Sample	Blood Glucose (mg/dL)			
	Day 0	Day 2	Day 4	Day 6
Positive control	167,0	147,6	160,6	151,8
Negative control	169,6	149,2	163,4	165,6
T1	144,4	168,6	154,4	152,6
T2	155,8	159,8	156,8	153,6
T3	140,0	139,8	198,0	196,2

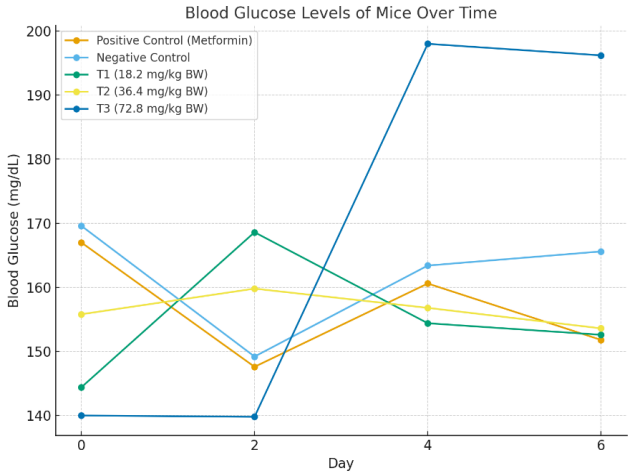

Figure 1. Blood glucose levels of alloxan-induced diabetic mice during 6 days of treatment. Mice were divided into five groups (n = 5 per group): negative control (untreated), positive control (metformin 10 mg/kg BW), and three treatment groups receiving red dragon fruit extract at doses of 18.2, 36.4, and 72.8 mg/kg BW. Statistical analysis using the Friedman test showed no significant differences among groups ($p > 0.05$).

Although the extract contained phytochemicals with known antidiabetic potential—such as flavonoids, tannins, alkaloids, and saponins—the lack of significant effect observed in this study may be affected by several factors. Previous research reported that the ethanol extract of red dragon fruit exerted significant antihyperglycemic activity at doses ≥200 mg/kg BW (Idris et al. 2023), suggesting that the doses tested here may not have reached the effective threshold. Moreover, treatment was carried out for 6 days, while other studies have shown that the antidiabetic effects of flavonoids often require 21-28 days of administration (Rahman et al. 2022). In addition, no quantitative analysis of phytochemicals was performed in this study, and flavonoid concentrations can vary depending on extraction methods and solvents (Pujiastuti & Zeba, 2021). If the active compound levels were relatively low, the pharmacological effects could also be limited.

Another factor to consider is the use of alloxan to induce diabetes. Alloxan is known for its unstable cytotoxicity and extensive β-cell damage, which can make glucose normalization more difficult even with bioactive treatments. Streptozotocin (STZ) is often recommended as a more stable and consistent alternative for diabetes induction (Samsul et al. 2020). Biological variability between animals may also have contributed, as indicated by the non-homogeneous distribution in some groups, which could mask potential effects in statistical analysis (Rahman et al. 2022).

Taken together, these findings suggest that under the tested conditions, red dragon fruit extract did not significantly reduce blood glucose levels in alloxan-induced diabetic mice. Nevertheless, the study provides useful preliminary data, and further research with optimized doses, longer treatment duration, and quantitative phytochemical analysis is recommended to better evaluate the antidiabetic potential of red dragon fruit.

CONCLUSIONS

In conclusion, qualitative phytochemical screening confirmed the presence of flavonoids, tannins, alkaloids, and saponins in red dragon fruit extract. However, the extract did not significantly reduce blood glucose levels in alloxan-induced mice at the tested doses and treatment duration. These findings suggest that further research using higher doses, longer treatment periods, and quantitative phytochemical analysis is required to better evaluate the antidiabetic potential of red dragon fruit.

Acknowledgements: The authors would like to express their sincere gratitude to the laboratory staff of the Research Laboratory and Animal Laboratory, Faculty of Medicine and Health Sciences, Warmadewa University, for their technical support and assistance during the experimental work. The support and contributions of colleagues and mentors are also gratefully acknowledged.

Authors' Contribution: I Gusti Agung Istri Mas Dianti Pratiwi contributed to study design and preparation of the initial manuscript draft. Ni Putu Ayu Dian Maharani and Ni Made Asthi Pramesti Kirana were responsible for extract preparation, phytochemical screening, and data analysis. Putu Nadya Asti Utari and I Gede Ngurah Bagus Arthayasa carried out the animal experiments and treatment protocol. Luh Gde Evayanti contributed through conceptual input and critical review of the final manuscript. Made Dharmesti Wijaya provided supervision, critical guidance throughout the research process, and substantial revisions to the manuscript. All

authors discussed the results together and approved the final version of the manuscript.

Competing Interest: The authors declare that there are no competing interests.

Funding: This research was funded by the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia through the Student Creativity Program (Program Kreativitas Mahasiswa/PKM) in 2024.

REFERENCES

Ali, M. K., Pearson-Stuttard, J., Selvin, E., & Gregg, E. W. (2022). Interpreting global trends in type 2 diabetes complications and mortality. *Diabetologia*, *65*(1), 3–13.

American Diabetes Association. (2024). 2. Classification and diagnosis of diabetes: Standards of Care in Diabetes—2024. *Diabetes Care, 47*(Supplement_1), S19–S34.

Arivalagan, M., Karunakaran, G., Roy, T. K., Dinsha, M., Sindhu, B. C., Shilpashree, V. M., Satisha, G. C., & Shivashankara, K. S. (2021). Biochemical and nutritional characterization of dragon fruit (Hylocereus species). *Food chemistry*, *353*.

Benson, M., Hossain, J., & Darmaun, D. (2023). Improved glycemic control either alone, or combined with antioxidant supplementation, fails to restore blood glutathione or markers of oxidative stress in adolescents with poorly controlled type 1 diabetes. *Nutrition research (New York, N.Y.)*, *117*, 83–90.

Harborne, J. B. (1998). Textbook of Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. 5th Edition. Chapman and Hall Ltd. London, 21-72.

Harreiter, J., & Roden, M. (2023). Diabetes mellitus – Definition, Classification, Diagnose, Screening und Prävention (Update 2023) [Diabetes mellitus: definition, classification, diagnosis, screening and prevention (Update 2023)]. *Wiener klinische Wochenschrift*, *135*(Suppl 1), 7–17.

Idris, Z., Yusuf, M., Sari, P.I. dan Susmihara. (2023). Efektifitas Ekstrak Etanol Buah Naga Merah *(Hylocereus polyrhizus)* pada Pengobatan Diabetes Mellitus Tipe 2. Jurnal Promotif Preventif: 6(3): 411.

International Diabetes Federation. (2025). Diabetes Facts & Figures. URL: https://idf.org/about-diabetes/diabetes-facts-figures/. Accessed 29 July 2025.

Liu, X., Wang, Y. & Zhang, L. (2023). Regeneration of pancreatic beta cells after alloxan-induced damage: A systematic review. Nature Scientific Reports, 13(4): 1-12.

Pitaloka D.Y. dan Juwariyah S. (2021). Efek Pemberian Buah Naga Merah *(Hylocereus polyrhizus)* Terhadap Penurunan Kadar Glukosa Darah Pada Penderita Diabetes Tipe 2. Manajemen Asuhan Keperawatan: 5(2): 97-103.

Pujiastuti, E., an Zeba, D.E. 2021. Perbandingan Kadar Flavonoid Total Ekstrak Etanol 70% dan 96% Kulit Buah Naga Merah (Hylocereus Polyrhizus) Dengan Spektrofotometri. *Cendekia Journal of Pharmacy*: 5(1): 28-43

Rahman, A., Putri, D.F., & Setiawan, H. (2022). Antidiabetic potential of *Hylocereus polyrhizus* peel ethanolic extract on alloxan-induced diabetic mice. *ResearchGate Journal of Phytomedicine*, 11(2): 45-52.

Ramadhan, I., & Sujono, T. A. 2025. Skrining Fitokimia Dan Uji Aktivitas Antioksidan Ekstrak Etanol Akar Bajakah (Spatholobus littoralis Hassk) Pada Tikus Putih (Rattus norvegicus) Yang Diinduksi Aloksan. *Usadha Journal of Pharmacy*, 102-115.

Rusip, G., Ilyas, S., et al., 2022. *The effect of ingestion of red dragon fruit extract on levels of malondialdehyde and superoxide dismutase after strenuous exercise in rats.* F1000Research / PMC. Available at: https://pmc.ncbi.nlm.nih.gov/articles/PMC9345267/ Accessed 30 January 2024.

Samsul, E., Soemardji, A. A., dan Kusmardiyani, S. (2020). Aktivitas Antidiabetes Serbuk Semut Jepang (Tenebrio molitor Linn.) pada Mencit Swiss Webster Jantan yang Diinduksi Aloksan. *Jurnal Sains dan Kesehatan*: 2(4): 298-302.

Woldekidan, S., Mulu, A., Ergetie, W., Teka, F., Meressa, A., Tadele, A., Abebe, A., Gemechu, W., Gemeda, N., Ashebir, R. and Sileshi, M., 2021. Evaluation of antihyperglycemic effect of extract of moringa stenopetala (Baker f.) aqueous leaves on alloxan-induced diabetic rats. *Diabetes, Metabolic Syndrome and Obesity*; 185-192.

THIS PAGE INTENTIONALLY LEFT BLANK