Trop J Nat Prod Res, October 2023; 7(10):4908-4911 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Evaluation of *Clitoria ternatea* L. Flower Extract in Preventing Complications of Diabetes Mellitus

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ARTICLE INFO

Article history:
Received 28 August 2023
Revised 16 September 2023
Accepted 07 October 2023
Published online 01 November 2023

ABSTRACT

Flavonoids and anthocyanins can be found in the *Clitoria ternatea* flower (*C. ternatea*). It has been demonstrated to lessen oxidative stress and to inhibit both the pancreatic enzyme α-amylase and the intestine's α-glucosidase. HbA1c is commonly used to detect glycemic levels, whereas malondialdehyde (MDA) is crucial for determining the potential risk of lipid peroxidation-related problems. This research assessed *C. ternatea* flower extract's (CTE) alter on fasting blood glucose, hemoglobin A1c (HbA1c), and MDA in *Wistar* rats with diabetes (DM) induced by streptozotocin-nicotinamide (STZ-NA). Thirty-six male *Wistar* rats were all used in this post-test-only control group experiment and allocated into six groups: standard control, DM control, DM+4,5 mg/kg acarbose, and DM+150 mg/kg CTE, DM+300 mg/kg CTE, and DM+600 mg/kg CTE, respectively. After 28 days of therapy, fasting blood glucose, HbA1c, and MDA plasma levels were assessed. DM+600 mg/kg CTE showed the lowest fasting blood glucose levels at 90.43±0.94 mg/dl. With a p-value of $p<0.05$, the Kruskal-Wallis test findings for fasting blood glucose and HbA1c levels demonstrated significant differences between the therapy groups. DM+600 mg/kg CTE showed the lowest levels of MDA of 3.13±0.09 nmol/ml and HbA1c of 26.04±0.13 ng/ml. One-way ANOVA findings for MDA levels had a p-value of $p<0.05$, indicating a significant difference between the treatment groups. According to the results, rats with diabetes induced by STZ-NA had lower fasting blood glucose levels, HbA1c, and MDA after receiving *C. ternatea* extract. *Clitoria ternatea* may have the ability to prevent DM problems.

Keywords: *Clitoria ternatea;* fasting blood glucose; hemoglobin A1c ; Malondialdehyde; Diabetes Mellitus; Acarbose.

Introduction

Diabetes mellitus type 2 (DM), a chronic metabolic condition, is now more common than ever globally.[1] Over the past three decades, DM patients have doubled.[2] Hyperglycemia, insulin resistance, and relative insulin deficiency are the characteristics of this disease.[1] A previous study found a high prevalence of glucose intolerance and undiagnosed DM in the population, highlighting the need for regular screening to reduce its incidence and complications in the short and long term.[3] The HbA1c test accurately measures chronic glycemic levels and assesses the risk of DM complications.[4] According to studies, oxidative stress, evidenced by high levels of biomarkers like MDA, has a significant impact on the development and complications of DM by encouraging lipid peroxidation, protein oxidation, and oxidative DNA damage.[5] Therefore, prevention and management of DM are critical to achieving therapeutic targets and preventing complications.[6]

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Citation: Dewi I, Chodidjah C, Atina H. Evaluation of *Clitoria ternatea* L. Flower Extract in Preventing Complications of Diabetes Mellitus**.** Trop J Nat Prod Res. 2023; 7(10):4908-4911. http://www.doi.org/10.26538/tjnpr/v7i10.28.

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Clitoria ternatea (*C. ternatea*) is utilized in traditional medicine worldwide, and its parts offer several benefits to humans. The flower petals of this plant contain antioxidants and antidiabetic properties, which can address hyperglycemic conditions.[7,8]

Studies have revealed that animals with alloxan-induced diabetes can lower fasting blood glucose levels and indicate antioxidant activity when administered *C. ternatea* flower extract.[9] Similarly, when consumed with sucrose, the flower powder of *C. ternatea* can increase plasma antioxidant capacity and improve postprandial glucose, insulin, and antioxidant status without lowering fasting blood glucose levels.[10] According to Chayaratanasin *et al.*, the aqueous extract of *C. ternatea* possesses strong antiglycation effects and antioxidant activity, as evidenced by its ability to prevent complications of diabetes mediated by Advanced Glycation End (AGEs) products, such as glycated hemoglobin.[11] The phenolic compounds, flavonoids, anthocyanins, and flavonol glycosides present in *C. ternatea* flower can also protect cells from oxidative stress and disrupt pancreatic α-amylase and intestinal α-glucosidase, resulting in a decrease in postprandial blood sugar.[12] In this investigation, the effectiveness of *C. ternatea* flower extract (CTE) on diabetic animal models' fasting blood glucose, HbA1c, and MDA levels was examined.

Materials and Methods

For 28 days, this research was accomplished at the Gadjah Mada University Center for Food and Nutrition Studies in Yogyakarta. The Medical Faculty's Ethical Committee of Universitas Islam Sultan Agung Semarang provided its permission regarding ethics. This study used 36 male *Wistar* rats, eight weeks old, weighing between 150 and 200 g, for the tests. The animals were acclimated for seven days on a

regular pellet diet with full access to water before being induced with STZ-NA.

Plant material and C. ternatea extract (CTE) preparation

C. ternatea flowers were collected locally and authenticated by Integrated Biomedical Laboratory Universitas Islam Sultan Agung Semarang, with specimen number FKSA-CT1-IX22. Flowers from *C. ternatea* were collected in June 2022. The collected flowers were dried in the oven at 40° C and ground into a fine powder. The 1 kg powdered flower sample was extracted with the maceration method using 96% ethanol and analyzed for the flavonoid content using qualitative methods.

Bioactive components

C. ternatea flowers positively contained flavonoids, tannins, saponins, and terpenoids. Meanwhile, the anthraquinone and alkaloid tests showed negative results.[12] To prepare the extract, selected fresh flowers were washed, dried at 40°C, blended until smooth, and sieved using a mesh. An extract was produced by macerating the sample in a 1:10 solution of 96% ethanol for four days, followed by filtering and concentration using a rotatory evaporator. *C. ternatea* flower extract was then suspended in 1% Carboxymethyl-Cellulose Nicotinamide (CMC-Na) for the CTE-DM treated groups, a final volume of 2 ml was administered at dosages of 150 mg/kg, 300 mg/kg, and 600 mg/kg.

Experimental design

The study involved 36 male *Wistar* rats. Six groups of six rats each were created out of the rats. The usual pellet and water *ad libitum* were given to each animal. The rats were adapted for seven days prior to the treatments. The standard control groups comprised six normal rats treated with 1% CMC-Na solution. DM rats were induced by a combination of streptozotocin and nicotinamide (STZ-NA). Injections of 45 mg/kg of streptozotocin dissolved in sodium citrate buffer (pH 4.5) and 110 mg/kg of nicotinamide in normal saline were given to the rats 15 minutes apart. Fasting blood glucose levels were determined after three days to allow for the induction of hyperglycemia and were found to be higher than 250 mg/dl. DM+4.5 mg/kg acarbose was used as standard therapy. CTE-treated groups were divided into three doses: 150, 300, and 600 mg/kg. The treatment was conducted for 28 days.[9]

Measurements

Using a blood sample, fasting blood glucose levels were assessed using the GOD-PAP enzymatic technique. The samples were collected on day 28; fasting blood glucose levels were then measured with a spectrophotometer UV-Vis (546 nm).[13] The HbA1c level in a blood sample was measured using column chromatography. For each sample, we used a separate column, and we subsequently collected the liquid that had been eluted from the column and contained the HbA1c. To determine total Hb, we combined hemolysate with a chemical reagent. Ultimately, HbA1c was measured using a spectrophotometer with a 415 nm wavelength.[14] HbA1c levels were measured on the 28th day. The Thiobarbituric Acid Reactive Substance (TBARs) test technique detected the MDA level. The sample taken is blood plasma. The measurements were based on the reaction between thiobarbiturate and MDA, which will form a pink chromogen. The absorbance was then assessed at a 523 nm wavelength.[15] MDA levels were measured on the 28th day.

Statistical analysis

The data were assessed using the mean (SD) standard deviation. The Kruskal-Wallis test was used to assess data on fasting blood glucose levels, and then the Mann-Whitney test. One-way ANOVA was used to analyze the data for the MDA and HbA1c levels, and a post hoc LSD test was then performed. A difference of $p<0.05$ was considered to be substantially different. The statistical analysis was done using IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, New York, USA).

Results and Discussion

Fasting blood glucose levels in the CTE group decreased with increasing doses (Figure 1). Figure 2 shows that the level of MDA decreased significantly in CTE-treated diabetic rats. The 600mg/kg dose of CTE caused a lower level of HbA1c (Figure 3). The four treatment groups' fasting blood glucose, MDA, and HbA1c levels given acarbose and different dose ranges of *C. ternatea* flower extract were lower than the diabetes control group. The group receiving 600 mg/kg of *C. ternatea* flower extract was the most effective for lowering fasting blood glucose, MDA, and HbA1c levels, and its effects were equivalent to those of the acarbose group (Table 1). Fasting blood glucose levels did not return to normal range after therapy.

Fasting blood glucose levels and HbA1c can be reduced with *C. ternatea* flower extract using 150 mg/kg, 300 mg/kg, and 600 mg/kg. Compared to the diabetic control group, these data showed a substantial difference. However, the most effective dose compared to other treatment groups is 600 mg/kg, although it could not reach normal levels. According to Manivannan *et al.*, *C. ternatea* flower extract of 300 mg/kg has metabolic effects as antidiabetic and hypoglycaemic; hence, it can improve the function of pancreatic cells and stimulate insulin release.[9]

Foods containing high sucrose levels increase postprandial blood glucose, leading to hyperglycemic conditions. High blood sugar levels trigger the production of excessive amounts of insulin, which are associated with metabolic syndrome, gestational diabetes, and type 2 diabetes. Intestinal α-glucosidase and pancreatic α-amylase are two carbohydrate enzymes that can be inhibited by *C. ternatea* flower extract. The study indicates that the flower extract of this flower can reduce postprandial blood glucose and sucrose-induced insulin response. Anthocyanins, which include delphinidin-3,5-glucoside, delphinidin-3-glucoside, malvidin-3-glucoside, kaempferol, p-coumaric acid, and six major ternatines (ternatine A1, A2, B1, B2, D1, and D2), are the primary phenolic components of *C. ternatea* flower extract. Intestinal α-glucosidase and pancreatic α-amylase are inhibited by anthocyanins.

The mechanism of the *C. ternatea* flower suppresses the activity of intestinal α- glucosidase enzymes, which is similar to acarbose. This substance in the digestive system functions as a fictitious carbohydrate and has the potential to block α-glucosidase enzymes present in the brush border of the gastrointestinal epithelium.[16,17] HbA1c results from non-enzymatic glycation that occurs when glucose in plasma binds covalently to hemoglobin. A spontaneous non-enzymatic event called glycation occurs when glucose is covalently bonded to hemoglobin at the amino terminus of the β-globin chain.

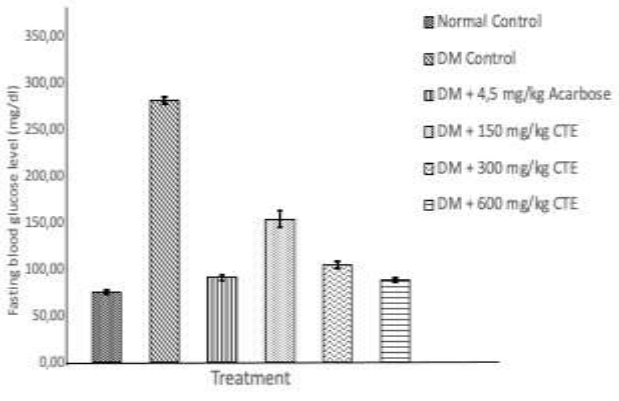

Figure 1: Fasting blood glucose levels in six groups showed significant different ($p<0,05$)

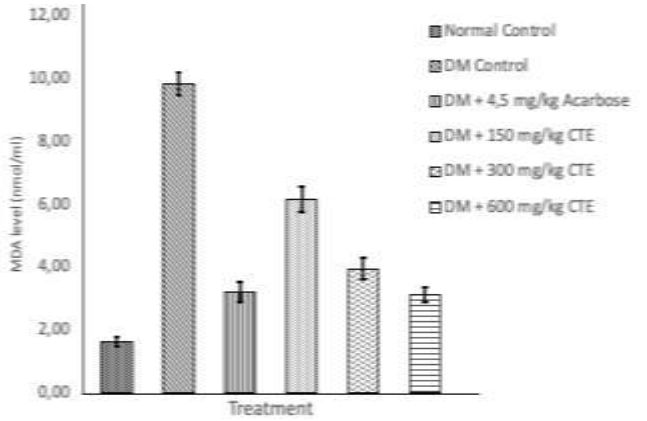

Figure 2: MDA levels in six groups showed significant different (p<0,05)

Glucose in red blood cells forms aldimine bonds with NH_2 of the valine beta chain and produces Amadori products with stable ketoamine bonds. Diabetes is characterized by an accumulation of Advanced Glycation End (AGEs) products resulting from this reaction, strongly associated with disease development and complications.[18] The binding of AGEs to their receptors might result in ROS (reactive oxygen species) generation and inflammatory cytokines, which may result in oxidative stress and inflammation.

It has been demonstrated that *C. ternatea* flowers have anti-glycation action, which helps eliminate free radicals and Methylglyoxal (MG) conjugates to protect against AGE-related disorders. The anthocyanins detected in *C. ternatea* flowers have been associated with various benefits, including antioxidative, anti-hyperglycemic, and anti-glycation properties. According to Chayaratanasin *et al.*, *C. ternatea* flower extract's direct carbonyl trapping and free radical scavenging properties reduce protein glycation and oxidative DNA damage caused by MG. Due to delphinidin derivatives and quercetin-3-rutinoside, which prevent the production of AGEs, *C. ternatea* flower extract can trap MG. Flavonoids, particularly delphinidin derivatives and flavonol glycosides, play an essential role in MG capture activity by *C. ternatea* flowers. Positions C-6 and C-8 on the A ring of flavonoids are the active sites for MG conjugation, while the C-5 hydroxyl group improves MG trapping efficiency. Flavonoid substances, particularly delphinidin derivatives and flavonol glycosides, which have active sites to capture MG and inhibit AGE receptors (RAGE), impact the MG capture activity of *C. ternatea* flowers.[11]

C. ternatea flower extract in this study reduced MDA levels as a marker of ROS. The most notable reduction in MDA levels was seen in the group that got 600 mg/kg of the extract; this impact was equivalent to that seen in the group that received acarbose. The study also shows that acarbose reduces lipid peroxidation and platelet activation in type 2 diabetic patients, improving postprandial epithelial dysfunction and lowering the risk of cardiovascular problems.[19]

Consumption of foods containing carbohydrates will produce metabolic byproducts in the form of ROS. High ROS production leads to an imbalance between oxidants and antioxidants, causing postprandial oxidative stress. MDA synthesis, a marker of lipid peroxidation caused by PUFA oxidation in cell membranes, was substantially associated with ROS overproduction. Excessive sucrose intake causes the formation of peroxidation markers with a decrease in plasma antioxidant capacity. Plasma antioxidant capacity and thiol group levels are maintained by the polyphenols in *C. ternatea* flower extract, which lowers plasma MDA levels.

According to Chusak *et al.*, healthy patients who ingested 1 g and 2 g of *C. ternatea* flower extract in sucrose had significantly lower plasma MDA levels, increased antioxidant status, and lower postprandial concentrations of glucose and insulin in the blood.[10] However, the current study did not investigate the pancreatic α-amylase and intestinal α-glucosidase enzymes from *C. ternatea* flower for their antioxidant and inhibitory properties. The limitation was the inability to measure MDA and HbA1c levels after induced with STZ-NA and before treatment with *C. ternatea* extract.

Conclusion

C. ternatea flower extracts showed antidiabetic effects, which prevent DM complications. Therefore, this study confirms the therapeutic effect of *C. ternatea* as a medicinal herb for a new therapy for DM.

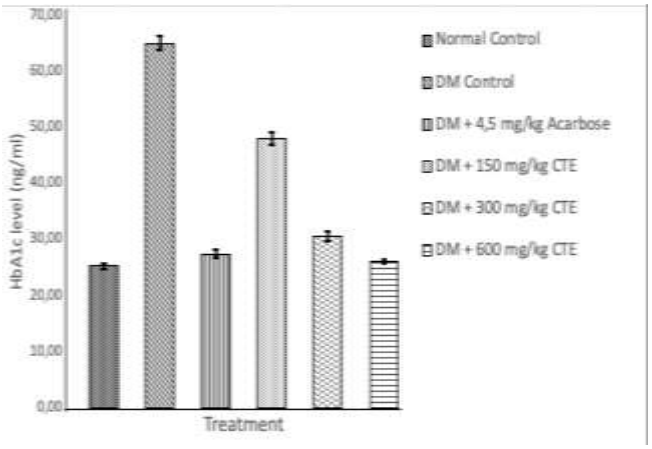

Figure 3: HbA1c levels in six groups showed significant different (p<0,05)

Table 1: Level of fasting blood glucose, MDA, and HbA1c in 6 treatment groups

Groups	Variables			
	Level of fasting blood glucose after STZ-NA induction (mg/dl)	**Level of fasting blood glucose after 28 days treatment (mg/dl)**	**MDA level (nmol/ml)**	**HbA1c (ng/ml)**
Normal control	74.51±0.75	76.11±0.75	1.61±0.06	25.31±0.19
DM control	278.12±3.80	280.82±3.51[a]	9.89±0.45[a]	64.73±1.01[a]
DM+ 4,5 mg/kg Acarbose	281.72±4.86	92.44±1.95	3.19±0.33	27.30±0.83
DM+150 mg/kg CTE	280.24±2.47	151.71±5.89[a]	6.18±0.46[a]	47.73±1.21[a]
DM+300 mg/kg CTE	276.71±2,75	104.07±2.72[a]	3.94±0.38[a]	30.69±0.79[a]
DM+600 mg/kg CTE	279.61±3.52	89.56±0.96	3.17±0.23	26.04±0.38[a]

Note: [a]Mean values were significantly different (p<0.05) compared to the DM+4.5 mg/kg acarbose as standard therapy

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors are grateful to Direktorat Riset, Teknologi, dan Pengabdian Masyarakat (DRTPM) Kementrian Pendidikan, Kebudayaan, Riset, dan Teknologi Indonesia for the PTM study grant with contract number 002/LL6/PB/AK.04/2022.

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