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Effectiveness of *Clitoria ternatea* L. Nanoherbal on Blood Glucose and Malondialdehyde Levels of Hyperglycemic Rats

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Abstract

Background: *Hyperglycemia induces excessive production of reactive oxygen species (ROS), leading to oxidative stress and lipid peroxidation characterized by increased malondialdehyde (MDA) levels. This study evaluated the effectiveness of butterfly pea flower nanoherbal formulated as alginate-based nanoparticles using the ionic gelation method in reducing blood glucose and MDA levels in hyperglycemic rats.* **Methodology:** *25 male Wistar rats (Rattus norvegicus) were divided into five groups: normal control (NC), metformin control (MC), and three nanoherbal treatment groups (NT1, NT2, and NT3) receiving doses of 12.5, 25, and 50 mg/kg body weight (BW), respectively. Hyperglycemia was induced by a single intraperitoneal injection of alloxan monohydrate (125 mg/kg BW). Blood glucose was measured on days 1, 14, and 28, while MDA levels were analyzed on day 28 using the TBARS method. The analysis of blood glucose levels was conducted using a Repeated Measures Two-Way ANOVA followed by a Bonferroni post hoc test, while MDA data were evaluated through a One-Way ANOVA with Duncan's multiple range test ($p < 0.05$).* **Findings:** *A significant, dose-dependent reduction in blood glucose levels was observed following nanoherbal treatment. The NT3 group (50 mg/kg BW) showed the greatest reduction, with mean glucose decreasing from 471.4 ± 142.00 mg/dL on day 1 to 146.4 ± 68.58 mg/dL on day 28. MDA levels also differed significantly, with NT3 showing 2.84 ± 0.19 nmol/mL, indicating lower oxidative stress.* **Contributions:** *These findings indicate that alginate-based butterfly pea nanoherbal can reduce blood glucose and MDA levels in hyperglycemic rats, suggesting its potential role in improving glycemic control and reducing oxidative stress under hyperglycemic conditions.*

Keywords: *Antioxidants; Clitoria ternatea; Hyperglycemia; Malondialdehyde; Nanoherbal*

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INTRODUCTION

Hyperglycemia is defined as a state in which blood glucose levels are elevated due to disruptions in insulin secretion, reduced sensitivity to insulin, or diminished glucose uptake by peripheral tissues (Galicia-Garcia et al., 2020). An elevated risk of diabetes mellitus and related metabolic disorders is strongly associated with this condition. According to American Diabetes Association (2019), One of the diagnostic criteria for hyperglycemia and diabetes mellitus is defined by blood glucose levels reaching or exceeding 200 mg/dL when measured two hours following an oral glucose tolerance test, while Perkumpulan Endokrinologi Indonesia (2019) establishes a similar diagnostic threshold as an indicator of impaired glucose tolerance. Chronic hyperglycemia triggers excessive production of reactive oxygen species (ROS), leading to oxidative stress and pancreatic β-cell damage through inflammatory mechanisms and lipid peroxidation (Volpe et al., 2018).

During conditions of oxidative stress, the formation of advanced glycation end-products (AGEs) is promoted by reactive oxygen species (ROS). These AGEs subsequently interact with their receptor (RAGE), leading to the activation of proinflammatory signaling pathways, including nuclear factor kappa B (NF-κB), which in turn intensifies cellular damage (Nsonwu-Anyanwu et al., 2019). Malondialdehyde (MDA), a final product of lipid peroxidation, is widely recognized as a key biomarker of oxidative injury, as its levels reflect the degree of cell membrane damage induced by ROS (Angelé-Martínez et al., 2022). Therefore, therapeutic approaches capable of reducing blood glucose levels while simultaneously suppressing oxidative stress are required to improve metabolic conditions under hyperglycemic states (Rehman & Akash, 2016).

The butterfly pea flower (*Clitoria ternatea* L.) has been widely recognized as a source of various bioactive constituents, such as flavonoids, anthocyanins, alkaloids, and saponins, which are associated with antioxidant, anti-inflammatory, and antihyperglycemic properties (Athallah et al., 2024). Lipid peroxidation can be inhibited and NF-κB activation suppressed by flavonoids, which also act as radical scavengers and contribute to the enhancement of endogenous antioxidant enzyme activity (Xu et al., 2022). Antidiabetic effects are exhibited by saponins through mechanisms involving increased insulin secretion, improved insulin sensitivity, and reduced intestinal glucose absorption (Lampa et al., 2018). The antidiabetic potential of butterfly pea flower extract has been supported by several in vivo investigations employing diabetic animal models. For instance, a significant reduction in blood glucose levels in alloxan-induced diabetic rats was reported following the administration of *C. ternatea* flower extract, highlighting its promise as a plant-derived therapeutic agent (Rajamanickam et al., 2015).

However, the effectiveness of conventional herbal extracts remains limited by low stability, solubility, and bioavailability of active compounds (Mishra et al., 2018). Advances in nanoherbal technology offer a promising strategy to enhance the efficacy of bioactive compounds by improving absorption, protecting them from enzymatic degradation, and enabling more controlled release (Yang et al., 2022). Previous studies have demonstrated that butterfly pea–based nanoparticles exhibit stronger biological activities than their conventional extracts. Several studies have explored nanoparticle

formulations of *C. ternatea* to improve its biological activity. For instance, Mobasher et al., (2023) developed chitosan nanoparticles containing *C. ternatea* extract and reported enhanced antidiabetic activity compared to the crude extract. Similarly, Sa et al., (2023) synthesized gold and cobalt nanoparticles using butterfly pea extract and observed increased antioxidant and antidiabetic properties. In addition, Alahmdi et al., (2022) reported The green synthesis of zinc oxide (ZnO) nanoparticles utilizing butterfly pea extract has been documented. Nevertheless, these investigations have largely emphasized nanoparticle fabrication or only conducted limited biological assessments, thereby underscoring the necessity for more comprehensive studies to evaluate the therapeutic efficacy of butterfly pea–based nanoformulations in diabetic conditions linked to oxidative stress.

Nevertheless, nanoherbal research on butterfly pea remains predominantly focused on metal-based and chitosan-based nanoparticles, which present limitations related to biocompatibility, stability, and potential long-term toxicity. To date, no studies have formulated *C. ternatea* nanoherbal using sodium alginate as an encapsulation matrix, despite alginate being recognized as a safe, biodegradable, and stable natural polymer suitable for ionic gelation techniques (Abourehab et al., 2022). Moreover, there has been no comprehensive evaluation of alginate-based butterfly pea flower nanoherbal on alloxan-induced hyperglycemic rats using both blood glucose and MDA levels as parameters. This highlights a critical research gap, considering the need for a more effective and safer herbal delivery system.

Based on this gap, the present study aimed to formulate butterfly pea flower nanoherbal using sodium alginate and calcium chloride as ionic gelation agents and to evaluate its effectiveness in reducing blood glucose and malondialdehyde (MDA) levels in alloxan-induced hyperglycemic rats. The findings of this study are expected to contribute to the development of a more effective, stable, and sustainable nano-based herbal therapy for managing oxidative stress and hyperglycemia.

METHOD

Experimental Design

A laboratory-based experimental approach was applied in this study using a Completely Randomized Design (CRD) with a post-test only control group scheme. The research was conducted over the period of June to September 2025. Animal care procedures and blood glucose assessments were carried out in the Biology Laboratory and the Animal Physiology Laboratory, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Negeri Semarang. The preparation of extracts and the formulation of butterfly pea flower nanoherbal were performed at the Chemical Research Laboratory Unit of FMIPA, Universitas Negeri Semarang. Measurement of malondialdehyde (MDA) levels was undertaken at the Integrated Research and Testing Laboratory, Universitas Gadjah Mada.

Plant Material and Extraction

Dried butterfly pea flowers (*Clitoria ternatea* L.) were obtained from a traditional herbal market in Bandungan, Ungaran, Central Java, Indonesia. The plant

material was identified based on its morphological characteristics according to standard botanical references for *Clitoria ternatea*.

Dried butterfly pea flowers (200 g) were first pulverized and passed through a 40-mesh sieve. Maceration was then carried out using 96% ethanol at a 1:3 ratio (600 mL) for 24 hours at ambient temperature, with agitation performed three times per day. The resulting solution was filtered through Whatman No. 1 filter paper, after which a second maceration step was conducted for an additional 24 hours using the same solvent. The collected filtrates were pooled and subsequently concentrated with a rotary evaporator. Final drying of the extract was achieved using a water bath maintained at 45°C. Preliminary phytochemical screening of the butterfly pea flower extract was conducted to detect the presence of major secondary metabolites, including flavonoids, phenolics, alkaloids, and saponins, using standard qualitative methods.

Nanoherbal Formulation

The ionic gelation method was employed for the preparation of the nanoherbal formulation. At the initial stage, 500 mg of extract was dissolved in 2.5 mL of 96% food-grade ethanol, then brought to a final volume of 50 mL with distilled water and homogenized. A volume of 1 mL of 0.1% sodium alginate solution was magnetically stirred until a homogeneous mixture was achieved, after which 1 mL of the extract solution was added and the mixture was agitated for 30 minutes at 1500 rpm. The cross-linking process was initiated by introducing 5 mL of 0.02% $CaCl_2$ solution, followed by further stirring for 60 minutes at the same speed. Subsequently, the suspension was sonicated for 60 minutes to achieve particle size reduction. The entire nanoparticle formulation procedure was conducted in triplicate to ensure consistency and reproducibility (Ariani & Purwanto, 2021). A volume of 1 mL of nanoparticles was analyzed using a Particle Size Analyzer (PSA; Horiba SZ-100) to determine particle size and confirm that it fell within the range of 50–500 nm (Samudra et al., 2021).

Experimental Animals

Male Wistar rats aged 8 weeks, with body weights ranging from 170 to 200 g, were utilized as experimental subjects. A 7-day acclimatization period was implemented prior to treatment under controlled conditions, including a temperature of 24–26°C, relative humidity of approximately 70%, and a 12-hour light–dark photoperiod, with free access to standard feed and water. The rats (n = 25) were randomly assigned into five groups (five rats per group), consisting of a normal control, a metformin-treated group (400 mg/kg BW) as a standard antidiabetic comparator (Radenković et al., 2016), and three groups receiving nanoherbal treatments, designated as NT1 (12.5 mg/kg BW), NT2 (25 mg/kg BW), and NT3 (50 mg/kg BW). Approval for the experimental procedures was obtained from the Health Research Ethics Committee, Faculty of Medicine, Universitas Diponegoro (Approval No. 132/EC/KEPK/FK-UNDIP/VI/2025).

Induction of Hyperglycemia

Alloxan induction and oral treatments were conducted after the 7-day acclimatization period. Prior to induction, the rats underwent fasting for 8–12 hours, after which alloxan monohydrate was administered intraperitoneally at a dose of 125 mg/kg BW. Hyperglycemic status was confirmed three days following induction by measuring blood glucose levels from the tail vein using a glucometer, with values ≥150 mg/dL indicating hyperglycemia. Treatments were administered for 28 days. The metformin control group received metformin, while the treatment groups received butterfly pea flower nanoherbal according to their respective doses. The normal control group received no treatment.

Biochemical Analysis

Following an 8–12 hour fasting period, blood glucose concentrations were assessed from tail vein samples using a glucometer on days 1, 14, and 28. For malondialdehyde (MDA) determination, plasma samples were obtained on day 28 through retro-orbital sinus collection. The quantification of MDA was carried out using the Thiobarbituric Acid Reactive Substances (TBARS) colorimetric assay according to Fauziah et al., (2018). In brief, plasma was combined with a thiobarbituric acid (TBA) reagent containing trichloroacetic acid (TCA) and hydrochloric acid, followed by incubation at 95°C for 30 minutes to facilitate the formation of the MDA–TBA complex. After cooling, the mixture was centrifuged, and the absorbance of the supernatant was measured at 532 nm using a spectrophotometer. MDA concentrations were subsequently determined using a standard calibration curve and expressed in nmol/mL.

Statistical Analysis

A Repeated Measures Two-Way ANOVA followed by Bonferroni post hoc testing was applied to analyze blood glucose data, while MDA values were examined using a One-Way ANOVA accompanied by Duncan's post hoc test. The statistical procedures were performed using IBM SPSS Statistics version 27 (IBM Corp., Armonk, NY, USA), with the level of significance set at $\alpha = 0.05$.

RESULT AND DISCUSSION

The results showed that blood glucose levels of rats on the first day after alloxan induction were within the hyperglycemic range, exceeding 150 mg/dL. This hyperglycemic status served as the basis for the homogeneity of baseline conditions across all treatment groups, except for the normal control group, and was consistent with the diagnostic criteria for hyperglycemia established by (American Diabetes Association (2019); Perkumpulan Endokrinologi Indonesia (2019).

The effectiveness of butterfly pea flower (*Clitoria ternatea* L.) nanoherbal in ameliorating hyperglycemic conditions in alloxan-induced male Wistar rats was assessed through blood glucose measurements. The induction of hyperglycemia by

alloxan is recognized to selectively impair pancreatic β-cells via the generation of reactive oxygen species (ROS), resulting in decreased insulin secretion and elevated blood glucose levels (González et al., 2023; Chen et al., 2025). Accordingly, blood glucose levels were utilized as the principal indicator of disturbances in glucose metabolism.

Repeated Measures ANOVA showed that blood glucose levels differed significantly across observation times ($p < 0.001$), indicating significant glycemic changes throughout the treatment period. Bonferroni post hoc analysis confirmed significant differences between day 1, day 14, and day 28 ($p < 0.01$). Meanwhile, One-Way ANOVA analysis of malondialdehyde (MDA) levels revealed significant differences among treatment groups ($p < 0.05$). Duncan's post hoc test confirmed that the medium- and high-dose groups exhibited significantly lower MDA levels compared with the positive control and low-dose groups, indicating a dose-dependent antioxidant response.

Blood glucose measurements on day 1 showed a clear distinction between the normal group and all alloxan-induced groups (Table 1). The normal group exhibited a blood glucose level of 93.0 ± 15.80 mg/dL, which was within the physiological range. In contrast, the positive control group demonstrated a markedly elevated blood glucose level of 374.6 ± 156.22 mg/dL. More severe hyperglycemic conditions were also observed in the treatment groups, with values of 432.8 ± 120.91 mg/dL in NT1, 522.0 ± 141.36 mg/dL in NT2, and 471.4 ± 142.00 mg/dL in NT3. These elevated glucose levels confirmed the successful induction of hyperglycemia by alloxan through pancreatic β-cell damage and disruption of glucose homeostasis (Utami et al., 2024).

Table 1. Mean Blood Glucose Levels of Rats (*Rattus norvegicus*)

Group	Blood Glucose Levels (mg/dL)		
	Day 1	Day 14	Day 28
Normal control	93.0 ± 15.80	92.6 ± 7.27	89.8 ± 7.33
Metformin control	374.6 ± 156.22	268.0 ± 156.22	178.6 ± 92.06
NT1	432.8 ± 120.91	375.6 ± 168.57	236.6 ± 96.78
NT2	522.0 ± 141.36	168.8 ± 48.84	155.0 ± 21.02
NT3	471.4 ± 142.00	313.0 ± 96.68	146.4 ± 68.58

Note: Data are presented as mean ± standard deviation. Statistical analysis was performed using repeated-measures ANOVA followed by Bonferroni post hoc test ($p < 0.05$). NT1 = nanoherbal butterfly pea 12.5 mg/kg BW; NT2 = 25 mg/kg BW; NT3 = 50 mg/kg BW.

Following treatment administration, reductions in blood glucose levels varied among groups. On day 28, the normal group maintained stable blood glucose levels at 89.8 ± 7.33 mg/dL. A reduction in blood glucose levels to 178.6 ± 92.06 mg/dL was observed in the positive control group receiving metformin, although these values still exceeded the normal range. The antihyperglycemic action of metformin is primarily mediated through the activation of AMP-activated protein kinase (AMPK), which leads to the inhibition of hepatic gluconeogenesis and the enhancement of glucose uptake in peripheral tissues without causing hypoglycemia (Cicih et al., 2022).

A greater decline in blood glucose levels was detected in the groups treated with butterfly pea flower nanoherbal, with the effect showing a dose-dependent pattern. Blood glucose levels in the NT1 group (12.5 mg/kg BW) were recorded at 236.6 ± 96.78 mg/dL, remaining within the hyperglycemic range. Conversely, lower values were obtained in the NT2 (25 mg/kg BW) and NT3 (50 mg/kg BW) groups, measuring 155.0 ± 21.02 mg/dL and 146.4 ± 68.58 mg/dL, respectively, both of which differed significantly from the NT1 group ($p < 0.05$). This pattern indicates that increasing doses of butterfly pea flower nanoherbal enhanced antihyperglycemic effects up to an optimal dose, consistent with previous reports on the antidiabetic activity of *Clitoria ternatea* (Sa et al., 2023; Pangondian et al., 2023).

The more optimal reduction in blood glucose levels observed in the NT2 and NT3 groups indicates that increasing doses of butterfly pea flower nanoherbal were associated with greater antihyperglycemic effects (Figure 1). However, the effectiveness of glucose reduction did not increase linearly with dose, as the NT3 group did not demonstrate substantially greater improvement compared to the NT2 group. This finding indicates the presence of an optimal effective dose, in which the medium dose was sufficient to elicit a maximal biological response in lowering blood glucose levels.

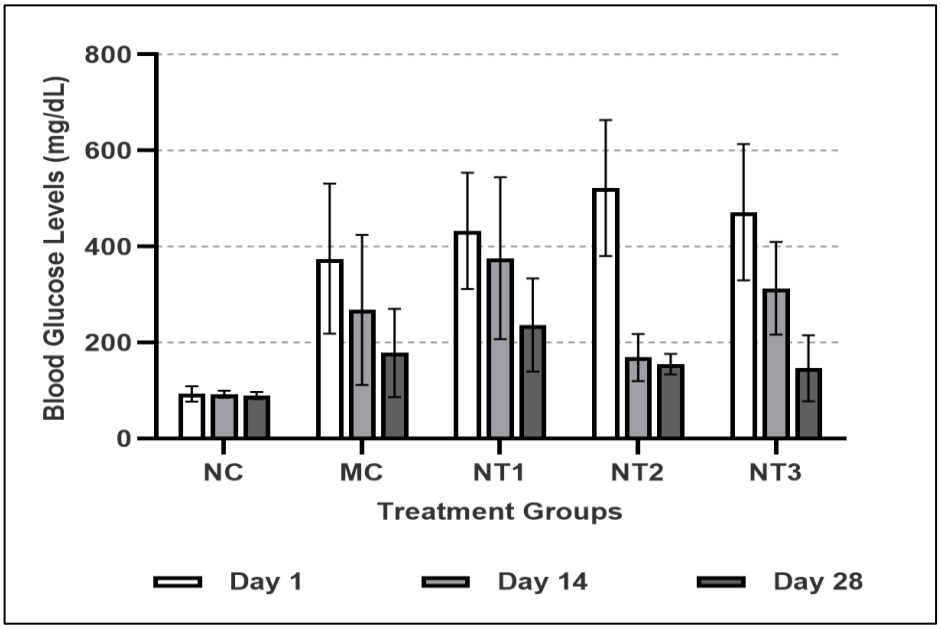

Figure 1. Changes in blood glucose levels of rats (*Rattus norvegicus*) on days 1, 14, and 28 during butterfly pea flower nanoherbal treatment. Values are presented as mean ± standard deviation (SD). Statistical significance was analyzed using repeated-measures ANOVA followed by Bonferroni post hoc test ($p < 0.05$).

The antihyperglycemic effect of butterfly pea flower nanoherbal can be explained by its major bioactive compounds, namely saponins and flavonoids. Saponins are known to exhibit mechanisms partially comparable to metformin by enhancing insulin sensitivity through activation of the AMPK pathway, suppressing hepatic gluconeogenesis, and promoting GLUT-4 translocation in muscle and adipose

tissues (El Barky et al., 2017). In addition, the amphiphilic properties of saponins allow interaction with the intestinal membrane, potentially reducing SGLT-1 transporter activity and thereby inhibiting glucose absorption (Athallah et al., 2024).

The restoration of metabolic energy balance is supported by improved glucose regulation, as indicated by the progressive decline in blood glucose levels approaching near-normal values in the medium- and high-dose treatment groups. These results are in agreement with earlier studies demonstrating that glycemic control in diabetic animal models can be improved through the administration of *Clitoria ternatea* L. extracts or their nanoformulations, primarily via enhanced glucose utilization and protective effects on pancreatic function (Simangunsong et al., 2023; Mobasher et al., (2023).

In addition to their role in glucose regulation, saponins and flavonoids also exhibit antioxidant activity that contributes to the control of oxidative stress. Chronic hyperglycemia is known to form a pathological cycle with oxidative stress, in which elevated blood glucose levels stimulate the generation of reactive oxygen species (ROS), while excessive ROS accumulation exacerbates insulin resistance and pancreatic β-cell damage (Widowati et al., 2023). This relationship is reflected in malondialdehyde (MDA) levels as an end product of lipid peroxidation.

In this study, groups with higher blood glucose levels also exhibited elevated MDA concentrations, as shown in (Table 2). The NT1 group, which had a day-28 blood glucose level of 236.6 ± 96.78 mg/dL, also showed the highest MDA level at 6.24 ± 0.44 nmol/mL. In contrast, the NT2 and NT3 groups, which demonstrated better reductions in blood glucose levels, also exhibited significantly lower MDA levels. The NT2 group showed an MDA level of 3.86 ± 0.29 nmol/mL, while the NT3 group exhibited an even lower MDA level of 2.84 ± 0.19 nmol/mL, approaching that of the normal group (0.98 ± 0.20 nmol/mL). This pattern is consistent with previous reports indicating that reductions in blood glucose levels are to be associated with decreased lipid peroxidation in experimental diabetic models (Angie et al., 2024; Widowati et al., 2024).

Table 2. Mean Malondialdehyde (MDA) Levels of Rats (*Rattus norvegicus*)

Group	MDA Levels (nmol/mL)
Normal control	0.98 ± 0.20^{a}
Metformin control	3.76 ± 0.12^{b}
NT1	6.24 ± 0.44^{c}
NT2	3.86 ± 0.29^{b}
NT3	2.84 ± 0.19^{b}

Note: Data are presented as mean ± standard deviation. Different superscript letters indicate significant differences among groups ($p < 0.05$). NT1 = nanoherbal butterfly pea 12.5 mg/kg BW; NT2 = 25 mg/kg BW; NT3 = 50 mg/kg BW.

As illustrated in Figure 2, MDA levels indicate that butterfly pea flower nanoherbal not only improves impaired glucose metabolism but also suppresses oxidative stress through the antioxidant activity of flavonoids. Flavonoids are known to neutralize reactive oxygen species (ROS) and activate the Nrf2 pathway as a primary mechanism of cellular antioxidant defense, thereby contributing to the suppression of

lipid peroxidation and oxidative stress (Suraweera et al., 2020). The synergistic interaction between the antihyperglycemic effects of saponins and the antioxidant activity of flavonoids enables the disruption of the pathological cycle between hyperglycemia and oxidative stress, as previously reported for nanoformulations of *Clitoria ternatea* L. (Mobasher et al., 2023).

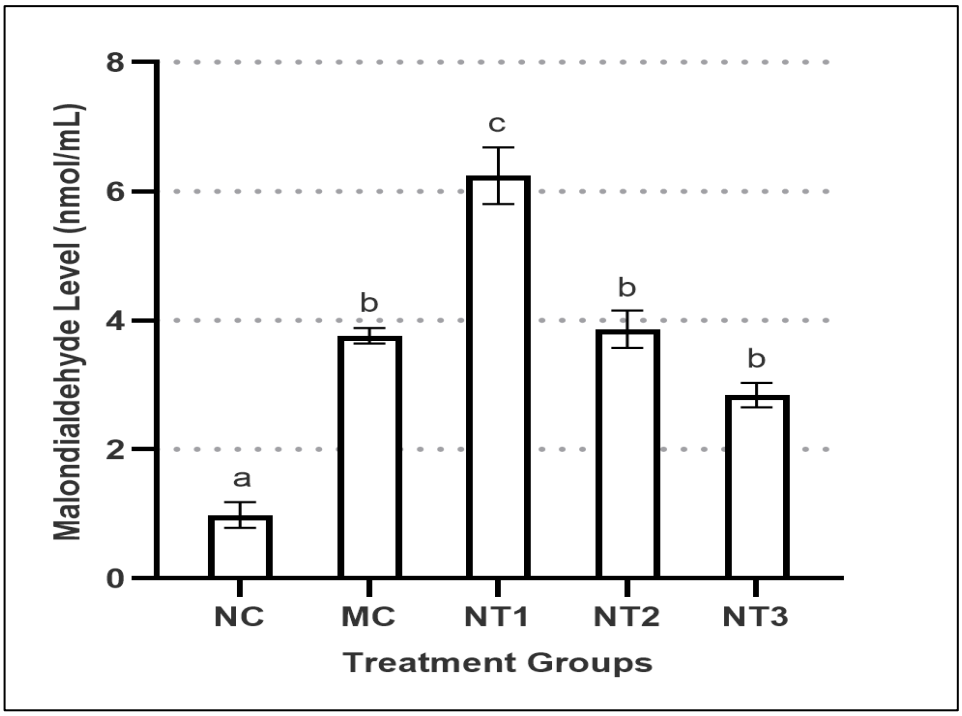

Figure 2. Malondialdehyde (MDA) levels of rats (*Rattus norvegicus*) after 28 days of butterfly pea flower nanoherbal treatment. Data are expressed as mean values accompanied by standard deviation (SD), and statistical differences among groups are indicated by distinct superscript letters ($P < 0.05$).

A strong association between metabolic dysregulation and oxidative stress was further supported by the observed relationship between decreased blood glucose levels and reduced MDA concentrations in this study. The scavenging of reactive oxygen species (ROS) and the suppression of AGE–RAGE and NF-κB signaling pathways are mediated by flavonoids, leading to attenuation of inflammatory responses and lipid peroxidation. Meanwhile, enhanced insulin sensitivity and protection of pancreatic β-cells against oxidative injury are attributed to saponins. Through the combined action of these bioactive compounds, the pathological cycle linking hyperglycemia and oxidative stress is disrupted, as evidenced by concurrent declines in blood glucose levels and MDA concentrations in the treated groups.

Increasing doses of butterfly pea flower nanoherbal exhibited differential effectiveness in improving hyperglycemia and oxidative stress. For the blood glucose parameter, dose escalation tended to be associated with reduced glucose levels, particularly at doses of 25–50 mg/kg BW. This relationship indicates the antihyperglycemic potential of butterfly pea flower nanoherbal, although the glucose-lowering response did not increase proportionally with dose. This observation is

supported by regression analysis results showing that the contribution of dose to variations in blood glucose levels remained limited (R^2 = 0.196), with a negative regression coefficient (B = −2.111), indicating that glucose reduction effects were moderate and influenced by other physiological factors, such as the extent of pancreatic β-cell damage caused by alloxan induction.

In contrast, the effectiveness of butterfly pea flower nanoherbal dosage on oxidative stress reduction exhibited a more consistent and robust pattern. Increasing doses were clearly associated with decreased MDA levels, particularly in the medium- and high-dose groups, reflecting a dose-dependent antioxidant response. The strength of this relationship was supported by regression analysis results indicating that most of the variation in MDA levels could be explained by the treatment (R^2 = 0.803), with a strong negative association, confirming that increasing nanoherbal doses effectively suppressed lipid peroxidation.

Based on these statistical analyses, the present study indicates that butterfly pea flower nanoherbal functions not only as an antihyperglycemic agent but also exhibits effective antioxidant activity in reducing oxidative stress, which is consistent with the findings reported by Zahra et al., (2024). The most pronounced effect was observed at the 50 mg/kg BW dose, at which reductions in blood glucose levels and MDA concentrations toward near-normal ranges were achieved, thereby supporting the potential application of butterfly pea flower nanoherbal as a complementary therapy that concurrently targets metabolic dysfunction and oxidative stress.

In summary, effective reductions in blood glucose levels and oxidative stress in alloxan-induced hyperglycemic rats were demonstrated by the butterfly pea flower nanoherbal (*Clitoria ternatea* L.). A dose-dependent response was observed up to a certain limit, with the 50 mg/kg BW dose yielding the most optimal outcome. The stability and bioavailability of saponins and flavonoids are presumed to be enhanced by the nanoherbal formulation, thereby improving their antihyperglycemic and antioxidant effects (Yu et al., 2024). Collectively, these results support the potential use of butterfly pea flower nanoherbal as a complementary phytotherapeutic agent capable of simultaneously ameliorating metabolic dysfunction and oxidative stress in diabetes mellitus.

CONCLUSION

A significant reduction in blood glucose levels in alloxan-induced hyperglycemic rats was demonstrated by the administration of butterfly pea flower nanoherbal (*Clitoria ternatea* L.). After 28 days of treatment, the NT3 group (50 mg/kg BW) showed the lowest blood glucose level (146.4 ± 68.58 mg/dL), followed by NT2 (155.0 ± 21.02 mg/dL), indicating a strong antihyperglycemic effect compared with the lower-dose treatment group NT1. In addition, nanoherbal administration also reduced oxidative stress, as indicated by lower malondialdehyde (MDA) levels in the NT3 group (2.84 ± 0.19 nmol/mL) compared to NT1 (6.24 ± 0.44 nmol/mL). These findings suggest that the antihyperglycemic effect of butterfly pea flower nanoherbal may be associated with its antioxidant activity, thereby facilitating a decrease in oxidative stress under hyperglycemic conditions. Nevertheless, the study was

constrained by a relatively limited sample size and the reliance on a single oxidative stress biomarker (MDA); therefore, further investigations incorporating additional biochemical and molecular indicators are needed to provide a more comprehensive understanding of the underlying mechanisms.

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REFERENCES

Abourehab, M. A. S., Rajendran, R. R., Singh, A., Pramanik, S., Shrivastav, P., Ansari, M. J., Manne, R., Amaral, L. S., & Deepak, A. (2022). Alginate as a Promising Biopolymer in Drug Delivery and Wound Healing: A Review of the State-of-the-Art. *International Journal of Molecular Sciences, 23*(16), 9035. https://doi.org/10.3390/ijms23169035

Alahmdi, M. I., Khasim, S., Vanaraj, S., Panneerselvam, C., Mahmoud, M. A. A., Mukhtar, S., Alsharif, M. A., Zidan, N. S., Abo-Dya, N. E., & Aldosari, O. F. (2022). Green Nanoarchitectonics of ZnO Nanoparticles from Clitoria ternatea Flower Extract for In Vitro Anticancer and Antibacterial Activity: Inhibits MCF-7 Cell Proliferation via Intrinsic Apoptotic Pathway. *Journal of Inorganic and Organometallic Polymers and Materials*, *32*(6), 2146–2159. https://doi.org/10.1007/s10904-022-02263-7

American Diabetes Association. (2019). Overview and Update: Standards of Medical Care in Diabetes 2019. *Diabetes Care*, *42*(Suppl. 1), S13–S28. https://doi.org/https://doi.org/10.2337/dc19-S002

Angelé-Martínez, C., Goncalves, L. C. P., Premi, S., Augusto, F. A., Palmatier, M. A., Amar, S. K., & Brash, D. E. (2022). Triplet-Energy Quenching Functions of Antioxidant Molecules. *Antioxidants*, *11*(2), 357. https://doi.org/10.3390/antiox11020357

Angie, E., Girsang, E., & Ikhtiari, R. (2024). Linking MDA Levels and Blood Glucose in Streptozotocin-Induced Rat Diabetes: Implications for Diabetic Complications and Therapeutic Strategies. *Jurnal Penelitian Pendidikan IPA*, *10*(6), 2898–2905. https://doi.org/10.29303/jppipa.v10i6.7220

Ariani, L. W., & Purwanto, U. R. E. (2021). Formulation of Nanoparticles from Hibiscus Leaf Extract (Hibiscus rosa-sinensis L.). *Intellectual Property Rights No. EC00202119945, In Repository STIFAR*.

https://repository.stifar.ac.id/Repository/article/view/303 **[*In Indonesian language*]**

Athallah, D. R., Rudiyanto, W., Musyabiq Wijaya, S., & Isti Angraini, D. (2024). Review Article: The Pharmacological Potential of Butterfly Pea (Clitoria ternatea). *Medula*, *14*(1), 1613–1619. **[*In Indonesian language*]**

Chen, X., Xie, N., Feng, L., Huang, Y., Wu, Y., Zhu, H., Tang, J., & Zhang, Y. (2025). Oxidative stress in diabetes mellitus and its complications: From pathophysiology to therapeutic strategies. *Chinese Medical Journal,* 138(1), 15–27. https://doi.org/10.1097/CM9.0000000000003230

Cicih, A., Aligita, W., & Susilawati, E. (2022). A Review: The pharmacokinetics and pharmacodynamics of metformin-herb interactions. *Jurnal Ilmiah Farmasi (Scientific Journal of Pharmacy)*, *18*(1), 13–25. http://journal.uii.ac.id/index.php/JIF **[*In Indonesian language*]**

El Barky, A., Hussein, S. A., Alm-Eldeen, A.-E., Hafez, A., & Mohamed, T. (2017). Review Diabetes Management Saponins and their potential role in diabetes mellitus. *Diabetes Manag*, *7*(1), 148–158.

Fauziah, P. N., Maskoen, A. M., Yuliati, T., & Widiarsih, E. (2018). Optimized steps in determination of malondialdehyde (MDA) standards on diagnostic of lipid peroxidation. *Padjadjaran Journal of Dentistry*, *30*(2), 136–139. https://doi.org/10.24198/pjd.vol30no2.18329

Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K. B., Ostolaza, H., & Martín, C. (2020). Pathophysiology of type 2 diabetes mellitus. *International Journal of Molecular Sciences, 21*(17), 1–34. https://doi.org/10.3390/ijms21176275

González, P., Lozano, P., Ros, G., & Solano, F. (2023). Hyperglycemia and Oxidative Stress: An Integral, Updated and Critical Overview of Their Metabolic Interconnections. *International Journal of Molecular Sciences, 24*(11), 9532. https://doi.org/10.3390/ijms24119352

Lampa, S., Alvarsson, J., McShane, S. A., Berg, A., Ahlberg, E., & Spjuth, O. (2018). Predicting off-target binding profiles with confidence using conformal prediction. *Frontiers in Pharmacology*, *9*, 1256. https://doi.org/10.3389/fphar.2018.01256

Mishra, V., Bansal, K. K., Verma, A., Yadav, N., Thakur, S., Sudhakar, K., & Rosenholm, J. M. (2018). Solid lipid nanoparticles: Emerging colloidal nano drug delivery systems. *Pharmaceutics, 10*(4), 191. https://doi.org/10.3390/pharmaceutics10040191

Mobasher, M. A., Baioumy, S. A., Alazzouni, A. S., Khayyat, A. I. A., Awad, N. S., Abdel-Hakeem, M. A., & Al-Sowayan, N. S. (2023). Clitoria ternatea extract-loaded chitosan nanoparticles ameliorate diabetes and oxidative stress in diabetic rats. *Indian Journal of Biochemistry and Biophysics*, *60*(7), 501–515. https://doi.org/10.56042/ijbb.v60i7.4140

Nsonwu-Anyanwu, A. C., Nsonwu, M. C., & Usoro, C. A. O. (2019). Hypoglycemic Agents and Changes in Oxidative Stress Indices, Electrolytes, and

Cardiovascular Risk Factors in Type 2 Diabetes. *Dubai Diabetes and Endocrinology Journal*, *25*(3–4), 118–126. https://doi.org/10.1159/000500912

Pangondian, A., Rambe, R., Umaya, C., & Jambak, K. (2023). The Antidiabetic Potential of Blue Pea Flower Extract (Clitorea ternatea L.) in Male White Mice (Mus musculus). *Forte Journal*, *3*(2), 150–157. https://www.ojs.unhaj.ac.id/index.php/fj **[*In Indonesian language*]**

Perkumpulan Endokrinologi Indonesia. (2019). *Guidelines for the management and prevention of type 2 diabetes in adults in Indonesia*. PB Perkumpulan Endokrinologi Indonesia https://pbperkeni.or.id/. https://drive.google.com/drive/folders/1o0p2Yw72xR9DmojNjQ2v4sDK4oj0Vyi_?usp=sharing **[*In Indonesian language*]**

Radenković, M., Stojanović, M., & Prostran, M. (2016). Experimental diabetes induced by alloxan and streptozotocin: The current state of the art. *Journal of Pharmacological and Toxicological Methods*, *78*, 13–31. https://doi.org/10.1016/j.vascn.2015.11.004

Rajamanickam, M., Kalaivanan, P., & Sivagnanam, I. (2015). Evaluation of anti-oxidant and anti-diabetic activity of flower extract of Clitoria ternatea L. *Journal of Applied Pharmaceutical Science*, *5*(8), 131–138. https://doi.org/10.7324/JAPS.2015.50820

Rehman, K., & Akash, M. S. H. (2016). Mechanisms of inflammatory responses and development of insulin resistance: How are they interlinked?. *Journal of Biomedical Science*, *23*(1), 16. https://doi.org/10.1186/s12929-016-0303-y

Sa, N., Tejaswani, P., Pradhan, S. P., Alkhayer, K. A., Behera, A., & Sahu, P. K. (2023). Antidiabetic and antioxidant effect of magnetic and noble metal nanoparticles of Clitoria ternatea. *Journal of Drug Delivery Science and Technology*, *84*, 104521. https://doi.org/10.1016/j.jddst.2023.104521

Samudra, A. G., Ramadhani, N., Sani, F. K., Lestari, G., & Nugroho, B. H. (2021). Formulation of Chitosan Nanoparticles from Methanol Extract of Brown Seaweed (Sargassum hystrix) Using the Ionic Gelation Method. *Jurnal Ilmiah Manuntung*, *7*(1), 92–99. https://doi.org/10.51352/jim.v7i1.428 **[*In Indonesian language*]**

Simangunsong, E. M. V., Febriani, Y., Saputri, M., Arisa, D., & Zarqa Afifah, G. (2023). Effectiveness of butterfly pea ethanol extract on decreasing blood glucose levels of male mice. *Jambura journal of health science and research (JJHSR)*, *5*(2), 707–721. https://ejurnal.ung.ac.id/index.php/jjhsr/index

Suraweera, T. L., Vasantha Rupasinghe, H. P., Dellaire, G., & Xu, Z. (2020). Regulation of Nrf2/are pathway by dietary flavonoids: A friend or foe for cancer management?. *Antioxidants*, *9*(10), 1–44. https://doi.org/10.3390/antiox9100973

Utami, W., Laksono, Y. D., Setiawibowo, S. N. F., Sunarsih, E. S., Wulandari, F., & Rohana, E. (2024). Antidiabetic and antioxidant activity of Clitoria ternatea flower extracts and fractions on blood glucose and MDA in rats induced by

alloxan. *Pharmacy Education*, *24*(6), 21–27. https://doi.org/10.46542/pe.2024.246.2127

Volpe, C. M. O., Villar-Delfino, P. H., Dos Anjos, P. M. F., & Nogueira-Machado, J. A. (2018). Cellular death, reactive oxygen species (ROS) and diabetic complications review-Article. *Cell Death and Disease*, *9*(2), 119. https://doi.org/10.1038/s41419-017-0135-z

Widowati, W., Darsono, L., Lucianus, J., Setiabudi, E., Susang Obeng, S., Stefani, S., Wahyudianingsih, R., Reynaldo Tandibua, K., Gunawan, R., Riski Wijayanti, C., Novianto, A., Sari Widya Kusuma, H., & Rizal, R. (2023). Butterfly pea flower (Clitoria ternatea L.) extract displayed antidiabetic effect through antioxidant, anti-inflammatory, lower hepatic GSK-3β, and pancreatic glycogen on Diabetes Mellitus and dyslipidemia rat. *Journal of King Saud University - Science*, *35*(4), 102579. https://doi.org/10.1016/j.jksus.2023.102579

Widowati, W., Darsono, L., Utomo, H. S., Sabrina, A. H. N., Natariza, M. R., Valentinus Tarigan, A. C., Waluyo, N. W., Gleyriena, A. M., Siahaan, B. H., & Oktaviani, R. (2024). Antidiabetic and hepatoprotection effect of butterfly pea flower (Clitoria ternatea L.) through antioxidant, anti-inflammatory, lower LDH, ACP, AST, and ALT on diabetes mellitus and dyslipidemia rat. *Heliyon*, *10*(8), e29812. https://doi.org/10.1016/j.heliyon.2024.e29812

Xu, W., Lu, H., Yuan, Y., Deng, Z., Zheng, L., & Li, H. (2022). The Antioxidant and Anti-Inflammatory Effects of Flavonoids from Propolis via Nrf2 and NF-κB Pathways. *Foods*, *11*(16), 2439. https://doi.org/10.3390/foods11162439

Yang, T. L., Hsieh, C. M., Meng, L. J., Tsai, T., & Chen, C. T. (2022). Oleic Acid-Based Self Micro-Emulsifying Delivery System for Enhancing Antifungal Activities of Clotrimazole. *Pharmaceutics*, *14*(3), 478. https://doi.org/10.3390/pharmaceutics14030478

Yu, F., Yu, Q., Yin, N., Sun, G., Peng, Y., Zeng, Y., Sun, Y., Wang, X., & Zhang, H. (2024). In Vitro and In Vivo Evaluating Bioaccessibility, Bioavailability, and Antioxidant Activities of Butterfly Pea Flower Containing Bioactive Constitutes. *Foods*, *13*(10), 1485. https://doi.org/10.3390/foods13101485

Zahra, M., Abrahamse, H., & George, B. P. (2024). Flavonoids: Antioxidant Powerhouses and Their Role in Nanomedicine. *Antioxidants, 13*(8), 922. https://doi.org/10.3390/antiox13080922

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