Indexed by : DOAJ Google Crossref Dimension sinta GARUDA

J.Biomed.Transl.Res
ISSN: 2503-2178

JOURNAL OF BIOMEDICINE AND TRANSLATIONAL RESEARCH

Available online at JBTR website: https://jbtr.fk.undip.ac.id

Original Research Article

The Impact of Butterfly Pea Flower (*Clitoria ternatea L.*) Extract on Atherosclerosis Biomarker Profiles in Obese White Rats (*Rattus norvegicus L.*)

Karnirius Harefa[1*], Ahmad Hafizullah Ritonga[1], Barita Aritonang[1], Rahmad Gurusinga[1], Sri Wulan[1], Irmayani[1]

[1]*Institut Kesehatan Medistra Lubuk Pakam, Indonesia*

Article Info
History
Received: 16 Sept 2023
Accepted: 24 Apr 2024
Available: 30 Apr 2024

Abstract

Clitoria ternatea L., commonly known as butterfly pea flower, has gained attention as a potential agent in the prevention and treatment of atherosclerosis due to its rich content of bioactive compounds, such as anthocyanin, a type of flavonoid renowned for its potent antioxidant and anti-inflammatory properties, along with its ability to enhance the immune system.

Objective: This study aimed to elucidate the positive impact of butterfly pea flower extract on atherosclerosis biomarker profiles.

Methods: The approach framework used in this research was a true experimental laboratory with a Control Group Post-Test design. Obese male white rats were selected as the research subjects. Thirty-six obese white rats were randomly divided into six groups using the Completely Randomized Design (CRD) method. The data collected were VCAM and IL-6 levels from the blood serum of obese white rats tested using an ELISA photometer. The tunica intima thickness was measured using a microscope. Data were analyzed employing SPSS 18 software, utilizing one-way ANOVA statistical tests and post hoc Tukey tests.

Results: The research showed that with the increase in Butterfly Flower Extract (BPFE) dose, there was a consistently lower level of inflammatory biomarkers, such as VCAM-1 and IL-6, compared to positive controls and other variations, as well as tunica intima thickness was thinning than others. A dose of 600 mg/kg BW (P3 group) had VCAM-1 levels up to 30.40 ± 6.71 ng/mL, IL-6 levels up to 17.70 ± 8.29 ng/mL, and tunica intima thickness up to 3.18 ± 1.24 µm.

Conclusion: BPFE effectively lowers inflammatory biomarkers and thins the tunica intima thickness in obese white rats at 600 mg/kg BW dose. It may offer promising therapeutic potential in addressing atherosclerosis.

Keywords: *VCAM-1; IL-6; tunica intima thickness; butterfly pea flower extract.*
Permalink/ DOI: https://doi.org/10.14710/jbtr.v10i1.20281

INTRODUCTION

In the modern era, obesity has emerged as a complex multifactorial disease of increasing concern. It is characterized by excessive adipose tissue accumulation in the body, resulting from an imbalance between caloric intake and expenditure. This condition poses immediate physical risks and significantly elevates the risk of various severe diseases, with atherosclerosis often referred to as the 'silent killer' due to its asymptomatic nature until advanced stages.[1,2] Atherosclerosis, a highly hazardous arterial disease, is characterized by the progressive accumulation of atheromatous plaques on the arterial walls. This pathological process commences with the endothelial cell damage within the arteries, coupled with increased free radicals that oxidize low-density lipoproteins (LDL) into oxidized LDL (Ox-LDL). This initial step is a pivotal foundation for the subsequent stages of atherosclerosis.[3–5]

* Corresponding author:
E-mail: *karniharefa74@gmail.com*
(Karnirius Harefa)

Table 1. Sample Treatment Groups

Group	Sample Code	Treatment for 45 Days
Negative Control	NC	Given standard feed
Positive Control	PC	Given HFAD feed
Na-CMC	P0	Given HFAD feed with Na-CMC 1%
BPFE-200	P1	Given HFAD feed with Na-CMC 1% and BPFE at 200 mg/kg BW doses
BPFE-400	P2	Given HFAD feed with Na-CMC 1% and BPFE at 400 mg/kg BW doses
BPFE-600	P3	Given HFAD feed with Na-CMC 1% and BPFE at 600 mg/kg BW doses

Notes: BPFE = Butterfly Pea Flower Extract, HFAD = High-Fat Atherogenic Diet, Na-CMC = sodium carboxymethyl cellulose

Ox-LDL migrates to the sub-endothelial layer in conjunction with monocytes, transforming monocytes into macrophages, which subsequently phagocytize Ox-LDL, forming foam cells.[3,4] The accumulation of foam cells initiates the formation of fatty streaks within the arteries, consequently narrowing the arterial lumen and amplifying inflammation via the upregulation of pro-inflammatory cytokines, such as Vascular Cell Adhesion Molecule-1 (VCAM-1), Interleukin-6 (IL-6), and Tunica Intima (TI) thickening. This intricate cascade of events underscores the dangerous nature of atherosclerosis.[6]

Presently, the treatment of atherosclerosis predominantly relies on pharmacological interventions involving drugs such as simvastatin. Although practical, prolonged usage of simvastatin is associated with severe adverse effects, including myopathy and kidney failure, underscoring the imperative need for safer therapeutic alternatives. One promising alternative is traditional herbal medicine, specifically the extract derived from the butterfly pea flower (*Clitoria ternatea L.*). Butterfly pea flower extract is rich in bioactive compounds, primarily anthocyanins belonging to the flavonoid family, renowned for their potent antioxidant and anti-inflammatory properties. These compounds can fortify the immune system and mitigate inflammation within arterial walls.[7,8] Numerous prior investigations have lent credence to the therapeutic potential of butterfly pea flower extract in managing diverse ailments. For instance, Maneesai et al.[9] documented the efficacy of butterfly pea flower extract in ameliorating cardiovascular dysfunction and decreasing oxidative stress in nitric oxide-deficient hypertensive rats. Maulidy et al.[10] also observed that butterfly pea flower extract could reduce total cholesterol levels in high-fat diet-induced obese rats (*Rattus norvegicus L.*). Furthermore, Widowati et al.[11] research unveiled the promising utility of butterfly pea flower extract in treating diabetes mellitus and dyslipidemia.

The extraction process used the maceration method with ethanol as the solvent to obtain bioactive compounds from butterfly pea flowers. While maceration is effective, it has the drawback of being time-consuming and yielding low extraction efficiency. Therefore, a more efficient extraction approach, such as the sonication method, is needed. Sonication utilizes ultrasonic vibrations to facilitate the disruption of cell walls in the extracted sample, allowing the chemical components within to be removed more easily from the cells. Moreover, the sonication extraction process requires less time than conventional methods such as maceration and Soxhlet extraction.[12]

This study aimed to investigate the effect of butterfly pea flower extract (BPFE) administration on atherosclerosis biomarker analysis in obese rats, employing a more efficient extraction method to assist in identifying the therapeutic potential of butterfly pea flower extract in addressing atherosclerosis conditions in obese rats.

MATERIALS AND METHODS

Research Design

This study adopted a pure experimental design with a post-test-only control group approach to assess the impact of ethanol extract from butterfly pea flowers on obese male white rats. The research was conducted from March 2022 to February 2023 at the Organic Chemistry Laboratory and Pharmacology Laboratory, Faculty of Pharmacy, Institut Kesehatan Medistra Lubuk Pakam, Deli Serdang, Indonesia. This research has undergone ethical considerations and received approval from the Research Ethics Committee of Institut Kesehatan Medistra Lubuk Pakam (Ethical Clearance Number 027.D/KEP-MLP/III/2022).

Population and Samples

The research population was composed of obese male white rats (*Rattus norvegicus L.*). The sample consisted of 36 male white rats. Each sample had to meet precise inclusion criteria, which encompassed an initial body weight falling within the range of 180-200 grams, an age of 3-4 months at the study's commencement, a state of good health, and a Lee index > 300 as an indicator of obesity. Samples that did not survive during the study were excluded based on the predefined exclusion criteria. The research samples were allocated into six groups, each randomly comprising six white rats. Each group underwent specific treatment for 45 days. Further details regarding the treatments administered to each sample group can be found in Table 1.

Preparation of High-Fat Atherogenic Diet (HFAD)

The high-fat diet used to induce obesity in male white rats consisted of goat fat 10%, cholesterol 1%, quail egg yolk 5%, cooking oil 1%, and standard feed. The HFAD

was prepared by crushing the common feed using a grinder, sequentially adding goat fat 10%, cholesterol 1%, quail egg yolk 5%, and cooking oil 1% to the feed. The mixture was stirred until well-distributed, reshaped using a noodle-making machine, and baked in an oven until fully cooked.

Preparation of 1% Na-CMC Solution

Ten grams of sodium carboxymethyl cellulose (Na-CMC) were dissolved in 200 mL of distilled water, heated, and allowed to float on the surface of the distilled water for approximately 15 minutes while stirring until a transparent and homogeneous mass formed. Then, distilled water was added to the mass until 1000 mL was obtained while continuously stirring. Na-CMC served as a hanging or stabilizing agent to prepare BPFE suspension to administer to white rats, keeping BPFE dispersed in the liquid and not settling.

Preparation of Butterfly Pea Flower Extract (BPFE) Using the Sonication Method

The butterfly pea flowers (BPFE) of 2,000 g are thoroughly washed, dried under sunlight, placed in an oven at 105°C for 60 min to remove moisture, blended into a simplisia powder, and filtered through a 50-mesh sieve. The simplisia powder of 100 g was dissolved in 500 mL of ethanol 96% and introduced into an ultrasonic batch operating at 42 KHz for 60 min. Next, it was filtered, resulting in a liquid extract, which was subsequently concentrated using a rotary evaporator at a temperature of 40°C. It was then dried using a water bath to yield a butterfly pea flower extract.[13]

Preparation and Administration of Butterfly Pea Flower Extract Suspension

The BPFE suspension was prepared individually for each group at 200/400/600 mg/kg BW doses. The BPFE was calculated and mixed with a 1% Na-CMC solution to reach a volume of 42 mL, then stirred until homogeneous. This suspension was prepared for use over one week, with daily administration to white rats for seven days. The same preparation and administration of BPFE suspension were continued into the following week, lasting until day 45.

Phytochemical Screening of Butterfly Pea Flower Extract

The phytochemical screening of butterfly pea flower extract involved several methods. Firstly, for alkaloid analysis, 2 mL of the extract solution was evaporated until residue formation, followed by dissolution in 5 mL of hydrochloric acid 2N, which was then divided into two reaction tubes. Tube one received three drops of Dragendorff's reagent, and tube two received three drops of Mayer's reagent. Orange and yellow precipitates indicated the presence of alkaloids. For flavonoid analysis, 1 mL of the extract was dissolved in concentrated acetone with borax and oxalic acid, heated, and mixed with ether. Yellow fluorescence under 366 nm UV light indicated the presence of flavonoids. Saponin assessment involved vigorously mixing 10 mL of the extract and adding one drop of hydrochloric acid 2N, with persistent foam after 10 minutes indicating the presence of saponin. The examination for tannins was conducted by reacting the BPFE with a ferric chloride solution of 10%, resulting in the formation of a greenish-black color, which indicates the presence of tannins. Lastly, terpenoid analysis used the Lieberman-Burchard reaction: evaporating 2 mL of extract, dissolving the residue in 0.5 mL chloroform, adding 0.5 mL glacial acetic acid, and then 2 mL of concentrated sulfuric acid to observe a brown or purple ring at the interface, signifying the presence of terpenoids[13,14].

Measurement of Atherosclerosis Biomarker

This study measured atherosclerosis biomarkers, specifically the concentrations of VCAM-1 and IL-6 taken from blood serum and the tunica intima thickness taken from the aorta in obese white rats. VCAM-1 and IL-6 measurements were conducted using enzyme-linked immunosorbent assay (ELISA) kits. Final absorbance was assessed using an ELISA photometer from Bio-Rad (Model 680) and then converted to ng/mL. Tunica intima thickness was measured using a microscope equipped with an ocular micrometer.

Data Analysis

Data analysis was conducted using One-way ANOVA SPSS version 18. The significance level set was 95% ($\alpha = 0.05$). A *p*-value exceeding 0.05 signified the absence of a significant difference, while a *p*-value below 0.05 signified a considerable difference. The analysis results were continued with the Post Hoc Tukey test.

RESULTS

Phytochemical Screening Results of Butterfly Pea Flower Extract

The phytochemical screening results using various reagents demonstrate that the butterfly pea flower extract contains bioactive compounds such as flavonoids, saponins, tannins, and terpenoids. It is evident in Table 2.

Table 2. Phytochemical Screening Results of Butterfly Pea Flower Extract

No	Secondary metabolite compounds	Result
1	Alkaloids	-
2	Flavonoids	+
3	Saponins	+
4	Tannins	+
5	Terpenoids	+

Notes: (+) = contained in the extract

Atherosclerosis Biomarkers Analysis of VCAM-1 in Obese White Rats

Obesity occurs due to the accumulation of excess fat in adipose tissue, causing atherosclerosis. Atherosclerosis triggers an inflammatory response, causing an increase in VCAM-1 levels. The impact of BPFE on VCAM-1 levels is presented in Table 3.

Table 3. V-CAM Levels in Obese White Rats

Group	VCAM-1 Level (ng/mL)	*ρ*-value
NC	28.33 ± 6.31	0.009
PC	112.90 ± 51.44	
P0	108.30 ± 56.09	
P1	95.05 ± 14.58	
P2	73.00 ± 42.12	
P3	30.40 ± 6.71	

Notes: NC = Negative Control, PC = Positive Control, P0 = Na-CMC, P1 = Na-CMC + BPFE-200, P2 = Na-CMC-BPFE-400, and P3 = Na-CMC+BPFE-600

Table 3 reveals that the mean VCAM-1 levels in the NC group were 28.33 ± 6.31 ng/mL, PC group were 112.90 ± 51.44 ng/mL, and P0 group were 108.30 ± 56.09 ng/mL. This study demonstrated that the higher the BPFE dose, the more significant the reduction in VCAM-1 levels. The 600 mg/kg BW dose (P3 group) showed a lower VCAM-1 level of 30.40 ± 6.71 ng/mL. The analysis demonstrated a significant difference among the treatment groups (ρ-value of 0.009), indicating that BPFE affected VCAM-1 levels in obese rats.

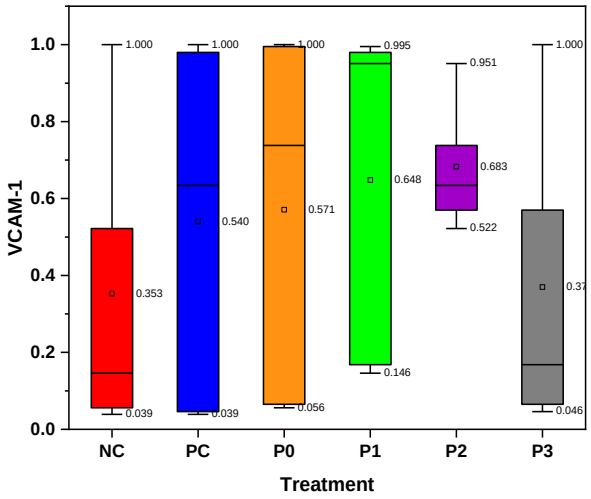

Figure 1. Tukey Box Plot of VCAM-1 in Obese White Rats

Tukey box plot (Figure 1) showed there were significant differences in the PC – P3 group (ρ = 0.046, ρ < 0.05) with an average difference in the VCAM-1 level of 82,500. Meanwhile, the other groups did not show significant differences in VCAM-1 levels. These results indicate that differences in VCAM-1 levels only occurred in certain groups, while other groups did not show significant differences.

Atherosclerosis Biomarkers Analysis of IL-6 in Obese White Rats

Obesity occurs because excess fat accumulation causes atherosclerosis, which, in turn, triggers an inflammatory response, resulting in increased IL-6 levels. The impact of BPFE administration on IL-6 levels in obese white rats is presented in Table 4.

Table 4. IL-6 Levels in Obese White Rats after BPFE Administration

Sample Code	IL-6 Level (ng/mL)	*ρ*-value
NC	22.90 ± 4.22	0.006
PC	95.65 ± 10.86	
P0	85.28 ± 65.69	
P1	55.75 ± 15.91	
P2	44.25 ± 20.63	
P3	17.70 ± 8.29	

Notes: NC = Negative Control, PC = Positive Control, P0 = Na-CMC, P1 = Na-CMC + BPFE-200, P2 = Na-CMC-BPFE-400, and P3 = Na-CMC+BPFE-600

Table 4 illustrates that the initial IL-6 levels were highest in the PC and P0 groups, while the NC group had the lowest. However, when the BPFE was given to obese rats, the IL-6 levels dropped. The most significant drop occurred in the P3 group, which got the highest extract dose. Statistical analysis confirmed that the BPFE significantly impacted IL-6 levels in obese rats, with IL-6 levels measuring 17.70 ± 8.29 ng/mL, which means there was a significant difference among the treatment groups (ρ-value of 0.006), indicating that BPFE affected IL-6 levels in obese rats.

In the Tukey box plot of IL-6 levels (Figure 2), there were significant differences in three groups, namely, the PC – P3 group (ρ = 0.016) with an average difference of 77,950, and group P0 – P3 (ρ = 0.044) with an average difference of 67.575. Meanwhile, the other groups did not show significant differences in IL-6 levels. These results indicated differences in IL-6 levels in certain groups, while other groups did not show significant differences.

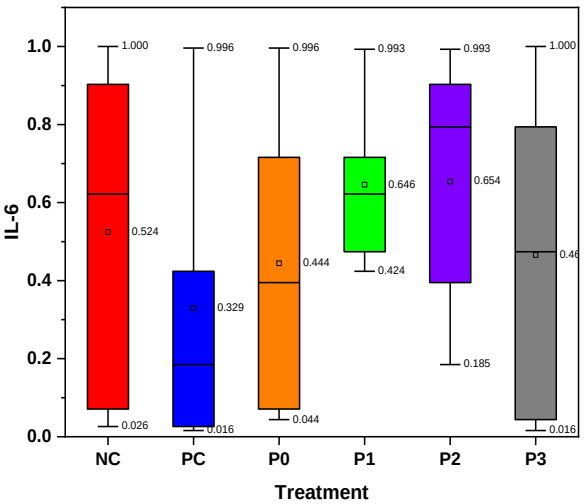

Figure 2. Tukey Box Plot of IL-6 in Obese White Rats

Atherosclerosis Biomarkers Analysis of Tunica Intima in Obese White Rats

Atherosclerosis, caused by fat accumulation due to obesity, can trigger an inflammatory response that increases tunica intima thickness. Histological results of the aortic tunica intima in obese white rats are presented in Figure 3.

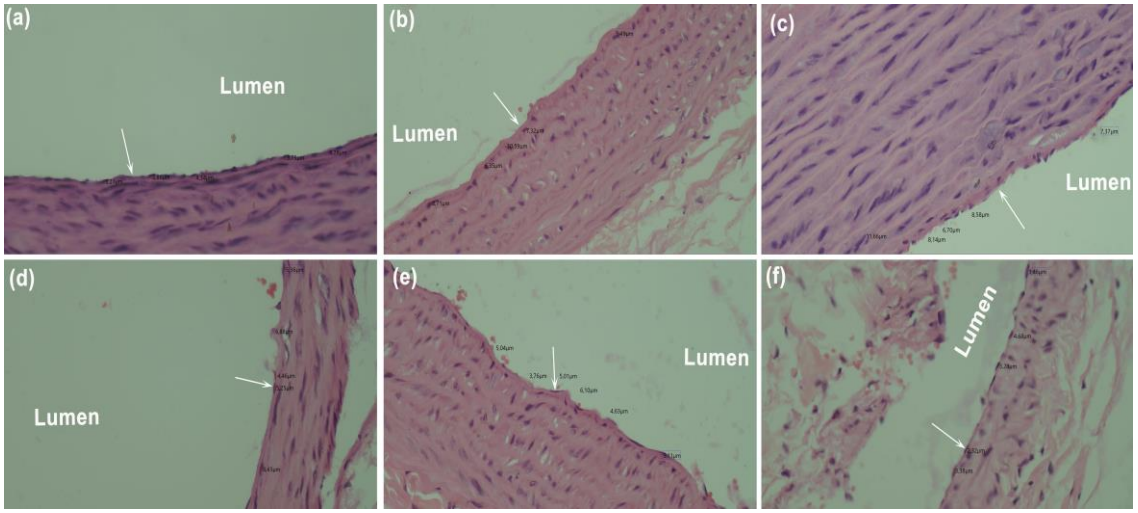

Figure 3. Histology of the Aortic Tunica Intima in the Obese White Rat of (a) NC, (b) PC, (c) P0, (d) P1, (e) P2, and (f) P3

Table 5. Tunica Intima Thickening in Obese White Rats after BPFE Administration

Group	Sample Code	Tunica Intima Thickness (µm)	*ρ*-value
Negative Control	NC	4.77 ± 2.00	0.001
Positive Control	PC	7.94 ± 0.61	
Na-CMC	P0	6.92 ± 1.93	
BPFE-200	P1	6.49 ± 1.05	
BPFE-400	P2	4.87 ± 0.73	
BPFE-600	P3	3.18 ± 1.24	

The NC group (Figure 3A) fed a standard diet showed aortic structures without atherosclerotic lesions. The endothelial and smooth muscle cell layers remained intact and organized with an intimal thickness of 4.77 ± 2.00 µm. The PC group (Figure 3B) was exposed to HFAD with apparent atherosclerotic lesions characterized by the accumulation of macrophages, inflammatory cells, foam cells, fat accumulation in smooth muscle cells, and endothelial damage[15]. The thickness of the tunica intima in the PC group was 7.94 ± 0.61 µm. The P0 group (Figure 3C) showed a slight improvement in the aortic structure compared with the PC group, but some damage remained. The P1 group (Figure 3D) showed further progress in the aortic structure. The P2 group (Figure 3E) showed that the aortic structure continued improving. The P3 group (Figure 3F) showed that the aortic structure was restored without atherosclerotic lesions, accumulation of macrophages, foam cells, or accumulation of intracellular fat in smooth muscle cells. The endothelial and smooth muscle cell layers showed organized characteristics with an intimal thickness of 3.18 ± 1.24 µm.

The effects of BPFE administration on tunica intima thickness in obese white rats are displayed in Table 5.

Table 5 shows that the NC group has an intima wall thickness of 4.75 ± 1.98 µm, while the PC group has a thickness of 7.92 ± 0.66 µm, and the P0 group has a thickness of 6.94 ± 1.99 µm. The PC group showed the thickest average intimal wall thickness, while the P3 group had the thinnest average tunica intima thickness. After administering BPFE to obese white rats, the thickness of the tunica intima became thinner, whereas the P3 group at a dose of 600 mg/kg BW had a tunica intima thickness of 3.18 ± 1.24 µm. Further statistical analysis showed significant differences between treatment groups ($\rho < 0.05$) with a ρ-value of 0.001, indicating that BPFE affected the thickness of the intima in obese white rats. Next, data pairs that differ significantly or not are presented in Figure 4.

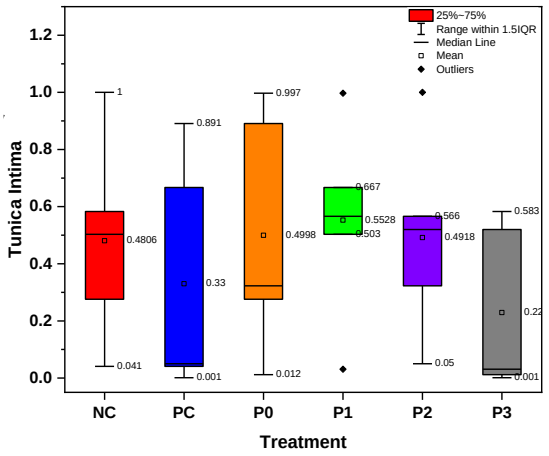

Figure 4. Tukey Box Plot of Tunica Intima in Obese White Rats

In the Tukey box plot of tunica intima thickness (Figure 4), there were some significant differences, namely between the PC – P3 group (p = 0.001) with an average difference in the tunica intima thickness of 4.765 µm, between the P0 – P3 (ρ = 0.012) with an average

difference in the tunica intima thickness of 3,740 μm, and between group P1 – P3 (ρ = 0.031) with an average difference of the tunica intima thickness 3.310 μm. Meanwhile, the other groups did not show significant differences in tunica intima thickness. The results of this study indicated significant differences in Tunica Intima thickness in certain groups, and these findings can be the basis for further research to understand the clinical implications. Additionally, the results of this study also noted differences in VCAM-1 and IL-6 levels between other groups, highlighting the complexity of the relationship in the context of this study.

DISCUSSION

Phytochemical Evaluation of Butterfly Pea Flower Extract

Phytochemical screening of butterfly pea flower extract (Table 2) identified key bioactive compounds: flavonoids, saponins, tannins, and terpenoids. These findings align with Jeyaraj et al.[16] research on butterfly pea flowers, which reported similar compounds. When exposed to the Lieberman-Burchard reagent, the extract exhibited intense yellow fluorescence under ultraviolet light, indicative of flavonoids, consistent with the Kumar & More[17] study. Furthermore, the extract's reaction with 2N hydrochloric acid produced a significant foaming response, confirming the presence of saponins. This observation echoes the findings of Manjula et al.[18], who reported on the polar glycoside and nonpolar steroid compounds of saponins. In addition, the extract's interaction with $FeCl_3$ reagent produced a dark green color, signifying tannin presence, correlating with Lijon et al.[19], that tannins' phenolic compounds are water-soluble and polar. Lastly, a brownish ring formed when the extract reacted with the Lieberman-Burchard reagent, indicative of terpenoid presence, in line with the findings of Ginting et al.[20]. Terpenoids, characterized by long hydrocarbon chains (C30), exhibit nonpolar characteristics and possess cyclic structures, such as alcohols, aldehydes, or carboxylic acids with -OH groups, rendering them semi-polar. These diverse bioactive compounds in butterfly pea flower extract hold promise for various potential health benefits and applications.

Impact of Butterfly Pea Flower Extract on VCAM-1 Levels in Obese Rats

The statistical analysis revealed a significant difference between the positive control and the administration of BPFE-200, BPFE-400, and BPFE-600, indicating that BPFE for 45 caused VCAM-1 to be lower than the positive control. The highest VCAM-1 levels were observed in the control group fed with HFAD or positive control groups. The administration of BPFE lowers the VCAM-1 level, where the higher the extract dose, the lower the VCAM-1 level. The results of the analysis showed that the BPFE-600 and the positive control showed significant differences in VCAM-1 levels. This finding aligns with research by Maneesai et al.[9], who reported that BPFE, which contains flavonoids, effectively improved cardiovascular dysfunction and oxidative stress due to a reduction in VCAM-1 levels in nitric oxide-deficient hypertensive rats. The lower VCAM-1 levels can be attributed to anthocyanin compounds in butterfly pea flower extract, a flavonoid known for its antioxidant and anti-inflammatory effects. Anthocyanins effectively inhibit enzymes involved in the inflammatory process, arachidonic acid metabolism, and LDL oxidation. This result is corroborated by the studies conducted by prior studies.[21,22]

Impact of Butterfly Pea Flower Extract on IL-6 Levels in Obese Rats

Our study showed that administration of BPFE-200, BPFE, 400, and BPFE-600 significantly lower IL-6 levels compared with positive controls. This finding aligns with research conducted by Widowati et al.[11], who reported that BPFE contains a bioactive flavonoid compound that functions as an antioxidant and anti-inflammatory so that it can lower IL-6 levels in experimental rats with diabetes mellitus and dyslipidemia. Muniroh et al.[23] also reported that BPFE has an anti-inflammatory function in leprosy, significantly reducing IL-6 levels. The reduction in IL-6 levels can be attributed to bioactive flavonoids in BPFE, which act as antioxidants and anti-inflammatory agents. Flavonoids can capture free radicals, inhibit LDL oxidation, and protect endothelial cell integrity. This finding aligns with research conducted by prior studies[24]. Maneesai et al.[9] also reported that the antioxidant properties of flavonoids prevent LDL oxidation and protect cell structures. Other studies by Rakha et al.[15] and Yamin et al.[25] also reported that flavonoids directly capture free radicals and stabilize reactive oxygen species (ROS) due to their hydroxyl groups, supporting the results of this study. The most significant results are observed at the BPFE-600 dose, as this dose possesses the optimal concentration to stimulate the desired biological response. According to Maneesai et al.[9], variability in this response may be influenced by factors such as dosage, administration time, rat health conditions, and the variability in BPFE composition. The BPFE holds promising therapeutic potential in addressing atherosclerosis. The section contains various bioactive compounds, primarily flavonoids such as anthocyanin, known for their antioxidant and anti-inflammatory properties. Flavonoids can protect arterial walls, inhibit LDL oxidation, and lower inflammation, all of which play roles in the prevention and treatment of atherosclerosis.

Impact of Butterfly Pea Flower Extract on Tunica Intima Thickness in the Obese Rats

The outcomes signify the significant impact of BPFE administration on aortic thickness, with the most effective dose for reducing tunica intima thickness being 600 mg/kg BW/day. This reduction can be attributed to flavonoids in BPFE, which function as antioxidants, inhibit LDL oxidation by macrophages, mitigate aortic thickening, and stimulate cellular immunity to prevent foam cell formation and smooth muscle cell proliferation within the tunica intima. These findings are consistent with studies by Ji et al.[26] and Ahmed,[27] which reported that flavonoids act as antioxidants and thins tunica intima thickness. Additionally, flavonoids exert anti-inflammatory effects by inhibiting prostaglandin synthesis through cyclooxygenase inhibition, preventing the conversion of arachidonic acid into prostaglandins.

One of the weaknesses of this study is the relatively short duration of intervention in the animal model, and the BPFE dosage difference was quite wide (i.e., 200 mg/kg) instead of a smaller dosage difference (for instance, 100mg/kg). There is a possibility that the dosage can be reduced is a smaller concentration difference or longer intervention duration is applied. Additionally, while the results suggest promising therapeutic potential, the long-term effects and safety of using BPFE in humans must be explored in clinical trials. The implication of this research provides valuable insights into the potential of BPFE as a natural remedy for atherosclerosis and related cardiovascular issues. The ability of BPFE to effectively lower inflammatory biomarkers and improve aortic health in obese rats suggests its therapeutic promise. This research sets the stage for further exploration of BPFE as a complementary approach to human cardiovascular health, offering a potentially safer and more natural alternative to traditional medications.

CONCLUSION

The butterfly pea flower (Clitoria ternatea L.) extract exhibits significant potential in reducing the levels of atherosclerosis biomarkers, including VCAM-1 and IL-6 concentration and intimal tunica thickness in the aorta of obese male white rats. The research findings indicate that administering butterfly pea flower extract for 45 days lowers VCAM-1 and IL-6 levels and thins the tunica intima thickness in obese white rats. The most effective dose in reducing intimal tunica thickness is 600 mg/kg body weight daily.

This butterfly pea flower extract effectively treats various diseases and can be a safer therapeutic alternative than promising pharmacological drugs such as simvastatin. Although not statistically significant, the reduction in levels of VCAM-1, IL-6, and tunica intima with the use of Na-CMC needs to be studied more deeply regarding the effect of Na-CMC on atherosclerosis biomarker analysis. It is necessary to validate these findings and identify more detailed mechanisms behind the positive impact of butterfly pea flower extract on atherosclerosis. Further research is needed to validate these findings and determine the more complex mechanisms behind the positive effects of butterfly pea flower extract on atherosclerosis.

ACKNOWLEDGMENTS

We want to thank the Institut Kesehatan Medistra Lubuk Pakam for their generosity in providing laboratory facilities and all the necessary materials and equipment.

REFERENCES

1. Hall KD, Farooqi IS, Friedman JM, Klein S, Loos RJF, Mangelsdorf DJ, et al. The energy balance model of obesity: beyond calories in, calories out. Am J Clin Nutr. 2022;115(5):1243–54. Available from: https://doi.org/10.1093/ajcn/nqac031
2. Febriani RT, Soesetidjo A, Tiyas FW. Consumption of fat, protein, and carbohydrate among adolescent with overweight/obesity. J Matern Child Heal. 2019;4(2):70–6. Available from: https://doi.org/10.26911/thejmch.2019.04.02.02
3. Khatana C, Saini NK, Chakrabarti S, Saini V, Sharma A, Saini R V, et al. Mechanistic insights into the oxidized low-density lipoprotein-induced atherosclerosis. Oxid Med Cell Longev. 2020;2020:1–14. Available from: https://doi.org/10.1155/2020/5245308
4. Liu M, Samant S, Vasa CH, Pedrigi RM, Oguz UM, Ryu S, et al. Co-culture models of endothelial cells, macrophages, and vascular smooth muscle cells for the study of the natural history of atherosclerosis. PLoS One. 2023;18(1):1–22. Available from: https://doi.org/10.1371/journal.pone.0280385
5. Amanda Q, Widyastiti NS, Prajoko YW, Maharani N, Bahrudin U. Cigarette Smoke Exposure, but Not High Fat Diet, is Able to Induce Atherosclerosis in Wild-Type Rats. J Biomed Transl Res [Internet]. 2022 Dec 30;8(3):130–7. Available from: https://doi.org/10.14710/jbtr.v8i3.15744
6. Kloc M, Kubiak JZ, Ghobrial RM. Macrophage-, Dendritic-, Smooth Muscle-, Endothelium-, and Stem Cells-Derived Foam Cells in Atherosclerosis. Int J Mol Sci. 2022;23(22):14154. Available from: https://doi.org/10.3390/ijms232214154
7. Mehmood A, Ishaq M, Zhao L, Yaqoob S, Safdar B, Nadeem M, et al. Impact of ultrasound and conventional extraction techniques on bioactive compounds and biological activities of blue butterfly pea flower (Clitoria ternatea L.). Ultrason Sonochem. 2019;51:12–9. Available from: https://doi.org/10.1016/j.ultsonch.2018.10.013
8. Kuswandari F, Sinaga E, Nurbaiti N, Husni A. Analysis of Total Phenols, Total Flavonoids and Anthocyanin Levels in Blue Pea Flowers (Clitoria ternatea L). J Trop Biodivers. 2022;2(3):152–9.
9. Maneesai P, Iampanichakul M, Chaihongsa N, Poasakate A, Potue P, Rattanakanokchai S, et al. Butterfly pea flower (Clitoria ternatea Linn.) extract ameliorates cardiovascular dysfunction and oxidative stress in nitric oxide-deficient hypertensive rats. Antioxidants. 2021;10(4):523. Available from: https://doi.org/10.3390/antiox10040523
10. Maulidy WH, Arifa Mustika A, Mukono IS. The Effect of Butterfly Pea (Clitoria Ternatea) Extract on Reducing Total Cholesterol Levels in Rattus norvegicus with the Hypercholesterolemia Model. Int J Res Publ. 2022;115(1):626–34. Available from: https://doi.org/10.47119/IJRP1001151122022434 9
11. Widowati W, Darsono L, Lucianus J, Setiabudi E, Obeng SS, Stefani S, et al. 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. J King Saud Univ. 2023;35(4):102579. Available from: https://doi.org/10.1016/j.jksus.2023.102579

12. Syafa'atullah AQ, Amira A, Hidayati S, Mahfud M. Anthocyanin from butterfly pea flowers (Clitoria ternatea) by ultrasonic-assisted extraction. In: AIP Conference Proceedings. AIP Publishing; 2020. Available from: https://doi.org/10.1063/5.0005289
13. Rahma K, Nurcahyanti O. Therapeutic Effect of Red Spinach (Amaranthus tricolor L.) Extract on Pancreatic MDA Levels Rats (Rattus norvegicus) Exposed to MLD-STZ. J Biomed Transl Res. 2021 Dec 31;7(3):129–33. Available from: https://doi.org/10.14710/jbtr.v7i3.12536
14. Rahma K, Purnomo FO. Potential Effects of Ipomoea reptans Poir. Extract on LDL, HDL levels and liver Histopathology. J Biomed Transl Res. 2023 Aug 31;9(2):61–7. Available from: https://doi.org/10.14710/jbtr.v9i2.16984
15. Rakha A, Umar N, Rabail R, Butt MS, Kieliszek M, Hassoun A, et al. Anti-inflammatory and anti-allergic potential of dietary flavonoids: A review. Biomed Pharmacother. 2022;156:113945. Available from: https://doi.org/10.1016/j.biopha.2022.113945
16. Jeyaraj EJ, Lim YY, Choo WS. Extraction methods of butterfly pea (Clitoria ternatea) flower and biological activities of its phytochemicals. J Food Sci Technol. 2021;58(6):2054–67. Available from: https://doi.org/10.1007/s13197-020-04745-3
17. Kumar M, More D. Phytochemical analysis and bioactivity of selected medicinal plant of butterfly-pea (Clitoria ternatea L.) used by Kolam tribe Addjoing region of Telangana and Maharashtra states. Pharma Innov. 2019;8(1):417–21.
18. Manjula P, Mohan CH, Sreekanth D, Keerthi B, Devi BP. Phytochemical analysis of Clitoria ternatea Linn., a valuable medicinal plant. J Indian Bot Soc. 2013;92(3and4):173–8.
19. Lijon MB, Meghla NS, Jahedi E, Rahman MA, Hossain I. Phytochemistry and pharmacological activities of Clitoria ternatea. Int J Nat Soc Sci. 2017;4(1):1–10.
20. Ginting EE, Rumanti RM, Savira D, Ginting P, Marbun N, Leny L. In Vivo study of Antidiabetic Activity from Ethanol Extract of Clitoria ternatea L. Flower. J Drug Deliv Ther. 2022;12(6):4–9. Available from: https://doi.org/10.22270/jddt.v12i6.5759
21. Martínez-Coria H, Arrieta-Cruz I, Gutiérrez-Juárez R, López-Valdés HE. Anti-Inflammatory Effects of Flavonoids in Common Neurological Disorders Associated with Aging. Int J Mol Sci. 2023;24(5):4297. Available from: https://doi.org/10.3390/ijms24054297
22. Yi L, Ma S, Ren D. Phytochemistry and bioactivity of Citrus flavonoids: a focus on antioxidant, anti-inflammatory, anticancer and cardiovascular protection activities. Phytochem Rev. 2017;16:479–511. Available from: https://doi.org/10.1007/s11101-017-9497-1
23. Muniroh M, Kusumaningrum N, Farmaditya EPM, Yuniati R. Clitoria ternatea L. Extract as Adjuvant Therapy on Reducing IL–6 Levels in Reversal Reaction. Biosaintifika J Biol Biol Educ. 2023;15(1). Available from: https://doi.org/10.15294/biosaintifika.v15i1.39808
24. Saiki P, Kawano Y, Nakajima Y, Van Griensven LJLD, Miyazaki K. Novel and stable dual-color IL-6 and IL-10 reporters derived from RAW 264.7 for anti-inflammation screening of natural products. Int J Mol Sci. 2019;20(18):4620. Available from: https://doi.org/10.3390/ijms20184620
25. Yamin AR, Sabarudin HN, Kasmawati H. Antioxidant activity assay and determination of phenolic and flavonoid content of Libho (Ficus Septica Burm. F) fruits. Open J Chem. 2022;8(1):8–13. Available from: https://doi.org/10.17352/ojc.000029
26. Ji HS, Li H, Mo EJ, Kim UH, Kim YH, Park HY, et al. Low-density lipoprotein-antioxidant flavonoids and a phenolic ester from Plectranthus hadiensis var. tomentosus. Appl Biol Chem. 2019;62(1):1–12. Available from: https://doi.org/10.1186/s13765-019-0464-y
27. Ahmed A. Flavonoids and cardiovascular risk factors: a review. Pharmadvances. 2021;3(3):523–49. Available from: https://doi.org/10.36118/pharmadvances.2021.11