Ashdin Publishing
Journal of Drug and Alcohol Research
Vol. 11 (2022), Article ID 236211, 7 pages

Research Article

The Effect of *Clitorea ternatea* Flower Extract towards Interleukin 1-Beta, Nitric Oxide and Aorta Intima-Media Thickness in the Early Stage of Atherosclerosis: *In Vivo* Study with Atherosclerosis Rats Model

Onna Januaresty[1], Teuku Heriansyah[2*], Henni vanda[3], Fauzul Husna[4], Novita[2] and Maha Fitra[2]

[1]Residency Cardiology and Vascular Educational Program, Faculty of Medicine, Universitas Syiah Kuala, Aceh, Indonesia
[2]Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Syiah Kuala, Aceh, Indonesia
[3]Laboratory of Pharmacology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Aceh, Indonesia
[4]Department of Pharmacology, Faculty of Medicine, Universitas Syiah Kuala, Aceh, Indonesia

[*]Address Correspondence to Teuku Heriansyah, E-mail: teuku_hery@unsyiah.ac.id

Received: 30 November 2022; Manuscript No: JDAR-22-82112; **Editor assigned:** 02 December 2022; PreQC No: JDAR-22-82112 (PQ); **Reviewed:** 16 December 2022; QC No: JDAR-22-82112; **Revised:** 21 December 2022; Manuscript No: JDAR-22-82112 (R); **Published:** 28 December 2022; **DOI:** 10.4303/JDAR/236211

Copyright © 2022 Teuku Heriansyah, et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

Cardiovascular disease is caused by many things, especially atherosclerotic plaque. The process involves cytokines as a complex inflammatory response in the formation of atherosclerotic plaques. Interleukin 1-β (IL-1β) is a potent pro-inflammatory cytokine. It is also affected by oxidative stress. *Clitorea ternatea* was a plant in Indonesia that could be used as anti-inflammatory. The purpose of this study was to determine potential ability of *Clitorea ternatea* flower extract to reduce pro-inflammatory cytokine (IL-1β) expression, to increase antioxidant activity (Nitric Oxide), and to decrease tunica intima-media thickness in aortic histopathology *in vivo* related with early stage of atherosclerosis. This study was an experimental study and only posttest with control group design, using male white *Rattus norvegicus Wistarstrain* which divided into 5 groups, namely 2 control groups (dyslipidemic rats and normal rats) and 3 treatment groups (dyslipidemic rats with *Clitorea ternatea* flower extract 500 mg/kg/day p.o., Dyslipidemic rats with atorvastatin 40 mg/day p.o, Dyslipidemic rats with *Clitorea ternatea* flower extract 500 mg/kg/day p.o. and atorvastatin 40 mg/day p.o.). Parameters observed were IL-1β, nitric oxide, and aortic intima-media thickness. Data were analyzed using One Way ANOVA with 95% of confidence interval. The administration of 500 mg/kg flower extract significantly decreased IL-1β, increased nitric oxide, and decreased aortic intima-media tunica thickness. Conclusions: *Clitorea ternatea* flower extract could reduce IL-1β levels, increase nitric oxide and decrease tunica intima media thickness in aortic histopathology ($p<0.05$), and *Clitorea ternatea* flower extract had similar potential with atorvastatin as control agent ($p>0.05$).

Keywords: *Clitorea ternatea*; Interleukin-1β; nitric oxide; Tunica intima-media thickness

Introduction

Heart and blood vessel disease is one of the contributors to morbidity and mortality. Annually, it is estimated that 17 million peoples in general die from heart and blood vessel disease, especially from heart attacks and strokes [1]. Cardiovascular disease could be caused by many things, mainly due to the development of atherosclerotic plaques. Atherosclerosis is a condition of accumulation of cells, cholesterol and extracellular matrix that causes hardening of the arterial walls [2]. This process involves cytokines as a complex inflammatory response in the formation of atherosclerotic plaques [3]. Interleukin 1β (IL-1β) is a strong pro-inflammatory cytokine [4]. IL-1β has an effect on human smooth muscle cells, is able to induce the production of autocrine platelet growth factor that stimulates smooth muscle proliferation and is able to induce the expression of other genes including endothelial cells and smooth muscle cells [5]. Oxidative stress is also known to be involved in the process of atherosclerosis [6]. Oxidative stress is caused by the imbalance of homeostatic mechanisms, imbalance between antioxidant power and activity of free radicals such as Reactive Oxygen Species (ROS), nitrogen and halogens [7]. Nitric oxide (NO) plays an important role in fighting oxidative stress at the cellular level. In addition, NO also plays a role in maintaining haemostasis in the form of vasodilation, inhibition of platelet adhesion and platelet aggregation, inhibition of smooth muscle cell proliferation and is able to stimulate the expression of protective pro-

teins. So that NO is considered as an agent to see the effect/degree of inhibition of oxidative stress on tissues [8].

Nowadays, the prevention of cardiovascular disease in primary prevention is the initial basis for the initial management of cardiovascular disease by utilizing and optimization natural resources [9]. Telang flower (*Clitorea ternatea*) apart from being an ornamental plant, this plant had long been known as food coloring, medicinal plant, and various *in vitro* studies had shown that telang flower (*Clitorea ternatea*) had pharmacological potential as an antioxidant and anti-inflammatory [10]. This effect was caused by the active compounds of telang flower (*Clitorea ternatea*) namely flavonoids, saponins, terpenoids, and tannins. Telang flower also had a strong category of antioxidant activity [11,12].

Materials and Methods

Ethic

This research had passed the ethical test by the Medical Research Ethics Commission, Faculty of Veterinary Medicine, Universitas Syiah Kuala with agreement file number 138/KEPH/XII/2021.

Plant collection and identification

Telang flower (*Clitorea ternatea*) used was a purple variety originating from Banda Aceh, Indonesia, in December 2021 at coordinates of 5˚32'28.3" North 95˚19'06.5" East which had been identified at Faculty of Mathematics and Science Nature, Department of Biology, Universitas Syiah Kuala (USK) with letter number B/664/UN11.1.8.4/TA.00.04/2020.

Extraction

The dried powdered of *Clitorea ternatea* flowers were macerated using 95% ethanol for three days. Then the evaporation was carried out with a rotary vacuum evaporator and the heating process was carried out at a temperature of 45˚C.

Preliminary phytochemical analysis

Phytochemical analysis was performed on the extracts, namely total phenolic test, total flavonoid test, antioxidant capacity test using the DPPH method (2, 2-Diphenyl-1-Pikrylhydrazil) and anthocyanin level test. Phytochemical analysis was performed at Chemistry Education Laboratory, Faculty of Teacher Training and Education, Universitas Syiah Kuala.

Animals

This study used male Rattus norvegicus strain Wistar rats (n=25), which were obtained from animal husbandry at Faculty of Veterinary Medicine, Universitas Syiah Kuala, aged 5 weeks and body weight of 70 grams-100 grams. All rats were handled and examined for health, placed in each cage at a controlled temperature (23 ± 1˚C), given with feed of 10% body weight and available water ad libitum.

Diet-induced hyperlipidemia in rats

The method of Gani et al. (2013) was used to produce diet-induced hyperlipidemia [13]. Atherogenic diet contained vitamin D3, 0.2% cholic acid, 2% egg yolk, 5% goat fat and 92.8% standard feed. The standard feed used in this study was standard commercial feed, namely pellets (Hi-Gro 552, Medan, Indonesia). The composition of Hi-Gro consists of 16%-18% protein, 4% fat, 12% ash content, 8% fiber, and 12% water content.

Study design

Rats aged 4 weeks underwent an adaptation process for 1 week, then the research process began. This research was divided into 2 stages. The first stage was carried out for 8 weeks to induce atherosclerosis in animals [13]. A total 25 rats aged 5 weeks with a body weight of 70 g-100 g were divided into 2 groups (for 8 weeks). The second stage started after 8 weeks, the rats were divided into 5 groups that received treatment for 20 days, namely:

- **Negative control group (NC):** Normal rats that received regular feed and 0.5% Carboxymethyl Cellulose Sodium (Na CMC)
- **Positive control group (PC):** Dyslipidemic rats that received atherogenic diet and 0.5% Na CMC [13].
- **DLC group:** Dyslipidemia rats that received atherogenic diet, 0.5% Na CMC and ethanol extract of *Clitorea ternatea* flower with a dose of 500 mg/KgBW/day p.o.
- **DLA group:** Dyslipidemic rats that received atherogenic diet, 0.5% Na CMC and 40 mg/day atorvastatin (as an establish agent) p.o. multiplied by conversion factor For the given dose to rats, we converted the dose of simvastatin based on the conversion table of Laurence and Bacharach (1984) [14].
- **DLAC group:** Dyslipidemic rats that received atherogenic diet, 0.5% Na CMC, *Clitorea ternatea* flower ethanol extract at a dose of 500 mg/KgBW/day and atorvastatin at a dose of 40 mg/day multiplied by a conversion factor p.o.

The number of animals used was 5 rats per group based on the Federer formula. At the end of the study, blood was taken from the ophthalmic vein in the orbital sinus for examination of High-Density Lipoprotein Cholesterol (HDL-C) and Low-Density Lipoprotein Cholesterol (LDL-C). Euthanasia was performed by injecting ketamine 15 mg/kgBW-20 mg/kgBW intra peritoneal, and cervical dislocation was performed. Then 3 cc of blood were taken from the heart then placed in a venojec. Blood plasma samples were centrifuged at 3000 rpm for 10 minutes, stored immediately at –20˚C. Plasma was used for the examination of HDL-C, LDL-C, IL-1β and NO. The rest of the rat tissue was cremated in an incinerator. The aorta was separated and fixed in 10% neutral buffer formalin (NBF). The aorta

was dehydrated with alcohol 70%, alcohol 80%, alcohol 90% alcohol, absolute alcohol I and absolute alcohol II. Then proceed to the next step, namely clearing process with xylol, paraffin infiltration process, and Hematoxylin Eosin Staining process.

Levels of LDL-C and HDL-C

After termination of the study, blood sample were collected in vacutainer tubes with EDTA. Then, the levels of HDL-C and LDL-C were examined by employing a chemistry analyzer (mindray). The result was determined in mg/dl.

Levels of nitric oxide and interleukin 1-β

Measurement of the amount of Nitric oxide and Interleukin 1β in blood plasma were conducted using ELISA Kit rat Nitric oxide (Elabsciece E-BC-K035-M) and ELISA Kit rat Interleukin 1β (Elabsciece E-EL-R0012). Competitive ELISA begun with an antigen coating. A total of 100 µL Standard and sample were taken and inserted into the well. Samples (except empty wells) were incubated at 37°C for 1 hour. Then 90 µL of substrate was added into each well and incubated for 30 minutes at 37°C. The results on the microplate reader were read at a wavelength of 450 nm.

The thickness of tunica intima media

Tunica intima media thickness was measured by observing the aortic preparation. The aortic preparations were first scanned with a microscope (Meiji, MT5000, New York) with 200x magnification, then viewed and measured using ImageJ software. The thickness of tunica intima media in each sample was measured in five zones, in the same angle and the average of the five zones was calculated.

Statistical analysis

Homogeneity test of the data was carried out by Levene's test. If the variation between groups was homogeneous ($p>0.05$), then proceed with One-Way Analysis of Variance (ANOVA) test analysis ($\propto=0.05$), if there was a significant difference, then proceed with post hoc test ($\propto=0.05$). This statistical test was conducted using Statistical Product and Service Solutions software (SPSS) 22 (IBM Cooperation, New York, NY).

Results

Phytochemical analysis

Qualitatively, based on phytochemical tests, ethanol extract of *Clitoria ternatea* flower used in this study was positive contained terpenoids, flavonoids, phenolics and tannins. While the negative results were alkaloids, steroids and saponins. Quantitatively, total phenolic test result was 7,841 ± 72.16 mg equivalent of Gallic Acid/gram extract, total flavonoid test result was 40.44 ± 0.56 (mg cathecin equivalent/gram extract), and antioxidant capacity using the DPPH (2, 2-Diphenyl-1-Pikrylhydrazil) method showed that the half maximal inhibitory concentration (IC_{50}) value of *Clitorea ternatea* flower extract was very good (5.37), close to Ascorbic Acid as standard (6.21). Meanwhile, the results of anthocyanin level test using pH difference method showed that anthocyanin content (ml/L) was 521.005 ml/L.

Basic characteristics

In this study, there was no significant difference in food intake and weight gain among the different groups observed (**Table 1**).

Table 1: Effect of different treatment on weight gain and food intake

Characteristics	Groups (Mean ± SD)				
	NC	PC	DLA	DLC	DLAC
N	5	5	5	5	5
Early Body weight (gram)	75.2 ± 3.96	86.2 ± 9.83	74.6 ± 13.75	74.8 ± 12.93	94.8 ± 4.65
Final Body weight (gram)	198 ± 7.74	198.2 ± 16.6	194.4 ± 48.8	202 ± 8.6	205.2 ± 24
Increasing of Body weight (gram)	122 ± 9.83	112 ± 19.06	119 ± 38.9	121 ± 9.24	110 ± 20.9
Food Intake in First Month (gram)	10.2 ± 1.09	12 ± 1.58	12 ± 1.73	11.2 ± 1.09	12.2 ± 0.44
Food Intake in Second Month (gram)	16.6 ± 1.51	16.4 ± 0.54	17.2 ± 0.83	16.2 ± 0.83	15.6 ± 1.51
Food Intake in Third Month (gram)	18.8 ± 0.44	17.6 ± 1.94	17.8 ± 2.58	18.6 ± 0.54	19.2 ± 1.64

Note. NC, Negative control group; PC, Positive control group; DLA, Dyslipidemia+atorvastatin group; DLC, Dyslipidemia+*Clitorea ternatea* group; DLAC, Dyslipidemia+Atorvastatin+*Clitorea ternatea* group.

Levels of LDL-C and HDL-C

There was a significant increase in serum levels of LDL-C in the PC compared with the NC. There was a significant decrease in serum levels of HDL-C in the PC compared with the NC. All the treatment groups produced a significant decrease in serum level of LDL-C and increase in serum level of HDL-C (**Table 2**).

Table 2: Effect of *Clitorea ternatea* on level of LDL-C dan HDL-C

Profile Lipid	Groups				
	NC	PC	DLA	DLC	DLAC
N	5	5	5	5	5
LDL-C After Intervention (mg/dl)	16.2 ± 1.92*	64.4 ± 2.60**	19.8 ± 0.83*	22.8 ± 4.0*	17.2 ± 1.48*
HDL-C After Intervention (mg/dl)	36.6 ± 1.51*	26.8 ± 0.83**	35.2± 0.83*	33 ± 3.5*	36 ± 1.3*

Note. NC, Negative control group; PC, Positive control group; DLA, Dyslipidemia+atorvastatin group; DLC, Dyslipidemia+*Clitorea ternatea* group; DLAC, Dyslipidemia+Atorvastatin+*Clitorea ternatea* group; *compared with positive control (p<0,05), ** compared with negative control (p<0,05).

Interleukin 1-β levels

Clitorea ternatea flower extract DLC group was similar to atorvastatin (DLA) group in reducing IL1-β levels. This was indicated by a statistically significant decrease in IL1-β levels compared to positive control (p=0.000). The decrease of IL1-β levels in DLA compared to PC was higher (p=0.000) than the decrease of IL1-β levels in DLC (p=0.000) compared to PC. However, the post hoc test results showed that the difference was not significant (p=0.806). The group that was given both (DLAC) was also able to lower IL1-β levels compared with PC (p=0.000). However, DLAC group did not show any significant difference compared to those who were given *Clitorea ternatea* flower extract alone or atorvastatin alone (**Table 3**).

Table 3: Effect of *Clitorea ternatea* on level of IL1-β

Groups	N	IL 1-β (pg/mL)	p‡
		Mean ± SD	
NC	5	85.44 ± 8.27*	*p*<0.05 (0.000)
PC	5	116.75 ± 13.14**	
DLA	5	72.39 ± 1.36*	
DLC	5	80.94 ± 0.95*	
DLAC	5	68.14 ± 5.08*	

Note. NC, Negative control group; PC, Positive control group; DLA, Dyslipidemia+atorvastatin group; DLC, Dyslipidemia+*Clitorea ternatea* group; DLAC, Dyslipidemia+Atorvastatin+*Clitorea ternatea* group; *compared with positive control (*p*<0,05), ** compared with negative control (*p*<0,05); ‡ One Way ANOVA test.

Nitric oxide levels

The flower extract group of *Clitorea ternatea* (DLC) had similar result with DLA group in increasing NO level. This was indicated by a statistically significant increase in NO levels compared to positive controls (p=0.005). The increase of NO levels in DLA compared with PC was higher (p=0.000) than the increase of NO levels in *Clitorea ternatea* flower extract compared with PC (p=0.005). However, the results of the post hoc test showed that the difference was not significant (p=0.332). The group that was given both (DLAC) was also able to increase NO levels compared with PC (p=0.011). However, DLAC group did not show any significant difference compared to those given *Clitorea ternatea* flower extract alone or atorvastatin alone (**Table 4**).

Table 4: Results of Nitric Oxide Level

Groups	N	NO (µmol/L)	*p*‡
		Rata-rata ± SD	
NC	5	30.02 ± 3.78*	*p*<0.05 (0.000)
PC	5	21.78 ± 1.72**	
DLA	5	38.26 ± 2.57*	
DLC	5	32.38 ± 5.06*	
DLAC	5	31.59 ± 5.74*	

Note. NC, Negative control group; PC, Positive control group; DLA, Dyslipidemia+atorvastatin group; DLC, Dyslipidemia+*Clitorea ternatea* group; DLAC, Dyslipidemia+Atorvastatin+*Clitorea ternatea* group; *compared with positive control (*p*<0,05), ** compared with negative control (*p*<0,05); ‡ One Way ANOVA test.

The thickness of tunica intima and tunica media aorta

Based on quantitative data that was analyzed from histopathological images, it was shown that the flower extract group of *Clitorea ternatea* and atorvastatin (DLAC) had smaller intima and media tunica layer sizes than the *Clitorea ternatea* flower extract group, the atorvastatin group and the normal group. Meanwhile the positive control group had a thicker layer than the other groups (**Table 5**). One Way ANOVA statistical analysis between the treatment groups showed a significant difference (**Figure 1**).

Table 5: The thickness of the layers of the tunica intima and tunica media of the aorta

Groups	N	Tunica Intima Media thickness (µm)	$p^{\ddagger}$
		Mean ± SD	
NC	5	49.4 ± 8.29*	p<0.05 (0.000)
PC	5	93.2 ± 15.40**	
DLA	5	61.0 ± 2.00*	
DLC	5	66.20 ± 6.40*	
DLAC	5	59.2 ± 2.58*	

Note. NC, Negative control group; PC, Positive control group; DLA, Dyslipidemia+atorvastatin group; DLC, Dyslipidemia+*Clitorea ternatea* group; DLAC, Dyslipidemia+Atorvastatin+*Clitorea ternatea* group; *compared with positive control (p<0,05), ** compared with negative control (p<0,05); ‡ One Way ANOVA test.

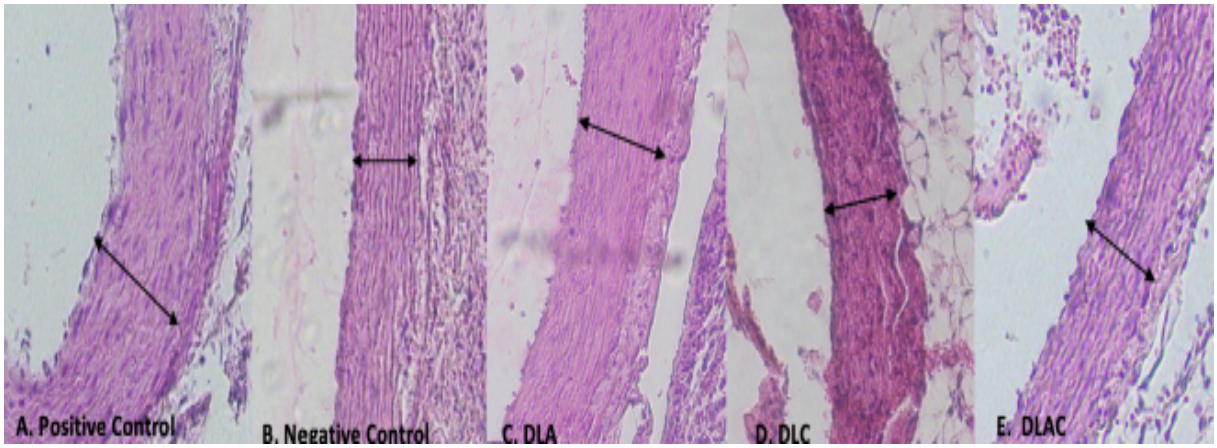

Figure 1: A. Positive control group, B. Negative control group, C. Dyslipidemia group + atorvastatin, D. Dyslipidemia group + *Clitorea ternatea* flower ethanol extract, E. Dyslipidemia group + *Clitorea ternatea* flower ethanol extract + Atorvastatin

Discussion

The prevention of cardiovascular disease in primary prevention is the initial basis for the initial management of cardiovascular disease by utilizing and optimization natural resources [9]. This study was the first *in vivo* study on male white rats *Rattus norvegicus Wistarstrain* in atherosclerosis model which assessed the anti-inflammatory and antioxidant activity of ethanol extract of purple variant *Clitorea ternatea* flower.

Based on phytochemical tests carried out in this study, it was known that flower extract of *Clitorea ternatea* contained several types of active compounds, namely Tannins, Terpenoids, Flavonoids, and Phenolics. Based on DPPH assay, it was found that *Clitorea ternatea* flower extract used in this study contained antioxidants. A compound was said to have a very strong antioxidant activity if the IC_{50} value was less than 50 g/ml, strong for IC_{50} was 50 g/ml-100 g/ml, while moderate for IC_{50} value was 151 µg/ml-200 µg/ml [15]. The results of antioxidant activity test showed that IC_{50} value of telang flower extract was 5.37 g/mL and IC_{50} value of Vitamin C was 6.31. This indicated that *Clitorea ternatea* flower extract from Banda Aceh (Indonesia) which used in this study had a very strong antioxidant activity, nearly similar with Vitamin C as a control.

In this study, it was shown that *Clitorea ternatea* flower extract decreased IL-1β levels in dyslipidemic rats with p<0.05. Post hoc analysis showed that there was no statistically significant difference in the concentration of IL1-β reduction between the administration of *Clitorea ternatea* flower extract and atorvastatin as standard agent. And from this study, it was shown that NO levels were increased upon administration of *Clitorea ternatea* flower extract in the group of dyslipidemic rats (p<0.05), and no statistical differences compared to the dyslipidemic group treated with atorvastatin (p>0.05). This means that flower extract of *Clitorea ternatea* (DLC) had similar potential benefit with atorvastatin group (DLA) to reduce inflammatory and against oxidative stress. Administration of combination both of them (DLAC) also showed the same effect, but there were no statistical differences compared with *Clitorea ternatea* alone or atorvastatin alone (p>0.05).

These results in line with *in vitro* studies, which showed

that anthocyanins in *Clitorea ternatea* flower had the ability to fight oxidized lipids. Active compounds of *Clitorea ternatea* were kaempferol 3-neohesperidoside (462.63 mg/100 g), caffeoylmalic acid (137.59 mg/100 g), and kaempferol 3-(2G-rhamnosylrutinoside) (129.28 mg/100 g) [16]. Flower extract of *Clitorea ternatea* also contained antocyanin which significantly reduced inflammatory markers in rat paw edema compared to positive controls. At the gene level, flower extract of *Clitorea ternatea* may had a protective effect against inflammation through the suppressor gene PI3Ks [17]. A previous study had similar result. The results showed that aqueous extract of *Clitorea ternatea* blue petals flower contained bioactive compounds such as anthocyanins that had anti-inflammatory effect, could reduce oxidative stress, could reduce plasma leptin, free fatty acids, low density lipoprotein cholesterol levels and liver malondialdehyde content in C57BL/6J male rats fed with high fat and high fructose diet [18]. In addition, the seeds and roots of *Clitorea ternatea* had been shown *in vivo* to have antihyperlipidemic activity and the ability to repair the body's natural antioxidants compared to atorvastatin [19].

The advantage of *in vivo* research was the ability to observe tissues [20]. In this study, it was shown that flower extract of *Clitorea ternatea* decreased the thickness of the tunica intima media compared to positive control ($p<0.05$).

However, the drawback of this study was that it did not differentiate between the doses of the administration *Clitorea ternatea* extract in the treatment group.

Conclusion

The administration of ethanol extract of *Clitorea ternatea* purple variant flower for 20 days was able to reduce the expression of pro-inflammatory cytokines (IL-1β), to increase antioxidant activity (NO) and to reduce the thickness of the tunica intima and tunica media of aorta. These effects had similarity with atorvastatin as a control agent.

Acknowledgements

None.

Conflict of Interest

Authors have no conflict of interest to declare.

References

1. World Health Organization (WHO) (2020) Data and Statistics.
2. E. Galkina, K. Ley, Immune and inflammatory mechanisms of atherosclerosis, Annu Rev Immunol, 27(2009), 165–197.
3. D. Tousoulis, E. Oikonomou, E.K. Economou, F. Crea, J.C. Kaski, Inflammatory cytokines in atherosclerosis: Current therapeutic approaches, Eur Heart J, 37(2016), 1723–1732.
4. F.J. Sheedy, K.J Moore, IL-1 signaling in atherosclerosis: Sibling rivalry, Nat Immunol, 14(2013), 1030–1032.
5. C.A. Dinarello, Biologic basis for interleukin-l in disease, Blood, 87(1996), 2095-2147.
6. U. Förstermann, N. Xia, H. Li, Roles of vascular oxidative stress and nitric oxide in the pathogenesis of atherosclerosis, Circ Res, 120(2017), 713–735.
7. X. Yang, Y. Li, X. Ren, X. Zhang, D. Hu, et al. Oxidative stress-mediated atherosclerosis: Mechanisms and therapies. Front Physiol, 8(2017), 600.
8. C. Napoli, F. de Nigris, S. Williams-Ignarro, O. Pignalosa, V. Sica, et al. Nitric oxide and atherosclerosis: An update, Nitric Oxide, 15(2006), 265–279.
9. E. Yu, V.S. Malik, F.B. Hu, Cardiovascular disease prevention by diet modification: JACC health promotion series, J Am Coll Cardiol, 72(2018), 914–926.
10. C.E. Purba, Kembang Telang (*Clitoria ternatea L.*): Pemanfaatan dan Bioaktivitas, Jurnal EduMatSains, 4 (2020), 111-124.
11. E. Cahyaningsih, P.E.S.K. Yuda, P. Santoso Skrining fitokimia dan uji aktivitas antioksidan ekstrak etanol bunga telang (*Clitoria ternatea l.*) dengan metode spektrofotometri UV-VIS. J Ilm Medicam, 5(2019), 51–57.
12. D. Andriani, L. Murtisiwi, Uji aktivitas antioksidan ekstrak etanol 70% bunga telang (*Clitoria ternatea L*) dari daerah Sleman dengan metode DPPH. Pharmacon J Farm Indones. 17(2020), 70–76.
13. N. Gani, L.I. Momuat, M.M. Pitoi, profil lipida plasma tikus wistar yang hiperkolesterolemia pada pemberian gedi merah (*Abelmoschus manihot L.*), 2(2013), 44-49.
14. D.R. Laurence, A.L. Bacharach, Evaluation of drug activities, Elsevier, 2(1964), 891-900
15. P. Molyneux, The use of the stable free radical diphenylpicryl- hydrazyl (DPPH) for estimating antioxidant activity, 26(2004), 210-219.
16. A.S.L. Prado, Y. Shen, R. Ardoin, L.F. Osorio, J. Cardona, et al. Effects of different solvents on total phenolic and total anthocyanin contents of *Clitoria ternatea L* . petal and their anti-cholesterol oxidation capabilities, Int J Food Sci Technol, 54(2019), 424–431.
17. Yanti, D Sabella, A.W. Gunawan, B.W. Lay, *Clitoria ternatea* anthocyanin extract suppresses inflammation in carrageenaninduced rat paw edema via down-regulating genes of phosphoinositide 3-kinase signaling pathway, Food Res, 4(2020), 1357–1362.
18. Y. Wang, T. Liu, Y. Xie, N. Li, Y. Liu, et al. *Clitoria*

ternatea blue petal extract protects against obesity, oxidative stress, and inflammation induced by a high-fat, high-fructose diet in C57BL/6 mice, Food Res Int. 162(2022), 112008.

19. Y.B. Solanki, S.M. Jain, Antihyperlipidemic activity of *Clitoria ternatea* and Vigna mungo in rats, Pharm Biol, 48(2010), 915–923.

20. S.K. Doke, S.C. Dhawale, Alternatives to animal testing: A review, Saudi Pharm J, 23(2015), 223–229.