INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY RESEARCH AND ANALYSIS

ISSN(print): 2643-9840, ISSN(online): 2643-9875

Volume 06 Issue 12 December 2023

DOI: 10.47191/ijmra/v6-i12-75, Impact Factor: 7.022

Page No. 6044-6049

The Effect of Bitter Melon Extract on Cholesterol, IL-6, and Malondialdehyde Serum Levels

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ABSTRACT: Hypercholesterolemia is a condition by an increase total cholesterol and Low Density Lipoprotein (LDL) cholesterol levels, which can lead the secretion of proinflammatory cytokines Interleukin-6 (IL-6) through the activation of the NF-kB pathway oxidative stress, and increase in Malondialdehyde (MDA) levels. Studies showed that the extract of bitter melon contains flavonoids as antioxidants can reduce cholesterol levels. To find out the effect of bitter melon extract on total cholesterol levels, IL-6, and MDA in hypercholesterolemic rats. Experimental research with Post Test Only Group Design. The research subjects were 25 wistar rats divided into 5 groups. Group K(N) normal rats, K(-) were only given a hypercholesterol diet for 28 days, groups K(+), P1, and P2 were given a hypercholesterol diet for 14 days followed by 14 days of hypercholesterol diet along with simvastatin at 0.09 mg/kgBW, bitter melon extract at 150 mg/kgBW, and combination of bitter melon extract at 75 mg/kgBW and simvastatin at 0.045 mg/kgBW. On the 29th day the rats were examined. The results of the Shapiro-Wilk and Levene tests showed that the data were normally distributed and homogenous. One-Way ANOVA and Tukey test was found that the levels of cholesterol, IL-6, and MDA in the P1, P2, and P3 hypercholesterolemic rat groups underwent significantly ($p<0.05$) compared to the control group. The extract of bitter melon, simvastatin, and the combination of simvastatin and bitter melon extract have effect on reducing cholesterol levels, IL-6, and MDA in hypercholesterolemic rats.

KEYWORDS: Bitter Melon, Interleukin-6, Malondialdehyde, Total Cholesterol, Simvastatin

I. INTRODUCTION

Hypercholesterolemia is a condition characterized by an increase in total cholesterol and an increase in Low-Density Lipoprotein (LDL) cholesterol (Kumar, Singh and Dhakal, 2017). Uncontrolled cholesterol levels are a risk factor for stroke, atherosclerosis, and atherogenic(Destiana and Timan, 2018; Vijayan *et al.*, 2018). Stroke is one of the leading causes of death in Indonesia and can significantly reduce the quality of life for the affected individuals (Destiana and Timan, 2018; Vijayan *et al.*, 2018; Saraswati and Khariri, 2021). The increase of fats in the blood will activate the NF-κB signaling pathway, which can trigger macrophages to secrete proinflammatory cytokines, including Interleukin-6 (IL-6), leading to systemic inflammation (Destiana and Timan, 2018; Vijayan *et al.*, 2018). Hypercholesterolemia can result in changes in the physical properties of cell membranes, facilitating the leakage of Reactive Oxygen Species (ROS), which causes an increase in lipid peroxidation and lipid membrane damage. Unsaturated fatty acids undergoing peroxidation are toxic and can produce Malondialdehyde (MDA), which serves as a marker of lipid peroxidation and the occurrence of oxidative stress (Kumar, Singh and Dhakal, 2017).

Bitter melon extract containing polysaccharides, flavonoids, and saponins, plays a role as an antilipidemic, antioxidant, hepatoprotector, and anti-inflammatory agent that acts through the mechanisms of Peroxisome Proliferator-Activated Receptor (PPAR-α), β-Hydroxy β-methylglutaryl-CoA (HMG-CoA reductase), AMP-activated protein kinase (AMPK), and inhibits the Nuclear Factor Kappa B (NF-κB) signaling pathway. Previous studies have extensively examined about the reduction of hypercholesterolemia with combinations of bitter melon extract and other extracts, the effect of bitter melon in reducing triglyceride levels and anti-inflammation, as well as the effect of bitter melon juice on MDA levels (Fernández-Real *et al.*, 2000; Jia *et al.*, 2017; Saraswati and Khariri, 2021). Pharmacological treatment with simvastatin operates through similar mechanisms to a bitter melon extract in lowering cholesterol levels. This study examined the effect of bitter melon extract at a dose of 150 mg/kgBW on total cholesterol levels, IL-6, and MDA, comparing it with simvastatin therapy in hypercholesterolemic rats.

II. MATERIAL AND METHODS

This research was conducted in the laboratory of the center for food and nutrition studies at Gadjah Mada University from 12 June 2023 to 17 July 2023. This research has gone through an ethical review test from the Ethics Committee of Medical Faculty of Sultan Agung University (No.210/VI/2023 Komisi Bioetik). This research used 25 male white Wistar strain rats and they were divided into 5 groups that have already met the inclusion criteria. The group of rats was then divided into (1) normal group: healthy rats on standard feed without any treatment, (2) Control -: rats were given hypercholesterol only feed for 28 days, (3) Control +: rats were given hypercholesterol feed for 14 days, then for the next 14 days were given hypercholesterol feed and simvastatin at a dose of 0.09 mg/kgBW. (4) treatment 1: Rats were given hypercholesterol feed for 14 days, then for the next 14 days, they were given hypercholesterol feed and bitter melon extract at a dose of 150 mg/kgBW, (5) Rats were given hypercholesterol food for 14 days, then for the next 14 days, they were given hypercholesterol feed and bitter melon extract at a dose of 75 mg/kg BW and simvastatin 0.045 mg/kg BW. The hypercholesterol feed in this research was quail egg yolks 10 mg/Kg/BW/day. The sampling was carried out on the 29th day.

Total cholesterol blood samples were taken twice on the fifteenth day and the twenty-ninth day. Blood cholesterol levels were measured by taking blood through the retro-orbital plexus. Total cholesterol levels were measured by using the CHOD-PAP method (Rahma and Syauqy, 2013; Kusuma *et al.*, 2017). Rats were declared hypercholesterolemic if cholesterol levels were >88 mg/dL (Kusuma *et al.*, 2017). IL-6 levels were measured by using the ELISA method, blood samples were taken on the twenty-ninth day through the retro-orbital plexus using a hematocrit tube. The results of the Elisa reader reading contained standard values, then the standard values and absorbance results obtained were made into a standard curve, and the results of IL-6 levels in pg/ml were obtained (Darwin, Afriani and Hanam, 2016). MDA levels were measured by using the Thiobarbituric Acid-Reacting Substances (TBARS) method with a wave length of 532 nm (Tubagus, Momuat and Pontoh, 2015; Rahmawati *et al.*, 2022). Normal MDA levels were between 0.12-1.71 nmol/ml. with an average of 0.26 nmol/ml. After the twenty-ninth day, blood was collected from the rats through the retro-orbital plexus. The MDA absorbance value was measured which was then calibrated using the Tetra Metoxy Propane (TMP) curve. Then the MDA levels were obtained in nmol/ml (Dixon *et al.*, 1998; Purwantyastuti, 2000).

Data on average levels of cholesterol, IL-6, and MDA were presented descriptively in tabular form. The data obtained were processed using computerized methods, and the analysis was performed using SPSS 21.0 for Windows. The data were then tested for normality with the Shapiro Wilk test and homogeneity test with the Levene's test. The distribution of data on cholesterol, IL-6, and MDA levels obtained normal and homogeneous results, so the One Way Annova test was conducted ($p<0.05$) then continued with the post hoc test with the Tukey test.

III. RESULT

A study of bitter melon extract on cholesterol, IL-6, and MDA levels in male rats with a high cholesterol diet showed the results shown in Figure 1 and Table 1.

Table 1. Mean serum of Cholesterol, IL-6 and MDA levels

Groups (Mean)	Cholesterol levels mg/dl Day 15	Total	Cholesterol Total levels mg/dl Day 29	IL-6 levels nmol/ml Day 29	MDA levels (pg / ml) Day 29
K N	79,12		80,93	25,19	0,92
K (-)	189,71		191,19	62,09	10,24
K (+)	188,09		129,11	32,59	3,85
P 1	187,06		101,47	27,97	1,14
P 2	189,41		112,28	29,65	1,98

Table 1 showed that the lowest average total cholesterol levels were in the normal control group, followed sequentially by treatment group 1, treatment group 2, control group +, and lastly, the control group -. Based on the Shapiro-Wilk test, all groups' cholesterol levels indicated a normal distribution data ($P > 0.05$), and the homogeneity test using Levene's Test resulted in homogeneity ($p > 0.05$). Data analysis was continued using One-Way ANOVA test and Tukey's test. The One-Way ANOVA test results indicated a significant difference between the groups ($p = 0.000$). The results of Tukey test showed that the cholesterol levels between any two groups had a significant difference in all groups ($p < 0.05$).

The IL-6 levels were lower in group 1 (P1) after being given a high cholesterol diet and bitter melon extract at a dose of 150 mg/kgBW/day compared to groups K-, K+, and P2. Based on the Shapiro-Wilk normality test, all groups' IL-6 levels showed a

normal distribution of data (P > 0.05), and the homogeneity test using Levene's Test showed a homogenous distribution of data (p > 0.05). Data analysis was continued with One-Way ANOVA test, which indicated a significant difference between the groups (p = 0.000). Subsequently, the data was further analyzed with Tukey's test, which revealed a significant difference in all groups (p < 0.05).

The lowest average MDA levels after being given a high cholesterol diet were in treatment group 1, rats were only given bitter melon extract at a dose of 150 mg/kgBW/day. Tests for normality and homogeneity of MDA levels in this research showed that the data were normally distributed (P>0.05) and homogeneous (p>0.05). The results of the One Way Anova test showed a significant difference between the treatment group and the control group (p=0.000). Tukey's test showed that MDA levels between groups had significant differences in almost all groups (p<0.05), except treatment group 1 (P 1) against the normal control group (K N) and vice versa K N against P 1 with a significant value of 0.807 (p >0.05). Tukey's test on the MDA levels of group P 1 on K N was not significant or there was no difference because P 1 in rats given 150 mg/kgBW/day of bitter melon extract which were given high cholesterol feed had the lowest effect similar to MDA levels on K N (normal rats without being fed high cholesterol feed).

Table 2. Analysis mean serum of Cholesterol, IL-6 and MDA levels

		Uji *Oneway Anova*		
		Sum of Squares	*Mean Square*	*Sig*
Kolesterol	*Between Groups*	351852565.360	87963141.340	
	Within Groups	3582866.400	179143.320	.000
	Total	355435431.760		
IL-6	*Between Groups*	3001301.440	750325.360	
	Within Groups	19452.000	972.600	.000
	Total	3020753.440		
MDA	*Between Groups*	45643569.040	11410892.260	
	Within Groups	107504.800	5375.240	.000
	Total	45751073.840		
****sig p < .05**				

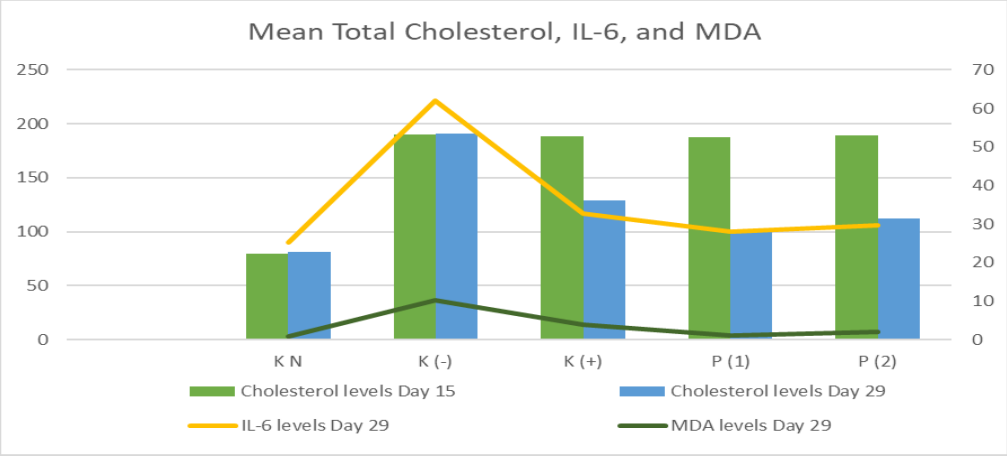

Figure 1. Mean Total of Cholesterol, IL-6, and MDA serum levels

IV. DISCUSSION

The K-, K+, P1, and P2 groups in this study showed hypercholesterolemic rats (cholesterol levels >88 mg/dL) with total IL-6 levels, and MDA levels were higher after being given a high cholesterol diet of 10 mL/kgBW orally for 14 days compared to the KN group. This was in accordance with Kusuma's research (2017) that hypercholesterolemia feed in experimental animals using quail egg yolk as much as 10 ml/kg for 14 days can cause an increase in cholesterol levels beyond the normal threshold (47-88 mg/kg). dl) (Binmowyna *et al.*, 2021). Aprilia's (2018) study stated that there was an increase in LDL and MDA cholesterol levels in rats after being given a high cholesterol diet for 14 days. Wulandari(Wulandari, Padaga and Herawati, 2012) in his research stated that high cholesterol feed can increase free radicals which can be seen from increased levels of MDA. Sarihati's research(2020) also showed an increase in IL-6 levels in rats given a high cholesterol diet. This proves that a high cholesterol diet can trigger an inflammatory process mediated by IL-6.

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Wahjuni (2015) stated that the increase in cholesterol levels in rats given a hypercholesterolemia diet induced the production of reactive oxygen species in adipose tissue and liver. Free radicals that increased in the body then took structural components and atomic electrons which cause a chain reaction resulting in ROS(Wahjuni, 2015). Assessment of lipid peroxidation and antioxidant activity in bitter melon extract can be seen through MDA levels in hypercholesterolemic rats. This research showed that MDA levels in the treatment group were lower than the K- group. This was in accordance with research conducted by Andiani7 that there were significantly lower changes in MDA levels in rats given a high cholesterol diet and bitter melon extract. Maulidia(2021) in her research stated that a high cholesterol diet can affect the inflammatory process which is characterized by higher IL-6 levels.

Isnawati (2014) in her research also mentioned that the content of bitter melon in total cholesterol levels was significantly lower in rats given a high cholesterol diet. Chaturvedi's research (2004) gave bitter melon extract for 30 days and significantly reduced triglycerides, LDL and increased HDL in diabetic rats fed a high-fat diet. However, this research was not in line with research conducted by Rita103 on mice fed high cholesterol diet and bitter melon juice at a dose of 0.5 ml/40 gBW. The results of the study did not show significantly lower cholesterol levels.

The control group + who was given simvastatin therapy on a high cholesterol diet experienced a significant decrease because simvastatin works by inhibiting the HMG-CoA reductase enzyme, increasing the affinity of the LDL receptor, increasing the rate of LDL catabolism, extraction of LDL precursors in the liver and resulting in a decrease in LDL in plasma. This research was in line with research conducted by Artha(2017) that there was a decrease in cholesterol levels in mice on a high cholesterol diet given simvastatin. Decreased cholesterol levels with simvastatin through the inhibition mechanism of HMG-CoA which is the main enzyme for cholesterol synthesis.

The average levels of cholesterol, IL-6, and MDA in the treatment group 1 were the lowest levels compared to the other treatment groups. This research was in line with Andiani (2018) that bitter melon extract can influence lower MDA levels in male white Wistar rats (*Rattus norvegicus*) fed a high-fat diet. Bitter melon extract can reduce cholesterol through many pathways, including increasing the activity of the enzyme Cholesterol 7 alpha-hydroxylase (CYP7A1) by converting cholesterol into bile acids which are then excreted by the body (Zeng *et al.*, 2018). The flavonoid compounds in bitter melon can inhibit the effects of the HMG-CoA reductase enzyme in the body in reducing cholesterol synthesis (Yu *et al.*, 2015). Lutein in bitter melon plays a role in reducing cholesterol levels by capturing free radicals so that it does not cause LDL oxidation (Kurniawaty and Liani, 2013). The polysaccharides in bitter melon extract can trap fat in the small intestine, thus reducing cholesterol levels in the blood by up to 5 % or more, can bind bile salts (cholesterol end product), and excreted along with feces.

Treatment group 2 with a combination of bitter melon extract 75mg/kgBB and simvastatin 0.045mg/kgBB experienced significantly lower changes in total cholesterol, IL-6, and MDA levels but the average value of the results was higher than treatment group 1. This is suspected because the dose of bitter melon extract given was half the dose of bitter melon extract and half the dose of simvastatin. Simorangki's research (2021) stated that using a dose of 150 mg/kgBW/day bitter melon extract is effective in reducing cholesterol levels. Simorangkir (2021) stated that a dose of simvastatin for humans is 10 mg/day, whereas in this research, the combined dose of simvastatin given was only 0.045 mg/kgBW, which is equivalent to a dose of 5 mg/day in humans.

Based on the research results obtained and the theoretical studies that have been described, the hypothesis of bitter melon extract can reduce cholesterol, IL-6 and MDA levels on high cholesterol diet rats has been proven. However, this research only conducted qualitative phytochemical testing of flavonoids. Further researches can conduct quantitative phytochemical testing of other compounds in bitter melon extract.

V. CONCLUSIONS

The extract of bitter melon, simvastatin, and the combination of simvastatin and bitter melon extract have effect on reducing cholesterol levels, IL-6, and MDA in hypercholesterolemic rats.

ACKNOWLEDGEMENT

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. The author would like to thank to everyone who have provided criticism and suggestion during the process of preparing the manuscript

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