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Isolation and Protection Effects of Methyl Gallate from Surian Leaves (*Toona sureni* BL Merr) Against Atherosclerosis of Hypercholesterolemic Rat.

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ABSTRACT

A study on the protection effect of methyl gallate (MG) isolated from Toona sureni leaves against atherosclerosis has been carried out. Rats with high blood cholesterol level were obtained by feeding with high fat diet and inducing propyl thiouracil (PTU). MG was administered orally at the doses of 5, 10, and 20 mg/kg. Histopathologic examination was conducted to measure the aortic wall thickness (AWT) and to observe any improvement of endothelial layer. The study showed that MG at doses of 5 and 10 mg/kg exhibited protection against the atherosclerosis indicated by its ability to prevent the thickening of aortic wall and damage of endothelial layer. This study concludes that MG isolated from Toona sureni leaves exhibits a protective effect against atherosclerosis in hypercholesterolemic rats.

Keywords: methyl gallate, Toona sureni, aterosklerosis, aortic wall thickness, endothelial layer

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INTRODUCTION

Atherosclerosis is an increasing problem around the globe and is a risk factor for coronary heart disease (CHD) which is one of leading causes of death. The World Health Organization (WHO) reported more than 63% of deaths are related to cardiovascular diseases [1]. It has been projected that these diseases will become the leading cause of both death and disability by 2020 [2]. A basic health survey conducted by National Ministry of Health, Republic of Indonesia in 2007 showed that the incidence of coronary heart disease was 7.2% among Indonesian people [3].

Atherosclerosis is a change in the intima layer of arteries, especially the large arteries, which is the accumulation of fat called plaque. This plaque is growing and may cause arteries to lose its elasticity, causing the lumen become narrower and may block the flow of blood into tissues. The risk factors for atherosclerosis can be divided into factors that can be controlled and those that can not be controlled. High blood cholesterol level is classified to factor that can be controlled. Recent study revealed that an increase of blood cholesterol levels above 180 mg/dL would also increase the risk for atherosclerosis. The disease might come even faster if the blood cholesterol level reach over 240 mg/dL [4,5,6].

High blood cholesterol level causes low density liporotein (LDL) molecules to be easily oxidized [6,7,8,9]. The oxidized LDL molecules are attached to the wall of blood vessels and infiltrate into the intima. The molecules will be phagocyted by macrophages (monocytes that have entered into the intima) to form the foam cells [10]. These cells will be mutually bonded to form clumps that grow bigger and narrowing the lumen of blood vessels. This situation gets worsen because when the macrophages ingest the oxidized LDL, the growth factors are produced which stimulate tunica media of smooth muscle cells to infiltrate into the intima layer and then proliferate, causing the lumen to get even narrowed [4]. Nowadays, several classes of conventional medicines are available to prevent the formation of atherosclerosis, but the side effects of these drugs are troublesome, thus the experts are trying to find prospective plant materials as medicines.

One potential natural ingredient to prevent endothelial cell dysfunction is *Toona sureni* (locally known as surian) that contains chelate substances. From the results of previous studies, the leaves of surian has been recognized to contain flavonoids quercetin, terpenoids/tetranortriterpenoid which are surenon and surenin [11], steroids, carotenoids, and methyl gallate (MG) [12]. Our unpublished work on the ethyl acetate fraction of *T. sureni* leaves showed antioxidant effects by means of DPPH method.

The present study is a pure experimental using male white rats (Rattus norvegicus) by means of posttest-only control group design. This study aimed to examine the protection effects of MG from *T. sureni* leaves to the occurrence of atherosclerosis in hypercholesterolemic rats.

MATERIALS AND METHODS

Methyl gallate preparation

The leaves of the plant identified as *Toona sureni* was collected from Padang Panjang, West Sumatra, Indonesia. The ethyl acetate fraction of the leaves was separated by column chromatography by Step Gradients Polarity with eluent composition as follows: 150 ml n-hexane 100%, n-hexane and ethyl acetate with various ratios; and 350 ml of methanol 100%. Each separated fractions produced by such procedure was monitored by TLC using stationary phase of silica gel 60 GF254 plate and mobile phase from combination of n-hexane, ethyl acetate and methanol with different degrees of polarity.

Characterization of Methyl Gallate

MG isolated from *T. sureni* underwent organoleptic examination, chemical constituent examination, determination of melting poin, TLC examination and inspection of purity, the physicochemical determination that included ultraviolet (UV), Infrared (IR), ^{1}HNMR, and ^{13}C NMR spectroscopy.

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Animal Preparation

A number of 25 Wistar rats weighing 200-250 grams were acclimatized under experimental condition for one week prior to experimental procedures. The rats were fed with high cholesterol diet that consisted of standard meal, beef tallow and egg yolk (3 : 1 : 0.25) in addition to propyl thio-uracil (PTU) 3% solution (0.25% v/m) administered orally for the first week before receiving the treatment. The rats were divided into 5 groups: three groups receiving 5, 10, and 20 mg/kg of MG; vehicle control group; and normal group (given normal diet). Each groups continued to receive the diet and the treatment for 60 days. At the end of the procedure, all rats were sacrificed and the hearts and aorta were taken to observe [13,14]. All procedures were approved by The Committee of Research Ethics, Andalas University, Indonesia.

Examination of atherosclerosis lesions

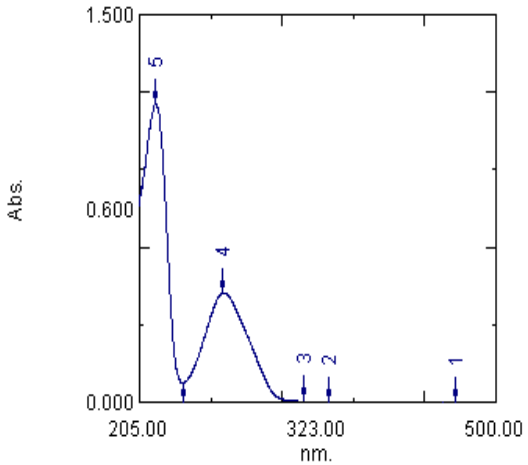
The aortic wall thickness (AWT) of each rats was measured at 6 different locations by using micrometer. Another parameter, degree of endothelial damage of the aortic wall, was examined visually through microscopic evaluation. The cell preparations were made by Haemotoxylin-Eosin staining to determine the degree of cell damage and the occurrence of proliferation of the smooth muscle. The degree was made by scoring the damage: 1 for normal cells; 2 for mild severity; 3 for moderate severity, and 4 for high severity. Both AWT and degree of endothelial damage were stated as mean ± SD.

Data analysis

Data of AWT were analyzed by one-way ANOVA followed by Bonferroni's post-hoc comparisons test. The significance level were taken at $P < 0.05$. While the degree of endothelial cell damage was analyzed by Chi - Square followed by Wilcoxon Signed Ranks Test.

RESULTS AND DISCUSSION

The compound isolated from *T. sureni* leaves was identified as methyl gallate (MG). The compound was white crystal-shaped with a melting point of 181-184^{o}C. The TLC plate gave retention factor of 0.44 from the compound. The maximum wavelength from UV-Vis spectrophotometry was 271 nm with absorbance of 0.67, while the standard MG comparator exhibited maximum wavelength at 275 nm.



No.	P/V	Wavelength	Abs.	D
1	↑	466.00	-0.001	
2	↑	361.80	0.003	
3	↑	341.20	0.004	
4	↑	274.00	0.424	
5	↑	218.00	1.155	
6	↓	456.40	-0.003	
7	↓	240.80	0.074	

Figure 1: UV spectra of isolated compound from *T. sureni* leaves

The result of Fourier Transform Infrared Spectroscopy (FTIR) spectrum analysis of polyphenol compounds from *T sureni* leaves was shown in Figure 2. The stretching frequencies were happening at wavelength 3414 (O-H); 2933 (C-H); 1693 (C=O); and 1619 (C=C, benzene ring). These numbers were quite close to the IR spectrum of MG comparator (Table 3).

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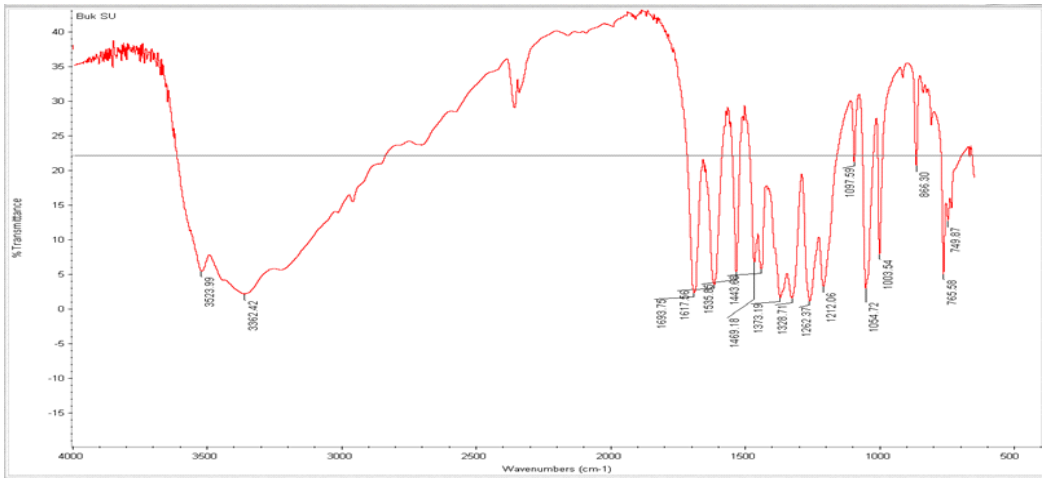

Figure 2: FTIR spectra of isolated compound from *T. sureni* leaves

Table 1. ^{13}CNMR spectrum data of compound isolated from ethyl acetate fraction of *T. sureni* leaves

No	Isolated Compound		MG comparator	
	^{13}CNMR δ (ppm)	^{1}HNMR δ (ppm)	^{13}CNMR δ (ppm)	^{1}HNMR δ (ppm)
1.	145.2	-	144.62	-
2.	137.9	-	137.24	-
3.	120.8	-	120.59	-
4.	109.0	7.188	109.15	7.10
OCH_3	51.2	3.763	51.43	3.76
C=O	166.6	-	166.93	-
OH	-	8.217	-	8.07

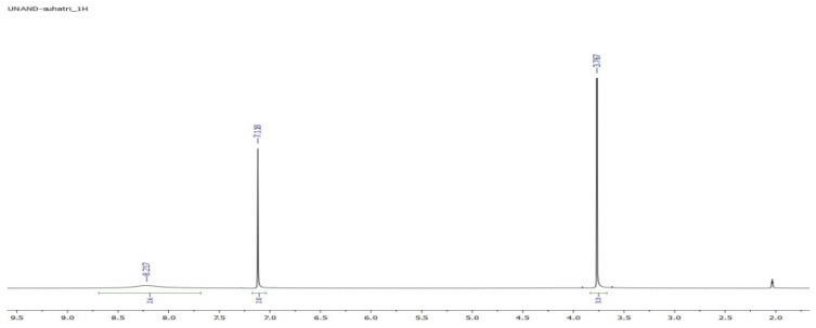

Figure 3: 1HNMR spectra of isolated compound from *T. sureni* leaves

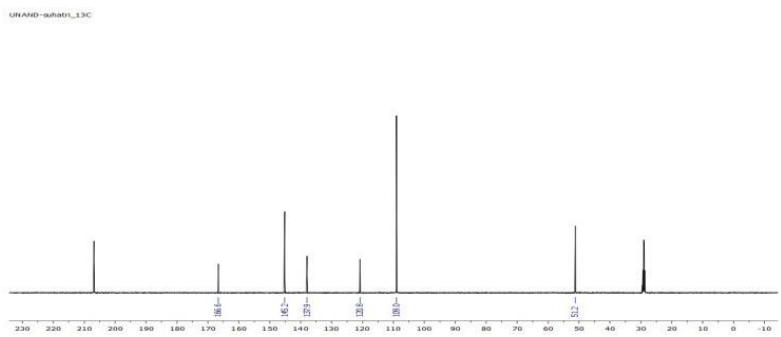

Figure 4: ^{13}C NMR spectra of isolated compound from *T. sureni* leaves

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Aortic Wall Thickness (AWT)

The results showed that administration of MG from *T. sureni* significantly influenced the AWT of the animals. The extract at doses of 5 and 10 mg/kg decreased AWT approaching normal group level (negative control). These two doses did not show a significant difference of AWT as compared with normal animal (P>0.05). On the other hand, higher dose (20 mg/kg) did not show a better effect to protect the aortic wall from thickening, where the AWT of this group was not different with untreated control. MG dose at 5 and 10 mg/kg could prevent the progression of atherosclerosis characterized by absence of proliferation of smooth muscle cells of aorta blood vessel [8].

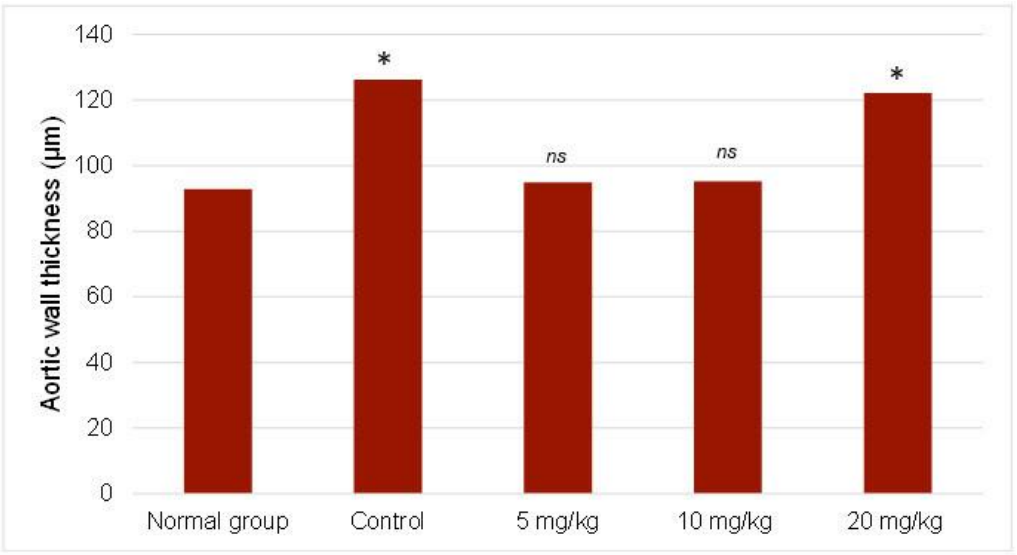

Figure 5: Aortic wall thickness (AWT) of animals treated with methyl gallate from *T. sureni* leaves (significance marks are relative to normal group: ns=not significant; * P<0.05)

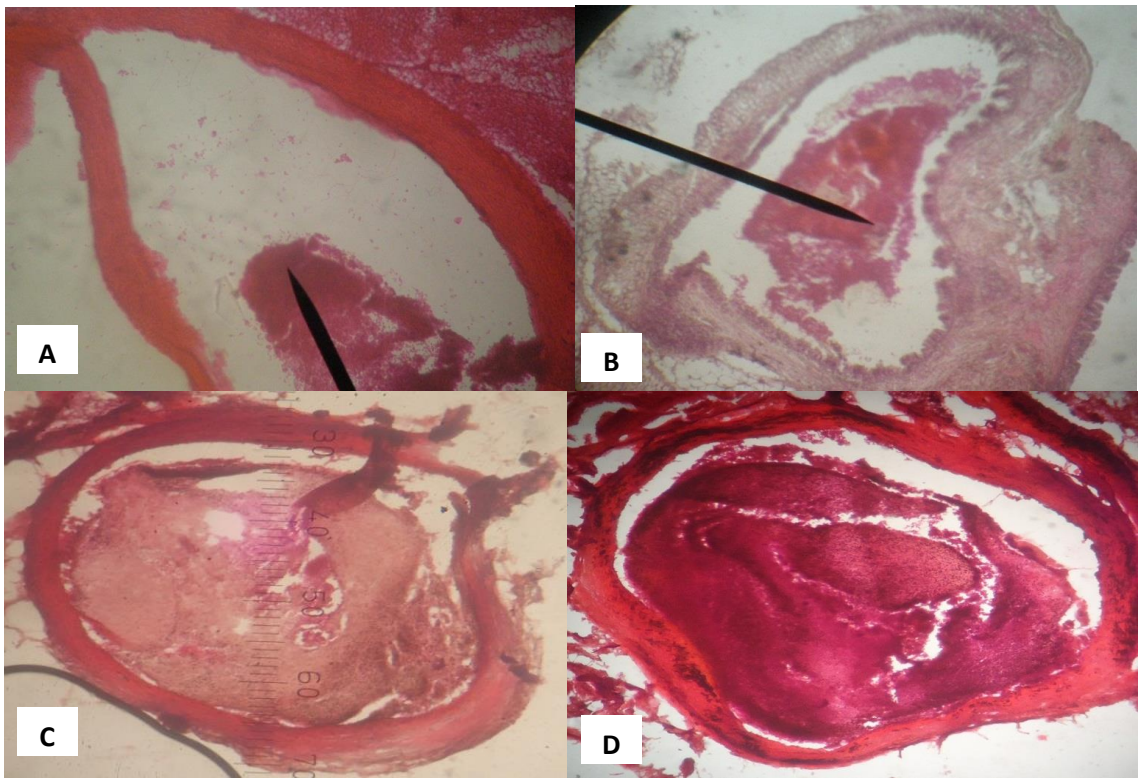

Figure 6: Microphotographs of histologic section of rat aorta after 60 days of treatment (A: normal group, B: hypercolesterolemic control, C: treated with MG 5 mg/kg, and D: treated with MG 10 mg/kg (D).

The surrounding area of aortic endothelial lining of blood vessels of animals treated with 5 and 10 mg/kg of MG had degree of damage of 8.2 and 11.4, respectively. Visual observation of the endothelial layer of this group revealed that tunica intima remained intact and uninterrupted. These scores were lower than those in control group that did not receive MG treatment (scored 22.1). The microphotograph showed extensive damage on the lining of endothelial layer of this group (fig. 6). On the other hand, the normal group was definitely scored 5 for the degree of damage and showed a clear and intact endothelial lining. The improvement of the cell lining achieved by MG at doses of 5 and 10 mg/kg was probably promoted by its antioxidant activity that prevent the oxidation of LDL, a process that may cause atherosclerosis. MG didn't seem to improve the endothelial layer at bigger dose (scored 18.3), a little lower than those in control. This dose was assumed to reach to toxic level in the animal. MG is well known for its antioxidant activity, where it might turn into prooxidant state in bigger doses [15].

CONCLUSION

Methyl gallate (MG) isolated from *Toona sureni* leaves at the doses of 5 and 10 mg/kg exhibits excellent protection effect against atherosclerosis characterized by preventing the thickening of aortic wall and damage of endothelial layer. Higher dose at 20 mg/kg could not prevent the progression of atherosclerosis. Clinical examination for further use of this potential natural compound is strongly suggested.

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