

HYPOCHOLESTEROLEMIC EFFECTS OF METHANOL EXTRACT FROM *Avicennia alba* LEAVES IN ATHEROSCLEROTIC-INDUCED NEW ZEALAND WHITE RABBIT

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ABSTRACT

Avicennia alba from the family of Avicenniaceae locally known as “api-api putih”, is one of the pioneer species of mangrove tree and widely distributed in the coastal region of Southeast Asia. A previous study reported that the leaves and fruits from *Avicennia* sp. including *Avicennia alba* were used in the treatment of skin ulcer, wound and tumor as traditional medicine. This present study was designed to determine the effects of the methanol (MeOH) extract from *Avicennia alba* leaves as a hypocholesterolemic agent. This study was carried out by using eighteen adult male New Zealand White rabbits which were divided into three groups: group I – Control (N), group II – 1% cholesterol diet (C), group III – 1% cholesterol diet + MeOH extract of *Avicennia alba* leaves (AV). This experiment was designed for 10 weeks. Body weight and blood sample were taken on week 0, week 5, and week 10 for biochemical measurements. At the end of the experiment, rabbits were sacrificed and the aorta were removed for intimal lesion analysis using Sudan IV staining. The high density lipoprotein cholesterol (HDL-C) of AV group had increased significantly from week 0 to week 10 compared to N group and C group. From the result of intimal lesion analysis, MeOH extract from *Avicennia alba* leaves reduced the development of aortic atherosclerosis. Therefore, dietary with MeOH extract from *Avicennia alba* leaves show the potential as hypocholesterolemic agent and reduced the atherosclerotic plaque formation in New Zealand White rabbit supplied with high cholesterol diet.

ABSTRAK

Avicennia alba daripada keluarga Avicenniaceae yang dikenali sebagai api-api putih, adalah salah satu pokok bakau perintis yang tersebar luas di pesisir wilayah Asia Tenggara. Kajian sebelum ini menunjukkan bahawa daun dan buah *Avicennia* sp. termasuk *A. alba* telah digunakan dalam perubatan tradisional bagi merawat radang kulit, luka dan tumor. Kajian yang sedang dijalankan adalah bertujuan untuk melihat kesan ekstrak metanol (MeOH) dari daun *Avicennia alba* sebagai agen hipokolesterolemik. Kajian ini dijalankan dengan menggunakan lapan belas ekor arnab putih New Zealand dewasa jantan yang dibahagikan kepada tiga kumpulan: kumpulan I – kawalan normal (N), kumpulan II - 1% diet kolesterol (C), kumpulan III - 1% diet kolesterol + ekstrak MeOH daun *Avicennia alba* (AV). Kajian ini dijalankan selama 10 minggu. Berat badan dan sampel darah diambil pada minggu 0, minggu ke-5, dan minggu ke-10 untuk ujian biokimia. Pada minggu ke-10, arnab dikorbankan dan aorta diambil untuk analisis pemendapan lipid pada lapisan intima menggunakan pewarnaan Sudan IV. Paras kolesterol HDL telah menunjukkan peningkatan yang signifikan daripada minggu 0 hingga minggu ke-10 berbanding kumpulan N dan kumpulan C. Keputusan daripada pewarnaan ini pula menunjukkan ekstrak MeOH dari daun *A. alba* dapat mengurangkan pembentukan plak aterosklerotik pada dinding aorta. Oleh yang demikian, diet bersama ekstrak MeOH dari daun *A. alba* menunjukkan potensi sebagai agen hipokolesterolemik dan mengurangkan pembentukan plak atresklerotik dalam arnab putih New Zealand yang diberikan diet tinggi kolesterol.

Key words: *Avicennia alba*, atherosclerosis, hypocholesterolemic, intimal lesion

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INTRODUCTION

Atherosclerosis is a condition when fat, cholesterol or other substance deposit along the artery walls and build up hard substances called plaque. If this condition continues, it may cause high blood pressure, heart attack, stroke, and sudden cardiac death. Many studies and documentation had shown the relationship between hypercholesterolemia and coronary heart diseases. Coronary heart disease is a pathologic condition associated with abnormalities of high plasma cholesterol concentration that increase the total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) but inversely for the concentration of high density lipoprotein cholesterol (HDL-C). Hypercholesterolemia is a condition where cholesterol level is high in the blood and is also asymptomatic leading to atherosclerosis. HDL-C was reported to possess atheroprotective properties and protection against oxidation of LDL-C (Tsompanidi *et al.*, 2010).

Avicennia sp. was previously reported to be used in the traditional medicine for ulcer, wound and skin ulcer treatment (Chan & Raihan, 1991). There were studies on *A. marina* reported to have non-toxic activity after sub chronic treatments (Ali & Bashir, 1998), exhibit marked inhibitory effect on mouse skin tumor promotion (Itigowa *et al.*, 2001) and as an agent of hypocholesterolemia (Mohamed *et al.*, 2007). The present study is aimed to determine the hypocholesterolemic effect of methanol extract from *A. alba* leaves and its effect on atherosclerotic-induced rabbits.

MATERIALS AND METHODS

Plant Materials

Leaves of *A. alba* were obtained from Kuala Selangor and were dried at 37°C for four days. The defatted leaves were extracted with chloroform to remove chlorophyll and were extracted with methanol using soxhlet apparatus. Then, the methanol (MeOH) extract was concentrated using rotary evaporator and dried into powder using freeze dryer. Eighteen adult male New Zealand White rabbits with average body weight of 2.0 – 2.7 kg were obtained from the animal house, Faculty of Medicine, University of Malaya.

Experimental animals

Eighteen male New Zealand White rabbits were acclimatized for one week in a single cage. The animals were divided into three groups of six: group I – Control (normal rabbit chow) (N), group II – 1% cholesterol diet (C), group III – 1% cholesterol diet + MeOH extract of *A. alba* leaves (AV). This experiment was designed for 10 weeks. Blood

samples were taken prior to treatment on week 0, week 5, and week 10 for lipid profile analysis. Blood samples were drawn from the marginal ear lobe of rabbit into EDTA coated blood tube and centrifuged at 3500rpm for 10min. At the end of the experiment (week 10), rabbits were sacrificed and the aorta were removed for assessment of atherosclerotic plaques using Sudan IV staining.

Lipid profile analysis

Total cholesterol, triglyceride, and high density lipoprotein cholesterol (HDL-C) was measured using commercial kit from Siemens and analysed on ADVIA® 1200 Chemistry System. Low density lipoprotein cholesterol (LDL-C) was calculated using Friedewald equation (Friedewald & Levy, 1972).

Quantification of atherosclerotic plaque

The aorta was removed immediately and washed with normal saline to remove blood clot and opened into longitudinal section to expose the lumen. Atherosclerotic plaque was stained with Sudan IV staining (Prasad, 1997) to quantify lipid deposits. The intimal lesion area was measured from a photograph of the aorta and calculated as a percentage of intimal lesion area per aortic surface.

Statistical analysis

All the data were analysed by one-way ANOVA using SPSS Statistics 17.0. Bonferroni post-test was used to determine significance of difference between groups and the significant value was set at $P < 0.05$.

RESULTS AND DISCUSSION

Lipid profile analysis – (Triglycerides- TG)

There is a significant difference between C and N groups in week 5 and no significant difference between the AV and N groups. Nevertheless, the TG level of AV group was increased significantly at week 10 compared to N group and C group (Table 1).

Total cholesterol (TC)

A total of 1% cholesterol diet showed the development of hypercholesterolemia in C group and AV group after week 5 and 10 compared to N group. Studies on animal models were reported to develop atherosclerosis when prolonged with high cholesterol diet (Semenkovich, 1998; Zulkhairi *et al.*, 2008). TC was significantly different between C group and AV group in both weeks; week 5 and week 10 compared to N group. Supplementation with AV extract maintained the TC level after week 5, however, lower than C group in both weeks.

Table 1. Serum lipid levels in atherosclerotic-induced rabbits prior to the experiment, week 5, and at week 10. (N- Control group (normal diet); C- 1% cholesterol diet; AV- 1% cholesterol diet + methanol extract of *A. alba* leaves)

Group (n=6)	LIPID PROFILE (Mean \pm SD)											
	Triglyceride (mmol/L)			Total Cholesterol (mmol/L)			HDL Cholesterol (mmol/L)			LDL Cholesterol (mmol/L)		
	Week 0	Week 5	Week 10	Week 0	Week 5	Week 10	Week 0	Week 5	Week 10	Week 0	Week 5	Week 10
N	0.43 \pm 0.15	0.55 \pm 0.23	0.57 \pm 0.20	0.95 \pm 0.39	1.08 \pm 0.33	0.83 \pm 0.27	0.62 \pm 0.25	0.68 \pm 0.24	0.56 \pm 0.25	0.14 \pm 0.13	0.16 \pm 0.13	0.01 \pm 0.06
C	0.42 \pm 0.12	1.7 \pm 0.70*	2.08 \pm 2.86	1.47 \pm 0.93	24.48 \pm 0.93*	25.98 \pm 1.41*	0.67 \pm 0.08	0.68 \pm 0.24	0.47 \pm 0.05	0.29 \pm 0.19	23.03 \pm 0.77*	25.53 \pm 1.05*
AV	0.39 \pm 0.12	0.63 \pm 0.18	1.67 \pm 1.23	1.02 \pm 0.38	20.63 \pm 3.41*	21.77 \pm 4.61*	0.58 \pm 0.15	0.72 \pm 0.15	0.86 \pm 0.22	0.26 \pm 0.23	19.63 \pm 3.29*	20.15 \pm 3.06*

Data presented as the Mean \pm SD. Significantly different from N group: * P<0.05.

High density lipoprotein cholesterol (HDL-C)

No significant difference was observed on the HDL level between AV group and C group compared to N group during the experiment. Nevertheless, the HDL-C level of AV group had increased significantly after week 10. Supplementation with AV extract showed the increase in HDL-C level which is correlated with slower progression of atherosclerotic plaque.

Low density lipoprotein cholesterol (LDL-C)

LDL-C level was similar in all groups at the beginning of the experiment but significantly different (P<0.05) in C group and AV group at week 5 and week 10 compared to N group that remained at low level until the end of the experiment. LDL-C level of C group increased significantly in week 5 and week 10 but LDL-C level of AV group only increased significantly in week 5 and remained at the same level after week 10. LDL-C level in C

group is much higher than AV group in week 5 and week 10. AV extract showed the hypocholesterolemic effect by the increased HDL-C level and the maintained LDL-C level after week 10.

Quantification of atherosclerotic plaque

Figure 1 showed that there was intimal lesion area of atherosclerotic plaque in the aorta lumens. There were 33% atherosclerotic plaques per lumen of aorta surface on C group and only 8% of atherosclerotic plaque for the AV group but there were no atherosclerotic plaques on the lumen surface of the normal group. From this study, it showed that cholesterol fed diet caused formation of atheromatous plaque on the aorta surface. Supplementation with *A. alba* showed less formation of atheromatous plaque due to increasing of HDL which is prevented from the formation of the atheromatous plaque.

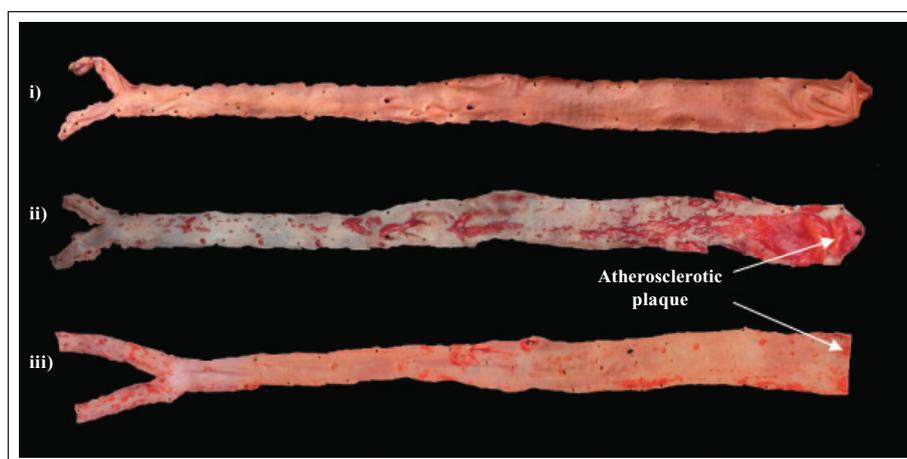


Fig. 1. Intimal lesion area of the aorta from the different groups of rabbits stained with Sudan IV (i- Control group (normal diet); ii- 1% cholesterol diet; iii- 1% cholesterol diet + methanol extract of *A. alba* leaves).

From this study, 1% cholesterol fed to the animals generated hypercholesterolemia in rabbits which is supported by the previous study (Zulkhairi *et al.*, 2008) that showed prolonged high cholesterol diet was one of the factors in the development of atherosclerosis. The present study showed that MeOH extract from *A. alba* can exhibit the HDL-C level and inhibit the progression of LDL-C level in the atherosclerotic-induced rabbits. There is also lower percentage of athero-formation in 1% cholesterol diet group treated with MeOH extract from *A. alba* leaves. It is concluded that the administration of MeOH extract from *A. alba* leaves has the potential as a hypocholesterolemic agent and reduce the atherosclerotic formation in New Zealand white rabbits.

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