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Original Research Article

Anti-atherogenic effect of *Channa striatus* fish extract in high cholesterol-fed rabbits

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Abstract

Purpose: *To determine the effect of aqueous extract of Channa striatus on plasma lipids concentrations, pro-inflammatory cytokines levels, adhesion molecules and arterial plaque formation in cholesterol-fed rabbits.*

Methods: *New Zealand rabbits (n = 30, females) were divided into five groups of six rabbits each as follows: cholesterol control group (0.5 % cholesterol); normal control group (normal diet), positive control group (0.5 % cholesterol + 5 mg/kg atorvastatin); and treatment groups (0.5% cholesterol + 250 mg/kg or 500 mg/kg C. striatus extracts, respectively) for 6 weeks. Blood was taken every 2 weeks and analyzed for total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), triglyceride (TG), tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β). Atherogenic index (AI) was calculated based on LDL:HDL ratio.*

Results: *Cholesterol feeding for 6 weeks resulted in significantly increased TC, LDL, HDL, AI, TG, TNF-α and IL-1β when compared to C. striatus-fed rabbits. Serum LDL was 26.34 ± 1.43 mmol/L in C. striatus group to 30.52 ± 0.82 mmol/L in cholesterol group ($p < 0.05$). C. striatus extract (250 mg/kg) increased HDL by approximately 400 % (6.23 ± 1.76 mmol/L from 0.72 ± 0.18 mmol/L), decreased AI value (4.6 in C. striatus group to 10.4 in cholesterol group). Also, IL-1β and TNF-α concentration also significantly ($p < 0.05$) reduced with C. striatus administration. Interestingly, down-regulation of ICAM-1 and VCAM-1 expression and reduced plaque formation were observed with C. striatus feeding.*

Conclusion: *Channa striatus extract is effective in reducing atherogenesis. This could be due to the high HDL level produced in 250 mg/kg dose group, and strongly suggests the significance of HDL in preventing atherogenesis even if other lipid profiles remain unchanged.*

Keywords: *Channa striatus, Hypercholesterolemia, Pro-inflammatory cytokines, Adhesion molecules, Atherogenic index (AI)*

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INTRODUCTION

Increased levels of circulating native low-density lipoprotein (LDL) and pro-inflammatory cytokines like interleukin-1β and tumor necrosis factor-α are two factors that contribute to the development of atherosclerosis [1]. Native LDL can be modified by reactive oxygen species to oxidized LDL which has lysophosphatidylcholine, a potent chemoattractant for macrophages,

through acetylation. Also, both interleukin-1β and tumor necrosis factor-α, primarily secreted by monocytes, have been reported as mediators of adhesion molecule expression during inflammatory stimulation [2]. Enhanced oxidative stress and pro-inflammatory cytokines up-regulate the expression of vascular cell adhesion molecules-1 (VCAM-1) and intercellular cell adhesion molecules-1 (ICAM-1) on the surface of vascular endothelial cells, which is required for the development of atherosclerotic lesions. VCAM-1 and ICAM-1 in turn promote the recruitment and transendothelial migration of circulating monocytes to the endothelium, eventually leading to progression of vascular diseases [3].

Channa striatus or "Haruan" in Malay, a snakehead fish, is a well-known natural remedy that has long been used for its wound healing properties and is considered a very good source of healthy food due to its high content of fatty and amino acids [4]. Aqueous extract of *C. striatus* has been shown to possess anti-inflammatory and analgesic activities when tested against a chronic inflammation model [5]. Also, its methanol extract has been shown to possess wound-healing properties [5]. High ω-3 & ω-6 polyunsaturated fatty acids (PUFA) of fish have been shown to have positive effects on cardiovascular diseases [6]. Therefore, *C. striatus* is believed to have the potentials to prevent the initial stages of inflammation associated with atherosclerosis through suppression of expression of both adhesion molecules. The present study evaluated the effect of *C. striatus* extract on cholesterol-fed rabbits to determine the changes in adhesion molecule expression which have a strong correlation with the cytokine network and hypercholesterolemia.

EXPERIMENTAL

Preparation of *C. striatus* extract

Live *C. striatus* was obtained from a lake in the Selangor area, Malaysia. Internal organs of the fish were removed and the flesh of the fish with intact skin was then filleted and cleaned. The fish extract was prepared using an electrical pressure cooker according to the method of Zuraini *et al* [7] with some modifications. Briefly, fish fillets were boiled in a ratio of 1 kg fillets to 2 liters of distilled water for 30 min. Next, the extract was instantly filtered and the fillets were discarded. The filtered extract was frozen at -20 °C and then freeze-dried to obtain a solid extract in powdered form.

Ethical matters

Thirty New Zealand White rabbits (female, 2 – 2.5 kg) were procured from the Animal House of the Faculty of Medicine and Health Sciences, Universiti Putra, Malaysia. All experimental procedures were carried out in strict compliance with the Animal Care and Use Committee (ACUC), Faculty of Medicine and Health Sciences, UPM regulations (approval no. UPM/FPSK/PADS/BR-UUH/00288), and complied with international guidelines for animal studies.

Study design and administration of extract

The rabbits were randomly divided into five groups of six animals per group. Each rabbit was weighed and reared in an individual cage. In the first week, all the rabbits were fed 100 g pellets (normal diet) daily in order to allow adaptation to confinement and estimate the number of pellets consumed daily. For the following six weeks, both *C. striatus* treatment groups (A and B) were fed with pellets containing 0.5 % cholesterol plus 250 or 500 mg/kg *C. striatus* extract (reconstituted using distilled water) daily by force-feeding. The normal control group (D) was fed continuously with normal diet, the cholesterol control group (C) was fed pellets containing 0.5 % cholesterol and the positive control group (E) was fed pellets containing 0.5 % cholesterol plus 5 mg/kg atorvastatin drug once daily, orally. The weight changes in all experimental rabbits were monitored bi-weekly.

Preparation of cholesterol-enriched diet

Pure cholesterol (95 %, powdered) was obtained from Sigma Chemicals (St. Louis, MO, USA). To prepare the cholesterol-enriched pellet, the powder was first dissolved in chloroform and then mixed with a certain quantity of intact pellets. Next, the cholesterol-enriched pellets were dried in the fume hood until the odor of chloroform was not detected. The cholesterol-rich diet was prepared every two weeks to reduce the accumulation of oxidized lipids [8].

Blood collection, centrifugation and analysis

Blood samples were drawn from the rats after one week of adaptation to determine the plasma lipid profile and pro-inflammatory cytokines. Approximately 15 mL of blood was taken from the ear vein of each rabbit and decanted into an EDTA tube and a plain tube. Thereafter, blood sampling was performed every two weeks following treatment. Blood samples in EDTA or plain tubes were then centrifuged at 3000 rpm for

10 min to obtain either plasma or serum, respectively. The serum and plasma samples were instantly stored in Eppendorf tubes and kept frozen at -20 ^{o}C until analysis. Plasma lipid profile – total cholesterol (TC), HDL and LDL – were determined enzymatically using a 902 Hitachi Chemical Analyzer and Roche® reagents. Serum levels of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) were determined using ELISA kits (Anogen, Mississauga, ON, Canada) according to the manufacturer's instructions. Atherogenic index (AI) of each animal group was calculated by applying the formula LDL/ HDL [7].

Immunohistochemistry

After six weeks of treatment, all experimental rabbits were sacrificed by an overdose of ethyl ether and the aorta tissues were collected for ICAM-1 and VCAM-1 expression analysis via immunohistochemistry staining. All aorta tissues were first fixed in 10 % formalin solution and then subjected to tissue processing and tissue embedding to obtain an aorta tissue block. Serial aorta cross-sections were cut on a microtome and placed on poly-L-lysine slides. The slides were heated in an oven at 60 °C for 20 min and then deparaffinized. Endogenous peroxidase activity was blocked with 0.3 % hydrogen peroxide in TBS for 1 h. The slides were incubated in 3 % bovine serum albumin in TBS solution (pH 7.6) for 1 hour to block nonspecific binding sites. Thereafter, they were incubated with primary antibodies at 1:100 dilution (Santa Cruz Biotechnology, Santa Cruz, CA USA) for 20 min at room temperature. Following a TBS rinse, the slides were incubated with a goat anti-rabbit Ig conjugated to peroxidase-labeled polymer at 1:500 dilution (DAKO EnVision, Carpinteria, CA, USA). Bound antibodies were visualized with diaminobenzidine (DAB substrate- chromogen solution, DAKO). The slides were further rinsed with distilled water and then counterstained with hematoxylin for 10 seconds. Slides were cleaned under running water for about 5 - 10 min, dehydrated, mounted with coverslips and viewed under the microscope for the detection of adhesion molecules. Grading was performed for assessing ICAM-1 and VCAM-1 expression in rabbit aortic rings. Briefly, a score of 5 is an aorta with thick stained rings; 4 are thin completely stained rings; 3 is thick irregular patches of stain; 2 represent thin irregular patches; 1 is for spotty staining and 0 has no staining.

Sudan IV and Oil red O staining

Except for the thoracic aorta for ICAM, VCAM, and oil red O staining, Sudan IV staining of the aorta was performed on the inner surface from the aortic arch to the iliac bifurcation. The extent of staining was evaluated by measuring both the total area and the stained area using Image-Pro Plus software (Rockville, Maryland, USA). The Oil red O staining of the histological sections processed was performed using the method of Oh *et al* [9].

Statistical analysis

All data obtained were presented as mean ± SD and analyzed using the GLM-univariate analysis of variance (SPSS version 25.0). The lipid profile and pro-inflammatory cytokines were compared among and within treatment groups to determine the differences. For *post-Hoc* test, a Duncan-New Multiple Range Test was further carried out to determine a specific difference between treatment groups and weeks if GLM test showed or revealed a significant overall difference at p value < 0.05.

RESULTS

Effect of *C. striatus* aqueous extract on plasma lipid profile in high cholesterol-fed rabbits

The mean changes in plasma lipid profiles of normal-fed and cholesterol-fed rabbits treated with 250 and 500 mg/kg *C. striatus* aqueous extracts are illustrated in Table 1. Cholesterol-feeding significantly increased ($p < 0.05$) the TC, LDL and HDL in group C starting from week 2 to week 6. Atherogenic index (AI) of each group is shown in Figure 1. Initial values of AI at week 0 were almost similar in all groups. In the following 6 weeks, AI remained unchanged in group D but increased significantly in group C at weeks 2, 4 and 6 ($p < 0.05$). Also, the AI was significantly reduced in Group A compared to Group C ($p < 0.05$) at weeks 2, 4 and 6. Group E also revealed a significant reduction of AI ($p < 0.05$) compared to C ($p < 0.05$) at weeks 2 and 6, although the reduction was only apparent but not statistically significant at week 4 ($p > 0.05$).

There were no significant differences ($p > 0.05$) between groups B and C (0.5 %) during week 2 and week 4. Conversely, Group B showed a significantly high value of AI compared to C by week 6 ($p < 0.05$). There were no significant differences ($p > 0.05$) between groups E and A at weeks 2 and 4 in terms of AI lowering. However, group A showed a significantly smaller AI compared to group E ($p < 0.05$) at week 6. Thus, this indicates that 250 mg/kg *C. striatus* extract showed a comparable effect to the commercial

anti-lipidemic drug atorvastatin or even better effects in atherogenic protection.

Sudan IV and Oil red O staining analysis

Interestingly, as shown in Figure 2, *C. straitus* at 250 mg/kg (Group A) reduced significantly ($p < 0.05$) the fatty streak formation (expressed as the percentage area of the atherosclerotic lesions on the inner surface) by 17.5 ± 3.5 % when compared to 500 mg/kg *C. straitus* (Group B) 47.2 ± 5.7 %, Control hypercholesterolemic rats (Group C) 58.9 ± 6.35 %, normal controls (Group D) 5.3 ± 1.9 % and atorvastatin treatment (Group E) 15.3 ± 2.9 %, respectively. Histologically, when stained with Oil red O, similar results were observed (Figure 3) whereby the atherosclerotic plaques were reduced by treatment of rabbits with C*. straitus* at 250 mg/kg.

Table 1: Changes in plasma lipid profile of rabbits fed with normal diet or 0.5 % cholesterol

Week		*C. striatus* extract		Cholesterol Control (Group C)	Normal Control (Group D)	Atorvastatin (Group E)
		250 mg/kg (Group A)	*500 mg/kg (Group B)*			
0	TC	1.35±0.07	1.44±0.20	1.35±0.05	1.42±0.07	1.33±0.05
	LDL	0.32±0.11	0.34±0.12	0.27±0.08	0.38±0.15	0.30±0.10
	HDL	0.72±0.18	0.48±0.14	0.43±0.13	0.50±0.16	0.50±0.13
	TG	3.02±0.43	2.58±0.32	2.53±0.39	2.02±0.85	2.80±0.43
2	TC	$14.46±0.60^{b}$	$0.18±3.76^{c}$	$19.59±2.16^{c}$	$0.52±0.20^{a}$	$2.61±2.49^{b}$
	LDL	$10.66±1.59^{b,c}$	$13.40±2.36^{c}$	$11.32±1.83^{b}$	$0.25±0.07^{a}$	$8.84±1.76^{b}$
	HDL	$2.04±0.21^{c}$	$1.94±0.37^{c}$	$1.66±0.23^{b}$	$0.38±0.10^{a}$	$1.47±0.12^{b}$
	TG	$2.92±0.71^{b}$	$2.69±0.50^{b}$	$2.46±0.25^{a}$	$1.50±0.49^{a}$	$1.29±0.18^{b}$
4	TC	$21.51±4.05^{b}$	$34.81±2.12^{d}$	$29.14±3.61^{c}$	$1.40±0.09^{a}$	$24.99±0.96^{b}$
	LDL	$16.43±3.25^{b}$	$23.69±2.18^{c}$	$20.64±2.44^{c}$	$0.28±0.03^{a}$	$17.11±1.20^{b}$
	HDL	$2.59±0.34^{b,c}$	$3.26±0.98^{c}$	$2.29±0.28^{b}$	$0.47±0.14^{a}$	$2.19±0.19^{b}$
	TG	$2.39±0.29^{a}$	$4.76±0.45^{c}$	$2.55±0.46^{a}$	$2.15±0.29^{a}$	$3.18±0.20^{b}$
6	TC	$31.39±0.13^{b}$	$36.79±0.24^{d}$	$33.40±0.16^{c}$	$0.73±0.16^{a}$	$31.61±0.31^{b}$
	LDL	$29.71±0.83^{c}$	$26.34±1.43^{d}$	$30.52±0.82^{c}$	$0.52±0.09^{a}$	$21.15±2.34^{b}$
	HDL	$6.23±1.76^{c}$	$2.70±0.36^{b}$	$3.08±0.24^{b}$	$0.51±0.14^{a}$	$3.76±0.70^{b}$
	TG	$6.21±0.79^{c}$	$4.07±1.37^{b}$	$5.49±1.53^{c}$	$2.88±0.33^{a}$	$5.46±0.25^{c}$

Note: Values are expressed in mean ± SD (n=6). Superscripts a - d indicates statistically significant differences ($p < 0.05$) in the same row

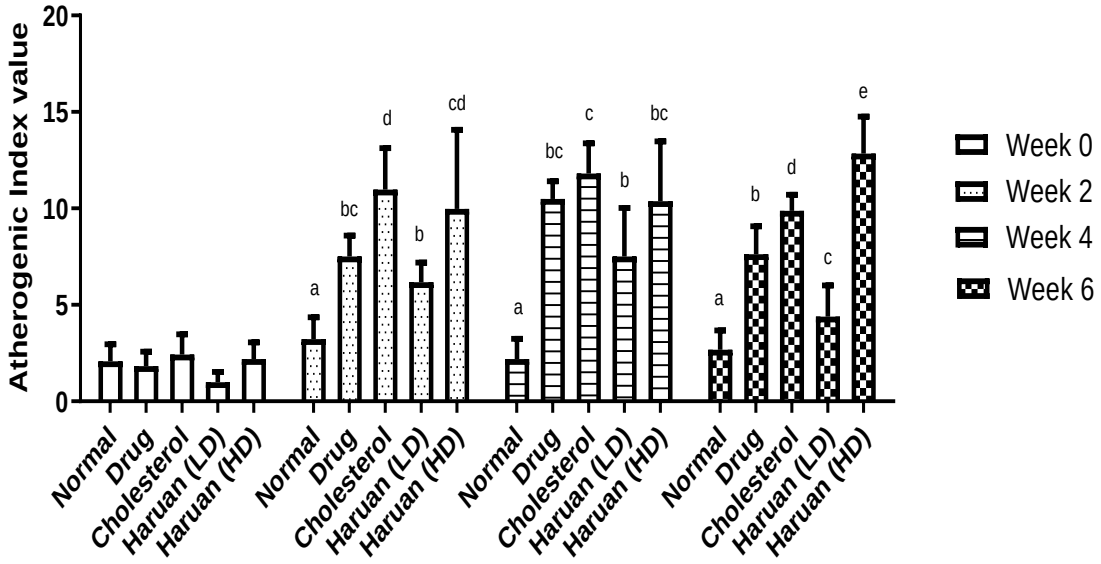

Figure 1: Atherogenic index (AI) of rabbits fed with normal diet or 0.5 % cholesterol. ***Key:*** Superscripts a - e indicate statistically significant differences ($p < 0.05$) compared to other treatment groups in a particular week. Haruan = *C. striatus*

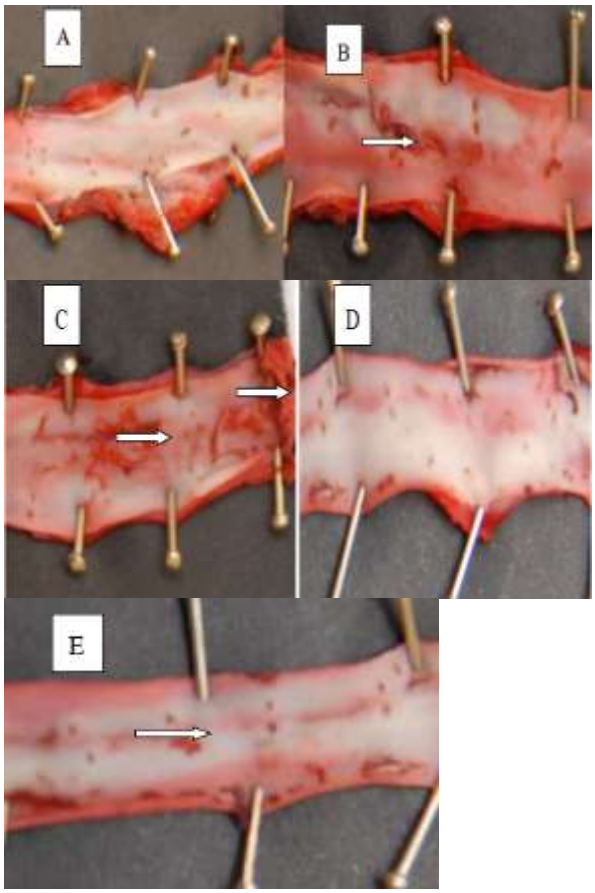

Figure 2: Sudan IV stained aorta sections of rats fed with normal diet or 0.5% cholesterol. ***Key:*** A: Sudan IV staining of 0.5 % cholesterol + 250 mg/kg *C. striatus* extract-treated group; B: Sudan IV staining of 0.5 % cholesterol + 500 mg/kg *C. striatus* extract-treated group; C: Sudan IV staining of 0.5 % cholesterol control group; D: Sudan IV staining of normal control group; E: Sudan IV staining of 0.5 % cholesterol + 5 mg/kg atorvastatin positive control group. Lesions (red stain/arrow) with high deposition in hypercholesterolemic rats (C). Normal Control showed no deposition. The plague deposition is minimal in the Positive Controls (E) and *C. striatus* low dose (A). However, high dose of *C. striatus* did not suppress the deposition of plague (B)

Effect of aqueous extract of *C. Straitus* on serum pro-inflammatory cytokine levels

As shown in Figure 4 and Figure 5, the TNF-α and IL-1β levels were significantly reduced in Groups E, A and B compared to C ($p < 0.05$). Statistical analysis showed that 500 mg/kg *C. striatus* extract produced the best effect in TNF-α lowering but its effect on IL-1β lowering was not as good as 250 mg/kg *C. striatus* extract and 5 mg/kg atorvastatin. There were no significant differences between groups E and A ($p > 0.05$), thus suggesting that both treatment groups showed similar effect in TNF-α and IL-1β reduction.

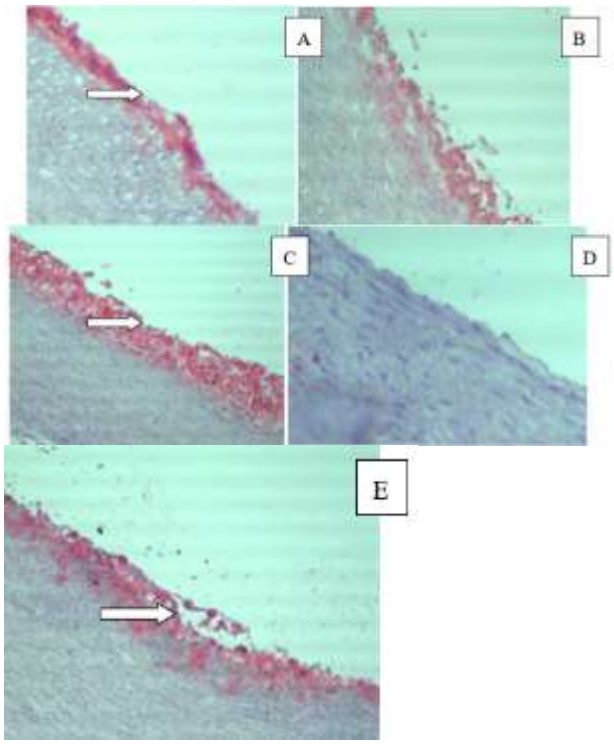

Figure 3: Oil red O stained aorta sections of rats fed with normal diet or 0.5 % cholesterol. A: Oil Red O staining of 0.5 % cholesterol + 250 mg/kg *C. striatus* extract-treated group; B: Oil Red O staining of 0.5 % cholesterol + 500 mg/kg *C. striatus* extract-treated group; C: Oil Red O staining of 0.5 % cholesterol control group; D: Oil Red O staining of normal control group; E: Oil Red O staining of 0.5 % cholesterol + 5 mg/kg Atorvastatin positive control group. Atherosclerotic plaques (red stain/arrow) are relatively uniform with high deposition in hypercholesterolemic rats (C). Normal Control showed no plaque. The plague deposition is minimal in the positive Controls (E) and C. *striatus* low dose (A). However, high dose of *C. striatus* did not suppress the deposition of plaque (B). Magnification 100×

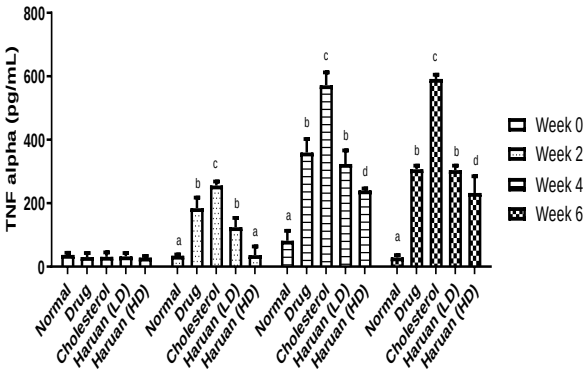

Figure 4: The TNF-α (pg/mL) of rabbits fed with normal diet or 0.5 % cholesterol. ***Key:*** a - d indicates statistically significant difference ($p < 0.05$) when compared to other treatment groups at a particular week. Haruan = *C. striatus*

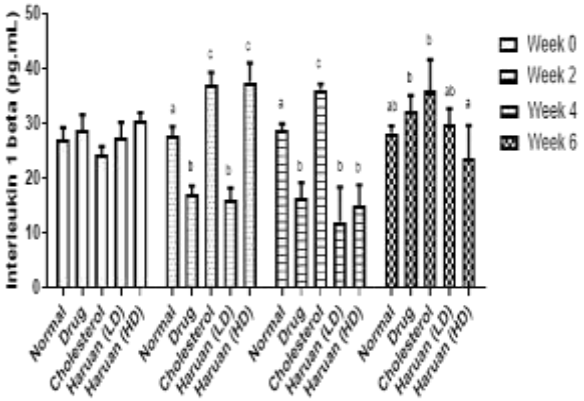

Figure 5: Serum IL-1β (pg/mL) of rabbits fed with normal diet or 0.5 % cholesterol. a-d indicate statistically significant difference (p < 0.05), when compared to other treatment groups in a particular week. Haruan = *C. striatus*

Effect of *C. straitus* on the expression analysis of ICAM-1 and VCAM-1

The scoring values are shown in Figures 6 and 7. Figures 8A-8E (ICAM-1) and Figures 9A-9E (VCAM-1) shows the expression patterns of these two proteins in aortic endothelial cells for each group. Based on comparison of the intensity and distribution of brown color staining, the expression of ICAM-1 and VCAM-1 was determined under microscopic visualization. The VCAM-1 and ICAM-1 proteins were predominantly expressed by endothelial cell. Microscopic analysis showed that aortic ICAM-1 and VCAM-1 expression was up-regulated by hypercholesterolemia. The normal control (NC) showed approximately no ICAM-1 and VCAM-1 expression.

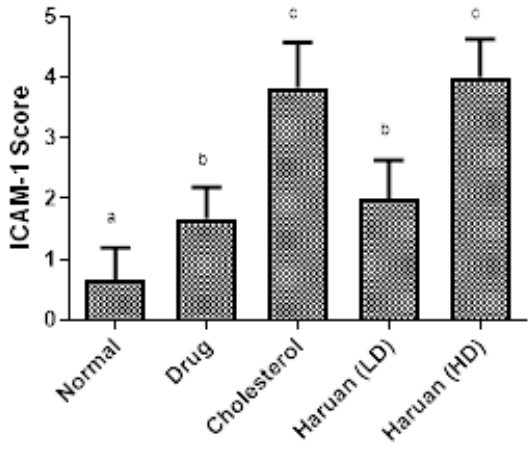

Figure 6: The scoring values of ICAM-1 expressions. a-d indicate statistically significant differences (p < 0.05), when compared to other treatment groups

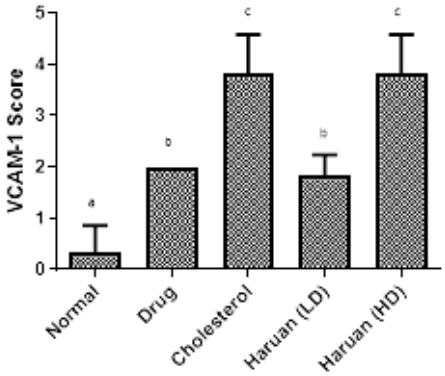

Figure 7: The scoring values of VCAM-1 expression in five animal groups. ***Key:*** a - d indicate statistically significant differences (p < 0.05), when compared to other treatment groups. Haruan = *C. striatus*

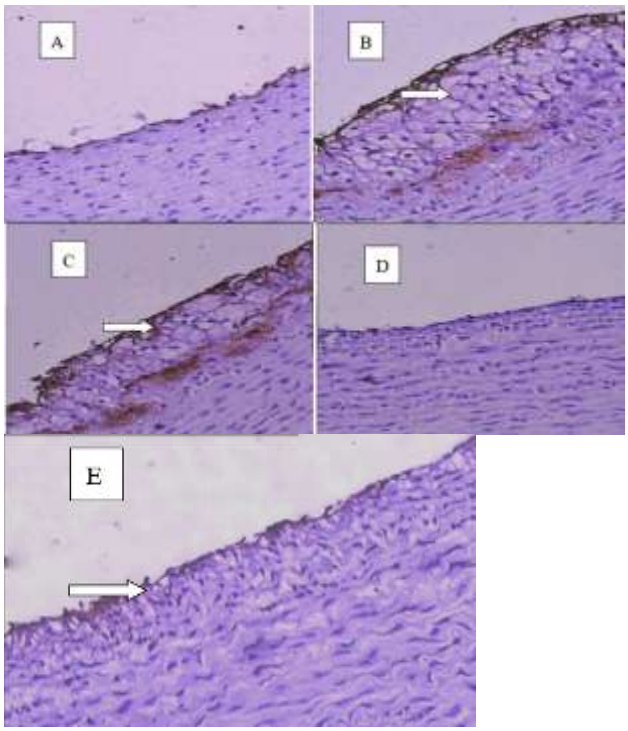

Figure 8: ICAM-1 expression in aorta of rats. A: ICAM-1 expression of 0.5 % cholesterol + 250 mg/kg *C. striatus* extract-treated group; B: ICAM-1 expression of 0.5 % cholesterol + 500 mg/kg C. striatus extract-treated group; C: ICAM-1 expression of 0.5 % cholesterol control group; D: ICAM-1 expression of normal control group; E: ICAM-1 expression of 0.5 % cholesterol + 5 mg/kg atorvastatin positive control group ICAM-1 expression (brown stain/arrow) was up-regulated by hypercholesterolemia (C). Normal Control showed no ICAM-1 expression. The ICAM-1 expression is down-regulated in the Positive Controls (E) and C. striatus low dose (A). However, high dose of C. striatus did not suppress the expression of ICAM-1 (B). Magnification 200×

The ICAM-1 and VCAM-1 expression were significantly down-regulated in groups E and A which showed relatively small scoring values

compared to group C ($p < 0.05$). Group B did not show apparent suppression in the expression of ICAM-1 and VCAM-1 and revealed no statistically significant differences compared to group C ($p > 0.05$). As a result, it is suggested that ICAM-1 and VCAM-1 expression was nearly same in groups C and B. There were no significant differences between groups E and A ($p > 0.05$), suggesting that both treatment groups showed comparable effects in down-regulation or suppression of ICAM-1 and VCAM-1 expression.

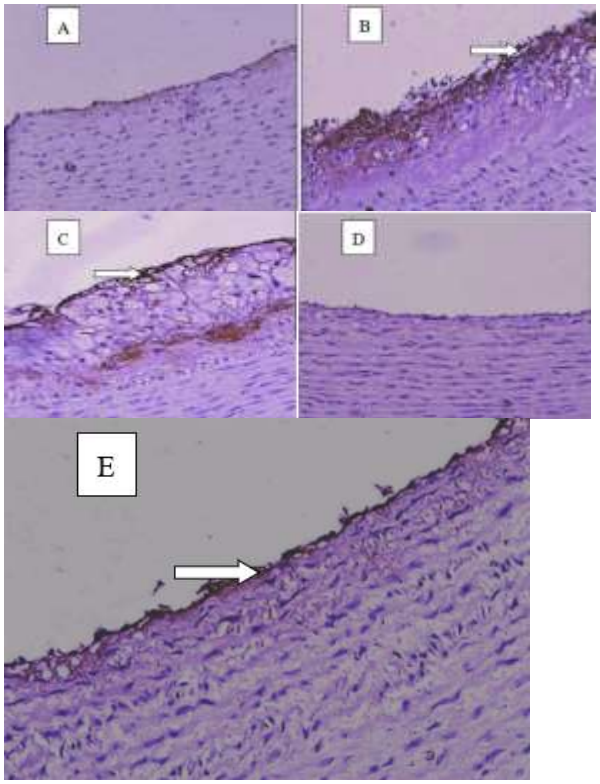

Figure 9: VCAM-1 expression in aorta of rats. A: VCAM-1 expression of 0.5 % cholesterol + 250 mg/kg *C. striatus* extract-treated group. B: VCAM-1 expression of 0.5 % cholesterol + 500 mg/kg *C. striatus* extract-treated group. C: VCAM-1 expression of 0.5 % cholesterol control group. D: VCAM-1 expression of normal control group. E: VCAM-1 expression of 0.5 % cholesterol + 5 mg/kg atorvastatin positive control group. VCAM-1 expression (brown stain/arrow) was up-regulated by hypercholesterolemia (C). Normal Control showed no VCAM-1 expression. The VCAM-1 expression is down-regulated in the Positive Controls (E) and *C. striatus* low dose (A). However, high dose of *C. striatus* did not suppress the expression of VCAM-1 (B). Magnification 200×

DISCUSSION

Previous reports have shown that high cholesterol increases the generation of reactive oxygen species (ROS) by smooth muscle and vascular endothelial cells. These ROS can then cause endothelial dysfunction and LDL oxidation, which may play a role in atherogenesis. A high level of LDL and its oxidation contributes to the recruitment of circulating monocytes into the arterial intima [10]. *In vivo*, oxidized LDL (Ox-LDL) particles are chemotactic, attract monocytes and elicit inflammatory responses by increasing adhesion molecule expression or through production of cytokines such as IL-1□ [10]. Foam cell production and lipid deposition in the artery wall are caused by the transformation of recruited monocytes into macrophages and the subsequent uptake of Ox-LDL into the intima. The current investigation showed that atherosclerotic aortic lesions and severe hypercholesterolemia were produced in rabbits fed an atherogenic diet.

In the present study, cholesterol-fed rabbits showed an increase in the plasma TC level to 33.40 mmol/L at the end of experiment as well as a simultaneous elevation of LDL and LDL/HDL ratio in each group. Normal plasma TC levels for New Zealand White rabbits were in the range of 0.78 – 1.68 mmol/L, with young animals of less than 3 kg body weight, usually at the upper part of this range [11]. Nachtigal *et al* reported that rabbits fed with a cholesterol-enriched diet showed a steady rise in serum concentrations of TC by almost 30-fold in comparison to rabbits given a normal diet during a study on the application of stereological methods for the quantification of VCAM-1 and ICAM-1 expression in early stages of rabbit atherogenesis [11]. This finding is in agreement with previous research that showed a high-cholesterol diet produced an increase in plasma TC, LDL and LDL/HDL ratio in atherosclerotic rabbits [11]. In this study, the administration of 250 mg/kg *C. striatus* extract significantly reduced plasma TC levels throughout the experiments in cholesterol-fed rabbits. Furthermore, rats fed 250 mg/kg *C. striatus* extract showed a significant reduction of plasma LDL level at week 2 as well as an increase in plasma HDL at weeks 2 and 6 compared to group C.

Transporting cholesterol from the liver to tissues where it is incorporated into cell membranes is the primary function of low-density lipoprotein (LDL). The understanding that LDL cholesterol causes atherosclerosis and subsequent heart disease is a fundamental of modern medicine. Oleic acid, a particular monounsaturated fatty acid (MUFA) found in *C. striatus* extract, has been reported to lower LDL cholesterol, suppress oxidative LDL *in vitro* and reduce macrophage uptake of plasma ox-LDL [7,12]. Besides, high percentages of amino acids within *C. striatus* extract such as aspartic acid and glutamic acid have been reported to increase HDL-C in

hypercholesterolemic rabbit studies [12,13]. High-density lipoproteins (HDLs) exert various potentially anti-atherogenic properties due to their ability to drive a process called "reverse cholesterol transport" (RCT). The RCT explains how cholesterol is transported to the liver and other steroidogenic organs by HDL-mediated efflux from non-hepatic cells. There, it is utilized for the synthesis of lipoproteins, bile acids, vitamin D and steroid hormones. [14]. Indeed, group A showed significant AI lowering compared to group C. Fish oils were found to inhibit atherosclerosis development in cholesterol-fed rabbits [14]. An essential component of *C. striatus* extract, polyunsaturated fatty acids (PUFA) [7], has been shown to have protective effects against cardiovascular diseases [7]. The ω-3: ω-6 ratio of *C. striatus* was 1:1.82 [7], which would contribute to a healthy human diet based on the ratio (1:1-1:1.5) recommended by Osman *et al* [15].

Indeed, ω-3 PUFAs, which are mainly found in fatty fish such as docosahexaenoic acid (DHA), have been reported to have preventive effects on human coronary artery disease [15]. The results from previous studies corroborate the present finding associated with an effect of 250 mg/kg *C. striatus* extract on plasma lipid profiles. Fish oil also affects the metabolism of inflammatory mediator molecules like interleukins and tumor necrosis factor-α, [7], thus believed to play a role in atherogenesis and plaque stability [15]. *Channa striatus* was discovered to be the most potent inhibitor of transudative (42.9 % inhibition) and proliferative (31.1 % inhibition) components of chronic inflammation [5].

De Caterina *et al* reported that both omega-6 and omega-3 fatty acids have anti-inflammatory properties that suppress the atherogenic activation of vascular endothelial cells [16]. The generation of adhesion molecules, chemokines and interleukins by endothelial cells, essential mediators that drive the atherosclerotic process, is inhibited by both PUFA types. Both doses of 250 and 500 mg/kg *C. striatus* extract significantly reduced the level of pro-inflammatory cytokines (TNF-α and IL-β) in cholesterol-fed rabbits. This finding shows the capability of *Channa striatus* extract to suppress the inflammatory response.

Li *et al* reported that an atherogenic diet treatment generated hypercholesterolemia in rabbits, which after six weeks led to an increase in the expression of VCAM-1 and ICAM-1 as well as a build-up of macrophages in the aortas [17]. Intimal monocytes/macrophages have been reported at lesion-predisposed sites of normal rabbits. Consistent with the present results, the expression of VCAM-1 and ICAM-1 by aortic endothelium in normal animals may result in occasional recruitment of monocytes into the intima. These intimal cells may contribute to enhanced recruitment to the arterial intima of circulating monocytes after the initiation of hypercholesterolemia. The generation of chemokines and inflammatory cytokines after the absorption of oxidized lipoproteins and conversion into foam cells is one possible explanation for this. Therefore, a backdrop for the establishment of atherosclerotic lesions may be provided by the localized expression of VCAM-1 and ICAM-1 in the aortic endothelium of normal animals [17].

The mechanism of anti-atherogenic effect of omega-3 fatty acids has been recently proposed. It is believed to alter the metabolism of adhesion molecules such as VCAM-1 and ICAM-1 [11]. An *in vitro* model study of the early steps in atherogenesis demonstrated that ω-3 fatty acid, DHA, significantly inhibited events involving endothelial activation, including VCAM-1 and, to a lesser extent, ICAM-1 after stimulation with factors such as IL-1β and TNF-α [17]. Weber *et al*. also demonstrated that DHA inhibits the nuclear factor kappa B (NF-κB) system of transcription factors, which controls the expression of adhesion molecules and coordination of leukocyte-specific chemo-attractants upon cytokine stimulation [18]. Furthermore, the inhibitory effect of oleic acid on endothelial activation has proven *in vitro* coupling with reduction of VCAM-1 mRNA [11], meanwhile, dietary aspartic acid and glutamic acid were discovered to prevent fatty streak initiation in cholesterol-fed rabbits [17].

The present analysis revealed aortic VCAM-1 and ICAM-1 expression were up-regulated by hypercholesterolemia and there was significant down-regulation of expression in both adhesion molecules in the rabbits administered 250 mg/kg *Channa striatus* extract and 5 mg/kg atorvastatin. Therefore, reduction of plasma TC level, LDL level, pro-inflammatory cytokines concentration and down-regulation of both VCAM-1 and ICAM-1 expression may contribute to atherogenic attenuation. The result of this study shows that the atherogenic prevention effects of 250 mg/kg *C. striatus* extract are similar to those of 5 mg/kg atorvastatin. This finding supports the anti-atherogenic effects of *C. striatus* extract at 250 mg/kg but not at 500 mg/kg, which did not show any desirable reduction of plasma TC level and LDL level, suppression of AI value and down-regulation of both adhesion molecules' expression compared to the cholesterol control

group (Group C). Some studies of supplementation in ω-3 PUFA-rich oils reported no difference while some others found a decrease or an increase in susceptibility of LDL particles to oxidation 17]. Dissimilarities in experimental design and supplementation dosage may explain these inconsistent results. Furthermore, a high dietary intake of ω-6 PUFAs (arachidonic acid and linoleic acid) found in *C. striatus* extract has been shown to promote LDL oxidation and enhance atherogenesis [7,17]. Arachidonic acid is a long-chain (ω-6) fatty acid (20:4(ω-6)) that results from the elongation and desaturation of linoleic acid (18:2(ω-6)) or directly from the diet [18]. Excess arachidonic acid was shown to be unhealthy because it promotes inflammation. Arachidonic acid produces eicosanoids, which control physiological processes that may raise the risk of coronary heart disease. However, it has been observed that an upper limit of 3 % of energy can be consumed as linoleic acid to prevent potential negative effects from excessive arachidonic acid (20:4n-6) and its eicosanoid derivatives [7].

CONCLUSION

Low doses of *C. striatus* reduce atherogenesis by increasing the proportion of HDL, decreasing the AI value, as well as IL-1 β and TNF- α concentrations, down-regulating the expression of ICAM-1 and VCAM-1, thus finally reducing the plaque formation in arteries. These results suggest that *C. striatus* aqueous extract improves cholesterol profile and prevents arteriosclerosis, thus making it a potential source of molecules with anti-arteriosclerosis properties.

DECLARATIONS

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Ethical approval

All experimental procedures were carried out in strict compliance with the Animal Care and Use Committee (ACUC) of the Faculty of Medicine and Health Sciences, UPM regulations (approval no. UPM/FPSK/PADS/BR-UUH/00288), and complied with international guidelines for animal studies.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Pang Chong Yee and Manraj Singh Cheema performed the experiments in this study. Yong Yoke Keong and Zuraini Ahmad reviewed the manuscript and the study design. Muhammad Nazrul Hakim and Zuraini Ahmad drafted the manuscript and supervised the study.

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