PHARMACIA

Pharmacia 71: 1–8
DOI 10.3897/pharmacia.71.e106817

Research Article

Amstirdam coffee ameliorates Lp-PLA2 and the inflammatory response in an atherosclerosis mice

Wirdatun Nafisah[1,2], Annia Zhafarina Dalilati[1], Yuyun Ika Christina[1], Mochammad Fitri Atho'illah[1], Muhaimin Rifa'i[1,3], Tengku Natasha Eleena Tengku Ahmad Noor[4,5], Alexander Patera Nugraha[6,7]

1 *Department of Biology, Brawijaya University, Malang, Indonesia*
2 *Bioinformatic Research Center, Indonesian Institute of Bioinformatics (INBIO), Malang, Indonesia*
3 *Center of Biosystem Study, LPPM of Brawijaya University, Malang, Indonesia*
4 *Membership of Faculty of Dental Surgery, Royal Collage of Surgeon, Edinburgh University, Edinburgh, UK*
5 *Military Dental Officer of Royal Medical and Dental Corps, Malaysian Armed Forces, Semenggo Camp, Kuching, Serawak, Malaysia*
6 *Department of Orthodontics, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia*
7 *Immunology Study Program, Postgraduate School, Universitas Airlangga, Surabaya, Indonesia*

Corresponding author: Alexander Patera Nugraha (alexander.patera.nugraha@fkg.unair.ac.id)

Received 23 May 2023 ♦ **Accepted** 12 September 2023 ♦ **Published** 4 January 2024

Citation: Nafisah W, Dalilati AZ, Christina YI, Atho'illah MF, Rifa'i M, Noor TNETA, Nugraha AP (2024) *Amstirdam* coffee ameliorates Lp-PLA2 and the inflammatory response in an atherosclerosis mice. Pharmacia 71: 1–8. https://doi.org/10.3897/pharmacia.71.e106817

Abstract

Coffee is a kind of daily beverage, and its correlation with cardiovascular disease and atherosclerosis is still debatable. The aim of this study is to investigate the effects of *Amstirdam* coffee extract (ACE) on lipoprotein-associated phospholipase A2 (Lp-PLA2) and the inflammatory response in atherosclerosis mouse models. The study used 25 Swiss male mice for five groups (n = 5): healthy mice fed a normal diet (N); mice fed a high-fat, high-fructose diet (HFFD); mice fed HFFD and treated with ACE at doses of 104 (D1), 520 (D2), and 5200 mg/kg BW (D3). The levels of Lp-PLA2, regulatory T cells (Tregs) ($CD4^+CD25^+CD62L^+$, $CD4^+CD25^+IL\text{-}10^+$, CD4+CD25+TGF-+), IL-10 (CD4+IL-10+), and TGF-B (CD4+TGF+) were analyzed using a flow cytometer. Histological analysis of the mouse aorta was done by hematoxylin and eosin (HE) staining. This study indicated a significant increase in total cholesterol (TC), triglyceride (TG), LDL, and Lp-PLA2 levels in the HFFD group. HFFD also reduced HDL, IL-10, and TGF produced by CD4 and Tregs compared with the normal group. ACE at all doses significantly reduced Lp-PLA2 levels compared with the HFFD group ($p < 0.05$). Interestingly, the administration of 520 mg/kg BW ACE (D2) increased the production of IL-10 significantly compared to other doses ($p < 0.05$). The D3 group possessed a high TGF- production and Treg expression level significantly different between groups ($p < 0.05$). Foam cells were mostly found in the aorta of the HFFD group compared to the normal and ACE treatment groups. This study suggested that ACE could reduce Lp-PLA2 enzyme activity and foam cell formation through the immunosuppressive activity of IL-10 and TGF cytokines.

Keywords

Atherosclerosis, Coffee, Immunosuppressive, Inflammation, Lp-PLA2, Medicine

Introduction

Atherosclerosis is an inflammatory disease that occurs in blood vessels, mainly arteries, and is related to the body's high levels of lipids and metabolic disorders. Atherosclerosis can lead to ischemic heart disease (IHD) and ischemic stroke (IS), which are the main factors in cardiovascular disease (CVD) (Kristanti et al. 2022). Based on Kim (2021), CVD is one of the leading causes of death and disability, which is continuously increasing. The characteristics of atherosclerosis are lipid accumulation, fibrous substances, and artery calcification (Jebari-Benslaiman et al. 2022). The accumulation of lipids, particularly LDL, in the blood leads to endothelial dysfunction, known as the first line of atherogenesis (Kotlyarov 2021). LDL then infiltrates endothelial cells, penetrates the tunica intima, and causes the production of anion superoxide and free radicals. Anion superoxide and free radicals can induce oxidized low-density lipoprotein (ox-LDL) and stress oxidation (Marchio et al. 2019). Ox-LDL tends to be attractive to macrophages through monocyte differentiation, which is mostly found in atheromatous plaque and leads to the formation of foam cells (Ganesan et al. 2018).

The lipoprotein-associated phospholipase A2 (Lp-PLA2) enzyme can be produced by macrophages and neutrophils in atherosclerosis plaque and has been incorporated for assessing the risk of cardiovascular disease (Cai et al. 2015). Lp-PLA2 is a biomarker specific for vascular inflammation due to its association with LDL in the blood (Ngen et al. 2017). This enzyme is commonly used to predict the prognosis of atherosclerosis. The development and progression of atherosclerosis depend on the role of immunomodulation due to vascular inflammation of atherosclerosis mediated by an autoimmune response to self-antigens such as ox-LDL in the vascular wall (Yamashita et al. 2015). Regulatory T cells reported can reduce inflammation and inhibit plaque formation by suppressing atherogenic T cells (Pastrana 2013) by contact-dependent suppression or by interleukin (IL)-10 and transforming growth factor beta (TGF-B) cytokines production (Li et al. 2013). Therefore, regulatory T cells show a promising effect for the treatment of atherosclerosis.

Coffee is a kind of non-alcoholic beverage, and its correlation with cardiovascular disease and atherosclerosis is still debatable. Studies reported that coffee consumption and the risk of cardiovascular disease had a positive correlation (Liu et al. 2013; Grioni et al. 2015). Several studies demonstrated no association (Floegel et al. 2012) or were even conversely associated (Ding et al. 2014, 2015). Thus, this study aims to investigate the effect of *Amstirdam* coffee extract (ACE) on Lp-PLA2 reduction and the immunosuppressive activity of Tregs.

Materials and methods

This study was conducted in the Physiology, Structure, and Animal Development Laboratory, Department of Biology, Brawijaya University, Malang, Indonesia, in accordance with the guidelines of EU Directive 2010/63/EU for animal experiments and approved by the Committee of Animal Care and Use, Institute of Bioscience, Brawijaya University (no. 1152-KEP-UB). A completely randomized design was used as the experimental design, with five groups and five mice in each group. There were two main groups: the normal group (N) and the high-fat, high-fructose diet (HFFD) group. HFFD was prepared on site in the department which contained 8% duck egg yolk, 17% beef tallow, 30% fructose, and 0.2% cholic acid (Oroli et al. 2019). The HHFD induction was done for 5 months which was then separated into 4 groups: HFFD mice without treatment (HFFD), HFFD mice receiving *Amstirdam* coffee extract (ACE) with three dose variations, such as 104 mg/kg BW (D1), 520 mg/kg BW (D2), and 5200 mg/kg BW (D3) for 2 weeks. The duration of the whole experiment was 5 months and 2 weeks.

Male mice of the strain Swiss (aged 7–8 weeks) were obtained from Laboratorium Penelitian dan Pengujian Terpadu(LPPT), Gadjah Mada University, Yogyakarta, Indonesia. Male mice were used due to their being more responsive to a high-fat diet, which led to a higher level of fat and lipid serum (Tóth et al. 2021). All animal models were cared for in the Experimental Animal Center, Physiology, Structure, and Animal Development Laboratory, Brawijaya University, and placed in the animal chamber with 12/12 dark/light cycles. After one week of acclimation, all animals were divided into two main groups: the normal group (5 mice) and the HFFD group (20 mice). Later, the HFFD mouse group would be treated with coffee extract in three different dosages orally. The animal care was appropriate to the protocol and procedure in the Molecular Biology Laboratory and the Physiology, Structure, and Animal Development Laboratory.

Amstirdam coffee is one of the Robusta planted in the Malang area. Coffee was diluted in aquadest with a 1:10 g/mL ratio, then heated until it reached 80 ˚C and incubated for 2 hours at the same temperature using a water bath (Memmert WNB 45). After 2 hours of incubation, the solution was filtered using filter paper, then stored in bottles and frozen in the deep freezer (-70 °C) for three days or until it was frozen. The frozen solution was then dried using the freeze-drying method (Alpha 1–2 LD plus). The dosage is based on the average coffee consumption of most people (60 kg body weight) of 2 mg twice a day. This dosage was then converted into mice's dosage based on the FDA table, and it became the absolute dose, D2 = 520 mg/kg. We modify the absolute dose to have dose variation with a low dose, D1 = 104 mg/kg (D2/5), and a high dose, D3 = 5200 mg/kg (D2×10).

The lipid profiles (total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL)) were evaluated using Lipid ProTM (Infopia Co., Ltd.) according to the manufacturer's instructions in the last week of the treatment. Next, the mice's blood for the assay was taken from the tail vein to evaluate the serum concentration of TC, triglyceride TG,

HDL, and LDL. The atherogenic indices, including the atherogenic index of plasma (AIP), atherogenic coefficient (AC), cardiac risk ratio (CRR), and cardioprotective index (CPI), were evaluated using the following equation (Atho'illah et al. 2017):

- Atherogenic index of plasma (AIP) = Log [TG/HDL];
- Atherogenic coefficient (AC) = [(TC-HDL)/HDL];
- Cardiac risk ratio (CRR) = [TC/HDL);
- Cardioprotective index (CPI) = [HDL/LDL].

At the end of the experiment, all animals were sacrificed, and the spleens were isolated for antibody staining and flow cytometry analysis. The spleen was crushed and mixed with phosphate buffer saline (PBS, Gibco) until homogeneous. Homogenate was then centrifuged (HERMLE Z 326 K) at 2500 rpm at 10 °C for 5 minutes. The pellet was resuspended in 1 mL of PBS and distributed to a microtube for antibody staining. Antibody staining was done with extracellular and intracellular antibodies. Extracellular antibodies such as fluorescein isothiocyanate (FITC)-conjugated anti-mouse CD4 (BioLegend), phycoerythrin (PE)-conjugated anti-rat CD25 (BioLegend), and CD11b were added to the cell for 50 l and incubated for 20 minutes. Intracellular antibodies such as PAFAH Polyclonal Antibody-conjugated Cyanin (Cy) 5.5, Cross Reactive Species: Human, Mouse, Rat (Bioss) to evaluate the enzymatic activity of Lp-PLA2 from CD11b, PE/Cy7-conjugated anti-mouse IL-10 (BioLegend), and TGF beta 1 Polyclonal Antibody-conjugated Cy3, Cross Reactive Species: Human, Mouse, Rat (Bios Additional reagents were needed before adding intracellular antibodies, including Fixation Buffer (BioLegend) for 50 second and incubated for 20 minutes and Perm Wash Buffer (BioLegend) for 400 second and then centrifuged before staining intracellular antibodies. After being stained, the cells were incubated for 20 minutes and then added to PBS for flow cytometry analysis.

The preparation of aortic histopathology was done to evaluate the accumulation of foam cells in the aortic tissue. Aortas were isolated from the mice and then fixed using 10% formalin. The preparation was done using the paraffin method and stained with H&E stain. The outcome of flow cytometry was analyzed using FlowJo v10 for Windows (FlowJo LLC, Ashland, OR). The data was then analyzed using one-way ANOVA and continued with the Duncan Multiple Range Test (DMRT) at a significance level of 5%. These tests were carried out using GraphPad Prism 8 (GraphPad Software Inc., La Jolla, CA).

Results and discussion

TC, TG, HDL, and LDL had significant increases ($p < 0.05$) in HFFD mice compared to the normal group. Our result showed that LDL, TG, and TC in HFFD mice increased by around 1.5–1.7 folds compared to normal mice. ACE administration improves TC, TG, HDL, and LDL in HFFD mice significantly more than in HFFD mice alone. It was unexpected that ACE administration at a higher dose did not significantly differ from the HFFD group (Table 1). Interestingly, the AIP, AC, and CRR were elevated in HFFD mice compared to normal mice, while the CPI declined. The ACE administration reduced AIP, AC, and CRR significantly ($p< 0.05$) compared to HFFD mice. The atherogenic indices are beneficial to assess the risk of cardiovascular disease development. Our study showed that ACE administration could improve CPI in HFFD mice.

Lp-PLA2 is a typical marker of atherogenesis, characterized by the formation of atheroma plaque in the vascular wall. In atherosclerosis, Lp-PLA2 is produced by macrophages ($CD11b^+$) that infiltrate into the sub-endothelial layer of the vascular wall. We observed that HFFD enhanced the production of Lp-PLA2 from macrophages in the spleen significantly (p 0.05), as seen in Fig. 1. This study indicated a significant increase in Lp-PLA2 production from $CD11b^+$ cells in the HFFD group compared to the normal group (31.24% vs. 9.75%) ($p< 0.05$). After treatment with ACE for two weeks, the production of Lp-PLA2 by $CD11b^+$ cells were significantly decreased in all doses (D1: 16.75%, D2: 12.81%, D3: 18.51%) (Fig. 1b). The Lp-PLA2 level produced by $CD11b^+$ cells in D2 was close to that of the normal group.

The accumulation of foam cells in the mouse aorta was correlated with the expression of Lp-PLA2 production. As seen in Fig. 2, the aorta of HFFD mice showed relatively high foam cell accumulation, mainly found in tunica

Table 1. ACE improves the lipid profile of HFFD mice.

Parameters	N	HFFD	D1	D2	D3
TC (mg/dL)	$112^{a} \pm 10.79$	$187^{c} \pm 7.0$	$100^{a} \pm 5.63$	$141^{b} \pm 5.47$	$175^{c} \pm 9.42$
TG (mg/dL)	$77^{a} \pm 17.62$	$123^{c} \pm 5.20$	$112^{bc} \pm 10.85$	$96^{ab} \pm 9.85$	$99^{abc} \pm 20.59$
HDL (mg/dL)	$45^{b} \pm 6.11$	$33^{a} \pm 6.36$	$75^{c} \pm 3.66$	$54^{bc} \pm 6.5$	$60^{c} \pm 6.86$
LDL (mg/dL)	$61^{a} \pm 10.5$	$91^{b} \pm 2.48$	$56^{a} \pm 8.57$	$59^{a} \pm 6.63$	$84^{b} \pm 4.42$
AIP	$0.23^{a} \pm 0.04$	$0.58^{b} \pm 0.11$	$0.17^{a} \pm 0.02$	$0.24^{a} \pm 0.1$	$0.21^{a} \pm 0.05$
AC	$1.38^{b} \pm 0.18$	$4.17^{d} \pm 0.05$	$0.33^{a} \pm 0.14$	$1.61^{bc} \pm 0.21$	$1.93^{c} \pm 0.32$
CRR	$2.38^{a} \pm 0.18$	$5.17^{d} \pm 0.05$	$1.33^{a} \pm 0.14$	$2.61^{bc} \pm 0.21$	$2.93^{c} \pm 0.32$
CPI	$0.74^{b} \pm 0.03$	$0.37^{a} \pm 0.07$	$1.35^{c} \pm 0.2$	$0.94^{b} \pm 0.22$	$0.72^{b} \pm 0.08$

Information: TC = total cholesterol; TG = triglyceride; HDL = high-density lipoprotein; LDL = low-density lipoprotein; AIP = atherogenic index of plasma; AC = atherogenic coefficient; CRR = cardiac risk ratio; cardioprotective index. N: normal-fed mice (non-high-fat-fructose diet); HFFD: high-fat-fructose diet mice (w/o administration of ACE); D1: HFFD mice receiving ACE 104 mg/kg body weight; D2: HFFD mice receiving ACE 520 mg/kg body weight; D3: HFFD mice receiving ACE 5200 mg/kg body weight. The different notation on the chart was considered significantly different for each group at $p < 0.05$, and vice versa on the DMRT post hoc test.

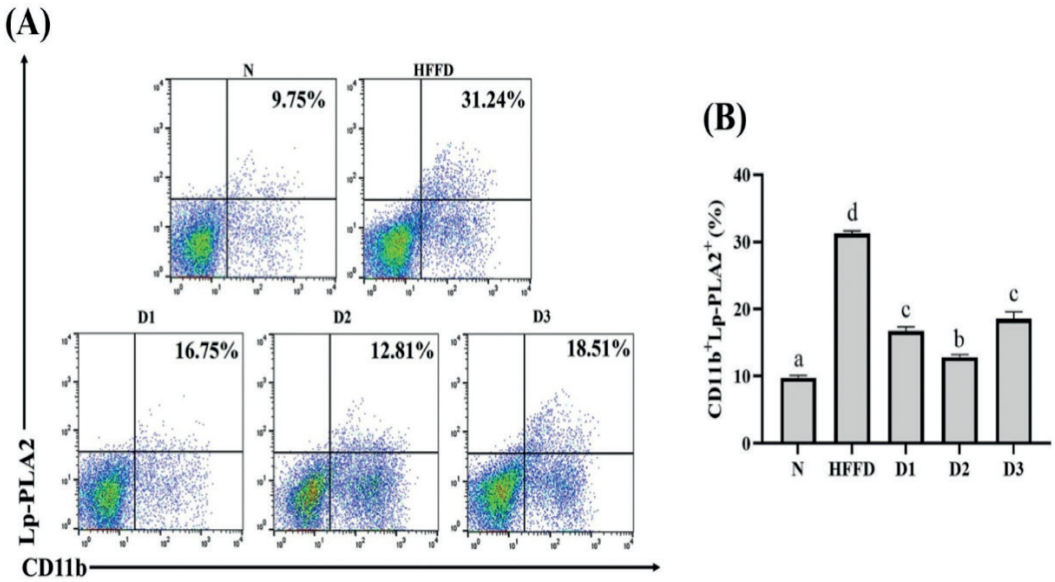

Figure 1. Reduction of Lp-PLA2 production after ACE treatment in mice fed a high-fat, high-fructose diet. The expression of Lp-PLA2 production in mice fed with HFFD and administered ACE was determined from flow cytometry analysis (Fig. 1A). The percentage of Lp-PLA2 production in mice fed with HFFD and administered ACE The data are mean SD (n = 5). N: normal-fed mice (non-high-fat-fructose diet); HFFD: high-fat-fructose diet mice (w/o administration of ACE); D1: HFFD mice receiving ACE 104 mg/kg body weight; D2: HFFD mice receiving ACE 520 mg/gram BW; D3: HFFD mice receiving ACE 5200 mg/kg BW. The different notation on the chart was considered significantly different for each group at $p< 0.05$ and vice versa on the DMRT post hoc test.

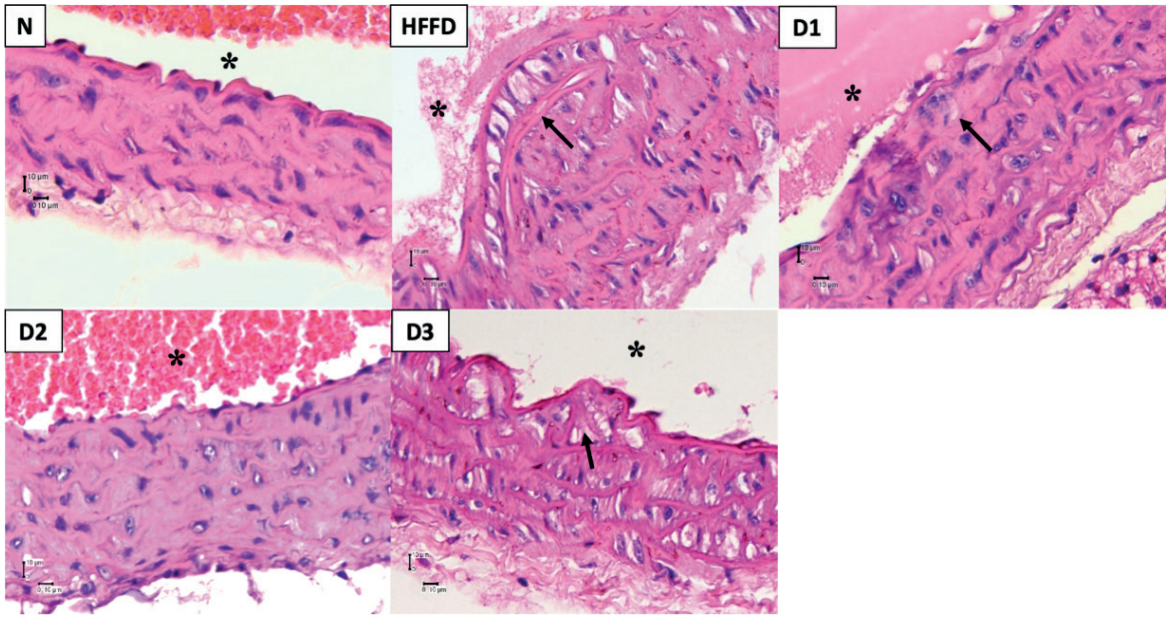

Figure 2. ACE administration reduced foam cells in aorta histopathology (M = 400×) in mice fed a high-fat, high-fructose diet for 5 months. The black arrow shows the accumulation of foam cells in the tunica media, and the asterisk (*) shows the lumen of the aorta. N: normal-fed mice (non-high-fat-fructose diet); HFFD: high-fat-fructose diet mice (w/o administration of ACE); D1: HFFD mice receiving ACE 104 mg/kg body weight; D2: HFFD mice receiving ACE 520 mg/kg body weight; D3: HFFD mice receiving ACE 5200 mg/kg body weight.

media, compared to normal mice (pointed by the arrow). The tunica media also formed a bulge into the lumen that caused the narrowing of the vascular wall. The accumulation of foam cells in the aortic wall tends to decrease after treatment with ACE for two weeks, particularly in D1 and D2. The aortic wall thickness also tends to decrease close to the normal condition compared to the HFFD mice without ACE treatment.

The reduction of LDL after administering ACE showed a correlation between a good prognosis (shown by the reduction of aortic wall thickness) and the pathogenesis of atherosclerosis (Table 1). LDL-C reduction has also been implicated in the reduction of foam cell formation, as shown in Fig. 2. One factor that affects Lp-PLA2 enzyme activity is lipid levels. Our study found both the reduction of Lp-PLA2 activity and LDL-C level related to the

reduction of foam cell formation (Table 1 and Fig. 2) and the high level of LDL-C and Lp-PLA2 produced by macrophages in D3 over the two other doses related to more foam cell formation (Table 1 and Fig. 2).

Tregs have a potential effect on suppressing pro-atherogenic agents by releasing anti-inflammatory cytokines or inhibiting pro-inflammatory cells such as Th1 cells and macrophages. The previous study showed that HFFD caused the decline of Treg subsets. The lowest level of Nave Tregs was found in the HFFD group (33.83%) (Fig. 3). It was indicated that HFFD decreased the number of naive Tregs significantly (p 0.05) compared to the normal group (72.51%). As treated by ACE, the level of naive Tregs increased in all doses (D1: 58.34%, D2: 50.57%, D3: 69.43%). As shown in Fig. 3, the highest level of the relative number of naive Tregs after treatment with ACE was found in D3, close to the normal condition (p 0.05). Interestingly, the reduction of nave Tregs was also followed by the reduction of IL-10 (Fig. 3B) and TGF (Fig. 3C) expressed by Tregs. Our result demonstrated that HFFD also caused a significant reduction of IL-10 and TGF- (p 0.05) (Fig. 3E, F). ACE administration improved naive Tregs and their products (IL-10 and TGF- cytokines).

Anti-inflammatory cytokines IL-10 and TGF- are produced by various cells; one is a $CD4^+$ T cell. HFFD was reported to alter $CD4^+$ T cells to become effector cells and secrete massive pro-inflammatory cytokines (Arifah et al. 2020). IL-10 has a significant role in inhibiting the production of pro-inflammatory cytokines such as TNF-, IFN-, and IL-1, while TGF-B maintains the homeostasis of T cells in the site of inflammation (Nur'aini et al. 2019). We observed that HFFD mice showed the lowest levels of both IL-10 (Fig. 4) and TGF-B (Fig. 5) produced by $CD4^+$ T cells compared to the normal group (p 0.05). The administration of ACE and $IL\text{-}10^+CD4^+$ tends to increase at D2

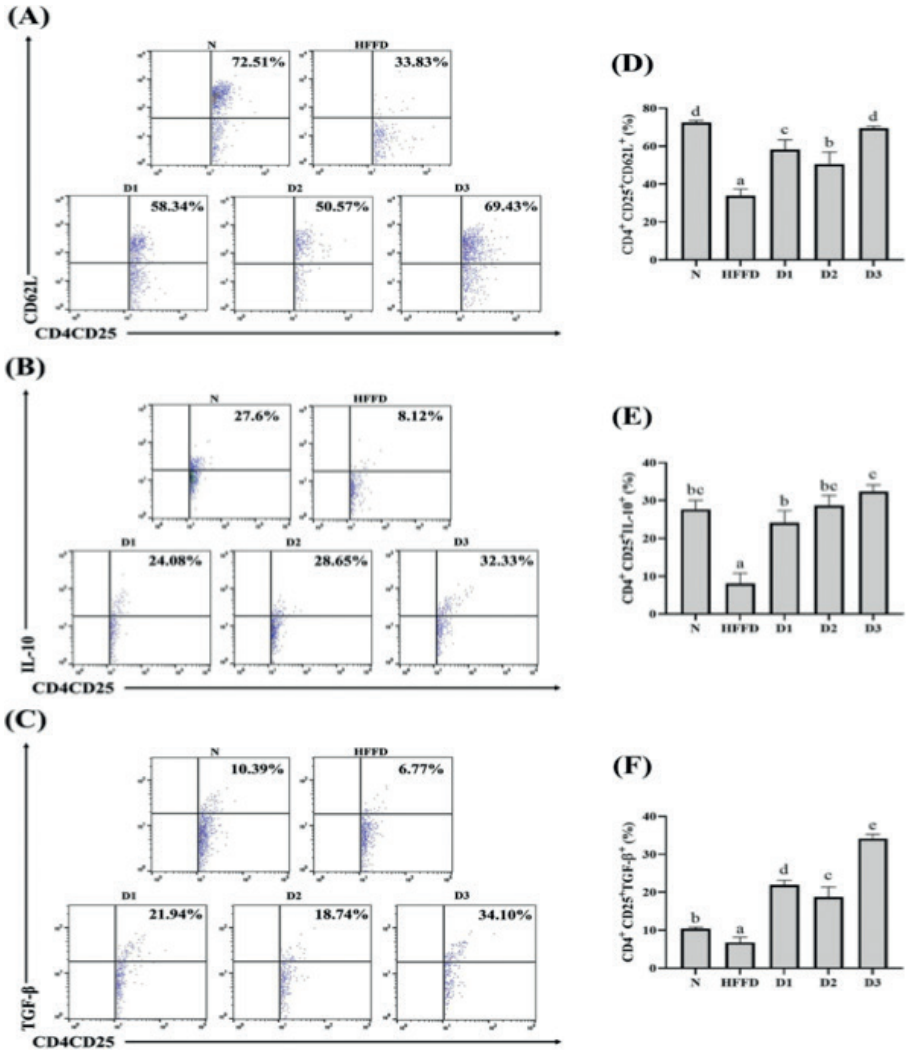

Figure 3. ACE administration increased the level of regulatory T cells in mice fed a high-fat, high-fructose diet for 5 months. The level of regulatory T cell **A.** $CD4^+CD25^+CD62L^+$ subsets, **B.** $CD4^+CD25^+IL\text{-}10^+$ subsets, and **C.** CD4+CD25+TGF-+subsets of mice fed with HFFD and administration of ACE from flow cytometry analysis. The percentage of regulatory T cell **D.** $CD4^+CD25^+CD62L^+$ subsets, **E.** $CD4^+CD25^+IL\text{-}10^+$ subsets, and **F.** CD4+CD25+TGF-+ subsets of mice fed with HFFD and administered ACE The data are mean SD (n = 5). N: normal-fed mice (non-high-fat-fructose diet); HFFD: high-fat-fructose diet mice (w/o administration of ACE); D1: HFFD mice receiving ACE 104 mg/kg body weight; D2: HFFD mice receiving ACE 520 mg/kg body weight; D3: HFFD mice receiving ACE 5200 mg/kg body weight. The different notation on the chart was considered significantly different for each group at $p<0.05$ and vice versa on the DMRT post hoc test.

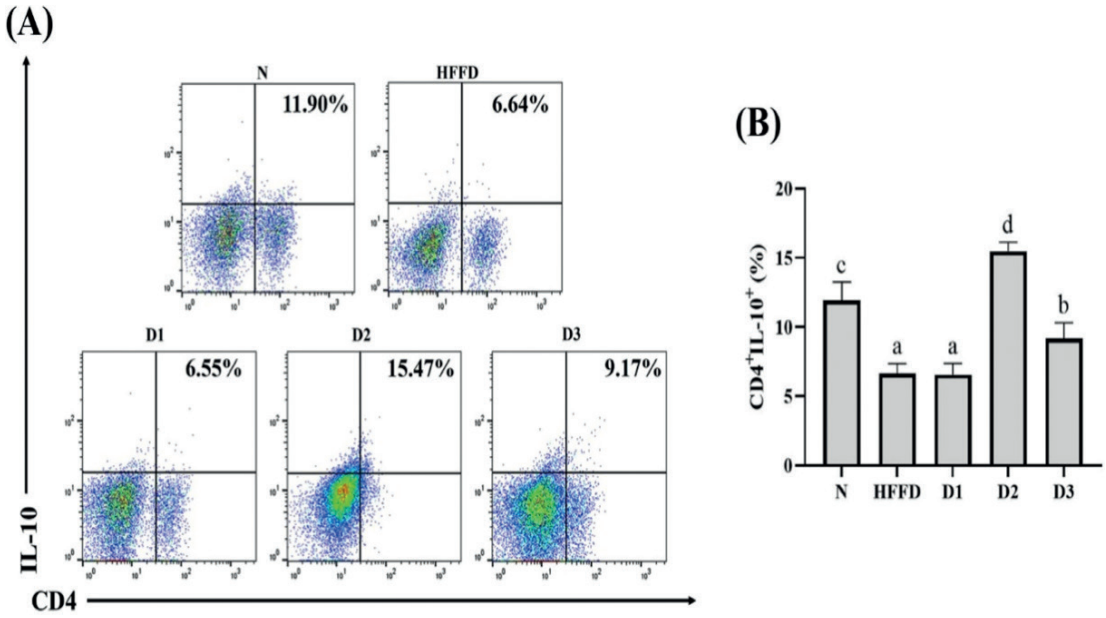

Figure 4. Administration of ACE increased IL-10 production ($CD4^+IL\text{-}10^+$) in mice fed with a high-fat, high-fructose diet for 5 months. The expression of $CD4^+IL\text{-}10^+$ in mice fed with HFFD and administered ACE was determined by flow cytometry analysis (Fig. 4E). The percentage of regulatory cells ($CD4^+IL\text{-}10^+$) in mice fed with HFFD and administered ACE (Fig. 4F) The data are mean SD (n = 5). N: normal-fed mice (non-high-fat-fructose diet); HFFD: high-fat-fructose diet mice (w/o administration of ACE); D1: HFFD mice receiving ACE 104 mg/kg body weight; D2: HFFD mice receiving ACE 520 mg/kg body weight; D3: HFFD mice receiving ACE 5200 mg/kg body weight. The different notation on the chart was considered significantly different for each group at $p < 0.05$ and vice versa on the DMRT pos hoc test.

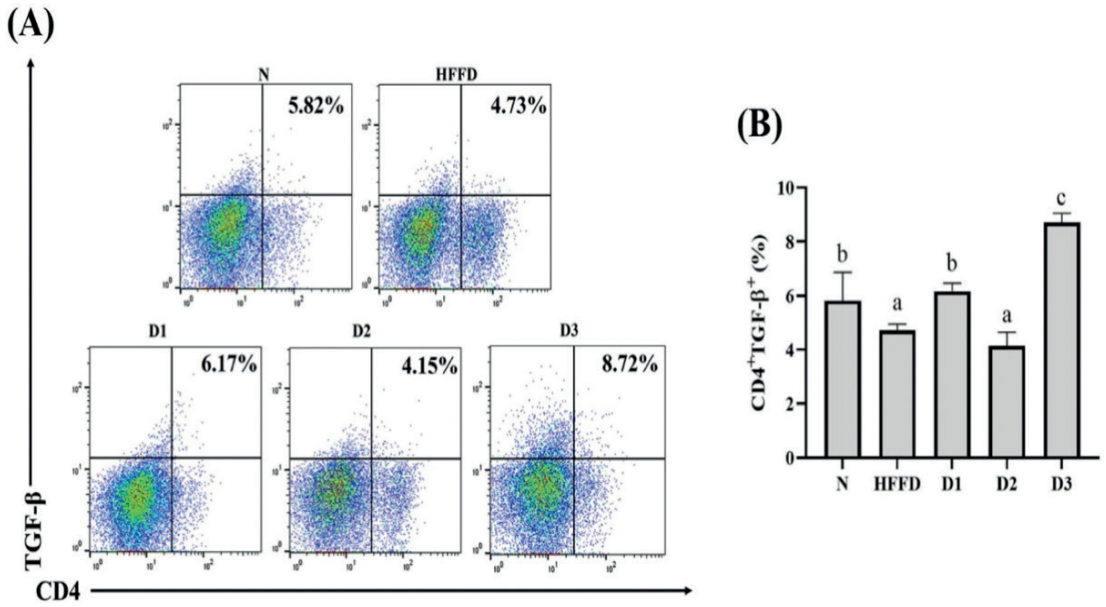

Figure 5. The effect of ACE increased TGF-β production ($CD4^+TGF\text{-}\beta^+$) in mice fed with a high fat-fructose diet for 5 months. The expression of TGF-β ($CD4^+TGF\text{-}\beta^+$) of mice fed with HFFD and administration of ACE from flow cytometry analysis (Fig. 5G). The percentage of regulatory ($CD4^+IL\text{-}10^+$) of mice fed with HFFD and administration of ACE (Fig. 5H). Data are mean ± SD (n=5). N: normal fed mice (non-high fat-fructose diet), HFFD: high fat-fructose diet mice (w/o administration of ACE), D1: HFFD mice receiving ACE 104 mg/kg body weight, D2: HFFD mice receiving ACE 520 mg/kg BW, D3: HFFD mice receiving ACE 5200 mg/kg BW. The different notation on the chart was considered significantly different for each group at $p < 0.05$ and vice versa on DMRT post hoc test.

(15.47%) and D3 (9.17%) (p 0.05). The administration of ACE also increased TGF-B $+CD4^+$ cell levels significantly ($p< 0.05$), particularly at D1 (6.17%) and D3 (8.72%).

The induction of HFFD caused the formation of plaque in the aortic wall and disrupted the immune system to support the pathogenesis of atherosclerosis. The dietary fat will be metabolized through the exogenous or endogenous pathway, resulting in fatty acids and triglycerides elevating blood circulation (Srivastava et al. 2000). Besides, the lipid profile, including LDL and HDL, is involved in

the mechanism of atherosclerosis pathogenesis. Accumulation of LDL activates the TLR (toll-like receptor) pathway and inhibits the activation of the transcription factor LXR-RXR (Liver X Receptor-Retinoid X Receptor) that is responsible for the process of cells cholesterol efflux to HDL particles or ApoA-1 to transport back to the liver (Tall and Yvan-Charvet 2015). In the event of LDL transport failure to the liver, the infiltration of LDL into sub-endothelial space increases (Emini Veseli et al. 2017). LDL accumulation undergoes oxidative modification, progressively forming oxidized LDL (ox-LDL) and inducing an inflammation response. It is characterized by overexpression of chemotactic proteins, including monocyte chemoattractant protein-1 (MCP-1) and adhesion molecules (vascular cell adhesion molecule-1 (VCAM-1), E-selectin, and P-selectin). Adhesion molecules promote blood-containing monocyte infiltration into the arterial wall. Monocytes differentiate into macrophages, which then phagocytes ox-LDL, form foam cells, and contribute to plaque development (Buscemi et al. 2010).

Our study found that ACE treatment improved the lipid profile and aortic wall histology of mice, except in D3. It can be assumed that its high dose causes endothelial dysfunction and increases the level of Lp-PLA2 in dose 3. In line with that, the high compound of caffeine can be toxic and cause endothelial dysfunction, as shown by a study on healthy subjects. The study found a reduction of FMD (flow-mediated dilation) as figured in endothelial function performance after ingestion of caffeinated coffee (Matsuda et al. 2011). However, the previous study demonstrated that the right dose of caffeine or polyphenol from coffee down-regulated sterol regulatory element-binding protein (SREBP)-1, fatty acid synthase (FAS), and acetyl-CoA carboxylase (ACC), which have pivotal roles in fatty acid synthesis (Vitaglione et al. 2019). The blockade of genes that contribute to fatty acid synthesis will lead to fatty liver improvement. The previous study demonstrated that coffee consumption increased liver LXR and up-regulated both ATP-binding cassette subfamily A1 (ABCA1) and ATP-binding cassette subfamily G1 (ABCG1) gene expression (Vitaglione et al. 2019; Zhang et al. 2022). As we stated before, LXR- maintained cholesterol homeostasis, thus elevating the cholesterol metabolism through two main transporters, ABCA-1 and ABCG-1 (Maiolino 2015).

The release of lysophosphatidylcholine and oxidized fatty acids through the phospholipid substrate on the LDL-C surface can trigger inflammatory cascades (Cimmino et al. 2017). Inflammation causes the recruitment of lymphocytes, T cells, monocytes, and other immunocompetent cells to atherosclerotic plaque that can induce pro-atherogenic activity (Cimmino et al. 2017). Inflammation can be solved by the immunosuppressive activity of T cells such as $CD4^+CD25^+$Treg. Several mechanisms of action by which $CD4^+CD25^+$Treg can suppress immune cells and inhibit inflammation exist, such as (1) the production of immunoregulatory cytokines such as TGF, IL-10, and IL-35; (2) the production of granzyme and perforin; (3) the inhibition of proliferative responses to interrupt metabolism via the IL-2 receptor, cAMP-mediated metabolic pathways, and the A2 adenosine receptor; and (4) the modulation of function and maturation of DCs via their interaction (Arce-Sillas et al. 2016). Suppression of $CD4^+CD25^+$Treg can be done by cell-to-cell contact by granzyme and perforin production. The level of $CD4^+CD25^+$Treg found in this study shows immunosuppressive activity due to a higher level of $CD4^+CD25^+$Treg in the treatment group.

This study demonstrated a possible mechanism for the anti-atherosclerosis effect of ACE through the enhancement of the immunosuppressive activity of Tregs. Since the level of nave Treg was increased after ACE treatment, we hypothesized that Treg suppressed macrophages and pro-atherogenic cells through the production of IL-10 and TGF-. The suppression of these cells correlated with the reduction of foam cell formation and Lp-PLA2 enzyme levels. The inhibition of proinflammatory cytokines implies that ACE may improve the inflammatory state induced by HFFD. However, the results of this study are limited to atherosclerosis animal model with limited investigation methods. Further study is urgently needed to elucidate the role of *Amstirdam* coffee ameliorates Lp-PLA2 in human with various investigation method and randomize clinical trial setting.

Conclusion

Amstirdam coffee extract improves the lipid profile in HFFD mice and reduces Lp-PLA2 enzyme activity and foam cell formation through the immunosuppressive activity of Treg and the anti-inflammatory cytokines IL-10 and TGF-B.

Acknowledgements

The author would like to thank the collaboration between Universitas Airlangga and Universitas Brawijaya in this study.

References

Arce-Sillas A, Álvarez-Luquín DD, Tamaya-Domínguez B, Gomez-Fuentes S, Trejo-García A, Melo-Salas M, Cárdenas G, Rodríguez-Ramírez J, Adalid-Peralta L (2016) Regulatory T cells: Molecular actions on effector cells in immune regulation. Journal of Immunology Research 2016: e1720827. https://doi.org/10.1155/2016/1720827

Arifah SN, Atho'illah MF, Lukiati B, Lestari SR (2020) Herbal medicine from single clove garlic oil extract ameliorates hepatic steatosis and oxidative status in high fat diet mice. Malaysian Journal of Medical Sciences 27(1): 46–56. https://doi.org/10.21315/mjms2020.27.1.5

Atho'illah MF, Widyarti S, Rifa'i M (2017) Elicited soybean (*Glycine max* L.) Extract improves regulatory t cell activity in high fat-fructose diet mice. 2nd International conference on composite materials and material engineering (Iccmme 2017). AIP Conference Proceedings 1844: 020004. https://doi.org/10.1063/1.4983415

Buscemi S, Verga S, Batsis JA, Donatelli M, Tranchina MR, Belmonte S, Mattina A, Re A, Cerasola G (2010) Acute effects of coffee on endothelial function in healthy subjects. European Journal of Clinical Nutrition 64(5): 483–489. https://doi.org/10.1038/ejcn.2010.9

Cai A, Li G, Chen J, Li X, Li L, Zhou Y (2015) Increased serum level of Lp-PLA2 is independently associated with the severity of coronary artery diseases: A cross-sectional study of Chinese population. BMC Cardiovascular Disorders 26(15): 1–14. https://doi.org/10.1186/s12872-015-0001-9

Cimmino G, Loffredo FS, Morello A, D'Elia S, De Palma R, Cirillo P, Golino P (2017) Immune-inflammatory activation in acute coronary syndromes: A look into the heart of unstable coronary plaque. Current Cardiology Reviews 13(2): 110–117. https://doi.org/10.2174/1573403X12666161014093812

Ding M, Bhupathiraju SN, Satija A, van Dam RM, Hu FB (2014) Long-term coffee consumption and risk of cardiovascular disease: A systematic review and a dose-response meta-analysis of prospective cohort studies. Circulation 129(6): 643–659. https://doi.org/10.1161/CIRCULATIONAHA.113.005925

Emini Veseli B, Perrotta P, De Meyer GRA, Roth L, Van der Donckt C, Martinet W, De Meyer GRY (2017) Animal models of atherosclerosis. European Journal of Pharmacology 816: 3–13. https://doi.org/10.1016/j.ejphar.2017.05.010

Floegel A, Pischon T, Bergmann MM, Teucher B, Kaaks R, Boeing H (2012) Coffee consumption and risk of chronic disease in the European Prospective Investigation into Cancer and Nutrition (EPIC)-germany study. The American Journal of Clinical Nutrition 95(4): 901–908. https://doi.org/10.3945/ajcn.111.023648

Ganesan R, Henkels KM, Wrenshall LE, Kanaho Y, Di Paolo G, Frohman MA, Gomez-Cambronero J (2018) Oxidized LDL phagocytosis during foam cell formation in atherosclerotic plaques relies on a PLD2-CD36 functional interdependence. Journal of Leukocyte Biology 103(5): 867–883. https://doi.org/10.3945/ajcn.111.023648

Grioni S, Agnoli C, Sieri S, Pala V, Ricceri F, Masala G, Saieva C, Panico S, Mattiello A, Chiodini P, Tumino R, Frasca G, Iacoviello L, de Curtis A, Vineis P, Krogh V (2015) Espresso coffee consumption and risk of coronary heart disease in a large Italian cohort. PLoS ONE 10(5): e0126550. https://doi.org/10.1371/journal.pone.0126550

Jebari-Benslaiman S, Galicia-García U, Larrea-Sebal A, Olaetxea JR, Alloza I, Vandenbroeck K, Benito-Vicente A, Martín C (2022) Pathophysiology of Atherosclerosis. International Journal of Molecular Sciences 23(6): e3346. https://doi.org/10.3390/ijms23063346

Kim HC (2021) Epidemiology of cardiovascular disease and its risk factors in Korea. Global Health & Medicine 3(3): 134–141. https://doi.org/10.35772/ghm.2021.01008

Kotlyarov S (2021) Diversity of Lipid Function in Atherogenesis: A Focus on Endothelial Mechanobiology. International Journal of Molecular Sciences 22(21): e11545. https://doi.org/10.3390/ijms222111545

Kristanti RA, Bramantoro T, Soesilawati P, Hariyani N, Suryadinata A, Purwanto B, Nugraha AP, Noor TNEBTA (2022) Inflammatory cytokines affecting cardiovascular function: a scoping review [version 1; peer review: awaiting peer review]. F1000Research 11: e1078. https://doi.org/10.12688/f1000research.122390.1

Li Q, Wang Y, Yu F, Wang YM, Zhang C, Hu C, Wu Z, Xu X, Hu S (2013) Peripheral Th17/Treg imbalance in patients with atherosclerotic cerebral infarction. International Journal of Clinical and Experimental Pathology 6(6): 1015–1027.

Liu J, Sui X, Lavie CJ, Hebert JR, Earnest CP, Zhang J, Blair SN (2013) Association of coffee consumption with all-cause and cardiovascular disease mortality. Mayo Clinic Proceedings 88(10): 1066–1074. https://doi.org/10.1016/j.mayocp.2013.06.020

Maiolino G, Bisogni V, Rossitto G, Rossi GP (2015) Lipoprotein-associated phospholipase A2 prognostic role in atherosclerotic complications. World Journal of Cardiology 7(10): 609–620. https://doi.org/10.4330/wjc.v7.i10.609

Marchio P, Guerra-Ojeda S, Vila JM, Aldasoro M, Victor VM, Mauricio MD (2019) Targeting early atherosclerosis: A focus on oxidative stress and inflammation. Oxidative Medicine and Cellular Longevity 2019: e8563845. https://doi.org/10.1155/2019/8563845

Matsuda Y, Kobayashi M, Yamauchi R, Ojika M, Hiramitsu M, Inoue T, Katagiri T, Murai A, Horio F (2011) Coffee and caffeine improve insulin sensitivity and glucose tolerance in C57BL/6J mice fed a high-fat diet. Bioscience, Biotechnology, and Biochemistry 75(12): 2309–2315. https://doi.org/10.1271/bbb.110452

Nur'aini FD, Rahayu S, Rifa'i M (2019) Anti-inflammatory activity of elicited soybean (*Glycine max*) extract on Balb/C mice (*Mus musculus*) with high-fat and -fructose diet. Central European Journal of Immunology 44(1): 7–14. https://doi.org/10.5114/ceji.2019.84010

Öngen B, Kalkan Uçar S, Levent E, Azarsız E, Koloğlu T, Çoker M, Sözmen E, Sağın FG (2017) Lipoprotein-associated phospholipase A_2: a new marker to determine cardiovascular risk in hypercholesterolemic dyslipidaemic children. Annals of Clinical Biochemistry 54(5): 539–547. https://doi.org/10.1177/0004563216671338

Oršolić N, Landeka Jurčević I, Đikić D, Rogić D, Odeh D, Balta V, Perak Junaković E, Terzić S, Jutrić D (2019) Effect of propolis on diet-induced hyperlipidemia and atherogenic indices in mice. Antioxidants 8(6): e156. https://doi.org/10.3390/antiox8060156

Pastrana JL, Sha X, Virtue A, Mai J, Cueto R, Lee IA, Wang H, Yang XF (2012) Regulatory T cells and atherosclerosis. Journal of Clinical and Experimental Cardiology(Suppl 12): 1–2. https://doi.org/10.4172/2155-9880.S12-002

Srivastava RA, Srivastava N, Averna M (2000) Dietary cholic acid lowers plasma levels of mouse and human apolipoprotein A-I primarily via a transcriptional mechanism. European Journal of Biochemistry 267(13): 4272–4280. https://doi.org/10.1046/j.1432-1033.2000.01473.x

Tall AR, Yvan-Charvet L (2015) Cholesterol, inflammation and innate immunity. Nature Reviews Immunology 15(2): 104–116. https://doi.org/10.1038/nri3793

Tóth ME, Dukay B, Péter M, Balogh G, Szűcs G, Zvara Á, Szebeni GJ, Hajdu P, Sárközy M, Puskás LG, Török Z, Csont T, Vígh L, Sántha M (2021) Male and female animals respond differently to high-fat diet and regular exercise training in a mouse model of hyperlipidemia. Int International Journal of Molecular Sciences 22(8): e4198. https://doi.org/10.3390/ijms22084198

Yamashita T, Sasaki N, Kasahara K, Hirata K (2015) Anti-inflammatory and immune-modulatory therapies for preventing atherosclerotic cardiovascular disease. Journal of Cardiology 66(1): 1–8. https://doi.org/10.1016/j.jjcc.2015.02.002

Zhang H, Lianto P, Li W, Xu M, Moore JB, Thorne JL (2022) Associations between liver X receptor polymorphisms and blood lipids: A systematic review and meta-analysis. Steroids 185: e109057. https://doi.org/10.1016/j.steroids.2022.109057