

Efficacy of Oral Curcuminoid Fraction from *Curcuma xanthorrhiza* and Curcuminoid Cider in High-cholesterol Fed Rats

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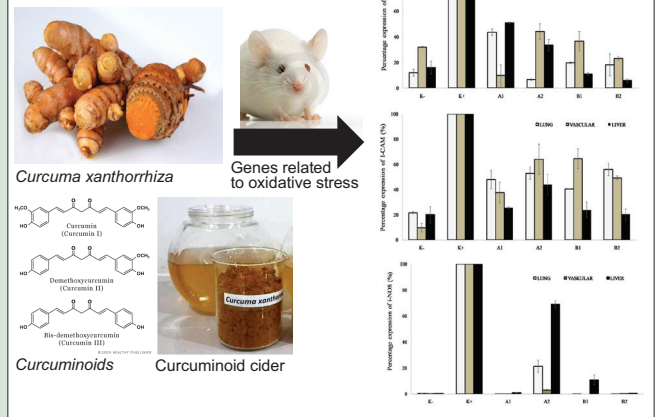
ABSTRACT

Background: Hypercholesterolemia is one of the most important risk factors for atherosclerosis and subsequent cardiovascular diseases. **Objective:** The present work was aimed to study the efficacy of curcuminoid fraction from *Curcuma xanthorrhiza* and its curcuminoid cider in reducing blood cholesterol level and four genes related to oxidative stress, including cluster of differentiation 44 (CD44), intercellular adhesion molecule 1 (ICAM-1), inducible nitric oxide synthase (iNOS), and lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) in high-cholesterol fed rats *in vivo*. **Materials and Methods:** Twenty-four male Sprague-Dawley rats were divided into six groups, namely normal group diet, high-cholesterol diet (HCD) 2%, HCD + 100 mg/kg b.w. curcuminoid fraction, HCD + 300 mg/kg b.w. curcuminoid fraction, HCD + cider 1% v/v, and HCD + curcuminoid cider 2% v/v for 4 weeks. Total cholesterol levels were measured at day 1, 14, and 28. Vascular tissues and organs from lung and liver were collected for RNA extraction, followed by quantitative analysis using real-time polymerase chain reaction (PCR). **Results:** Our results demonstrated that among all the treatment groups, curcuminoid cider at 2% v/v significantly lowered total cholesterol level compared to those of positive control. Real-time PCR data showed both curcuminoid fractions (100 and 300 mg/kg) and curcuminoid cider (1 and 2% v/v) inhibited the gene expression of CD44, ICAM-1, iNOS, and LOX-1, indicating their hypocholesterolemic effects via attenuating genes related to oxidative stress in rats *in vivo*. **Conclusion:** Oral administration of curcuminoid fraction and its cider product may exert potential inhibitory effects on oxidative stress related-genes for preventing hypercholesterolemia-induced atherosclerosis *in vivo*. **Key words:** Atherosclerosis, curcuminoid cider, curcuminoid fraction, genes related to oxidative stress, hypercholesterolemia

SUMMARY

- Curcuminoid and its cider significantly inhibited the gene expression of CD44, ICAM-1, iNOS, and LOX-1 in rats *in vivo*
- Curcuminoid and its cider suppressed oxidative stress-related genes inducing formation of atherosclerosis
- Curcuminoid and its cider may offer cardioprotective effect for preventing hypercholesterolemia-induced atherosclerosis

PICTORIAL ABSTRACT



Abbreviations Used: ROS: Reactive oxygen species, NO: Nitric oxide, NOS: NO synthase, NADPH: Nicotinamide adenine dinucleotide phosphate, CD44: Cluster of differentiation 44, ICAM-1: Intercellular adhesion molecule 1, iNOS: inducible NOS, LOX-1: lectin-like oxidized LDL receptor-1, HMG-CoA: 3-hydroxy-3-methylglutaryl-coenzyme A, 5-HMF: 5-hydroxymethylfurfural, HCD: High-cholesterol diet

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INTRODUCTION

Hypercholesterolemia is one of the metabolic disorders characterized by high serum levels of low-density lipoprotein (LDL) cholesterol and blood cholesterol. As a result, cholesterol level and triglycerides metabolism affect oxidative stress mediators and promote the production of reactive oxygen species (ROS) by various mechanisms, which leads to increased lipid peroxidation.^[1] Oxidative stress is an imbalance condition between oxidants and antioxidants in favor of the oxidants, potentially leading to cell damages. Atherosclerosis is a condition of high oxidative stress characterized by lipid and protein oxidation in the vascular walls. Overproduction of ROS under pathophysiological conditions forms an integral part of the development of cardiovascular disease and in particular atherosclerosis. Several characteristics, such as endothelial dysfunction and the loss of nitric oxide (NO) activity, may occur in the development of atherosclerosis and determine further vascular complications.

ROS belongs to the major mediator of signaling pathways that underlie vascular inflammation in atherogenesis, starting from the initiation of fatty streak development, through lesion progression, to ultimate plaque rupture. Several studies demonstrated that ROS released from nicotinamide adenine dinucleotide phosphate (NADPH) oxidase,

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myeloperoxidase, xanthine oxidase, lipoxygenase, NO synthase (NOS), and heightened ROS production from dysfunctional mitochondrial respiratory chain plays a key role in atherosclerosis and other vascular diseases.^[2] Curcuminoids and sesquiterpenoids as major compounds found in the rhizomes of *Curcuma longa* (turmeric) and *Curcuma xanthorrhiza* (temulawak) have been widely used as dietary spice and folk medicine for reducing swelling and symptoms of osteoarthritis and rheumatoid arthritis such as pain and inflammation. It is reported that curcuminoids are known to have anti-oxidative, anti-carcinogenic, and anti-inflammatory activities.^[3] This study was aimed to examine the efficacy of curcuminoid fraction from *C. xanthorrhiza* and curcuminoid cider products on modulating lipid profiles and inhibiting the expression of several genes related to oxidative stress inducing atherosclerotic plaque formation in rats *in vivo*.

MATERIALS AND METHODS

Preparation of curcuminoid extract from *Curcuma xanthorrhiza*

One kilogram of fresh plant materials from *C. xanthorrhiza* was purchased from a local market in Bogor, Indonesia. Fresh rhizomes were cleaned, sliced, and freeze-dried for 3 days. Afterward, the dried rhizomes were blended and filtered to obtain the same size particle powder. The powder of *C. xanthorrhiza* was macerated with 80% ethanol (1:4, w/v) at room temperature for 2 days. The supernatant was combined and concentrated under reduced pressure at 45°C using a rotary vacuum evaporator. The crude extract was then freeze-dried until a constant weight was obtained to afford the maceration extract.

Preparation of curcuminoid cider from *Curcuma xanthorrhiza*

Curcuminoid fraction from *C. xanthorrhiza* was dissolved in hot water with final concentration of 1% and 2% (w/v). *Acetobacter xylinum* culture was added to curcuminoid solution with ratios of 2:1 and 4:1, sugar of 2.5% was added to provide nutrients for the culture growth as well as providing taste and aroma to the cider product. Furthermore, the solution was filtered and incubated for 14 days at 30°C. The cider was further pasteurized at 50–60°C for 20 min and then filtered to obtain the functional food product curcuminoid cider. Cider products were tested for its efficacy *in vivo*.

Identification of chemical compounds in curcuminoid fraction and curcuminoid cider

Curcuminoid extract and curcuminoid cider were analyzed using pyrolysis gas chromatography mass spectrometry (Py-GC/MS). Py-GC/MS QP2010 (Shimadzu, Duisburg, Germany) was carried out to analyze the chemical compounds found in each sample. Sample was injected to the capillary column (Phase Rtx-5MS) with a film thickness of 60 m × 0.25 mm ID. Pyrolysis temperature was set to 280°C. Helium was used as the carrier gas.^[4]

Table 1: Treatment of the group rats diets

Group	Treatment
K ⁻	Normal diet
K ⁺	HCD
A1	HCD plus administration of 1% v/v curcuminoid cider
A2	HCD plus administration of 2% v/v curcuminoid cider
B1	HCD plus administration of curcuminoid extract 100 mg/kg b.w.
B2	HCD plus administration of curcuminoid extract 300 mg/kg b.w.

HCD: High-cholesterol diet

Preparation of animals

The experiment was conducted on 24 male Sprague-Dawley rats weighing approximately 140 g and adapted to the laboratory environment until stable condition for 1 week. The animals were divided into six groups: Negative control (K⁻) was fed a standard diet; the remaining five received hypercholesterolemic diets [Table 1] for 2 weeks. The rats were maintained in individual cages, under controlled temperature, humidity and illumination conditions, with water and diet *ad libitum* for 2 weeks. Body weight, total blood cholesterol, LDL, and high-density lipoprotein (HDL) level were recorded weekly. At the end of 4 weeks, the animals were sacrificed. Blood was collected through the retro-orbital puncture, and tissue organs (lung, vascular, and liver) were collected in RNA later solution. These organs were sectioned for quantitative real-time polymerase chain reaction (qRT-PCR). The protocol was approved by the Animal Care and Use Committee of the Faculty of Veterinary, Bogor Agricultural University, Bogor, Indonesia.

Determination of genes related to oxidative stress using quantitative real-time polymerase chain reaction

Analysis of qRT-PCR was used to quantify the expressions of four genes related to oxidative stress, including cluster of differentiation 44 (CD44), intercellular adhesion molecule 1 (ICAM-1), inducible NOS (iNOS), and lectin-like oxidized LDL receptor-1 (LOX-1).^[5] RNA was isolated from the rats' tissue organs (heart, vascular, and liver) using TRIzol[®] reagent (Life Technologies, California, US) according to the manufacturer's protocol. cDNA was synthesized using iScript One-Step RT-PCR Kit with SYBR[®] Green and KAPA SYBR[®] FAST qPCR Kit for analysis by RT-PCR with beta-actin as internal control. Primer sequences for RT-PCR were listed in Table 2.

Statistical analysis

The duplicate experiments were performed throughout this study. All data are presented as the mean ± standard deviation. The significant difference between control and treated groups was statistically analyzed by the *t*-test ($P \leq 0.05$).

RESULTS

Identification of chemical compounds in curcuminoid fraction and curcuminoid cider

The curcuminoid fraction obtained was 11.5 g from 1 kg *C. xanthorrhiza* rhizome. The Py-GC/MS chromatogram of the rhizome extract of *C. xanthorrhiza* is shown in Figure 1. Twenty-five peaks were obtained; all chemical constituents were characterized and identified [Table 3]. The components were grouped into main classes: Sesquiterpenoid (67.85%), phenolic (7.73%), alkene (7.66%), monoterpene (5.35%), benzene (2.63%), ester (0.97%), diterpene (0.55%), fatty acid (2.14%), benzyhydrol (2.18%), and others (2.94%).

Furthermore, GC-MS chromatogram of the curcuminoid cider is shown in Figure 2. Forty peaks were obtained; all the chemical compounds were characterized and identified [Table 4]. Chromatogram demonstrated that components were grouped into main classes: Furfural derivatives (25.75%), cycloalkanes (21.8%), organic acids (17.63%), benzopyrones (10.39%), benzene (4.41%), phenolics (4%), and others (16.05%).

Body weight and lipid profiling in rats

After 2 weeks of high-cholesterol diet (HCD) treatment, five groups showed higher body weight in relation to negative control. Serum levels of cholesterol increased in all the experimental groups after 2 weeks and

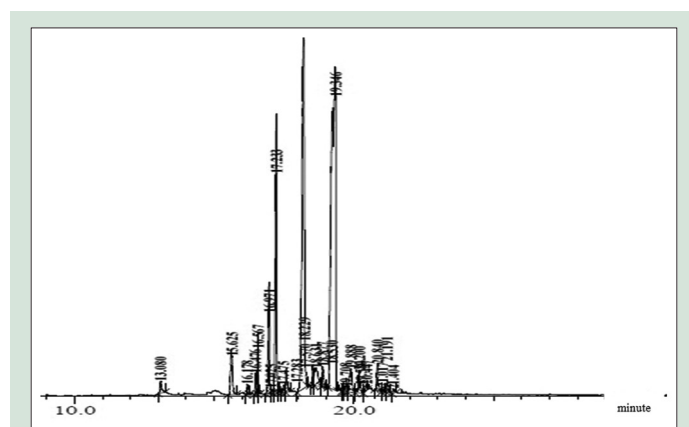
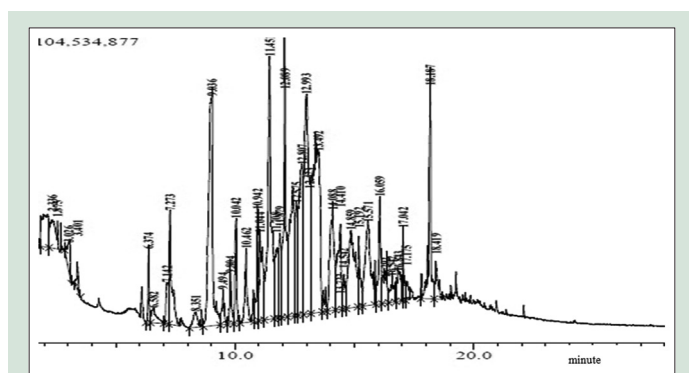
Table 2: Oligonucleotide primer for quantitative real-time polymerase chain reaction

Target gene	Forward primer	Reverse primer
CD44	GCTAAGACTGAGGTCTTTGGAG	GGAGGGATGCCAAGATGATAAG
ICAM-1	CCATGTGCTATATGGTCCTCAC	TACTCCCACGGGAGCTAAA
i-NOS	GGCTGAAATCCCCTCCAGAAT	CCTCTTGCTTTGACCCAGTAG
LOX-1	CCCTCACCTGGAAGCTAAAC	TCTCTGGGACTCCTCTGAATA
Beta actin	GTGCTATGTTGCCCTAGACTTC	TAGAGCCAGGGCAGTAAT

CD44: Cluster of differentiation 44; ICAM-1: Intercellular adhesion molecule-1; i-NOS: Inducible nitric oxide synthase; LOX-1: Lectin-like oxidized low-density lipoprotein receptor-1

Table 3: Identification of chemical compounds in curcuminoid fraction from *Curcuma xanthorrhiza*

Peak	Concentration	Name	Molecular formula	Molecular weight	Compound nature
1	2.04	Phenol, 4-methoxy- (cas) hqmm	C ₇ H ₈ O ₂	124.14	Phenolic
2	7.66	2,7-dioxa-tricyclo[4.4.0.0 3,8]deca-4,9-diene	C ₈ H ₈ O ₂	136.15	Alkene
3	0.62	Alpha-humulene	C ₁₅ H ₂₄	204.35	Sesquiterpene
4	2.63	Benzene, (2-propenyloxy)- (cas) phenyl allyl ether	C ₉ H ₁₀ O	134.18	Benzene
5	2.23	Cyclopentanemalononitrile			
6	6.92	Zingiberene	C ₁₅ H ₂₄	204.35	Sesquiterpene
7	0.7				
8	8.96	Aromadendrene	C ₁₅ H ₂₄	204.35	Sesquiterpene
9	0.97	Dihydrocarvyl acetate	C ₁₂ H ₂₀ O ₂	196.29	Ester
10	0.84	Alpha-humulene	C ₁₅ H ₂₄	204.35	Sesquiterpene
11	2.7	Alpha-humulene	C ₁₅ H ₂₄	204.35	Sesquiterpene
12	15.98	Patchoulane	C ₁₅ H ₂₆	204.35	Sesquiterpene
13	2.18	Perilla alcohol	C ₁₀ H ₁₆ O	152.23	Benzhydrol
14	5.7	Alpha.-humulene	C ₁₅ H ₂₄	204.35	Sesquiterpene
15	2.78	Trans-caryophyllene	C ₁₅ H ₂₄	204.35	Sesquiterpene
16	19.23	8b, 13:13,17-diepoxy-14,15-bisnorlabdane	C ₁₈ H ₃₀ O ₂	278.42	Sesquiterpene
17	0.55	Naphthalene, 1,2,3,4-tetrahydro-5,8-dimethyl-1-octyl- (cas) 5,8-dimethyl-	C ₂₀ H ₃₂	272.37	Diterpene
18	1.67	9,12,15-Octadecatrienoic acid, methyl ester (cas) Methyl 9,12,15-octadecatri	C ₁₉ H ₃₂ O ₂	292.5	Fatty acid
19	5.35	1,3-decadiyne (cas)	C ₁₀ H ₁₄	134.22	Monoterpene
20	3.45	Cis, cis, trans-3,3,6,6,9,9-hexamethyl-tetracyclo[6.1.0.0 (2,4).0 (5,7)]	C ₁₅ H ₂₄	204.35	Sesquiterpene
21	0.89	Cholesta-8,24-dien-3-ol, 4-methyl-, (3.beta.,4.alpha.)- (cas) 4.alpha.-methyl	C ₂₈ H ₄₆ O		Phenolic
22	3.5	7,7a-dimethyl-3a, 4,5,7a-tetrahydro-3h-benzofuran-2-one	C ₁₀ H ₁₄ O ₂	166.22	Phenolic
23	0.47	3,6-Octadecadienoic acid, methyl ester (cas) methyl-3,6-octadecadiyn	C ₁₉ H ₃₀ O ₂	290.44	Fatty acid ester
24	1.3	Tricyclo[5.1.0.0 (2,4)]octane-5-carboxylic acid, 3,3,8,8-tetramethyl-, methyl ester	C ₁₄ H ₂₂ O ₂	222.32	Phenolic
25	0.67	Linderazulene	C ₁₅ H ₁₄ O	201.27	Sesquiterpene


Figure 1: Pyrolysis gas chromatography mass spectrometry chromatogram of curcuminoid fraction from *Curcuma xanthorrhiza*

Figure 2: Pyrolysis gas chromatography mass spectrometry chromatogram of curcuminoid cider

decreased after 2 weeks administration of curcuminoid fraction and cider [Figure 3]. Interestingly, our results showed that there were no significant effects on LDL and HDL values after treatment with both samples. Then, curcuminoid fraction and its cider only affected cholesterol total, but not LDL and HDL values.

Determination of genes related to oxidative stress using quantitative real-time polymerase chain reaction

The expression of CD44, ICAM-1, iNOS, and LOX-1 mRNAs in various rat organs (lung, vascular, and liver) showed different patterns after treated with curcuminoid fraction and cider product. At all concentrations,

Table 4: Identification of chemical compounds in curcuminoid cider

Peak	Concentration	Name	Molecular formula	Molecular weight	Compound nature
1	1.86	Furan, 2-methyl- (cas) 2-methylfuran	C ₅ H ₆ O	82.1	Furfural derivative
2	1.47	1,2-diethylidiborane-d10	C ₄ H ₁₁ B	69.941	Borane
3	0.74	Acetic acid (cas) ethylic acid	C ₂ H ₄ O ₂	60.05	Organic acid
4	0.34	Propanoic acid, 2-oxo- (cas) pyruvic acid	C ₃ H ₄ O ₃	88.06	Organic acid
5	0.67	2-furancarboxaldehyde (casW) furfural	C ₅ H ₄ O ₂	96.08	Furfural derivative
6	0.6				
7	0.91	2-furanmethanol (cas) furfuryl alcohol	C ₅ H ₆ O ₂	98.1	Furfural derivative
8	2.23	2 (3h)-furanone, 5-methyl- (cas) 5-methyl-2-oxo-2,3-dihydrofuran	C ₅ H ₆ O ₂	98.1	Furfural derivative
9	0.59	3-heptyne-2-one	C ₇ H ₁₀ O	110.17	Aldehyde
10	8.29	Cycloheptane (cas)	C ₇ H ₁₄	98.19	Cycloalkanes
11	0.66	2-cyclopenten-1-one, 3-methyl- (cas) 3-methyl-2-cyclopentenone	C ₆ H ₈ O	96.13	Cycloalkanes
12	0.8	Carbamic acid, phenyl ester (cas) phenyl carbamate	C ₇ H ₇ NO ₂	137.14	Urethane
13	1.58	2h-pyran-2,6 (3h)-dione (cas) glutaconic anhydride	C ₅ H ₆ O ₄	130.1	Organic acid
14	1.33	Cyclobutane, 1,2-diethyl-, trans- (cas) 1,2-diethyl-cyclobutane	C ₈ H ₁₆	112.21	Cycloalkanes
15	1.28	Furan, 2,2'-methylenebis- (cas) 2-furfurylfuran	C ₉ H ₈ O ₂	148.16	Furfural derivative
16	2.33	Phenol, 2-methyl- (cas) o-cresol	C ₇ H ₈ O	108.14	Phenolic
17	7.34	2-methyl-1,3-cyclohexanedione	C ₇ H ₁₀ O ₂	126.15	Cycloalkanes
18	1.86	1,2-propanediol, 3-chloro- (cas) 3-chloro-1,2-propanediol	C ₃ H ₇ ClO ₂	110.54	
19	1.67	Phenol, 3,5-dimethyl- (cas) 3,5-xyleneol	C ₈ H ₁₀ O	122.16	Phenolic
20	4.46	Furan, 2-(2-furanylmethyl)-5-methyl- (cas) 5-methyl-2-furfurylfuran	C ₁₀ H ₁₀ O ₂	162.18	Furfural derivative
21	5.43	3-methyl-furan-2-carboxylic acid	C ₆ H ₆ O ₃	126.11	Organic acid
22	2.13	3-hexenoic acid	C ₆ H ₁₀ O ₂	114.14	Organic acid
23	5.69	4,4-d2-3-hydroxy-cyclopentene	C ₅ H ₆ O ₂	98.1	Furfural derivative
24	8.65	2-furancarboxaldehyde, 5-(hydroxymethyl)- (cas) HMF	C ₆ H ₆ O ₃	126.11	Furfural derivative
25	10.39	2h-1-benzopyran-2-one, 3-methyl- (cas) 3-methylcoumarin	C ₁₀ H ₈ O ₂	160.17	Benzopyrone
26	0.37				
27	3.57	Butanedioic acid (cas) succinic acid	C ₄ H ₆ O ₄	118.09	Organic acid
28	2.58	Benzene, 2-propenyl- (cas) allylbenzene	C ₉ H ₁₀	118.17	Benzene
29	0.71				
30	4.18	Oxirane, 2-butyl-3-methyl-, cis- (cas)	C ₇ H ₁₄ O	225.19	Cycloalkanes
31	1.05				
32	3.84	2,4-hexadienedioic acid (cas) muconic acid	C ₆ H ₆ O ₄	142.11	Organic acid
33	1.87	3-(tetrahydro-pyran-2-yloxy)-9-oxa-bicyclo[3.3.1]nonan-2-ol	C ₁₃ H ₂₂ O ₄	242.31	Diacetyl derivative
34	1.17	Benzene, 1-cyclopropyl-2-nitro- (cas) o-nitrocyclopropylbenzene	C ₉ H ₉ NO ₂	163.17	Benzene
35	0.66	2-propen-1-ol, 3-phenyl- (cas) cinnamyl alcohol	C ₉ H ₁₀ O	118.17	Benzene
36	0.91	Aflatoxicol h1			
37	0.67	1-nonen-3-yne (cas)	C ₉ H ₁₄	122.21	
38	0.37	3,5-octadien-2-one, (e, e)- (cas) e, e-3,5-octadien-2-one	C ₈ H ₁₂ O	124.18	
39	3.93	Caffeine	C ₈ H ₁₀ N ₄ O ₂	194.19	Caffeine
40	0.85	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3beta.,17beta.)	C ₂₂ H ₃₂ O ₂	328.49	

HMF: 5-hydroxymethylfurfural

curcuminoid fraction and cider significantly inhibited >50% of gene expression of CD44, ICAM-1, iNOS, and LOX-1 [Figure 4], indicating that both samples had potential effect to reduce oxidative stress-related genes inducing the formation of atherosclerotic plaque.

DISCUSSION

Spices not only make our food palatable, but also give beneficial pharmacological effects when used in judicious quantities. Even though they cannot totally displace the medications, they may be used in conjunction with medications to have better therapeutic potential to minimize the drug dose with little or no toxic effects as compared to synthetic chemicals and thereby be most cost effective. Many studies showed that the usage of alternative herbal medications such as curcumin have antihypercholesterolemic effects in Triton WR1339-induced hypercholesterolemia. Triton WR1339 (Tyloxapol) is widely used to

induce hypercholesterolemia in animal models.^[6] It is noted that the hypocholesterolemic effect of curcumin was attributed to its stimulatory effect on hepatic cholesterol-7 α -hydroxylase enzyme, an enzyme that regulates cholesterol catabolism.^[7]

Curcumin was also reported to modulate 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity that lead to the decrease in the levels of serum and liver cholesterol, triglycerides, and free fatty acid.^[8] Moreover, a study by Ramirez-Tortosa *et al.* also described about hypocholesterolemic mechanism of turmeric extract in rabbits fed by a HCD as an experimental atherosclerotic model.^[9] The mechanism was assumed through increased cholesterol excretion in the gall bladder together with decreased saturation of biliary cholesterol and increased fat excretion in the feces. Therefore, oral administration of turmeric extract significantly inhibited LDL oxidation and exerted hypocholesterolemic potential in rabbits.

Our data showed that Py-GC/MS analysis of curcuminoid fraction from *C. xanthorrhiza* rhizome revealed the presence of 25 components

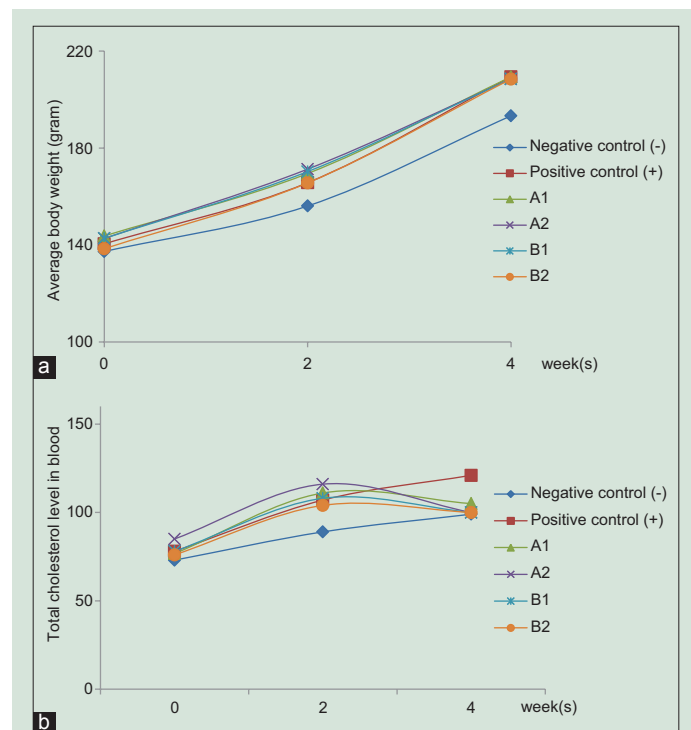


Figure 3: Diagram of average body weights (a) and total cholesterol level in blood (b). Six groups of male Sprague-Dawley rats were divided into: normal group diet (negative control), high-cholesterol diet 2% (positive control), high-cholesterol diet + 1% v/v cider (A1), high-cholesterol diet + 2% v/v cider (A2), high-cholesterol diet + 100 mg/kg b.w. (B1), and high-cholesterol diet + 300 mg/kg b.w. (B2) at 8 weeks of age (0 week), and body weight gain after 2 weeks (high-fed cholesterol) and 4 weeks (after treatment). Data were shown as mean \pm standard deviation from duplicate experiments

[Figure 1 and Table 3]. Major compound was identified as sesquiterpenoids (~67.85%). This study is in linear with a study by He *et al.* which reported that sesquiterpenoids are the major constituents of turmeric oil from *C. longa*.^[10] Curcuminoids are primarily accumulated in the rhizomes of *C. longa*. The contents of curcuminoids in *C. longa* rhizomes vary with varieties, locations, sources, and cultivation conditions. To date, at least 235 compounds, primarily terpenoids compounds and phenolics, have been identified from the species, including 22 diarylheptanoids and diarylheptanoids, eight phenylpropanes and other phenolic compounds, 68 monoterpenes, 109 sesquiterpenes, five diterpenes, three triterpenoids, four sterols, two alkaloids, and 14 other compounds.

Curcuminoids and essential oils are the major bioactive ingredients with various bioactivities *in vitro* and *in vivo* bioassays. Dried turmeric rhizomes usually yield 1.5–5% essential oils, which are dominated by sesquiterpenes and are responsible for its aromatic taste and smell.^[11] In terms of its bioefficacy, curcumin regulates the expression of genes related to energy metabolic process and fat deposition, thus decreasing the quantity of intra-cellular lipids. In adipose tissues (body fat), curcumin suppresses angiogenesis, which is essential for tissue growth and together with the effects on lipid metabolic process in adipocytes. Curcumin as an anti-obesity agent lowers body fat and reduces weight.^[12] Furthermore, our results also demonstrated that curcuminoid cider product consisted of 40 chemical compounds [Figure 2 and Table 4], and the major substances from the cider were identified as 3-methylcoumarin (10.39%) and 5-hydroxymethylfurfural (5-HMF; 8.65%). Both compounds have been widely reported for contributing to the aroma of fermented beverages.^[13] Coumarin is a fragrant organic chemical compound in the benzopyrone chemical class. Many coumarins and their derivatives have the special ability to scavenge ROS and show lipid lowering potentials. It has been known that coumarins also exert dual ability to scavenge ROS and modulate HMG-CoA reductase in HepG2 cell line via ROS-induced dysregulation of enzyme activity.^[14]

The 5-HMF, a product of the famous Maillard reaction, is mainly generated by acid-catalyzed thermal dehydration of fructose and identified as a flavoring substance in a wide variety of heat-processed products. It

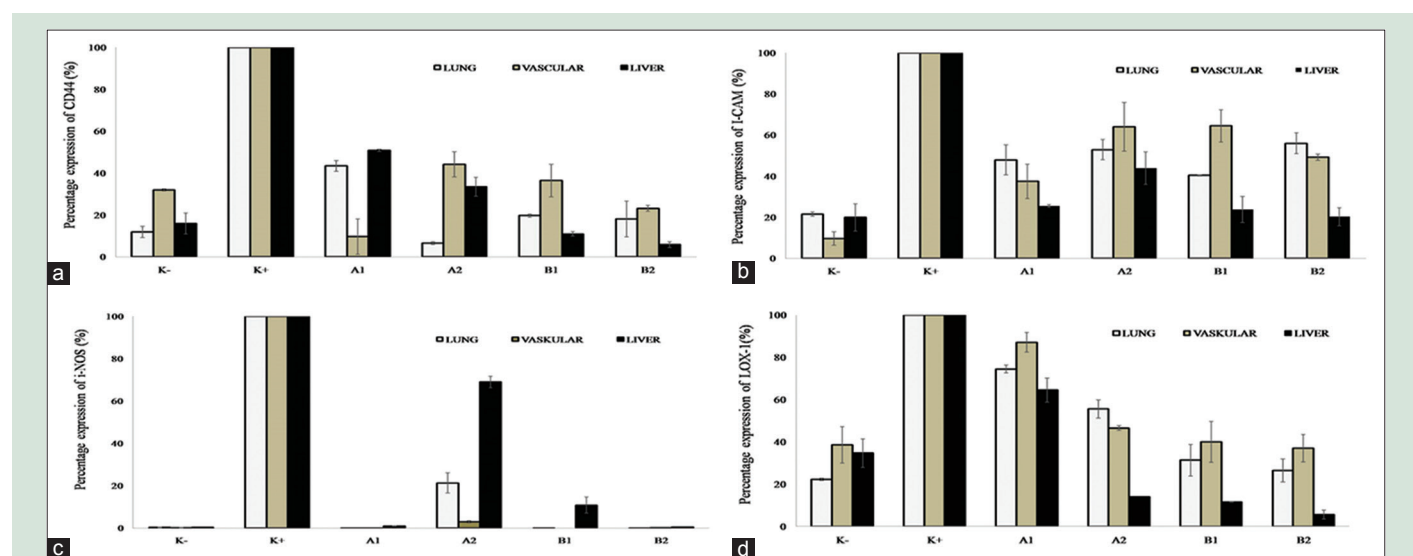


Figure 4: Percentage expression of cluster of differentiation 44 (a), intercellular adhesion molecule-1 (b), inducible nitric oxide synthase (c), lectin-like oxidized low-density lipoprotein receptor (d), and genes in various rat organs (lung, vascular, and liver). Six groups of male Sprague-Dawley rats were divided into: normal group diet (negative control), high-cholesterol diet 2% (positive control), high-cholesterol diet + 1% v/v cider (A1), high-cholesterol diet + 2% v/v cider (A2), high-cholesterol diet + 100 mg/kg b.w. (B1), and high-cholesterol diet + 300 mg/kg b.w. (B2). Data were shown as mean \pm standard deviation from duplicate experiments. $P < 0.05$ against positive control

has been reported that 5-HMF has favorable biological effects, such as antioxidant activity, anti-hypoxia, and inhibition of sickling of red blood cells.^[15-17] Mechanism of hypoxic injury contains oxidative stress process and 5-HMF can protect hepatocytes against damages induced by H₂O₂ *in vitro*.^[18,19] Other compounds such as furfural derivative (furan) as a potential carcinogen can be induced by heat from sugars, ascorbic acid, and fatty acids during cider fermentation process. Furan levels tend to be greater in foods that are heat processed in cans or jars, since the furan that forms cannot escape from the closed container.^[20]

Initial body weight of the animals was approximately 140 g for all groups. After 2 weeks of diet treatment, body weight increased more rapidly in high-cholesterol fed diet groups compared to normal diet groups [Figure 3a]. As a consequence, the percentage of weight gain increased compared with that in control negative group (K⁻) and the total blood cholesterol level in the HCD group was significantly higher. After 2 weeks administration of curcuminoid fraction and cider, total cholesterol levels from all animals fed with the treatment group (A1, A2, B1, and B2) were found to be effectively decreased [Figure 3b]. Oral administration of 2% curcuminoid cider (A2) caused significant elevation in reducing total cholesterol level in blood. Unfortunately, lipid profiles showed that administration of curcuminoid fraction and its cider at various doses did not affect significant change in decreasing LDL and increasing HDL compared to those of control group (data not shown). Previous study reported that the deposition of cholesterol in the liver decreased with the increasing dosage of curcumin in the diet. Deposition was maximum with dietary cholesterol and no curcumin and minimum when diets included curcumin.^[21] Signs with any tendency for the total cholesterol level to rise due to faulty dieting or defective metabolism can be reversed by the ingestion of curcuminoid fraction.

In this study, we quantified whether the treatment of curcuminoid fraction and its cider modulated the expression of several genes related to stress oxidative (CD44, ICAM-1, iNOS, and LOX-1) in various rat organs using qRT-PCR [Figure 4]. Our results showed that among all genes, the expression of CD44 mRNA after sample treatments (curcuminoid fraction and cider) in lung, vascular, and liver exerted fluctuative results [Figure 4a]. Overall, both curcuminoid fraction and cider may offer significant protection against stress oxidative effects via attenuating CD44, ICAM-1, iNOS, and LOX-1 genes expression [Figure 4a-d]. Previous study described that curcumin was known as a downregulator of stem cell marker CD44 in colon cancer and suppressed the increased intensity of ICAM-1 expression in the diabetic rats.^[22,23] At low concentration, curcumin inhibited NO production by suppressing the expression of iNOS mRNA and protein induction in macrophages.^[24]

Our results showed that curcuminoid fraction at 300 mg/kg body weight (B2) reduced the expression of CD44, ICAM-1, and iNOS mRNAs in the concentration-independent manner [Figure 4a-c]. CD44 is expressed on both inflammatory and vascular cells and it mediates the adhesion of T-lymphocytes to endothelium and smooth muscle cells (SMCs), release of inflammatory mediators from macrophages and T-lymphocytes, and proliferation of vascular SMCs. The principal ligand of CD44 is hyaluronan, a major glycosaminoglycan constituent of extracellular matrix, which is upregulated within atherosclerotic lesions.^[25]

ICAM-1 is one of the immunoglobulin superfamilies of endothelial adhesion molecules, participates in leukocyte adhesion to the endothelium and plays an important role in all stages of atherosclerosis.^[26] ICAM-1 and most of the other adhesion molecules can be regulated by cytokines. ICAM-1 was the most prominent adhesion molecule in lesion-prone sites; its expression appeared to be independent of plasma cholesterol levels. NO is an important biological mediator in the living organism that is synthesized from L-arginine using NADPH and molecular oxygen. NO is a short-lived free radical and a very small

compound that diffuses freely within cells from its site of formation to its site of action. However, the overproduction of NO which is catalyzed by iNOS, a soluble enzyme and active in its dimeric form, is cytotoxic and lead cell to oxidative stress condition.^[27]

Treatment of curcuminoid fraction and cider also significantly reduced the expression of LOX-1 genes in a concentration-dependent manner. Several studies showed that curcumin inhibited LOX-1 and suppressed the inflammatory response *in vitro* and *in vivo*.^[28] LOX-1 expression is highly correlated with atherosclerotic plaque instability.^[24] In strong contrast, deletion of LOX-1 reduced atherogenesis in LDLR knockout mice, fed with a HCD.^[29] Furthermore, human genetic studies link LOX-1 polymorphisms to cardiovascular disease susceptibility.^[30]

CONCLUSION

Oral administration of curcuminoid fraction from *C. xanthorrhiza* and curcuminoid cider exerted promising hypocholesterolemic effects through inhibition of genes related to oxidative stress (CD44, ICAM-1, iNOS, and LOX) in rats *in vivo*. Therefore, curcuminoid fraction and its cider may offer cardioprotective effect for preventing hypercholesterolemia-induced atherosclerosis *in vivo*.

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Conflicts of interest

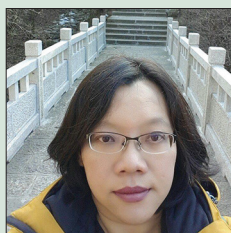
There are no conflicts of interest.

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