J Intercult Ethnopharmacol 2013; 2(1):53-56 *ISSN:2146-8397*

GESDAV

Journal of Intercultural Ethnopharmacology

available at www.scopemed.org

Original Research

Potential effects of xanthone on inflammation status in atherosclerotic rats

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Received: December 03, 2012

Accepted: December 25, 2012

Published Online: January 18, 2013

DOI : 10.5455/jice.20121225012655

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Keywords: Xanthone; inflammation; NF-κB p65; TNF-α; NO; hypercholesterol diet.

Summary
Aim: To clarify an effect of xanthones on inflammation status in hypercholesterolemic rats.
Method: A total of 32 Wistar rats were divided into four groups (n=8), including control, hypercholesterolemic diet groups, hypercholesterolemic diet + xanthone at dose 35; 70; and 140 mg/kg body weight (mg/kgBW). Control group received standard diet for 60 days. Hypercholesterolemic diet group received standard diet plus yellow egg, sheep oil, cholic acid, and pig oil for 60 days per oral. Analysis of nuclear factor-kappa beta p65/p50 distribution and tumor necrosis factor-alpha level, was done using enzyme linked immunosorbent assay technique. Analysis of nitric oxide level was done by colorimetric technique using spectrophotometer.
Results: Hypercholesterolemic diet significantly increased nuclear factor-kappa beta p65/p50 distribution, tumor necrosis factor-alpha level, and nitric oxide level compared with the control group ($p<0.05$). Xanthone decreased nuclear factor-kappa beta p65/p50 distribution in line with the distribution at standard diet at doses 70 and 140 mg/kg body weight. Xanthone significantly decreased tumor necrosis factor-alpha level compared with atherosclerotic diet group at all doses, although it did not reach the level at standard diet. Xanthone decreased nitric oxide level, reaching the level at standard diet in all doses.
Conclusion: Xanthone has antiinflammatory potentials by inhibiting distribution of nuclear factor-kappa beta p65/p50, decreasing tumor necrosis factor-alpha and nitric oxide level.

INTRODUCTION

The mangosteen fruit is known as the queen of the fruit due to its delicious taste and pleasant aroma. This plant originates from Southeast Asia and distributed in Thailand, India, Sri Lanka, Myanmar, Malaysia, the Philippines, China, Indonesia and other tropical countries [1]. In the United States, mangosteen product is now widely available due to the perception of the public as a supplement that can improve health. Mangosteen juice repredents a food supplement of the primary agricultural products, which tops the sales in 2005 [2].

Mangosteen rind has been used as a traditional medicine in Southeast Asia for many years, to cure diarrhea, dysentery, skin infections, chronic wounds, suppuration, leukorrhea, and gonorrhea [1, 3]. Various studies have revealed the potential effects of active ingredients from the mangosteen rind, among others, as antioxidants [4], antibacterial, antifungal, antimalarial, anti-inflammatory [5], and the cytotoxic activities of human immunodeficiency virus (HIV) inhibitor, aromatase inhibitors and quinone reductase activity inductor [6].

Anti-inflammatory potential of Garcinia mangostana (GM) was evidenced in the research by Yamakuni et al.,

[7] in which garsinon B (10μM) reduced the release of prostaglandind-E_2 (PGE_2) by 30%, which was induced by A23187 in C6 rat glioma cells. Garsinon B (20μM) also decreases the activation of nuclear factor-kappa beta p65/p50 (NF-κB p65/p50) which is induced by lipopolysaccharide (LPS) by 30%. Research by Chonmawang et al., [4] proved that GM can reduce the production of tumor necrosis factor-alpha (TNF-α) on inflammation caused by Propionibacterium acne.

The mangosteen fruit is a rich source of phenolic compounds. Various phenolic compounds in mangosteen include xanthone, tannins, and anthocyanins. Of these phenolic compounds, only xanthone is most frequently investigated [8]. Xanthone compounds are soluble in alcohol, ether, acetone, chloroform, and ethyl acetate, while flavonoids and polyphenols are soluble in water and other polar solvents [1,3]. Xanthone core is known as 9-xanthenone or dibenzo-γ-pyrone which is simetric. Xanthone is classified into five groups: (a) simple oxygenated xanton; (b) xanthone glycosides (c) prenylated xanthone*;* (d) xanthonolignoids and (e) other xanthones. Biological activities of xanthone is related to tricyclics but vary depending on the position of various substituents [9].

No various studies above revealed the potential effects of xanthone on inflammatory status in atherosclerotic mouse models. Therefore, this study will attempt to analyze the potential effects of xanthone on levels inflammation in atherosclerotic rats model. The hypothesis of this study is that ethanol extract of mangosteen rind can reduce levels of NF-κB p65/p50, TNF-α, and nitric oxide (NO) levels in atherosclerotic rats.

MATERIALS AND METHODS

Subjects

Subjects of the research were Rattus norvegicus obtained from the Laboratory of Pharmacology of the Faculty of Medicine, University of Brawijaya, Malang. The rats used here were male rats, aged 6-8 weeks, weight 100-150 grams, and kept in a cage with an open vent. Before the treatment, the mice were acclimatized for 2 weeks. Rats were kept in single system cages, each cage contains 1 rat. This cage is a plastic cage measuring 45 cm x 35.5 cm x 14.5 cm with a lid made of woven wires. The bottom of cage is rice husk, which was changed every three days. The research has been approved by the Research Ethics Committee of the Medical Faculty, University of Brawijaya, Malang.

Feed and xanthone supplementation

The feed given every day was feed for adult rats with composition of the Comfeed PAR-S, high protein flour, and water. This composition is the composition of the feed for standard diet. Weight of feed given per rat is ± 40 grams per day and replaced daily. For hypercholesterolemic diet, the composition of feed ingredients consist Comfeed PAR-S, flour, egg yolks, goat oil, cholic acid and pig oil. Xanthone used here is the xanthone isolates of Nacalai Tesque products.

The study groups consisted of a group of rats that received a standard diet, the rats that received the high-fat diet, as well as groups of rats that received the high-fat diet + xanthone diet (35, 70, and 140 mg/kg body weight (mg/kgBW)). The treatment was carried out for 60 days. Each treatment group consisted of eight rats for a total of rats from all groups were 32 rats.

Sampling

The sample was aorta after 60 days of treatment. Aortas were removed by putting rats in a jar containing cotton already soaked with ether for inhalation anesthesia. When the rats were already anesthetized, they were dissected to by opening the abdomen to the thorax. Once the heart was seen clearly, blood was collected by 5 mL syringe in their ventricles slowly. Once the blood was already removed, the aorta was cut and then stored in cold temperature and subject to imunohistochemistry (NF-κB p65/p50), enzyme linked immunosorbent (ELISA) assay (TNF-α) and spectrophotometric analysis (NO). All procedures were done according to instruction of kit manufactures.

Statistical analysis

Data are presented as mean ± standard deviation and differences between groups were analyzed using Analysis of Variance (ANOVA) test using Statistical Package for Social and Science (SPSS) 16.0 software. $p < 0.05$ was considered statistically significant.

RESULTS

ANOVA test of the distribution of NF-κB p65/p50 in the various treatment groups found significant differences ($p < 0.001$). Post Hoc Test found significant increase in the distribution of NF-κB p65/p50 between the standard diet groups compared with the hypercholesterolemic diet group ($p < 0.001$). Mann Whitney test showed significant decrease in distribution of NF-κB p65/p50 between the hypercholesterolemic diet group compared with hypercholesterolemic diet groups + xanthone isolates of 35 mg/kgBW ($p<0.001$); between hypercholesterolemic diet group compared with the hypercholesterolemic diet group + xanthone isolates of 70 mg/kgBW ($p<0.001$); between hypercholesterolemic diet group compared with hypercholesterolemic diet group + xanthone isolates of 140 mg/kgBW ($p<0.001$). Mann Whitney test found no significant differences

between standard diet groups compared with the hypercholesterolemic diet group + xanthone isolates of 70 mg/kgBW (p=0.183), and between standard diet groups compared with hypercholesterolemic diet group + xanthone isolates of 140 mg/kgBW (p=0.229).

Kruskall-Wallis test on TNF-α levels in the various treatment groups found significant differences ($p < 0.001$). Mann Whitney test found significant increase in the distribution of TNF-α between the standard diet groups compared with the hypercholesterolemic diet group (p=0.009). Mann Whitney test showed significant decreases in level of TNF-α between the hypercholesterolemic diet group compared with hypercholesterolemic diet groups + xanthone isolates of 35 mg/kgBW (p=0.009); between hypercholesterolemic diet group compared with the hypercholesterolemic diet group + xanthone isolates of 70 mg/kgBW (p=0.009); between hypercholesterolemic diet group compared with hypercholesterolemic diet group + xanthone isolates of 140 mg/kgBW (p=0.009).

Analysis of the levels of NO in the various treatment groups with the Kruskall-Wallis test found significant differences ($p < 0.001$). Mann Whitney test found significantly increase in NO levels between standard diet groups compared with the hypercholesterolemic diet (p=0.009); between standard diet groups compared with hypercholesterolemic diet + xanthone isolated of 35 mg/kgBW (p=0.009), between the standard diet group compared with the hypercholesterolemic diet + xanthone isolates of 70 mg/kgBW (p=0.009); between standard diet groups compared with the hypercholesterolemic diet + xanthone isolates of 140 mg/kgBW (p=0.009).

Mann Whitney U test showed significant decreases in NO level between the hypercholesterolemic diet group compared with hypercholesterolemic diet groups + xanthone isolates of 35 mg/kgBW ($p < 0.001$); between hypercholesterolemic diet group compared with the hypercholesterolemic diet group + xanthone isolates of 70 mg/kgBW (p=0.009); between hypercholesterolemic diet group compared with hypercholesterolemic diet group + xanthone isolates of 140 mg/kgBW (p=0.009). I was found no significant decrease in levels of NO between the cholesterolemic diet group + xanthone isolates of 35 mg/kgBW compared with hypercholesterolemic diet + xanthone isolates of 140 mg/kgBW (p=0.602); between cholesterolemic diet + xanthone isolates of 70 mg/kgBW compared with the hypercholesterolemic diet + xanthone isolates of 140 mg/kgBW (p=0.530).

DISCUSSION

Inflammation is a pathophysiological processes mediated by various signaling molecules produced mainly by leukocytes, macrophages, and plasma cells [10]. Inflammation is a predictor of cardiovascular disease and considered as precursors of metabolic syndrome. Various treatments were administered to manage inflammation. Steroid is the best medicine used to treat acute inflammation, but has side effects when used for long periods of time, such as lowering the resistance of infection. Non-steroidal anti-inflammatory drugs are also used to treat inflammation, but they bring about side effects such as gastrointestinal bleeding [11].

In this study, xanthone isolates are able to reduce the distribution of NF-κB p65/p50 starting at a dose of 35 mg/kgBW and reached a standard diet at dose levels of 70 mg/kgBW and 140 mg/kgBW. The mechanism of inhibition of NF-κB p65/p50 distribution is caused by the blockade of kappa-B kinase inhibitor activity. This is based on the research by Udani et al., [11] which has proved the potential effects of the mangosteen as anti-inflammatory substance through the inhibition mechanism of cyclooxygenase, changes in arachidonic acid into prostaglandin E_2, blockade of kappa-B kinase activity and inhibition of rat foot edema. Moreover, inhibition of NF-κB p65/p50 distribution will impede the genomic process of NF-κB p65/p50 which is characterized by a decrease in its protein product. This is evidenced by a decrease in production of TNF-α.

Table 1. Distribution of NF-κB p65/p50 and TNF-αNO levels in some groups

	Standard diets	Hypercholesterolemic diet + xanthone isolates			
Distribution & Levels		0 mg/kg BW	35 mg/kg BW	70 mg/kg BW	140 mg/kg BW
NF-κB p65/p50	5.40±2.88	19.20±1.92[a]	12.20±2.59[ab]	7.40±2.51[bc]	3.60±1.14[bcd]
TNF-α (ng/mL)	14.06±0.31	42.93±0.75[a]	23.55±1.13[ab]	25.84±4.15[ab]	17.55±1.03[abcd]
NO (mmol/L)	0.74±0.15	6.95±0.58[a]	1.47±0.19[ab]	1.09±0.11[abc]	1.59±0.60[ab]

NF-κB p65/p50: nuclear factor kappa beta p65/p50; TNF-α: tumor necrosis factor-alpha; mg/kg BW: miligram/kilogram body weight; ng/mL: nanogram/mililiter; mmol/L:milimol/liter;
[a]There was significant difference in standard diet group ($p<0.05$); [b]There was significant difference in hypercholesterolemic diet + xanthone of 0 mg/kg bw ($p<0.05$); [c]There was significant difference in hypercholesterolemic diet + xanthone of 35 mg/kg bw ($p<0.05$); [d]There was significant difference in hypercholesterolemic diet + xanthone of 70 mg/kg bw ($p<0.05$)

The mechanism of this decrease takes place through inhibition of NF-κB p65/p50 translocation to the nucleus characterized by a decrease in the distribution of NF-κB p65/p50. The decline in NF-κB p65/p50 distribution will cause a decrease in mRNA expression of TNF-α. The research by Chonmawang et al., [4] have shown that the GM extract at a dose of 50 mg/mL can inhibit the formation of TNF-α from peripheral blood mononuclear cells induced by Propionebacterium acne at 94.59%.

Macrophages play an important role in producing proinflammatory molecules of nitric oxide. Nitric oxide synthesized by the enzyme inducible nitric oxide synthase (iNOS) is an acute and chronic inflammatory mediators [10]. The study by Chen et al.[5] has proven mechanism of α-mangostin dan γ-mangostin as anti-inflammation in vitro. In RAW 264.7 cells exposed to LPS, α-mangostin dan γ-mangostin could inhibit the production of NO and PGE_2. The research by Tewtrakul et al., also found that extracts of mangosteen can lower levels of NO in RAW 264.7 cells exposed to LPS, which was better than α-mangostin dan γ-mangostin. In this study, reduced levels of NO is caused by the capability of xanthone to inhibit the expression of iNOS [12]. This is supported by Chen et al., saying that inhibition of NO production occurs through inhibition of iNOS expression rather than through inhibition of iNOS activity in RAW 264.7 cells exposed to LPS [5].

CONCLUSION

Xanthone has a good anti-inflammatory potential through the inhibition of NF-κB p65/p50 distribution, decreased levels of TNF-α and decreased levels of NO in aorta of atherosclerosis rats.

ACKNOWLEDGMENT

The author are thankful to Dr. Nurdiana for permission to acces Laboratory of Pharmacology and to Mrs. Husnul Khotimah, S.Si., M.Kes for her valuable technical expertise. Author also thank to all technician in Laboratory of Pharmacology (Mrs. Ferida and Mr. Moch Abuhari) for technical helping in this study.

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