

Mangosteen Pericarp Inhibits Nuclear Factor κ B (NF- κ B) Activation and Reduces Expression of ICAM-1 in High Cholesterol Diet Rat

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Background: Atherosclerosis is widely viewed as an inflammatory disease with hypercholesterolemia being a dominant underlying risk factor. This study aimed to determine the effect of mangosteen pericarp in inhibition of NF- κ B activation and ICAM-1 expression in rat fed with high cholesterol.

Methods and Results: Various doses of crude extract mangosteen pericarp were administered to the high fat diet wistar rats and the activity of NF- κ B measured by immunohistochemistry to assess nuclear NF- κ B expression and the ICAM-1 expression. The high fat diet resulted significant increased serum LDL levels. Increased nuclear NF- κ B activation and ICAM-1 expression were also observed in high fat diet rats in concurrence with increased serum LDL. The inhibitory effect on NF- κ B activity and ICAM-1 expression was observed when 400 mg of mangosteen pericarp crude extract was administered and even showed a higher inhibitory effect in 800 mg of mangosteen pericarp treated rats. The 800 mg extract treatment resulted in decreased ICAM-1 expression similar to those of non high fat rats.

Conclusion: The administration of 800 mg mangosteen pericarp crude extract significantly inhibited NF- κ B activation and reversed the expression of ICAM-1 to the normal level in high cholesterol diet rats.

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Keywords: High fat rat, mangosteen pericarp, NF- κ B, ICAM-1

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Kulit Manggis Menghambat Aktivasi Nuclear Factor κ B (NF- κ B) dan Menurunkan Ekspresi ICAM-1 pada Tikus dengan Diet Tinggi Kolesterol

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Latar belakang: Aterosklerosis secara luas dipandang sebagai penyakit inflamasi dengan hiperkolesterolemia menjadi faktor risiko dominan yang mendasari. Penelitian ini bertujuan untuk mengetahui pengaruh kulit manggis dalam penghambatan aktivasi NF- κ B dan ekspresi ICAM-1 pada tikus yang diberi pakan tinggi kolesterol.

Metode dan Hasil: Berbagai dosis ekstrak kasar kulit manggis diberikan pada tikus wistar dengan pakan tinggi lemak dan aktivasi NF- κ B diukur dengan imunohistokimia untuk menilai ekspresi NF- κ B inti sel dan ekspresi ICAM-1. Pakan tinggi lemak mengakibatkan peningkatan kadar LDL serum yang signifikan. Peningkatan aktivasi NF- κ B dan ekspresi ICAM-1 juga diamati pada tikus diet tinggi lemak sesuai dengan peningkatan serum LDL. Efek penghambatan pada aktivasi NF- κ B dan ekspresi ICAM-1 dapat diamati pada pemberian ekstrak kasar kulit manggis 400 mg dan bahkan menunjukkan efek penghambatan lebih tinggi pada tikus yang diberi ekstrak kulit manggis dengan dosis 800 mg. Pemberian ekstrak kulit manggis 800 mg menghasilkan penurunan ekspresi ICAM-1 mirip dengan tikus yang tidak diberi pakan tinggi lemak.

Kesimpulan: Pemberian ekstrak kasar kulit manggis 800 mg menghambat aktivasi NF- κ B secara signifikan dan menurunkan ekspresi ICAM-1 ke tingkat normal pada tikus dengan pakan tinggi lemak.

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Kata kunci : tikus dengan pakan tinggi lemak, kulit manggis, NF- κ B, ICAM-1

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Atherosclerosis, the major cause of death from cardiovascular disease in industrialized countries, is characterized by the progressive accumulation of lipid and fibrous depositions in the vessel wall of large arteries.^{1,2} Despite considerable therapeutic advances over the past 50 years, cardiovascular disease is the leading cause of death worldwide. This is mainly a result of the increasing prevalence of atherosclerosis, and, above all, the widespread under-recognition and under treatment of individuals with risk factors for atherosclerosis with hypercholesterolemia being a dominant underlying risk factor.³ It is

believed to be initiated by retention of LDL particles in the lesion-prone areas, which is followed by monocyte recruitment and their differentiation into cholesterol-laden macrophage foam cells. Excessive cholesterol accumulation in macrophages exaggerates innate immune response that is manifested by up regulated production and secretion of inflammatory cytokines and chemokines, thus dramatically amplifying initial signal originated from the injured artery.⁴

Molecular pathogenesis researches are warranted to dissect the mechanism and explore the possible inhibitory ways by a new effective drug. One alternative a candidate anti inflammatory agent is mangosteen pericarp which contains many antioxidant substances including xanthone.⁵

This study therefore aimed to examine whether mangosteen pericarp inhibits inflammatory processes through the transcription factor NF-κB. The expression of ICAM -1, an adhesion molecular target of NF-κB, was also measured to see the consequence of NF-κB inhibition.

Materials and Methods

Thirty 3-4 month-old healthy male *Rattus novvergicus* wistar strain (150- 300 gram) were randomly divided into 5 groups. 4 groups fed with high cholesterol diet and 1 group fed with non high cholesterol (as a control negative group). The compound of diet consist of 67% comfeed PAR-S, 33% of the flour, and water sparingly (*normal diet*), and *hipercholesterol diet* consists of 50% comfeed PAR-S, 25% flour, 2% cholesterol, 0.2% cholic acid, 5% oil (lard), and water. Mangosteen pericarp crude extract were administered by gastric sonde route. Among 4 groups of high cholesterol rats, 3 groups were treated with 200 mg/kg body weight (kbw), 400 mg/kbw and 800 mg/kbw of mangosteen pericarp crude extract, respectively. 1 group of high fat diet rats without treatment functioned as a positive control group. After 12 weeks treatment, NF-κB activation and ICAM-1 expression was checked by immunohistochemistry methods, respectively.

Mangosteen crude extract preparation: *First, Drying Process* wash started by washing the hull of mangosteen cleanly, cutting in small size and finally keeping in the 80 degrees oven or with the sun hot until dry (moisture free), *second, The process of extraction:* Once dry, puree with a blender until smooth, weigh as much as 100 grams of dried sample (mangosteen hull)

into the erlenmeyer glass size 1 liter, then soak with volume 900 ml of ethanol up, shake until completely mixed (\pm 30 minutes), let sit for 1 night until it settles, and *third, The process of evaporation:* take the top layer of a mixture of ethanol with active substances that have been fetched, put in the evaporation tube 1 liter, attach evaporation tube in evaporator, fill water bath with water until it is full, all pairs of sets of tools including rotary evaporator, heater water bath (set up to 90 degrees), let the solution of ethanol with active substance splitting existing in the pumpkin, wait until the flow stops dripping on ethanol reservoir tube (\pm 1.5 to 2 hours for 1 tube), the results obtained roughly 1/3 of the dry natural materials, put extraction results in a plastic bottle, store in freezer.

Immunohistochemistry using the monoclonal anti-ICAM-1

Cells are washed with HEPES buffer for 30 minutes and fixation with methanol for 5 minutes, dry and wash with PBS pH 7.4, application of 3% H₂O₂ for 10 minutes and wash with PBS pH 7.4. Bloking using serum 5% FBS containing 0.25% Triton X-100 and incubation for 1 hour at room temperature. Wash with PBS pH 7.4 give drops with antiGoat IgG (secondary antibodies) biotin labeled and incubation for 1 hour, wash with PBS pH 7.4 and give drops with SA-HRP for 40 minutes, then wash with PBS pH 7.4 and apply for HRP substrate, i.e. DAB (Diamono Benzidine). Counterstain with Mayer hematoxilin for 10 minutes, rinse with tap water and washing with dH₂O. Drain and shut the cover glass.

Observe under the microscope with magnification up to 200 x until 100 endothelial cells is achieved by three different laboratory personnel who are experienced. Expression of ICAM-1 in endothelial cells are seen in the brown color on the cell membrane endothelial cell. Note the number of endothelial cells that are brown in between the endothelial cells of 100.

Observation on the expression of NF-κB activation Aortic Tissue by Immunohistochemistry

Slide washed with PBS pH 7.4 one time for 5 minutes, endogenous bloking using 3% peroxide H₂O₂ for 20 minutes, wash using PBS, pH 7.4, three times for 5 minute, bloking using unspesifik protein 5% FBS containing 0.25% Triton X-100, wash using PBS,

pH 7.4, three times for 5 minutes. Incubation using monoclonal antibodies of NF-κB activation (p65) (LabVision), during the 60 minutes, wash using PBS, pH 7.4, three times for 5 minutes, incubation using anti mouse HRP conjugated for 40 minutes. Wash using PBS, pH 7.4, three times for 5 minutes. Give drops with a DAB (Diamino Benzidine) and incubation for 10 minutes. Wash using PBS, pH 7.4, three times for 5 minutes, wash using dH₂O, for 5 minutes. The counterstaining using Mayer Hematoxilen incubated for 10 minutes and wash using tap water, rinse using dH₂O and dried. Mounting using entelan and cover with cover glass, and observe the light microscope.

Definitions

Normal Diet: food consists of 67% comfeed PAR-S, 33% of the flour, and water sparingly. *Hypercholesterol Diet :* Food consists of 50% comfeed PAR-S, 25% flour, 2% cholesterol, 0.2% cholic acid, 5% oil (lard), and water.

Serum LDL cholesterol: total cholesterol levels in the blood of every mg/dl, is called hypercholesterolemia if taken more than 2 times the levels of control.

Translocation of NF-κB activation: endothelial cell number which is express p65 (NF-κB activation) in the cytoplasm and nucleus of the endothelial cells that are painted with immunohistochemistry method on one cell well disc. It said if the number of p65 translocations in the cell nucleus more than in the cytoplasm. Endothelial cells that express p65 NF-κB activation marked with a brown color in the cytoplasm and the nucleus. Measured using a semiquantitative method (endothelial cell number that brown among 100 endothelial cells) and viewed with a light microscope (nikon) at 200 x magnification.

Expression of ICAM-1: The number of endothelial cells that express ICAM-1 protein in the cytoplasm of endothelial cells that were painted by immunohistochemistry methods on one well disc. Endothelial cells

expressing ICAM-1 is characterized by brown color on the cell membrane. Measurement of endothelial cells expressing ICAM-1 using a semiquantitative method (the number of endothelial cells brown among the 100 endothelial cells) and viewed with a light microscope (Nikon) at 200X magnification.

Statistical Analysis

Data analysis, which were performed on One way ANOVA, and continued with Post Hoc Analyses, to examine the effect of mangosteen pericarp with variety doses about NF-κB activation and ICAM-1 expression on high cholesterol diet treatment group, significance level 0.05. Analyses were performed with SPSS version 17.0 for Windows (SPSS, Inc, Chicago, Illinois).

Results

Figure 1. In the negative control group (A) occurs the expression of NF-κB activation (arrow), the positive control group who received high-cholesterol diet (B) increased expression of NF-κB activation (arrows). On treatment with mangosteen peel extract dose of 200 mg/kg (C) is still there appeared to be increased expression of NF-κB activation. But at a dose of 400 mg / kg (D) and the dose of 800 mg / kg (E) began to appear decreased expression of NF-κB activation.

Figure 2. In the negative control group (A) occurs the expression of ICAM-1 (arrow), the positive control group who received high-cholesterol diet (B) increased expression of ICAM-1 (arrows). On treatment with mangosteen peel extract dose of 200 mg/kg (C) is still there appeared to be increased expression of ICAM -1. But at a dose of 400 mg/kg (D) and the dose of 800 mg / kg (E) began to appear decreased expression of ICAM -1.

Table 2. In the negative control group occurred expression of NF-κB activation with the lowest value

Table 1. Mean of Cholesterol, Triglycerides, LDL and HDL level

Treatment	n	Cholesterol		TG		LDL		HDL	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Negative Control	6	113	10.353	94	5.366	47.33	9.973	46.83	1.472
Positive Control	6	165.66	19.356	143	8.672	108	17.765	28.33	2.316
High-cholesterol diet + mangosteen peel extract 200 mg/kg	6	152.66	7.685	134.33	4.320	92.66	7.118	33.00	3.346
High-cholesterol diet + mangosteen peel extract 400 mg/kg	6	140.83	8.658	128.33	4.131	79.83	8.976	35.33	2.160
High-cholesterol diet + mangosteen peel extract 800 mg/kg	6	132.33	5.680	120.66	6.439	61.33	7.312	46.833	2.994

(29.67), whereas the positive control group who received high-cholesterol diet increased the mean expression of NF-κB activation, with the highest yields (71.83). On treatment with mangosteen peel extract dose of 200 mg/kg was still an increase in the average expression of NF-κB activation (71.00), and with in-

creasing doses starting to look a decrease in the average expression of NF-κB activation, at doses of 400 mg/kg (56.67) and at doses of 800 mg/kg (39.00).

Table 3. In the negative control group occurred expression of ICAM -1 with the lowest value (20.50), whereas the positive control group who received

Table 2. Mean NF-κB activation in aortic endothelial cells.

Treatment	n	Mean	SD
Negative Control	6	29.67	6.121
Positive Control	6	71.83	4.708
High-cholesterol diet+ mangosteen peel extract 200 mg/kg	6	71.00	2.530
High-cholesterol diet+ mangosteen peel extract 400 mg/kg	6	56.67	6.088
High-cholesterol diet+ mangosteen peel extract 800 mg/kg	6	39.00	6.164

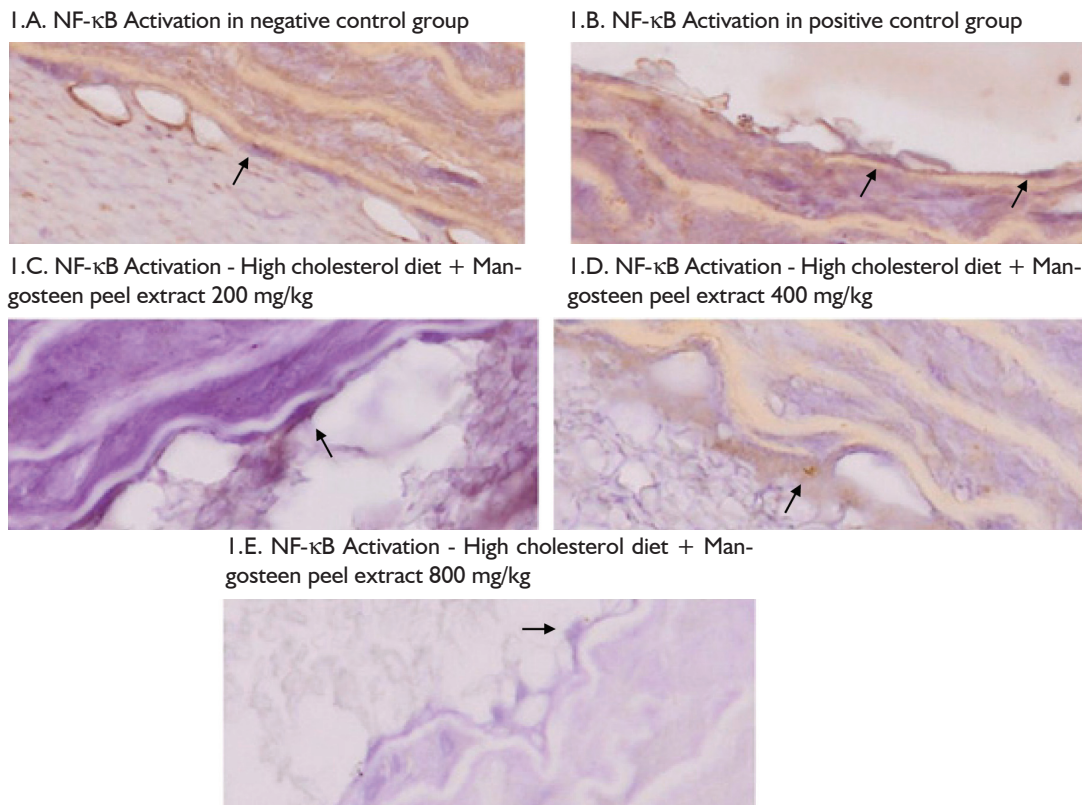


Figure 1. A, B, C, D, E Activation of NF-κB Expression in aortic endothelial cells.

Table 3. Mean ICAM-1 expression in aortic endothelial cells.

Treatment	n	Mean	SD
Negative Control	6	20.50	3.271
Positive Control	6	51.33	8.664
High-cholesterol diet + mangosteen peel extract 200 mg/kg	6	45.67	4.457
High-cholesterol diet+ mangosteen peel extract 400 mg/kg	6	37.50	6.091
High-cholesterol diet+ mangosteen peel extract 800 mg/kg	6	26.67	3.077

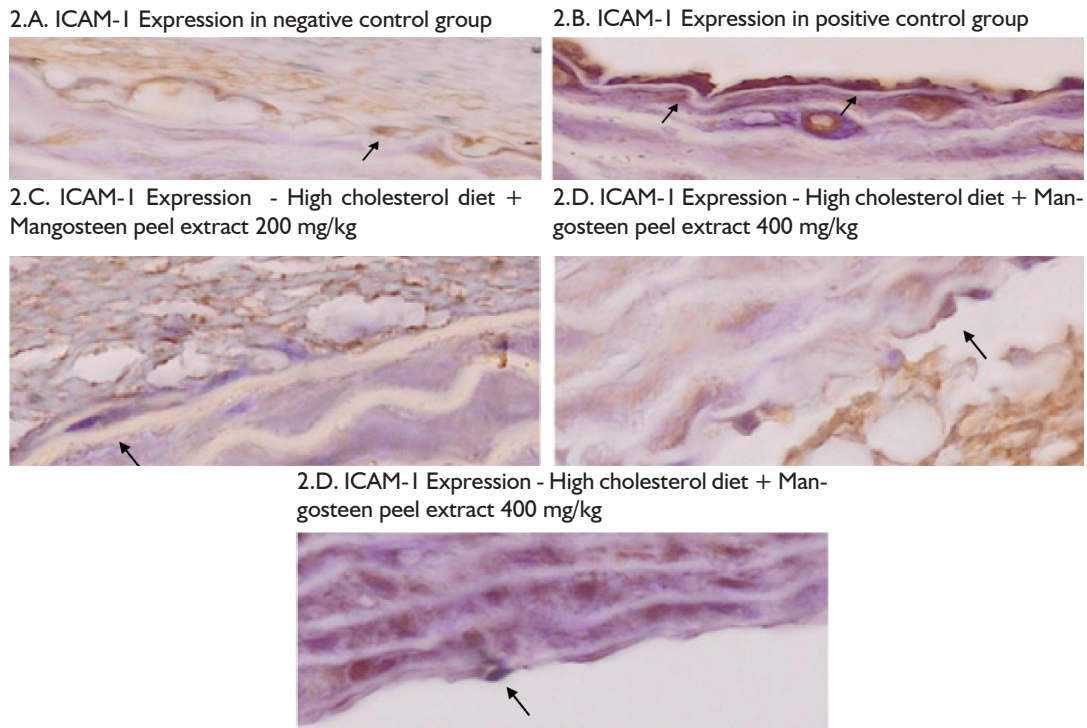


Figure 2. A, B, C, D, E - ICAM-1 Expression in aortic endothelial cells.

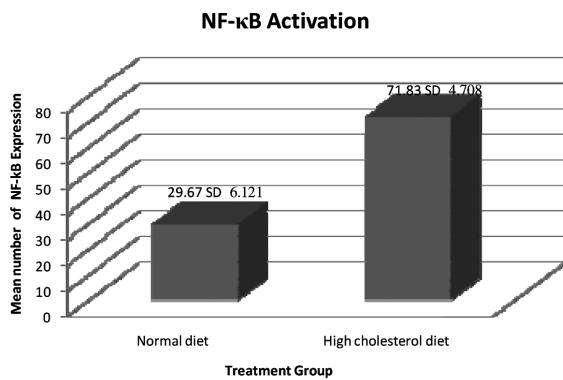


Figure 3. Graph of the effect of high dietary cholesterol on the activation of NF-κB in aortic endothelial cells. Data expressed as average number, n = 6, significant if the value of p <0.05.

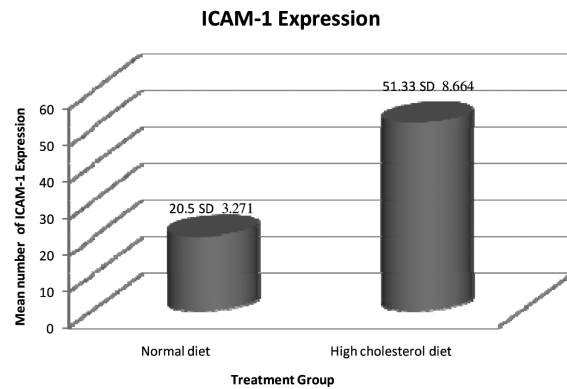


Figure 4. Graph of the effect of high dietary cholesterol on the activation of ICAM-1 in aortic endothelial cells. Data expressed as average number, n = 6, significant if the value of p <0.05.

high-cholesterol diet increased the mean expression of ICAM -1, with the highest yields (51.33). On treatment with mangosteen peel extract dose of 200 mg/kg was still an increase in the mean expression of ICAM-1 (45.67), and with increasing doses starting to look a decrease in the mean expression of ICAM-1, at doses of 400 mg/kg (37.50) and at doses of 800 mg/kg (26.67).

Effect of high dietary cholesterol on the activation of NF-κB in the positive control than the negative control

After the test conducted by One way ANOVA and Post Hoc analysis, as found in Figure 3 obtained the result that there are significant differences in the expression of NF-κB activation between the mice fed a cholesterol

Table 4. Antioxidant properties of *G. mangostana*.

G. mangostana extracts and/or xanthone	References
• The methanol extract of the fruit hulls of GML showed DPPH scavenging activity	Yoshikawa et al. (1994) Williams et al. (1995) Fan and Su (1997)
• α-Mangostin inhibited copper-induced LDL oxidation in vitro	Mahabusarakam et al. (2000)
• α and γ-Mangostin showed antioxidant activity using the ferric thiocyanate method	Leong and Shui (2002) Moongkarndi et al. Garcia et al. (2005)
• The copper-induced LDL oxidation in vitro was inhibited by α-mangostin and by prenylated xanthenes derived from this xanthone	Jung et al. (2006) Weecharangsan et al. (2006) Chomnawang et al. (2007)
• Methanolic extract of the edible portion of GML exhibited antioxidant activity using DPPH and ABTS assays	Haruenkit et al. (2007) Devi Sampath and Vijayaraghavan (2007) Chin et al. (2008)
• The crude methanol extract of pericarp from GML ameliorated the intracellular production of ROS in SKBR3 cells (2004a)	
• The pericarp extract of GML was able to scavenge HO• and effective to inhibit lipid peroxidation	
• Several xanthenes showed scavenging ONOO ⁻ ability in vitro	
• The aqueous and ethanolic extracts of the pericarp of GML present DPPH scavenging activity and protects neuroblastoma cell line NG108-15 from H ₂ O ₂ cytotoxicity	
• The ethanolic extract of GM showed antioxidant activity against DPPH radicals and reduced the ROS production of PML	
• Mangosteen-fruit showed antioxidant activity against DPPH and ABTS radicals and prevents the decrease in antioxidant activity induced by a cholesterol supplemented diet in rats	
• α-Mangostin showed protective effect against isoproterenol-induced oxidative damage and myocardial injury in rats	
• γ-Mangostin showed HO•-scavenging activity	

diet (positive control group) with rat not given a diet high in cholesterol (negative control group), (positive control: 71.83; negative control : 29.67; p = 0.000).

In **Figure 4** obtained the result that there are significant differences in ICAM-1 expression between rat fed a cholesterol diet (positive control group) with mice not given a high cholesterol diet (negative control), (positive control : 51.33; negative control : 20.50; p = 0.000).

Effect of various doses of mangosteen peel extracts on NF-κB activation and ICAM -1 expression in aortic endothelial cells of mice that were given high-cholesterol diet

Based on the ANOVA test shows that there is a significant difference in effect between the five treatment groups on the expression of NF-κB activation, and expression of ICAM-1 (p-value = 0.000).

Based on the results of Post Hoc test seen that the addition of mangosteen peel extracts on the mice fed high-cholesterol diet lowers the expression of NF-κB activation and ICAM-1. Dose of 400 mg/kg of mangosteen peel extract has the effect of decreased expression of NF-κB (p-value = 0.000) and ICAM-1 (p-value = 0.000) when compared with positive control group. Dose of 800 mg/kg of mangosteen peel extract has the effect of decreased expression of NF-κB (p-value = 0.000) and ICAM -1 (p-value = 0.000) when compared with positive control group.

Thus concluded that the addition of mangosteen peel extract of 800 mg/kg in mice fed a high cholesterol diet is the best dose to reduce the expression of NF-κB activation and expression of ICAM-1, although the inhibitory effect of ICAM -1 expression and activation NF-κB has been significant with a dose of 400 mg/kg with significant results.

Discussion

Dyslipidemia is marked by elevated levels of plasma cholesterol and (or) triglycerides (TG) or low levels of high-density lipoprotein (HDL) that contribute to the development of atherosclerosis.⁶

Atherosclerosis is a chronic disease of the arterial wall where both innate and adaptive immunoinflammatory mechanisms are involved. Inflammation is central at all stages of atherosclerosis. It is implicated in the formation of early fatty streaks, when the en-

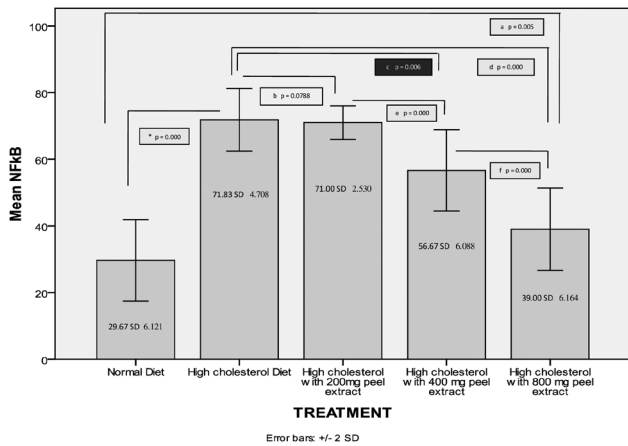


Figure 5. Graph Effect of mangosteen peel extract on NF-κB activation in endothelial cells aorta rats fed high-cholesterol diet. Data expressed in mean number of endothelial cells expressing NF-κB activation, n = 6, significant when the p-value <0.05.

- * Normal diet vs High cholesterol diet p = 0.000
- a Normal diet vs (High cholesterol diet with 800 mg extract) p = 0.005
- b High cholesterol diet vs (High cholesterol diet with 200 mg extract) p = 0.0788
- c High cholesterol diet vs (High cholesterol diet with 400 mg extract) p = 0.006
- d High cholesterol diet vs (High cholesterol diet with 800 mg extract) p = 0.000
- e (High cholesterol diet with 200 mg extract) vs (High cholesterol diet with 400 mg extract) p = 0.000
- f (High cholesterol diet with 400 mg extract) vs (High cholesterol diet with 800 mg extract) p = 0.000

endothelium is activated and expresses chemokines and adhesion molecules leading to monocyte/lymphocyte recruitment and infiltration into the subendothelium. It also acts at the onset of adverse clinical vascular events, when activated cells within the plaque secrete matrix proteases that degrade extracellular matrix proteins and weaken the fibrous cap, leading to rupture and thrombus formation. Cells involved in the atherosclerotic process secrete and are activated by soluble factors, known as cytokines.⁷

LDL oxidation has a key role in the activation and facilitation of atherogenesis. The signals in the form of oxidized lipoproteins and reactive oxygen species (ROS) lead to the activation of transcription factors such as nuclear factor-κB (NF-κB) that are involved in the regulation of expression of ICAM-1.⁸

Many studies have shown that free radicals cause oxidative damage to fats, and nucleic acids. Antioxidants seem very important in the prevention of degenerative diseases (including heart disease

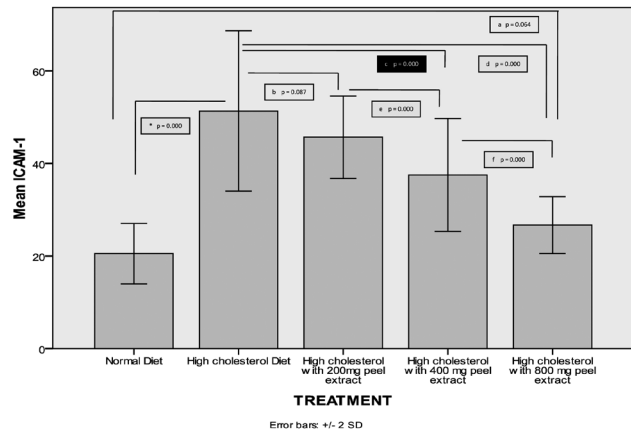


Figure 6. Graph Effect of mangosteen peel extract on the expression of ICAM-1 on endothelial cells aorta rats fed high-cholesterol diet. Data expressed in mean number of endothelial cells expressing ICAM -1, n = 6, significant when the p-value <0.05.

- * Normal diet vs High cholesterol diet p = 0.000
- a Normal diet vs (High cholesterol diet with 800 mg extract) p = 0.064
- b High cholesterol diet vs (High cholesterol diet with 200 mg extract) p = 0.087
- c High cholesterol diet vs (High cholesterol diet with 400 mg extract) p = 0.000
- d High cholesterol diet vs (High cholesterol diet with 800 mg extract) p = 0.000
- e (High cholesterol diet with 200 mg extract) vs (High cholesterol diet with 400 mg extract) p = 0.000
- f (High cholesterol diet with 400 mg extract) vs (High cholesterol diet with 800 mg extract) p = 0.000

and blood vessels), because it can inhibit the formation of the substrate chain reaction that can be oxidized.⁹

In vitro studies had explained that activation of oxLDL-mediated endothelial LOX-1-linked signaling pathway causing pro-inflammatory responses. LOX-1 activation can stimulate ROS production and activate nuclear factor κB (NF-κB). Activation of NF-κB and translocation into cell nucleus, result in increase of pro-inflammatory and adhesion molecules coding genes, i.e. tumor necrosis factor-α, ICAM-1, and VCAM-1.¹⁰

Oxygen radicals react readily with cellular phospholipids and proteins, causing lipid peroxidation and oxidation of thiol groups with subsequent alteration of membrane ultrastructure and dysfunction of various cellular proteins. The antioxidants are known to interfere with the free radical formation, and antioxidant reserve and enzyme capacity are significantly reduced following ischemia and reperfusion.⁵

Several studies have shown that antioxidant nutrients and (or) natural medicine positively modulate the susceptibility of LDL to oxidation and enhance the antiatherogenic properties of HDL, thus playing an important role in the prevention of cardiovascular diseases.¹¹

It has been reported that some free radical scavengers and antioxidants prevent arrhythmias and cardiac injury induced by myocardial ischemia-reperfusion. Xanthones have been described as strong scavengers of free radicals and antioxidants. They exhibit concentration-dependent scavenging activity toward superoxide anions, hydroxyl and peroxy radicals.⁵

The fruit hull of mangosteen, *G. mangostana*, has been widely used as an anti-inflammatory medicine in Southeast Asia for many years.¹¹

Mangostin contained in mangosteen peel, that is an effective inhibitor of LDL peroxidation in vitro as indicated by lagtime to oxidation and malondialdehyde production. Derivatives of the parent compound have been produced and may offer further benefits and therapeutic potential.¹²

Flavonoids and quinones have received the most attention as phenolic antioxidant derivatives and much is known about the structural requirements for antioxidant activity. Therefore, so many other phenolic compounds such as xanthone have been shown to act as scavengers of various oxidizing species; superoxide anion (O_2^-), hydroxy and peroxy-radicals.⁸

Evidence that antioxidant micronutrients potentially reduce the risk of CHD comes from four major sources. First, studies of antioxidant supplementation in animal models of atherosclerosis have generally shown a reduction in disease. Second, many studies have now shown that antioxidant supplementation in healthy subjects or patients with CHD can reduce levels of free radical damage products and protect LDL against oxidation. Third, large scale epidemiological studies generally show that low intakes of antioxidants are associated with increased cardiovascular risk after correcting for other risk factors. Thus, there is a plausible case supported by experimental studies, animal experiments, and epidemiology linking oxidative stress and atherosclerosis. The key test of such a hypothesis is whether increased antioxidant intake can be shown to prevent the clinical manifestations of atherosclerosis in humans. Several published randomised studies have now considered this issue, and others are currently ongoing. Early results have not been encouraging.¹⁴

There is substantial evidence to suggest that xanthones and xanthone derivatives may be potentially useful as pharmacological agents in the treatment or prevention of cardiovascular diseases, including ischemic heart disease, atherosclerosis and hypertension. The protective effects of xanthones in the cardiovascular system may be due to their antioxidant, anti-inflammatory, platelet aggregation inhibitory, antithrombotic and or vasorelaxant activities. In particular, the antagonism of endogenous NOS inhibitors by xanthones may represent the basis for improved endothelial function and for reduction of events associated with atherosclerosis. However, the precise effects of xanthones need to be further elucidated in animal experiments in vivo and in humans. Moreover, pharmacokinetics, toxicity and structural optimization of xanthones should also be explored.⁵

Conclusion

There is an increased activation of NF-κB and increased expression of ICAM-1 significantly ($p < 0.05$) in rat fed high cholesterol diet (positive control) compared to negative control. There are inhibition to activation of NF-κB and decreased expression of ICAM-1 in rat fed high cholesterol diet with mangosteen peel extract in various doses. The 400 mg and 800 mg dose of mangosteen peel can inhibit NF-κB and ICAM-1 significantly.

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