

EFFECT OF ETHANOL EXTRACT OF MANGOSTEEN PEEL ON SERUM MALONDIALDEHYDE AND LEUKOTRIENE B4 LEVELS IN MALE WHITE RATS (*Rattus norvegicus*) WISTAR STRAIN EXPOSED WITH ELECTRIC CIGARETTE SMOKE

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ABSTRACT

This study aimed to analyze the administration of mangosteen peel ethanol extract at different doses can reduce levels of malondialdehyde (MDA) and leukotriene B4 (LTB4) serum in male white rats (Rattus norvegicus) Wistar strains exposed to electric cigarette smoke. Electric cigarette induces mitochondrial ROS production and triggers oxidative stress. Antioxidants are needed by the body if there are many free radicals, for example mangosteen peel. This laboratory experimental study uses a randomized post-test only control group design. Thirty male white rats were divided into 5 groups randomly (KK, KR, P1, P2, P3). KK given 0.5% Na-CMC, KR given smoke and 0.5% Na-CMC, P1, P2, P3 given smoke and mangosteen peel ethanol extract 100, 200, 300 mg/kg weight. Data were tested for normality and homogeneity tests. Data distribution was found not normal. Data were tested by Kruskal-Wallis Test and Mann-Whitney. Kruskal-Wallis Test results showed significant differences in MDA and LTB4 levels in each experimental group (KS, KR, P1, P2 and P3), $p < 0.05$. Mann-Whitney test results showed a significant difference in the MDA levels of the KR against P3 and in the LTB4 level of the KR against P2 ($p < 0.05$). The conclusion of the study was the administration of mangosteen peel ethanol extract can reduce MDA and LTB4 levels serum in male white rats (Rattus norvegicus) Wistar strains exposed to electric cigarette smoke.

Keywords: Mangosteen; MDA; LTB4; electric cigarette

ABSTRAK

Penelitian ini bertujuan untuk menganalisis pemberian ekstrak etanol kulit manggis pada dosis yang berbeda dapat mengurangi kadar malondialdehyde (MDA) dan serum leukotriene B4 (LTB4) pada tikus putih jantan (Rattus norvegicus) strain Wistar yang terpapar asap rokok listrik. Rokok listrik menginduksi produksi ROS mitokondria dan memicu stres oksidatif. Antioksidan dibutuhkan oleh tubuh jika ada banyak radikal bebas, misalnya kulit manggis. Penelitian eksperimental laboratorium ini menggunakan desain kelompok kontrol uji acak saja. Tiga puluh tikus putih jantan dibagi menjadi 5 kelompok secara acak (KK, KR, P1, P2, P3). KK diberikan 0,5% Na-CMC, KR diberikan asap dan 0,5% Na-CMC, P1, P2, P3 diberikan asap dan ekstrak etanol kulit manggis 100, 200, 300 mg/kg berat. Data diuji untuk uji normalitas dan homogenitas. Distribusi data ditemukan tidak normal. Data diuji dengan Uji Kruskal-Wallis dan Mann-Whitney. Hasil Uji Kruskal-Wallis menunjukkan perbedaan yang signifikan dalam kadar MDA dan LTB4 pada setiap kelompok eksperimen (KS, KR, P1, P2 dan P3), $p < 0,05$. Hasil uji Mann-Whitney menunjukkan perbedaan yang signifikan dalam kadar MDA KR terhadap P3 dan pada LTB4 kadar KR terhadap P2 ($p < 0,05$). Kesimpulan dari penelitian ini adalah pemberian ekstrak etanol kulit manggis dapat menurunkan kadar MDA dan LTB4 serum pada tikus putih jantan (Rattus norvegicus) galur Wistar yang terpapar asap rokok listrik.

Kata kunci: Buah manggis; MDA; LTB4; rokok elektrik

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INTRODUCTION

Mangosteen peel is rich in antioxidant bioactive compounds, namely, polyphenolic compounds, including xanthenes, anthocyanins (Kanti & Syed 2009), proanthocyanidins (Raharjo & Santoso, 2013)

(Santoso 2015) and catechin (Hiranrangsee et al 2016). About 50 types of xanthone compounds were successfully isolated from mangosteen peel (Darmawansyih 2014). Xanthenes on mangosteen peel are also antidiabetic, anti-inflammatory (Wiwin 2010), anticancer (Akao et al 2008), anti-bacterial and

antifungal (Hermawan 2016). Xanthenes are soluble in alcohol and oil and are not soluble in water (Sumarny et al 2015).

Anthocyanins are good antioxidants to prevent or reduce the risk of disease. The content of anthocyanins in mangosteen peel is very high (Chaovanalikit and Mingmuang, 2007) (Hiranrangsee et al., 2016). Anthocyanins in the mangosteen peel consist of cyaniding-sophoroside, cyanidingglucoside-pentoside, cyaniding-glucoside, and cyaniding glucoside-X (X indicates a compound that is not identified and is incompatible with general sugar residues). Cyanidin-3-sophoroside and cyanidin-3-glucoside are the main anthocyanin compounds in mangosteen peel and are the only ones that increase with the development of a darker skin color (Palapola et al 2009). The content of anthocyanin extracted by maceration of mangosteen pericarp dried was 21.19 ± 0.49 mg Cyn-3-Glu/100 g (Hiranrangsee et al 2016) and 1403 ± 122.9 mg Cy-3-Sop/100 g (Palapola et al 2009). Cyanidin-3-glucoside has been shown to have a large oxygen radical absorbance capacity (ORAC) in vitro (Lee et al 2017).

This anthocyanin compound also shows biological activity as antidiabetic, antibacterial, anti-inflammatory, anti-cancer and anti-tumor (Hiranrangsee et al 2016). Anthocyanins are soluble in water and most organic solvents (Khoo et al., 2017).

The way anthocyanin works as an antioxidant through direct and indirect pathways. The direct pathway is anthocyanin directly scavenging free radicals and thus reduces oxidative stress. The indirect pathway involves the downregulation of cell proliferation and apoptosis, namely the downregulation of the activity of the enzyme cyclooxygenase (COX-1 and COX-2). These enzymes catalyze the formation of leukotrienes, prostacyclin, prostaglandins (PG), and thromboxan (Khoo et al 2017).

Electric cigarettes are marketed as an alternative product if one day conventional cigarettes are not permitted (Kennedy et al., 2017). The use of e-cigarettes has increased in recent years; an increase in the prevalence of adults using e-cigarettes in Indonesia in 2011 by 0.3%, Italy in 2013 by 1.2%, Greece in 2013 by 1.9% and Poland in 2010-2011 by 5.9% (Breland et al., 2017). Farsalinos research in 2015 found that the dose of particles caused by electric cigarettes is the same as the dose of particles caused by conventional cigarettes. Electric cigarette smoke shows oxidant and reactivity of reactive oxygen species that are similar to conventional cigarette smoke (Pisinger 2014).

Learner et al. in 2015 found that electronic cigarette aerosols and copper nanoparticles induced the

production of mitochondrial ROS, mitochondrial stress (reducing the stability of electron transport OxPhos chain (ETC) complex IV subunits) and DNA fragmentation in lung fibroblasts. Acrolein has been shown to trigger oxidative stress and inflammation, which results in loss of integrity of the endothelial cell barrier in the lungs. Propylene glycol, glycerin, and methanol have been shown to increase hydrogen peroxide production. Flavoring additives from cinnamon rolls stimulate increased production of IL-8 inflammatory cytokines in pulmonary fibroblasts (Cai and Wang, 2017).

Examination of electron paramagnetic/spin resonance or electron paramagnetic/spin resonance (EPR/ESR) shows the production of free radicals from the results of the evaporation of electric cigarettes, namely increased lipid peroxidation in pulmonary homogenates (assessed from thiobarbituric reactive acid or thiobarbituric acid reactive substances (TBARS)) mice exposed to electric cigarettes. A 58% increase in the number of macrophages in bronchoalveolar lavage (LAB) 24 hours after final exposure (twice per day for two weeks) accompanied by an increase in IL-6 production. This results in reduced defense against bacteria and viruses. In healthy human subjects, the use of e-cigarettes triggers oxidative stress, decreased nitric oxide, and endothelial/vascular dysfunction resulting in impaired blood flow (dilatation). Moheimani et al in 2015 reported the occurrence of oxidative stress and increased sympathetic activity of the heart in e-cigarette users. This is a risk factor for cardiovascular disease. Oxidative stress due to exposure to e-cigarettes can trigger cardiopulmonary pathogenesis, neurodegenerative disorders and cancer (Cai & Wang 2017).

Oxidants due to exposure to electric cigarettes can trigger the pathogenesis of lung disease, for example, chronic obstructive pulmonary disease (COPD) (Cai and Wang, 2017). Chronic obstructive pulmonary disease (COPD) is one of the leading causes of chronic morbidity and mortality worldwide. Chronic obstructive pulmonary disease is currently the fourth largest cause of death in the world but is predicted to be the 3rd leading cause of death in 2020 (Gerald and Bailey, 2018). World health organization (WHO) said COPD is the fourth leading cause of death in the world (Oemiati 2013). Based on data from Basic Health Research (Rikesdas) in 2013, the prevalence of COPD disease in Indonesia reached 3.7% (Indonesian Ministry of Health's Health Research and Development Agency, 2013). The main risk factors for COPD are tobacco smoking (Gerald & Bailey 2018) although other factors such as occupational factors are often exposed to hazardous materials/gases (coal, silica, cotton, heavy

metals) and air pollution (carbon and sulfur dioxide formed when burning fossil fuels of coal or diesel oil) also affects the occurrence of COPD (Hermawan 2016).

Lipid peroxidation is a major consequence of oxidative stress and a cause of oxidative damage. Malondialdehyde (MDA) is a lipid peroxidation product that has been recognized as an oxidative stress biomarker (Safyudin & Subandrate 2016). Increased MDA concentration is used as a biomarker of oxidative stress in various health problems such as cancer, psychiatric disorders, chronic obstructive pulmonary disease, asthma, or cardiovascular disease (Khoubnasabjafari et al 2015).

Interleukin-8 and Leukotrien B4 (LTB4) are known as neutrophil chemotactic factors which will activate and recruit neutrophils into the airway. Leukotriene B4 is preferred in experimental research to reflect the process that occurs in humans because only LTB4 is owned by humans and rat (*Rattus norvegicus*) Wistar strains. Leukotrienes B4 contribute to many inflammatory diseases, including COPD (O'Dwyer et al., 2015). High levels of LTB4 in plasma show a strong proinflammatory response and serve to identify the risk of developing lung complications (Auner et al 2012).

This study aims to analyze the administration of mangosteen peel ethanol extract at different doses can reduce levels of malondialdehyde (MDA) and leukotriene B4 (LTB4) serum in male white rats (*Rattus norvegicus*) Wistar strains exposed to electric cigarette smoke for 4 weeks.

MATERIALS AND METHODS

Experimental research using a randomized post-test only control group design. The independent variables of the study were mangosteen peel extract and electric cigarette smoke. The dependent variables of the study were MDA and Leukotriene B4 serum.

The study used samples of experimental animals total 30 male white rats (*Rattus norvegicus*) Wistar strains with criteria for age 2-3 months, body weight 170-180 g healthy physical. Acclimatization of laboratory conditions is carried out for 7 days. The weight of all mice was weighed after acclimatization. Experimental animals were divided into 5 groups randomly (1 group containing 6 mice), Group 1 was the negative control group/without exposure to electric cigarette smoke and was given a placebo (KK), Group 2 was the group given exposure to electric cigarette smoke and given a placebo (KR), Group 3 is a treatment group that is given exposure to electric cigarette smoke and mangosteen

peel ethanol extract at a dose of 100 mg/kg BW/day (P1), Group 4 is a treatment group that is given exposure to electric cigarette smoke and ethanol extract of mangosteen peel with dose of 200 mg/kg body weight/day (P2), Group 5 is the treatment group given exposure to electric cigarette smoke and ethanol extract of mangosteen peels with a dose of 300 mg/kg body weight/day (P3).

Exposure to e-cigarette smoke is given from day 1 to day 28. The liquid cigarette smoke is evaporated/heated to form smoke. Each group of rats (except the KK group) was put into a hollow glass box. The box is connected to the connection of the rubber eagle and the iron pipe which has 2 ends. The first end is connected to the hole of the glass box and the second end is connected to the solder which functions to vaporize the e-cigarette liquid. The entire body of the animal is exposed to electric cigarette smoke. Negative control group rats were exposed to free air. One group of rats was exposed to e-cigarette smoke once a day with a dose of e-cigarette liquid 8 drops/x every day for 4 weeks (Glynos et al., 2018). The composition of e-cigarettes used in this research is propylene glycol 30%, vegetable glycerin 70%, flavorings/flavor 40%, sweetener.

Provision of placebo and ethanol extract of mangosteen peel is given on days 15-28. Provision is done 1 hour before being exposed to electric cigarette smoke. Group KK and KR was given a 5% Na-CMC O solution (placebo). Group P1, P2 and P3 were given ethanol extract of mangosteen peel at a dose of 100,200,300mg/kgBB/day in 5% Na-CMC O solution. Placebo and ethanol extract of mangosteen rind in Na-CMC O solution, 5% given orally through gastric sonde. On the 29th day, rats in all groups were terminated. Blood is drawn through intra-cardiac puncture using a 5 ml syringe and a 23G needle. Blood is stored in a vacuum tube with a gold cap (without EDTA) for centrifuge to obtain serum and parameter checks. Serum MDA levels were examined using thiobarbituric acid (TBA) reagents. Serum Leukotriene B4 levels were measured by a competitive Elisa method using the Rat Leukotriene B4 ELISA Kit from Elabscience, Inc. with catalog number E-EL-006.

Data of serum MDA and LTB4 levels were calculated on average (mean) and standard deviation (SD) and were performed normality tests with one sample Kalmogorov-Smirnov and homogeneity tests with Levene. Normal distribution uses the Anova one-way test and the LSD (Least Significance Difference) test. Abnormal data distribution was tested using the Kruskal-Wallis Test and the Mann-Whitney Test.

RESULTS

Serum MDA levels in each group can be seen in Table 1, while serum LTB4 levels in each group can be seen in Table 2. The data were processed in SPSS software (Statistical Package for the Social Sciences) version 20.0 and calculated the mean (mean) and standard deviation (SD) and conducted a normality test with Shapiro Wilk. The results of mean and standard

deviation (SD) analysis of serum MDA and LTB4 levels can be seen in Table 3.

Table 4 shows the p value <0.05 for each parameter measured and concluded that the data were not normally distributed at each of the examined levels. Data analysis using non-parametric test, the Kruskal-Wallis Test. The results of the Kruskal-Wallis Test can be seen in table 5.

Table 1. Serum MDA levels in each group of experimental animals

Sample	MDA (nmol/ml)				
	KK	KR	P1	P2	P3
1	12.072	41.539	8.976	8.976	8.288
2	13.218	13.218	13.448	14.594	9.090
3	11.613	33.513	15.282	6.797	8.861
4	9.090	37.182	19.410	13.562	9.778
5	14.480	20.556	12.416	12.760	10.696
6	11.384	31.679	3.357	7.027	8.747

Table 2. Serum LTB4 levels in each group of experimental animals

Sample	LTB4 (pg/ml)				
	KK	KR	P1	P2	P3
1	113.80	293.80	239.30	87.20	186.40
2	158.40	443.30	122.90	164.70	112.00
3	117.10	380.50	141.90	238.60	236.00
4	128.30	438.30	293.30	220.90	283.30
5	263.80	190.00	109.10	99.00	390.00
6	218.70	664.60	340.00	325.50	315.00

Table 3. Mean and standard deviations of serum MDA and LTB4

Level	Mean ± standard deviations
MDA	14.72 ± 9.28
LTB4	243.86 ± 130.78

Table 4. Test for normality of serum MDA and LTB4 levels

Level	Shapiro Wilk		
	Statistic	Df	p
MDA	0.766	30	0.000
LTB4	0.900	30	0.008

Table 5. Kruskal-Wallis Test for serum MDA and LTB4

Level	Kruskal-Wallis		
	Statistic	Df	p
MDA	14.509	4	0.006*
LTB4	10.099	4	0.039*

*p<0.05 → significant differences

Table 6. Mann-Whitney Test for MDA and LTB4 levels

		MDA	LTB4
		p	p
KK	KR	0.004*	0.009*
	P1	0.7	0.6
	P2	0.7	0.82
	P3	0.009*	0.18
KR	P1	0.015*	0.03*
	P2	0.009*	0.03*
	P3	0.002*	0.09
P1	P2	0.59	0.59
	P3	0.18	0.59
P2	P3	0.7	0.3

*p<0.05 → significant differences

Table 6 shows the significant differences in the MDA levels of the KK group against the MDA levels of the KR and P3 groups and the significant differences in the MDA levels of the KR group against the P1, P2 and P3 groups. Table 6 also shows a significant difference in the LTB4 levels of the KK group against the LTB4 levels of the KR group and a significant difference in the LTB4 levels of the KR group against groups P1 and P2.

DISCUSSION

Electrical smoking can cause COPD disease because exposure to e-cigarette smoke increases oxidative stress, triggers inflammation, interferes with autophagy and reduces resistance to bacteria and viruses (Cai and Wang, 2017).

Malondialdehyde (MDA)

Malondialdehyde (MDA) is used as an indicator of lipid peroxidation resulting from oxidative stress (Herlina et al 2017). Malondialdehyde is formed by autoxidation and enzyme degradation of polyunsaturated fatty acids in cells. The secondary end product of oxidation of these polyunsaturated fatty acids reacts with two molecules of thiobarbituric acid (TBA) through acid-catalyzed nucleophilic-addition reaction to produce pink-pink chromagen with maximum absorbance at 532 nm (Hodges et al 1999).

Reactive oxygen species (ROS) are typical free radicals that are produced during oxidative stress. The best known ROS signaling is through the respiratory chain. This involves the electron transfer reaction pathway, where the enzyme superoxide dismutase (SOD) produces hydrogen peroxide (H₂O₂) in the mitochondria. In complexes I and III in the mitochondria produce hydroxyl radicals (·OH)

produced by the Fenton reaction ($H_2O_2 + Fe^{2+} + H^+ \rightarrow \cdot OH + OH^- + Fe^{3+}$). Anthocyanin is a reducing agent in the electron transfer reaction pathway (direct path). This direct pathway consists of 2 pathways, the first pathway is the "attack" of the hydroxyl group of the B-ring of the theanthocyanin structure and the second is the "attack" of the ionic ion on the C-ring (Khoo et al., 2017). The indirect pathway is via the nuclear factor- κ B signaling pathway, NF- κ B which is the main link between a large number of metabolic signals and inflammation. Nuclear factor- κ B is a transcription factor that is sensitive to oxidative stress and inflammation itself. Nuclear factor- κ B is expressed everywhere by cells and is located in the cytoplasm, where it is in an inactive form (Vendrame & Klimis-zacas 2015).

Stressors (including oxidative stress) activate nuclear factors (NF) - κ B, which results in an increase in the expression of proinflammatory cytokines for example interleukin (IL) -6 and tumor necrosis factor (TNF)- α . This modulates the expression of systemic inflammatory mediators (e.g., C-reactive protein (CRP) and inhibits anti-inflammatory mediators such as adiponectin. Nuclear factor- κ B activation also increases the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2). In conditions where proinflammation, excess nitric oxide (NO) is converted to highly reactive peroxynitrite (while COX-2 leads to the formation of proinflammatory prostases and vasoconstriction such as PGE₂ and PGF₂ α . Reduction of oxidative stress by anthocyanins results in the activation of NF- κ B being inhibited so that it inhibits the entire cascade of proinflammation and vasoconstriction such as PGE₂ and PGF₂ α (Vendrame & Klimis-zacas 2015). This mechanism results in a weak inflammatory response. This was proven in an in vivo study that showed that lipopolysaccharide-induced mice fed 10% blueberries (containing anthocyanin) had reduced TNF-

α and IL-6 protein and mRNA expression in serum compared with controls (Khoo et al 2017).

Table 6 shows a significant difference in the MDA levels of the KS group against the MDA levels of the KR and P3 groups and a significant difference in the MDA levels of the KR group against groups P1, P2 and P3. This shows that serum MDA levels from the KR group were significantly higher than the control group (KS) and the treatment group (P1, P2 and P3). The most significant difference (the smallest p value) was found in the MDA levels of the KR group against the MDA levels of the P3 group ($p = 0.002$). This shows that serum MDA levels decreased most significantly at a dose of 300mg/kg BW/day. Anthocyanin in the ethanol extract of mangosteen rind can reduce serum MDA levels in rats exposed to electric cigarette smoke.

Leukotriene B4 (LTB4)

Leukotriene B4 (LTB4) is a pro-inflammatory mediator that is synthesized in myeloid cells and is derived from arachidonic acid. Leukotriene B4 (LTB4) synthesis is enhanced by inflammatory mediators including endotoxin, complement fragments, tumor necrosis factor and interleukin. Increased intracellular free calcium in response to various inflammatory stimulations activates phospholipase A2 in the nuclear envelope cleaving arachidonic acid (AA) of phospholipids (Crooks & Stockley 1998). Arachidonic acid (AA) which has been released by phospholipase A2 undergoes oxygenation at C-5 position by 5-lipoxygenase (5-LOX) to form 5 (S)-hydroperoxy-6E, 8Z, 11Z, 14Z eicosatetraenoic acid [5 (S)-HpETE], and subsequently to form leukotriene A4 (LTA4). 5(S)-hydroperoxy-6E, 8Z, 11Z, 14Z eicosatetraenoic acid [5(S)-HpETE] is further hydrolyzed by LTA4 hydrolase to produce LTB4 (Saeki & Yokomizo 2017).

The availability of AA depends on the level of activation of membrane phospholipase and the rate of reincorporation of AA to membrane phospholipid. Increased AA release is stimulated by various signaling molecules including endotoxin, C5a, TNF α , IL-1, GM-CSF, PAF (platelet activating factor), neutrophil elastase and LTB4 itself. All of these stimuli increase LTB4 production but all these stimuli can be inhibited by anthocyanin antioxidant properties both through direct and indirect channels (in the malondialdehyde section) (Saeki & Yokomizo 2017).

Table 6 shows a significant difference in the LTB4 levels of the KS group against the LTB4 levels of the KR group and a significant difference in the LTB4 levels of the KR group against groups P1 and P2. The most significant difference (the smallest p value) was

found in the LTB4 level of the KS group against the LTB4 level in the KR group ($p = 0.009$). This shows that serum LTB4 levels from the KR group were significantly higher than the control group (KS) and the treatment group (P1 and P2) and the serum LTB4 levels decreased significantly when the dose of the mangosteen rind ethanol extract was increased at a dose of 200mg/kg BW /day. At a dose of 300mg/kgBW/day there was no significant difference compared to a dose of 200mg/kgBW/day, i.e., LTB4 levels in the P2 group against LTB4 levels in the P3 group ($p > 0.05$). Anthocyanin in the ethanol extract of mangosteen rind can reduce serum LTB4 levels in rats exposed to e-cigarette smoke at a dose of 200mg/kgBW/day.

CONCLUSION

The conclusion based on the results of the current study is that the administration of mangosteen peel ethanol extract at a dose of 100 mg/kg BW/day, 200mg/kg BW/day and 300mg/kg BW/day can reduce serum MDA and LTB4 levels in rats exposed to electric cigarette smoke for 4 weeks.

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