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Nanoemulsion of Turmeric in VCO Inhibit the Progressivity of Lung Fibrosis due to Cigarette Exposure

(Nanoemulsi Kunyit dalam VCO Merencat Perkembangan Fibrosis Paru-Paru Akibat Pendedahan Rokok)

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ABSTRACT

Pulmonary fibrosis is a form of lung damage caused by chronic inflammation. One of the causes is cigarette smoke exposure, which can damage cilia and epithelial cells, that is able to stimulate oxidative stress as well. The inflammatory response by inflammatory cells triggers release of inflammatory mediators, for example, TNF-α. Increased levels of TNF-α indicate a high inflammatory process and a high risk of pulmonary fibrosis. Nanoemulsion of turmeric extract in VCO contains curcumin, which can suppress the secretion and expression of TNF-α through several pathways. This study is aimed to analyze the inhibitory effect of turmeric extract nanoemulsion in VCO on pulmonary fibrosis in an inflammatory way. In this study, 40 white rats were used and divided into five groups; K0 was negative controls group, K1 was exposed to smoke from two cigarettes/day for 42 days, K2 received 0.3 mL dose of nanoemulsion + cigarette exposure, K3 received 0.6 mL dose of nanoemulsion + cigarette exposure and K4 received dexamethasone (0.2 mg/kgBW) + cigarette exposure. Furthermore, plasma TNF-α levels taken from cardiac blood and histopathological preparations (HE, MA) were made from the right lung. One-way ANOVA test was used to analyze plasma TNF-α levels, the Kruskal-Wallis test was used to analyze fibrosis degree scoring based on Aschroft Modification Scale and the correlation test was analyzed by Spearman test. The results showed that 0.3 mL of turmeric extract nanoemulsion in VCO had the best inhibitory effect on progressivity tissue damage and pulmonary fibrosis.

Keywords: Chronic respiratory disease; cigarette; curcumin; nanoemulsion

ABSTRAK

Fibrosis paru adalah satu bentuk kerosakan paru-paru yang disebabkan oleh keradangan kronik. Salah satu puncanya ialah pendedahan kepada asap rokok. Keradangan akibat asap rokok boleh merosakkan silia dan sel epitelium dan ia juga boleh merangsang tekanan oksidatif. Tindak balas keradangan oleh sel radang mencetuskan pembebasan mediator keradangan, contohnya, TNF-α. Peningkatan tahap TNF-α menunjukkan proses keradangan yang tinggi dan risiko tinggi fibrosis paru. Nanoemulsi ekstrak kunyit dalam VCO mengandungi kurkumin yang dapat menyekat rembesan dan pengekspresan TNF-α melalui beberapa laluan. Kajian ini bertujuan untuk menganalisis potensi perencatan nanoemulsi ekstrak kunyit dalam VCO pada fibrosis paru melalui keradangan. Dalam kajian ini, 40 ekor tikus putih telah digunakan dan dibahagikan kepada lima kumpulan dengan K0 adalah kumpulan kawalan negatif, K1 didedahkan kepada asap daripada dua batang rokok/hari selama 42 hari, K2 menerima 0.3 mL dos nanoemulsi + asap rokok, K3 menerima 0.6 mL dos nanoemulsi + asap rokok dan K4 menerima deksametason (0.2 mg/kgBB) + asap rokok. Tambahan pula, kadar TNF-α plasma diambil daripada darah jantung dan persediaan histopatologi (HE, MA) dibuat daripada paru-paru kanan. Ujian ANOVA sehala digunakan untuk menganalisis kadar TNF-α plasma, ujian Kruskal-Wallis digunakan untuk menganalisis pemarkahan darjah fibrosis berdasarkan skala pengubahsuaian Aschorft dan ujian korelasi dianalisis dengan ujian Spearman. Keputusan menunjukkan bahawa 0.3 mL nanoemulsi ekstrak kunyit dalam VCO mempunyai kesan perencatan terbaik terhadap kemajuan kerosakan jaringan dan fibrosis paru.

Kata kunci: Kurkumin; nanoemulsi; penyakit pernafasan kronik; rokok

Introduction

Pulmonary fibrosis is a chronic lung disease characterized by scar tissue due to the increased proliferation of fibroblasts or the accumulation of extracellular matrix (Desdiani et al. 2020; Todd, Luzina & Atamas 2012). Pulmonary fibrosis could impair function and damage lung tissue, which is why it should be prevented (Wulandari et al. 2013). Three conditions can form pulmonary fibrosis: Inflammation, oxidative stress, and the role of procoagulant lung (Todd, Luzina & Atamas 2012). Lung tissue inflammation can be triggered by cigarette smoke because it contains many irritating chemicals. Cigarette smoke inhaled and enters the respiratory tract can trigger an inflammatory cell response. Inflammatory cells that play a major role are alveolar macrophages. Macrophages will phagocytize foreign bodies and release several inflammatory cytokines, one of them is Tumor Necrosis Factor Alpha (TNF-α). These cytokines can activate NF-κB and increase the expression of TGFβ-1 as the main fibrosis mediator (Sullivan et al. 2009; Supriono, Pratomo & Praja 2018). Microscopically, pulmonary fibrosis shows hyperplasia or metaplasia of epithelial cells, thickening of the interalveolar septum due to collagen deposition, and increased inflammatory cell infiltration (Gawda et al. 2020; Maharaj, Shimbori & Kolb 2013).

Turmeric has been widely used as herbal medicine in Asian countries. It can treat some diseases, such as biliary disorder, inflammation, cough, diabetic wounds, liver disorder and rheumatism (Bae, Park & Lee 2018; Bagchi 2012). Turmeric contains the most curcumin compounds compared to other Zingiberaceae families (Ramli et al. 2019). Curcumin has many benefits, including anti-inflammatory and anti-fibrotic agents (Bae, Park & Lee 2018; Huang et al. 2016; Rasool et al. 2020). Based on the study of Zhang et al. (2011), curcumin can reduce inflammation and collagen accumulation in rat lungs. It can also suppress the secretion of proinflammatory cytokines, including TNF-α (Huang et al. 2016). Oral curcumin has a bad pharmacokinetic profile, such as low levels of body absorption, which makes it necessary to combine curcumin with other compounds that can improve its pharmacokinetic profile (Cas & Ghidoni 2019; Flora et al. 2013; Ramli et al. 2019). According to Sephapour et al. (2018), a combination of curcumin and vegetable oil (coconut oil) can increase the solubility and absorption of curcumin since they share the same polarity. Because of this, the researchers want to demonstrate the ability of turmeric extract nanoemulsion in VCO to inhibit the progression of pulmonary fibrosis caused by cigarette smoke exposure (through the inflammatory way).

Materials and Methods

This study is a true experimental study using an animal model (Post Test Only with Control Group Design). The animal model research was conducted in March - May 2022 at the Experimental Animal Laboratory, Department of Medical Physiology and Biochemistry, Faculty of Medicine, Airlangga University, Surabaya, Indonesia. This research has been submitted to the Health Research Ethics Committee and declared ethically feasible in accordance with the Certificate of Eligibility for Ethics No.33/EC/KEPK/FKUA/2022.

Nanoemulsion of turmeric extract

Nanoemulsion is made of turmeric extract dissolved in Virgin Coconut Oil (VCO) with the following formula: 1.5% thick turmeric extract, 5% VCO, 40% tween80, 20% propylene glycol, and 33.5% aquadest ad 100 mL. All of the materials were homogenized with high-speed homogenizer for 15-20 min (1200 rpm). Determination of the turmeric extract nanoemulsion dose given to the animal model based on Rasool et al. (2020) and Vasconcelos et al. (2020) is 0.3 mL/200 g and 0.6 mL/200 g.

Animal model

The samples of this research were white male Wistar rats (3 months) with a body weight of about 180-200 grams and in good health. The number of samples were 40, which were randomly divided into five groups, so that eight rats were obtained per group. K0 as a negative control group, K1 as a positive control group, K2 and K3 were given turmeric extract nanoemulsion in VCO orally at a dose of 0.3 mL/200 g/day and 0.6 mL/200 g/day, and K4 was given dexamethasone tablet (0.2 mg/kgBW/day). Turmeric extract nanoemulsion in VCO and dexamethasone were administered 1 hour before being exposed to cigarette smoke for 49 days. K1, K2, K3, and K4 were exposed to *kretek* cigarette smoke (nicotine of 2.3 mg and 39 mg of tar) for two cigarettes/30 min for 42 days. On the 59th day, all rats were terminated, then the lungs and cardiac blood were taken.

Laboratory analysis method

Blood samples were used to examine plasma TNF-α levels using the ELISA method (Bioenzy Mouse TNF-α

ELISA kit). The right lung was used for histopathological preparations with Hematoxylin-Eosin (HE) and Malory Azan (MA) staining. The HE staining was used to observe the lung histological structure, inflammatory cell infiltration, atelectasis, and septal thickening. The MA staining was used to determine pulmonary fibrosis degree according to Ashcroft modified scale. Aschroft modified scale (Hubner et al. 2008), consisting of 0-8 fibrosis degree as follows 0 = no fibrosis found in most of the alveolar septum and normal lung structure; 1 = there is limited fibrosis of the alveolar septum (septum thickening 3× than normal) and most of the alveoli are enlarged; 2 = marked fibrosis in the alveolar septum (septum thickening 3× than normal) and some of the alveoli appear enlarged; 3 = the walls of the alveoli begin to appear fibrotic with thickening of the interalveolar septum 3× from normal and some of the alveoli are enlarged; 4 = Alveolar septal thickness varies and a single fibrotic mass (<10% visual field) is found in the lung structures; 5 = the thickness of the alveolar septum varies and there are confluent fibrotic masses (10-50% of the visual field), as well as extensive structural damage; 6 = the thickness of the alveolar septum varies and is mostly deformed, a large fibrotic mass is found (>50% visual field) and extensive damage to the lung structure is found; 7 = alveolar septum is not shaped, the alveoli appear to be fused with fibrotic masses, but air bubbles are still found; 8 = the alveolar septum is amorphous and there is complete obliteration with fibrotic masses (no air bubbles).

STATISTIC ANALYSIS METHOD

The result of plasma TNF-a levels was analyzed using One Way Anova test. The HE observations were described based on inflammatory cell infiltration, atelectasis, and septal thickening. The score data of fibrosis degree was analyzed using Kruskal Wallis test. The correlation between plasma TNF-a levels and pulmonary fibrosis degree was analyzed using Spearman correlation test.

RESULTS AND DISCUSSION

This study used an animal model (40 Wistar rats), and then, four rats were dropped out (Each rat from K1, K2, K3, and K4), which makes the final number of samples of terminated and examined rats were 36.

Pulmonary histopathological observations on Haematoxylin-Eosin (HE) staining were aimed to evaluate several parameters of lung inflammation in rat models, including structure changes of the alveoli as described by the enlarged of alveoli or atelectasis, as well as thickening of the interalveolar septum due to inflammatory cell infiltration. Interalveolar septal thickening is also characterized by connective tissue deposition (fibrosis) (Klopfeisch 2013; Zeldin et al. 2001).

Based on Table 1, the negative control group (K0) showed a normal-shaped alveoli structure, and the interalveolar septum was not thickened and demarcated between alveoli. The most severe damage to lung structure occurred in the K1 (exposed to cigarette smoke). This group also showed a lot of inflammatory cell infiltration. Meanwhile, in the nanoemulsion or dexamethasone treatment group (K2, K3, and K4), the least damaged lung structure was found in the K2 group (getting 0.3 mL/200g/day nanoemulsion).

The inflammatory process occurred can be observed by measuring proinflammatory cytokines; one of them is TNF- α. Table 2 presents the average results of plasma TNF-α levels for each group of rats. The lowest average value is K0 (77.5 ng/L), and then the highest average value is K1 (129.5 ng/L). The average value of plasma TNF-α levels in the other groups, which are K2, K3, and K4, were 79.3; 93.3, and 82.0 ng/L. The results of statistical analysis with One-Way ANOVA test obtained a p-value = 0.000 ($p < 0.05$), meaning that there were differences in plasma TNF-α levels between groups of cigarette smoke exposure and given turmeric extract nanoemulsion in VCO.

In the K2 group, group of rats that received 0.3 mL/200g/day of turmeric extract nanoemulsion, the average value of plasma TNF-α levels was the lowest and close to the value in the K0 group. This demonstrates that using a nanoemulsion of turmeric extract in VCO can reduce the production of TNF-α levels due to the inflammatory process caused by cigarette smoke exposure.

The inflammatory process caused by chronic cigarette smoke exposure can cause lung structures to deteriorate, such as fibrosis. Scarring caused by extracellular matrix deposition causes pulmonary fibrosis. Pulmonary fibrosis can be evaluated through histopathological preparations with Mallory-Azan (MA) staining. Microscopically, fibrosis is indicated by a thickening of the interalveolar septum, so that the boundaries between alveoli appear widened. Besides that, fibrosis can also appear like fibrotic mass (mass formation contains connective tissue (collagen) deposition with minimal lymphocytes).

Based on Table 3, in the negative control group (K0), 75% of the samples did not find any thickening of

the septum characterized by fibrosis (fibrosis degree = 0) (Figure 1). Meanwhile, in the positive control group (K1), 71% of the samples had extensive fibrosis formation (10-50%) accompanied by varying thickening of the alveolar septum (fibrosis degree = 5) (Figure 1). The nanoemulsion (P1) or dexamethasone (P3) treatment group showed almost the same results; 43% of the samples belonged to 3rd fibrosis degree (septal thickening is more than three times normal, the structure of the alveoli is partially enlarged) (Figure 1).

The Kruskal-Wallis test shows a significant value (p) = 0.000 that there is a significant difference in the mean value of fibrosis degree in 2 or more groups (Table 4). Furthermore, to find out which groups have differences, the Post Hoc Mann-Whitney test is continued with the results presented in Table 5. The groups that showed significant differences were K0-K1; K0-K2; K0-K3; K0-K4; K1-K2; K1-K3; K1-K4; K2-K3, and K3-K4, while the group with no significant difference was K2-K4.

The results of the Spearman correlation test between plasma TNF-α levels and the fibrosis degree (Table 6) showed that both had a unidirectional and a quite strong relationship ($r > 0.5$-0.75; $p<0.01$), which means that increased plasma TNF-α levels can increase fibrosis degree scores too.

TABLE 1. Interpretation of lung histopathology observations (HE 10x, 40x)

Experimental animal group	Histopathological observation results
Negative Control (K0)	The formation of the reticular alveoli and interalveolar septum looks normal. There is no septal thickening or atelectasis formation
Positive Control (K1)	The shape of the alveoli varies. There is a widening of the alveoli and a large area of atelectasis (>60%), and the alveolar septum appears thickened due to a large number of inflammatory cell infiltration (+++)
Nanoemulsion 0.3 mL/200g/day + cigarette smoke exposure (K2)	The formation of the alveoli varies. The formation of widening of the alveoli and atelectasis is found in <30% of the area. The interalveolar septum is thickened due to inflammatory cell infiltration (+)
Nanoemulsion 0.6 mL/200g/day + cigarette smoke exposure (K3)	The shape of the alveoli varies. The interalveolar septum is thickened due to inflammatory cell infiltration (++). There is a widening of the alveoli and atelectasis (30-60% of the area)
Dexamethasone tablet 0.2 mg/kgBW/ day + cigarette smoke exposure (K4)	The shape of the alveoli varies. The interalveolar septum is thickened due to inflammatory cell infiltration (++). There is a widening of the alveoli and atelectasis (30-60% of the area)

TABLE 2. Results of plasma TNF-α Levels

Experimental animal group	Total (n)	Plasma TNF-α level (ng/L)
K0	8	77.51[b]
K1	7	129.56[a]
K2	7	79.37[b]
K3	7	93.0[b]
K4	7	82.0[b]

Significant value of One Way Anova test p=0,000 (p<0,05), [a]Post Hoc LSD test compared with the K0 group (p value <0.05), [b]Post Hoc LSD test compared with the K1 group (p value <0.05)

TABLE 3. Results of fibrosis degree scoring in each group of experimental animals

Experimental animal group	Fibrosis degree									Total
	0	1	2	3	4	5	6	7	8	
K0	75%	25%								100%
K1					29%	71%				100%
K2	14%	29%	14%	43%						100%
K3			14%	29%	43%	14%				100%
K4			28.50%	43%	28.50%					100%

TABLE 4. Kruskal-Wallis analysis test results of fibrosis degree data

Experimental animal group	Total (n)	Rating average
K0	8	5.38
K1	7	31.64
K2	7	13.57
K3	7	23.86
K4	7	19.93

*Asymp. Sig : 0.000

TABLE 5. Mann-Whitney post hoc test results of fibrosis degree data

	K0	K1	K2	K3	K4
K0					
K1	0.001*				
K2	0.009*	0.001*			
K3	0.001*	0.020*	0.018*		
K4	0.001*	0.003*	0.082	0.0253*	

*shows p value <0.05

TABLE 6. Spearman correlation test results of plasma TNF-α levels and fibrosis degree

Parameter	Correlation coefficient	Sig. (2-tailed)
Plasma TNF-α levels Fibrosis degree	0.576**	0.000

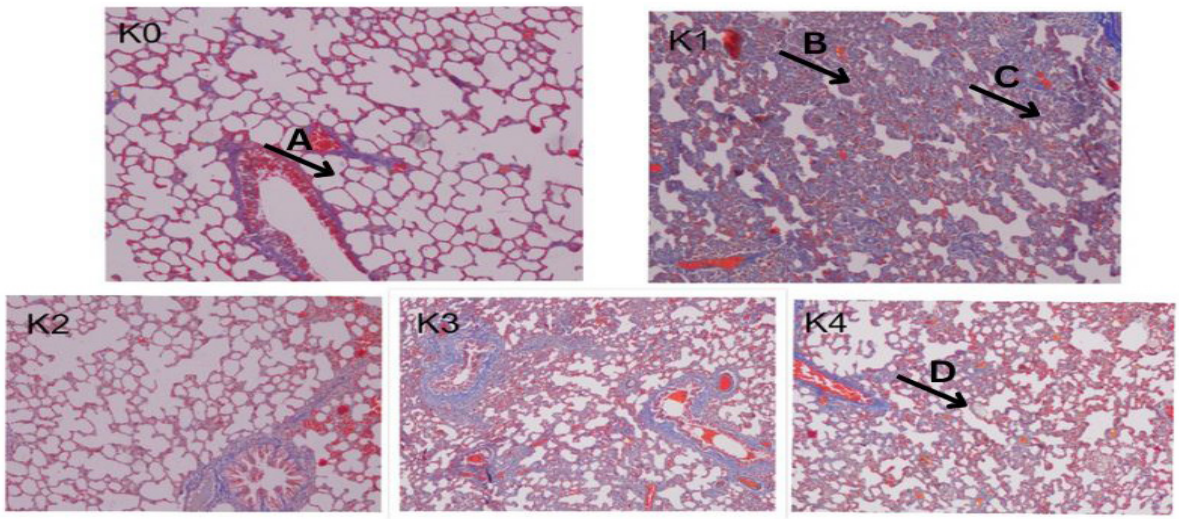

FIGURE 1. Histopathological description of each group of experimental animals (MA, 100x)

*Description: A = Normal Alveoli, B = Thickened Interalveoli Septum,
C = Atelectasis, D = Widening of Alveoli

In this study, one animal model from the K1, K3, and K4 groups each dropped out due to death caused by hypoxia, while one animal model from the K2 group dropped out due to burns because of cigarette fire. Cigarette smoke is known to contain chemicals that are harmful to the body, one of which is carbon monoxide (CO). Carbon monoxide has an affinity for hemoglobin 250 times higher than oxygen, so more carboxyhemoglobin is formed. As a result, oxygen that enters the respiratory tract is unable to be transported and distributed to the tissues, resulting in tissue hypoxia (Ercan, Ilbamis & Tasci 2021). This condition is supported by a study conducted by Dorey et al. (2020) that smokers' carboxyhemoglobin levels will increase after consuming 1-3 cigarettes, because it can trigger the cellular hypoxia.

Cigarette smoke also contains free radicals and irritating chemicals, which can cause oxidative stress, damage to tissue structures, inflammation, and pulmonary fibrosis. Oxidative stress in the lungs can trigger the activation of inflammatory genes, protease inactivation, stimulation of mucus production, and plasma exudation (Maulidiyah & Amin 2015). According to a study by Napanggala (2015) and Salawati (2016), inhaling cigarette smoke is said to damage the cilia of the respiratory tract, resulting in a buildup of mucus that causes infection and inflammation. In addition, cigarette smoke exposure will trigger repeated injury to epithelial cells (especially type II pneumocytes) so that cells undergo apoptosis. This can disrupt the interaction of epithelial cells with fibroblasts, affecting the imperfect remodeling process and causing pulmonary fibrosis (Wuyts et al. 2013).

Inflammation is the body's response to tissue damage caused by oxidative stress, infection, or other factors that can lead to fibrosis (Medzhitov 2010). Lung tissue damage can trigger an inflammatory response that involves the natural, adaptive immune system and activation of alveolar epithelial cells, endothelial cells, and fibroblasts (Lopes et al. 2013; Lugg et al. 2021; Yudhawati & Prasetyo 2018). When observed microscopically, the inflammatory response manifests itself as infiltration of inflammatory cells in the extracellular matrix of the interalveolar septum, resulting in septum thickening (Profita et al. 2010).

Cigarette smoke also contains free radicals that trigger oxidative stress that can activate NF-κB, so that there is an increase in the transcription of inflammatory mediators such as IL-2, IL-6, IL-8, and TNF-α. The most secreted inflammatory mediator is TNF-α, in which it can be secreted by various kinds of cells such as macrophages, monocytes, PMN cells, T cells, and mast cells (Cho et al. 2007). TNF-α plays a major role in the stimulation of the inflammatory response, cell adhesion and trans-endothelial migration, and the production of other cytokines (Wuyts et al. 2013).

In this study, cigarette smoke exposure triggered inflammation in the lungs of animal model, which was characterized by an increase in plasma TNF-α levels (Table 2) and changes in histopathological features by the thickening of the interalveolar septum due to infiltration of inflammatory cells and collagen deposition in group K1 (Table 1). This is aligned with a research by Basyigit et al. (2010), who exposed white Wistar strain rats to cigarette smoke and discovered that TNF-α levels is increased, particularly in the COPD control group.

According to Sullivan et al. (2009), a high TNF-α level can increase the risk of pulmonary fibrosis; this is in accordance with the results of the correlation test in this study which showed a positive relationship between plasma TNF-α levels and the pulmonary fibrosis degree (Table 6). TNF-α triggers pulmonary fibrosis through increased expression of the TGFß1 gene. TGFβ1 is the primary profibrotic mediator, which stimulating extracellular matrix synthesis and inducing fibroblast differentiation into myofibroblasts (Todd, Luzina & Atamas 2012). The mechanism for increasing TGFβ1 expression occurs through the pathway of increasing activation of transcription factor activator protein-1 (AP-1) and increasing TGFß1 mRNA stability through the ERK-MAPK pathway (Sullivan et al. 2009).

It has been proven that dexamethasone or nanoemulsion therapy has a protective effect against pulmonary fibrosis, inflammation, and damage to tissue structure. In this study, the administration of turmeric extract nanoemulsion in VCO at a dose of 0.3 mL/200g/day showed the most potent protective effect. The administration of dexamethasone tablet 0.2 mg/kgBW/day (K4) has a protective effect that is almost the same as the administration of nanoemulsion at a dose of 0.3 mL/200g/day (K2); this is evidenced by the results of statistical analysis tests, which show there is no significant difference between them (Tables 2 & 5). Dexamethasone is a corticosteroid drug with an anti-inflammatory effect that is commonly used to treat pneumonia cases, such as Chronic Obstructive Pulmonary Disease (COPD) caused by cigarette smoke exposure (Aggarwal et al. 2011).

The interesting thing from this research, the experimental animal group that received a higher dose of nanoemulsion treatment of 0.6 mL/200g/day (K3) showed a decrease in the effectiveness of anti-inflammatory and antifibrotic activity compared to the nanoemulsion group at 0.3 mL/200g/day (K2) (Tables 2 & 3). This is most likely caused by the polyphenolic chemicals in the turmeric extract nanoemulsion in the VCO, which have pro-oxidant characteristics. According to a study by Yordi et al. (2012), polyphenol compounds are antioxidants with a variety of health benefits, but at high doses, they can change the action of polyphenols to make them pro-oxidants. The pro-oxidant activity of polyphenolic compounds occurs based on the presence of phenoxyl radicals which easily bind to oxygen and produce O_2, H_2O_2, quinones, and semiquinones (Eghbaliferiz & Irfansashi 2016). High amount of polyphenol compounds can also form complexes with transition metal ions (Fe and Cu). The impact of the formation of pro-oxidant activity from polyphenolic compounds is mitochondrial dysfunction, lipid peroxidation, DNA damage, and apoptosis (Eghbaliferiz & Irfansashi 2016; Yordi et al. 2012).

The main component of the turmeric extract nanoemulsion in VCO is curcumin, a polyphenolic molecule with anti-inflammatory properties, and is frequently utilized in herbal medicine. The manufacture of turmeric extract nanoemulsion preparations with VCO solvent is aimed to improve the pharmacokinetics of curcumin by increasing the stability of digestive enzymes, thereby increase the oral bioavailability ninefold (Sari et al. 2015; Yu & Huang 2012). According to Damin, Alam and Sarro (2017), the main component of VCO is a medium chain of saturated fatty acids, such as lauric acid and caprylic acid. Due to their similar polarity, the fatty acids in VCO are anticipated to boost curcumin's solubility and absorption (Sephapour et al. 2018).

The mechanism of curcumin as an anti-inflammatory works by influencing several signaling pathways and inhibiting the production of inflammatory mediators (Peng et al. 2021). This is in line with studies on animal model conducted by Jain et al. (2009), which stated that curcumin is able to reduce levels of several inflammatory mediators, including TNF-α. The suppression of TNF-α production mostly occurs at the transcriptional level, which is played by curcumin as an anti-inflammatory. Curcumin can inhibit specific acetyltransferase P300/CREB, which resulted in the decreasing of histone/non-histone protein acetylation and histone acetyltransferase-dependent chromatin transcription. In addition, curcumin can affect the function of the TNF-α promoter through the promoter methylation process as well (Aggarwal et al. 2013; Balasubramanyam et al. 2004). TNF-α expression can be reduced by curcumin by inhibiting numerous signaling pathways, particularly the NF-κB pathway. In addition, curcumin can also inhibit the TLRs, JNK, AP-1, and MAPK pathways (Aggarwal et

al. 2013; Cho et al. 2007). Inhibition of NF-κB activation causes NF-κB failure in entering the nucleus, so that there is no transcription of inflammatory mediators, as well as the decreasing of TNF-α and TNF-α production.. Based on the molecular docking study by Gupta et al. (2011) and Wua et al. (2010), curcumin affects the TNF-α signaling pathway by binding protein ligands directly to three areas at once. Based on the explanation above, it shows that curcumin is a strong and potent inhibitor in preventing the secretion and expression of TNF-α through a complex mechanism.

Conclusion

This research showed that the preparation of turmeric extract nanoemulsion in VCO can increase the anti-inflammatory and antifibrotic activity of curcumin. Turmeric extract nanoemulsion in VCO at a dose of 0.3 mL/200g/day had the most potent inhibitory effect on inflammation and pulmonary fibrosis due to cigarette smoke exposure of two cigarettes/day for 42 days.

References

Aggarwal, B.B., Prasad, S., Reuter, S., Kannappan, R., Yadev, V.R., Park, B., Kim, J.H., Gupta, S.C., Phromnoi, K., Sundaram, C., Prasad, S., Chaturvedi, M.M. & Sung, B. 2011. Identification of level anti-inflammatory agents from Ayuverdic medicine for prevention of chronic disease. *Curr. Drug Targets* 12: 1595-1653.

Aggarwal, B.B., Gupta, S.C. & Sung, B. 2013. Curcumin: An orally bioavailable blocker of TNF and other pro-inflammatory biomarkers. *British Journal of Pharmacology* 169: 1672-1692.

Bae, M., Park, Y.K. & Lee, J.Y. 2018. Food components with antifibrotic activity and implication in the prevention of liver disease. *Journal of Nutritional Biochemistry* 55: 1-11.

Bagchi, A. 2012. Extraction of curcumin. *Journal of Enviromental Science Toxicology and Food Technology* 1(3): 1-16.

Balasubramanyam, K., Varier, R.A., Altaf, M., Swaminathan, V., Siddappa, N.B., Ranga, U. & Kundu, T.K. 2004. Curcumin, a novel p300/CREB-binding protein-specific inhibitor of acetyltransferase, represses the acetylation of histone/nonhistone proteins and histone acetyltransferase-dependent chromatin transcription. *J. Biol. Chem.* 279(49): 51163-51711.

Basyigit, I., Sahin, M., Sahin, D., Yildiz, F., Boyaci, H., Sirvanci, S. & Ercan, F. 2010. Anti-inflammatory effect of montelukast on smoke-induced lung injury in rats. *Multidisciplinary Respiratory Medicine* 5: 1-9.

Cas, D.M. & Ghidoni, R. 2019. Dietary curcumin: Correlation between bioavailability and health potential. *Nutrients* 9: 1-14.

Cho, J.W., Lee, K.S. & Kim, C.W. 2007. Curcumin attenuates the expression of IL-1β, IL-6, and TNF-α, as well as cyclin E in TNF-α, treated HaCat cells; NF-kB and MAPKs as potential upstream targets. *International Journal of Molecular Medicine* 19(3): 469-474.

Damin, S.H., Alam, N. & Sarro, D. 2017. Characteristics of virgin coconut oil (VCO) harvested at various altitudes where it grows. *Journal of Argotekbis* 5(04): 431-440.

Desdiani, Iris Rengganis, Samsuridjal Djauzi, Agus Setiyono, Mohammad Sadikin, Sri Widia A Jusman, Nurjati Siregar, Suradi & Putri C. Eyanoer. 2020. Green tea extract reduces the area of rat lung fibrosis. *Indonesian Pathology Magazine* 29(1): 15-24.

Dorey, A., Scheerlinck, P., Nguyen, H. & Albertson, B.G.T. 2020. Acute and chronic carbon monoxide toxicity from tobacco smoking. *Military Medicine* 185(1): 61-67.

Eghbaliferiz, S. & Iranshahi, M. 2016. Prooxidant activity of polyphenols, flavonoids, anthocyanins, and carotenoids: An updated review of mechanism and catalyzing metals. *Phytotherapy Research* 30(9): 1379-1391.

Ercan, E., Ilbamis, M.S. & Tasci, U. 2021. Effect of smoking on acute hypobaric hypoxia tolerance. *Hamidiye Med J.* 2(1): 37-42.

Flora, G., Gupta, D. & Tiwari, A. 2013. Nanocurcumin: A promising therapeutic advancement over native curcumin. *Crit. Rev. The Drug Carrier Syst.* 30(4): 331-336.

Gawda, E.W., Wrzos, P.C., Zarobkiewicz, M.K., Chlapek, K. & Jedrych, B.J. 2020. Lung histological alterations in rats exposed to cigarette smoke and electronic cigarette vapor. *Experimental and Therapeutic Medicine* 19: 2826-2832.

Gupta, S.C., Prasad, S., Kim, J.H., Patchva, S., Webb, L.J., Priyadarsini, I.K. & Aggarwal, B.B. 2011. Multitargeting by curcumin as revealed by molecular interaction studies. *Nat. Prod. Rep*. 28(12): 1937-1955.

Huang, R., Liu, Y., Xiong, Y., Wu, H., Wang, G., Sun, Z., Chen, J., Yan, X., Pan, Z., Xia, J., Zhang, Z., Wang, J. & Wu, C. 2016. Curcumin protects against liver fibrosis by attenuating infiltration of grh1i monocytes through inhibition of monocyte chemoattractant protein. *Discov. Med.* 21(118): 447-457.

Hubner, R.H., Gitter, W., El Mokhtari, N.E., Mathiak, M., Both, M. & Bolte, H. 2008. Standardized quantification of pulmonary fibrosis in histological samples. *Biotechnique* 44: 507-517.

Jacob, A., Wu, R., Zhou, M. & Wang, P. 2007. Mechanism of the anti-inflammatory effect of curcumin: PPAR-γ activation. *PPAR Research* 2007: 89369.

Jain, K.S., Rains, J., Croad, J., Larson B. & Jones, K. 2009. Curcumin supplementation lowers TNF-α, IL-6, IL-8, and MCP-1 secretion in high glucose-treated cultured monocytes and blood levels of TNF-α, IL-6, MCP-1, glucose, and glycosylated hemoglobin in diabetic rats. *Antioxidant & Redox Signaling* 11(2): 241-249.

Klopfleisch, R. 2013. Multiparametric and semiquantitative scoring systems for the evaluation of mouse model histopathology. *BMC Vet. Res.* 9(123): 1-15.

Lopez, A.G., Thiago, S.F., Renata, T.N., Manuella, L., Karla, M.P.P., Ari, M.S., Ricardo, M.B., Antonio, J.R.S., Samuel, S.V. & Luis, C.P. 2013. The antioxidant action of propolis on mouse warp exposed to short-term cigarette smoke. *Bioorganic and Medicinal Chemistry* 21(24): 7570-7577.

Lugg, S.T., Scott, A., Parekh, D., Naidu, B. & Thickett, D.R. 2021. Cigarette smoke exposure and alveolar macrophages: A mechanism for lung disease. *Thorax* 77(1): 94-101.

Maharaj, S., Shimbori, C. & Kolb, M. 2013. Fibrocytes in pulmonary fibrosis: A brief synopsis. *Eur. Respir. Rev.* 22: 552-557.

Maulidiyah, N. & Amin, M. 2015. Respiratory biomarkers in lung disease. *Respiration Journal* 1(2): 67-71.

Medzhitov, R. 2010. Inflammation 2010: New adventures of an old flame. *Cells* 140(6): 771-776.

Napanggala, A. 2015. Chronic obstructive pulmonary disease with pleural effusion and grade I hypertension. *Medula Journal* 4(2): 1-6.

Peng, Y., Ao, M., Dong, B., Jiang, Y., Yu, L., Chen, Z., Hu, C. & Xu, R. 2021. Anti-inflammatory effects of curcumin in the inflammatory diseases: Status, limitations, and countermeasures. *Drug Design, Development and Therapy* 15: 4503-4525.

Profita, M., Sala, A., Bonanno, A., Riccobono, L., Ferraro, M., La Grutta, S., Albano, G.D., Montalbano, A.M. & Gjomarkaj, M. 2010. Chronic obstructive pulmonary disease and neutrophile infiltration: Role of cigarette smoke and cyclooxygenase products. *Am. J. Physiol. Lung Cell Mol. Physiol.* 298: 261-269.

Ramli, N., Rifa'i, Y., Wunas, J. & Yustianus, R.R. 2019. Curcumin content in an extract of some rhizomes from the Zingiberaceae family. *Journal of Pharmaceutical and Medicinal Sciences* 4(1): 15-19.

Rasool, S.T., Alavala, R.R., Kulandaivelu, U. & Sreeharsa, N. 2020. Non-invasive delivery of nano emulsified sesame oil extract of turmeric attenuates lung inflammation. *Pharmaceutics* 12(1206): 1-15.

Salawati, L. 2016. The relationship of smoking with the degree of chronic obstructive pulmonary disease. *Syiah Kuala Medical Journal* 16(3): 165-169.

Sari, T.P., Mann, B., Kumar, R., Singh, R.R.B., Rajan, S., Minaxi, B. & Athira, S. 2015. Preparation and characterization of nanoemulsion encapsulating curcumin. *Food Hydrocolloid* 43: 540-546.

Sephapour, S., Selamat, J., Manap, M.Y.A., Khatib, A. & Razis, A.F.A. 2018. Comparative analysis of the chemical composition, antioxidant activity, and quantitative characterization of some phenolic compounds in selected herbs and spices in different solvent extraction systems. *Molecules* 23(402): 1-7.

Sullivan, D.E., Ferris, M., Nguyen, H., Abboud, E. & Brody, A.R. 2009. TNF-a induces tgfb1 expression in lung fibroblasts at the transcriptional level via AP-1 activation. *J. Cell Mol. Med.* 13(08): 1866-1876.

Supriono, Pratomo, B. & Praja, D.I. 2018. Effect of curcumin on nf-kb levels and degree of liver fibrosis in liver fibrosis rats. *Journal of Internal Medicine* 5(4): 174-183.

Todd, N.W., Luzina, I.G. & Atamas, S.P. 2012. Cellular and molecular mechanisms of pulmonary fibrosis. *Fibrogenesis and Tissue Repair* 5(11): 1-24.

Vasconcelos, L.H.C., Silva, M.C.C., Costa, A.C., de Oliveira, G.A., de Souza, I.L.L., Righetti, R.F., Queiroga, F.R., Cardoso, G.A., Silva, A.S., da Silva, P.M., Vieira, G.C., de F.L.C., Tibério, I., Madruga, M.S., de A Cavalcante, F. & da Silva, B.A. 2020. Virgin coconut oil supplementation prevent airway hyperreactivity of guinea pigs with chronic llergic lung inflammation by antioxidant mechanism. *Oxidative Medicine and Cellular Longevity* 2020: 5148503.

Wua, S.T., Sun, J.C., Lee, K. & Sun, Y. 2010. Docking prediction for tumor necrosis factor- and five herbal inhibitors. *Int. J. Eng. Sci. Technol.* 2: 4263-4277.

Wulandari, R.D., Hadisaputro, S. & Suhartono. 2013. Various factors related to the incidence of pulmonary function disorders in the workspace (a case study of electroplating home industry workers in Talang sub-district, Tegal district. *Indonesian Environmental Health Journal* 12(1): 94-98.

Wuyts, W.A., Agostini, C., Antoniou, K.M., Bouros, D., Chambers, R.C., Cottin, V., Egan, J.J., Lambrecht, B.N., Lories, R., Parfrey, H., Prasse, A., Robalo-Cordeiro, C., Verbeken, E., Verschakelen, J.A., Wells, A.U. & Verleden, G.M. 2013. The pathogenesis of pulmonary fibrosis: A moving target. *European Respiratory Journal* 41(5): 1207-1218.

Yordi, E.G., Perez, E.M., Matos, M.J. & Villares, E.U. 2012. Antioxidant and prooxidant effects of polyphenolic compounds and structure-activity relationship evidence. *Nutrition, Well-Being, and Health.* https://cdn.intechopen.com/pdfs/29974.pdf

Yu, H. & Huang, Q. 2012. Improving the oral bioavailability of curcumin using novel organelle-based nanoemulsions. *J. Agric. Food Chem.* 30(60): 5373-5379.

Yudhawati, R. & Prasetyo, Y.D. 2018. Immunopathogenesis of chronic obstructive pulmonary disease. *Journal of Respiration* 4(1): 19-25.

Zeldin, C.D., Lenane, C.W., Chulada, P., Bradbury, A., Scarborough, P.E., Roggli, V., Langenbach, R. & Schwartz, D.A. 2001. Airway inflammation and responsiveness in prostaglandin H synthase-deficient mice exposed to bacterial lipopolysaccharide. *American Journal of Respiratory Cell and Molecular Biology* 25: 457-464.

Zhang, D., Huang, C., Yang, C., Liu, R.J., Wang, J., Niu, J. & Bromme, D. 2011. Antifibrotic effects of curcumin are associated with overexpression of cathepsins and bleomycin-treated mice and human fibroblasts. *Respiratory Research* 12(1): 1-12.

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