

## ORIGINAL ARTICLE

# Potential of Nanocurcumin on Cytokine Storm through Decreased IL-6 and TNF- $\alpha$ Expression

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## ABSTRACT

**Introduction:** Patients infected with covid-19 coronavirus and even influenza can die because of an overreaction of the immune system called cytokine storms. Previous research has said that turmeric (curcumin) has the ability to suppress viral infection condition where cytokine increase and continuing by increased of cytokine storm. This condition showed that curcumin potentially to using for healing Ebola patients. Curcumin seems to have very low bioavailability on our human body. In order to increase absorption, nanocurcumin which has smaller particle substrate was used. Our previous research has said that nanocurcumin increase mitochondrial biogenesis in skeletal muscles. This study aims to look at the effect of nanocurcumin administration on IL-6 and TNF- $\alpha$  which increase on high intensity endurance exercise. **Methods:** Wistar rats 10 weeks old used on this experiment and entered into non-exercise and exercise groups. Furthermore, every group divided with control and nanocurcumin. Curcumin doses was one hundred mg/ kilogram/day peroral on both groups for 28 days to known effect of nanocurcumin itself and nanocurcumin together with exercise. Two hours swimming exercise per-day used to determine effect of endurance training. Western blotting (WB) was used to detect of proteins expression IL-6 and TNF- $\alpha$ . **Results:** Compared to non-exercise group, exercise group showed enhance IL-6 and TNF- $\alpha$  protein expression and interested that nanocurcumin significant abolish this effect. **Conclusion:** Taken together, these results suggest that nanocurcumin treatment ability decreased inflammation involved on endurance training induced cytokine storm and potentially to be one of candidate drugs to decrease cytokine storm condition.

**Keywords:** Nanocurcumin, Cytokine Storm, IL-6, TNF- $\alpha$

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## INTRODUCTION

Unlikely the previous coronavirus families for example outbreak on 2002 known as Severe Acute Respiratory Syndrome (SARS) and also viruses diseases which transferred to human from infected dromedary camels as name as Middle East Respiratory Syndrome (MERS), the another viral named covid-19 (corona) which potentially attacks many human organs. Indeed, the covid-19 outbreak has infected and killed above two millions people in the world. Generally, covid-19 begins

and ends in the lungs; because, just like the influenza, coronavirus is a respiratory disease. Novel coronavirus quickly attacks human lung cells. The cell protector comes from two classes: one that makes mucus, while the other is hair-like cilia. Mucus helps maintain lung tissue from pathogens and ensures respiratory organs do not dry out. Meanwhile, cilia cells clear 'debris' such as pollen or viruses. Researchers from the US explained that SARS seems to infect and kill cilia cells, which then peel off and fill the patient's airways with debris and fluid (1). He suspected that the same thing happened to the new coronavirus. At that time, the patient is against the viruses. Fearful of the presence of an attacking virus, the body will protect lung tissue in order to release damage and healing lung tissue. A number of important studies conducted in China said that patients who died from

covid-19, could be caused by their own immune system. The deaths were caused by covid-19, not from the virus itself, but as a result of cytokine storm syndrome (2). During cytokine storms, the body's excessive immune response can damage healthy pulmonary organ tissue. It causes acute respiratory diseases and furthermore can damaged failure many organs. The patient in another study after being infected with coronavirus then developed cytokine storm syndrome in his body, apparently ironically had a genetic immune defect. Thus, resulting in an uncontrolled immune response. In treating cytokine storms caused by other diseases, such as viral infections or other autoimmune diseases, the mortality rate of patients increase above 27 percent. In case of covid-19 with mild symptoms, it may turn into a more severe infection, if the patient is known to have this cytokine storm syndrome. This cytokine storm syndrome causes a pathological state of the body's overactive immune response that leads to fatal multi-organ dysfunction syndrome (MODS).

Nowadays, herbal plants are widely used by the public as one of the ways to help prevent coronavirus infection or covid-19. Some herbs that are commonly consumed by the public are turmeric or curcumin. Previous researchers indicated that receptor angiotensin converting enzyme 2 (ace2) seem look like to be the important regulation on plays a role in turmeric (3). Ace2 can be in fixed form (attached to cells) and soluble (not attached to cells). Research on curcumin compounds (as a single or pure compound) reportedly increased ace2 in mouse test animals, but there has been no direct link study to coronavirus infection (4). Other studies have suggested that curcumin has excellent potential in Ebola virus therapy as well as other viral infections through its ability to suppress cytokine release and cytokine storms as evidenced by the suppression of parameters included as Interleukin-6 (IL-6), Interleukin-8 (IL-8) and Tumours Necrosis Factor Alpha (TNF- $\alpha$ ) (5,6). The suppression of these inflammatory parameters will further cause mitochondrial biogenesis through AMPK and PGC-1 $\alpha$  activation pathways, especially in lung organs so that lung mitochondrial cells become better (7).

Nowadays, agriculture and food system has high impact on revolutionary technology, one is known as Nanotechnology (8). This technology in order to developing and building gears, make substances considered extremely small for example enzymes or receptor to be smaller than the human body cells or almost between 100 to 10,000 times (9). Curcumin was knows as the substrate with low bioavailability. In order to increase absorption of curcumin by the body, we use nanocurcumin on this current study. Purposed of the current research is to determine whether nanocurcumin has the ability to suppress inflammatory parameter protein such as IL-6 and TNF- $\alpha$  as marker for cytokine storm which increase on exercise conditions in order to get the same conditions on the covid-19 patients

(10). We hypothesises that nanocurcumin have ability to decrease IL-6 and TNF- $\alpha$  and this current study result with impacted to help treatment maintain for covid-19 patients.

## MATERIALS AND METHODS

### Animals

This experiment can be continuing to investigated since approved by the ethics committee of Universitas Kristen Maranatha (No: 098/KEP/VII/2020). Adult rat aged 10 weeks were used on this experiment. Wistar rats were placed in room with air-conditioned facilitation and face to light by setting 12 hours light and 12 hours dark. Diet was given ad libitum. Animals were entered into two groups of non-exercise and non-exercise groups and each group was further divided into control groups and groups with got nanocurcumin (100 milligram/kg-body weight/day). All animals get treatment through oral pathways for 24 days and for control group get fluid equal to treatment.

### Exercise treatment

The exercise treatment was given by swimming exercise for 2 hours which dividing into 4 sets with the duration of each set is 30 minutes and given a break of 5 minutes at each of set. After the first 30 minutes a load of two percentage from body weight was given to the rats by wrapping the animals body by a plastic cable. The swimming pool is 50 cm deep and the swimming area is 200 cm<sup>2</sup>/rat. Animal exercise for 24 days with frequency 6 times a week for 4 weeks.

### Western blotting analyses

After completion of the treatment period, rats injected by 50 mg Pentobarbital every 100 mg of body weight to anaesthetized. After that, for the examination of protein expression, gastrocnemius muscle was quickly taken and isolated. The tissue is washed and stored in cool NaCl solution, previously cleaned from other tissues, after that immediately put in liquid nitrogen (21). Generally 40 mg gastrocnemius muscle was homogenized in Buffer A which made from 250 mM sucrose, 10 mM NaCl, 3 mM MgCl<sub>2</sub>, 1 mmol/l dithiothreitol (DTT) and place on 500  $\mu$ l of ice-cold for 30 second, after that 1 mM phenylmethylsulphonyl fluoride (PMSF), and 2  $\mu$ l protease inhibitor cocktail added on Buffer A. Centrifuged used at 500 g at 4°C in order to get supernatant on the homogenized procedure. This conditions knows as the whole fraction. Furthermore, The remaining pellet was resuspended in 500  $\mu$ l of ice-cold buffer B which contains of 50 mM Tris, pH 7.5, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 50 mM NaF, 5 mM Na pyrophosphate, 50 mM MgCl<sub>2</sub>, 10% glycerol, 1% Triton X-100, 1 mM PMSF, and after that 2  $\mu$ l tissue protease inhibitor cocktail added on that buffer). The buffer incubated with mixing for every 1 minutes on cold condition for 10 minutes. The resuspended pellet which get from this protocol take on centrifuge and spin

at 3100 g at 4°C for 5 or 6 minutes..

Analysis using WB follows previously described. Briefly, SDS-PAGE gel 12.5 % used to be place for loading equal protein amounts of samples included IL-6 and TNF- $\alpha$ , and continuing by transferred to membrane which build from a polyvinylidene fluoride (PVDF). Blocking buffer used to incubation place for the membrane and subsequently with IL-6 (Cell Signaling Technology, Danvers, MA, USA), TNF- $\alpha$  (Calbiochem, San Diego, CA, USA), GAPDH (Abcam, Cambridge, England), antibodies on refrigerator 40C degree for overnight. Furthermore, membrane continuing by incubated on secondary antibody, after that membrane washed 3 times for 10 minutes every time washing and the analysing protein signal used by Amersham ECL western blotting detection reagent chemiluminescence (ECL) (GE Healthcare, Piscataway, NJ, USA). In order to quantified analysed intensity we used Image J from NIH (Maryland, USA).

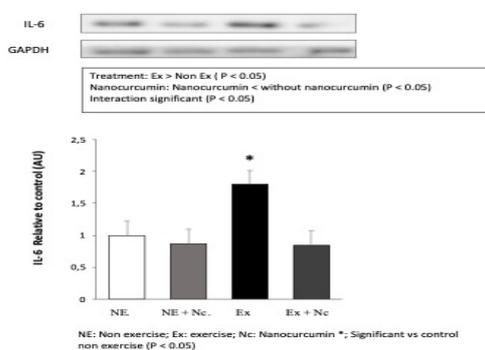
### Statistical analysis

In order to investigated effect of curcumin together with exercise and non-exercise inhibitors together with curcumin on IL-6 and TNF- $\alpha$  expression, we used A two-way ANOVA and continuing by The Tukey-Kramer post-hoc test to assess significance between experimental groups. All data are showed on mean  $\pm$  standard deviation (SD) and P values for this statistically calculation were  $< 0.05$ .

## RESULTS

### Nanocurcumin treatment decrease IL-6 expression on exercise group

As shown in Fig. 1, on the exercise group, nanocurcumin treatment decrease the effect of eTR to increase IL-6 on skeletal muscle (Fig. 1), suggesting that nanocurcumin could decrease inflammation marker on skeletal muscle.

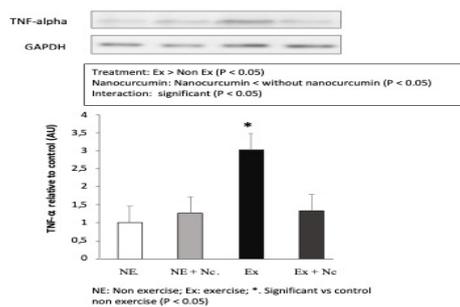


**Fig. 1: Immunoblots for markers of IL-6 Expression**  
Values are expressed as the mean  $\pm$  SD, n = 6. \*: significant difference from Control non exercise (p < 0.05).

### Nanocurcumin treatment decrease TNF- $\alpha$ expression on exercise group.

As shown in Fig. 2, on the exercise group, nanocurcumin treatment decrease the effect of eTR to increase TNF- $\alpha$  on

skeletal muscle (Fig. 2), suggesting that nanocurcumin could decrease inflammation marker on skeletal muscle.



**Fig. 2: Immunoblots for markers of TNF- $\alpha$  Expression**  
Values are expressed as the mean  $\pm$  SD, n = 6. \*: significant difference from Control non exercise (p < 0.05).

## DISCUSSION

Our current result indicated that IL-6 and TNF- $\alpha$  increase by endurance exercise (Fig 1 and 2). This result is indeed similar with that of the previous study which showed that performed the exercise enhance pro-inflammatory IL-6 and TNF- $\alpha$ . The exercises conducted in this study tended to have high intensity, where according to Bernecker et al, 2013 (11). High intensity exercise led to enhance parameter inflammation IL-6 and TNF- $\alpha$ . This result indicated that process of regular physical activity is recommended to reduce death (12). On the other side, long endurance sport such as long distance running included marathon can be trigger for the heart attack diseases (13). Currently, the mechanisms exercise correlation with cardiovascular diseases (e.g. increased of biomarker cardiac such as troponin) are not really understood as well as other diseases. One reason is leak of systemic inflammation. Parallel with this, previous data showed correlation between endothelial dysfunction and inflammation. Inflammatory cytokines indicated potentially to be an important way which involve in plaque disorders and increase thrombosis conditions after plaque disorders (14). Other previous study also suggested that cardiac dysfunction included acute myocardial infarction (AMI) and myocarditis tend increasing TNF- $\alpha$  (15). Collaboration between ligands of heterotrimeric and also one until two receptor on cell surface included (TNFR1 and TNFR2) will starting regulated production of TNF- $\alpha$  in macrophages and continuing by regulated of the TNF- $\alpha$  response (16). The local increase in TNF- $\alpha$  causes of inflammation sign. Furthermore, high systemic TNF- $\alpha$  levels produce shock signs (17). The data suggest that TNF- $\alpha$  inflammatory cytokines and also IL-6 plasma enhance after a long running event include marathon after regulation of leukocytes. This condition, especially TNF- $\alpha$ , was involve on a higher risk for heart diseases (18).

Furthermore, our current result showed that nanocurcumin has an ability to decrease the effect of endurance exercise to increase inflammation marker IL-6 and TNF- $\alpha$  (Fig 1 and 2). This result indicated that

nanocurcumin potentially to decrease inflammation which happen on endurance exercise. Cytokine are family of intercellular molecular which involve on regulated inflammation and immune respond. Actually, increasing of inflammation marker on cell suggest that body making the physiological protective response. However, the increasing of this response on the other hand, releasing cytokine into the circulation which change be self-destructive, pathogenic even can be worse conditions for the host. Previous study showed that Inteleukin-6, Interleukin-8 and also linterleukin-10 increase on sport event included marathon, long running and also triathlon (10). Indeed, this conditions can enhance the negative effect for the host included delay onset muscle soreness, muscle damage because leucocytosis conditions (10). Parallel with these current study result, previous investigation determined and give suggestion that curcumin supplementation decrease TNF- $\alpha$  and IL-6 blood level and potentially to reduce conditions of vascular inflammation in diabetes (19). Furthermore, curcumin also seem look like to similar effect to decrease cytokine transcription and inflammatory marker response on endurance exercise with inhibit NF-kB and activator protein1 (6). Based on the above statement, we speculated that curcumin potentially to inhibit inflammation marker on skeletal muscle included IL-6 and TNF- $\alpha$ . In order to increase bioavailability of curcumin we used nano technology with ball mill methods to making nanocurcumin. Indeed, our current result showed potentially of nanocurcumin to decrease cytokine storm. However, some limitations arise when we conduct this experiment, including the difficulty of obtaining animals samples which infected by covid-19 to completed the results of this study, but these conditions would be challenge to continuing the research in the future.

## CONCLUSION

Our current result indicated that nanocurcumin ability to decrease inflammation marker Interleukin-6 (IL-6) and Tumours Necrosis Factor alpha (TNF- $\alpha$ ) and furthermore tend decreased cytokine storm. This study showed that nanocurcumin has a potential to be used by patients with covid-19 in order to decrease cytokine storm on their conditions.

## ACKNOWLEDGMENTS

This research was supported by Directorate General of Higher Education through Penelitian Dasar Unggulan Perguruan Tinggi (PD-UPT).

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