International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 13, Special Issue 3, 2021

Original Article

TURMERIC EXTRACT POTENTIAL INHIBIT INFLAMMATORY MARKER IN LPS-STIMULATED MARCOPHAGE CELLS

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Received: 10 Aug 2020, Revised and Accepted: 04 Oct 2020

ABSTRACT

Objective: Inflammation can be induced by microbiological, chemical, physical factors and plays roles in inflammatory diseases. Turmeric (*Curcuma longa* L.) has been widely used to provide a diverse array of biological activities, including anti-inflammatory, antimicrobial, also antioxidant. The Turmeric extract (TE) anti-inflammatory potential was conducted using a Lipopolysaccharide (LPS)-induced RAW264.7 macrophage cell line by inhibiting inflammatory mediators especially IL-6, PGE-2, IL-1β, COX-2, TNF-α, iNOS, also NO level.

Methods: The TE safe concentration in LPS-induced macrophage cell line was measured using MTS assay for further assay. The inflammatory markers (IL-6, PGE-2, COX-2, IL-1β, TNF-α, iNOS, NO) were measured using ELISA assay and NO by the nitrate/nitrite colorimetric assay in LPS-induced RAW264.7 cell line. LPS induced inflammatory marker by increasing inflammatory marker (IL-6, PGE-2, COX-2, IL-1β, TNF-α, iNOS, NO).

Results: TE with 100 to 25 µg/ml, caused a significant reduction of cells viability, reaching only 30.27 % live cells. TE with lower concentrations (7.5; 5; 2.5 µg/ml) had no cytotoxic effect on macrophage cells (viability 117.31-131.08 %). LPS induction caused an increase in inflammatory cytokines IL-1β, PGE-2, IL-6, COX-2, TNF-α as well as iNOS and NO. Turmeric extract caused the reduction of the inflammatory cytokines in a dose-dependent manner.

Conclusion: The research resulted that TE has anti-inflammatory activity by decreasing IL-6, PGE-2, COX-2, IL-1β, TNF-α, iNOS, and NO level on LPS-induced RAW264.7 cells.

Keywords: Inflammation, Turmeric, TNFα, IL-6, IL-1β

DOI: https://dx.doi.org/10.22159/ijap.2021.v13s3.01 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Inflammation is the host natural defence against pathogen infections, which also can be induced by microbiological, chemical, physical factors [1-3]. Inflammation involves several events such as changes in blood flows and vascular permeability, activation and migrations of leukocytes, and synthesis of local inflammatory mediators. The inflammatory mediators such as interleukin (IL)-1β, prostaglandin E2 (PGE2), IL-6, tumor necrosis factor-alpha (TNF-α), cyclooxygenase-2 (COX-2), and nitric oxide (NO) has been observed as the primary response to inflammation [4, 5]. Prolonged exposure to these inflammatory mediators may induce acute and/or chronic inflammatory responses in the organs such as heart, lung, brain, and reproductive systems and potentially leading to tissues damage [4, 6, 7]. Increased level of TNF-α elicited platelet activation when during inflammation the complement is activated on the surface of platelet [8, 9] meanwhile burst of IL-1β are involved in acute attack of systemic or local inflammation [10] and IL-6 trans signal mediate inflammation from acute to chronic [11]. To treat the inflammatory related disease, abundance research has been conducted to find potential anti-inflammatory compounds, including plants-derived compounds. Turmeric (*Curcuma longa* L) has been used as traditional therapy over the years, including in the ayuverdic medication. Turmeric has been provided scientifically against such human ailments [12]. Turmeric contains active compounds, classified as curcuminoids, consist of curcumin (77%), bisdemethoxycurcumin (3%) and demethoxycurcumin (17%). Curcumin has been stated as major biologically active components having anti-bacterial, anti-diabetic, anti-oxidant, anti-inflammatory activities, lowering cholesterol [13].

The widely used as inflammatory cells model *in vitro* was murine macrophage cell line (RAW 264.7). While lipopolysaccharide (LPS) as bacterial metabolite is one of the well-studied stimulus to induce inflammatory mediators' secretion. In the current study, we focused on the turmeric extract (TE) potential anti-inflammatory activity by modulating the pro-inflammatory molecules production (TNF-α, PGE-2, IL-1β, iNOS, IL-6, COX-2, and NO).

MATERIALS AND METHODS

Materials

Turmeric (*C. longa* L.) rhizome was collected from farmer plantation in Bogor, West Java, Indonesia. The murine macrophage cell line (RAW 264.7, ATCC TIB-71) was obtained from Biomolecular and Biomedical Research Center, Aretha Medika Utama, Bandung, Indonesia. Dulbecco's Modified Eagle Medium (DMEM) (from Biowest, L0104), 10% fetal bovine serum (FBS) (from Biowest, S1810-500), 1% Antibiotic/ antimycotic (ABAM, from Biowest, L0010-100), 1% Nanomycopulitine (from Biowest, L-X16-010), and 0.1% Gentamicin (from Gibco 15750078). Bovine Standard Albumin (BSA) (from Sigma Aldrich, A9576). Quick Start Dye Reagent 1X (from Biorad, 5000205). LPS from *Escherichia coli* (Sigma Aldrich, L2880), MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (from Abcam, ab197010), Elisa Kit mouse IL-1β (E-EL-M0037), mouse TNF-α (E-EL-M0049), mouse COX-2 (E-EL-M0959), mouse IL-6 (E-EL-M0044), PGE-2 (E-EL-0034), mouse iNOS (E-EL-M0696), NO Colorimetric Assay (E-BC-K035-M).

Methods

Extraction process

Turmeric (*Curcuma longa* L.) rhizome was identified by herbarium staff of Scholl of Life Sciences, Bandung Institute of Technology,

Bandung, West Java, Indonesia. One kilogram of dried rhizome powder was macerated using distilled ethanol, filtered, and evaporated by rotary evaporator (Zhengzhou Well-known, RE-201D). The turmeric extract (TE) in the form of paste was stored at-20 °C until further use [14].

Cells culture

Cells that used in this study (murine macrophage cell line also known as RAW 264.7) was cultured in DMEM, ABAM, FBS, Gentamicin, and Nanomycopulitine then incubated at 37 °C in a humidified atmosphere with 5% CO_2. When the cells was at 80% confluency, it was harvested using cells scrapper [15-18].

Cells viability measurements

MTS assay was used to measured murine macrophage cell line viability. In 96 well-plates, cells ($5x10^3$ cells/well) were planted in 180 µl growth medium also extract with various concentrations (100; 75; 50; 25; 7,5; 5; 2.5; 0 µg/ml) as much as 20 µl, then incubated in 37 °C with CO_2 5% for 24 h. Then the reagent (MTS) was added into each well and incubated at the same condition for 3 h. At the wavelength of 490 nm in a microplate reader (Multiskan Go, Thermo Scientific), the absorbance was read using and cells viability was calculated [10-14].

LPS-induced RAW cells and treatments

In 6 well plate, cells ($5x10^5$ cells/well) were planted and incubated for 24 h. It was treated with TE and induced by LPS. Six treatments that used for this research: (1) the negative control (without induced by LPS); (2) positive control (induced by 1 µg/ml LPS); (3) macrophage cells that induced by 1 µg/ml LPS and treated by TE at 7.5 µg/ml; (4) macrophage cells that induced by 1 µg/ml LPS and treatetd by TE at 5 µg/ml; (5) macrophage cells that induced by 1 µg/ml LPS and treated by TE 2.5 µg/ml. It was incubated for another 24 h. After that, the conditioned medium was collected. It was centrifuged and collected the cell-free supernatant that used for measure the COX-2, IL-1β, TNF-α, IL-6, PGE-2, iNOS, and NO level [15-19].

Measurement of IL-1β, TNF-α, COX-2, IL-6, PGE-2, and iNOS level

ElabScience Elisa Kit was used to measure the IL-1β, TNF-α, COX-2, IL-6, PGE-2, iNOS level used under manufacture protocols [15-22].

Measurement of NO level

The nitrite associated with NO production was measured using NO Colorimetric Assay that performed based on manufacture protocols. The Sodium Nitrite Standard curve was determined to measure the nitrite quantity [15-17].

Statistical analysis

SPSS software (IBM SPSS 22) were used to statistically analysed the data. The statistical significance among treatments was evaluated using One-way Analysis of Variance (ANOVA) followed by Tukey HSD post hoc test with $p<0,05$.

RESULTS AND DISCUSSION

The cytotoxic effect of TE toward macrophage cells were done to determine the safe extract concentration. TE with 100 to 25 µg/ml, caused a significant reduction of cells viability, reaching only 30.27 % live cells. The lower concentrations of TE (2.5; 5; 7.5 µg/ml) was showed no cytotoxic effect on macrophage cells (viability 117.31-131.08 %) (fig. 1).

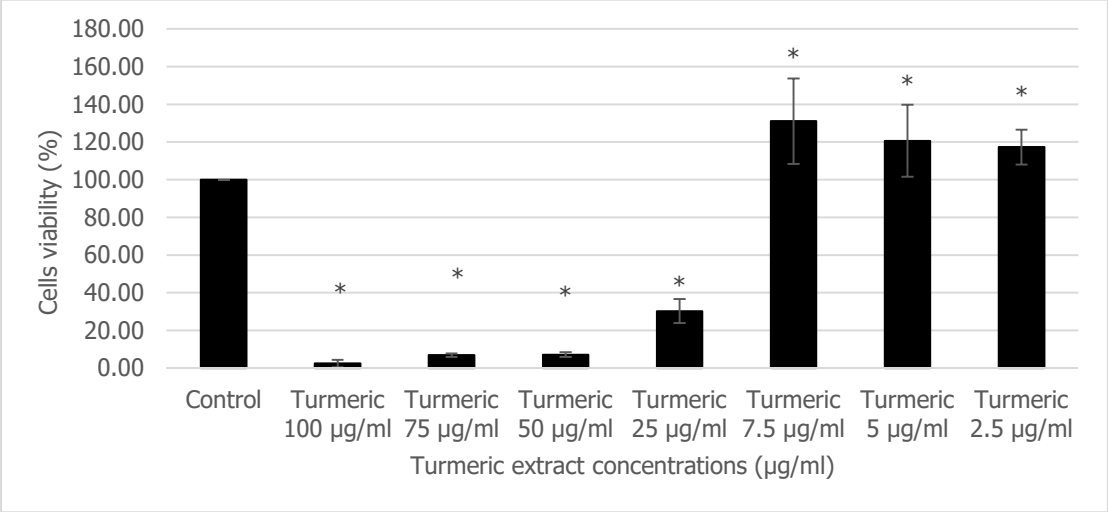
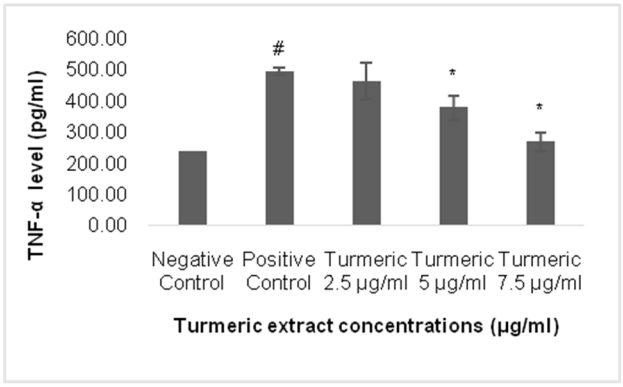


Fig. 1: Effect of turmeric extract on RAW 264.7 cells viability,
The data was presented as mean+standard deviation that has been analysed by using Tukey HSD post hoc test at $p<0.05$. Single asterisk (*) shows significant differences compared to control.

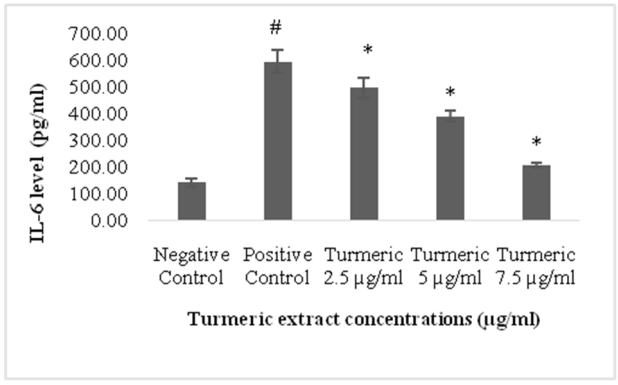
LPS induction caused increase in inflammatory cytokines such as TNF-α (fig. 2a), IL-6 (fig. 2b), IL1-β (fig. 2c), PGE-2 (fig. 2d), COX-2 (fig. 2e) as well as iNOS (fig. 2f) and NO (fig. 2g). TE reduced the inflammatory cytokines in dose-dependent manner. The main mechanism of TE action is by curcumin content which has been shown in various inflammatory models such as macrophage [24, 25, 32], microglial cells [26, 28, 29, 31] induced by LPS, and macrophage cells induced by adipocyte conditioned medium from obesity patients [27, 30].

LPS induces macrophage activation through extracellular region of Toll-Like receptor 4 (TLR4) [33]. The activated TLR4 will activate cascades events in the cytoplasm which leads to MAPK (JNK, ERK1/2, p38) pathway activation. The phosphorylated MAPKs causes phosphorylation of inhibitory factor kappa B (IkB) which leads to nuclear factor-kappa B (NFkB) activation, followed by its translocation to the nucleus. NFκB is a ubiquitous transcription factor that controls genes expression involved in apoptosis, immune responses, and cell cycle [34].

Exposure LPS to cells leads the dimerization of NFκB which leads to inflammatory related genes activation such as IL-1β, TNF-α, PGE-2, IL-6, iNOS, also COX-2 [35, 36]. TNF-α and IL-1β is the innate immune response mediator which leads to other inflammatory cytokines secretion. TNF-α induces leukocytes adhesion molecules expression, leading to diapedesis through vascular endothelial cells. IL-6 mainly acts in acute response and activation of lymphocytes' antibody secretion [23, 35, 36] JNK signaling cascade regulates LPS-stimulated iNOS expression [35]. Meanwhile iNOS is an enzyme that produces NO [5]. NO is synthesized and released into the endothelial cells and mediates vasodilatation which helps leukocytes migration to the site of infection [5].



(a)



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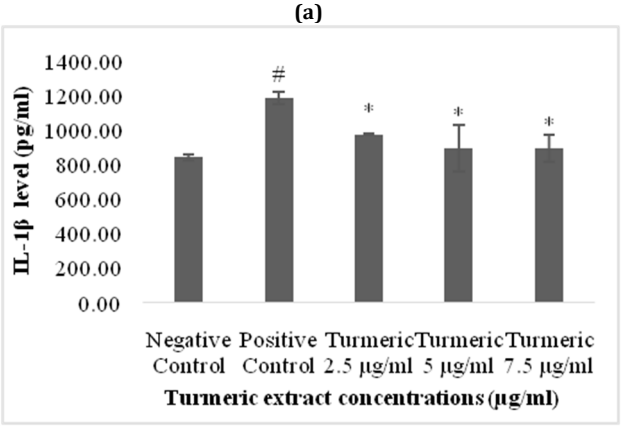

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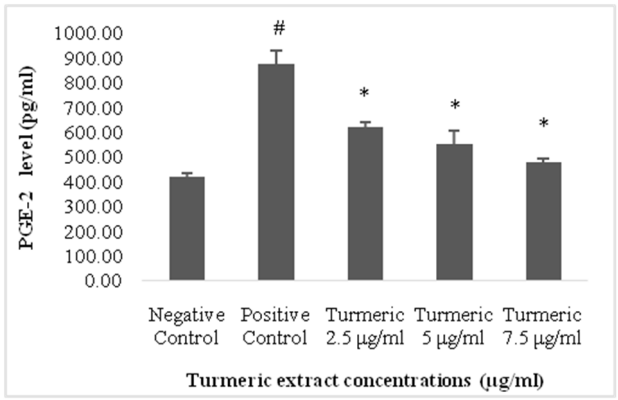

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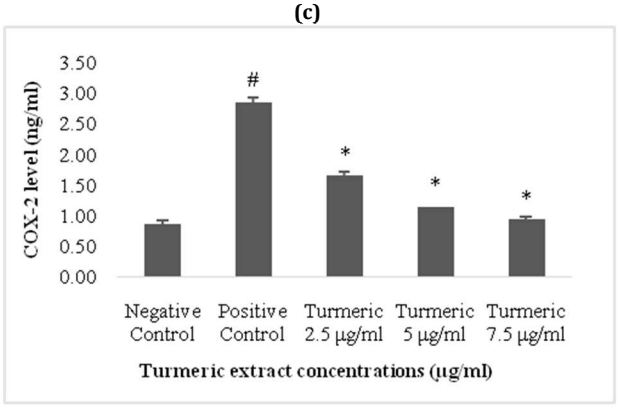

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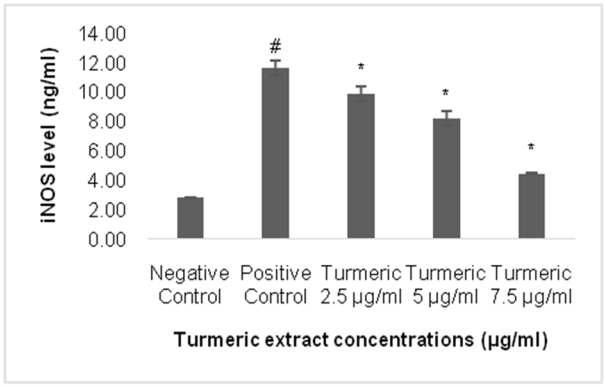
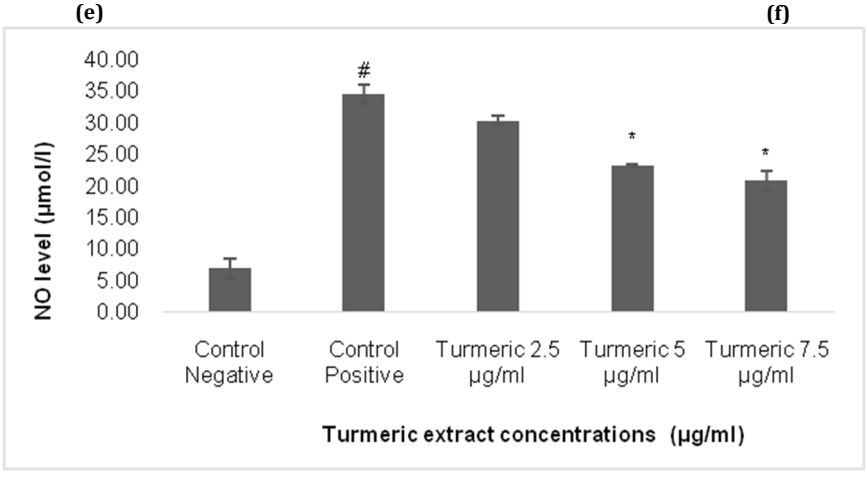


(f)



(g)

Fig. 2: Turmeric extract effect in LPS-stimulated macrophage cells toward inflammatory marker, data is presented as mean+standard deviation that has been analysed using Tukey HSD post hoc test ($p<0.05$). Single asterisk symbol (*) shows significant differences among treatment compared to positive control and hashtag symbol (#) shows significant differences between positive control and negative control. All groups were conducted in triplicate. (a) TNFα level, (b) IL-6 level, (c) IL-1β level, (d) PGE-2 level, (c) COX-2 level, (f) iNOS level, (g) NO level

Turmeric extract mainly acts through curcumin which has been widely studied and has been known to have anti-inflammatory properties through several molecules inhibition. Curcumin causes inhibition of inflammatory-related kinases such as JNK, ERK, MAPK, transcription factors such as NFκB, enzymes such as iNOS, as well as the inflammatory cytokines such as IL-6, TNF-α, and IL-1 [37]. Our results suggest that TE could inhibit the LPS-induced inflammatory marker including NO, PGE-2, TNF-α, IL-1β, COX-2, IL1-β, IL-6, iNOS level. We proposed the mechanism of action anti-inflammatory property of TE based on our result and literature review in fig. 3.

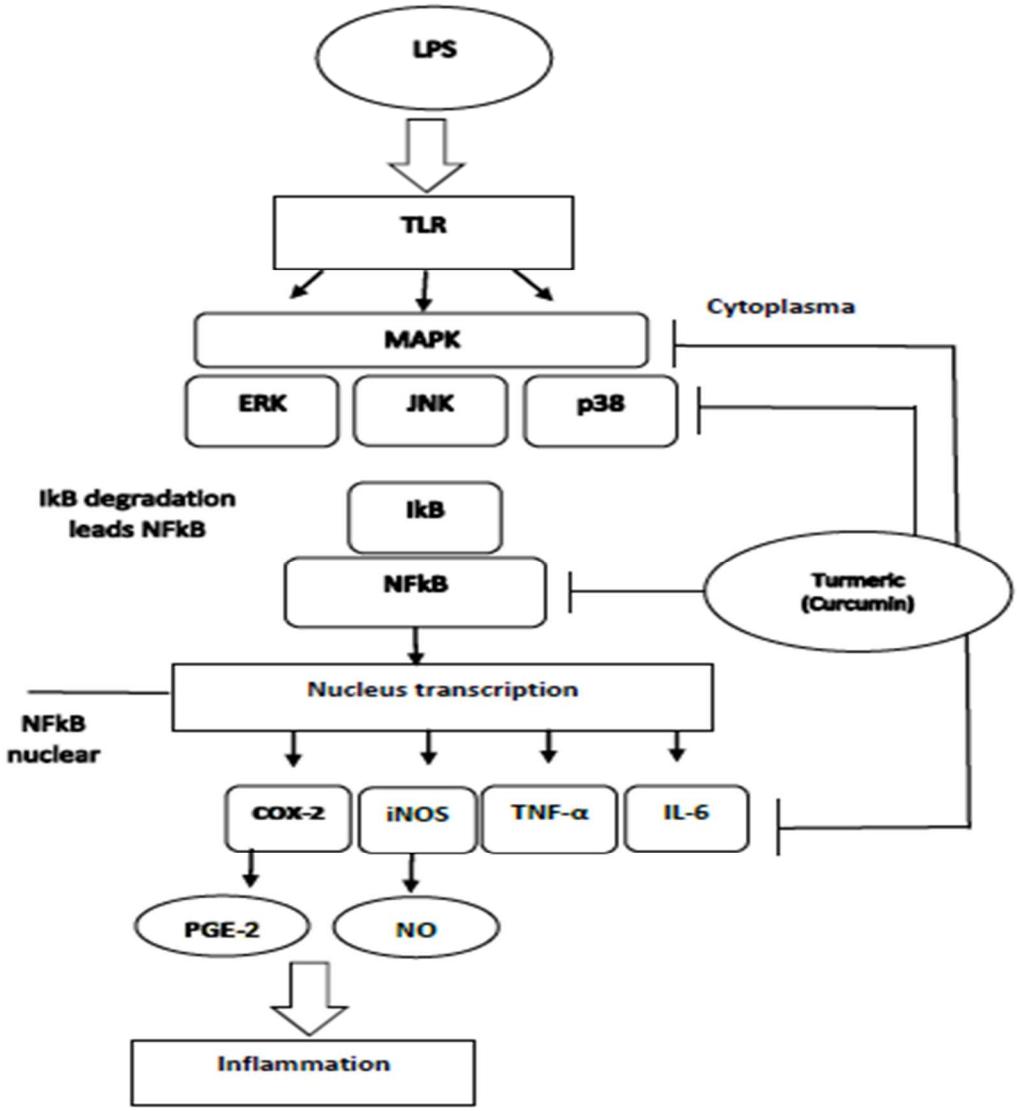


Fig. 3: Proposed mechanism of LPS-induced inflammation inhibition by turmeric extract

CONCLUSION

Turmeric extract has a potential as anti-inflammatory by decreasing TNF-α, IL-6, PGE-2, COX-2, IL-1β, NO and iNOS. While this research could be used as a reference for anti-inflammatory activity of turmeric *in vitro* research, further research by *in vivo* and clinical trial is needed for confirmation of the result.

ACKNOWLEDGEMENT

The authors gratefully acknowledge to Biomolecular and Biomedical Research Center, Aretha Medika Utama, Bandung, West Java, Indonesia for financial support, laboratory facilities and research method. We also thank to Aditya Rinaldy, Muhamad Aldi Maulana from Aretha Medika Utama Biomolecular and Biomedical Research Center for their valuable assistance.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

There are no conflicts of interest.

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