The Activity of Virgin Coconut Oil to Increase Proliferation and COX-2 Expression towards NIH 3T3 Cell Line

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Keywords: VCO, Acid Value, %FFA, NIH 3T3, Proliferation Cell, Percent of Wound Closed, Expression of COX-2.

Abstract: This research aims to investigate the effect of VCO (Virgin Coconut Oil) to increase the activity of NIH 3T3 cell proliferation and COX-2 expression. The sample used were VCO, and acid value was determined. Proliferation was appraised using the MTT method. Furthermore, wound healing activity assays were established with a microscopic system, and expression of COX-2 was determined using RT-PCR. Acid value and % FFA of VCO are 1.07±0.01 and 0.51±0.02. VCO 62.5µg/mL with viability cells were 107.758±0.45%; 104.45±0.48% and 104.45±0.48% after 24h, 48h, and 72 h incubation, cell migration in the wound healing assay after 24 h and 48 h incubation (49.11±0.09% and 74.82±0.22%), and increase in expression of COX-2 (Control=1; and VCO=1.21). The results explain that VCO supply potent proliferation output. This study is planned to appraise of wound closure activity of VCO on the scratched monolayer of NIH 3T3 cell line.

1 INTRODUCTION

Wound healing is a complicated process involving many cells consisting of four phases namely the phases of hemostasis, inflammation, proliferation, and remodeling (Stamm, et al., 2016). The hemostasis phase is the beginning of the wound healing process by involving platelets (Rodrigues, et al., 2016). During the inflammatory phase, fibroblasts function as cytokine secretions, and growth factors to activate the body's defense system (Ridiandries, et al., 2018). During the proliferation and remodeling phases, fibroblasts are important for granulating and reorganizing tissues of the extracellular matrix (Ariffin and Hasham, 2016).

Wound healing is associated with bacterial contamination in the wound area (Ariffin and Hasham, 2016). The ultimate aim of wound healing is to restore the functional properties of the leather and prevent infection (Ariffin and Hasham, 2016).

The COX enzyme consists of 2 isoenzymes such as COX-1, COX-2 and COX-3 (COX-1 variants) (Chandrasekharan, et al., 2002). COX-2 plays a role in the process of angiogenesis The expression of COX-2 affects the process of migration, angiogenesis, and proliferation of fibroblasts (Futagami, et al., 2002).The process of angiogenesis, proliferation, and migration of fibroblasts is very important in wound healing. The expression of COX-2 affects the process of proliferation, angiogenesis, and migration of fibroblasts (Futagami, et al., 2002).

This research aims to evaluate the effect of VCO (Virgin Coconut Oil) in increasing the activity of cell proliferation, COX-2 expression, and wound healing migration NIH 3T3 cells.

2 MATERIALS AND METHODS

2.1 Materials

VCO (Palem Mustika®, Indonesia) and all chemicals and reagents that were used in this study were of analytical grade. NIH 3T3 cells were gotten from Institute of Pharmacy, Gajah Mada University. NIH 3T3 cells were modified in Dulbecco's modified Eagle's medium added with 10% Fetal bovine serum and saved 37^0C with a CO^2 provide of 5%.

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2.2 Methods

2.2.1 Acid Value Determination

Five (5) g VCO was weighed and procedure titration carried out, then the acid value and free fatty acid (FFA) percentage of HVCO was calculated as previously described (Margata, et al., 2018; Silalahi, et al., 2016).

2.2.2 Proliferative Activity

HVCO (1000 µg/mL to 15.625 µg/mL in *co-solvent* DMSO (Sigma) was submitted for proliferative test. In that way, NIH3T3 cell line (58.5 x10^4cells/mL) was grown in DMEM complete medium. After 24; 48 and 72 h treatment, MTT assay was performed and cell viability was counted to determine the proliferative activity (Harahap, et al., 2018).

2.2.3 Wound Healing Migration Assay

The migration assay was carried out with NIH3T3 cells were seeded at 5x104cells/well in 24-well plates and saved for 24 h at 37ºC. Cultured cells were washed up with PBS and added culture media which containing 0.5% FBS and saved for 24 h. Scratch was done in the bottom center of the well within cell layer using yellow tip. Cell residues in the plate were washed up with PBS and treated with HVCO and incubated for 48 h at 37ºC and documented under the inverted microscope against cell migration rapidity after 0, 24, and 48 h. The space from scratch treatment between control and treatment cultur cell was measured using Image J software and defined as cell migration area (Freiesleben, et al., 2017; Harahap, et al., 2018).

2.2.4 Expression of COX-2

NIH3T3 cells (5x10^4 cells/well) were planted into 6-well plate and saved for 24 h and RNA extraction followed Harahap, et al., 2018. The supernatant was divided and used for RNA extraction (Genaid, USA) and RNA concentration was determined by spectrophotometric method (Nanodrop) and stored at -80ºC until used. Complementary DNA (cDNA) was synthesized from 3.0 µg total RNA using RT-PCR kit (Toyobo, Japan) to make final volume of 20 µL using random primers based on the manufacturer's instructions. RT-PCR was carried out in AB 7500 Fast (ABI, USA). The reaction mixture consisted of GoTaq Green (12.5 µL) (Promega), 1.0 µL of cDNA 1 µL forward primers, 1 µL reverse primers, and 9.5 µL ddH_2O to make a total volume of 25 µL. β-actin was used as internal reference control. The PCR primers were used for β-actin (F: 5'-gtc gta cca ctg gca ttg t-3'; R: 5'-cag ctg tgg tga agc t-3'), Cox-2 (F: 5'-cca gca ctt cac gca tca gt-3'; R: 5'-acg ctg tct agc cag agt ttc ag-3'). The PCR condition were comprised of first incubation at 95ºC for 2 minutes, 95ºC for 30 sec, annealing at 55ºC 30 sec, extension at 72^0 for 1 minute, and 35 cycles. The PCR products were detected by electrophoresis in 2% agarose gels, and added gel red 10 µL. Then, they were visualized with gel doc (Li, et al., 2017; Harahap, et al., 2018).

2.2.5 Statistic Analysis

The results were served as means ± SD. The statistical analysis was carried out by using SPSS edition 21.

3 RESULT

3.1 Acid Value and %FFA of VCO

Acid value is 1.07±0.01 mg NaOH/g oil and free fatty acids (FFA) is 0.51±0.02%.

3.2 Proliferative Activity

To appraise the effect of VCO to increase the quantity of cells by stimulating cell proliferation. The percentage of viable cells after treatment and incubation for 24h, 48h, and 72h (107.76±0.45; 107.94±0.45; 104.45±0.48) showed the stimulation effect of VCO towards proliferation of NIH3T3 cells. The effect of VCO is given in Figure 1.

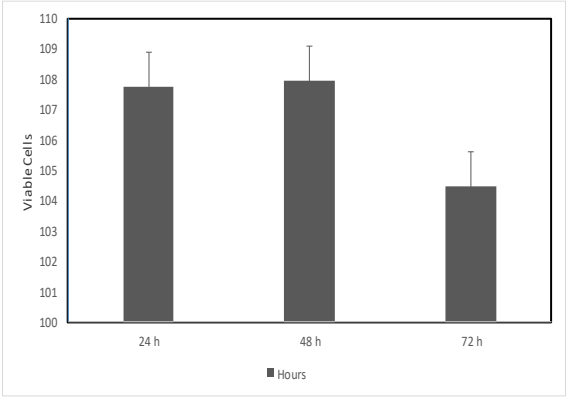

Figure 1: Percentage of Viable Cells of NIH3T3 Cells were Treated by VCO 62.5 µg/mL for 24; 48 and 72 h and Measured Viable Cells.

3.3 Wound Healing Migration

The scratch wound healing assay was done to evaluate the influence of VCO on NIH3T3 migration. The wound healing migration of VCO is given in Figure 2. A little wound repair was observed in wells with VCO at 62.5 µg/mL after 24 and 48h incubation with 49.11 ± 0.09% and 74.82 ± 0.22% respectively closure area.

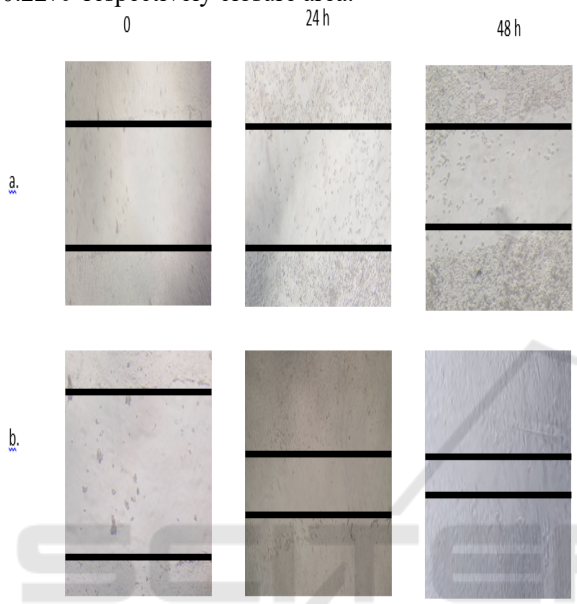

Figure 2: Wound Healing Migration Assay. NIH3T3 cells were treated by VCO for 0; 24 and 48h and measured the closure area. (a) control cells; (b) VCO 62.5 µg/mL.

3.4 COX-2 Expression

Two steps RT-PCR were used to evaluate COX-2 expression in NIH3T3 cells after the treatment with VCO. VCO showed a significant up-regulatory effect on the expression of COX-2. The COX-2 expression is given in Figure 3.

4 DISCUSSION

Acid value is defined as mg NaOH used to neutralize FFA contained in 1 g of fats or oils to indicate the amount of FFA in one gram fats or oils (Silalahi, et al., 2016).

Lauric acid is antibacterial and anti-inflammatory agent that able to overcome skin problems (Ariffin and Hasham, 2016). Lauric acid can decrease the time for complete epithelialization, because lauric acid can increase proliferation cells and migration cells (Ariffin and Hasham, 2016).

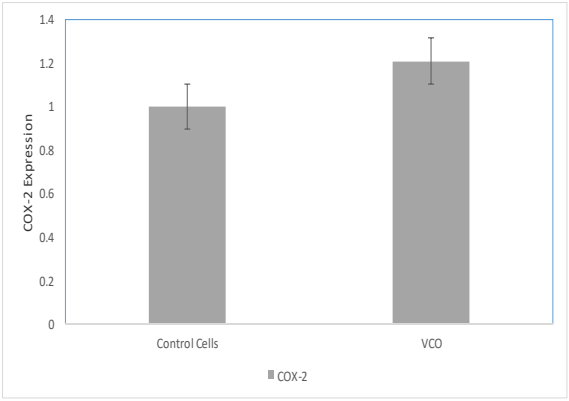

Figure 3: COX-2 Expression (a) control cells; (b)VCO 62.5 µg/mL.

Thourghout wound healing process, cells at the wound side migrate, and proliferate, caused to re-epithelialization of the wound side. Migration of NIH 3T3 fibroblasts was evaluate with wound healing assay. Lauric acid can increase proliferation cells and migration cells (Ariffin and Hasham, 2016). Cell migration activity in the VCO group is faster than control group. Lauric acid can increase proliferation cells and migration cells. Lauric acid is found in VCO that stimulate cells to migrate. So the percentage of VCO group cell migration is faster than control group.

Cyclooxygenase-2 (COX-2) is an inducible enzyme which plays a critical role in multiple pathophysiological processes including atherosclerosis, inflammation, tumorigenesis, tissue injury, and angiogenesis (Futagami, et al., 2002). COX-2 protein and mRNA were expressed primarily in the head and basal layers of the epidermal wound edges, which are arranged of proliferative and migratory cells (Futagami, et al., 2002). In the Ebeling, et al study, COX-2 is one of the wound healing parameters. Pentacyclic triterpene and botulin are active compounds of birch bark extract. They influence the inflammatory phase of wound healing by upregulating chemokines, proinflammatory cytokines, and cyclooxygenase-2 (COX-2) in human primary keratinocytes. COX-2 and IL-6 that their mRNA enlargement is due to a mRNA stabilizing effect, a process in which p38 MAPK and HuR (human antigen R) are primarily involved (Ebeling, et al., 2014). In this study, Lauric acid increase of expression COX-2 which mediated angiogenesis and migration NIH 3T3 cell Two steps RT-PCR were used to evaluated COX-2 expression in NIH3T3 cells after the treatment with VCO.

5 CONCLUSION

VCO can increase proliferation cells, COX-2 expression, and accelerate wound closure. So that VCO has wound healing activities.

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