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Virgin coconut oil compared to corn oil in world health organization formula on glutathione, TNF-α, and body weight gain in severe malnourished Wistar rat

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ABSTRACT

Background: Severe malnutrition remains a major killer of children under five years old. On such condition, there is a lack of antioxidants, an increase in free radical agents and pro-inflammatory cytokine. They prolong a hyper catabolic state, hindering an increase in weight. Virgin coconut oil (VCO) contains medium chain triglyceride and antioxidants. It is expected to solve that problem compared to corn oil. There has been no published study about VCO usage for severe malnutrition.

Objective: The aim of this study was to find out the influence of VCO compared to corn oil for severe malnutrition treatment in GSH level, TNF-α level, and weight gain.

Methods: It was a laboratory experiment study, using a randomized post-test control group design, to compare the treatment effect of the VCO vs corn oil in Formula 75 and Formula 100 in the treatment of severe malnourished rat. Severe malnourished rats were divided randomly into 2 groups. One group was given F75 and F100 with VCO content (Group A) and another group was given F75 and F100 with corn oil content (group B). Both groups were treated for 28 days.

Results: There were 19 rats in each group. One rat from group B died before the study ended. An analysis was done for the last 37 rats. There was no significant difference in the mean of weight gain, 73.45 g (SD 20.08) and 68.97 g (SD 11.49) for group A and B respectively (p=0.41). There was no significant difference in the GSH levels (p=0.70), where group A is 1.35 pg/g (SD (0.74) and group B 1.27 pg/g (SD 0.48). TNF-α level in group A (1087.2 pg/g (320.00-2525.46)) was higher than group B (711.32 pg/g (403.19-2400.91)) but not statistically significant (p=0.08).

Conclusion: There is no significant difference in GSH and TNF-α level in the liver and body weight gain for VCO and corn oil groups. Virgin coconut oil can be one of the alternative vegetable oils that can be utilized in treating severe malnourishment.

Keywords: severe malnutrition, VCO, GSH, TNF-α

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INTRODUCTION

Severe malnutrition is the most cause of death in children under five years old. About 20 million children all around the world suffer from severe malnutrition. Most of them are from South Asia and SubSahara Africa. The children with severe malnutrition mortality rate is 5-20 times higher than the well-nourished. About one million children per year died because of severe malnutrition.[1]

The antioxidants concentration (plasma glutathione and vitamin E) decreases in kwashiorkor.[2] This condition is the result, not the etiology of kwashiorkor.[3] Liver total glutathione (GSH) in severe malnourished rat decrease 65% compared to well-nourished rat.[4] This condition is similar to inflammation state. Liver GSH level has an inverse relationship with liver NF-KB activation level, increase IL-1β and TNF-α transcription.[5] Glutathione depletion could increase liver lipid peroxidation and damage the liver capacity to inactivate reactive oxygen species (ROS) which is the main stimulator of cytokine production.[5,6] GSH depletion could be a result of cytokine activation. Therefore, it is difficult to differentiate the primary from the secondary change of GSH.[4]

A prolonged production of Tumor necrosis factor-α (TNF-α) can cause muscle and fat wasting (cachexia). TNF-α induces appetite depression and depressed lipoprotein lipase synthesis, an enzyme for releasing fatty liver from lipoprotein circulation.[7] If this hyper catabolic state prolongs, it will have a detrimental effect.[8] Increasing body weight will be difficult.

Nutrition treatment has a significant role in severe malnutrition. The WHO Formula 75 (F75) and Formula 100 (F100) are used for treating severe malnutrition. One component of F75 and F100 is vegetable oil. In this study, we used virgin coconut oil (VCO) to be compared with corn oil.

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In Indonesia, many coconut trees are available, especially in rural area. VCO can easily be made at home from a coconut. Therefore, it is more accessible for the Indonesian. VCO contains medium chain triglyceride (MCT) and polyphenolic acid. It was postulated of having its role to solve depressing antioxidant level and lipid malabsorption's in severe malnutrition and modify immunity respond in this condition. The aim of this study is to observe whether VCO can better repair immune system and achieve body weight gain compared to corn oil in WHO Formula.

METHODS

An experimental study using a control group and a post-test only was designed to observe whether VCO could increase liver GSH, decrease liver TNF-α, and increase body weight of male Wistar rat with severe malnutrition. This study was approved by the Research Ethical Committee of Udayana University Faculty of Medicine in conjunction with the teaching hospital, Sanglah General Hospital, Denpasar, Bali, Indonesia. As many as 38 rats were employed in this study. We used four-week-old rats. They were adapted for one week, then treated becoming severe malnutrition by feeding a low protein diet (5% protein) *ad libitum* for three weeks. The low protein diet consisted of 61.5% *β cornstarch*, 5% *mild casein*, 10% *α potato starch*, 8% *cellulose powder*, 6% *soybean oil*, 3.5% mineral, 5% sugar, 1 % multivitamin. After they became severe malnourished they were divided into two groups randomly. The first group was treated with F75 and F100 (contain VCO), and the second group treated with F75 and F100 (contain corn oil) for 28 days. The composition of F75 and F100 are listed in Table 1. Formula 75 was given 416.7 kilocalories per kg of bodyweight per day (kcal/kgBW/d) on the first day. They were fed F100 with doses 416.7 kcal/kgBW/d on the second day. In the following days, F100 dose was increased gradually every day as many as 41.7 kcal/kgBW/d until 916.7 kcal/kgBW/d was reached. After the dose achieved 916.7 kcal/kgBW/d, it was continued until 28 days from the first day of F75 treatment. The body weight was measured every week.

In the first five days of treatment, all rats were given a prophylaxis antibiotic (cefixime 33.3 mg/kgBW/d, divided in two). In the 29th day of treatment, the rats were neutralized using ketamine hydrochloride 40 mg/kg and xylazine 2.5 mg/kg per intravenous. The livers were taken and washed using phosphate buffer saline (PBS). The livers were stored in a -80^{o}C refrigerator until were examined for GSH and TNF-α liver. GSH kit used for this study was Total Glutathione (tGSH) Microplate Assay from Oxford Biomedical Research. TNF-α kit used for this study was TNF-alpha Platinum Elisa eBioscience Cat. No. BMS622 to measure TNF-α quantitatively. The body weight was measured using a digital scale ALK-231 (capacity of 500 g, accuracy 0.1 g).

T-test or Mann-Whitney-U-test was performed to analyze the difference between the two treatment with α = 0.05.

Table 1 F75 and F100 Compositions

Ingredients	F75	F100
Skim milk powder (g)	25	85
Sugar (g)	100	50
Vegetable oil (VCO or corn oil) (g)	30	60
Eletrolyt solution (ml)	20	20
calori (kcal)	750	1000

Table 2 Sample Characteristics

Characteristic	Group A (VCO) in gram n=19	Group B (corn oil) in gram n=18	p
Body weight before severe malnutrition mean (SD)	74.79 (8.78)	74.63 (8.40)	0.953
Body weight when severely malnutrished median (min-max)	58.3 (50.00-84.20)	59.90 (45.90-83.00)	0.903

RESULTS

After 3 weeks of low protein diet, the rats became severe malnourished based on their age. The rats became thin, with muscle atrophy, sparse hair, and edema similar to kwashiorkor in a human being. In the beginning of our study, the characteristics of the sample were similar. One rat died before the study had ended. The remaining 37 rats were analyzed (Table 2).

The mean of body weight gain was 73.45 g (SD 20.08) in group A, and 68.97 g (SD 10.43) in group B (p=0.414). The mean of daily body weight gain was 2.62 g (SD 0.72) in group A and 2.46 g (SD 0.41) in group B. The mean difference in the daily body weight gain was also not statistically significant (p=0.48).

After 28 days of VCO/corn oil treatment, the GSH and TNF-α in group A were higher than group B, but the differences were not statistically significant (Table 3).

DISCUSSION

Nutrition treatment in severe malnutrition has an important role. It can control the oxidative stress in such condition where lack of antioxidant occurs. A study which compared the effect of VCO to copra oil, olive oil, and sunflower oil

Table 3 VCO and Corn Oil Effect on GSH, TNF-α, and Body Weight Gain

Variables	Group A (VCO) n=19	Group B (Corn Oil) n=18	p
GSH level (pg/g) mean (SD)	1.35 (0.74)	1.27 (0.48)	0.703
TNF-α level (pg/g) median (range)	1087.2 (320.00-2525.46)	711.32 (403.19-2400.91)	0.083
Body weight gain (g) mean (SD)	73.45 (20.08)	68.97 (11.49)	0.414
Body weight gain perday (g) mean (SD)	2.62 (0.72)	2.46 (0.41)	0.416

on endogenous antioxidant and paraoxonase-1 activity against oxidative stress in rats, found that VCO antioxidant repair status was best among them (statistically significant). VCO provides the highest increase in catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase activity in rat liver, heart, and kidney. In addition, when compared to others, VCO prevents oxidative stress better by decreasing lipid peroxidation formation and oxidative protein product like MDA, hydroperoxide, conjugated dienes, and protein carbonyl in serum and tissue. A wet processing of VCO maintains a decent amount of unsaponifiable active biologic component like polyphenol (84 mg per 100 g oil) and tocopherol (33,12 µg per 100 g oil).[9]

There was a study using corn oil in pig that examine the effect of oxidized corn oil with or without antioxidant addition (vitamin E). This study determined the tissue oxidative status and the quality of meat product. The activity of glutathione peroxidase in the liver was higher in fresh corn oil group, although it was not significant between the group fed on oxidized corn oil with and without added antioxidant, and the group using fresh corn oil with and without added antioxidant. The glutathione peroxidase activity in serum was highest in the group given fresh corn oil added antioxidant. Oxidized corn oil impaired growth in pig and created oxidative stress. By adding antioxidant, the negative effect of oxidized corn oil can be reduced by reducing protein oxidation.[10]

In our study, the GSH liver level was higher in VCO group than corn oil group. But, this finding is not significant (p=0.327). Corn oil used in this study is a commercially packed corn oil added with antioxidant (vitamin E 23% recommended daily allowance for diet 2000 kcal) and not oxidized (not being heated before use). VCO used for this study came from a wet processing which maintained the amount of unsaponifiable active biologic components like polyphenol and tocopherol. The endogenous antioxidant level in VCO group was higher than in corn oil group, although the difference was not significant.

There are immune parameter changes in severe malnutrition, an increase in Th-2 response (high IL-4 and IL-10 level, low IL-2, IL-12, and IFN-γ). In the other hand, the increase of IL-6 and TNF-α level could be related to infection. Mechanism of this changes has not been cleared yet. If it is caused by lack of nutrient, a question may arise as for why it do not influence all cytokines. Infection could have influence this changes. But, malnutrition itself is independent of immune function changes. An intracellular receptor, mammalian target of Rapamycin (mTOR) exists almost in all type cell. This receptor responses to nutrient concentrate around the cell and the cell metabolism adapts to the available nutrients. Immune cell used mTOR to regulate an activation. The available nutrients could determine whether or not the immune cell should be activated, and whether the T cell is to be differentiated to proinflammatory phenotype or to develop a tolerance. Nutrient function in microenvironment is developed from building material, and it is a becoming signal transducer molecule. This mechanism could be involved in this anomaly of immunology parameter in malnutrition.[11]

TNF-α is a pleiotropic cytokine. It induces cellular responses such as proliferation, inflammation mediator production, and cell death. TNF-α has an important role in septic shock and wasting syndrome. In the liver, TNF-α is involved in the pathophysiology of viral hepatitis, alcoholic and nonalcoholic fatty liver disease, and ischemia-reperfusion injury. TNF-α has dichotomy role in liver tissue, as cell death mediator and in inducing hepatocyte proliferation and liver regeneration.[12] In rat liver, TNF-α induces both of that responses depends on a different physiologic condition. It stimulates hepatocyte proliferation after hepatotectomy and induces cell death in hepatotoxic impairment. In normal condition, hepatocyte resists to TNF-α toxicity. It depends on TNF-α signaling ability to regulate cell protective gene. Activation of transcription factor NF-KB cellular is important in inducing cell resistant to TNF-α toxicity.[13]

In this study, TNF-α level in liver was higher in VCO group than in corn oil group, but not statistically significant. It may be because of the insignificance of GSH level between these groups. Or, it could be because both of the groups were in the same stage of treatment, the rehabilitation stage. In

rehabilitation stage, there is a repairing mechanism, where TNF-α has a role. Because TNF-α in the liver acts as cell death mediator. In contrast, it can also induce hepatocyte proliferation and regeneration depends on different physiologic condition.[12] In bone fracture, TNF-α expression has a biphasic pattern, the first peak occurs at the beginning of fracture repairing, and the second peak occurs at chondrogenesis transition to osteogenesis during the endochondrial maturation. TNF-α promotes migration of muscle-derived stromal cells (MDSC) and osteogenic differentiation at a low dose. Adding 1 ng/mL TNF-α at fracture area can faster the healing process.[14] In the healing phase of severe malnutrition, tt could be having the same response.

Many factors can influence body weight gain in severe malnutrition. The quantity, the type, and the quality of the diet, or infection can influence the weight gain. [15,16] Those factors will impact on the immunological condition in severe malnutrition. One of those is TNF-α because TNF-α depends on the antioxidant availability (GSH). In this study, the body weight gain difference was not statistically significant. It could be because the GSH and TNF-α difference were also not significant.

CONCLUSION

There was no difference between VCO and corn oil group in GSH, TNF-α level in the liver, and body weight gains. But, since coconut trees are plenty in Indonesia, and VCO is easy to make at home, VCO can be one of the alternative vegetables oil that can be used for a severely malnourished child.

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