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Effect of Virgin Coconut Oil Compared to Corn Oil in World Health Organization Formula on Malondialdehyde Expression in Intestine of Severe Acute Malnutrition Wistar Rats



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ABSTRACT

Background: In severe acute malnutrition, oxidative stress is occurred and it cause dysfunction of various tissue, including intestine. Intestinal function is impaired and altered epithelial transport. It may be related with intestinal oxidative stress. In other hands, virgin coconut oil (VCO) contain a lot of antioxidant properties which is can be solved that problem.

Objective: This study aimed to investigate the oxidative stress in intestine after given virgin coconut oil compared to corn oil in rehabilitation stage of severe acute malnutrition treatment of wistar rats.

Methods: A post-test only control group design experimental study was done to investigate effect of VCO compare to corn oil on malondialdehyde (MDA) expression in intestine in male wistar rats with severe acute malnutrition. Thirty eight severe acute malnutrition

male wistar rats were used in this study, which was divided by two groups. Group A was feeding by WHO formula (F75 and F100) that contain VCO, group B was feeding by WHO Formula that contain corn oil. This formula was taken for 28 days and intestinal tissue of the wistar rats will be analyzed.

Results: At the end of this study, one rat was died (Group B), and 37 rats were analyzed for MDA expression in intestine. Mean of MDA expression in Group A was 1.45% (SD = 0.70 %) and Group B was 1.51% (SD = 0.54%). There was no mean differences for MDA expression between Group A and Group B (p for T-test was 0.71, mean difference -0.06 (95% CI = -0.48-0.36).

Conclusion: In rehabilitation phase of treatment of severe acute malnutrition wistar rats, there is no differences in MDA expression in intestinal epithelial between VCO and corn oil.

Keywords: Severe acute malnutrition, intestine MDA expression, virgin coconut oil

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INTRODUCTION

Twenty million preschool-age children suffer from severe acute malnutrition, mostly from African and South-East Asia with significant mortality rate.¹ In severe acute malnutrition, oxidative stress occurred and it cause dysfunction of various tissue, including intestine.² Intestinal function is impaired and it shall alter epithelial transport. This process was related to intestinal oxidative stress.³

World Health Organization (WHO) recommendation for dietary management in severe acute malnutrition is diet with combination of dried skimmed milk, sugar, vegetables oil, and mineral mix in adequate proportion with supplemented multivitamin,¹ without special suggestion of specific vegetables oil nature.

In the application of WHO formula, corn oil is often used because of common society sense that corn oil is one of healthy oil. This oil is rich of polyunsaturated fatty acid, omega 6, precursor of pro-inflammatory mediators which are could induce the increasing of oxidative stress.⁴ While,

severe malnutrition state is an oxidative stress condition. It suggests us to searching for another alternative vegetables oil being used.

Virgin coconut oil (VCO) is oil produced from fresh meat coconut, milk coconut residue, rich in medium chain fatty acids, polyphenols, and it has antioxidant property.⁵ It is suggested that VCO with antioxidant property can solve the problem of intestinal oxidative stress in severe acute malnutrition and become specific oil to completed the WHO's formulas. Thus, this study aimed to investigate the oxidative stress state in intestinal epithelial after given VCO compared to corn oil in rehabilitation stage of severe acute malnutrition treatment of wistar rats.

METHODS

This research was post-test only control group design experimental study to investigate the effect of VCO compare to corn oil on malondialdehyde (MDA) expression in intestinal epithelial of male wistar rats with severe acute malnutrition. It had approved by Medical Faculty of Udayana

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There were 38 four-week old male wistar rats used in this study. After one week being adapted, they were treated to be severe acute malnutrition by feeding low protein diet (5% protein) *ad libitum* for three weeks. Low protein diet to be given was consisted of 61.5% β cornstarch, 5% sugar, 10% α potato starch, 8% cellulose powder, 5% mild casein, 6% soybean oil, 3.5% mineral, and 1 % multivitamin. After becoming severe acute malnutrition, they were divided randomly by two groups, Group A and Group B. Group A were treated with F75 and F100 (contain VCO), and Group B treated with F75 and F100 (contain corn oil) for 28 days. Composition of F75 and F100 are listed in Table 1. Formula 75 was given 416.7kcal/kg bodyweight/day (kcal/kgbw/d) in the first day. In the second day of treatment, they were feeding by F100 with doses 416.7kcal/kgbw/d, and in the following days, F100 dose were increased by 41.7 kcal/kgbw/d up to 916.7 kcal/kgbw/d. After that continued this dose until fulfill 28 days of treatment.

Prophylaxis antibiotic (cefixime 33.3 mg/kgbw/d, divided by two) was given for 5 days. In the 29th day of treatment, the rats were sacrificed using ketamine hydrochloride 40 mg/kg xylazine 2.5 mg/kg intravenously.⁷

Intestine were taken and washed by using NaCl 0.9% and fixation with buffer formalin. Paraffin-embedded serial sections of intestine were deparaffinized with xylol (2 × 5 minutes), rehydrated with ethanol (100%, 95%, 90%, 80%, 70% respectively for each in 5 minutes), than wash with Phosphate Buffer Saline (PBS) for 3 × 2 minutes in pH 7.4. And then react with citrate buffer for 20 minutes in 95°C. Soak in 3% H₂O₂ for 20 in room temperature. Wash with PBS 3 × 2 minutes. Incubate the cytology slide with antibody Santa Cruz anti MDA antibody 1:100 for 18 hours in 4°C, then wash with PBS pH 7.4 for 3 × 5 minutes. Added secondary antibody with biotin labeled (Anti rabbit Ig G - Biotin Labeled) and incubated for 30 minutes in room temperature. Washed by PBS pH 7.4 for 3 × 5 minutes. StreptAvidin-Horseradish Peroxidase (SA-HRP) was added for 20 minutes in room temperature. Wash with PBS pH 7.4 for 3 × 5 minutes. We

applied DAB (DiamonoBenzidine) for 5 minutes in room temperature, wash with aquabidest for 3 × 5 minutes, then counterstaining with Mayer Hematoxiline for 2 minutes and wash with water flow. Dried by wind flow, then mounting and covered with coverslip. Counted cell that stained by MDA immunohistochemistry (IHC) in 400X magnified microscope with formula:

$$\% \text{ cell MDA stained} = \frac{\text{number of cells that MDA stained}}{\text{number of whole cell}} \times 100\%$$

Normality test was done and if data distribution was normal, T-test was performed to determine whether there was a significant mean difference between Group A and Group B.

RESULTS

Before the end of this study, one rat was died (from Group B). Intestine samples from the last 37 rats were examined for MDA expression (Figure 1).

Test of normality (Shapiro-Wilk) for each of group had $p > 0.05$ (Group A = 0.074; Group B = 0.15). Mean of MDA expression in Group A was 1.45% (SD = 0.70 %) and Group B was 1.51% (SD = 0.54%). There was no mean differences for MDA expression between Group A and Group B (p for T-test was 0.71, mean difference -0.06 (95% CI = -0.48-0.36).

DISCUSSION

Free radical reactions have shown implication in malnutrition. Reactive oxygen intermediates production increase, and lead to oxidative stress. A product of lipid peroxidation (MDA) increase and could influence apoptosis, contribute to pathophysiology of malnutrition.⁸ Intestinal function is impaired in malnutrition.³ Glucose absorption is impaired and it correlate with oxidative stress.⁸ Oxidative stress is a component of gastrointestinal

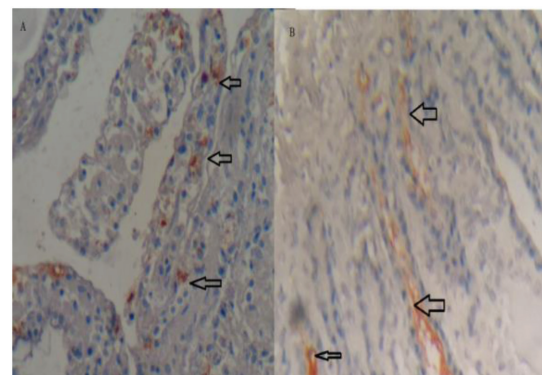


Figure 1 Intestine MDA Immunohistochemistry for Group A and Group B. Lipid peroxidation (MDA) was shown by arrow (brown stained)

Table 1 Composition of F75 and F100⁶

| Substances | F75 | F100 |
|-------------------------------------|-----|------|
| Skim milk powder (g) | 25 | 85 |
| Sugar (g) | 100 | 50 |
| Vegetable oil (VCO or corn oil) (g) | 30 | 60 |
| Elektrolyt solution (ml) | 20 | 20 |
| calorie (kcal) | 750 | 1000 |

injury, and malnutrition may reduce antioxidant defense. Malnutrition induces intestinal free radical damage and changes epithelial transport. Oxidative stress may contribute to the intestinal dysfunction associated with malnutrition.³

Palm oil is as effective as soybean oil in promoting weight gain, but it given better intestinal function reparation than soybean oil. Composition of vegetables oil in the diet change intestinal function and cell cycle by oxidative stress mechanism.² polyunsaturated fatty acids may have an important role in intestinal repair in chronic diarrhea with protein energy malnutrition, especially those in the n-3 series (omega-3). Fish Oil diet improved pathology score of histological and ultra-structural analysis. Chronic diarrhea depletes antioxidant defense in rat intestine, but fish oil and purified phospholipids from pig brain can increase GSH levels in colon and some antioxidant activities vary according to the source of fatty acids.¹⁰ Another family of polyunsaturated fatty acid, omega-6, is precursor of proinflammation⁴ that may lead to increase oxidative stress.

After supplementation of antioxidant for one month, MDA level decreased and erythrocyte superoxide dismutase capacity increase.⁸ This study was post-test only control group design experimental study. We did not have pretest data for intestine MDA expression. After treated with VCO or corn oil for 28 days, MDA expressions in intestine were lower in VCO Group than Corn Oil Group, but it was not statistically significant. Corn oil can stimulate antioxidant by giving oxidative stress, then intestinal tissue will produce antioxidant to against that oxidative stress.^{11,12} In this study, intestines were obtained after rats in final rehabilitation phase when their body already can produce antioxidant if there oxidative stress. This may lead to the results of this study are not statistically significant between both of groups. Further studies are needed to know whether the same result is obtained if we check intestine MDA expression in stabilizing and transition phase of severe malnutrition treatment.

CONCLUSION

As conclusion of this study, there are no differences in intestine MDA Expression after treatment with

VCO or corn oil in WHO formula in rehabilitation phase of severe malnutrition treatment.

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