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JOURNAL HISTORY

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Based on the decision of Asosiasi Departemen Reproduksi Veteriner Indonesia (ADERVI) or the Indonesian Association of Department of Veterinary Reproduction (IADVR) meeting on December 11th, 2019 in Surabaya, the publication of the OVOZOA e-journal starting in 2020 in collaboration with ADERVI. The Memorandum of Agreement between the Dean of the Faculty of Veterinary Medicine, Universitas Airlangga and the Chairperson of ADERVI, Number (FKH unair): 1445/UN3.1.6/KS/2020 and Number (ADERVI): 04/ADERVI/V/2020 signed on May 4th, 2020. Since March 27th, 2021 OVOZOA Journal of Animal Reproduction has also collaborated with the Indonesian Animal Reproduction Association (Asosiasi Reproduksi Hewan Indonesia, ARHI). Starting from Volume 9 – 2020, OVOZOA completes its name as the OVOZOA Journal of Animal Reproduction. It contains the publication of scientific work of lecturers, researchers, and practitioners of ADERVI members in the field of Veterinary Reproduction and Animal Reproduction Biotechnology. Ovozoa Journal of Animal Reproduction is published at <https://e-journal.unair.ac.id/OVZ/index> periodically three times a year: April, August, and December.

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Original article

Virgin coconut oil protected the diameter and the epithelium thickness of seminiferous tubules of mice (*Mus musculus*) from oral ethanol induction**Vishantheni S Ravi^{1*}, Soeharsono Soeharsono², Hani Plumeriastuti³,
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ABSTRACT

This study investigated the preventive effect of virgin coconut oil (VCO) on the epithelium thickness and the diameter of the seminiferous tubules induced by ethanol in mice (*Mus musculus*). This study used 20 male mice as the experimental animal which were divided into five groups with four mice in each group. Negative control (C-) was given 2% Tween and aquadest, while positive control (C+) was given 2% Tween and 33% ethanol. T1, T2, and T3 were respectively given 0.09, 0.19, and 0.37 mL/kg bw VCO and 33% ethanol (0.2 mL/kg bw). VCO was given orally for 39 days, and ethanol was given orally seven days later for 32 days. Ethanol was administered two hours after the VCO administration. Analysis of variance followed by Tukey's range test on the epithelium thickness and the diameter of the seminiferous tubules showed significant differences ($p < 0.05$) of C+ group from the other groups. Whereas, there was no significant difference ($p > 0.05$) was found among C-, T1, T2 and T3 group. The result concluded that VCO could protect the testis of mice from the damage caused by ethanol.

Keywords: alcoholism, male fertility, reproductive health, spermatozoa, testis histology

INTRODUCTION

Liquor is generally devoured in mixed refreshments everywhere throughout the world, and the liquor-related issue is winding up progressively to significant reasons for morbidity and mortality (Ye *et al.*, 2021). The constituents of each alcoholic beverage can be divided into major, minor, or trace components. The major constituents usually consist of ethanol and water. Ethanol is present in alcoholic beverages due to

the fermentation of carbohydrates with yeast (Cacho and Lopez, 2005). Ethanol is a little atom solvent in both water and lipids. It can pervade all body tissues and influence the fundamental organ (Koob *et al.*, 2021). However, excessive alcohol consumption has been proved to cause a risk of male infertility (Van Heertum and Rossi, 2017).

Infertility is the inability of a couple to have a child even after one year of unprotected, frequent sexual intercourse. The male is solely

responsible for about 20% of cases and contributes to another 30% to 40% of all infertility cases (Leslie *et al.*, 2021). It is estimated that infertility affects 8-12% of couples globally, with a male factor being a primary or contributing cause in approximately 50% of couples. Causes of male subfertility are very high but can be related to congenital, acquired, or idiopathic factors that impair spermatogenesis in the seminiferous tubules (Agarwal *et al.*, 2021). Ethanol is responsible for oxidative stress markers in the animal model of prolonged alcohol consumption (Kołota *et al.*, 2020). Thereby, they need the antioxidant for oxidative stress due to alcohol consumption.

VCO is a blend of triglycerides containing just short and medium-chain saturated fatty acids (92%) and unsaturated fatty acids (8%) (Eyres *et al.*, 2016). It comprises many vitamins and minerals, known to be an antioxidant. VCO is an edible oil with numerous applications for humankind and has a remarkable role in food, medicine, cosmetics, and nanotechnology (Satheesh, 2015). Coconut oil has been generally utilized from the beginning of time for its restorative worth and has served human as significant nourishment for a great many years (Boateng *et al.*, 2016). The potential benefit of VCO is preventing or enhancing different biological conditions due to the active polyphenol components shown (Illam *et al.*, 2021). VCO showed antioxidant activity based on β -carotene bleaching method analysis (Wiyani *et al.*, 2020).

Administration of VCO showed a therapeutic effect on animals treated with ethanol orally though improved the antioxidant status by decreasing MDA levels and altering lipid profile levels to near normal (Dosumu *et al.*, 2014). However, there has been no publication on the use of VCO as a preventive for fertility disorders in male animal models. Hereinafter, this study aimed to determine the effect of VCO on the diameter and the epithelium thickness of seminiferous tubules in the testis of ethanol-induced male mice (*Mus musculus*) as an animal model.

MATERIALS AND METHODS

This study was conducted at the Experimental Animal Laboratory, while the histopathological testis slides were prepared at the Division of Veterinary Pathology, Faculty of Veterinary Medicine, Universitas Airlangga. The study's ethical approval was obtained from the Animal Care and Use Committee (ACUC) Faculty of Veterinary Medicine, Universitas Airlangga No. 2.KE.204.12.2019.

Experimental animals

This study used 30 male mice (*Mus musculus*) aged 2-3 months with an average body weight of 21 grams. The mice were purchased from Pusat Veterinary Pharma, Surabaya. All mice were given an exposition for adaptation for one week before treatment.

Treatment of mice

The mice were divided randomly into five groups. The animals of each group were treated as follows. The C- group was the negative control group. This group was given access to food and water ad libitum, and no VCO nor ethanol was given. Two percent Tween and aquadest were given as replacement. The C+ group was given 33% ethanol. This group did not receive VCO treatment, instead 2% Tween was given. T1, T2, and T3 group was respectively given 0.09, 0.19, and 0.37 mL/kg bw VCO and 33% ethanol. VCO was given first for 39 days, and ethanol was given for the last 32 days (starting seven days later). In this study, processed food alcohol (ethanol) was used in the dosage of 0.2 mL/kg bw. These treatments were conducted orally every day in the morning. After the completion of treatments, all mice were sacrificed. Testis were dissected and fixed for histological specimens with hematoxylin-eosin staining.

Measurement of variables

The changes in the diameter and the thickness of seminiferous tubules were observed microscopically through histopathology preparation with HE staining and measured using the image raster version 3.0. Diameter and the thickness of seminiferous tubules were observed in five different fields using a Nikon Eclipse

microscope with the magnification of 100x for the diameter and 400x for the thickness.

Diameter of seminiferous tubules

The seminiferous tubules' diameter was measured using a micrometer from a software called image raster with a 100x magnification for a total of five random views. The roundest seminiferous tubules in each view were measured (Prastyaningtyas *et al.*, 2021).

Epithelium thickness of of seminiferous tubules

The epithelium thickness of seminiferous tubules was measured using a micrometer from image raster with a 400x magnification for a total of five views, just as the diameter previously. The epithelium thickness was then measured from the spermatogonium near the basement membrane of the seminiferous tubules until the spermatid (Wurlina *et al.*, 2021).

Data analysis

The analysis of variance was used in this study, followed by Tukey's range test at a 95% level of confidence in Statistical Product and Service Solutions (SPSS) version 23 International Business Machines (IBM) Corporation (Armonk, New York, USA).

RESULTS

This study aimed to determine the VCO would have a positive effect as prevention towards the testis of ethanol-induced male mice (*Mus musculus*).

Diameter of seminiferous tubules

The diameter of seminiferous tubules of mice in the C+ group (treated ethanol only) was lower ($p < 0.05$) than those of the C- group (normal mice). Meanwhile, the diameter of seminiferous tubules of mice among T1, T2, and T3 groups (received different dosages of VCO as a preventive treatment followed by ethanol induction) and those of the C- group showed no significant difference ($p > 0.05$) (Table 1).

Epithelium thickness of seminiferous tubules

The epithelium thickness of the seminiferous tubules of mice in the C+ group (treated ethanol

only) group was significantly ($p < 0.05$) lower than those of the other groups. The preventive administration of VCO on mice ethanol-treated (T1, T2, and T3 groups) were significantly different ($p < 0.05$) from those of C+ group (treated ethanol only), and not significantly different from those of the C- group (normal mice) (Table 1).

Table 1 The diameter and the epithelium thickness (means \pm SD) of seminiferous tubules of mice (*Mus musculus*) after oral treatment with virgin coconut oil (VCO) and ethanol

	diameter	epithelium thickness
C-	255.84 \pm 13.96 ^b	93.10 \pm 10.65 ^b
C+	171.22 \pm 19.65 ^a	52.22 \pm 11.35 ^a
T1	229.24 \pm 18.24 ^b	65.84 \pm 12.51 ^b
T2	233.52 \pm 15.97 ^b	86.64 \pm 10.76 ^b
T3	249.06 \pm 23.87 ^b	77.13 \pm 14.60 ^b

C-: mice were given 2% Tween and aquadest; C+: mice were given 2% Tween and 33% ethanol; T1, T2, T3: mice were respectively given 0.09, 0.19 and 0.37 mL/kg bw VCO as a prevention and followed by 33% ethanol; VCO was given for 39 days, while ethanol was given for the last 32 days at a dosage of 0.2 mL/kg bw; all treatments were given orally every day; ethanol was administered two hours after VCO.

DISCUSSION

Mice treated with ethanol showed significantly higher malondialdehyde (MDA) levels. The antioxidant defense such as glutathione (GSH) and catalase activities of treated animals with alcohol was lower than the normal animals, also sperm number, motility, and serum testosterone levels were also significantly reduced (Dosumu *et al.*, 2014). Most studies have shown that alcohol diminished gonadotropin-releasing hormone (GnRH) secretion and delays puberty. Glial transforming growth factor β 1 (TGF β 1) played a role in glial-neuronal communications facilitating prepubertal GnRH secretion (Srivastava *et al.*, 2014). Studies on animals have proved that alcohol intake had a deadly effect on testis (Dosumu *et al.*, 2014). The Sertoli cells were usually the first testicular cells to be insulted by

ethanol consumption (Figueiro *et al.*, 2017). Generally, the relationship between ethanol consumption and infertility was dose-dependent (Kim *et al.*, 2003).

Different studies have shown that in mice, the consumption of alcohol caused degeneration of sperm cells, decreased lumen of seminiferous tubules, testicular atrophy, and increased apoptosis rate (Duca *et al.*, 2019). Studies have shown that anything more than eight drinks in a week has been caused to decrease productivity in men, and the most common reported case was teratozoospermia using the sperm parameter. The threshold level of alcohol consumption to adversely affect the parameters remained unclear. Indeed, the effect of ethanol on human spermatogenesis might seem to be dose-dependent. It is said from a European study that more than 10,000 couples were found to be having high alcohol consumption, which was more significant than eight drinks in a week was found to be low infertility; however, moderate intake was not. Based on the study, there was evidence that alcohol intake and infertility are linked to the high level of consumption (Sheynkin *et al.*, 2013).

Over-consuming ethanol was known to damage organs, included testis. It was known that ethanol generated the alcohol metabolism that produces a toxin called acetaldehyde after the alcohol dehydrogenase (ADH) activity. This indirectly caused injuries to other organs and promotes lipid peroxidation. There was the presence of ADH in the interstitial cells and seminiferous tubules in the testis. The intake of ethanol caused oxidative injury by enhancing the production of free radicals, which automatically decreased the antioxidant level (Ballway *et al.*, 2021). A past study proved that ethanol affected the anterior pituitary gland, which caused the production of LH and FSH to be lower. Ethanol impeded the secretion of LH and FSH, which was far worse than lowering the production. It was proven that 16 weeks later, after ethanol, GnRH significantly decreased as well, and chronic alcohol consumption affected the hypothalamic cell, which then resulted in decreased hormones. Leydig cells were responsible for hormone synthesis, whereas the Sertoli cell was responsible for the maintenance and nutrients of the testis. The Leydig cell, which secretes

progesterone, was also affected. This showed that ethanol affected the testis tissue and the hormones, and the Sertoli cell was the first cell in the seminiferous tubules to be affected by ethanol. Polyphenol and tocotrienol, known as vitamin E, helped in protecting the cells from damage (Oremosu *et al.*, 2014). The reducing number of plasma testosterone levels corresponded with the decreasing responsiveness to human chorionic gonadotropin, suggesting the damage of Leydig cells caused by ethanol consumption. The usual cause of the level of LH in alcoholic men was normal levels because estrogen and androgen contribute to the feedback regulation of LH. In a much recent study, there was a significant reduction in the follicle-stimulating hormone (FSH), LH, and plasma testosterone concentrations (Duca *et al.*, 2019).

Alcohol could cause a significant change in semen volume, motility, and sperm concentration. There were significant morphological abnormalities that have been seen in semen samples of men that consume an excessive amount of alcohol. Chronic exposure was then dealt with by reducing the diameter of the seminiferous tubules and the germinal epithelium (Muthusami *et al.*, 2005). Generally, in men and male animals, ethanol exposure had been said to be with lower serum testosterone levels, an increasing level of plasma sex hormone, and an increased estradiol level. It was proven that the consumption of ethanol excessively caused a decrease in the diameter, degeneration of sperm cells and germ cells, and the thinning of seminiferous tubules. It was indicated that alcohol damage happens due to the testicular interstitial cells and seminiferous tubules, especially the peritubular wall of the latter (Giannessi *et al.*, 2015). It was reported that excessive alcohol intake resulted in testicular atrophy and gonadal failure. The effect of alcohol on testis could be from the pituitary gland or directly on seminiferous tubules due to its metabolism (Condorelli *et al.*, 2015).

The diameter and epithelium thickness of seminiferous tubules were indivisible because of the structure of the seminiferous tubules which composed of germinal cells and somatic cells that make up the germinative epithelium (Hayati, 2011). Based on a few studies, the decrease of seminiferous tubules' diameter occurred due to the

destruction of germinal cells from germinative epithelium, which eventually decreased the epithelium thickness of the seminiferous tubules. The decrease in diameter and epithelium thickness might also occur due to the low number of cells that make up seminiferous tubules due to apoptosis (En *et al.*, 2020).

Administration of VCO improved the antioxidant status by decreasing MDA levels and altering lipid profile levels close to normal levels, and sperm count, motility, and serum testosterone levels were increased compared with the alcohol only (Dosumu *et al.*, 2014). VCO was believed to be more beneficial than copra oil because of its extraction, which retained so much more biologically active components like vitamin E and polyphenols (Wallace, 2019). There were notwithstanding expanded testosterone levels, coconut crude oil (CNO) decreased the danger of prostatic cancer by fundamentally decreasing increment in prostate to the bodyweight proportion instigated by testosterone, which has incited the examination (De Lourdes *et al.*, 2007). VCO was known to be listed as a valuable source by many authors as a potent medicine. Medicinal plants have been used from the early era many thousand years ago as modern medicine was not available as easy as it is now. VCO had been proven to promote health because of phytochemical constituents such as polyphenols and vitamin E that could boost the antioxidant defense and help with oxidative stress and lipid peroxidation (Illam *et al.*, 2021). The antioxidant properties that VCO demonstrated might decrease oxidative stress and prevent tissue damage in the testis induced with ethanol. It also protected the metabolic pathway of the testosterone against lipid peroxidation, which then prevented the suppression of testosterone (Ogedengbe *et al.*, 2016). It was consistent with Dosumu and coworkers' (2012) study that proved VCO did help prevent the damage of testis because of ethanol by inhibiting oxidative stress and lipid peroxidation.

The results of this study showed that the administration of 33% ethanol at a dosage of 0.2 mL/kg bw caused a decrease in the diameter and thickness of the seminiferous tubule epithelium compared to normal rats, which could be related to the decreasing level of testosterone that eventually impaired spermatogenesis (Dosumu

et al., 2014), and resulted in a thinner epithelium and subsequently a decrease in the diameter of the seminiferous tubules. In addition, all dosages of VCO given resulted in diameter and epithelium thickness of the seminiferous tubules that were not different from normal mice (Table 1). This proved that the treatment of VCO starting from 0.09 mL/kg bw responded well as a preventive to the induction of ethanol because the polyphenols and tricophenol contained in VCO prevented the damage from oxidative stress, which was consistent with the study of Dosumu and coworkers (2010). In this study, VCO was given a week before ethanol induction. It is not known whether the same response will be obtained if VCO is given at the same time or after ethanol induction.

CONCLUSION

Treatment with VCO protected alcohol-induced damage to the testis of mice. Further study is needed to measure sperm quality and the malondialdehyde (MDA) level in the testis to determine the oxidative stress level.

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Journals

Nakamura K, Kusama K, Ideta A, Imakawa K, Hori M. 2020. IFNT-independent Effects of Intrauterine Extracellular Vesicles (EVs) in Cattle. *Reproduction* 159: 503-11.

Windeyer MC, Gamsjager L. 2019. Vaccinating Calves in the Face of Maternal Antibodies:

Challenges and Opportunities. *Review Vet Clin North Am Food Anim Pract.* 35: 557-73.

Books

Gordon I. 2003. *Laboratory Production of Cattle Embryos*. 2nd Ed. CABI Publishing. UK

Chapter in books

Parkinson TJ, Vermunt Jos J, Noakes DE. 2019. Maternal Dystocia: Causes and Treatment. In: Noakes DE, Parkinson TJ, England GCW (Ed). *Veterinary Reproduction and Obstetrics*. 10th Ed. Elsevier, UK. 236-49.

Thesis/Dissertation

Utama S. 2012. *Monitoring and Assessment of Nuclear Transfer Pregnancies using Maternal*

Pregnancy Recognition Proteins. PhD. Thesis. Monash University. Melbourne, Australia.

Internet

Akhir N. 2020. Title of article from the internet. <http://www.link>. access date.

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