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SPERM QUALITY AND TESTICULAR STRUCTURE OF *MUS MUSCULUS* AFTER *GARCINIA MANGOSTANA* L. PERICARP EXTRACT ADMINISTRATION IN DIFFERENT POLARITY

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ABSTRACT: This study was aimed to compare the effect of various doses of *Garcinia mangostana*pericarp extract using different solvent polarity on mice sperm quality and testicular structure after being exposed to 2-Methoxyethanol. As much as thirty-two mice of BALB/C strain divided into 8 groups (n=4/group); two control groups (negative and positive) and six treatment groups given various extract polarities (nonpolar, semipolar, and polar) anddoses. All treatment was subcutaneously injected daily for 40 days (2-ME for 5 days and *Garcinia*extract for 35 days). The result showed that *Garcinia mangostana* pericarp extract on various solvent polarities and doses were able to significantly decreased DNA fragmentation compared to that of positive control group. Other sperm qualities and testosterone level on mice given 0.4 mg/kg of polar extract was found to be higher than the other treatment group. In negative control group, the structureand size of testicle appeared to be thicker. Lumen was also filled with sperm compared to treatment groups. It can be concluded that the administration of polar solvent extract of *Garcinia mangostana* pericarp at low dose (0.4 mg/Kg) was able to repair decreasing mice sperm quality and testicle structure after 2-ME exposure.

KEYWORDS :*Garcinia mangostana*, 2-Methoxyethanol, sperm quality, testicular structure, DNA fragmentation

INTRODUCTION

Garcinia mangostana is mostly used as medicinal plant because of antioxidative properties of active compounds found in the plant. Active compound used for medicinal purpose which was extracted from its pericarp was called *xanthone*. In the male reproduction system, xanthone was found to be able to improve sperm quality. Previous study showed that 25 and 50 mg/kg doses of methanol extract from mangosteen pericarp could increase mice spermatogenic cells quantity and sperm quality which were previously exposed to 2-ME, but at higher dose of 100 mg/kg or more, on the contrary, it could increase *malondialdehide* (MDA) level and decrease the number of embryos conceived[1]. From previous result, *Garcinia* extract at lower dose was able to improve spermatogenic cells quantity and sperm quality. This improvement on male reproductive system after being exposed to toxic compounds means that Garcinia contained active compounds with antioxidative properties. However, at higher doses, the extract could cause decreasing spermatogenic cells quantity and sperm quality instead, thus it was

assumed that at *Garcinia* extract at higher doses had oxidative properties.

The study was then continued to investigate xanthon compound groups which could dissolved into polar, semi polar and nonpolar solvents. Then the active compounds contained in various polarities of *Garcinia* extract affecting sperm quality were investigated. *Garcinia* contained numerous active compounds such as alkaloid, glycoside, flavonoid, saponin, tannin and polyphenol, also steroid or triterpenoid. Those which dissolved into polar solvents were saponins, alkaloids, flavonoid, triterpenoid, tannin, xanthone (α-mangostin), and polyphenol. Xanthone is a natural chemical substance related to flavonoid and belongs to phenol compounds (polyphenolic) group. Meanwhile, compounds such as gartanin santon, 1-isomangostin, 1-isomangostin hydrate, 3-isomangostin and 3-isomangostin hydrate was dissolved into nonpolar solvents (n-heksana)[2].

In male reproduction, sperm quality is determined by various factors, such as sperm maturity which occurred during its transport from testicle to female reproductive tract. Structural and biochemical changes were found on the sperm, such as morphological, histochemical, physiological and cellular metabolism. Metabolic alteration in testicle and epididymis could affect quality and function of the sperm. In addition. testosterone level in blood serum also determined sperm quality. Testosterone produced by Leydig cell was needed for spermatogenesis and maturity process. Reducing testosterones level could cause spermatogenesis failure at meiosis stage[3]. The inhibition on spermatogenesis could decrease the amount of spermatogenic cells in seminiferous tubule and diameters and thickness of seminiferous tubules.

The quantity of spermatogenic cells and sperm quality in male reproductive system was affected by toxic substance 2-*methoxyethanol* (2-ME). 2-Methoxyethanol, also known as ethylene glycol monomethyl ether, is a clear liquid with an ether-like odour. It was used mainly as an anti-icing agent in jet fuels and in chemical processing, industrial coatings and other industry application, including electronic manufacturing, hydraulic fluid, and pharmaceutical manufacturing. 2-methoxyethanol is a toxic substance harmful to the health. Prevous studies revealed a wide range of toxic effects caused by 2-ME, such as decrease spermatogenesis and sperm qualities. One of the purposes of this study was to determine the effect of Garcinia extract in male reproductive system. Further research needed to be conducted to investigate active compound contained in Garcinia that affects reproductive health. This could be done by testing various solvents of active compounds i.e nonpolar, semipolar and polar fractions.

MATERIALS AND METHODS

Health ethics committee of Universitas Airlangga approved the experimental protocols and animal procedures in the current study (Process No. 318/EC/KEPK/FKUA/2014).

Animals

As much as 32 mice of BALB/C strain, aged 8±1 week and weighted 25±5 g, were used in current study. Mice were divided into 8 groups; 2 control groups consisted of negative control which was given 0.1 ml 0.05% *Carboxy Methyl Cellulose* (CMC) for 35 days and positive control given 200 mg/kg 2-*methoxyethanol* for 5 days and 0.1 ml 0.05% CMC for 35 days subcutaneously and six treatment groups given 200 mg/kg 2-*methoxyethanol* for 5 days and *Garcinia* extract with various polarity and doseas following: non polar extract (0.6 and 3 mg/kg), semi polar extract (4 and 20 mg/kg) and polarextract (0.4 and 2 mg/kg dose) dissolved into 0.05% CMC subcutaneously for 35 days.

Preparation for Garcinia mangostanaextract

Garcinia mangostana were bought at local market in Surabaya and authenticated using voucher specimen from herbarium of Department of Biology, Universitas Airlangga. Extract was prepared by using the method of[4] with modifications, using various solvents with different polarities (non polar (n-hexane), semi polar (ethyl acetate) and polar solvent (methanol). Pericarp was peeled from the fruit, cut into small pieces and crushed using household blender. Paste obtained was then dried and extracted twice using solvent at room temperature with continuous agitation. Solvent was removed using rotary evaporator under reduced pressure at 60°C. Water was removed by freeze drying for 12 h. From approximately 1000 g of *Garcinia mangostana* pericarp, 293g solid material was obtained, giving 29.3% yield. Extract powder was stored at -70°C until used for bioassays. Dried extract was reconstituted in 0.05% carboxyl methyl cellulose (CMC) for subcutaneous treatments.

Testicle preparations

Testicle slides were prepared using paraffin method organ first fixed in neutral buffered form alin and then stained using Harry's hematoxylin and eosin. Spermatogenic cells observed were spermatogonia, spermatocyte and oval spermatide inside round seminiferous tubule and were at 7thstage of epitelium cycle[5]. Observation conducted by using light microscope with 400 x magnifications.

Diameter of seminiferous tubule was measured from cross section of tubules diameter, from one edge of basement membrane to other edge. Epithelium thickness of seminiferous tubules was measured from edge of basement membrane to lumen tubule (µm). Diameter and thickness of seminiferous tubules epithelium was measured using light

microscope equipped with micrometer at 400x magnification.

Sperm collection

Sperm was collected from cauda epididymal testicle whose fat was first removed. Epididymal fluid was then diluted to 2 mL pre-warmed (37°C) normal saline. The suspension was used to analize sperm quality (DNA fragmentation, viability, membrane integrity, morphology, motility and quantity). This technique was used to collect mice's sperm because it was easier and faster to obtain sperm suspension[5].

Sperm DNA fragmentation

Evaluation of DNA fragmentation on sperm was conducted using *Acridine orange* (AO) staining. One drop of sperm suspension was placed on to object glass, smeared and airdried at room temperature. Smear was then fixed using Carnoy`s solution (methanol: glacial acetic acid, 3:1 v/v) for a night. The airdried slide was then put into AO absorbent solution consisted of 10 ml of 1% AO in 40 ml 0.1M citric acid and 2.5 ml 0.3M $Na_2HPO_4.7H_2O$ (Merck, Germany) with pH 2.5 and then stored in light-tight chamber at room temperature. Staining was conducted using 1% (100 mg/ml) AO (Sigma) for 10 minutes. After stained, slide was then rinsed using distilled water and airdried. As much as 100 sperms were measured using flouresence microscope (Olympus). Sperm with normal DNA (*double stranded*) was colored green, meanwhile sperm with fragmented DNA was colored orange or red[6].

Sperm viability

Sperm viability was evaluated by smearing sperm suspension stained with Eosin (1%)-Nigrosin (10%). Evaluation was conducted using light microscopy (100xmagnification). Inviable sperm would absorb the stain, while the viable ones would not absorb the stain. As much as 100 sperms (live and dead) were observed and then presented as percentage[1].

HOS test

Sperm membrane integrity was investigated using *hypoosmotic swelling test* (HOS), by mixing sperm suspension into hypoosmotic solution and incubated at anaerob condition (added with $NaHCO_3$ powder) for 60 minutes. Smear was then prepared and stained using Eosin-Nigrosin. Sperm membrane integrity was measured from percentage of swelling and unswelling sperm[1].

Sperm morphology

Evaluation was conducted by preparing smear of sperm suspension. One drop of sperm suspension was put onto object glass, added with one drop of Eosin and Nigrosin solution respectively. Mixture was homogenized and smeared, then airdried for 1-2 minutes. Morphology of 100 sperm cells was observed (%) byusing light microscope (400x magnification) with 10 times repetition. Abnormal sperm morphology showed abnormality on head, neck and tail part, as well as cytoplasm residue in the form of *cytoplasmic droplet*[1].

Spermmotility

Evaluation of sperm motility (µm/second) was conducted by observing one drop of sperm suspension into concave object glass and sealed with glass sealer. Motility rate from 200 sperm was measured by using inverted microscope with 100x magnification. Observation of sperm motility duration (minutes) was conducted by putting one drop of suspension onto concave object glass and sealed with glass sealer. Initial time of sperm movement until it ceased was recorded. Sperm with active forward movement was observed[1].

Sperm concentration

Measurement of sperm concentration was conducted by using hemocytometer. One ml of sperm suspension was inserted into hemocytometer then the quantity of sperm was calculated by using light microscope (400x magnifications)[1].

Testosteroneassays

Testosteron concentration was analyzed by *radioimmunoassay* (RIA) method. 0.25 ml mice blood was taken from left ventricle by using syringe, then the blood was kept for 2 hours at room temperature. Blood was then centrifuged at 3000 rpm for 10 minutes. Serum was collected and stored into microtube at -20^0 C before analysis. Testosterone level was measured by adding 1 ml of Jodium 131 (^{131}I) testosterone isotope onto each tube contained 50 µL serum. The mixture was homogenized by using vortex for 5-10 minutes and incubated for 3 hours at room temperature. Decant was then produced and testosterone level was measured by using gamma rays from Jodium 131 (^{131}I) testosterone isotope.

Statistical analysis

Comparison of spermatogenic cells total count, tubules measurement, sperm DNA fragmentation, sperm quality (sperm motility, morphology, viability, plasma membrane integrity and concentration) and testosterone concentration was performed statistically using ANOVA followed by LSD. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Histology of mice testicular

Result showed difference on seminiferous tubule cross section between controls and treatment groups. On negative control, epithelium of seminiferous tubule appeared to be thick, filled with well-arranged spermatogenic cells from basement membrane to lumen appeared as following: spermatogonia, spermatocyte, and oval spermatide. Lumen was also filled with sperm (Figure 1A). Based on the study, administration of 2-ME on positive control decreased quantity of spermatogonia, spermatocyte and oval spermatide (Figure1B). It was revealed that 2-ME was toxic and strong oxidant which could hamper development of spermatogenic cells.

At low dose, group given nonpolar fraction (Figure1C), semi polar (Figure1E)

and polar (Figure1G) had higher average quantity of spermatogenic cells and seminiferoustubule than groups of non polar (Figure1D), semi polar (Figure1F) and polar (Figure1H) at high doses. It is shown from the arrangement of spermatogenic cells that appeared denser, the increase in spermatogenic cells quantity, and size of tubule that appears thicker.

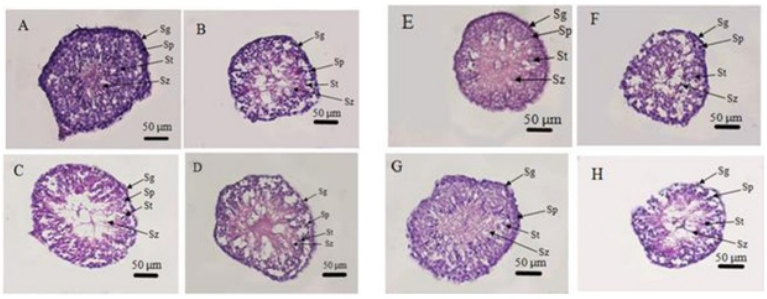

Figure1. Cross section of *Musmu sculus* tubules. A: negative control; B: positive control; C & D: nonpolar fractions (low and high doses); E & F: semipolar fractions (low and high doses); G &H: polar fractions (low and high doses);Sg: Spermatogonia; Sp: Spermatocyte; St: Oval spermatide; Sz: Sperm; 400x magnification; HE staining.

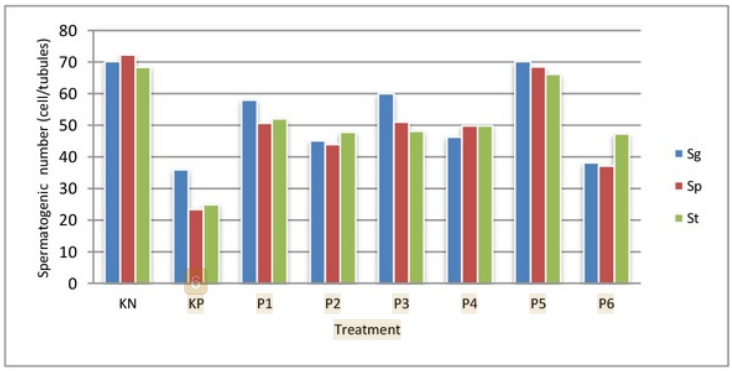

Figure2. Histogram of spermatogenic number (Sg, spermatogonium; Sp, spermatocyte, and St, oval spermatide) on control and treatment group with various doses. Kn = negative control; Kp: Positive control (2-ME); P1: non polar (low dose); P2: non polar (high dose); P3: semi polar (low dose); P4: semi polar (high dose); P5: polar (low dose); P6: polar (high dose)

From the observation, it was found that spermatogonia quantity on negative control had the highest number however it was significantly different from negative control and other fraction groups, except that of polar fraction group at low dose

(Figure2). Average spermatogonia number at low dose was higher than other treatment group of each fraction at high dose. Negative control had the highest number of spermatogonia quantity. Spermatocyte number was also found highest on negative control and only slightly different from low dose polar fraction group, but significantly different from other fraction group. Spermatocyte number on fraction group at low dose was higher than of that given higher dose.

Spermatocyte and oval spermatide quantity showed significant difference between negative control and other group, except for group given polar fraction with low dose. Second highest number was from group given polar fraction at low dose. Second highest number was from group given polar fraction at low dose which had higher oval spermatide count compared to other treatment group.

Diameter of tubule

Diameter was measured from opposite basement membrane range of cross-sectioned tubule in micrometer (µm). Result showed difference among the treatment groups. Negative control had highest diameter compared to positive control and other treatment groups. Negative control showed significant difference from positive control which had smallest tubule diameter. Seminiferous tubule on negative control or on normal tubule micro anatomy showed layered arrangement of spermatogenic cells according to the development stages from basement membrane, spermatogonia, spermatocyte and oval spermatide (stage VII). Normal arrangement of spermatogenic cells caused diameter and epithelial thickness of seminiferous tubule to be higher. In addition, size of seminiferous tubule was bigger due to more spermatogenic cells filling the epithelium of seminiferous tubule. Positive control given 2-ME without pericarp fraction showed disarrangement and sparse spermatogenic cells. This condition caused the diameter and epithelial thickness of seminiferous tubule to be reduced. Epithelium of seminiferous tubule was measured by using micrometer (µm) from the edge of basement membrane to the adjacent area of tubulus lumen. Epithelial thickness of negative control appearedto be higher than of positive control and was slightly different from polar fraction group at low dose. Positive control had the lowest thickness and was slightly different from non polar group (at high dose). Polar group (at low dose) had second highest tubular epithelial thickness (Figure 3).

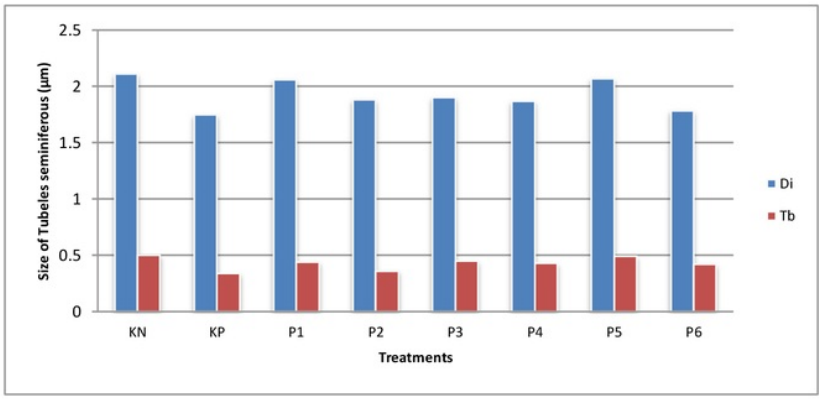

Figure3. Histogram of diameter (Di) and epithelial thickness (Tb) of mice's seminiferous tubule on control groups and treatment groups with various doses and polarity. KN = negative control; KP: positive control (2-ME); P1, P3, P5: non polar, semi polar, polar fractions at low doses and P2, P4, P6: non polar, semi polar, and polar fractions at high doses

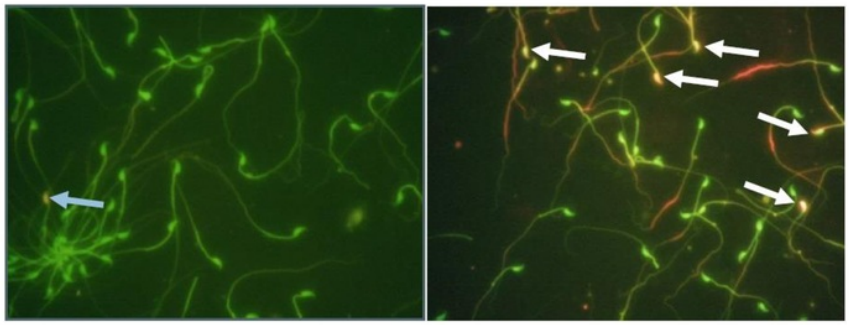

Figure4. Staining sperm using *Acridine Orange,* fragmented DNA sperm (yellow-orange, shown in arrow), normal DNAsperm (green), 100x magnification

Table 1.Spermquality on control and treatment groupgiven various polarity and dose of *Garcinia mangostana* pericarp extract after 2-ME exposure

Sperm Quality	Negative control	Positive control (200 mg/kg 2-ME)	Doses and fraction variation ofGarcinia mangostana pericarp extract					
			Nonpolar		Semipolar		Polar	
			Low dose(0.6 mg/kg)	High dose (3 mg/kg)	Low dose (4 mg/kg)	High dose(20mg/ kg)	Low dose(0.4 mg/kg)	High dose (2 mg/kg)
DNA fragmentation (%)	2.7±0.8^{a}	63.8±1.7^{f}	9.3±1.1^{c}	42.4±7.0^{e}	14.8±5.3^{cd}	13.7±2.2^{d}	5.8±0.9^{b}	18.3±3.7^{d}
Viability (%)	85.8 ± 1.8^{c}	24.8 ± 3.1^{a}	45.4 ± 0.8^{bc}	30.6 ± 3.3^{a}	46.7 ± 1^{c}	44.1 ± 0.6^{b}	83.9 ± 0.4^{d}	44.9 ± 1.1^{bc}
Membrane Integrity (%)	81.0 ± 3.5^{c}	23.9 ± 1.5^{a}	36.2 ± 1.2^{b}	25.4 ± 0.4^{a}	37.6 ± 2.5^{b}	25.6 ± 0.9^{a}	63.1 ± 2.6^{d}	32.8 ± 36^{c}
Morphology (%)	91.0±1.1^{d}	52.7±5.7^{a}	67.7±9.5^{bc}	66.1±2.9^{ab}	79.7±5.1^{cd}	64.6±2.4^{ab}	90.6±1.8^{d}	70.7±2.1^{bc}
Motility Rate (µm/s)	57.2±9.5^{bc}	30.9±1.4^{a}	58.3±6.7^{bc}	54.8±0.6^{c}	63.8±1.4^{c}	53.8±1.4^{c}	74.7±3.3^{d}	54.3±3.9^{b}
Motility duration (minutes)	15.2 ± 1.9^{t}	5.5 ± 1.9^{a}	6.1 ± 2.8^{a}	5.8 ± 1^{a}	7.2 ± 1^{a}	7 ± 1.9^{a}	16.1 ± 4.4^{b}	12 ± 3.6^{b}
Quantity (10^{8}cell/ml)	4.9±0.4^{bc}	2.9±0.8^{a}	4.5±0.6^{b}	4.1±0.2^{b}	4.6±0.6^{b}	4.2±0.7^{b}	5.8±1.1^{c}	4.2±0.9^{b}
Testosterone (mg/ml)	0,5±0,2^{a}	0,2±0,1^{b}	0,6±0,4^{ab}	0,2±0,1^{b}	0,9±0,5^{a}	0,3±0,1^{b}	0,7±0,2^{a}	0,3±0,2^{b}

Quality of Mice's Sperm

Result of sperm quality evaluation was presented in Table 1. DNA fragmentation in negative control had the lowest percentage, followed by group given low dose polar fraction and other treatment groups. Highest DNA fragmentation was found on positive control (63.8%) compared to negative control (2.7%) and other treatment groups. Administration of 200 mg/kg2-MEon positive control group could significantly increased sperm DNA fragmentation.

Staining using Acridine orange was able to distinguish sperm with normal DNA and fragmented DNA (Figure 4). Sperm with normal DNA was colored green, while sperm with fragmented DNA was colored yellow-orange.

Administration of pericarp fraction with various polarity and dose revealed significant decrease in DNA fragmentation percentage compared to positive control group. Group given nonpolar fraction at low dose and high dose reduced DNA fragmentation from 63.8 %to 9,3 and 42.4% respectively; semipolar fraction at low and high doseswas also able to reduce DNA fragmentation from 63.8% to 14.8 and 13.7 % respectively; polar fraction at low and high doses could also significantly reduced DNA fragmentation from 63.8% to 5.8 and18.3%. Treatment with various polarity and dose was able to reduce DNA

fragmentation, however extract administration could not able to restore impaired sperm back into its normal condition as seen on negative control (2,7 %).

On sperm viability (Table 1), it could be seen that positive control had lowest number (24.8%), thus was not significantly different from group given nonpolar fraction (30.6%). Meanwhile, negative control had highest percentage (85.8%) which was significantly different from rest of the groups. Table1showed that various mangosteen pericarp fraction at low doses was able to repair damaged sperm viability due to 2-MEexposure, in comparison to those at higher dose.

Lowest number of sperm membrane integritywas found on positive control (23.9%), although it was not significantly different from group given high dose of nonpolar fraction (25.4%). Highest number was found in negative control (81%) (Table1). The number of sperm membrane integrity on group given low doses nonpolar, semi polar and polar fractions showed that it was able to repair the damaged sperm membrane integrity due to 2-ME exposure compared to other groups with high dose of treatment. It indicated that at low dose, mangosteen pericarpof each fraction was suitable to improve the quality of damaged sperm membrane integrity caused by 2-ME exposure.

Morphology of normal sperm was indicated by its complete parts. such as crescent shaped head with bulging posterior and straight tail. Meanwhile morphology of abnormal sperm indicated by head part did not shaped like hook, curved and broken tail, also residue of *cytoplasmic droplet* found on cell membrane. The number of normal sperm morphology on positive control was significantly different from other group. However, treatment group at low dose showed higher normal morphology sperm count compared to other fraction groups at high dose. Group given low dose polar fraction also showed higher motility rate and duration compared to other treatment groups.

Testosterone level showed significant difference between positive control (0.2±0.1 mg/ml) and negative control (0.5±0.2 mg/ml), also between low doses of non polar, semi polar and polar fraction groups, but the result showed no significant difference among the groups given high doses of non polar,semi polar and polar fractions.

Testicular histology

Based on current study, 2-ME administration on positive control decreased the quantity of spermatogenic cells compared to negative control. It was revealed that 2-ME was a toxic and oxidant which was able to inhibit the development of spermatogenic cells. While it had been known to possess teratogenic properties, this compound was also toxic to male reproductive organ, testicles was especially sentitive to the exposure. Inside the body,

2-ME was found to be able to increase *reactive oxygen species* (ROS) level, thus it was able to damage of rat hepatocite cell[5].

At low dose, extract variation was able to increase the quantity of spermatogenic cells and seminiferous tubule size compared to high doses. It was shown that spermatogenic cells increased quantitatively and size of seminiferous tubule was also elevating. Result showed that spermatogonia cell count on negative control was higher than that of positive control and different from group of polar extract at low dose. Cell count was significantly different on negative control compared to other group, except that of polar group at low dose (Figure 3). Spermatogonia number at low dose was higher than other group given extractathigh doses. The quantity of spermatocyte was also found highest on negative control and at group given polar extract at low dose, both of which was significantly different from other group. The quantity of spermatocyte on low dose was found to be higher than that of higher dose.

Spermatocyte and oval spermatide count showed significant difference between negative control and other group, except for group given polar extract at low dose. Second highest was found on polar group (low dose) which had higher of oval spermatide compared to other groups. Recovery of spermatogenic cells on this treatment was occured due to active agent (xanthone) contained in the extract functioning as antioxidant[2]. This antioxidant compound was able to prevent impairment because of ROS in all vital components of the cell[5]. It was reported that this compound was able to react with reactive oxigent compound because it possessed one or two hydroxyl group of aromatic ring taking roleas hydrogen donor. This process occurred via hydrogen atom removal by antioxidative compound, thus free radicalwas able to captured one electron released from antioxidative compound. Xanthone compound was able to bond with unstable free radical inside the body to prevent degeneration process of the cells. X*anthone* was categorized as strong antioxidant which was able to increase testosterone production as the most important hormone involved in sperm production and maturity in seminiferous tubule[7]. Thus, the damage of cell membrane caused by free radicals could be prevented that the relationship between hormones involved in spermatogenesis process and its receptor was not interrupted.

The disposition of 2-ME inside the body was through blood vessel travelling to testicles. This compound was able to form a bond with Sertoli cell receptor, hampering protein synthesis needed for cell division of spermatogonia. Decreasing spermatogonia cells quantity would also cause reduce on spermatocyte and spermatide number because cells inside seminiferous tubule were associated with one another via *Blood Testis Barrier*. Decrease in primary

spermatocyte cells number was also supported by[8] which stated that primary spermatocyte was very sensitive towards external condition and tend to be damaged after first stage of meiosis, when the crossover between homologous chromosomes was happened. At spermatogonial stage, cell also undergone apoptosis but the number was low. It was why basement membrane role on maintaining spermatogenesis was very important, thus at spermatogonial stage, cell had higher resistance toward apoptosis.

Diameter of seminiferous tubule

Diameter measurement was performed by on the cross section of tubule's diameter of each opposite basement membrane and calculating the average number in micrometer (µm). Result showed difference was present among treatment groups. Negative control had highest tubule diameter compared to positive control and treatment groups. Negative control was significantly different from positive control, which had lowest diameter. Seminiferous tubule on negative control or normal seminiferous tubule showed layered arrangement of spermatogenic cells according to the development stages starting from basement membrane, spermatogonia, spermatocyte andoval spermatide (stage VII). Normal arrangement of spermatogenic cells causeddiameter and epithelial thickness of seminiferous tubule became higher. In addition, seminiferous tubule size was bigger due to more spermatogenic cells filled epithelium of seminiferous tubule. Positive control given 2-ME without extract showed disarrangement and sparse spermatogenic cells. This condition lowers the diameter and epithelial thickness of seminiferous tubule.

Epithelium of seminiferous tubule was measured by using micrometer (µm) from the edge of basement membrane to the adjacent area of lumen tubulus. Epithelium from negative control was appeared thicker than positive control and slightly different from polar fraction group (at low dose). Positive control had the lowest average thickness and was slightly different from non polar group (at high dose). Polar group (at low dose) had second highest tubular epithelial thickness. After Garciniain polar extract administration at low dose, seminiferous tubule size had the closest condition to normal compared to the other extract. Activity of polar Garcinia extract needed to prevent oxidation process, which was 50% higher than other semipolar or polar fractions.

A reduce in seminiferous tubule's size (diameter and epithelial thickness) was presumably occurred due to estrogenic. In the seminiferous cords of estrogen-deprived, the basement membrane appeared fragmented, the germ cells and Sertoli cells appeared disorganized[9] and saponin contained in *Garcinia*[10]. Saponin was used for steroidal hormone synthesis and also used as estrogen contraceptive. Reducing

tubule diameter was presumably due to estrogenic properties of saponin contained on mangosteen extract, thus bonds was formed with estrogen receptor on anterior hypophysis. Estrogen produced from aromatization reaction of testosterone hormone was important as inhibitor in FSH control. Physiologically, hypothalamus secreted GnRH to stimulate FSH and LH release from anterior hypophysis. However, the bond between mangosteen compound, saponin compound and estrogen receptor inhibited FSH and LH secretion by anterior hypophysis. It would also lead to reducing testosterone secretion by Leydig cells. Moreover, inhibition of FSH secretion from anterior hypophysis gland would cause reduce on ABP secretion from Sertoli cells as well. Thus, testosterone level admitted into seminiferous tubule was also lowered. Reduce on FSH and testosterone level was found to interruptspermatogenesis process and cause atrophy of spermatogenic cells.

Sperm quality

Result of sperm quality evaluation was presented in Table 1. Lowest DNA fragmentation was found from negative control, followed by group given low dose polar extract and other groups. Highest DNA fragmentation was from positive control (63.8%) in comparison to negative control (2.7%). Administration of 200 mg/kg 2-ME on positive control was able to significantly increase sperm DNA fragmentation.

Staining using Acridine orange distinguished sperm between normal DNA and fragmented DNA. Sperm with normal DNA was colored green, while sperm with fragmented DNA would be appeared as yellow-orange.

Administration of extract in various polarity and dose revealed significant decrease DNA fragmentation compared to positive control. Groups given nonpolar at low and high dose was found to be able to reduce DNA fragmentation from 63.8 % to 9.3 and 42.4% respectively; semipolar at low and high dose also reduced DNA fragmentation from 63.8% to 14.8 and 13.7 % respectively; while polar extract at low and high dose was able to significantly reduce DNA fragmentation from 63.8% to 5.8 and 18.3 %. Treatment with various polarity and dose could reduce fragmented DNA; however extract administration was not capable to restore DNA condition into normal as seen on negative control (2.7 %).

On sperm viability (Table 1), positive control had the lowest percentage (24.8%), and it was not significantly different from group given nonpolar extract (30.6%). Meanwhile, negative control had highest percentage (85.8%) which was significantly different from the rest of groups. Table 1 showed that Garcinia extract variation at low dose was able to repair damaged sperm viability due to 2-ME exposure, in comparison to those given higher doses.

Lowest percentage of sperm membrane integrity was observed from positive control (23.9%) compared to other treatment group, although the result was not significantly different with mice given high dose of nonpolar fraction (25.4%). The highest percentage was found in negative control (81%) (Table1). Sperm membrane integrity on mice given low dose of nonpolar, semi polar and polar fractions indicated that the extract could repair damaged sperm membrane integrity due to 2-ME exposure compared groups given high dose extract. It indicated that Garciniaextract of each group was optimal at lower dose to improve the quality of damaged sperm membrane integritycaused by 2-ME exposure.

Morphology of normal sperm was shown by its complete parts, including crescent-shaped head with bulging posterior and straight tail. Meanwhile, abnormal sperm morphology indicated by head part which did not shaped like hook, curved and broken tail, besides residue of *cytoplasmic droplet* in cell membrane. The number of normal sperm morphology on positive control was significantly different from other groups. However, treatment groups at low doses showed higher normal morphology percentage compared to other groups at high doses. Mice given low dose polar extract also showed higher value of motility rate and duration compared to other treatment group.

From (Table 1), testosterone level showed significant difference between positive control (0.2±0.1 mg/ml) and negative control (0.5±0.2 mg/ml), also between low doses of non polar, semi polar and polar groups, but no significant difference was found among groups given high doses of non polar, semi polar,and polar. Testosterone level was decreased after 2-ME exposure because 2-ME was found to be metabolized into methoxyacetic acid (MAA) in liver, and it was able to affect gene expression of Sertoli and Leydig cells. Leydig cells were somatic cells in gonad served to synthesize testosterone after being stimulated by lutheinizing hormone, thus any changes on Leydig cells would inhibit testosterone secretion[11]. It can be concluded that the administration of Garciniapolar extract at low dose (0.4 mg/kg) was able to improve quality of spermandmice's testicular structure after being exposed to 2-methoxyethanol.

REFERENCES

1. Hayati, A., L. Karolina, N. D. Subani & R. Yudiwati. 2014. The potencial of *Garcinia mangostana* pericarp extract on spermatogenesis and sperm quality of mice (Mus musculus) after 2-methoxyethanol exposure. *Journal of Applied Environmental and Biological Science*. 4(4): 47-51.

2. Chaverri, J., N. Rodriguez, M. Ibarra, & J. Rojas. 2008. Medicinal properties of mangosteen. *Journal Food and Chemical Toxicology*. 46: 3227-3239.
3. Walker, W. H. 2011. Testosterone signaling and the regulation of spermatogenesis. *Spermatogenesis*. 1(2):116-120.
4. Adaramoye, O.A., I. Awogbindin & J.O. Okusaga. 2009. Effect of kolaviron, a biflavonoid complex from *Garcinia kola* seeds, on ethanol-induced oxidative stress in liver of adult wistar rats. *Journal of Medicinal Food*. 12(3): 584-590.
5. Hayati, A. 2011. Spermatologi, *Airlangga University Press*, Surabaya.
6. Chanapiwat, P., K. Kampon &T. Padet. 2010. The sperm DNA damage Rafter cryopreservation of bar semen in relation to post-thawed semen qualities, antioxidant supplementation and boars effects. *Thailand Journal Veterinary Medica*. 40(2):187-193.
7. Ralebona, N., C.R. Sewani & C. Nkeh. 2012. Effect of ethanolic extract of *Garcinia kola*on sexual behaviour and sperm parameters in male wistarrats. *African Journal of Pharmacy and Pharmacology*. 6(14): 1077–1082.
8. Johnson, M., B. Everitt. 1990. Essential in Reproduction. London: *Blackwell Science Pub Oxford*.
9. Albrecht. E.D., M.V. Lane , G.R. Marshall, I. Merchenthaler ,D. R.Simorangkir, C. R.Pohl, T.M. Plant & G.J. Pepe. 2009. Estrogen promotes germ cell and seminiferous tubule development in the baboon fetal testis. *Biol Reprod*, 81(2):406-14.
10. Chikerel, O.U, O.C. Gloria & E.D. Nnabuihe. 2015. The Effect of Ethanolic Extract of *Garcinia Kola* on the Sperm Parameters and Histology of the Testis of Male Wistar Rats. *Advances in Life Science and Technology*, (33).
11. Bagchi, G.,Y. Zhang & D.J. Waxman. Impact of methoxyacetic acid on mouse Leydig cell gene expression. *Reprod Biol Endocrinol*,8: 65.

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