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# The Potential of *Garciniamangostana* Pericarp Extract on Spermatogenesis and Sperm Quality of Mice (*Musmusculus)* After 2-Methoxyethanol Exposure

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## ABSTRACT

The objective of this research was to examine the effects of *Garcinia mangostana* pericarp extract on spermatogenesis and sperm quality of mice which has been exposed by 2-methoxyethanol (2-ME). This research used 24 male mice, which were divided into four groups: one control and three treatments with various dose of extract. The control were exposed by 200 mg/kg/day 2-ME for five days and 0,05% carboxy methyl cellulose for 35 days. The treatments were exposed by 200 mg/kg/day 2-ME for 5 days and various dose of extract (25, 50, and 100 mg/kg, respectively) for 35 days. The results indicated 25 and 50 mg/kg extracts could increased the number of spermatogenic cells (spermatogonia, spermatocytes, and oval spermatid) and sperm quality (motility and morphology) after 2-ME exposure. The sperm viability was increased after 25 mg/kg extract and decreased when dose was added, but malondialdehyde (MDA) level was decreasing on 50 mg/kg. In 100 mg/kg dose affects reduction on all parameters, except MDA level was increased. So, *G. mangostana* pericarp extract (low dose) intake improved the spermatogenesis and sperm quality, but high dose may lower the spermatogenesis and sperm quality of mice which have been exposed by 2-ME.

**KEY WORD:** *Garcinia mangostana*, spermatogenesis, sperm quality, malondialdehyde

## INTRODUCTION

Fertility is essential in determining organism survivability and its genetic diversity. In Indonesia, male infertility problem is increasing during the last decade. Based on the previous research, almost 12% of married couple in Indonesia becomes infertile after their second anniversary. The causes of infertility originates from 40% male, 40% female, 10% both of them, and 10% unknown factor. Testis, as a primary reproduction organ were very sensitive on the environment changes[1]. Changes in environmental, for example temperature, toxic materials would decrease testicular function may lead to toxicity and infertility. One of the factor to contribute on infertility was free radical. Free radical known as reactive oxygen species (ROS)includes superoxide, hydrogen peroxide, hydroxyl, and radical peroxide. Oxidative stress cause oxidative damage by ROS increasing that has been implicated in the cause of many diseases. There were caused by drugs, chemicals, and solvents which used in industry[2]. One of toxic chemicals and oxidants may cause testis dysfunction is 2-methoxyethanol (2-ME). Concentration of 200 mg/kg 2-ME may reduce sperm motility, morphology, viability, and membrane integrity of mice and then changing thecellmembraneultrastructure and mitochondria of sperm[3]. 2-ME cause damages on tubullus seminiferous, decreasing of spermatogonium, primary spermatocyte, and oval spermatocyte numbers and reducing the diameter and thickness of tubullus seminiferousepithelium[4]. 2-ME can enter the body either by inhalation of air containing 2-ME, ingestion of contaminated water, or by dermal contact. Inhalation of air containing high levels of 2-ME can lead to irritation of the upper respiratory tract, headache, drowsiness, dizziness and unconsciousness. Exposure to 2-ME over long periods of time can damage the male reproductive system, while exposure during pregnancy can harm the developing fetus. Inside the cells of living organism, this compound undergoes oxidation process. Oxidation of 2-ME eliminates two hydrogen molecules and uses released free oxygen to form free radicals. This intracellular oxidation process triggers formation of oxidant radicals which caused destruction and death of the cells[5].

Recently, there were many plants used as traditional medicine for decreasing free radicals. One of them was *G. mangostana.* This plant contains xanthones compounds that include mangostin, mangostenol, mangostinonA, mangostenon B, trapezifolixanthone, tovophyllin B, alpha-mangostin, beta-mangostin, garcinon B, mangostanol, flavonoid epicatechin, and gartanin[6]. In low dosage, this compound is very beneficial for health. Xanthones are compounds produced only from genus *Garcinia*.Among these compounds, most of previous researches reported that *α-mangostin, γ-mangostin*, and *garcinon-E* have pharmacological effect while *α-mangostin*and *γ-mangostin*have antioxidant effect[5]. Compounds in pericarp of *G. mangostana* have been reported to have biological activities including antioxidant and anti-inflammatory effects, and the major xanthone compounds in *G. mangostana* were α-mangostin and γ-mangostin[7]. Xanthonewas natural chemical

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substance which is categorized as polyphenolic compounds that can be used to cure variation diseases. Until now not known the effect of high doses and longtime of administration of extract pericarp *G mangostana* to male reproductive health, so it is necessary to investigate the side effects.

## MATERIALS AND METHODS

### A. Material

This research used 24 male mice, there were divided into four groups (one control and three treatment). The control was exposed by 200 mg/kg/day 2-ME for five days and 0,05% carboxymethyl cellulose (CMC) through sub-cutaneous for the next 35 days. The treatments were exposed by 200 mg/kg/day 2-ME for five days and extract of *G. mangostana* pericarp with various doses 25, 50, and 100 mg/kg/day respectively for 35 days.

### B. *G. mangostana*Pericarp Extraction

*G. mangostana* pericarp fifty grams were macerated in 200 ml methanol on 500 ml Erlenmeyer flask for 24 hours with several stirrings. Then, the solution was filtrated by using vacuum and filter papers to separate the dregs out of its filtrate. the filtrate was evaporated to vaporize the solvent, which results *G. mangostana* pericarp extract. The extract was weighed out and stored at 40° C [8].

### C. Spermatogenic Cells Measurement

Spermatogenic cells in this research are spermatogonia, spermatocyte cells, and oval spermatocytes of histological testis. The Spermatogenic cells was measured by counting the number of these cells in each tubulus seminiferouson seventh stage of epithelium cycle. The tubulus seminiferous used in this research are cycle-shaped with certain criteria of its constituent cells[2]. The examination was done by using microscope and takes five visual fields and is done five times each field.

### D. Sperm Collection

A day after treatment, mice were sacrificed and dissected to obtain its epididymis and testicular Epididymis and right-and-left testicles were cleared from its fat tissue. Then, epididymis and testicles are made suspension into two ml phosphate buffer saline. In order to separate spermatogenic cells out from other cells, separation process was done by using centrifugal separation 3000 rpm at 4° C for 15 minutes. Supernatant which contained spermatogenic cells were ready for the next tests.

### E. Semen Analysis

#### 1. Motility

Sperm motility was measured by one drop of sperm suspension on a concave object glass. The object was examined by using inverted digital microscope (Olympus, IX51) with 40 times magnification. Motility of 100 sperm was chosen as a random straight line velocity(SLV). Sperm motility (μm/second) was measured through 10 repetitions.

#### 2. Morphology

Sperm morphology slides were prepared by placing a drop of eosin-nigrosin,It was detected by staining and non staining on a glass slides. A drop of the suspension was placed on the slide near the stain drop. The spermatozoa suspension and stain were then mixed on the slide usingthe long edge of a glass stick, the mixture was spread ina thin film along the slide and allowed to air dry. Spermatozoa were classified as normal and abnormal (abnormal head, abnormal tail, cytoplasmic droplet on the head and distal cytoplasmic droplet on tail), andbent tail. Examine was used light microscope (typically using a 40x objective lens). Morphology percentage was measured through 10 repetitions.

#### 3. Viability

Viability examination was done if there are more than 50% immobilized sperm on a visual field. Firstly, sperm suspension was diluted in phosphate buffer saline. The nigrosin-stain stain produces a dark background on which the sperm stand out as lightly colored objects. Normal live sperm exclude the eosin stain and appear white in color, whereas dead sperm (i.e. those with loss of membrane integrity) take up eosin. Viability percentage was measured through 10 repetitions.

#### 4. Sperm MDA

MDA level was measured by examining spermatozoa taken from epididymis cauda. Sperm suspensions were separated from fats by adding 0.5 ml trichloroacetic acid 20% and centrifuged in 5000 rpm for 10 minute to accelerate protein sedimentation. 1000 μl sperm supernatant was mixed with 200 μl sodium thiobarbiturate 1% and 8.8 ml chloride acid in a measuring glass. The solution was incubated on a water bath for 135 minutes at 50°C. the colored solution which contains MDA was examined using spectrophotometer with wave-length 531.4 nm.

### F. Statistical Analysis

Sperm motility, morphology, viability and MDA level statistically analyzed using MANOVA. Comparison between treatment and control group were made by Least significance different (LSD) test used to identify the effect. Differences between groups were considered significant at $p<0.05$. For testis histological results, analyzed by description.

## RESULTS AND DISCUSSION

The result of microscopic observation indicated *G. mangostana* pericarp extract intake affected the numbers of spermatogonium, spermatocytes, and oval spermatid. Tubullus seminiferous examination on treatment groups given 25 and 50 mg/kg indicated increasing number of spermatogenic cells. Meanwhile, treatment group with 100 mg/kg intake indicated lower number of spermatogenic cells compared to control group (Table 1) and histological of testicle is described in Figure 1.

**Table 1: Average the numbers of spermatogenic cells after *G. mangostana* percarp extract intake in various doses**

| Combined Extract (mg/kg) | Spermatogenic number | | |
|---|---|---|---|
| | Spermatogonia | Spermatosit | Spermatid |
| **0, Control** | 67.0 ± 6.1 [a] | 58.7 ± 8.2 [a] | 41.0 ± 8.9 [a] |
| **25** | 75.5 ± 4.5 [b] | 68.8 ± 3.8 [b] | 53.6 ±11.2 [b] |
| **50** | 70.0 ± 2.5 [b] | 65.3 ± 3.1 [b] | 45.8 ± 4.9 [b] |
| **100** | 55.3 ± 4.5 [c] | 39.3 ± 9.9 [c] | 26.3 ± 7.5 [c] |

Note: numbers followed by the different letter at the same column means significantly different.

Based on Table 1, the numbers of spermatogonium, primary spermatocytes, and spermatid are increasing. Compared to the control group, the highest average was shown by treatment group with 25 mg/kg intake, followed by treatment group with 50 mg/kg intake and treatment group with 100 mg/kg is the lowest. This result suggests that 25 and 50 mg/kg *G. mangostana* pericarp extract intake could improve the number of spermatogenic cells after 2-ME exposure. However, it was assumed that 100 mg/kg was oxidant as 2-ME. Therefore, it decrease the number of spermatogenic cells in mice testicles.

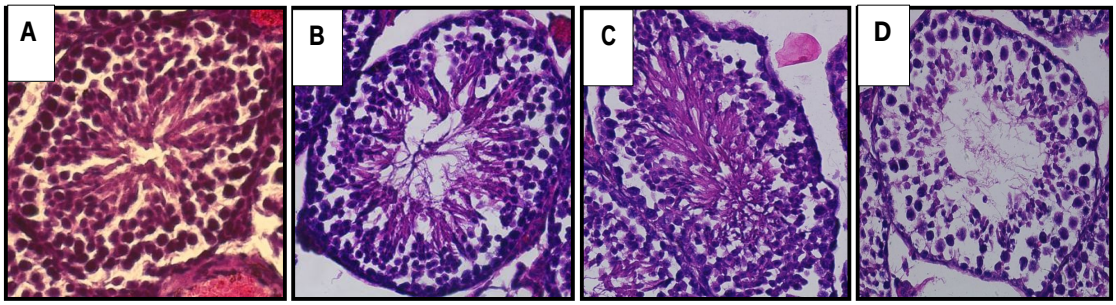


**Figure 1: Tubulus seminiferous, mice testis cross sectional (HE stain and 40x objective lens). A: control group with 2-ME 200 mg/kg. B, C, and D: treatment groups with 25, 50, and 100 mg/kg *G. mangostana* pericarp extract**

Based on Figure 1, there is association among spermatogonium, spermatocytes, and spermatid which composed in layers based on its development stage starting from basement membrane to lumen. Histological description of treatmentt groups which have been given 25 and 50 mg/kg of *G. mangostana* percarp extract indicated dense and organized composition of spermatogenic cells developmental stages: spermatogonia, spermatocytes, and spermatid showed in layers from basement membrane to lumen. Tubulluslumens seem to be full of sperm. Meanwhile, treatment group with 100 mg/kg dose intake indicated sparse and unorganized spermatogenic cells composition. It contains less spermatogonium, spermatocytes, and spermatid. Therefore, lumen seems to be less full than other groups.

Tubullus seminiferouswas one of the component which composes testicles (the other ones were connective tissue and blood veins). In tubullus seminiferous, sperm were produced as result of sequential germinal epithelium cells division. These cells form new cells which development leads to lumen of tubullus seminiferous[7].Micro-anatomy of normal tubullus seminiferous will describe spermatogenic cells association composed in layers based on its developmental stages started from basement membrane to lumen tubullus: spermatogonia, spermatocytes, and spermatid. Lumen seems to be full of spermatozoa. However, there were particular cases such as drug intake that would affect spermatogenesis process by inhibiting germinal epithelium division (development from germinal epithelium cells to sperm).

*G. mangostana* pericarpextract intake for 35 days affected the number of spermatogonium, primary spermatocyte, and oval spermatid which have been exposed by 2-ME (Table 1). The improvement on 2-ME exposed spermatogenic number was caused by *xanthone*compounds inside *G. mangostanae* pericarp extract which function as antioxidant. Small doses intake (25 and 50 mg/kg) were potentially functioning as antioxidant which help recovering tissuedamage. But, high dose intake (100 mg/kg) potentially functions as oxidant. It was indicated from the decreasing number of spermatogenic and sperm quality (Table 1 and Table 2).

As an antioxidant, *xanthone* contributes in cutting off free radical chained oxidation reaction. To inhibit oxidation reaction, there were also reduction reactions which involve hydrogen. Hydrogen involvement in these reduction reactions, can inhibit oxidation reaction between free radical and oxygen, therefore it promotes body balance. Meanwhile, xanthoneworks as oxidant by inhibiting aromatase functionality using γ-mangostin. Aromatase was the enzyme which catalyzes testosterone into estrogen. Hence, higher dose of mangosteen pericarp extract would improve testosterone level. Without hypophysis and hypothalamus negative feedbacks, high-level testosterone may disturb the function of testicles. The decreasing of spermatogenic number was caused by 2-ME exposure and high dose extract intake. The decreasing of spermatocyte cell number was caused by external factors. It tends to be damaged after prophase of the first meiosis when homologous chromosomes are crossing over. In this stage, nucleus and cytoplasm grow as the biggest cells. However, oxidant substances during spermatogenesis process affect spermatogenic cells. The decreasing number of spermatogenic is seen on treatment group with high dose intake. It may be caused by high dose extract intake for a long period; therefore the extract becomes oxidant which destructs cells.

**Sperm Quality**

The results of sperm quality of treatment and control groups were described in Table 2. In the table, it was stated that average sperm motility of control group is 3.60 μm/s while average motility of three treatment groups are 5.94; 5.17; and 3.50 μm/s.

**Table 2:Sperm quality average after various doses of *G. mangostana* pericarp extract intake**

| Combined Extract (mg/kg) | Sperm quality | | | |
|---|---|---|---|---|
| | Motility (μm/s) | Morphology (%) | Viability (%) | MDA (nmol/mg) |
| **0, Control** | 3.60±0.3 [a] | 40 ±6.7[a] | 43±7.4[a] | 19,193±6,118[a] |
| **25** | 5.94± 2.1[b] | 61±4.9 [b] | 72±3.5[b] | 19,563±2,206[a] |
| **50** | 5.17±1.4[b] | 54±5.8[b] | 48±6.4[a] | 14,140±12,540[b] |
| **100** | 3.50±0.1 [a] | 31±7.2[a] | 47±4.9[a] | 22,355±3,340[a] |

Note: numbers followed by the different letter at the same column means significantly different.

Based on Table 2, it was indicated that the best doses to improve mice sperm motility after 2-ME exposure are 25 and 50 mg/kg. Similarly, 25 and 50 mg/kg doses potentially increase the percentage of sperm morphology. However, the higher dose (100 mg/kg) decrease the sperm motility and normal morphology percentage. Sperm viability is increased at 25 mg/kg. However, it was got lower as the dose of extract increased. At 50 mg/kg dose intake, the MDA level of treatment groups was lower than control group. However, it was increased as the dose increased.

The sperm morphology was described on Figure 2. Headless sperm, broken-neck of sperm, and crooked-tail were often found during examination.

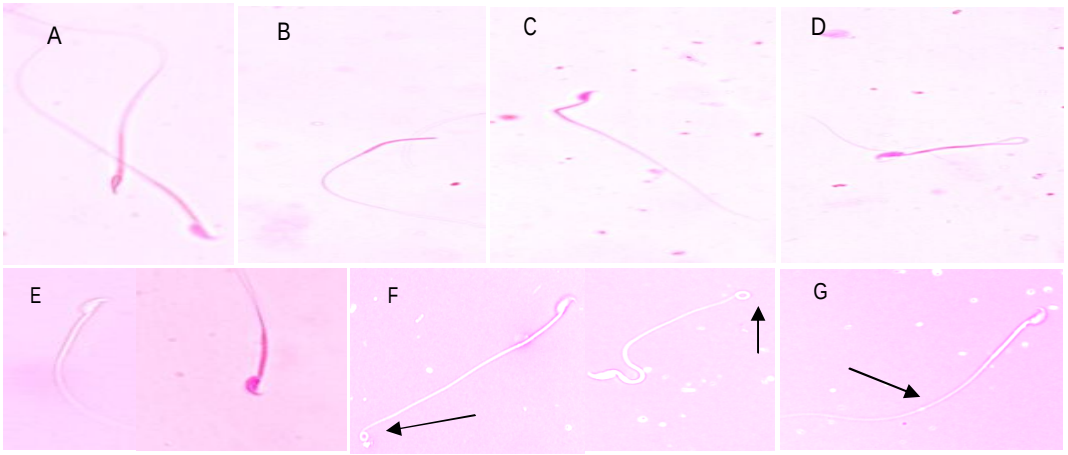


**Figure 2: Morphology and viability of mice sperm: A. normal sperm; B. headless; C. crooked neck; D. abnormal-tailed; E. live sperm (not stained) & dead sperm (take up the stain); F. good integrity; and G. low integrity**

The findings of this research suggest 25 mg/kg dose intake can improve sperm motility, morphological, and viability. These improvements are caused by xanthone compounds contained by extract that function as antioxidant which neutralizing free radicals (Pasaribu, et al. 2012). In contrary, at higher dose (100 mg/kg), this antioxidant does not work effectively because probably it becomes toxic and improving reactive oxygen species (ROS) level which cannot be neutralized by sperm antioxidant.

In this research, 50 mg/kg dose intake effectively reduced sperm MDA level of 2-ME exposed. However, the higher dose of extract intake may improve MDA level. It may be caused by high dose of extract intake for a long period may increase ROS level (marked by dead or sperm damaged) as indicated by high MDA level improvement.

In maintaining good sperm quality, the balance between enzymatic antioxidant and non-enzymatic antioxidant was needed[9]. Extract administration in various doses was expected to be able to improve sperm quality and to reduce MDA level caused by 2-ME exposure. Dose determination is based on active and sub-chronic toxicity examinations of mangosteen pericarp extract for 28 days. During this period,the extract did not indicate significant toxic effects. This condition commonly found on natural ingredients which contain multiple chemical compounds instead of singular compound. These components cooperate causing particular effects. However as the dose increased, more chemical compounds accumulated cause effect reduction[10,11].

Based on research data presented above, it can be concluded that certain dose *G. mangostana* pericarp extract given in certain period could improve the number of spermatogenic cells and sperm quality which leads to successful fertility process. But, the higher dose of extract given might reduce the number of spermatogenic and lower sperm quality which may lead to oligospermia or even azoospermia. When azoospermia condition reached, it can be said that high-dose of extract given in long period leads to infertility. Extract of *G. mangostana* in mice could oxidative stress in brain, too[12].

## CONCLUSION

*G. mangostana* percarp extract (low dose: 25 and 50 mg/kg) intake improved the number of spermatogenic cells, sperm quality (motility, morphology, viability), and lower MDA level of mice which have been exposed by 2-ME; but in high dose (100 and more than 100 mg/kg extract may lower the number of spermatogenic cells and sperm quality of mice which have been exposed by 2-ME.

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The authors declare that they have no conflicts of interest in the research.

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