

# The Administration of Moringa Seed Oil (Moringaoleifera L.) Inhibited the Decrease of Leydig Cell and Testosterone Level in Male Wistar Rats (*Rattusnorvegicus*) that are Exposed to Cigarette Smokes

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**Abstract:** *Moringaoleifera L. Kelor in Indonesian ,the ripe seed is believed to have anti-aging properties. Cigarette smoke is one of the free radicals responsible for aging process. Decrease in testosterone is one of the indicators of the occurrence of aging process. Moringa seed oil (MSO) contains a number of chemical substances: alkaloid, phenol, flavonoid, sterol, tannin, saponin, protein, mineral, vitamin A, C, and E. This study was conducted to explore the effects of MSO on testosterone level and generate histology description of Leydig cell in Wistar rats. The study was a true experimental study that applied posttest-only controlled group as its research design. The samples in this study were 32 male healthy Wistar rats aged 6 months with body weight of 200-230 grams, which were divided into 2 groups, with 16 rats each. The control group was administered with plasebo (aquadest) with 1 ml/100 grams bw/day dosage, while the treatment group was administered with MSO with 2ml/kg bw/day dosage immediately after being exposed to 4 sticks of cloves (kretek) cigarettes. The treatment was given once a day for 14 days. On day 22, the rats were euthanized, then measured testosterone level with ELISA method and testicle surgery was conducted to generate microscopic supply, calculate the number of Leydig cells. The analysis result of the Leydig cells calculation showed that the average total /1 HPF in the control group was  $20.33 \pm 4.19$  and in the treatment group was  $32.41 \pm 7.26$  with  $p \leq 0.001$ . The average value of testosterone level in the controlled group was  $2.237 \pm 0.401$  and the treatment group was  $2.938 \pm 0.326$ . With an independent sample t test, the number of Leydig cells in the treatment group was more significant ( $p \leq 0,001$ ) and testosterone level showed significantly higher in the treatment group ( $p \leq 0,001$ ). The concluded that administration of MSO potentially inhibits the decreased of Leydig cells and testosterone level in male Wistar rats that were exposed to cigarette smoke.*

**Keywords:** moringa seed oil, MSO, Leydig cell, testosterone level, exposure to cigarettesmoke

## 1. Introduction

The new concept in Anti Aging Medicine suggests that aging is preventable, inhibitable, and that one's condition can be reversed back to optimal condition. Aging process is influenced by internal and external factors. Internal factors are triggered by free radicals, decreasing hormone, glycosylation, methylation, and apoptosis processes, degrading immune system, and gene, while external factors are primarily triggered by unhealthy lifestyle, unhealthy diet, inappropriate habit, environmental pollution, stress, and poverty. Cigarette smoke plays role in aging process[1]. Cigarette smoke contains a number of hazardous chemical substance, namely nitrogen, ammonia, CO<sub>2</sub>, H<sub>2</sub>O, NO, SO, CO, nicotine, tar and metal[2]-[3]. These constitute free radicals that will cause oxidative damage in certain protein, lipid, and DNA[4]-[5]. Oxidative stress in testicle that is caused by tobacco product leads to a significant increase in peroxidation lipid, malondialdehyde (MDA), and decreasing rate of glutathione (GSH), catalase, and Superoxide Dismutase (SOD)[5]-[6]. Nicotine can work in GABA

neurons which will inhibit GnRH's activity that functions as a testosterone regulator through FSH and LH feedback mechanism[7]. The cadmium content causes distraction in adenylyl cyclase enzyme activities in the Leydig cell membrane, which in turn leads to the synthesis of testosterone hormone[8].

Testosterone do not only function in sexual and reproductive organs, but also influence muscle growth, development of bone mass, erythropoiesis, cognitive function, and life comfort. The aging in rats is associated with the decreasing concentration of testosterone in the serum which is caused by the decreasing production of testosterone by Leydig cell[9]-[10]. The low testosterone level might cause anatomic impairment as well as disfunction of sexual and reproductive organs. This condition is also experienced when aging process takes place[1]-[11].

Moringa seed oil contains alkaloid, phenol, flavonoid, organic acid, sterol, tannin, saponin, epicatechin, carotenoid, ascorbate, tocopherol, beta-sitosterol, moringin, essential amino acid Kaempferol, macro mineral and micro mineral,

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and unsaturated fat acid[12]-[13]-[14]. The high level of antioxidant that synergizes with other contents brings about protection effect on the Leydig cell steroidogenesis process, which is capable of repairing testicle's damage, stabilize testicle membrane, as well as increase testosterone level and number of Leydig cell in rats through the oxidative stress inhibition mechanism[15]-[16]-[17]-[18]-[19].

## 2. Material and Methods

### 2.1 Material

The subject of the study were 32 healthy Wistar strain male rats (*Rattus norvegicus*) aged 6 months, with weight ranging from 200 – 230 grams. Integrated Biomedical Laboratory Unit, Faculty of Medicine, Udayana University, Denpasar, Bali. The moringa seed oil was obtained from PT. MoringaOrganik Indonesia. Cloves cigarettes (*kretek*), aquadest, ketamine 10%, xylazine 2%, Hematoxylin-Eosin, ethanol, paraffin, Rat Testosterone and Elisa Kit were obtained from Bioassay Technology Laboratory.

### 2.2 Tool

Stainless steel screw press machine, rat scale, centrifuge, syringe, surgery set, embedding cassette, glass object and cover, blood tube, ointment pot, ELISA

### 2.3 Methods

Prior to being used in this study, the 32 rats were undergoing a 7-day acclimatization. On day 8, they were weighted to determine their average body weight, and thereafter were randomly divided into two groups, namely control group and treatment group. The control group was exposed to 4 sticks of cloves cigarettes and was administered with placebo aquadest at 1 ml/100 grams bw/day dosage, while treatment group was administered with moringa seed oil at 2 ml/kg bw/day dosage, immediately after being exposed to 4 sticks of cloves cigarettes once a day for 14 consecutive days. On day 22, the rats were euthanized, then measured testosterone level with ELISA method and testicle surgery was conducted to generate histology supply and calculate the number of Leydig cell.

**Observation on Leydig cell histology appearance:** The cell size was bigger, round-shaped or polygonal, with core being located in the center, and eosinophilic cytoplasm located between 3-4 seminiferous tubules. The number of Leydig cell refers to the average Leydig cell found in 5 interstitial areas which were explored through zigzag (Z-like) pattern under microscope with 400X magnification level.

**Measurement of testosterone hormone:** Vena blood was taken from the *medial canthus sinus orbitalis* approximately 1 ml amount, inserted into sterile Eppendorf tube, centrifuged with the speed of 3000 rpm for 10 minutes, and was then processed into a serum. 40 µl of serum sample was inserted into the sample tube, and added with 10 µl of anti-T body. 50 µl of Streptavidin-HRP was inserted into the serum sample tube and standard tube; the strip was shaken to mix the substance and was covered with a plastic

wrap, and was then put into an incubator for 60 minutes at 37°C. Subsequently it was examined using ELISA indirect method that was read on wave length of 450 nm using spectrophotometer.

### 2.4 Analysis Outcome

The data processing was conducted using SPSS. The data was analyzed using independent sample t-test.

## 3. Results

The analysis result of the Leydig cell calculation showed that the total average of /1 LPB in control group was  $20.33 \pm 4.19$  and that of treatment group was  $33.41 \pm 7.26$ . With an independent sample t-test, the examination of Leydig cell number with p value =  $< 0.001$  showed in the treatment group was more significant, that there was an actual difference between control group and treatment group.

The average testosterone level of control group was  $2.237 \pm 0.401$  and that of treatment group was  $2.938 \pm 0.326$ . With an independent sample t-test, the examination of testosterone level with p value =  $< 0.001$  showed testosterone levels were significantly higher in the treatment group, an actual difference between control group and treatment group.

## 4. Discussion

### Influence of Cigarette Smoke to Leydig Cell Number

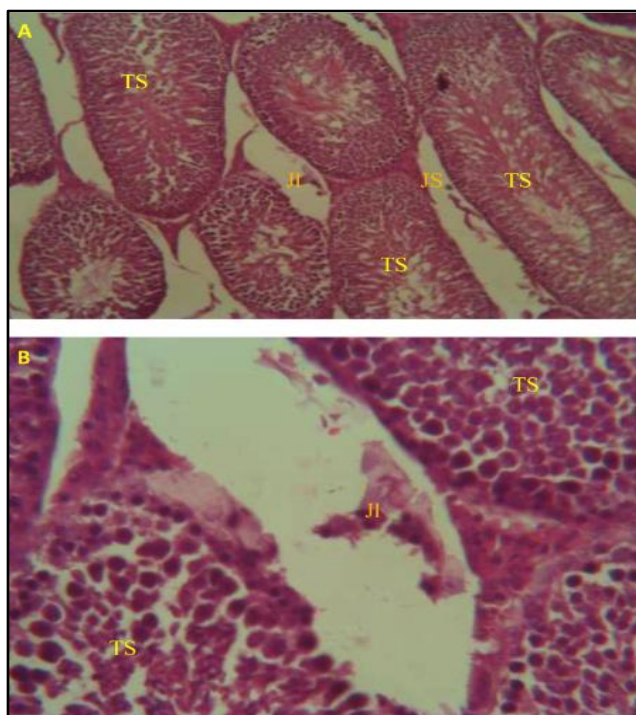
Cigarette smoke has been proven to contain hazardous chemical substances, be full of free radicals, and can induce the formation of oxidative stress in testicle. This causes a significant increase in lipid peroxidation, increase of malondialdehyde (MDA), disturbance to adenylyl cyclase enzyme activities, and decrease of glutathione (GSH), catalase, and Superoxide Dismutase (SOD). Testicle tissue is very vulnerable to oxidative stress due to extremely high oxygen use for cell division, which leads to decrease of intracellular antioxidant in Leydig cell's mitochondria, which in turn causes apoptosis in Leydig cell. Through the effect of luteinizing hormone (LH) in anterior hypothalamus - hypophysis, the increasing ROS will lead to inhibition of GnRH hormone, which causes decrease of LH hormone secretion, which in turn will hinder Leydig cell maturation, and eventually decreases the number of Leydig cell[5]-[6]-[7]-[8].

### Influence of Cigarette Smoke Exposure to Testosterone Level

Cigarette smoke content might cause oxidative stress in testicle, which leads to inhibition of testosterone hormone synthesis caused by decrease of steroidogenesis process in the testicle[20]. It is suspected that the cause is the decrease in number of Leydig cell, decrease in production of cyclic adenosine monophosphate (cAMP), decrease in production of steroidogenic acute regulatory (StAR) protein and P450<sub>sc</sub> (side-chain cleavage) which was an enzyme generated from the release of side-chain of cholesterol and formation of free radicals[21].

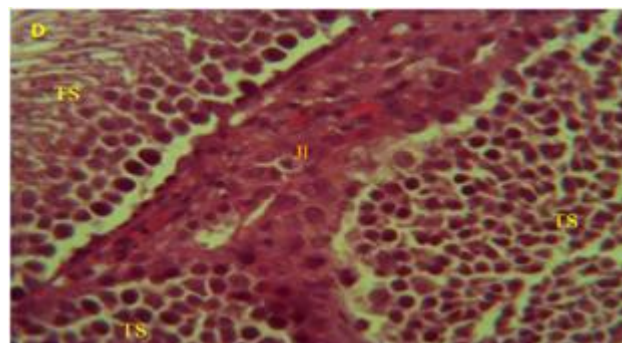
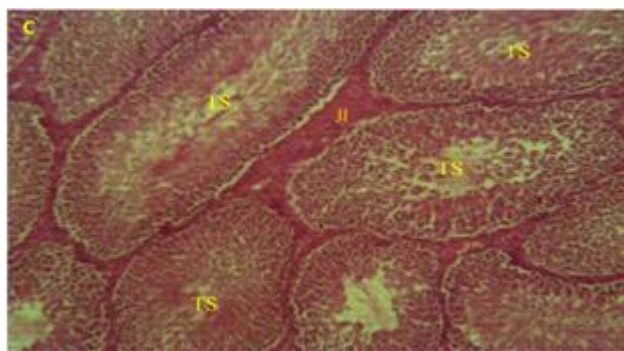
### Moringa Seed Oil's Influence on the Number of Leydig Cell and Testosterone Level

The number of Leydig cell and testosterone level with  $p$  value =  $< 0.001$  showed a visible difference between control group and treatment group. This is due to the fact that moringa seed oil contains alkaloid, phenol, flavonoid, organic acid, sterol, tannin, saponin, epicatechin, carotenoid, ascorbate, tocopherol, beta-sitosterol, moringin, essential amino acid, kaempferol, macro mineral, micro mineral, and unsaturated fat acid[12]-[13]-[14]. The high level of antioxidant that synergizes with other contents creates protection effect to the Leydig cell steroidogenesis process, can cure testicle damage, stabilize testicle membrane and increase testosterone level and number of Leydig cell in rats through oxidative stress inhibition mechanism[15]-[16]-[17]-[18]-[19].



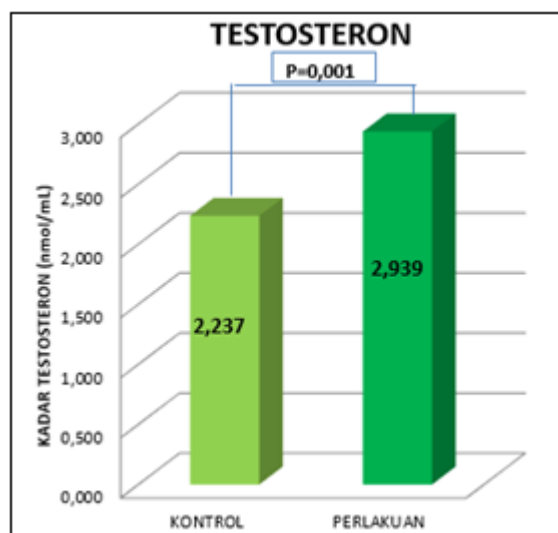
**Image 1:** Histology of the control group

A. Leydig cell in the interstitial tissue (JI) of rats testis inspected in a microscopic field at X 100. B. Leydig cell in the interstitial tissue of rats testis inspected in a microscopic field at X 400



**Image 2:** Histology of the treatment group

A. Leydig cell in the interstitial tissue (JI) of rats testis inspected in a microscopic field at X 100. B. Leydig cell in the interstitial tissue of rats testis inspected in a microscopic field at X 400



**Image 3:** Testosterone level in control group and treatment group

## 5. Conclusions

Moringa (*Moringaoleifera L.*) seed oil potentially inhibits the decreased of Leydig cells and testosterone level in male Wistar rats. It is necessary to conduct clinical test in order to identify the effectiveness of moringa seed oil in inhibiting aging process in humans, possibly with a longer duration or higher doses.

## 6. Ethical Clearance

This study has met the ethics-associated requirement and been granted the Animal Ethics Approval Certificate from Animal Ethics Committee, Faculty of Veterinary, Udayana University.

## 7. Conflict of interest

This thesis is independent from any conflict of interest.

## 8. Funding

This thesis is fully funded by the researcher.

## 9. Author Contributions

The writer contributes fully in all parts of the study activity from sample collection to data analysis under the supervision of a thesis supervisor.

## 10. Plagiarism free

This thesis is a self-work and contains no element of plagiarism.

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