

Unripe Papaya Seed Ethanol Extract (*Carica papaya*, Linn.) Inhibits FSH and LH of Male Mice (*Mus musculus*)

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Family planning is a program designed to control the rate of population growth extensively in the country of Indonesia. In order to avoid of potential destructive effects of population explosion, a nation-wide Family Planning Program has been adopted long-standing in Indonesia. As to make it successful, family planning would have to involve with participation of both males and females. It was aimed to prove that the ethanol extracts of a local plant, Bali papaya, have antifertility function via inhibiting the secretion of FSH and LH. Randomized post-test only control group design was selected for this study. 38 male mice (strain Balb-C) were used and randomly divided into two equal groups. Only 0.5 ml double-distilled water was applied orally for 36 days to controls; and 0.5 ml ethanol extract of unripe papaya seed with dose of 20 mg/20g BW/day for 36 days to treatments. At the end of the experimental duration, blood samples of each individuals of both of the groups were collected for FSH and LH measurement. FSH and LH levels in controls were recorded as 3.379 and 15.718 mIU/ml, respectively. In experimental animals, the level of FSH was declined to 2.053 mIU/ml, while LH was measured as 8.626 mIU/ml. The reduction of both of the hormone was probably related to the active substances of extract. Administration of the ethanol extract of papaya seed reduces the average level of FSH and LH ($p < 0.05$), significantly.

Keyword: Unripe papaya seed ethanol extract, FSH, LH, male mice.

Increase in population spurt and the quality of human resources are two things that should be the attention of country. Indonesia is always covered with high population density. Also, the welfare of the nation depends on the quality and competence of its human resources to sustain the nation's future. Indonesia is currently experiencing a population spurt of 1.49% every year, the population in Indonesia is now around 250

million people and is the country with the densest population number four in the world.¹

Statistical projection may predict Indonesia will have a population of more than 270 million in 2025, more than 285 million in 2035 and 290 million in 2045. Such population spurt will be a problem if not well controlled. The rate of population growth in Indonesia is still quite high, in order to avoid the population spurt

and the establishment of a happy and prosperous small family norm, the family planning program was established. Family planning is a national program in Indonesia, in order of family planning program to succeed, both men and women must participate. In fact, the family planning program is still dominated by women that is 95% of the total program participants. The reason for the low participation of men in family planning because male contraception that available is very limited in type. That's why the development of contraceptive technology should be more directed to men.^{1,2}

Research by Asmarinah *et al.* (2005) that gave papaya seed extract treatment 70-80 mg/ml *in vitro* found that papaya seed extract can significantly decrease sperm motility and spermatozoa integrity, and unable to penetrate into cervical sap. The current male contraceptives are very limited, it is necessary to strive for the development of the ideal male contraceptive drugs, one of them by looking for alternative materials from plants that have antifertility properties. Plants that have antifertility properties allegedly grow in Indonesia because Indonesia is a tropical country rich in various types of plants. The study of chloroform extract of papaya seeds as antifertility in white mice was reported to suppress spermatogenesis without showing toxic effects and libido disorders.³⁻⁷

Spermatogenesis is controlled by various afferent impulses in the central nervous system integrated into the hypothalamus region, which contain fibers and nerve cells rich in biogenic amines (norepinephrine and dopamine) that act as neurotransmitters that regulate gonadotropin-releasing hormone. While gonadotropin-releasing hormone (GnRH) will stimulate the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH). The control of spermatogenesis in the testes by the hypothalamus and anterior pituitary is known as the hypothalamus-pituitary-testis axis.² The hypothalamus is central to the integration of reproductive hormones, located below the cerebral ventricle, weighing 4 grams. The peptide-ergic nerve of the hypothalamus releases gonadotropin-releasing hormone. Gonadotropin-releasing hormone is a decapeptide containing ten amino acids secreted from the pre-optic and arcus nucleus of the hypothalamus. Gonadotropin-

releasing hormone is released in pulsatile and periodically. Once secreted, gonadotropin-releasing hormone has a short half-life of about 5-7 minutes in the portal bloodstream. Gonadotropin-releasing hormone is also present in other tissues, but its function is unknown. Follicle stimulating hormone and luteinizing hormone is a glycoprotein consisting of 2 subunits of polypeptide ring, \pm ring and ring.^{2,4}

This study was conducted to prove that the local Bali papaya ethanol extract has an antifertility by inhibiting the secretion of FSH and LH hormones and also look for possible alternative contraceptives for men.

MATERIAL AND METHOD

Study design

Randomized post-test only control group design was selected for this study, the experimental mouse was randomly divided into two different group. First group is the intervention group (I) consisting of 19 male mice, given oral ethanol extract of papaya seeds with a dose of 20 mg/ 20 grams body weight/day for 36 days. The second group is control group (C) consisting of 19 male mice given aquabidest per oral as much as 0.5 cc for 36 days. The intervention was given using feeding tube every day at 09.00–10.00 AM.

After 36 days of intervention, blood sample was collected from both group, blood sample was taken from sub conjunctive eye. Examination of LH and FSH hormone levels were done with ELISA method. All intervention and laboratory examination was done in Pharmacology Laboratory, Faculty of Medicine Udayana University, Bali-Indonesia.

Experimental Animal

The experimental animal of this study is male Balb C strain mice aged 12 weeks with body weight 20-22 gram. Plastic boxes with 40 cm \times 15 cm \times 10 cm dimension were used as an animal cage. The base of the cage was covered by husk, and the upper part was covered with wire gauze. The cage was cleaned and husk was replaced every two days. Feeding box was cleaned and filled every day, and the cage was kept clean and dry with a stable temperature. The mice were given the opportunity to adapt within a week with 12 h of bright and 12

h of dark. Mice are given water if necessary and standard pellets as much as 12–20 g per day. The sick mice were not used for the study.

Preparation of papaya seeds extract

The immature papaya fruit is opened and the seeds are taken. After the seeds are collected then put into hot ethanol until submerged approximately one minute with the aim of stopping the cell metabolism and enzymatic reactions. Papaya seed drained and then dried in open place with good air circulation and avoid direct contact with sunlight. This drying process lasts about two weeks. Papaya seeds that have been dried and then mashed to a powder obtained until obtained about 2.3 kg of papaya seed powder.

Dry powder from papaya seeds as much as 2.3 kg extracted by maceration using ethanol about 2.5 liters. After 24 hours the ethanol extraction and then separated by filtering added a new ethanol solvent for the subsequent extraction process. This extraction process is repeatedly done until all components of the secondary compound/metabolite are extracted (extraction is done seven times). All ethanol extracts obtained were evaporated with

vapor rotary evaporator until ethanol extract was obtained.

Statistical analysis

Normality test using was used to determine the normal distribution of FSH and LH values between the control group and the intervention group, the t-independent test was used to compare the mean between the FSH and LH values between the intervention group and the control group. The value is considered significant if $p < 0.05$.

RESULTS

This study used 19 male Balb C rats as intervention group (got 20 mg / 20 gr BW / day papaya seed extract) and 19 male rats of the same type as a control group (0.5 cc of aquabidest). Analysis of normality test of FSH and LH levels between control group and intervention group can be seen in table 1.

Based on table 1, all data has a normal distribution ($p > 0.05$), and then an independent t-test was used to compare the FSH and LH level between intervention group and control group.

Table 1. Normality Test

Variable	n	Mean ± SD	Kolmogorov Smirnov-Z test
FSH (mIU/mL)			
Intervention Group	19	2.05 ± 4.68	0.71*
Control Group	19	3.37 ± 5.71	0.20*
LH (mIU/mL)			
Intervention Group	19	8.62 ± 1.44	0.20*
Control Group	19	15.71 ± 2.09	0.20*

*Normal distribution ($p > 0.05$)

Table 2. Comparison of FSH and LH level between intervention and control group

Variable	n	Mean ± SD	t	p-value
FSH (mIU/mL)				
Intervention Group	19	2.05 ± 4.68	7.81	0.001*
Control Group	19	3.37 ± 5.71		
LH (mIU/mL)				
Intervention Group	19	8.62 ± 1.44	12.11	0.001*
Control Group	19	15.71 ± 2.09		

*significant ($p < 0.05$)

Comparison of FSH and LH level between both groups can be seen in table 2.

The mean FSH values in the intervention group were 2.05 ± 4.68 ng/mL and the control group was 3.37 ± 5.71 , through statistical analysis there was a significant difference in the FSH values between the intervention group and the control group ($p < 0.05$), the FSH value are higher in the control group (Table 2).

The mean value of LH in the intervention group was 8.62 ± 1.44 ng / mL and the control group was 15.71 ± 2.09 ng / mL, through statistical analysis there was a significant difference in the LH value between the intervention group and the control group ($p < 0.05$), the LH value is much higher in the control group then the intervention group (Table 2).

DISCUSSION

There was a significant difference in FSH and LH levels between the control group and the treatment group (Table 2). The decrease in the amount of the hormone FSH and LH is probably caused by the active ingredients contained in the extract of young papaya seeds such as steroid, triterpenoid, and alkaloids, where the substance is suspected to be antifertility. Active substances contained in papaya seeds can affect cytotoxic, anti-androgen or estrogenic effect.^{1,7} While Mendez *et al.* (2016) reported that the alkaloids contained in papaya seeds had cytotoxic effects. The papaya seed extracted by ethanol will contain chemicals such as alkaloid salts, quaternary alkaloid alkaloids, oxidized amines, anthocyanates, glycosides, tannin saponins and carbohydrates and alkaloid group antifertility substances.^{8,9}

The hormones estradiol and progesterone contained in papaya seed ethanol extract will inhibit the process of spermatogenesis by inhibiting the function of the hypothalamus gland and anterior pituitary function. These hypothalamic glandular alteration will cause binding of GnRH secretion, while in the anterior pituitary gland will cause the secretion of FSH and LH also inhibited. Estradiol will cause negative feedback to the hypothalamus and anterior pituitary, causing GnRH and gonadotropin (FSH and LH) hormones to be inhibited.¹⁰ While the hormone progesterone will inhibit the secretion of FSH resulting in

disruption of spermatogenesis process.^{10,11} GnRH serves to stimulate the secretion of FSH and LH, while the regulation of spermatogenesis done by FSH, LH and testosterone hormone. FSH and testosterone have very important role in the process of spermatogenesis because FSH and testosterone hormone showed synergistic work in some experiments manipulation of testicular function.¹¹

FSH will stimulate the manufacture of proteins that bind to androgens in the seminiferous tubules and stimulate the number of germ cells to be more sensitive to testosterone, where the hormone testosterone is indispensable in the process of spermatogenesis.¹¹ The effect of FSH on the process of spermatogenesis occurs through Sertoli cells, because on the surface of Sertoli cells located in the basal compartment contain FSH receptors. The function of FSH against Sertoli cells is to stimulate adenylcyclase to improve cyclic AMP, stimulate protein-kinases and conduct phosphorilase processes from proteins. Sertoli cell function is very vital in the process of spermatogenesis.^{2,12}

Sertoli cells function are to maintain and provide nutrition on the number of spermatogenic cells to be able to proliferate and deffreniate become spermatozoa. Besides serving as a feeder to the number of spermatogenic cells, this cell also produces growth factor, androgen binding protein (ABP).¹³ Growth factor is one of the most important factors in the regulation of spermatogenesis process.¹⁴ If there is an alteration on FSH level will cause disruption of Sertoli cell function, such phenomenon could causes the spermatogenesis process is disrupted as well. In the process of spermatogenesis other than testosterone, also required FSH and LH.¹³ FSH stimulates Sertoli cells to form androgen binding proteins (ABP). The hormone testosterone produced by Leydig cells will be transported by ABP with a very high concentration to the spermatogenesis site. FSH also stimulates the start of the spermatogenesis process while the hormone testosterone itself with high intra-testicular concentration will maintain the process of sepermatogenesis.^{14,18}

Androgen binding protein (ABP) is a necessary substance to transport testosterone into the lumen of the seminiferous tubule and spermatogenic cells. This hormone is required for metabolic activity and maturation of spermatozoa,

thus the hormone testosterone is indispensable in the continuity of spermatogenesis process. The influence of LH in spermatogenesis occurs through Leydig cells because on the surface of this Leydig cell there are many LH receptors. This LH will stimulate Leydig cells to produce testosterone. This testosterone will be bound by androgen binding protein (ABP) and carried to the target tissues within the seminiferous tubules to maintain the spermatogenesis process. If the hormone testosterone is altered then the spermatogenesis process will also be disrupted.¹⁶

These spermatogenic cell disorders may also be due to a decrease in the hormone gonadotrophin and testosterone hormones. The decrease in these hormones causes metabolic disorders in spermatogenic cells in the seminiferous tubules¹⁶. Spermatogenic cell metabolic disorders may also be caused by cytotoxic effects of alkaloids contained in papaya seeds, administration of cytotoxic substances such as alkaloids would cause spermatogenic cell metabolic disorders.^{17,18} The most important metabolism is glycolysis, which changes glucose to pyruvic acid, lactic acid and produces ATP. This ATP is the energy source of spermatogenic cells for protein synthesis process in seminiferous tubules. The presence of these metabolic disorders will cause spermatogenic cells to be damaged or degenerated and vacuoles occur eventually in lysis.²

The occurrence of spermatogenic cell decline and the occurrence of vacuolization in seminiferous tubules is caused by spermatogenic cells degeneration. The situation may be due to the decrease of glucose transport into the spermatogenic cells so that the cell lacks energy sources. Spermatogenesis can only take place well if the supply of nutrients through the testicular vascular system is in good condition, adequate hormone supply and normal germ cell development.¹⁴

The results of this study are also consistent with the research by Chinoy and Ranga (1984) who reported that grain extract of papaya seeds in male mice led to suppression of spermatogenesis process, reduced diameter of seminiferous tubules, and decreased the number of children compared to controls.¹⁵ Another study by Lohiya *et al.* (1999) reported that chloroform extract of papaya seeds

can suppress spermatogenesis without showing toxic effects and libido disorders.⁶

CONCLUSION

There was a significant difference to the FSH and LH parameters between control and intervention groups. Provision of unripe papaya seed ethanol extract decreased the average number of FSH and LH levels. Further research needs to be conducted about the toxicity test of the ethanol extract of young papaya seed. If the study on animals is sufficient and safe, it is necessary to perform another research on humans in order to seek a new alternative for male contraception.

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