

**Unripe Papaya (*Carica papaya* L.) Seed Hexane Fraction Extract
Inhibits Male Mice (*Mus musculus*) Spermatogenesis
Stronger Than Unripe Papaya Seed Methanolic Extract**

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Background: Men and women have the same rights and obligations in family planning program. Practically, participation in family planning program is still predominated by women while participation of men is still low. Low participation of men in family planning program is due to very limited choices in male contraceptive method. In recent years, studies have been refocused on investigating traditional plants as antifertility herbal medicine for men. Studies on antifertility effects of unripe papaya seeds have been done. However, similar studies on hexane fraction extract have not yet been done. In this study, unripe seeds were collected from local Balinese papaya (*Carica papaya*) fruits. **Objective:** This study was aimed to assess the effect of unripe papaya seeds hexane fraction extract on spermatogenesis and the testosterone level of male mice. Hexane fraction extract of unripe papaya seeds contains glycosides and triterpenoids, which is assumed to have an antifertility property, so it can be used as a male contraceptive, although the mechanism of action remains to be elucidated. **Method:** This study used pre-test and post-test control group design, using 30 male mice of balb C strain, 12 weeks of age, weighing 20-22 gram, which were randomly grouped into 3 groups, each consisting of 10 male mice. One control group (P0 = control group) was given double-distilled water, and two treatment groups were given hexane fraction extract of unripe *Carica papaya* seeds 20 mg/20gram/day, and methanolic extract of unripe *Carica papaya* seeds 20 mg/20 gram/day (P1 and P2, respectively). After 36 days of treatment, evaluation of the testes and blood of the male mice was conducted. **Results:** Data were analysed by normality test of Kolmogorov-Smirnov Goodness of Fit, homogeneity test, and Anova test. This study showed that spermatogonia A, primary pachytene spermatocytes, spermatid and Sertoli cells were decreased significantly ($p < 0,05$) but Leydig cells and testosterone levels were not decreased significantly ($p > 0,05$). **Conclusion:** It can be concluded that hexane fraction extract of unripe *Carica papaya* seeds can decrease the mean number of spermatogonia A cells, spermatocyte of primary pachytene spermatocytes, spermatid and Sertoli cells better than methanolic extract of unripe *Carica papaya* seeds.

Keywords: Spermatogenesis, testosterone, *Carica papaya*, methanolic, extract.

DOI: 10.15562/bmj.v5i2.224

Cite this Article: Satriyasa, B.2016. Unripe Papaya (*Carica papaya* L.) Seed Hexane Fraction Extract Inhibits Male Mice (*Mus musculus*) Spermatogenesis Stronger Than Unripe Papaya Seed Methanolic Extract. Bali Medical Journal 5(2): 93-96. DOI:10.15562/bmj.v5i2.224

INTRODUCTION

In the effort to prevent population explosion, Family Planning Program should involve the participation of both male and female individuals. In reality, however, the program is greatly dominated by female participants than their male counterparts.

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The low participation of men in Family Planning Program is partly due to the fact that only a limited number of male contraceptive devices are currently in existence. Therefore, it is extremely important that appropriate technology be developed to produce contraceptives that can be used specifically by men.¹

As male contraceptives are very limited in types, efforts to develop more ideal male contraceptive drugs is crucial; particularly the effort to find alternative effective substances derived from natural resources.

Unripe papaya (*Carica papaya* L.) seeds are known to be one of the natural substances that have

anti-fertility effect. The purpose of this study is to investigate whether hexane fraction of unripe papaya seed extract has a stronger effect to inhibit the process of spermatogenesis than the methanolic fraction. In this study, unripe seeds were collected from local Balinese papaya fruits.

STUDY METHOD

This study was an experimental research using *pre-test post-test control group design* (Campbell and Stanley, 1968). The study used 30 male mice strain Balb-C which were allocated by simple random sampling into three groups: control group (P0); treatment Group 1 (P1); treatment group 2 (P2). Anova analysis was also done to find out the average differences among groups and comparative analysis to find out the differences between control group and treatment groups. Significance level was determined with $\alpha \leq 0.05$

RESULTS

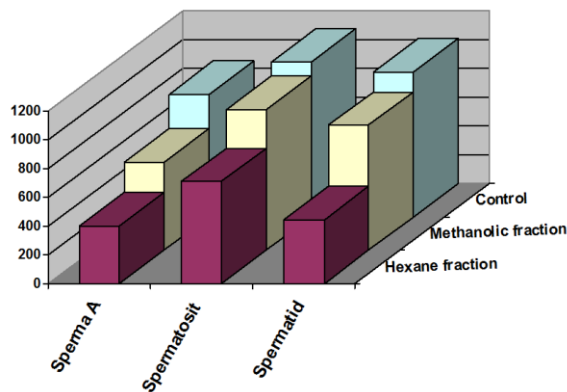


Figure 1

Histogram showing the levels of spermatogonia A, pachytene primary spermatocyte, spermatid, in Balb-C strain mice after treated with 0,5 ml hexane and methanolic fraction extract for 36 days.

DISCUSSION

The Effect of Unripe Papaya Seed Extract Fractions on The Number of Spermatogonia a Cells

The analysis of *one-way Anova* showed that the average number of Spermatogonia A cells in the control group was 852, treatment group 1 was 402, and treatment group 2 was 610.

The analysis of LSD (Least Significant Difference) showed that the number of spermatogonia A cells in the control group differed significantly from that of group 1 ($p=0.000$) and group 2 (0.002). Treatment group 1 was significantly different from treatment group 2 ($p=0.006$). The treatment group given hexane fraction extract of unripe papaya seeds showed greater decrease in the number of spermatogonia A cells than in the treatment group given methanolic extract of unripe papaya seeds.

The Effect of Unripe Papaya Seed Extract Fractions on the Number of Primary Pachytene Spermatocytes

The result of *one-way Anova* analysis showed that the number of primary pachiten spermatocyte cells in the control group was 1080, in treatment group 1 was 710 and in treatment group 2 was 980. The three groups showed significant differences ($p=0.000$).

The result of LSD (Least Significant Difference) analysis showed the number of primary pachiten spermatocyte cells in the control group differed significantly from that in the treatment group 1 ($p=0.000$) and group 2 ($p=0.002$). The difference was significant between both treatment groups ($p=0.000$). The treatment group treated with hexane fraction of unripe papaya seed extract showed a greater decrease of primary pachytene spermatocytes than the one treated with methanolic fraction of unripe papaya seed extract.

The Effect of Unripe Papaya Seed Extract Fractions on Average Number of Spermatid Cells

One-way Anova analysis showed that the average number of spermatid cells in the control group was 1010, in the treatment group 1 was 448, and in treatment group 2 was 870. The three groups clearly showed significant differences ($0,000$).

The result of LSD (Least Significant Difference) analysis showed significant differences between the control group and treatment group 1 ($p=0.000$), and between control group and treatment group 2 ($p=0.037$). The result of comparison between treatment group 1 and 2 was significant ($p=0.000$). Hexane fraction of unripe papaya seed extract administered to treatment group 1 reduced spermatid cell number more significantly than the methanolic fraction administered to group 2.

The Effect of Unripe Papaya Seed Extract Fractions on the Average Decrease of Spermatogonia a Cells

Reduction of spermatogonia A cells might be due to the active substances contained in the hexane fraction of unripe papaya seed extract (steroid and triterpenoid) and in the methanolic fraction of the same unripe papaya seed extract. These substances are thought to have anti-fertility properties.

Reduction of the number of spermatogonia A cells was probably due to the effect of estradiol (E2) as well as progesteron (P4) contained in the hexane extract. Both hormones are responsible in the inhibition of FSH and LH secretion. Estradiol supresses the hypothalamus and anterior hypophysis and inhibits GnRH and gonadotropin (FSH and LH).² On the other hand, progesteron

inhibits FSH secretion and causes disturbance in the process of spermatogenesis.³

Inhibition of FSH further disturbs the process of mitosis and poliferation of spermatogonia A cells, because FSH is vital in the later process. If there is disturbance in FSH, there disturbance will also occur in both spermatogonia and subsequent spermatogenesis process.

The Effect of Unripe Papaya Seed Extract Fractions on the Average Decrease of Primary Pachytene Spermatocytes

Decrease of the number of primary pachytene spermatocytes was suspected to be due to the effect of estradiol (E2) and progesteron (P4) contained in hexane fraction of the extract. Both hormones inhibit FSH and LH secretion. Estradiol supresses hypothalamus and anterior hypophysis, thus inhibits GnRH and gonadotropin (FSH and LH). FSH also has a very important role in enhancing the maturation stage and slowing meiosis of primary pachytene spermatocytes (Turek, 2005). Meanwhile, progesteron can inhibit FSH secretion, resulting in disturbance of spermatogenesis process.³

Spermatocytes may decrease in number because of the disturbance in Sertoli cells function, which initiate the decrease of lactate and piruvate supplies, known as energy sources for primary pachytene spermatocytes. Thus, the lack of lactate and piruvate will inhibit the synthesis and activity of lactate dehydrogenase enzyme (LDH-X) in primary pachytene spermatocytes, which finally will cause degeneration of the spermatocytes (Jutte et al., 1978). The conversion process of primary spermatocyte cells to secondary spermatocyte cells and the creation of spermatids is governed by testosterone and FSH.⁴ Reduction of FSH and testosterone will inhibit glucose transportation into spermatocyte cells and protein synthesis.

Lohiya et al. report that administration of chloroform extract of papaya seeds may lead to a significant decrease in spermatocyte cells, loss of cytoplasmic organelles and cell membranes damage.⁵ If spermatocytes are disrupted and degenerated, the cells will be phagocytized by Sertoli cells, thus reducing the number of spermatocyte cells. Administration of MCP 1 and ECP 1 (purified papaya seeds) for 90 days caused vacuolization and the absence of spermatocyte cells. It was reported that both substances (MCP 1 and ECP 1) were effective as contraceptives in male mice because they were reversible and cause no adverse effects.⁵ Meanwhile, Ucha et al. reported that oral administration of papaya seeds extract to male mice for 3 weeks caused the absence of spermatocytes and spermatozoas.⁶

The Effect of Unripe Papaya Seed Extract Fractions on the Average Decrease of Spermatid Cells

The reduction of spermatid cells number was probably caused by active substances in hexane fraction extract (steroid and triterpenoid) as well as in methanolic fraction extract (alkaloid) of local unripe Balinese papaya seeds, which were suspected to have anti-fertility effect. These substances also caused cytotoxic, anti-androgenic and estrogenic effects. Cytotoxic effect causes inhibition of the germinal cells metabolism.⁵

The decrease of spermatid cells may be caused by the inhibited function of Sertoli cells, which lead to lactate and piruvate supplies reduction. Lactate and piruvate are energy sources of spermatid cells.⁷ Spermatid cells can be decreased by several mechanisms such as disturbance in meiosis process, disturbance in primary process of spermiogenesis, the escape of spermatid into seminiferous tubules lumen and spermatid cells apoptosis.

FSH plays a role in regulating cytoskeletal structure of Sertoli cells, which is important for binding spermatid cells, while testosterone functions to regulate the association between ectoplasmic specialization (ES) and Sertoli cells through cell adhesion molecules (CAMs) in Sertoli cells. Reduction of FSH level damages the cytoskeletal structure of Sertoli cells, decreases the power spermatid cells adherence to Sertoli cells, which then causes the release of spermatid cells into the lumen of seminiferous tubules.⁸ FSH facilitates maturation of spermatid cells to become spermatozoa during the process of spermatogenesis. The decrease of FSH and testosterone level will inhibit the protein synthesis and finally initiate degeneration of the spermatid cells.

The result was similar to that of the study done by Lohiya et al. They reported that administration of chloroform extract of papaya seeds could cause significant decrease of spermatid cells, loss of cytoplasmic organelles and membrane disruption. Moreover, Ucha et al. found that oral administration of papaya seed extract to male mice caused absence of spermatid cells.^{5,6}

CONCLUSION

It can be conluded that hexane fraction of unripe papaya seed extract reduces spermatogonia A cells, primary pachytene spermatocytes, and spermatid cells significantly, and the reduction is greater than that caused by methanolic fraction of unripe papaya seed extract.

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