



Original Article

Antifertility effect of betel nut (*Areca catechu L*) in male rat

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ABSTRACT

Background: Betel nut (*Areca catechu L*) are traditional beverages in Jambi. Betel nut high dose has a cytotoxic effect on testis and other organs—limited data about the effect of betel nut as male antifertility.

Objective: This study aimed to determine the effect of infertility on betel nut use at a dose of 50 mg/kg body weight (BW) in male rats.

Methods: Ten male Sprague Dawney rats that had passed the fertility test, aged 2-3 months and weighing 150-200 grams were used in this study. The rat was grouped into two groups randomly. Group 1, as the negative control, were received aqua dest, and group 2 was treated by betel nut with dose 50 mg/kg BW for 35 days. When terminated, testis weight weighed using micro scales. Blood collected for measuring testosterone levels. Histopathology assessment of testis used Hematoxylin Eosin Staining and sperm counting from cauda epididymis.

Results: The weight of testis in the group received betel nut was lower than the control group. The histopathology of testis showed shrunk, reduced the diameter of seminiferous tubules, and like cytolytic lesions in the germinal layer—the total sperm number and progressive sperms also lower than the control group ($p < 0.05$). There was no abnormality in Leydig cell and interstitium tissue. Male rats after treatment could not impregnate the female rats.

Conclusion: Betel nut at dose 50 mg/kg BW have antifertility activity in male rats.

INTRODUCTION

Population growth is a severe problem worldwide, especially in developing countries such as Indonesia. Overpopulation can cause many problems with health. Contraception is one method to control population growth. There are female and male contraceptives. Male contraceptives are not popular enough than female contraceptives. Male contraceptives have been lower participation of males in family planning.¹

Some medicinal plants have antifertility effects and the potential for contraceptives in males.^{2,3} Extract of papaya seed showed an antifertility effect in mice by lowering sperm concentration, motility, and viability.^{4,5} Extract of *Stelechocarpus burahol* also has an antifertility effect in male mice⁶. Ethanol extract of *Dalbergia sissoo Roxb* stem bark has anti-spermatogenic activity,⁷ *Taraxacum officinale* also has anti-spermatogenic activity.⁸ Pan Masala, which consists of areca nut, catechu, lime, and cardamom, <https://doi.org/10.30595/medisains.v18i2.7588>

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caused sperm morphologic abnormality.⁹ Alkaloid from *Areca catechu* decreased human sperm motility in vitro study.¹⁰ Alcoholic extract of *Areca catechu* doses 300 and 600 mg/kg BW showed antifertility activity.¹¹ Other research also showed that the seed of *Areca catechu* in high doses could cause apoptosis in rats' testis tissue.^{12,13} Betel nut is a common plant in Sumatra. A man commonly consume this for a drink juice as a stamina booster. So, betel nut potentially developed as a male herbal contraceptive.

Herbal drugs are cheaper and safer as compared to synthetic drugs at the appropriate dose.¹⁴ But then, high dose of betel nut, about higher than 250 mg/kg BW, also cause damage in other organ.¹⁵⁻¹⁸ Data on antifertility of betel nut in lower doses were limited. Physiology and anatomical of the rat reproductive system are similar to that of humans. This study's objective is to find out the effect antifertility of betel nut at dose 50 mg/kg BW in rats.

METHOD

Study Design

This is an experimental animal study with the randomized post-test.

Study Site

All the studies carried out in the Biomedical Laboratory, Faculty of Medicine and Health Sciences, Jambi University, except for testosterone level testing were carried out in Jambi Province Health Laboratory.

Plant Material

Green colored raw betel nut seed was purchased from local areca catechu plantation. The betel nut was dried on the oven 50°C for 24 hours and then be powdered. The dose used is 50 mg/kg BW that dissolves in the 3 ml drinking water.

Animal Preparation and Experimental Procedure

Rats were obtained from the animal house of Biomedical Laboratory, Faculty of Medicine and Health Sciences, Jambi University. Ten male Sprague Dawley rats, age 2-3 months, and weight 150-200 gram were used in this study. As a preliminary study, we used the least number of animal samples, with only five rats in each group according to ethical review. After acclimated for seven days, Rats were divided into two groups by pure random. Group 1, as a negative control, were received aqua dest and group 2 treated by betel nut with dose 50 mg/kg BW for 35 days. Powder of betel nut was weighted according to the dose, dissolved with 1 mL aqua dest and given by feeding tube. Rats were housed in plastic cages in the room with 25°C temperature and 50-80% humidity with 12 hours cycle variation between the light and dark and given a standard diet and water ad libitum throughout the study.

Fertility Test

Female Sprague Dawley rats used for a fertility test. A test for testing the fertility of male rats to make pregnant of the female. We used female rats that had labored once before. The male and female mated for seven days before and after male rat treated by betel nut. Rats mated on day 29th, and the treatment continued until the day 35th. The number of litters that labored by the female rats was counted.

Testes Weight Measurement

After the treatment, at the day 36th, male rats were terminated by overdosed anesthesia drug and minor surgery performed to remove the testis. The testis is measured using micro scales.

Testosterone Level

After rat terminated, about 500 µL blood collected from the heart for measuring the level of testosterone. VIDAS Testosterone II used in this research. Analyses were carried out by standard quantitative ELFA technique according to the manufacturer's instructions.

Spermatozoa Analysis

Spermatozoa motility analysis assessed according to modification of the methods described earlier.¹⁹⁻²² Spermatozoa collected from the right caudal epididymis. Caudal epididymis was minced with anatomical scissors in 5 mL prewarmed physiological saline. Approximately 50µL of diluted sperm suspension transferred to each object-glass, and counting was done under a light microscope at ten fields with 400x magnification. Percentage of motile spermatozoa assessed using a graded semiquantitative scale; that is, No tail movement (non-motile), and progressive movement (motile). Total sperm counted by add several motile and non-motile spermatozoa.

Histopathology of Testis

The histopathological assessment used Haematoxylin Eosin stain. One anatomical pathologist assessed with a blind method. The testis' examination variable includes the diameter, percentage of the abnormal seminiferous tubule, and abnormal Leydig cell. The examination was done for 50 tubules in 5 fields. The severity of germ cell degeneration or depletion in seminiferous tubules were classified as minimal if < 5% of tubules affected; slight if 5-25% tubules affected, moderate if 25-50% tubules affected, marked if 50-75% tubules affected and severe >75% tubules affected severity/severely damaged.²³

Statistical Analysis

Data presented with percentage, median (minimum-maximum), mean ± SD, and analyzed with Mann Whitney test with a significant level at p <0.05.

Ethical Consideration

The ethics committee has approved this research of Medical and Health Sciences of Jambi University (No. B/400/UN21.8/PT.01.04/2019).

RESULTS

Effect of betel nut on reduced fertility on male rats shown with the number of litter labored by female rats after mating. The number of litters labored by female rats after mated with a male before treatment ranged from 0-8 litters. After male rats treated with betel nut, there were no litters that labored by females.

The betel nut mechanism on reduced male fertility was analyzed with weight testis, sperm analysis, histopathology of the testis, and testosterone level. The results of weight testis were shown in figure 1. There was a decrease in weight testis. The testis weight reduction after treatment is associated with reduced sperm total and increased atrophy seminiferous tubular. Sperm analysis was showed in figure 2-3, and the result of the histopathology of testis was shown in figure 5A-E. Results of testosterone level after treatment were shown in figure 4.

Results of the histopathological assessment of testis were showed in table 1 and figured 5. The diameter of the seminiferous tubule in the group treated by betel nut was significantly smaller than the control group ($p < 0.05$). The percentage of the abnormal seminiferous tubule in the group treated by betel nut also were significantly higher than the control group ($p < 0.05$). The severity grade of germ cell degeneration in seminiferous tubules is marked (52.40%). The Leydig cell in the group treated by betel nut was no different from the control group.

Table 1. Histopathological assessment of testes

Parameter	Mean \pm SD		p-value
	Control group	Betel nut group	
The diameter of seminiferous tubule	30.40 \pm 2.88	23.80 \pm 3.83	0.015
Percentage of abnormal seminiferous tubule	2.40 \pm 1.67	52.40 \pm 3.18	0.008
Percentage of abnormal Leydig cell	0.92 \pm 1.45	0.13 \pm 0.30	0.368

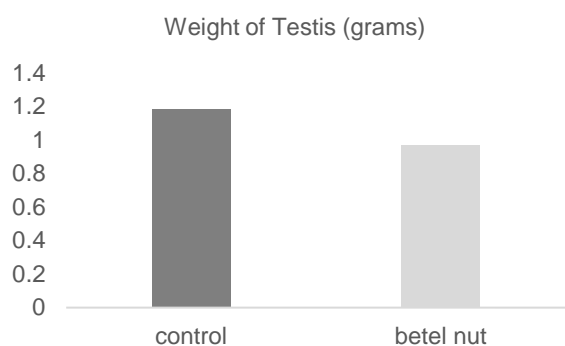


Figure 1. Comparison of weight testis between two groups. The mean weight testis of control groups (1.18 \pm 0.14) was higher than betel nut groups (0.96 \pm 0.47). This difference was not significant statistically ($p = 0.376$).

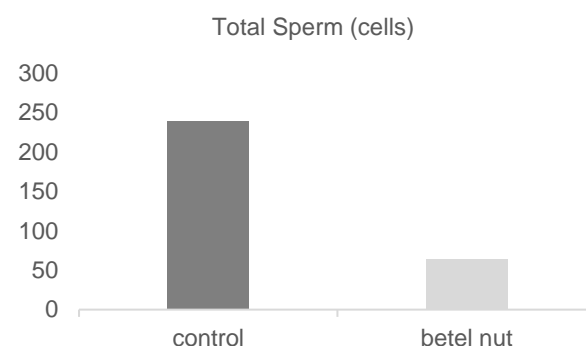


Figure 2. Comparison of total sperm between the two groups. The mean of total sperm in control groups (238.80 \pm 163.98) was higher than betel nut groups (63.60 \pm 39.55). This difference was significant statistically ($p = 0.016$).

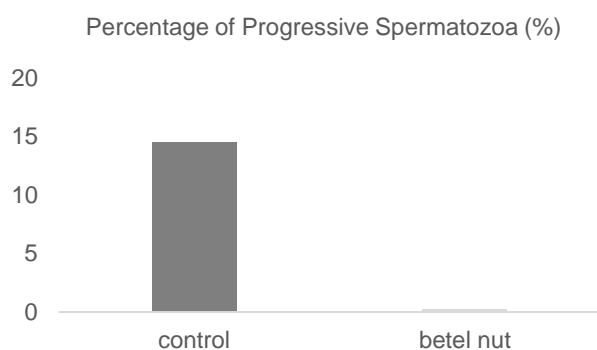


Figure 3. Comparison of progressive spermatozoa percentage between the two groups. The mean percentage of progressive spermatozoa in control groups (14.47 \pm 11.24) was higher than betel nut groups (0.25 \pm 0.55). This difference was significant statistically ($p = 0.007$).

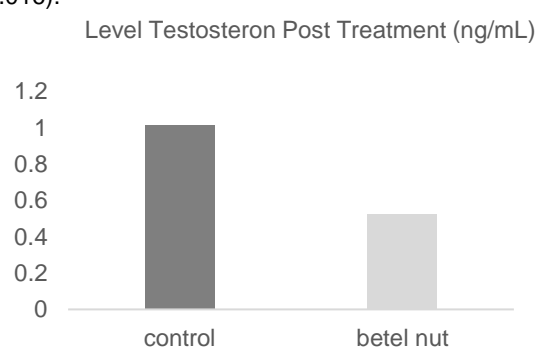


Figure 4. Comparison of testosterone level after treatment between two groups. The mean testosterone level after treatment in control groups (1.01 \pm 0.78) was higher than that of betel nut groups (0.52 \pm 0.38). This difference was not significant statistically ($p = 0.307$).

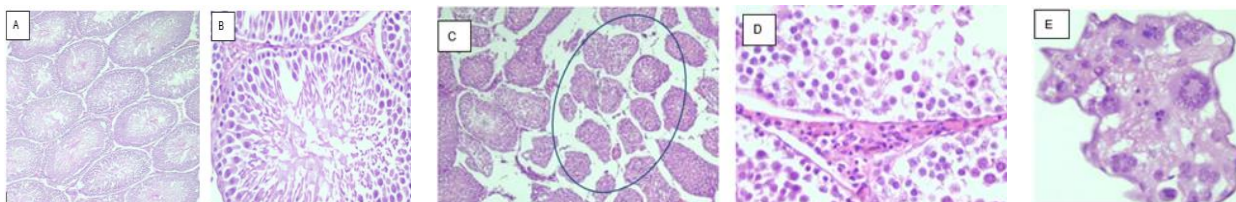


Figure 5. Histopathology of the testis. A and B are testis of the control group. C, D and E are testes of group treated betel nut. (A) Typical of seminiferous tubules at magnification 40x. (B) Typical of seminiferous tubules at magnification 400x. Seminiferous tubules are lined by Sertoli and germ cells arranged in layers and surrounded by a basement membrane consisting of an outer thin parietal epithelium overlying peritubular myoid cells, collagen and an inner layer of the extracellular matrix. (C) Abnormal of seminiferous tubules showed shrunken tubules at magnification 40x. (D) Abnormal of seminiferous tubules at magnification 400x. There is a loss of typical germ cell arrangement—healthy Leydig cells. Leydig cells have abundant, eosinophilic cytoplasm and massive, round nuclei with large nucleoli. (E) Increasing the number of vacuoles within the tubular.

DISCUSSION

Physiologic and histologic of rat testis are similar to humans. Testes composed of seminiferous tubules, Sertoli cells, germ cells, and Leydig cells. The interstitium between seminiferous tubules is composed of Leydig cells, rare inflammatory cells, and vessels. Leydig cells synthesize and secrete testosterone under the regulation of pituitary luteinizing hormone—spermatogenesis of rat for 52 days with cycle 12.9 days. Spermatogonia are the earliest stage of germ cell maturation located at the base of the tubules. During spermatogenesis, germ cells move from the periphery to the lumina of the tubules as they proceed through highly ordered and sequential stages of maturation.^{24,25}

In this study, there was evidence of the reduced number of litters labored by female rats who mated with a male rat that was given betel nuts. The weight of the testis in the group received betel nut was lower than the control group. The histopathology of testis in the group received betel nut showed atrophy and shrink of seminiferous tubules, like cytotoxic lesions in the germinal layer that evidence of reduction spermatogenesis. The counting of total spermatozoa and progressive spermatozoa significantly decreased than the control group. In this study, there was no abnormality in Leydig cell and interstitium tissue in rats after being treated by betel nut. The morphological appearances of the Leydig cell is not a sensitive indicator of Leydig function.²³ This study showed that testosterone levels in the group received betel nut was slightly lower than control groups. Reduced testosterone levels result in reduced accessory sex organ weights and impaired spermatogenesis. Although testicular atrophy can occur without first changes in testosterone.²³ The limitation of this study was that it had a low power size of the sample.

There are many plants with antifertility properties such as anti-spermatogenic activity, spermicidal/sperm immobilization effect^{26–28} or anti-androgenic activity.²⁷ The anti-spermatogenic activity results in the cessation of

spermatogenesis that is indicated by a decrease in sperm count and like cytolytic lesions in the germinal layer.^{26,27} The anti-androgenic activity is reflected by the regression and disintegration of the Leydig cell.²⁷ The result of histopathology and spermatozoa count in this study indicates that betel nut at dose 50 mg/kg BW has antifertility activity with anti spermatogenic activity. With hematoxylin-eosin staining, it difficult to distinguished the various stage of spermatogenesis in this study. A periodic acid Schiff can be used to distinguished the various stage use of spermatogenesis.²⁴ Knowing the kinetics of spermatogenesis will help identify the susceptible target population of cells.²⁹

The pattern of spermatogenesis disturbances can be particular and diagnostic of the mechanism of toxicity,^{29,30} but only seen during early development of the lesion, at a shorter duration of exposure up to 28 days.^{25,29} With a more extended period of dosing reduces the specificity of the pattern of spermatogenic disturbance as the tubules become depleted of more germ cells. Whether spontaneous or induced, death germ cells appear to occur predominantly through apoptosis, are tightly regulated by Sertoli cells^{23,31}. Many of dying cells do not have the classic morphologic appearance of apoptotic cells.²³

CONCLUSIONS AND RECOMMENDATION

A high dose of betel nut can damage rat testis so that it has potential as a male contraceptive. However, high dose betel nut also causes damage to other organs. This study found that betel nut at a lower dose, 50 mg/kg BW have an antifertility effect in male rats. Further study on markers of betel nut's mechanism action and toxicity profile is also needed.

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