Research Article

ANTIFERTILITY EFFECT OF *ARECA CATECHU* IN MALE ALBINO RATS

Sajala Kafle[1], Tara Shanbhag[1,*], Smita Shenoy[1], Arul Amuthan[2], Krishnananda Prabhu[3], Sheethal Mohan[4], Somayaji SN[5], Jyoti Shrestha[6]

1. Department of Pharmacology, Kasturba Medical College, Manipal University, Manipal, India.
2. Department of Pharmacology, Melaka Manipal Medical College, Manipal University, Manipal, India.
3. Department of Biochemistry, Kasturba Medical College, Manipal University, Manipal, India.
4. Department of Biochemistry, Melaka Manipal Medical College, Manipal University, Manipal, India.
5. Department of Anatomy, Melaka Manipal Medical College, Manipal University, Manipal, India.
6. *Department of Pharmacology, Kathmandu University School of Medical Sciences, Dhulikhel, Nepal.*

***Corresponding author's E-mail:** tarashanbhag777@gmail.com

Accepted on: 16-05-2011; **Finalized on:** 25-08-2011.

ABSTRACT

The aim of the present study was to evaluate the antifertility activity of *Areca catechu* in male albino rats. Alcoholic extract of *Areca catechu* was studied for antifertility activity at doses 300 and 600 mg/kg body weight. Fertility was assessed with mating test. Body weight and weight of the reproductive organs (testis and epididymis) were observed. Biochemical evaluation (total protein content and cholesterol level) and histopathological study (cellular dynamics) were performed on testes. There was a 50% reduction in fertility at 300 and 100% reduction at 600 mg doses. There was no significant change in the body weight and weight of the reproductive organs (testis and epididymis). Cholesterol in the testis was significantly ($p<0.01$) increased and total protein content was significantly ($p< 0.001$) decreased at both doses. Histology of the testis showed reduction in the number of secondary spermatocytes and spermatids. There was a reduction in the number of Leydig cells, increase in the diameter of seminiferous tubules with necrotic products and fluid collections at higher dose. Alcoholic extract of Areca catechu showed antifertility activity at 300 and 600mg/kg body weight doses.

Keywords: *Areca catechu,* antifertility, cholesterol, total protein, spermatogenesis.

INTRODUCTION

The fertility rate in a couple is influenced by several factors. These include age, sexually transmitted diseases, environmental toxins, coexistent disease states and various systemic disorders like chronic renal insufficiency, cirrhosis, malnutrition, etc.[1] Environmental factors like exposure to volatile organic solvents[2], alcohol consumption[3], smoking[4], areca nut chewing[5], etc. can lead to infertility.

Areca nut (*Areca catechu Linn.)* chewing is a practice of great antiquity in many parts of Asia, mainly India, Pakistan, Bangladesh and Sri Lanka. *Betel nut chewing is an established practice among Indians for the past 2000 years.* It is most commonly chewed as a constituent of betel quid which is a mixture of betel leaf, areca nut and slaked lime. It is estimated that currently there are several hundred million users of areca nut in the world. *Seeds of areca contain catechin, tannins (15%), gallic acid, fat, gum and alkaloids like arecoline (0.07%) and arecaine (1%). Arecaidine, guvacoline, guvacine and choline are present in trace amount.* Arecoline is the major alkaloid[6].

Dried areca nut has antibacterial, antioxidant, antiseptic, euphoriant and wound healing properties[5]. It also has hepatoprotective[7], hypoglycemic[8] and anti-ulcerogenic effects[9]. It was proved to have abortifacient, antifertility[10] and anti-implantation activities[11]. Pharmacological actions of arecoline resemble that of muscarine and pilocarpine. It violently stimulates the peristaltic movements of the intestines and bowels. It produces a marked constriction of bronchial smooth muscle which can be overcome by adrenaline and atropine. When dropped into the eye, 1% solution constricts the pupil like physostigmine. It is a powerful sialagogue, and stimulates the secretion of sweat in the same way as pilocarpine[6]. The mutagenicity of betel quid, arecoline (the main alkaloid in areca nut) [12], arecaidine (a metabolite of arecoline) and N – nitrosoguvacoline[13] has been reported.

A survey of literature revealed that antifertility effect of alcoholic extract of areca nut has not been documented in male albino rats. Hence this study was undertaken to evaluate the antifertility activity of areca nut in male albino rats of Wistar strain.

MATERIALS AND METHODS

Animals

Healthy, inbred, mature, male albino rats of proven fertility of Wistar strain weighing between 150-200 g were used. The study protocol was approved by institutional animal ethics committee, KMC Manipal (IAEC/KMC/20/2008-09). They were maintained under standard environmental conditions, with temperature (23 ± 2)° C and humidity 50 ± 5%. The animals were fed with standard rat diet (Amrut lab animal feed, Pranav Agro Industries Ltd, Sangli, Maharashtra) and water *ad libitum* throughout the study.

Method of preparation of crude extract of *Areca catechu*

The seeds of *Areca catechu* were purchased from local market and authenticated by the Professor of Botany, Mahatma Gandhi Memorial College, Udupi. Voucher

specimen was kept in the Department of Pharmacology, KMC, Manipal.

The nuts of *Areca catechu* were chopped into small pieces and dried under sun for a few days. The dried chopped nuts were powdered and defatted in petroleum ether. The residue was hot extracted in soxhlet apparatus using 400 ml of 70% ethanol for 5 cycles. The extract was filtered, lypophilised and concentrated over a water bath to obtain dry extract, termed as alcoholic extract [14]. The crude extract was stored in a desiccator. A suspension of the crude extract in 4% gum acacia was used for the study.

Acute toxicity study

Acute toxicity study was done in male rats weighing between 150-200 g. Rats were fasted overnight. They were divided into 6 groups of two animals each. The ethanolic extract of *Areca catechu* was administered orally in ascending and widely spaced doses viz. 10, 30, 100, 300, 1000, 3000 mg/kg. The animals were observed continuously for 2 hours and then occasionally for further 4 hours and finally overnight mortality was recorded[15].

No signs of toxicity were observed even with 3000 mg/kg of alcoholic extract of *Areca catechu*. So, two doses of extract were chosen for the study, 300 mg/kg corresponding to the 1/10th of the maximum tolerated dose (3000 mg/kg) and other was 600 mg/kg.

Antifertility study

Selection of animals for antifertility study: Thirty male rats of proven fertility and sixty female rats with regular estrus cycles were chosen for the study.

Confirmation of estrus cycle in female rat: This was done by vaginal smear method. Stages of estrus cycle of each female animal was determined by taking vaginal smears daily between 9 – 10 AM for 15 days. This 15 days smear observation was to cover three regular estrus cycles and those rats showing regular estrus cycles were chosen for the study.

Preparation of vaginal smear: Vaginal smear was prepared by introducing a drop of distilled water into the vagina with the help of a dropper and collecting it back. The collected discharge was placed on a clean slide that contained a drop of glycerin. A coverslip was placed gently over this and the smear was examined microscopically under low power for different type of cells.

If the majority of cells are leucocytes, the animal is labelled as in diestrus phase. In proestrus phase, leucocytes are rarely seen. Most of the cells present are parabasal cells, often with an irregular and shrunken appearance, but intermediary cells may also be observed. Presence of large number of nucleated cells indicates proestrus which lasts for 3-12 hours, hence the smear will be clear and dominate by cells. These consist of intermediary cells, superficial cells and anuclear (keratinized) cells. Metestrus phase lasts for about 21 hours and usually has many neutrophils and scattered squamous epithelial cells in the smear. As this stage progresses, more intermediary cells begin to appear. These are often small and dark. Parabasal cells can also be seen. However, large intermediary cells and leucocytes are also present[10].

Study design: The selected male rats were divided into three groups of ten animals each.

The drug treatment was as follows:

Group I - Control, received 2 ml of 2% gum acacia daily orally for 60 days.

Group II - Received ethanolic extract of *Areca catechu* 300 mg/kg orally daily for 60 days.

Group III - Received ethanolic extract of *Areca catechu* 600 mg/kg orally daily for 60 days.

Parameters assessed: After 55 days of drug treatment, male rats were cohabited with the female rats in the ratio of 1:2 from the day 56. On day 61 of treatment, that is 24 hours after the last dose, male rats were sacrificed by cervical dislocation. Body weight of control and drug treated rats were assessed prior to and every two weeks thereafter. Testis and epididymis were dissected out and weighed. One testis from each animal was processed for total protein and cholesterol estimation and the other testis was for histopathological study. Cholesterol was estimated by chod-pod method using manual kit (Aspen Laboratory Pvt. Ltd. Delhi). Total protein was also estimated by using the same kit. Values were expressed as milligram/gram of testis.

The number of female rats who delivered and the number of litters born were also counted.

Statistical analysis:

Values were expressed in mean ± SEM. Results were analysed by one way analysis of variance (ANOVA). Student's "*t*" test was also applied wherever necessary. A p value < 0.05 was considered significant.

RESULTS AND DISCUSSION

Changes in body weight

During the period of experiment (total 60 days), there was no significant change in body weight (Table 1).

Fertility in male rats

The fertility in male rat was assessed by calculating the number of litters delivered by the particular female rats which were previously co-habited with male rat in the ratio of 2:1. The male rats in control group showed 100% fertility. Treatment with *Areca catechu* extract at 300 mg/kg caused 50% reduction in fertility of rats. None of the rats were fertile after treatment with *Areca catechu* extract at 600 mg/kg daily for 60 days (Table 2).

Table 1: Effect of *Areca catechu* extract on body weight of male rats

Treatment	Initial weight (g)	Weight after 2 weeks (g)	Weight after 4 weeks (g)	Weight after 6 weeks (g)	Weight after 8 weeks (g)
Control	171 ± 12	167 ± 11	171 ± 13	168 ± 12	171 ± 12
Areca catechu 300 mg/kg	157 ± 3	156 ± 3	158 ± 3	160 ± 3	156 ± 2
Areca catechu 600 mg/kg	160 ± 4	158 ± 3	160 ± 3	159 ± 3	159 ± 4

Values are mean ± SEM; n=10 in each group

Table 2: Effect of *Areca catechu* extract on fertility in male rats

Treatment	No. of female rats cohabited with males (female: male ratio 2:1)	Number of litters	Fertility (%)
Control	20	75	100
Areca catechu 300 mg/kg	20	35	50
Areca catechu 600 mg/kg	20	0	0

Values are mean ± SEM; n = 10 in each group.

Table 3: Effect of *Areca catechu* on organ weight (testis and epididymis), and biochemical parameters in male rats

Treatment	Testis weight (mg/100 g of body weight)	Epididymis weight (mg/100 g of body weight)	Testis Cholesterol (mg/g of tissue)	Testis Protein (mg/g of tissue)
Control	1427.05 ± 10.3	382.60 ± 4.4	1.156 ± 0.311	98.3 ± 5.772
Areca catechu 300 mg/kg	1591.13 ± 7.8	505.67 ± 6.3	2.409 ± 0.110[a]	67.3 ± 11.34[b]
Areca catechu 600 mg/kg	1498.67 ± 5.6	419.56 ± 11.7	2.604 ± 0.970[a]	72.25 ± 18.675[b]

Values are mean ± SEM; n=10 in each group; a: $p < 0.01$ vs control (Student's "*t*" test); b: $p < 0.01$ vs control (Student's "*t*" test)

Organ weight and biochemical parameters

There was no significant change in weight of reproductive organs (testis and epididymis) in any of the groups. Cholesterol level in the testis was significantly ($p < 0.01$) increased in *Areca catechu* treated groups as compared to control. Protein content in the testis was significantly ($p < 0.01$) decreased with *Areca catechu* as compared with control (Table 3).

Histopathological changes in testes

Animals in control group showed normal spermatogenesis (Figure 1).

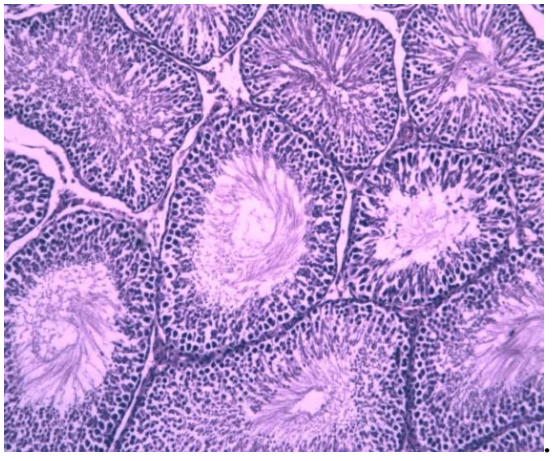

Figure 1: Section of rat testis in control group showing normal spermatogenesis (H&E,100x).

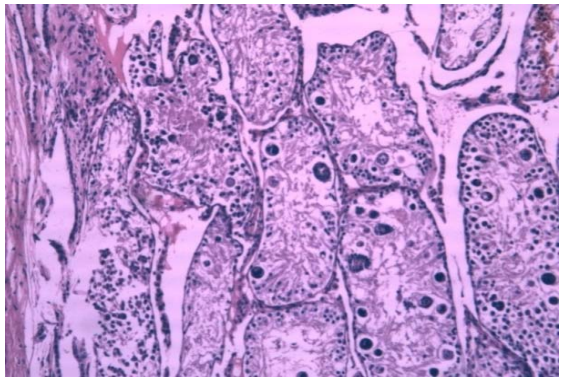

Figure 2: Section of rat testis treated with *Areca catechu* (300mg/kg) showing areas of apoptosis with reduced number of secondary spermatocytes and spermatids (H&E,100x).

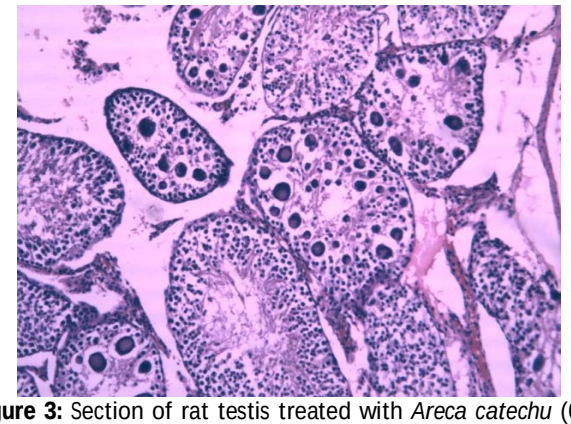

Figure 3: Section of rat testis treated with *Areca catechu* (600 mg/kg) showing reduced number of secondary spermatocytes, spermatids and Leydig cells. There are areas of degenerating cells and debris with increased diameter of seminiferous tubules (H&E,100x).

In *Areca catechu* treated groups, there was a reduction in the number of secondary spermatocytes and spermatids with areas of degenerating cells and debris. At 600 mg/kg dose, there were many areas of apoptosis, a reduction in the number of Leydig cells and increased diameter of seminiferous tubules with necrotic products and fluid collections, which may be due to the inflammatory reaction to the necrotic products (Figures 2, 3).

Discussion

Cholesterol is involved in steroidogenesis in testes. It is the most important precursor in synthesis of steroid hormones and its level is related to fertility of individuals[16]. Increased level of cholesterol may be due to decreased androgen production, which results in accumulation of cholesterol in testes and impaired spermatogenesis[17]. The protein synthesis and concentration in the testes and accessory sex organs are androgen dependent[18]. In our study, protein content in the testes was significantly reduced with *Areca catechu*, which may be due to low levels of testosterone. The findings are further supported by histopathological changes in the testes. Leydig cells were reduced which indicates the insufficiency of these cells to synthesize testosterone. The number of Leydig cells has a direct bearing on spermatogenesis[19]. The reduction in the number of secondary spermatocytes and spermatids may be due to insufficient amount of testosterone.

CONCLUSION

In conclusion, this study demonstrated the antifertility effect of alcoholic extract of *Areca catechu* in male albino rats of Wistar strain.

REFERENCES

1. David SG, Robert LB, William F, Crowley, Definitions of infertility and recurrent pregnancy loss, Fertility and Sterility, 89, 2008, 1603-1606.
2. Smith EM, Hammonds M, Clark MK, Kirchner HL, Fuortes L, Occupational exposure and risk of female infertility, J Occupat and Environ Med, 39(2), 1997, 138-146.
3. Grodstein F, Goldman MB, Cramer DW, Infertility in women and moderate alcohol use, Am J Public Health, 84(9), 1994, 1429-1432.
4. Cramer DW, Barbieri RL, Xu H, Reichart JKV, Determinants of basal follicle stimulating hormone levels in premenopausal women, J Clin Endocrinol Metab, 79(4), 1994, 1105-1109.
5. Azeez S, Amudhan S, Adiga S, Vasanth kumar A, Rao N, Udupa AL, Sanath H, Wound healing profile of *Areca catechu* extracts on different wound models in Wistar rats, Kuwait Medical Journal, 39 (1), 2007, 48-52.
6. Nadkarni KM, Indian Materia Medica, 2nd edition, Popular Book Department, Bombay, 1954, 219-221.
7. Pimolpan P, Saruth N, Rapepol B, Hepatoprotective potential of extracts from seeds of *Areca catechu* and Nutgalls of Quercus infectoria, Molecules, 14, 2009, 4987-5000.
8. Chempakam B, Hypoglycemic activity of arecoline in betel nut *Areca catechu* L, Indian J Exp Biol, 31, 1993, 474-475.
9. Reena RN, Anthikat, Michael A. Anti-ulcerogenic effect of *Areca catechu L*. in Sprague dawley rats, International Journal of Pharmaceutical Sciences and Research, 2(1), 2011, 179-184.
10. Shresta J , Shanbhag T, Shenoy S, Amuthan A, Prabhu K, Sharma S, Banerjee S, Kafle S, Antifertility and abortifacient actions of *Areca catechu* nut in female albino rats, Indian J Pharmacol, 42, 2010, 306-311.
11. Duke JA, Handbook of medicinal herbs, 2nd edition, CRC Press, Florida, 2002, 40-42.
12. Gupta PC, Pindborg JJ, Mehta FS, Comparison of carcinogenicity of betel quid with and without tobacco: an epidemiological review, Ecology of disease, 1, 1982, 213-219.
13. Jeng JH, Tsai CL, Hahn LJ, Yang PJ, Kuo YS and MY Kuo, Arecoline cytotoxicity on human oral mucosal fibroblasts related to cellular thiol and esterase activities, Food Chem Toxicol, 37(7), 1999, 751-6.
14. Padamja PN, Bairy KL, Kulkarni DR, Pro-healing effect of betel nut and its polyphenols, Fitoterapia, 65, 1994, 298-300.
15. Ghosh MN, Fundamental of experimental pharmacology, 3rd edition, Hilton and company, Kolkata, 2005, 193 – 194.
16. Eik-Nes KB, Hall PF, Isolation of dehydroepiandrosterone C14 from dogs infused with cholesterol C14 by the spermatic artery, Proc Soc Exp Biol Med, 3, 1962, 280-283.
17. Bedwal RS, Edwards MS, Katoch M, Bahuguna A, Dewan R, Histological and biochemical changes in testis of zinc deficient strain of mice, Indian J Exp Biol. 32, 1994, 243-247.
18. Brooks DE, Higgins SJ, Characterization and androgen dependence of proteins associated with luminal fluid and spermatozoa in the rat epididymis, J Reprod Fertil, 59, 1980, 363-375.
19. Gupta RS, Kumar P, Dixit VP, Dobhal MP, Antifertility study of the root extracts of the *Barleria prionitis* Linn. In male albino rats with special reference to testicular cell population dynamics, J Ethnopharmacol, 70, 2000, 111-117.

About Corresponding Author: Dr. Tara V Shanbhag

Tara V Shanbhag is serving as Professor at the Department of Pharmacology, Kasturba Medical College, Manipal University, Manipal, Karnataka. She has a teaching experience of 25 years and has been examiner to several universities. Dr. Shanbhag is an honorable recipient of the "Good Teacher" award. She also has to her credit several articles in national and international journals. She has also authored books – *Pharmacology: Prep Manual for Undergraduates* (Medical) and *Pharmacology for Dental Students* with Elsevier.