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REVIEW ARTICLE

Role of *Areca catechu* L. on reproductive physiology in Mice: a review

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Abstract

The *Areca catechu* palm tree, a medium-sized Arecaceae species, produces commercially valuable areca nut. Indians chew areca nuts for freshening their mouth breath. Chewing betel nuts has been a South Asia-Pacific practice for thousands of years. Meghalaya's Khasi tribe relies on kwai (areca nut) as a part of their hospitality and culture. It is decorated during Assam's Bihu festival and handed to notable people during felicitations. The Manipur's Meitei community require betel nuts for all religious rites, including birth, marriage, and death. Birth guests get kwa or betel nuts as a token of appreciation. It follows the main course at feasts. Kwa aids digestion. After a good dinner, Nagaland's Ao tribe consumes betel nuts. Every Mizoram paan shop sells kuhvahring (green areca nut), which visitors and guests are often requested to try. Arunachal Pradesh, Sikkim, and Tripura chew areca nuts for refreshment. Numerous research has examined *Areca catechu*'s dental health risks. This habit can harm reproductive as well as dental health. In the present review, an attempt has been made to summarise the ethnomedicinal, ayurvedic, and pharmacological opinions of *Areca catechu* L. kernel on reproductive physiology since there are few reviews on the detrimental effects of areca nuts on mice. Areca nuts affect the endocrine system, causing hypothyroidism, prostate hypertrophy, and infertility. The areca nut suppresses fertility, regulates the menstrual cycle, removes dysmenorrhea, prostate enlargement, menopausal symptoms, breast pain, delivery pain, and mild euphoria. During pregnancy, it causes premature birth and low birth weight. Thus, the *A. catechu* seed must be rigorously managed for the good of civilization because it is a hazardous and addictive substance that harms both male and female reproductive systems.

Keywords: *Areca catechu*; Betel Nut; Reproductive Physiology; Infertility; Fertility; Pregnancy.

1. Introduction

The medium-sized *Areca catechu* palm tree, a member of the Arecaceae family, is grown for its commercially important seed, the areca nut. Areca nut chewing is popular among Indians as a way to freshen their breath. From the South Asia to Pacific, chewing betel nuts has been a tradition, custom, or ritual for thousands of years (Singh A, 2020; Moss et al., 2022; Berger et al., 2016; Gupta and Warnakulasuriya 2002; Xiao et al., 2019). Areca nut chewers get used to the habit mainly due to social influence, stress, or lack of awareness (Athukorala et al., 2021). The Khasi tribe of Meghalaya in northeastern India relies heavily on kwai (areca nut). It is given as a token of honour during felicitations to men and women who have accomplished something deserving and used as decoration during the Bihu celebration in Assam. The Meitei people of Manipur require the betel nut for all religious ceremonies, including those for birth, marriage, and death. During birth rituals, guests receive kwa, or betel nuts. At feasts, it is provided after the main course. Kwa is thought to enhance digestion. Following a hearty meal, the Ao tribe of Nagaland chews betel nuts. Every neighbourhood paan shop in Mizoram sell what the Mizos refer to as kuhvahring (green betel nut), and visitors and guests are frequently invited to taste the betel nut. Betel nuts are typically chewed as a refreshment in other northeastern states like Arunachal Pradesh, Sikkim, and Tripura. Numerous studies have looked into the damaging effects of eating *Areca catechu* on oral health.

The risks connected with the practice, however, go beyond the mouth; they also pose a threat to reproductive health. An effort has been made to summarise the ethnomedicinal, ayurvedic, and pharmacological views of *Areca catechu* L. kernel on reproductive physiology since there are few reviews describing the detrimental effects of areca nuts on the reproductive physiology of mice. Areca nuts have an effect on the endocrine system, which can cause hypothyroidism, prostate enlargement, and infertility. The betel nut is well known for its ability to affect reproductive health by reducing fertility, regulating the menstrual cycle, and treating mild euphoric conditions as well as breast pain, menopausal symptoms, prostate enlargement, and dysmenorrhea. It also harms the foetus when used during pregnancy, leading to preterm birth and an unfavourable birth weight (Shrestha et al., 2010; Chang et al., 2004; Berger et al., 2016; García-Algar et al., 2005; Duke, 2002; Sinha and Rao 1985; Yang et al., 2008). *A. catechu* seed contains hazardous and addictive chemical that adversely affects both male and female reproductive systems directly or indirectly, hence its use must be strictly regulated for the benefit of the society.

2. Impact on male reproductive system

Consuming areca nuts causes morpho-functional alterations in male albino rats, such as interruption of sperm maturation, stimulation of hormone synthesis, and an increase in degenerative seminiferous tubules (Yuan et al., 2012; Zhou et al., 2014). Further, the release of testosterone and Leydig cell activity may both be stimulated by arecoline (present in betel nut extracts), according to a number of in vivo investigations done on rats. This may be due to the inhibition of pineal activity (Saha et al., 2007). Male rat's prostate glands have been observed to be regulated by betel nut alkaloids (BNAs), particularly arecoline (Saha et al., 2011). In one study, arecoline treatment specifically increased serum levels of the hormones gonadotropin and testosterone as well as the prostate gland's weight.

Further, arecoline oxidant modulates regulatory proteins to have diverse impacts on the cell cycle. Arecoline triggered cell cycle arrest in the G2/M phase in RWPE-1 and PC-3 as well as the G0/G1 phase in LNCaP cells. Arecoline altered the expression of cyclin-dependent kinase (CDK)-1, p21, cyclins B1 and D3, while having no impact on CDK2 or cyclin D1 expression in RWPE-1 cells. Arecoline lowered the expression of the proteins CDK1, CDK2, CDK4, p21, p27, and cyclin D1 and D3 in PC-3 cells while increasing the expression of the protein cyclin B1. Arecoline showed no effects on CDK1 or cyclin B1 expression in LNCaP cells, but it did reduce the expression of CDK2, CDK4, and cyclin D1 and increase the expression of p21, p27, and cyclin D3 (Shih et al., 2020).

2.1. Sperm movement

Arecoline, arecaidine, and guvacine, the three primary alkaloids found in areca nuts, all impact sperm motility. A very strong dose-dependent effect of arecoline resulted in the lowering of motile sperm. A decreased dose-dependent effect of arecaidine was responsible for the reduction of motile sperm. However, the possibility that guvacine will reduce motile sperm in a dose-dependent way is unknown. Areca nuts dramatically reduce sperm motility, sperm count, sperm abnormalities, and antioxidant enzyme activity, hence, long-term consumption of areca nuts may result in infertility in males (Wu et al., 2010; Zhou et al., 2014). According to reports, the structure that controls the motion of the flagella may be affected by the inflammatory response caused by COX-2 production, which could result in a reduction in sperm motility (Er et al., 2006). Another potential mechanism is that the injection of arecoline causes oxidative stress in the sperm that is brought on by reactive oxygen species (ROS) (Wu et al., 2010).

2.2. Role in spermatogenesis

Areca nuts affect the seminiferous tubule and spermatogenesis. Disorders in spermatogenesis and a reduction in the diameter of the seminiferous tubule are both brought on by tubular degeneration. Located in the seminiferous tubules, Sertoli cells and epithelial cells form the blood testicular barrier, which supports the spermatogenesis process. Arecoline can compromise the blood-testicular barrier by altering proteins involved in the development of tight junctions, according to in vitro research (Cheng et al., 2012). In the treatment group that received areca nuts, the histological image of the testes revealed empty tubules with no spermatogenic process (Taba and Kumar, 2022; Figure 1). Following treatment with areca nut, a reduction in testicular weight, seminiferous tubule number and shape, and spermatozoa motility has been noted. There are also reports of the dose-dependent effect of betel nut extract on testicular morphology with a higher dose of areca nut (40 mg/kg BW) displaying significantly smaller seminiferous tubule diameter, an increase in the degenerative tubule, and an increase in the percentage of abnormal spermatozoa morphology. The research also discovered various anomalies in the morphology of spermatozoa (Rahman et al, 2022; Zhou et al., 2014).

2.3. Role in prostate gland function

Areca nuts increase the amount of fructose and sialic acid in the coagulating gland and the development of androgen receptors in the prostate, which causes hyperplasia and hypertrophy and the issues associated with an enlarged prostate (Yang et al., 2004; Saha et al., 2007; Saha et al., 2011). Guvacine and arecaidine are less effective than arecoline at causing alterations in prostate cell proliferation. Further research is required to determine whether the acetylcholine receptor (AchR), which can also act as an arecoline receptor (Chang et al., 2007; Lin et al., 2008; Chou et al., 2008; Li et al., 2010; Hsu et al., 2010; Hsieh et al., 2011), is responsible for the effects of arecoline on both normal and cancerous prostate cells. Arecoline has different impacts on normal and malignant prostate cells cell cycles depending on the kind of cell.

2.4. Blood-Testis barrier function

The blood-testis barrier can be breached by paan masala (a betel quid combination) or its derivatives, which can have an impact on the spermatogenesis process (Mukherjee et al., 1991). Alkaloids may therefore be able to cross the blood-testis barrier in humans and hamper the process of spermatogenesis (Yuan et al., 2012).

3. Effect on female reproductive systems

Areca nuts are well recognised for their abortifacient, anti-implantation, antifertility, uterine inflammatory, and leucorrhea-inducing properties (Shrestha et al., 2010; Duke JA., 2002). All of these provide insight into the study of female health issues related to gonad physiology, particularly in regard to exposing the potential of

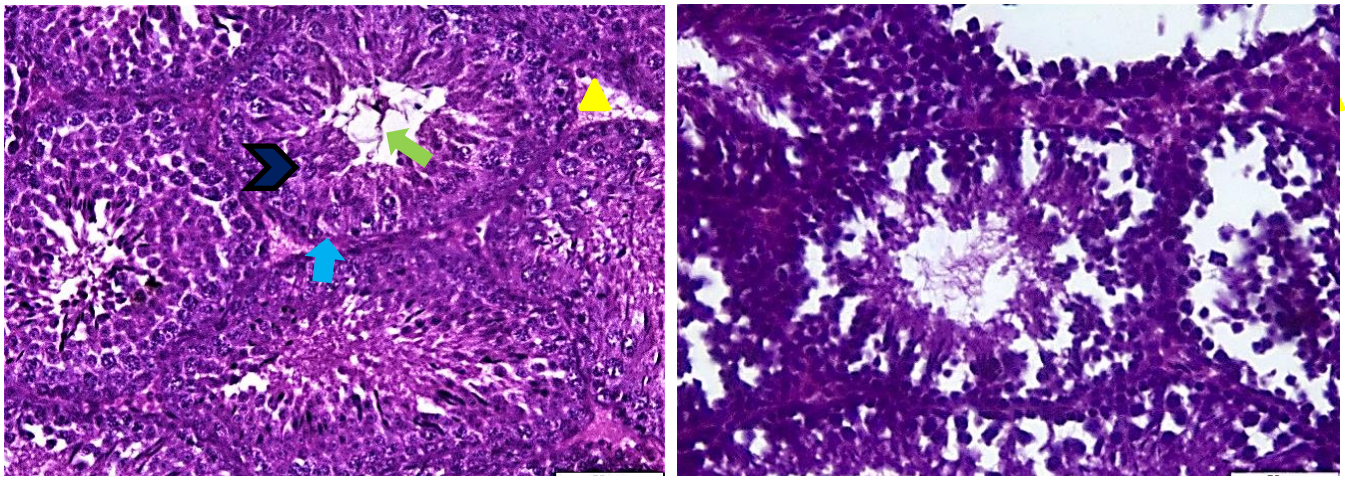

Figure 1. Representative images of transverse sections of adult male mice testes administered with extract of Areca nut (Image b) along with its control (Image a). Image a and b have scale bars = 50 µm showing seminiferous tubules of the testis (Taba and Kumar, Unpublished Observation).

Key: Seminiferous tubules ( ); Sermatogonial cell ( ); Spermatids ( ); Leydig cells ( )

the areca nut as a viable cause for female infertility. In regular female areca nut chewers, the levels of the feminine hormones progesterone and estradiol are unaffected, however, with increasing doses of betel nut extract, there is a decrease in the estradiol levels in the blood serum of female rats (Núñez-De La Mora et al., 2007; Aritonang et al 2020). Users of areca nuts experience worsened consequences of vitamin D insufficiency because areca nuts have an independent effect on 25(OH)ase, which results in lower serum calcitriol (Ogunkolade et al., 2006).

3.1. Changes in ovarian follicle development

According to a study, Graafian follicles, ruptured follicles, and corpus luteum were not present in approximately half of the ovarian sections while the remaining half of the ovarian section shared a similar histological structure compared to the control group (Shrestha et al., 2010). However, in our own study, we observed a significant demarcation in the ovarian histology (follicular development) when compared to the control group (Taba and Kumar, 2022, Unpublished Observation; Figure 2). Changes in the estrous cycle have also been observed in female mice with prolongation of the proestrus phase while a decrease in the duration of the estrus and metestrus phases. However, there was no change observed during the diestrus phase (Shrestha et al., 2010).

3.2. Impact of Areca Nut on pregnancy

Areca nut consumption during pregnancy increases the likelihood of low birth weight, low birth length, and preterm deliveries (García-Algar et al., 2005; Senn et al., 2009; Berger et al., 2016; Garg et al., 2014; Sinha and Rao 1985; Yang et al., 2008). Low doses of Areca nut produce eNOS-mediated dilation of the umbilical arteries, but higher doses cause endothelial cell differentiation to be arrested, leading to malfunction (Kuo et al., 2005). Heavy metals including lead, arsenic, and cadmium, which are detrimental to the baby when consumed by pregnant women, are more prevalent among betel quid chewers (Al-Rmalli et al., 2011). Perinatal exposure to areca nuts exposes the foetus to the adverse effects of carcinogens because the activity of the -SH enzyme leads to alteration in the level of malondialdehyde, and the cytochrome-450 (Singh et al., 1995). The survival rate of embryos treated with arecoline decreases as the arecoline concentration rises. Thus, the embryos treated with arecoline exhibit general development retardation, a slower heartbeat, and neonatal jaundice (Chang et al., 2004; de Costa and Griew, 1982).

Embryos treated with arecoline showed a dose-dependent overall developmental delay. According to tests, the general cytotoxic impact brought on by the depletion of intracellular thiols is what mostly causes the growth retardation caused by arecoline in embryos (Liu et al., 2011; Chang et al., 2004). With an increase in dosage, betel nut extract administration caused the pregnant dams to pass away. Aspirin and betel nut extract that were not treated proved to be more hazardous to the animals. The percentage frequency of resorptions (implantations without any discernible foetus) and dead, macerated foetuses increased noticeably in pregnant mothers exposed to aspirin or either species of betel nut, clearly confirming the embryotoxicity of the test materials. Higher doses of the betel nut extract also caused certain foetal skeletal abnormalities and a delay in ossification (Sinha and Rao, 1985). In a neonatal withdrawal syndrome study in an infant born to a regular betel nut-consuming woman, arecoline was found in the mother's placenta (LópezVilchez et al., 2006).

Areca chewing results in higher sister chromatid exchange in pregnant female than in non-chewing female (Ghost and Ghosh 1988). Arecoline, a major betel nut alkaloid, has already been reported to induce chromosomal aberrations in bone marrow cells of mice and also to interfere with nucleic acid metabolism (Shivapurkar and Bhinde, 1979). The fetotoxic effects of betel nut extract might result from 2 of its major alkaloids, arecoline and arecaidine, both of which have monofunctional methyl groups in their pyridine nuclei and can produce toxic as well as more active metabolites as 1-oxide 324 or as cysteine beta-alkylation adduct. These 2 alkaloids can obstruct DNA synthesis by directly crosslinking with DNA, similar to other DNA-crosslinking agents. It was also reported that the relaxation of the human umbilical artery and vein rings by arecoline is concentration-dependent; the more arecoline is present, the more pronounced the rings relaxation (Panigrahi and Rao 1982; Panigrahi and Rao 1983; Wary et al., 1988; Sundqvist et al., 1989; Wary et al., 1991; Kuo et al., 2005).

High doses of areca nut or prolonged use may cause endothelial dysfunction and related disorders because higher levels of arecoline impede the formation of endothelial cells (Kuo et al., 2005). It is believed that the main reasons pregnant women chew betel nuts are to avoid morning sickness, to avoid having bad breath, to satisfy a chewing habit, and to become hooked. Most of the women believed that chewing would not harm the unborn, which is not the case.

Chewing betel nuts regularly can disrupt a normal pregnancy or result in an abortion. Abortion may result from the corpus luteum or placenta secreting insufficient progesterone, a condition known as a luteal phase deficiency (Schorge et al., 2008). As a result, this may be the reason behind the sharp rise in the percentage of miscarriages and the drug effect noticed in women who consume areca.

4. Virucidal qualities

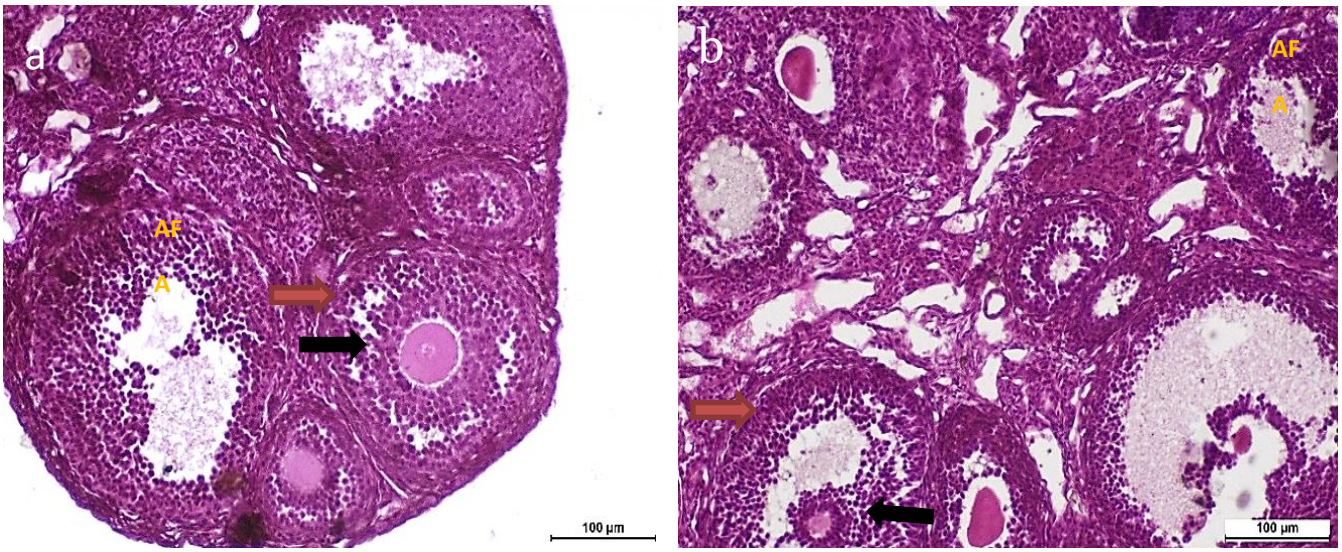

Figure 2. Representative images of transverse sections of adult female mice ovary administered with extract of Areca nut (Image b) along with its control (Image a). Image a and b have scale bars = 100 µm showing different stages of follicular development in the ovary (Taba and Kumar, Unpublished Observation). Key: (A) Antrum, (AF) Atretic follicle; Growing Secondary follicle (→); Granulosa cells (➡)

Extracts from areca nuts have also been examined for their antiviral properties. The studies conducted by various researchers on the applications of various plants (herbal medicines) for the prevention and treatment of AIDS and sexually transmitted illnesses (STDs) in men have been reviewed (Vermani et al., 2002).

It has been discovered that the areca nut extract's tannins and alkaloids have inhibitory effects on the STD viruses that cause HIV and herpes simplex virus (HSV-1) (Kurokawa et al., 2010; Marastoni et al., 2004). This action is caused by the procyanidine found in areca tannin B1. The viral development of the New Castle Disease Virus (NDV) and Egg Drop Syndrome Virus (EDS) grown in embryo cultures was likewise seen to be inhibited by the areca nut extract (Anthikat et al., 2009).

5. Role in endocrine hormones that are not reproductive

According to research on animals, arecoline increases the release of T3, T4, and thyroid stimulating hormone (TSH) when administered acutely; when administered in high doses, it stimulates the HPA axis, which is analogous to the stress response, and when used frequently, it results in hypothyroidism (Dasgupta et al., 2010). The plasma content of serotonin rises and melatonin falls after regular areca nut consumption (Saha et al., 2018). Arecoline influences the endocrine system and increases the level of endogenous corticotrophin-releasing hormone by stimulating the hypothalamic-pituitary-adrenal cortical axis and blocking the movement of calcium ions into the adrenal medulla pheochromocyte. It has been also reported that arecoline has a role in effecting immune system by stimulating and inhibiting the activity of adrenal and inhibit adrenal hormone in mice (Xiao et al., 2019; Dasgupta et al., 2010)

6. Policy concerns

Due to the growing market demand for herbal pharmaceuticals, the worldwide herbal drug sector is currently threatened by adulteration and replacement (Kumari et al., 2021). The lack of necessary species, comparable morphological appearance, incorrect handling, improper storage, purposeful substitution, and conflicts in vernacular nomenclature are a few other variables that either directly or indirectly contribute to the quality degradation of herbal medicines (Thakur et al., 2021). These actions ultimately result in the poor quality of herbal medicines (Chaudhary et al., 2021; Ekor, 2014). Additionally, it has an impact on the marketing efforts of conventional herbal treatments (Thakur et al., 2021). The WHO states that even if two drugs are derived from the same plant if more than 5% of the original medicine is blended with them, the product will be rejected (Katiyar et al., 2012). The poor quality of pharmaceuticals is partly a result of a lack of standardised methods. It misidentifies the original medication, which ultimately takes advantage of its use in the conventional medical system (Fokunang et al., 2011).

The manufacturers of paan masala have increased their mouth freshener-based surrogate advertising. A new generation of impressionable teenagers and children who chew areca nuts is being produced as a result of this. The Gutka enterprises had long before circumvented the law and operated almost unfettered for many years (Mangla, 1993). By outlawing the sale and manufacture of Gutka, numerous state governments in India have taken a significant step. These restrictions are currently in place in India's 20 states and three union territories. The economic burden of the enormous loss of human life and wealth brought on by the illness and mortality brought on by areca nut and paan masala addiction is far greater than the income produced by this sector. An areca nut control programme needs to be established by the government. It is time for tougher legislation to control the consumption of areca nuts and strict guidelines for producers to include pictorial warnings on their goods. Therefore, a Herbal Authentication System (HAS) that can act as a regulator and aid in raising the standard of the herbal drug trade must be created.

7. Discussion

Areca nuts are a highly addictive narcotic that is used by people of all ages in a number of different places across the globe. According to the current research findings, consuming betel nuts in any form, including their raw or processed forms, can lead to fetopathic and embryotoxic changes in albino mice (Sinha and Rao, 1985). In addition to being carcinogenic to the liver, oesophagus, pharynx, and oral cavity, it is also associated with a wide variety of unfavourable effects on the reproductive system of the human body (Senevirathna et al., 2023). Arecoline, the principal alkaloid found in areca nuts, is primarily responsible for these nuts' systemic effects. Areca nut consumption on a regular basis may result in hypothyroidism, an enlarged prostate, and an inability to conceive children. A research states that there was evidence of damaged testes, fewer and less mobile spermatozoa, and an overall increase in the number of abnormal spermatozoa (Garg et al., 2014; Zhou et al., 2014).

Areca nut chewing has been linked to an increased risk of premature birth and lower birth weight for babies born to their mothers (Chang et al., 2004; Berger et al., 2016). Due to the shorter duration of the estrus and metestrus phases, it is possible that the matured Graafian follicles or secondary follicles are not present or are not mature, preventing ovulation. The extension of the proestrus phase provides evidence that the delayed follicle maturation that occurred during the preovulatory phase was a contributing factor in the immaturity of the Graafian follicles. This was brought on by incongruity in the levels of endogenous steroids, proteins, and hormones, or the lack of availability of oestrogen, which is produced by the granulosa cells and is essential for the development and differentiation of the ovarian follicles. In the groups that consumed areca, transverse sections of the ovaries indicated the presence of fibrous, adipose, and primordial follicles in the ovarian stroma. These sections also revealed the presence of primary, secondary, and dilated blood arteries in the ovarian stroma. In addition to the findings discussed previously, Graafian follicles, burst follicles, and corpus luteum were discovered in the ovarian sections of the group that did not consume areca. These findings demonstrated that ovulation had already taken place and that there was no hormonal imbalance. The weight of the ovaries was significantly different in the groups that consumed areca and those that did not drink areca. This difference indicated that the ovary's stroma, follicles, and corpus luteum were less active. It is possible that this is due to a deficiency in gonadotrophic, steroidal, or both of these hormones. Cholesterol is the first step in the process of steroidogenesis, which occurs in the ovarian endocrine tissues. This process results in the production of estrogens, progestins, and androgens. The fact that areca consumers had a considerable rise in their ovarian cholesterol levels demonstrates that cholesterol is not required for steroidogenesis. (Shrestha et al., 2010; Aritonang et al., 2020).

According to these findings, people who ingest areca may be at risk for experiencing adverse effects on their gonadal processes. Because it is abundantly obvious that areca nuts are a chemical that is both harmful and addictive and that has an effect on the entirety of the human body, the use of areca nuts needs to be rigorously regulated for the welfare of human society.

8. Conclusion and prospects

The *Areca catechu*, a palm tree is grown for its commercially important seed, the areca nut. India produces the most areca nut, which is thought to be a reproductive medicinal plant used to control population growth. Over 6000 higher plant species are used in India's traditional healthcare system, making herbal medicines vital to human life. Tannins, gallic acids, oily matter, gum, and alkaloids such as arecoline, arecaine, guvacoline, and guvacine have been reported to be found in areca nuts. Chewing the combination of areca nut and betel leaf has been practised for thousands of years. Areca nut consumption has been linked to a variety of health risks, including preterm birth and low birth weight. Arecoline, a cytotoxic and genotoxic alkaloid with pharmacological properties similar to muscarine and pilocarpine, is a potential influencing alkaloid. Betel quid use in pregnancy has a number of negative effects on the unborn child, including an increase in preterm birth and a decrease in the baby's height and weight. Herbal remedies have long been used to reduce fertility, regulate the menstrual cycle, treat dysmenorrhea and

prostate enlargement, menopausal symptoms, breast pain, and pain during and after childbirth. In male albino rats, eating areca nuts caused morpho-functional changes such as sperm maturation interruption, hormone synthesis stimulation, and an increase in degenerative seminiferous tubules. Arecoline, arecaidine, and guvacine, three primary alkaloids found in areca nuts, have been shown to regulate male rats prostate glands, increase serum hormone levels, and modulate cell cycle regulatory proteins. It also has a strong dose-dependent effect on sperm motility and has the potential to compromise the blood-testicular barrier by altering proteins involved in tight junction development. These effects could be attributed to pineal activity inhibition. Areca nuts have been shown to reduce testicular weight, the number and shape of seminiferous tubules, and spermatozoa motility. They also increase the amount of fructose and sialic acid in the coagulating gland, as well as the development of androgen receptors on the prostate, resulting in hyperplasia and hypertrophy, as well as the problems associated with an enlarged prostate. More research is needed to determine whether arecoline's effects on both normal and cancerous prostate cells are mediated by the acetylcholine receptor (AchR). Therefore, despite only showing warning pictures in the gutka packets, the government needs to tighten the policies more strictly for the betterment of the society.

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Authors declarations

NT, MY, and PK conceptualized the article. NT wrote the first draft of the manuscript, while MY and PK edited the manuscript. All the authors read and approved the final version of the manuscript. PK supervised the work.

Conflict of interests

The authors declare that there is no conflict of interest.

References

Al-Rmalli SW, Jenkins RO and Haris PI. 2011. Betel quid chewing elevates human exposure to arsenic, cadmium and lead. Journal of Hazardous Materials 190(1-3): 69-74. doi:10.1016/j.jhazmat.2011.02.068

Anthikat RRN and Michael A. 2009. Study on the Areca nut for its antimicrobial properties. Journal of Young Pharmacists 1(1):42-5. doi:10.4103/0975-1483.51874

Aritonang TR, Natzir R, Sinrang AW, Massi MN, Hatta M and Kamelia. 2020. The effect of administration of extract from areca nut seeds (*Areca Catechu* L) on the estradiol and estrus cycle balb/C female rats. Journal of Physics: Conference Series. doi:10.1088/1742-6596/1477/6/062026

Athukorala IA, Tilakaratne WM and Jayasinghe RD. 2021. Areca nut chewing: initiation, addiction, and harmful effects emphasizing the barriers and importance of cessation. Journal of Addiction. doi:10.1155/2021/9967097

Berger KE, Masterson J, Mascardo J, Grapa J, Appanaitis I, Temengil E, Watson BM and Cash HL. 2016. The effects of chewing betel nut with tobacco and pre-pregnancy obesity on adverse birth outcomes among Palauan women. Maternal and Child Health Journal 20(8): 1696–1703. doi:10.1007/s10995-016-1972-6

Chang BE, Liao MH, Kuo MY and Chen CH. 2004. Developmental toxicity of arecoline, the major alkaloid in betel nuts, in zebrafish embryos. Birth Defects Research, Part A: Clinical and Molecular Teratology 70(1): 28–36. doi: 10.1002/bdra.10136

Chang EE, Miao ZF, Lee WJ, Chao HR, Li LA, Wang YF, Ko YC, Tsai FY, Yeh SC and Tsou TC. 2007. Arecoline inhibits the 2,3,7,8-tetrachlorodibenzo-pdioxin-induced cytochrome P450 1A1 activation in human hepatoma cells. Journal of Hazardous Materials 146(1-2): 356 - 361. doi: 10.1016/j.jhazmat.2006.12.035.

Cheng CY and Mruk DD. 2012. The blood-testis barrier and its implications for male contraception. Pharmacological Reviews 64(1): 16-64. doi: 10.1124/pr.110.002790

Chou WW, Guh JY, Tsai JF, Hwang CC, Chen HC, Huang JS, Yang YL, Hung WC and Chuang LY. 2008. Arecoline-induced growth arrest and p21 WAF1 expression are dependent on p53 in rat hepatocytes. Toxicology 243(1-2):1-10. doi: 10.1016/j.tox.2007.09.003

Choudhary S, Kumari I, Thakur S, Kaurav H and Chaudhary G. 2021. Brahmi (Bacopa Monnieri)– A potential ayurvedic cognitive enhancer and neuroprotective herb. International Journal of Ayurveda and Pharma Research 9(5): 41-49. https://doi.org/10.47070/ijapr.v9i5.1917

Choudhary S, Walia B and Chaudhary G. 2021. *Ferula asfoetida* (Hind): A review based upon its ayurvedic and pharmacological properties. International Journal of Pharmaceutical Sciences Review and Research 68(2):31-39. doi:10.47583/ijpsrr.2021.v68i02.006

Dasgupta R, Chatterji U, Nag T C, Chaudhuri-Sengupta S, Nag D and Maiti B R. 2010. Ultrastructural and hormonal modulations of the thyroid gland following arecoline treatment in albino mice. Molecular and Cellular Endocrinology 319(1-2):1–7. doi: 10.1016/j.mce.2010.01.005

de Costa C and Griew AR. 1982. Effects of betel chewing on pregnancy outcome. The Australian & New Zealand Journal of Obstetrics & Gynaecology 22(1): 22–24. doi:10.1111/j.1479-828x.1982.tb01392.x

Duke JA. 2002. Tropical medicinal plants. In: Handbook of Medicinal Herbs, 2nd ed. CRC Press (Florida). Pp: 72-73.

Ekor M. 2014. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Frontiers in Pharmacology 4: 177. doi: 10.3389/fphar.2013.00177

Er TK, Tsai EM, Tsai LY, Ko YC and Lee JN. 2006. In vitro effects of arecoline on sperm motility and cyclooxygenase-2 expression. The Journal of Toxicological Sciences 31(1) 75-82. doi: 10.2131/jts.31.75

Fokunang CN, Ndikum V, Tabi OY, Jiofack RB, Ngameni B, Guedje N M, Tembe-Fokunang EA, Tomkins P, Barkwan S, Kechia F, Asongalem E, Ngoupayou J, Torimiro NJ, Gonsu KH, Sielinou V, Ngadjui BT, Angwafor F, 3rd Nkongmeneck A, Abena OM, Ngogang J, Asonganyi T, Colizzi V, Lohoue J and Kamsu-Kom. 2011. Traditional medicine: past, present and future research and development prospects and integration in the national health system of Cameroon. African Journal of Traditional, complementary, and alternative medicines: AJTCAM 8(3): 284–295. https://doi.org/10.4314/ajtcam.v8i3.65276

García-Algar O, Vall O, Alameda F, Puig C, Pellegrini M, Pacifici R and Pichini S. 2005. Prenatal exposure to arecoline (areca nut alkaloid) and birth outcomes. Fetal and Neonatal Edition 90(3): F276–F277. doi: 10.1136/adc.2004.061325

Garg A, Chaturvedi P and Gupta PC. 2014. A review of the systemic adverse effects of areca nut or betel nut. Indian Journal of Medical and Paediatric Oncology 35(1): 3–9. doi: 10.4103/0971-5851.133702

Ghosh PK and Ghosh R. 1988. Effect of betel chewing on the frequency of sister chromatid exchanges in pregnant women and women using oral contraceptives. Cancer Genetics and Cytogenetics 32(2): 211-215. https://doi.org/10.1016/0165-4608(88)90283-x

Gupta PC and Warnakulasuriya S. 2002. Global epidemiology of areca nut usage. Addiction Biology 7(1): 77–83. doi:10.1080/13556210020091437

Hsieh TJ, Hsieh PC, Wu MT, Chang WC, Hsiao PJ, Lin KD, Chou PC and Shin SJ. 2011. Betel nut extract and arecoline block insulin signaling and lipid storage in 3T3-L1 adipocytes. Cell Biology and Toxicology 27(6):397-411. doi: 10.1007/s10565-011-9195-5

Hsu HF, Tsou TC, Chao HR, Shy CG, Kuo YT, Tsai FY, Yeh SC and Ko YC. 2010. Effects of arecoline on adipogenesis, lipolysis and glucose uptake of adipocytes-A possible role of betel-quid chewing in metabolic syndrome. Toxicology and Applied Pharmacology 245(3):370-377. doi: 10.1016/j.taap.2010.04.008

Katiyar C, Gupta A, Kanjilal S and Katiyar S. 2012. Drug discovery from plant sources: An integrated approach. Ayu Journal 33(1): 10–19. doi: 10.4103/0974-8520.100295

Kumari I, Kaurav H and Choudhary G. 2021. *Rubia cordifolia* (Manjishtha): A review based upon its ayurvedic and medicinal uses. Himalayan Journal of Health Sciences 6:17-28.

Kuo FC, Wu DC, Yuan SS, Hsiao KM, Wang YY, Yang YC and Lo YC. 2005. Effects of arecoline in relaxing human umbilical vessels and inhibiting endothelial cell growth. Journal of Perinatal Medicine 33(5): 399–405. doi:10.1515/JPM.2005.072

Kurokawa M, Shimizu T, Watanabe W and Shiraki K. 2010. Development of new antiviral agents from natural products. The Open Antimicrobial Agents Journal 2: 49-57.

Lambers DS and Clark KE. 1996. The maternal and fetal physiologic effects of nicotine. Seminars in Perinatology 20(2): 115-126. doi:10.1016/s0146-0005(96)80079-6

Li CB, Yang X, Tang WB, Liu CY and Xie DP. 2010. Arecoline excites the contraction of distal colonic smooth muscle strips in rats via the M3 receptor-extracellular Ca2+ influx-Ca2+ store release pathway. Canadian Journal of Physiology and Pharmacology 88(4): 439-447. doi: 10.1139/y10-024

Lin CF, Shiau TJ, Ko YC, Chen PH and Wang JD. 2008. Prevalence and determinants of biochemical dysfunction of the liver in Atayal aboriginal community of Taiwan: Is betel nut chewing a risk factor?. BMC Gastroenterology 8: 13. doi: 10.1186/1471-230X-8-13

Liu ST, Young GC, Lee YC and Chang YF. 2011. A preliminary report on the toxicity of arecoline on early pregnancy in mice. Food and Chemical Toxicology 49(1):144-148. doi:10.1016/j.fct.2010.10.009

López-Vilchez MA, Seidel V, Farré M, García-Algar O, Pichini S and Mur A. 2006. Areca-nut abuse and neonatal withdrawal syndrome. Pediatrics 117(1): e129–e131. doi:10.1542/peds.2005-0281

Mangla B. 1993. India: Betel nut warning. The Lancet 341(8848): 818-819. https://doi.org/10.1016/0140-6736(93)90581-Z

Marastoni M, Baldisserotto A, Canella A, Gavioli R, Risi CD, Pollini GP and Tomatis R. 2004. Arecoline tripeptide inhibitors of proteasome. Journal of Medicinal Chemistry 47(6): 1587–1590. https://doi.org/10.1021/jm0309102

Moss WJ. 2020. The seeds of ignorance — consequences of a booming betel-nut economy. The New England Journal of Medicine pp-387:1059-1061. doi: 10.1056/NEJMp2203571

Mukherjee A and Giri AK. 1991. Sister chromatid exchange induced by 'pan masala' (a betel quid ingredient) in male mice in vivo. Food and Chemical Toxicoogy 29(6): 401-403. https://doi.org/10.1016/0278-6915(91)90080-Q

Núñez-De La Mora A, Chatterton RT, Mateo ET, Jesmin F, and Bentley GR. 2007. Effect of chewing betel nut on measurements of salivary progesterone and estradiol. American Journal of Physical Anthropology 132(2) 311–315. doi: 10.1002/ajpa.20513

Ogunkolade WB, Boucher BJ, Bustin SA, Burrin JM, Noonan K, Mannan N and Hitman GA. 2006. Vitamin D metabolism in peripheral blood mononuclear cells is influenced by chewing "betel nut" (*Areca catechu*) and Vitamin D status. The Journal of Clinical Endocrinology & Metabolism 91 (7) 2612–2617. https://doi.org/10.1210/jc.2005-2750

Panigrahi GB and Rao AR. 1982. Chromosome-breaking ability of arecoline, a major betel-nut alkaloid, in mouse bone-marrow cells in vivo. Mutation Research Letters 103(2): 197–204. https://doi.org/10.1016/0165-7992(82)90029-x

Panigrahi GB and Rao AR. 1983. Influence of caffeine on arecoline-induced SCE in mouse bone-marrow cells in vivo. Mutation Research Letters 122(3-4): 347–353. https://doi.org/10.1016/0165-7992(83)90018-0

Puyathorn N, Sukonpan C, Toungsuwan P, Narakornwit W and Phaechamud T. 2022. Effect of arecoline, aqueous and methanolic areca nut extracts on rumen fluke. IOP Conference Series: Materials Science and Engineering. Pp. 2-3. doi:10.1088/1757-899X/1234/1/012019

Rahman AO, Dewi H, Geniro BB and Amdely EV. 2022. Evaluation of the antifertility effect and toxicity of *Areca* nut as oral contraceptive: study on male rats. Journal Kedokteran Brawijaya 32(1):13–18. doi: http://dx.doi.org/10.21776/ub.jkb.2022.032.01.3.

Saha I, Chatterjee A, Mondal A, Maiti BR and Chatterji U. 2011. Arecoline augments cellular proliferation in the prostate gland of male Wistar rats. Toxicology and Applied Pharmacology 255(2):160-168. doi: 10.1016/j.taap.2011.06.010

Saha I, Chatterji U, Chaudhuri-Sengupta S, Nag TC, Nag D, Banerjee S, and Maiti BR. 2007. Ultrastructural and hormonal changes in the pineal-testicular axis following arecoline administration in rats. Journal of Experimental Zoology. Part A, Ecological genetics and physiology 307(4):187-198. doi: 10.1002/jez.354

Saha I, Pradhan D, Chatterji U and Maiti BR. 2018. Arecoline cannot alter pineal-testicular responses to metabolic stress in wistar rats. Acta Endocrinologica 14(2): 175–183. doi: 10.4183/aeb.2018.175

Schorge JO, Schaffer JI, Halvorson LM, Hoffman BL, Corton MM and Hamid CA. 2008. *Williams Gynecology*, 3rd edition. The Mc Graw-Hill companies, China. Pp: 137-140.

Senevirathna K, Pradeep R, Jayasinghe YA, Jayawickrama SM, Illeperuma R, Warnakulasuriya S and Jayasinghe RD. 2023. Carcinogenic effects of areca nut and its metabolites: A review of the experimental evidence. Clinics and Practice 13(2): 326–346. https://doi.org/10.3390/clinpract13020030

Senn M, Baiwog F, Winmai J, Mueller I, Rogerson S and Senn N. 2009. Betel nut chewing during pregnancy, Madang province, Papua New Guinea. Drug and Alcohol Dependence 105(1-2): 126-131. doi: 10.1016/j.drugalcdep.2009.06.021

Shih LJ, Wang JY, Jheng JY, Siao AC, Lin YY, Tsuei YW, Kuo YC, Chuu CP and Kao YH. 2020. Betel nut arecoline induces different phases of growth arrest between normal and cancerous prostate cells through the reactive oxygen species pathway. International Journal of Molecular Sciences 21(23): 9219. doi: 10.3390/ijms21239219

Shivapurkar NM and Bhide SV. 1979. Effect of betel-nut constituents on nucleic acid metabolism. Indian Journal of Experimental Biology 17(10): 1141–1144.

Shrestha J, Shanbhag T, Shenoy S, Amuthan A, Prabhu K, Sharma S, Banerjee S and Kafle S. 2010. Antiovulatory and abortifacient effects of *Areca catechu* (betel nut) in female rats. Indian Journal of Pharmacology 42(5): 306–311. doi: 10.4103/0253-7613.70350.

Singh A and Rao AR. 1995. Effect of areca nut, a masticatory, on hepatic drug metabolizing enzymes –SH content and lipid peroxidation in lactating mothers and their suckling neonates. Cancer Letters 92(2):175-180. doi: 10.1016/0304-3835(95)03776-s

Singh A, Dikshit R and Chaturvedi P. 2020. Betel nut use: The south asian story. Substance use & misuse 55(9): 1545–1551. https://doi.org/10.1080/10826084.2020.1753772

Sinha A and Rao AR. 1985. Embryotoxicity of betel nuts in mice. Toxicology 37(3–4): 315-326. https://doi.org/10.1016/0300-483X(85)90095-2

Sundqvist K, Liu Y, Nair J, Bartsch H, Arvidson K and Grafström RC. 1989. Cytotoxic and genotoxic effects of areca nut-related compounds in cultured human buccal epithelial cells. Cancer Research 49(19):5294–5298.

Thakur S, Kaurav H and Chaudhary G. 2021. *Nardostachys jatamansi*: Importance of highly significant and endangered plant in ayurveda. International Journal of Research in Ayurveda and Pharmacy 12(3):124-130.

Thakur S, Walia B and Chaudhary G. 2021. Dalchini (*Cinnamomum zeylanicum*): a versatile spice with significant therapeutic potential. International Journal of Pharmaceutics and Drug Analysis 6:126-136. doi: 10.47957/ijpda.v9i2.467

Vermani K and Garg S. 2002. Herbal medicines for sexually transmitted diseases and AIDS. Journal of Ethnopharmacology 80(1): 49-66. https://doi.org/10.1016/S0378-8741(02)00009-0

Wang SW, Hwang GS, Chen TJ and Wang PS. 2008. Effects of arecoline on testosterone release in rats. American Journal of Physiology. Endocrinology and metabolism 295(2): E497–E504. doi:10.1152/ajpendo.00045.2008

Wary KK and Sharan RN. 1988. Aqueous extract of betel-nut of north-east India induces DNA-strand breaks and enhances rate of cell proliferation in vitro. Effects of betel-nut extract in vitro. Journal of Cancer Research and Clinical Oncology 114(6):579–582. https://doi.org/10.1007/BF00398180

Wary KK and Sharan RN. 1991. Cytotoxic and cytostatic effects of arecoline and sodium nitrite on human cells in vitro. International Journal of Cancer 47(3): 396–400. https://doi.org/10.1002/ijc.2910470315.

Wu PF, Chiang TA, Chen MT, Lee CP, Chen PH, Ko AM, Yang KJ, Chang PY, Ke DS and Ko YC. 2010. A characterization of the antioxidant enzyme activity and reproductive toxicity in male rats following sub-chronic exposure to areca nut extracts. Journal of Hazardous Materials 178(1-3): 541-546. doi: 10.1016/j.jhazmat.2010.01.118

Xiao Y, Yang Y, Yong J and Lu C. 2019. Chemical components and biological activities of *Areca catechu* L.. Biomedical Research and Reviews 3:1-4. doi : 10.15761/BRR.1000131

Yang MS, Chang FT, Chen SS, Lee CH and Ko YC. 1999. Betel quid chewing and risk of adverse pregnancy outcomes among aborigines in Southern Taiwan. Public Health 113(4):189-192. doi:10.1038/sj.ph.1900563

Yang MS, Lee CH, Chang SJ, Chung TC, Tsai EM, Ko AM and Ko YC. 2008. The effect of maternal betel quid exposure during pregnancy on adverse birth outcomes among aborigines in Taiwan. Drug and Alcohol Dependence 95(1–2):134-139. doi: 10.1016/j.drugalcdep.2008.01.003

Yang NY, Kaphle K, Wang PH, Jong DS, Wu LS, Lin JH. 2004. Effects of aqueous extracts of betel quid and its constituents on testosterone production by dispersed mouse interstitial cells. American Journal of Chinese Medicine 32(5):705-715. doi: 10.1142/S0192415X04002430

Yuan J, Yang D, Liang Y, Gao W, Ren Z, Zeng W, Wang B, Han J, and Guo D. 2012. Alkaloids from areca (betel) nuts and their effects on human sperm motility in vitro. Journal of Food Science 77(4): T70–T78. https://doi.org/10.1111/j.1750-3841.2012.02653.x

Zhou J, Sun Q, Yang Z and Zhang J. 2014. The hepatotoxicity and testicular toxicity induced by arecoline in mice and protective effects of vitamins C and E. The Korean Journal of Physiology & Pharmacology 18(2): 143-148. doi: 10.4196/kjpp.2014.18.2.143