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# Chemistry, metabolism and pharmacology of carcinogenic alkaloids present in areca nut and factors affecting their concentration

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ABSTRACT

Areca Nut (AN), the seed of tropical palm tree *Areca catechu,* is a widely chewed natural product with estimated 600 million users across the world. Various AN products, thriving in the market, portray 'Areca nut' or 'Supari' as mouth freshener and safe alternative to smokeless tobacco. Unfortunately, AN is identified as a Group 1 human carcinogen by International Agency for Research on Cancer (IARC). Wide variation in the level of alkaloids, broadly ranging from 2 to 10 mg/gm dry weight, is observed in diverse variety of AN sold worldwide. For the first time, various factors influencing the formation of carcinogenic alkaloids in AN at various stages, including during the growth, processing, and storage of the nut, are discussed. Current review illustrates the mechanism of cancer induction by areca alkaloids in humans and also compiles dose-dependent pharmacology and toxicology data of arecoline, the most potent carcinogenic alkaloid in AN. Careful monitoring of the arecoline content in AN can potentially be used as a tool in product surveillance studies to identify the variations in characteristics of various AN sample sold worldwide. The article will help to generate public awareness and sensitize the government bodies to initiate campaigns against AN use and addiction.

## 1. Introduction

### 1.1. Areca nut usage and its global distribution

Areca nut (AN) or Betel nut (BN) is the seed of tropical palm tree *Areca catechu* which is widely consumed across the globe for its stimulating properties (IARC Working Group on the Evaluation of Carcinogenic Risk to Humans, 2007; Strickland, 2002) (Fig. 1). AN chewing is a common practice in many regions in south Asia, south-east Asia, and the Asia Pacific region. Traditionally, it has played an important role in social customs, religious ceremonies and cultural rituals (Lee et al., 2011). The consumption of AN is indigenous to India, Sri Lanka, Bangladesh, Myanmar, Taiwan and islands in South Pacific (Johnson et al., 2018). Its use is also popular in parts of Thailand, Indonesia, Malaysia, Cambodia, Vietnam, Philippines, Laos, China (World Health Organization Regional Office for the Western Pacific. Geneva and WHO, 2012). It is estimated by the Food and Agriculture Organization (FAO) that Bangladesh has the world's largest agricultural base of AN production (IARC Working Group on the Evaluation of Carcinogenic Risk to Humans, 2007). A high prevalence of AN use, with or without tobacco, is also reported among the USA and UK Indo-Asian immigrants (Changrani et al., 2006). With the increased commercialization of areca products, the production of AN has increased several fold in most South Asian countries over the past four decades (Osborne et al., 2017).

A harsh truth is that AN is an addictive substance consumed in many parts of the world by the people of all age groups, including children. The World Health Organization (WHO) reported that about 600 million people, around the world, use AN in some form. AN is the fourth most popular mind-altering substance used in the world, only surpassed by nicotine, alcohol, and caffeine (Benegal et al., 2008; Pandya et al., 2009).

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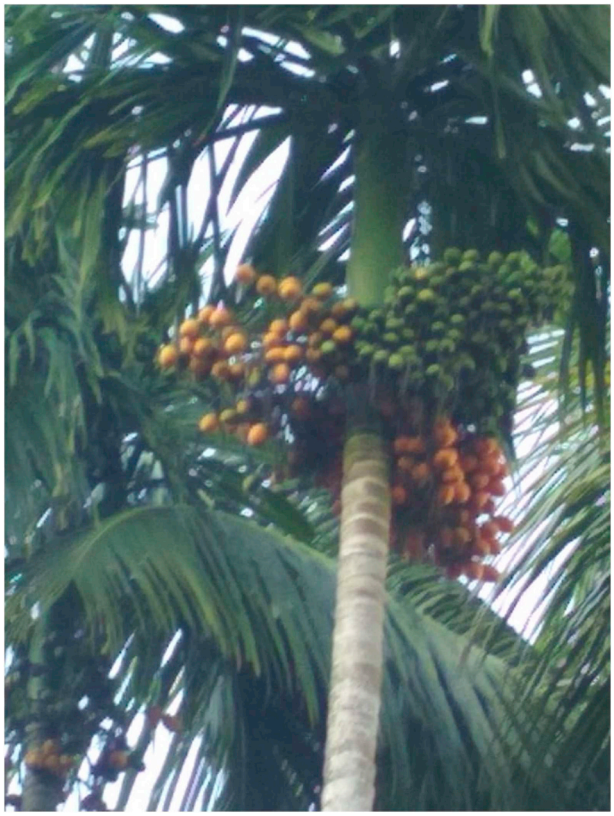

**Fig. 1. The *areca catechu* palm with drupe fruits** Source Sinha DN, Andaman Nicobar.

AN products are available in India at low prices, have packaging similar to smokeless tobacco, and are kept at stores and stalls frequented by children (Chande and Suba, 2016). This makes school children the most vulnerable group to initiate AN use (Rose et al., 2016). Many children who begin chewing habits with AN, especially sweetened and flavoured AN, usually advance to tobacco use. Thus, AN acts as a gateway product often leading to later tobacco use (Chandra and Mulla, 2007; Verma, 2011).

AN is used at different stages of maturity as the whole nut or in thin slices, in natural state or after processing in many forms (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2004). The nut can be used fresh, dried and cured by baking, roasting or sun-drying. In some eastern parts of India and Sri Lanka, Areca fruit is boiled and fermented by covering with mud to soften the nut for consumption. AN is also the basic ingredient of a variety of widely used chewed products across the world (Patidar et al., 2015). Betel quid, commonly used in India, contains AN, slaked lime, and various spices with or without tobacco wrapped in a fresh betel leaf. Tobacco or flavourful spices, such as cloves, cardamom, nutmeg, aniseed, coconut, sugar, syrups and fruit extracts, are combined with AN to enhance flavour (Baig et al., 2018). Various AN products are thriving in the marketplace under misleading advertisements portraying 'Areca nut' or 'Supari' as a safer alternative to tobacco products or marketed with an emphasis on its use as a mouth freshener. The most common AN-containing preparations are gutka and pan masala. Gutka is a dry, relatively non-perishable commercial preparation containing AN, slaked lime, catechu, condiments, and powdered tobacco (Niaz et al., 2017). Pan masala is the same mixture without added tobacco (Gupta and Warnakulasuriya, 2002). Chemistry of AN changes when it is mixed with tobacco and other flavouring agents, thereby further increasing its biological risk. However, we are limiting our article purely to AN.

### 1.2. Areca nut and health

Traditionally, the AN was believed to possess a number of medicinal properties ranging from anti-helminthic and astringent to an aphrodisiac, digestion improvement and psychomotor stimulant (Burton-Bradley, 1979). However, evidence of its health benefits is limited and unfortunately AN chewing is habit-forming, causes chemical dependency, and can lead to substance abuse (Chen et al., 2008; Chu, 2002; Winstock, 2002).

Use of AN along with tobacco is the major cause for the vast majority of oral cancers and oral potentially malignant disorders (OPMDs) in the developing world (Sankaranarayanan et al., 2015). Growing documented evidence reflects that apart from poor oral hygiene and bleeding of gums, regular AN users can face various other serious health problems (Bedi and Scully, 2013; Wollina et al., 2015). Chewing alters the oral bacteriome and microbiome (Hernandez et al., 2017). This may be the reason that as many as 5 million young Indians are estimated to have precancerous lesion condition as a result of the increased popularity of the habit of chewing AN products (Winstock, 2012). AN is a harmful addictive substance that has adverse effects on the whole human body and is carcinogenic to humans (Sharma, 2003).

Diverse type of cancers viz. oral, pharyngeal and oesophageal are associated with AN chewing (Chen et al., 2017; S. Gupta et al., 2018; Sharan et al., 2012). Chronic AN consumption can greatly increase the risk of heart attack (Khan et al., 2013; Yamada et al., 2013), type II diabetes (Hsu et al., 2010), chronic kidney disease (Wang et al., 2018), liver cirrhosis (Tsai et al., 2004), obesity (Lin et al., 2009), asthma (Taylor et al., 1992) and can causes hypothyroidism (Dasgupta et al., 2010), vitamin D deficiency (Ogunkolade et al., 2006) and interfere with the immune system (Yen et al., 2014). AN can effect sexual health in men (Huang and Jiann, 2017; Yuan et al., 2012) and women, who consume AN regularly, have more incidences of low birth weight and premature deliveries (Kumar, 2013; Kuo et al., 2005; Yen et al., 2006).

Seeing evidence of AN toxicity from the epidemiologic, animal, and mechanistic studies, the IARC has deemed AN (with or without tobacco) as group 1 carcinogen to humans (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2004). A monograph, "Betel-Quid and Areca-Nut Chewing" issued by the IARC-WHO summarized the epidemiological data associating areca use with oral diseases and cancer (WHO, 2012). An Areca Nut Data Base compiling 662 articles has been recently published (Thakur et al., 2019). However, in spite of the clear evidence that AN alone could be an independent major risk factor of various types of cancer, its usage is unabated across the globe as it is well tolerated in most cultures and people are not at all motivated to quit (Song et al., 2015). Lack of awareness about the carcinogenic potential of AN chewing among most of the consumers and even in many of the general practitioners could be the reason that the use of AN is continuing unabated worldwide (Lechner et al., 2019, 2018). Further, no regulations or serious strategy from the government bodies seems to exist in most of the countries to control the AN use (Kaur et al., 2017; Lee et al., 2018; Secretan et al., 2009).

A review by Garg et al. summarized that the systemic adverse effects of AN are mainly due to its principle alkaloid, arecoline (Garg et al., 2014). Studies revealed that arecoline has a psychoactive effect similar to nicotine (Horenstein et al., 2017), as is a partial agonist of nicotinic and muscarinic acetylcholine receptors. It evokes multiple effects on the central nervous system and causes an increase in the levels of monoamines such as noradrenaline as well as acetylcholine (Volgin et al., 2019).

The concentration of arecoline in AN samples is found to vary, greatly across the world, depending upon factors like geographical and climatic conditions of areca plant, age and processing methods of the AN (Gupta et al., 2018). Therefore, the current review discusses the global distribution of AN, its health effect and illustrates the cancer induction mechanism of its major alkaloid, arecoline in humans. The article highlights the wide variation in the level of carcinogenic

alkaloids content present in the diverse variety of AN sold worldwide. For the first time, various factors influencing the formation of carcinogenic alkaloids in AN at various stages such as during the growth, maturity of the nut, its processing and storage have been compiled. The article also presents comprehensive data on the dose-dependent pharmacology and toxicology of arecoline.

## 2. Chemical constituents of areca nut

AN is the seed of the endosperm of the fruit which is green when unripe and becomes yellow to orange when ripe. The seed or the nut is separated from the fibrous coloured pericarp. The nut has slightly bitter taste and characteristic astringency and its processing involves various stages like husking fresh raw fruit, removing the nut, drying nuts in the sun or with artificial heat, baking or roasting, boiling and fermentation. The presence of AN in products was determined using infrared spectral identification using Fourier Transform Infrared Spectroscopy (FT IR) with minimal sample preparation and rapid analysis time. FT IR has also been used to identify alkaline agents, tobacco species, and non-tobacco plant materials, such as spices (Stanfill et al., 2018, 2015, 2011). Lightweight handheld infrared spectral scanners that could be used to identify the AN constituent are now available. Chemical composition of AN have been extensively analysed and constantly reviewed. Major constituents in AN are carbohydrates, fats, proteins, crude fibre, polyphenols (flavanols and tannins), alkaloids and trace elements (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2004; Liu et al., 2017). *Shwetha Hr* et al. in 2019, analysed the major phytochemical constituents present in AN at different form of AN (Hr et al., 2019) (Table 1).

### 2.1. Polyphenols

Catechin, flavonoids, flavan-3,4-diols, leucocyanidins and hexahydroxyflavans are the prominent polyphenols found in AN (Sharan and Choudhury, 2010). Polyphenols are likely to contribute to the toxicity of AN. Proven carcinogens in tannins and polyphenols include safrole, hydroxychavicol, and catechins (Anand et al., 2014; Shah et al., 2012) The production and release of ROS occurs under alkaline conditions during the autoxidation of AN polyphenols, in the AN chewer's saliva. The ROS can be directly involved in the tumor initiation process, by inducing genotoxicity and gene mutation, or by attacking the salivary proteins and oral mucosa (Chen et al., 2017). Safrole metabolites, eugenol, and dihydroxy chavicol, found in the oral cavity, are regarded as a genotoxic carcinogen in the rat liver. These metabolites form DNA adducts *in-vitro* by $^{32}$P-post labelling assay. Huang et al. have reported that AN extract contains catechin-based procyanidins which range from dimers to decamers and polymers (Huang et al., 2010). During mastication, procyanidins become oxidized and confer the characteristic red colour in saliva, teeth and lips of AN chewers. The polyphenol content of the AN varies depending on the region of cultivation, degree of maturity, and method of processing. Polyphenol content is highest in unripe nuts with the content decreasing as the nut matures (Benegal et al., 2008; Sinor et al., 1990).

**Table 1**
Major constituents present in diverse forms of areca nuts.

| S no | Constituents concentration | Unripe nut | Ripe nut | Dried Nut | Roasted Nut |
|---|---|---|---|---|---|
| 1 | Total carbohydrate (in %) | 1.288 | 1.668 | 1.857 | 0.759 |
| 2 | Total Alkaloids (in %) | 0.062 | 0.140 | 0.061 | 0.064 |
| 3 | Total Protein (in %) | 0.040 | 0.080 | 0.031 | 0.045 |
| 4 | Total Tannin (in %) | 2.930 | 6.573 | 0.280 | 3.569 |
| 7 | Total Arecoline (in %) | 0.052 | 0.075 | 0.037 | 0.044 |
| 8 | Total copper (by wt.) | 2.07 | 3.31 | 3.63 | 3.30 |

### 2.2. Tannins

Tannins are specific types of polyphenols which are capable of precipitating proteins. The predominant tannin in AN is gallotannic acid, which is present in the outer part of the nut. Minor amounts of gallic acid, D-catechol and phiobatannin are also found in the inner part of the nut (Sharan and Choudhury, 2010). Roasted nuts possess the highest tannin content followed by raw than boiled nuts. In a study done by Awang, the tannin content of AN ranged from 5 to 41% in the roasted variety, 25% in the sun-dried variety, and 17% in the boiled variety (Awang, 1986).

### 2.3. Trace elements

AN also contains trace elements including sodium (Na), magnesium (Mg), calcium (Ca), vanadium (V), manganese (Mn) and copper (Cu) (Spyrou, 2001). Presence of trace elements in AN influence various biological activity in human. The copper content in raw and processed AN sample was found to be much higher than in any other nuts consumed by humans. The mean concentration of copper in samples of commercially available processed AN was upto 12.83 mg/kg which is 2.5 times higher than in raw AN which has mean as 3.64 mg/kg (Mathew et al., 2014). High levels of copper in AN are most likely linked to lysyl oxidase mediated fibrosis (Sharma et al., 2014). Moreover, the presence of arecoline enhances oxidation reduction potential of copper leading to increased cleavage of DNA which may generate an apoptotic response (Khan et al., 2015).

Arsenic metabolism is significantly associated with AN (Al-Rmalli et al., 2011). Studies found that arsenic increases the risk of skin lesions (Yu et al., 2016). A study by Faouzi et al. demonstrated that AN extract mobilizes $Ca^{2+}$ ions in various immune cell lines hence contribute to the inflammatory disorders in AN chewers (Faouzi et al., 2018).

### 2.4. Areca alkaloids

It has been well documented that the addictive and carcinogenic effect of AN is primarily due to its alkaloids (Volgin et al., 2019). There are four main alkaloids in AN, namely arecoline, arecaidine, guvacoline and guvacine. Further, it is evident that difference in the levels of areca alkaloids could potentially contribute to variations in addictive and carcinogenic potential across AN products (Liu et al., 2016). Analysis of a variety of processed AN across the world, by liquid chromatography-tandem mass-spectrometry (LC-MS/MS) method showed high variation in relative amounts of alkaloids (Jain et al., 2017). Guvacine being the most abundant (1.39–8.16 mg/g), followed by arecoline (0.64–2.22 mg/g), arecaidine (0.14–1.70 mg/g) and guvacoline (0.17–0.99 mg/g) (Table 2). However, the information on the levels of areca alkaloids in specific products is extremely scarce and there is an important gap in knowledge due to the great diversity of AN containing products.

Of all the AN-specific alkaloids, arecoline, a tertiary amine, is mainly responsible for parasympathetic and muscarinic effects and thus responsible for both the abuse liability and the carcinogenicity of AN (Lu et al., 2006). Arecoline, has deep brain penetration as evidenced by its numerous central nervous system effect (Chu, 2002). Arecoline's activity on nicotinic acetylcholine receptor (nAChR) is associated with addiction and may account for the habitual use of AN (Papke et al., 2015). The concentration of arecoline is reported to reach around 140 μg/ml during AN chewing. High levels of arecoline are present in the oral cavity even 10 min after the onset of AN chewing (Venkatesh et al., 2018).

## 3. Metabolism and Mechanism of Cancer Induction by an alkaloid

Though toxicity of AN alkaloids is effecting about one-sixth of the world's adult population, their metabolism is not yet fully explored

**Table 2**
Variation in alkaloid levels in areca nut-from India and China.

| Product | pH | % Moisture | Areca Nut Alkaloids (mg/g product dry weight) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Guvacine | Arecaidine | Guvacoline | Arecoline | Total Alkaloids |
| Dry areca nuts from Mumbai India | | | | | | | |
| Product 1-8 | 4.4–5.6 | 8.2–13.0 | 1.39–4.06 | 0.14–0.41 | 0.17–0.99 | 0.91–2.22 | 2.86–6.41 |
| Product 9 (Roasted) | 5.3 | 8.9 | 8.16 | 0.42 | 0.35 | 0.98 | 9.91 |
| Chinese Areca nut | | | | | | | |
| Product 1-3 | 7.9–8.3 | 17.9–20.2 | 2.05–4.49 | 0.64–1.46 | 0.18–0.40 | 1.07–1.44 | 4.52–7.20 |

(Gupta and Warnakulasuriya, 2002; Patterson et al., 2010).

Numerous and highly complex nitrosamine derivatives are formed from AN alkaloids in the presence of nitric oxide, generated by bacterial action, in mouth and stomach (Sharan and Choudhury, 2010). Nitrosation of major alkaloid, arecoline leads to four major N-Nitroso compounds that include N-Nitrosoguvacoline (NGL), N-Nitrosoguvacine (NGC), N-(Methylnitrosamino) propionitrile (MNPN), N-(Methylnitrosamino) propionaldehyde (MNPA) (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2004). Metabolite profile studies of mice urine by Giri et al., in 2006, revealed that arecoline is metabolized in the liver and kidneys forming several other compounds including arecaidine, arecoline N-oxide, arecaidine N-oxide, N-methyl nipecotic acid, N-methyl nipecotylglycine, arecaidinyl glycine, arecaidinyl glycerol, arecaidine mercapturic acid, arecoline mercapturic acid, and arecoline N-oxide mercapturic acid (Giri et al., 2006). Presence of these derivatives has been detected in the saliva and urine of AN chewers and is found to be the major cause of carcinogenesis (Nair et al., 1985).

Both arecoline and arecaidine are inter-convertible (Giri et al., 2007). N-oxidation of arecoline takes place mainly in the kidneys to form arecoline 1-oxide (Nery, 1971), which undergo glutathione conjugation combined with double bond reduction to form mercapturic acid in rats (Boucher and Mannan, 2002). MNPN is also shown to display carcinogenicity in *in-vitro* studies (Garg et al., 2014). It is proposed that MNPA may further generate N-(methylnitrosamino) 3-hydroxypropionaldehyde and N-(methanoyl nitrosamino) propionaldehyde derivatives, each of which in turn produces several diazohydroxide derivatives (Franke et al., 2015) (Fig. 2).

Slaked lime, an alkaline agent, is commonly consumed compound along with AN. Calcium hydroxide present in slaked lime favours hydrolyzation of arecoline and guvacoline to arecaidine and guvacine respectively. Altered salivary flow rates and pH in AN chewers, render the oral mucosa vulnerable to the toxic effects of AN (Rooban et al., 2006).

## 4. Factors affecting the concentrations of carcinogenic alkaloids in AN

Wide variation was seen in the levels of alkaloids in different type of commercially available nuts (Hr et al., 2019). Alkaloids content depends upon various factors like geographical location, maturity of areca fruit, processing method and storage. De-husked mature AN when dried in the sun for about 6–7 days, like Chali (whole), Parcha (cut in half) and Lylon (sliced) types, have arecoline content between 0.1 and 0.7%. While Nuli type, which uses immature nut, is found to contain higher levels of arecoline (0.6–0.9%) (Shivashankar et al., 1969). Various factors are discussed in the following section:

### 4.1. Geographical and climatic conditions

During the growth of the palm tree of AN, geographical and climatic conditions contribute to the observed variation in the constituents (IARC, 1985). Huang et al. reported that the contents of the four major alkaloids in fresh AN obtained from Darwin, and Australia, were somewhat higher than observed for Indian and Papua New Guinean type due to seasonal and geographical variations (Huang and McLeish, 1989). A study by Lin et al. in China showed that arecoline content in AN obtained from different producing areas was highly variable (Lin et al., 1992).

In China, commercially produced AN is commonly consumed as dried or cured fruit with husk. Levels of arecoline, arecaidine, and guvacine are highly variable in different AN samples and are highest in the dried kernel of AN over fresh kernels (Yuan et al., 2012). The content of arecoline in fresh kernel ranges between 2.17 and 7.64 mg/g while in the dried kernel it is between 4.6 and 17.57 mg/g. Wang et al., at Taiwan, detected arecoline as 1550 μg/g in mature AN (Wang et al., 2002), while in another study by Franke et al. in Guam, USA showed arecoline content as 595.3 μg/g in mature AN (Franke et al., 2015). This variation in arecoline content was suggested due to seasonal and/or geographical variations. Jantarat et al. found that the contents of arecoline in AN obtained from Northeast Thailand were in the 0.02–0.12% range whereas the contents of arecoline in areca nuts grown in China were in the 0.22–0.56% range (Jantarat et al., 2013). Thus, AN grown in temperate areas have different arecoline content than AN grown in tropical areas.

### 4.2. Stage of maturity of the nut

Arecoline level in the nut rises during the maturation period of AN but drops significantly in the matured nut. Young AN contains alkaloids in the order of abundance as arecoline > guvacine > guvacoline > arecaidine. Huang and McLeish reported that the contents of arecoline, arecaidine, guvacoline and guvacine in fresh AN seeds were approximately 0.30–0.63%, 0.31–0.66%, 0.03–0.06% and 0.19–0.72%, respectively (Huang and McLeish, 1989). Franke et al. in 2015, found significant differences in the total alkaloids and relative levels of individual alkaloids in the aqueous extract of young and mature areca nuts (Franke et al., 2015). As indicated in Table 3**,** levels of all alkaloids except arecaidine are higher in mature AN extract as compared to young AN extract. Aromdee et al. showed that the contents of arecoline in dried seed powder of unripe AN was 0.14% while it was 0.09% in ripe AN powder (Aromdee et al., 2003).

Thus, overall lower level of total alkaloids was observed in the young green nut compared to the mature nut. Arecoline being the major alkaloid in young nut while guvacine was the major alkaloid in the mature nut, with almost 3-fold higher concentration than arecoline.

Content of arecoline and total amounts of alkaloids in Areca fruit increased slightly in length (1.5–3.3 cm) and weight (1–5 g) but decreased at longer size (> 3.3 cm) and higher weight (> 5 g). Upside-down areca fruit i.e. growing upward (opposite to normal fruits, growing downward), contained a much higher amount of arecaidine content (4 mg/g of fresh wt.) than normal fresh unripe areca fruit (1.5 mg/g of fresh wt.) (Wang et al., 2002). Arecoline content was also found dependent on the shapes of AN, in the small round and large oblong nuts, arecoline concentration is 0.09%, in medium round nuts (0.04%), large round nuts (0.02%), medium oblong (0.12%) (Dutta

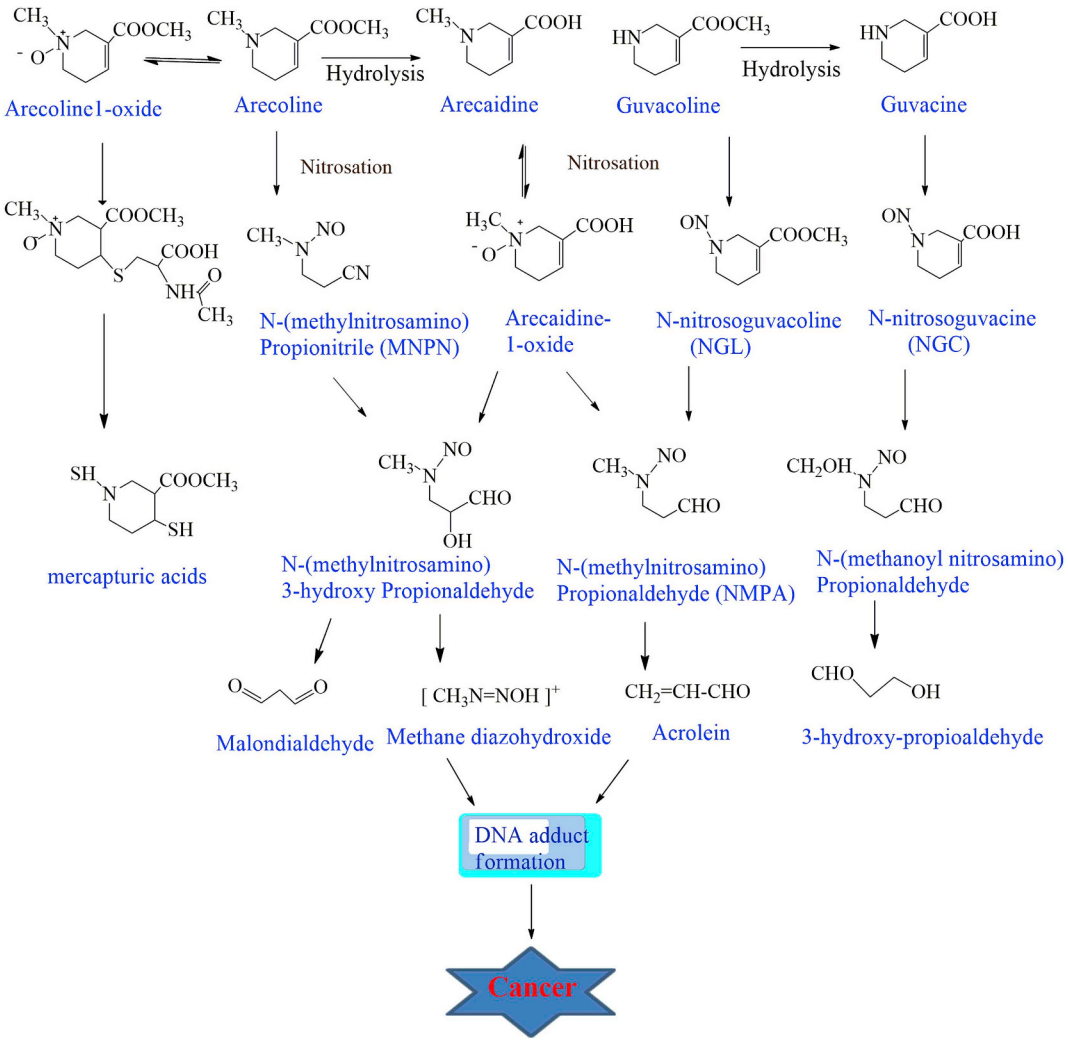


**Fig. 2.** Metabolism and mechanism of cancer induction by an alkaloid.

**Table 3**
Variation in alkaloid content in mature and young Areca Nut.

| S no. | Compound | Mature areca nut (μg/g) | Young green areca nut (μg/g) |
|---|---|---|---|
| 1 | Guvacine | 1871.4 | 302.9 |
| 2 | Arecoline | 595.3 | 339.1 |
| 3 | Guvacoline | 315.5 | 283.1 |
| 4 | Arecaidine | 47.7 | 93.2 |
| 5 | Total alkaloid | 707.5 | 254.6 |

et al., 2018). Developmental patterning and segregation study of different alkaloids distribution in AN by mass spectrometric methods revealed that in the ripened AN, arecoline, arecaidine, and guvacoline get segregated in the outer brown region of the nut while guvacine gets segregated in the inner white region. As the major fraction of the areca alkaloids stay in the brown region of the ripened AN, it could be safer to consume the white portion of the ripened nut (Srimany et al., 2016).

### 4.3. Processing methods

The processing treatments of the fresh nuts change the chemical composition, astringency, and the flavour of the nut. A proper processing method could help in reducing the carcinogenic chemicals present in AN as raw AN is more harmful for consumption by chewers than the processed AN (Rao and Das, 1989).

Wide variations in the arecoline content of AN have been demonstrated in commercially available nuts, ranging between 0 and 1.4% due to its processing method (Dutta et al., 2018). A study done by Awang stated that the arecoline content is reduced to 1.35% by sun-drying, to 1.29% by roasting, to 0.7% by soaking in water, and to 0.1% by boiling in water (Awang, 1988). The arecoline concentration is highest for the sun-dried raw variety followed by the roasted and the boiled variety (Angadi and Rao, 2011; Awang, 1986; Benegal et al., 2008). Arecoline content of charred AN is far lower than that of the raw AN (Djordjevic et al., 1993).

Dutta et al. in 2018, studied the levels of arecoline in various AN preparation consumed in India (raw, boiled, and roasted) along with commercially prepared sample of AN. The study showed that raw AN contains the highest concentration of arecoline (1.15 ± 0.008) followed by pan masala preparations (0.94 ± 0.006), least content in boiled AN (0.79 ± 0.009), while roasted variety exhibited an intermediate level (0.85 ± 0.007) (Dutta et al., 2018).

### 4.4. Storage conditions

Several age-old and crude methods are used to keep the nut moist for chewing like keeping the fresh ripe fruit in water or in pits. The husk contains easily fermentable substances like sugar and pectin which are easily attacked by microbes under humid conditions (Raisuddin and Misra, 1991). AN harvested prematurely or with high moisture content, if not dried and processed properly, are prone to attack by Aflatoxins, fungal toxins thereby inducing acute toxicity, carcinogenicity and

**Table 4**
Dose dependent pharmacology and toxicological data of arecoline.

| Sr. No. | Disease | Minimal toxic concentrationper mL | Results | Reference |
|---|---|---|---|---|
| 1 | Oral Precancerous conditions (OSF, OPMDs & leucoplakia) | ≥75 μg | Arecoline inhibited HaCaT epithelial cell proliferation, survival and cell cycle arrest in the G1/S phase, in a dose-dependent manner, | (Zhou et al., 2013) |
| | | 0.08 mM | Induced epithelial-mesenchymal transition (EMT)-related factors in primary human buccal mucosal fibroblasts | Zheng et al. (2015) |
| | | 239 μM | Induced vascular basic fibroblast growth factor contributing to OSF, by combining increased growth factor expression with endothelial necrosis, and thus driving fibroblast proliferation. | Ullah et al. (2015) |
| | | 0.2 mM | Activated latent transforming growth factor β1 via mitochondrial reactive oxygen species in buccal fibroblasts | (Hsieh et al., 2018) |
| | | 5 μg | Promoted keratinocyte migration and induced invasion, thereby supporting malignant transformation | Moutasim et al. (2011) |
| | | 10 μg | Increased stress-inducible protein, HO-1 expression in vivo | Tsai et al. (2009) |
| | | 20 μg | Generated reactive oxygen species and caused cell cycle arrest at the G1/G0 phase in HaCaT cells | Thangjam and Kondaiah (2009) |
| | | 50 μg | Significantly elevated TIMP-1 protein and mRNA expression in buccal mucosal fibroblasts | Shieh et al. (2003) |
| | | 0.8–1.2 mM | Impaired T cell activation and induction of PGE2, TNF-alpha and IL-6 production | Jeng et al. (2003) |
| | | 50 μg | Upregulated type I plasminogen activator inhibitor | Yang et al. (2003) |
| | | 10 μg | Induced c-jun protooncogene expression on oral mucosal fibroblasts. | Ho et al. (2000) |
| | | 0.4 mM | Differentially induced the dysregulation of cell cycle control, GSH level and intracellular $H_2O_2$ production | Chang et al. (2001) |
| | | 20 μg | Induced myofibroblast trans differentiation from human buccal mucosal fibroblasts | Chang et al. (2014) |
| | | 20 μg | Affected the collagen metabolism of fibroblasts | Xia et al. (2009) |
| | | 166 μg | Induced genotoxicity by delay in the cell cycle | Chatterjee and Deb (1999) |
| | | 0.5 mM | Activated the ATM/ATR kinase and Induces phosphorylated p-p53 p21(WAF1) protein expression in rat hepatocytes | Chou et al. (2009) |
| | | 80 μg | Increased β-catenin expression, correlated with the tumour size and clinical stage in human oral epithelial cells | Lee et al. (2012) |
| | | 5 μg | Increased epithelial-mesenchymal transition (EMT) transcriptional protein levels (Twist expression) in human primary buccal mucosal fibroblasts | Lee et al. (2016) |
| | | 200 μM | Decreased expression of monoamine oxidase gene in cancerous tissues | Chen et al. (2014a) |
| | | 200 μM | Increased fibrotic related genes by arecoline N-oxide in mice oral keratinocytes | Kuo et al. (2015) |
| | | 100 μM | Promoted the proliferation and invasion of oral squamous cell carcinoma by downregulated miR329 and miR410 expressions | Shiah et al. (2014) |
| 2 | Oral Cancer (OSCC) | 10 μg | Increased cell surface markers and transcription factors, epithelial-mesenchymal trans-differentiation, chemoresistance, migration invasiveness independent growth and *in-vivo* tumour growth in human oral epithelial (OE) cells | Wang et al. (2016) |
| | | 12.5 μg | Low-dose induced cell proliferation, ATM promoter activity, and DNA repair. High-dose induced cell cycle arrest, apoptosis, and DNA damage. | Tu et al. (2019) |
| | | 0.025 μg | Suppressed epithelial cell viability through the Akt/mTOR signalling pathway | Gu et al. (2019) |
| | | 0.4 mM | Reduced cell survival | Chen et al. (2014b) |
| | | 10 μg | Elevated carbonic anhydrase IX (CAIX) expression in normal buccal mucosa fibroblasts | Yang et al. (2014) |
| | | 0.3 mM | Induced disruption of expression and localization of the tight junctional protein in human endometrial Ishikawa cells | Giri et al. (2010) |
| | | 0.1 or 0.2 mM | Suppressed DNA repair in human keratinocytes (HaCaT cells) | Huang et al. (2016) |
| | | 8.77 mM | Arecoline N-oxide upregulated Caspase-8 Expression, in Oral Hyperplastic Lesions of Mice | Chang et al. (2017) |
| 3 | Endometrial adenocarcinoma | 0.1 mM | Induced cytotoxicity, DNA damage, G0/G1 cell cycle arrest in rat hepatocytes | Chou et al. (2008) |
| 4 | Lung cancer | 10 μM | Increased cell migration ability of A549 Lung Cancer Cells through EGFR/Src/FAK Pathway activation | Chang et al. (2019) |
| | | 1.7 μ g | Exhibited cytotoxicity | Abbas et al. (2018) |
| 5 | Head and neck squamous cell carcinoma | 130 nM | Increased epidermal growth factor receptor (EGFR) expression. | Yang et al. (2018) |
| 6 | Breast cancer (MCF-7 cells) | 0.1 μM | Inhibited proliferation and induced apoptosis | Feng et al. (2016) |

several other adverse effects on human health (Bhat et al., 2017). Arecoline content of AN decreases significantly when the nuts are dried and stored with husk as whole nut as compared to fresh mature nut (Bhat, 2016). Better preservation techniques like steeping the nut in mixed preservative solution should be utilized to control the growth of micro-organisms. Use of proper drying yards, proper spreading and turning of nuts during drying and avoiding moisture can greatly reduce the formation of carcinogenic alkaloids in AN. Further storage of nuts in air tight bags also minimizes the fungal infection.

## 5. Dose-dependent pharmacology and toxicology of arecoline

Studies have shown that AN users have persistent background salivary arecoline levels long after chewing, whereas concentrations achieved are highly variable and consistent with a role in oral pre-malignancy and malignancy (Venkatesh et al., 2018). A study by Tseng et al. showed that arecoline induced cell cycle arrest, apoptosis, and cytotoxicity to human endothelial cells (Tseng et al., 2012). Arecoline stimulates collagen in cultured cells at concentration as low as 0.1 μg/ml and is cytotoxic above 10 μg/ml (Cox et al., 2010). *S*alivary concentration of arecoline in AN chewers typically range from 40 to 400 μM, and 90% of AN chewers showed residual levels of at least 400 nM prior to chewing (Papke et al., 2015).

Arecoline induced the generation of reactive oxygen species and suppresses HaCaT cell proliferation, through cell cycle regulatory molecules (Thangjam and Kondaiah, 2009; Zhou et al., 2013). Many previous studies had shown that arecoline induces epithelial mesenchymal transition, vascular basic fibroblast growth proliferation and c-jun proto-oncogene expression; promotes keratinocyte and myofibroblast migration and invasion; activates latent transforming growth factor β1 in a dose-dependent manner in oral leucoplakia (OL), submucous fibrosis (OSF) and potentially malignant disorders (OPMDs) (Lee et al., 2016; Ullah et al., 2015; Zheng et al., 2015; Chang et al., 2014; Moutasim et al., 2011; Ho et al., 2000).

Various *in-vivo* and *in-vitro* studies in recent years have confirmed that arecoline induced cell cycle arrest and DNA damage, increased transcriptional proteins (Tu et al., 2019; Wang et al., 2016), suppressed epithelial cell viability through the Akt/mTOR signalling pathway at a minimum concentration of 12.5 μg/ml, 10 μg/ml and 0.025 μg/ml, respectively in oral cancer (Chen et al., 2014b; Gu et al., 2019). Another study showed that 1.7 μg of arecoline exhibited cytotoxicity in lung cancer cell line (Abbas et al., 2018). In a breast cancer cell line study, arecoline, at a concentration of 100 μM/l inhibited the proliferation and induced the apoptosis of MCF-7 cells (Feng et al., 2016). The pharmacology and toxicology of arecoline with respect to its doses is presented in Table 4. Arecoline at concentration between 50 and 200 μM could induce neuronal apoptotic death by attenuating antioxidant defence and enhancing oxidative stress (Shih et al., 2010).

## 6. Conclusions and road ahead

Being a customary and traditional commodity with some perceived health benefits, AN use is socially acceptable among all sections of society, including women and children, especially in South East Asian Regional (SEAR) countries. Sufficient evidences from the large-scale epidemiological and experimental studies over the last 40 years have shown that AN alone or with tobacco or lime is found to be deleterious to human health. As AN has been designated as a IARC Group 1 carcinogen, consumption of AN in any form should be treated as a “neglected global public health emergency”.

Of the numerous compounds present in AN, arecoline is the primary psychoactive ingredient, comparable to nicotine which contributes to the histologic changes in the mucosa, inducing oral submucous fibrosis (OSMF) in humans. There is a common synergistic link between arecoline and the molecular mediators of nicotine addiction as arecoline and nicotine both act on the same receptor proteins in the brain. Partial agonist therapies may also be used to aid AN cessation activity. This points to the possibility that drugs like cytisine or varenicline which are used to break nicotine dependence could also be effective to break the habit of AN use.

Variations in the levels of alkaloids content in AN could potentially contribute to the variations in the addictive and carcinogenic potential of AN and its preparations. Various factors including the age of the AN, its processing method, and storage conditions can influence the levels of carcinogenic constituents especially arecoline in finished AN-containing products. Labels of all manufactured AN preparation must contain a manufacturing code, expiration date, processing method, and storage conditions along with all its constituents and their associated health hazards. Similar to tobacco products, scientific evidence-based evaluation of all AN preparation is crucial to reduce the burden of AN induced fatal diseases. Pertaining to high degree of addictiveness associated, the use of AN, especially among adolescents, should be discouraged by preventive efforts. There is an urgent need to sensitize paan shop vendors about the repercussions of AN consumption and persuade them not to sell AN product to minors. Behavioural support and counselling by health professionals could be effective for abstinence from AN use.

The California Environment Protection Agency (CalEPA) has marked AN as a carcinogen and toxic product while US FDA has also restricted the import and banned inter-state transport of AN. Canada has already banned sale of AN products. Taiwan, where AN use is high, is also working hard to tackle this menace by providing subsidy to farmers for growing alternate crops and also plan to impose health tax on AN. To ensure food safety in AN import, in 2018, the Food Safety and Standards Authority of India (FSSAI) has prescribed standards nut in its Food Safety and Standards Regulations (Food Products Standards and Food Additives) 2011. In addition, it set limits of aflatoxin in AN through an amendment in the Food Safety and Standards (Contaminants, Toxins & Residues) Regulations, 2011. Many countries in SEAR like Bhutan, Thailand, Nepal, Myanmar have initiated steps to ban or regulate smokeless tobacco, but the region lacks effective policies and strategies to tackle AN menace and also initiate comprehensive national AN control program to reduce the burden of cancers due to AN use and to save millions of lives.

In conclusion, it is suggested that careful monitoring of the arecoline content can potentially be used as a tool in product surveillance studies to identify the similarities or differences in carcinogenic or toxic characteristics of various AN samples.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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