Review Article

ROLE OF ARECA NUT AND ITS COMMERCIAL PRODUCTS IN ORAL SUBMUCOUS FIBROSIS- A REVIEW

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ABSTRACT:
Potentially malignant disorders (PMD) like oral submucous fibrosis (OSMF) often precede Oral Squamous cell carcinoma (OSCC). OSMF is etiologically related to chewing of areca nut (betel nut) and its commercial products, a habit prevalent in India and South-East Asia. The increased prevalence of OSMF in the last two decades or so corresponds with the increased processing and commercialization of areca nut products. This review aims to provide an insight into the production of raw areca nut, the chemical constituents and its various processed forms. Importantly, the role of specific constituents in the pathogenesis of OSMF is also discussed.
Key words: Oral submucous fibrosis, Areca nut, Commercial products

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INTRODUCTION

Oral Squamous cell carcinoma (OSCC) is the most common cancer of the oral cavity and represents about 90% of all oral malignancies.[1] Several factors like tobacco, areca nut, alcohol, genetic predisposition and hormonal factors are suspected as possible risk factors. It is a global health problem with increasing incidence and mortality rates; around 300,000 patients are annually estimated to have oral cancer worldwide.[2] Oral squamous cell carcinoma (OSCC) is more common in the South and Southeast Asian countries in contrast to western society.[3] In India, because of cultural, ethnic, geographic factors and the popularity of addictive habits, the frequency of oral cancer is high; as 4 in 10 of all cancers are oral cancers. Annually 130,000 people succumb to oral cancer in India which translates into approximately 14 deaths per hour. Recently, a trend has been observed towards increased incidence of oral cancer among young adults.[4] The concept of a two-step process of cancer development in the oral mucosa, i.e, the initial presence of a precursor lesion subsequently developing into cancer, is well established. Thus, most OSCC are usually preceded by certain changes in oral mucosa. These warning lesions are referred as potentially malignant disorders (PMD). Oral submucous fibrosis (OSMF) is one of the commonest PMD found in oral mucosa.[5] The malignant transformation OSMF ranges from 3 to 19%.[6] Areca nut chewing has been causally related to OSMF. Current evidence suggest that, along with alkaloids in areca nut, the high copper content in the nut play an important role in the pathogenesis of OSMF.[7] This review aims to highlight the basic facts about the areca nut and its commercial products which are the main risk factors for OSMF.

ARECA NUT PRODUCTION IN INDIA

The areca nut palm (Areca catechu) is cultivated mainly in India, Malaysia, Polynesia, Micronesia, and most places in the South Pacific Islands.[8] It is a long slender, single trunked palm which can grow up to the height of 15 meters and crowned with 6 to 9 palm fronds. This tropical palm tree yield fruit twice a year, which are ovoid or oblong with a pointed apex, measuring 3–5 cm in length and 2–4 cm in diameter. The outer surface is green when unripe (Figure 1) and orange-yellow when ripe (Figure 2). The nut is the seed (endosperm) found within the fruit, mottled gray to brown in color with white markings.[9]

Figure 1: Unripe areca nuts

India is the largest producer of areca nut producing nearly half of global areca nut production. India ranks first in both area (58%) and production (53%) of arecanut. It is estimated that nearly ten million people depend on areca nut industry for their livelihood in India. Karnataka, Kerala, Assam, West Bengal, and Tamil Nadu are the important Indian states growing areca nut.[10]

Figure 2: Ripe areca nuts

HISTORY OF ARECA NUT CHEWING

Chewing of areca nut is an ancient custom in India, several parts of south-east Asia, the south Pacific islands and Taiwan. This practice dates back several thousand years and is deeply related to the tradition and culture of the population. References to the areca nut appear in ancient Greek, Sanskrit, and Chinese literature as early as the 1st century BC. There are innumerable references to areca nut palm in the Sanskrit manuscripts and its usage has been mentioned as food, medicine and for social and religious ceremonies. The most important reference is 'Anjana Charitra' (Sisy Mayana 1300 BC), where the reference has been made to groups of areca nut palms full of inflorescence and branches presenting an exquisite appearance.[11]

ARECA NUT USAGE- CURRENT SCENARIO

Areca nut is the fourth most commonly used social drug, ranking after nicotine, ethanol and caffeine. It is possibly the second most consumed carcinogen after tobacco in the Indian subcontinent. In India, areca nut is chewed for variety of reasons such as stress reliever, mouth freshener, concentration improver and a digestive following food. Being an addictive substance, its withdrawal symptoms are mood swings, anxiety, and irritability, loss of concentration, sleep disturbance and craving.[12]

Table 1: Shows the major constituents of a betel quid[15]

	Constituent	Origin/preparation
1	Areca nut	Unripe/ripe Whole/sliced Raw/roasted/sun dried Boiled/soaked in water Fermented (under mud)
2	*Piper betle* L.	Fresh leaf Inflorescence Stem
3	Slaked lime	From coral From shell fish From quarried lime stone
4	Tobacco	Sun dried Fermented Boiled with molasses Perfumed Concentrated extract (kiwam)
5	Catechu (extracted from)	• Heartwood of *Acacia catechu* or *A. suma* • Leaves of *Uncaria gambier* • Bark of Lithocarpus polystachya (nang ko)
6	Spices	Cloves Cardamom Aniseed (± sugar coat)
7	Sweeteners	Coconut Dried dates
8	Essences	Rose essence Menthol Mint Rose petals

Table 2: Showing the Chemical composition of areca nut at two maturity levels[19]

Constituents nut	Green (unripe)	Ripe nut
Moisture content	69.4–74.1	38.9–56.7
Total polysaccharides	17.3–23.0	17.8–25.7
Crude protein	6.7–9.4	6.2–7.5
Fat	8.1–12.0	9.5–15.1
Crude fibre	8.2–9.8	11.4–15.4
Polyphenols	17.2–29.8	11.1–17.8
Arecoline	0.11–0.14	0.12–0.24
Ash	1.2–2.5	1.1–1.5

Percentage based on dry weight except for moisture

ARECA NUT AND ITS COMMERCIAL PRODUCTS

Areca nut may be used fresh or it may be dried and cured before use, by sun-drying, baking or roasting. Areca preparations and specific ingredients vary by cultural group and individual user. The nut may be consumed alone or as a betel quid (BQ) in combination with betel leaf and slaked lime and may contain other substances like tobacco, catechu, spices or sweeteners. It is the basic ingredient of a variety of widely used chewed products including betel quid.[8] A betel quid (synonymous with 'pan' or 'paan') generally contains betel leaf (piper betle), areca nut and slaked lime, and may contain tobacco. Other substances, particularly catechu and spices, including cardamom, saffron, cloves, aniseed, turmeric, mustard or sweeteners, are added according to local preferences. In addition, some of the main ingredients (tobacco, areca nut) can be used by themselves or in various combinations without the use of betel leaf.[9] A consensus workshop held in 1996 recommended that the term 'quid' should be defined as a substance, or mixture of substances, placed in the mouth, usually containing at least one of the two basic ingredients, tobacco or arecanut, in raw or any manufactured or processed form.[13] It is recommended that, when the term 'betel quid' is used, other ingredients used to make up the quid be specified. A betel quid is often formulated to an individual's wishes with selected ingredients. The major constituents of a betel quid are listed in Table 1. Thus BQ can be of the following combinations:

i) Areca nut alone, without any betel leaf, slaked lime or tobacco
ii) Chewing tobacco without any areca nut
iii) Areca nut with betel leaf and any other ingredients except tobacco (betel quid without tobacco)
iv) Areca nut with betel leaf and any other ingredients including tobacco (betel quid with tobacco).

Still others prefer commercially manufactured dry areca products (e.g. Paan masala, Supari), which are convenient and imperishable mixtures that allow for widespread use.

Commercial betel quid substitutes

A variety of packaged areca products are now available in several countries. Notably,

i) Pakku or Supari- They are packaged areca nut which is processed and flavored
ii) Pan masala is basically a preparation of areca nut, catechu, cardamom, lime, and a number of natural and artificial perfuming and flavoring materials.
iii) Gutka is a variant of pan masala, which in addition to these ingredients contain flavored chewing tobacco. Both products are often sweetened to enhance the taste.[9]

Numerous commercially produced mixtures containing some or all of these ingredients are also available in various parts of the world. With the emergence of commercial pan masala and gutka about three decades ago, not only did the Indian market witness a massive growth in the sales of smokeless tobacco and areca nut products, but also a huge worldwide export market developed.[14] The packaging revolution has made these products portable, cheap, and convenient, with the added advantage of a long shelf-life. These products are exported to all countries where Asian migrants live.[9] Global estimates report up to 600 million chewers.[15]

Chemical constituents

The major constituents of the areca nut are carbohydrates, fats, proteins, crude fibre, polyphenols (flavonols and tannins), alkaloids and mineral matter. Polyphenols (flavonols, tannins) constitute a large proportion of the dry weight of the nut and are responsible for the astringent taste of the nut. It contain at least 9 structurally related pyridine alkaloids including arecoline, arecaidine, arecaine, arecolidine, guvacine, isoguvacine, guvacoline, and coniine. They are very important biologically and have a simulating effect.[16,17,18,19] The ranges in concentration of the chemical constituents of areca nut are given in Table 2.

Polyphenols (flavonols, tannins) constitute a large proportion of the dry weight of the nut. The ranges in concentration of polyphenols in raw nuts are shown in Table 2. Its content in areca nut may vary depending on the degree of maturity and its processing method. The tannin content is highest in unripe areca nuts and decreases significantly with increasing maturity.[16] The roasted nut possesses the highest average content of tannins, ranging from 5 to 41% (mean, 21.4%); the average tannin content of sun-dried nuts is 25%; and the lowest levels are seen in boiled nuts, which contain 17%.[20]

Alkaloids: Among the chemical constituents, alkaloids are the most important biologically. The nut has been shown to contain at least 9 structurally related pyridine alkaloids, of which four (arecoline, arecaidine, guvacine and guvacoline) have been conclusively identified in biochemical studies.[16,21,22] Arecoline is generally the main alkaloid. The ranges in concentration of arecoline in raw nuts are given in Table 2.

Wide variations in the arecoline content of areca nut have been demonstrated in commercially available nuts, ranging between 0 and 1.4%.[23,24] Arecoline content is reduced following processing of the nut. The content is reduced from 1.4% 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.[25] The practice of boiling the nut in a liquor obtained from the previous year's boiling is designed to increase the alkaloid content of treated nuts.[24]

Elemental composition: Concentrations of sodium, magnesium, chlorine calcium, vanadium, manganese, copper and bromine were measured in areca nut, pan masala and other chewing materials available in the United Kingdom.[26] In view of possible fibrogenic, mutagenic and toxic effects of areca nut, the copper content in samples of raw and processed areca nut was analysed and reported to be much higher than that found most frequently in other nuts consumed by humans.[27] The mean concentration of copper in samples of processed, commercially available areca nut was 18 ± 8.7 µg/g.[28] In an Indian Food Report, the copper content of processed areca nut was found to be 2.5 times that of the raw nut.[29] Study conducted by Shakya et al.[30] (14.9-18.3 mg kg^{-1}) also revealed higher copper levels in commercial areca nut products. Dhaware et al.[31] in their study stated that among the smokeless tobacco products, higher copper content was observed in the commercial preparations containing areca nut. A study conducted by us which compared copper content in natural raw areca nuts and commercial areca nut products showed that the copper content in commercial product was significantly higher than the raw areca nuts.[32]

ROLE OF CHEMICAL CONSTITUENTS IN THE PATHOGENESIS OF OSMF

The chewing habit varies among individuals, but usually the areca nut or commercial products are placed in the buccal vestibule for about 15 min to an hour and repeated five to six times a day. The alkaloids and flavonoids from the areca nut are absorbed and undergoes metabolism. These alkaloids undergo nitrosation and give rise to N-nitrosamines, which might have a cytotoxic effect on cells.[33]

Additionally, these constituents of areca nut and their metabolites are a source of constant irritation to oral tissues. Furthermore, the microtrauma produced by the friction of rough of areca nut fragments also facilitates the diffusion of alkaloids and flavonoids into the subepithelial connective tissue, resulting in juxtaepithelial inflammatory cell infiltration. [34] Over a period of time, due to persistent habit, chronic inflammation sets in at the site. Inflammation is characterized by the presence of activated T cells, macrophages which produces various chemical mediators of inflammation, especially prostaglandins (PGs).[35] Cytokines like interleukin-6, tumor necrosis factor (TNF) and interferon ά along with growth factors like transforming growth factor β (TGF-β) are synthesized at the site of inflammation.[36,37] Thus persistent mucosal inflammation is the crucial event for the initiation of fibrosis. TGF-β is a key regulator of extra cellular matrix (ECM) assembly and remodeling. The two main pathways regulated by TGF-β are collagen production pathway and collagen degradation pathway, along with the chemical constituents present in areca nut.

Collagen production pathway as regulated by TGF- β

TGF- β is a growth factor and it activates the procollagen genes, resulting in production of more pro-collagen. It also induces the secretion of procollagen C-proteinase (PCP) and procollagen N-proteinase (PNP), both of which are required for the conversion of pro-collagen to collagen fibrils. In OSMF, there is increased cross-linking of the collagen, resulting in increased insoluble form. This is facilitated by increased activity and production of a key enzyme – lysyl oxidase (LOX). PCP/ bone morphogenetic protein1 (BMP1) and increased copper (Cu) in areca nut stimulate LOX activity, a key player in the pathogenesis of this disease. The flavonoids increase cross-linking in the collagen fibers. These steps results in increased collagen production.[38]

Collagen Degradation Pathway as Regulated by TGF- β

TGF-β activates the genes for tissue inhibitor of matrix Metalloproteinase (TIMP); thereby more TIMP is formed. This inhibits the activated collagenase enzyme that is necessary for the degradation of collagen. It also activates the gene for plasminogen activator inhibitor (PAI), which is an inhibitor of plasminogen activator, thus plasmin formation is significantly reduced. Plasmin is required for the conversion of pro collagenase to active form of collagenase. The flavonoids of areca nut also inhibit the collagenase activity. A reduction in the activity and levels of collagenase results in a decrease in collagen degradation. [38]

Up-regulation of LOX

Copper acts by up regulating LOX activity, which is the key enzyme in collagen metabolic pathway. The LOX activity is important for formation of insoluble collagen due to cross-linking. The process of cross-linking gives tensile strength and mechanical properties to the fibers as well as makes the collagen fibers resistant to proteolysis. The LOX is dependent on copper for its functional activity. [39] Removal of copper leads to a catalytically inactive apoenzyme. During the biosynthesis of LOX, copper is incorporated into LOX. [40] Apart from copper, LOX also contains another co-factor, a covalently bound carbonyl prosthetic group – lysine tyrosylquinone (LTQ). [41] The LTQ is essential for the reaction mechanism of LOX, i.e. in the formation of cross-links in the collagen fibers. [42] Copper has been suggested to play a structural role in stabilizing the LTQ. [43] During the process of cross-linking, copper plays an important role in reoxidizing the reduced enzyme facilitating the completion of the catalytic cycle. [41]

CONCLUSION

The alkaloids, flavanoids and trace elements like copper from areca nut plays important role in the pathogenesis of OSMF. Thus association between areca nut/commercial areca products and OSMF is well established. Eradication of the habit through various initiatives has not quite succeeded in reducing the incidence and prevalence of the disorder. In fact the incidence only seems to be increasing, especially in the younger population. With this scenario it is only prudent to consider that if eradication of the habit is not working, then blunting causative substance or element could prove to be beneficial in bringing down the number of cases. The maximum permissible levels for the above mentioned contents in raw arecanut used for commercial products and in the products can be evaluated and curtailed by further research and standardization.

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