Arecanut as an emerging etiology of oral cancers in India

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ABSTRACT

Arecanut (AN) usage is widespread in Asian countries, especially India and Taiwan. The incidence of oral cancer is increasing day by day, but there is no exponential increase with tobacco usage. Especially in the country like Taiwan where betel quid mostly do not contain tobacco, AN can be correlated with the increased incidence of cancer. There are different studies in the literature about AN and oral cancer but none of them have concluded with the definite pathway for carcinogenesis. The present paper includes reviews of the literature for AN and oral cancer and summarizes the possible mechanisms associated with AN-induced carcinogenesis; and we have also tried to propose pathway of carcinogenesis.

Key words: Arecanut, Asian countries, betel quid, carcinogenesis, oral cancer

INTRODUCTION

Burden of oral malignant disease and premature death related to that is the burning issue in Asian countries. The association of betel quid with cancer could be concluded almost 100 years back; from the pre-Christian era, the records could be traced and was used as medical as well as psychosomatic substance as a breath refresher, digestive agent, worm expellant, aphrodisiac, and to maintain stamina.[1,2] In the recent era, the usage of betel quid was reintroduced almost 400 years when it was introduced from European traders.[1] The increased incidence of cancer in the recent population can be due to the change in the method of usage, i.e., keeping at particular site rather than rapid chewing and swallowing of all the contents, thereby decreasing direct contact time with the oral mucosa. The use of betel quid has become culturally accepted practice in India, which has now become a public health problem.[3,4]

The concept about role of arecanut (AN) as etiology for oral cancer emerged from Taiwan, where 10% of the population is pure AN chewer and 80% of the preparations do not contain tobacco; on the other side, most of the quid preparation in India contains tobacco.[4]

Tobacco has become a social nuisance now; so, most of the people have switched over to other nontobacco-containing products such as pan-masala that contain AN and lime with other condiments. The other side of the coin is that most of the people including medical professionals are unaware about the side effects of AN: Carcinogenicity and addiction. There are a few in vitro and in vivo studies as well as review articles in the literature stating the role of AN as a carcinogen, but exact carcinogenic pathway has not been clarified yet.[1] This paper intends to present the role of AN as carcinogen, suggest a carcinogenic pathway, and reviews the literature.

AN industry counts almost 300 crore every year; there are 200 billion users; it is openly sold and advertised all over public places without warning.[3] State of California-Environmental protection agency, Office of environmental health hazard assessment-Safe drinking water and toxic enforcement act of 1986 has considered AN as carcinogenic agent in February, 2006.[6] The incidence of oral submucous fibrosis (OSF) from betel nut rages form 0.9 to 4.7% in China, whereas in the India, that is almost up to 4.0 to 10%.[7] and malignant transformation rate of 7.6% in an Indian cohort over a period of 17 years; while in Pakistan, the rate is quite more.[8,9]

In 1969, the International Agency for Research on Cancer (IARC) initiated a program on the evaluation of the
carcinogenic risk of chemicals to human beings, involving the production of critically evaluated monographs on individual chemicals. With Supplement 6 (IARC, 1987a), the title of the series was modified from *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans* to *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*. The criteria established in 1971 to evaluate carcinogenic risk to human beings were adopted by the working groups whose deliberations resulted in the first 16 volumes of the *IARC Monographs* series. Those criteria were subsequently updated by further ad hoc working groups.[10,11]

In 2003, IARC has considered AN as group 1 human carcinogen. The 2004 monograph includes evaluation of working groups, working procedures, exposure data, etc. The monographs include composition of different substances, industrial packages, geographic region-wise consumption, regulation and legislation, studies on cancer in human and experimental models, physiologic and toxic effects.[10,11]

**WHAT IS ARECANUT?**

AN (*areca catechu*—an endosperm (nut/fruit) from tropical tree *Areca catechu Linnaeus*) is the fourth commonly used psychoactive substance chewed as an aid to digestion and as stimulant, either used alone or added with different tobacco or nontobacco substances to make different combinations. AN is a part of betel quid commonly consumed in Asian countries.[12] “betal nut” is a wrong terminology commonly used for AN; betel tree do not contain fruits but contain only leaves—betel leaves.

AN is known to produce mutagenic and genotoxic effects on tissues of body which may lead to various neoplastic and preneoplastic lesions.[7,13] Commission on cancer (COC) has first considered carcinogenesis of AN in 1993-1994.[13] The target cells of AN are oral fibroblast/myofibroblasts and keratinocytes.[14]

Different types of AN-containing commercially available preparations are available; most of the quids available in India contain tobacco [Table 1]. AN may be used as[11,12] unripe/ripe, whole/sliced, raw/roasted/sun dried, boiled/soaked in water, or fermented (under mud). Depending upon the type of curing, there are many types of AN. Marked reductions in the chemical constituents (carcinogens) were observed when the AN was subjected to soaking and boiling.[11,13] Boiled nut is due to change in arecanut extract (ANE) composition.[13] In contrast, van Wyk stated that boiled nut contained highest amount of ANE[16] (contents of *Areca nut*—[Table 2]).

**EPIDEMIOLOGIC STUDIES**

AN is consumed widespread in Asia. In countries like India, Pakistan, Bangladesh, Sri Lanka, tobacco is often added, and consumption is higher in women. However, in countries

| Table 1: Composition of the different types of chewing substances[10,11] |
|-----------------|-----------------|-----------------|---------------|---------------|-----------------|
|                | Arecanut*       | Betel†          | Catechu*      | Tobacco†      | Slaked lime     |
|                | Leaf            | Inflorescence   | Stem‡         |               |                 |
| Areca          | X               |                 |               |               |                 |
| Betel-quid without tobacco | X            | X               | (X)'          | X             |
| Betel-quid with tobacco | X             |                 | (X)'          | X             |
| Gutka          | X               | X               | X             | X             |
| Pan masalag†   | X               |                 | X             | X             |
| Khaini         | X               |                 | X             | X             |
| Mawa           | X               |                 | X             | X             |
| Mainpuri tobacco | X             |                 | X             | X             |
| Lao-hwa (Taiwan) | X            |                 | X             | X             |
| Betel-quid (Taiwan) | X          |                 | X             | X             |
| Stem quid (Taiwan) | X            |                 | X             | X             |
| Naswar         | X               |                 | X             | X             |
| Zarda          |                 |                 |               | X             |

May be used unripe, raw, or processed by baking, roasting, or baking with sweetening, flavoring, and decorative agents; 'in place of the leaf, the inflorescence or its stem may also be used; 'stem of inflorescence; ‘in powdered or paste form; ‘in flaked, powdered, or paste form, with or without processing, with or without sweetening; ‘) means optional; †used in unripe form

| Table 2: Contents of arecanut[11,12] |
|-----------------|-----------------|-----------------|---------------|---------------|
| 5.41%           | Tannins         |                 |               |
| 11.1-29.8%      | Polyphenols     |                 |               |
| 0.15-0.67%      | Alkaloids       |                 |               |

[arecoline (7.5 mg/g weight), arecaidine (1.5 mg/g weight), guvacoline (2.0 mg/g weight), and guvacine (2.9 mg/g weight), arecoline]
like Taiwan (China) (10% of the population is AN users), Hainan (southern China), and Papua New Guinea (80% of the population is AN users), tobacco is never added. Studies done by Dayal 1978 (1.5% in Ahmedabad mill workers), Gupta 1996 (0.5% in Mumbai), and Daftary 1980 (0.7% in Ernakulam) have reported very less percentage of pure AN chewers. However, on contrast, studies by Chakraborty 1990 (11.4% West Bengal) and Shah 2002 (28.9% Pakistan primary school children) have shown higher rate of consumption.[11]

On the other hand, Taiwanese studies have shown higher AN consumption rate of almost 50%: Tang 1997 (China Hunan 20.3% AN, 15.1% AN with smoking), Ko 1992 (Taiwan 42.1% AN), Yang et al. 2001 (Taiwan 47.8% chewing only, total 69.5%).[11]

There are so many Indian studies reported in the literature but most of them have studied betel-quid that mostly contain tobacco in Indian scenario. However, some international studies have studied pure AN and there are two meta-analysis. The AN in quid along with tobacco may play synergistic role for carcinogenesis [Table 3].

## PSYCHOACTIVE PROPERTIES

AN is the fourth commonly used psychoactive substance used worldwide, after tobacco, alcohol, and caffeine-containing beverages. AN quid chewing has claimed to produce a sense of well being, euphoria, warm sensations of the body, sweating, salivation, palpitation and heightened alertness, tolerance to hunger, and increased capacity and stamina to

| Table 3: Epidemiologic studies |
|-------------------------------|
| Country | Preparation studied | Investigator | Cases | Details |
| India 1959 | Betel-quid and smoking and alcohol | Shanta and Krishnamurthi[17] | 206 (8.7%) SCC 278 (51.8%) controls | Carcinoma: Tongue (188) Buccal floor (40) |
| India 1962 | Betel-quid and smoking and alcohol | Chandra[18] | 181 (25%) SCC 326 (22%) controls | OR 1.3 |
| India, Sri Lanka 1966 | Betel-quid and smoking and alcohol | Hiramaya[19] | 40 (33%) SCC 142 (28%) controls | OR 1.2 |
| Pakistan 1876 | – | Jafarey[20] | 118 (34%) SCC 190 (6%) controls | OR 3.6 |
| India 1989 | Betel-quid and smoking and alcohol | Sankaranarayanan et al.[21,22] | 115M, 233F SCC 115M, 233F controls | OR 15, M, 2.2F |
| India 1990 | – | Nandakumar et al.[23] | 5 (60%), 102 (70%) SCC 51 (4%), 195 (22%) controls | OR 28.2, OR 6.9 adjusted for smoking |
| Taiwan 1995 | Betel-quid and smoking and alcohol | Ko[24] | 40 (83%) SCC 160 (24%) controls | OR 58.4 |
| Taiwan 1996 | – | Lu[25] | 32 (13%) SCC 152 (8%) controls | OR 1.7 |
| India 2000 | Betel-quid | Dikshit[26] | 100 erythroplakia 47773 control | OR 19.8 |
| India 2000 | Chewing and alcohol | Hashibe et al.[27] | 47773 control | OR 19.8 |
| Pakistan 2000 | Smoking, drinking, paan chewing and oral hygiene | Merchant[28] | 79 (53%) SCC 149 (11%) controls | OR 9.9 |
| India 2002 | Smoking, drinking, paan chewing and oral hygiene | Balaram[29] | 142 (11%) SCC 283 (3%) controls | OR 4.2 |
| Taiwan 2002 | HPV infection, betel-quid chewing, and cigarette smoking | Chen[30] | 22 (86%) SCC 29 (30%) controls | OR 17.1 |
| India 2003 | Tobacco smoking, chewing and alcohol drinking | Znaor[31] | 90 SCC 90 controls | OR mouth- 2.6, tongue- 1.7 |
| India 2004 | – | Chitra et al.[32] | 90 SCC 90 controls | OR 2.8 |
| Papua New Guinea 2007 | Betel-quid not containing tobacco- meta-analysis | Thomas S J[33] | 143 SCC 477 controls | OR: Chewing 2.03, heavy chewers 2.47, smokers 2.63, heaviest smokers 4.63 |
| Mainland China 2007 | Betel-quid | Zhang X[34] | Hainan fresh Hunan fresh- high prevalence of AN chewing | OSF 0.9-4.4% |
| Papua New Guinea 2008 | Betel-quid not containing tobacco- meta-analysis | Thomas S J[35] | 197 cases, 1282 controls | Prevalence OL 11.7% |

(%) describe percentage of betel-quid chewer without tobacco, OR - odds ratio, OL - oral leukoplakia, OSC - oral squamous-cell carcinoma, OSF - oral submucous fibrosis
work. All these neurological effects suggest that chewing AN quid influences central and autonomic nervous system at various levels.[12] These effects of AN are habit and dose related and is stronger for fresh or occasional chewers than in habitual chewers. Different people have reported different studies regarding this property.[11]

AN psychosis was originally described about 25 years ago in Papua New Guineans by Burton-Bradley (1977). He described how traditional healers challenged victims with so-called betel nut to induce insanity as a part of their diagnostic strategy.[11, 36] [Table 4].

**Pathogenesis of Carcinoma**

Emerging evidence indicates that sustained stress exposure induces epigenetic reprogramming of some mammalian cells, thereby increasing mutation rate to accelerate adaptation to stressful environments.[47] ANE has been shown to be mutagenic and genotoxic in a variety of short-term assay systems.

Oral carcinogenesis is a complex, multi-step process that includes initiation, promotion, and progression and is thought to be resulting from the progressive accumulation of genetic lesions after long-term betel-quid (BQ) exposure.[46] Interaction between presumed carcinogens and cellular macromolecules such as DNA, proteins, and lipids is the most important and decisive event of the chemical carcinogenesis.[48, 49] Toxicity studies relating to AN-containing polyphenols and tannins are not conclusive, with both carcinogenic and anticarcinogenic effects being reported.[46] Thus, the target organs for tumorigenesis by AN extract and AN polyphenols may be different.[46]

**Carcinogens in Areca Nut**

The contents that are proven as carcinogens are tannins, some of the polyphenols: Safrole, hydroxychavicol, and catechins, and most of the alkaloids. Some constituents of betel leaf are known to have antimutagenic effects; hydroxychavicol, eugenol.

When the alkaloids are compared on a weight basis with the extract, no single agent has detectable effects on the cells at concentrations of the extract that cause decrease colony survival and DNA single-strand breaks. Therefore, additive or synergistic effects could be considered among the alkaloid.[31]

**Table 4: Psychoactive properties**

| Name                      | Year | Description                             |
|---------------------------|------|-----------------------------------------|
| Schneider, 1986(20)       |      | Pleasure-giving substance               |
| Norton, 1998(20)          |      | Psychostimulating effects               |
| Chu, 2001, 2002(26-40)    |      | Stimulant effects                       |
| Burton-Bradley 1977(9)    |      | Betel nut psychosis in papua            |
| Cawte, 1985(4)            |      | Altered status of consciousness and intoxication in Melanesia |
| Pickwell et al., 1996(4)  |      | Betelmania in Cambodian women living in USA |
| Burton-Bradley, 1978(40)  | Talonu, 1989(40) | Habituation and addiction               |
| Winstock et al. 2000(44)  |      | Dependence syndrome                     |
| Kuruppuarachchi and Williams, 2003(43) |      | Higher frequency of consumption in schizophrenic patients of Sri Lanka |
| Behari M, Sharma AK, Changkakoti S, Sharma N, Pandey RM(43) |      | Meige’s syndrome in India               |

Alkaloids

Lime is commonly consumed compound along with AN. In the presence of lime (calcium hydroxide), arecoline and guvacoline are hydrolyzed to arecaidine and guvacine. Bacterial enzyme nitrite reductase from denitrifying (Pseudomonas) and non-denitrifying (E. coli, Proteus) bacteria aids in catalysis of nitrosation of secondary amines.[52] and poor oral hygiene also play a role. Thiocyanate in the oral cavity, catechu, and lime also act as a catalyst at pH 9.5. Enhanced by Fe2+, Fe3+, Cu2+, and inhibited by Mn2+.[10] The formation occurs through autoxidation, redox cycling via quinone/semiquinone radical, and iron-catalyzed Haber–Weiss and Fenton reactions.[10]

Arecoline is parasympathomimetic while arecaidine lacks that action. Arecaidine is more potent, cytotoxic, and mutagenic and is tumor promoter. In vitro, this action is prevented by antioxidants such as Glutathione, N- Acetyl L-Cysteine. Arecoline is de-esterified in liver while other compounds are excreted in urine. The metabolic interconversion of arecoline and arecoline 1-oxide is possible.[53] ANE increase salivary flow and decrease pH that may render tissue to more cytotoxic effects.[1]

Nitrosation of arecoline leads to four N-Nitroso compounds: N-Nitrosoguvacoline (NGCO), N-Nitroso guvacine, 3 (Methylnitrosamino) propionitrile (MNPN), 3 (Methylnitrosamino) propionaldehyde.[1, 11, 13] These nitroso compounds have been detected in the saliva of AN chewers and are thought to be the culprit.
of carcinogenesis. Among all these compounds, NGCO is the most significant one. In in vitro studies, MNPN has also shown carcinogenicity.[11,13]

**Polyphenols**

Polyphenols are likely to contribute to the marked toxicity of the extract. Safrole is also a major component extracted from betel-quid preparation in Taiwan. Its metabolites found in the oral cavity are eugenol and dihydroxychavicol. That had been extensively studied showing DNA adducts formation in vitro by 32P-postlabeling assay, regarded as a genotoxic carcinogen in the rat liver. Eugenol, a major polyphenol of betel-quid, is cytotoxic to human buccal mucosal fibroblasts by decreasing cellular ATP level and lipid peroxidation. A recent report further suggests role of safrole in oral carcinogenesis, by demonstrating safrole forms, safrole-DNA adducts in human oral tissue following betel-quid chewing.[54]

In contrast, according to some studies, hydroxychavicol and eugenol extracted from betel leaf have antimutagenic effects against dimethylbenzanthracene-induced mutagenesis.[55,56]

**MODE OF ACTION**

**Host defense modulation glutathione**

Glutathione is tripeptide involved in detoxification of toxic electrophilic xenobiotics, is reducing agent and antioxidant, and is responsible for cell cycle and thermoregulation.[13]

ANE and polyphenols increase glutathione; while arecoline decrease glutathione; and both decrease protein–sulfhydryl (SH) content. Protein–SH is important for cell division and differentiation and many carcinogens inhibit protein–SH as part of carcinogenesis.[57] ANE decreases GST (glutathione S transferase) and acid soluble sulfhydryl (–SH) levels; while, increases cytochrome b, and P-450 levels in mice.[58] Thus, they impair host defense.

ANE and arecoline increases PgE2, IL-6, TNF-β in CD4 and CD8 cells, thereby causing impaired T cell activation. In keratinoblasts (KB) cells, these causes COX2 expression and inflammation that leads to decreased cell growth and cell cycle arrest and apoptosis [Figure 1].[59]

**Inflammatory mediators prostaglandins**

ANE activate mitogen-activated protein kinase superfamily (ERK, c-JNK, p38) and transcription factor NF-kB in oral keratinocytes that are important signaling elements. ANE did not act on EGF receptor signaling system but blockage of NF-kB activation leads to ANE-modulated COX-2 upregulation.[60] But COX-2 mRNA and protein expression upregulation are reversible and can be inhibited by indomethacin and aspirin. Thus, it is not the main pathway.[61] Arecoline induces COX-2 expression in sperm cells in dose-dependent manner and decrease motility.[62]

**REACTIVE OXYGEN SPECIES**

Various AN constituents may generate reactive oxygen species (ROS) (O₂, H₂O₂, OH) in the presence of lime but catechin fraction is the most active producer. Fe2+ had additive effect, while Mg2+ has marked inhibitory effect.[63] ROS are responsible for oxidative DNA base tissue damage. ROS can be detected by presence of o- and m-tyrosine in saliva of chewer.[64] ANE induces micronuclei and cytokinesis failure in ovary cells in vitro. These changes are associated with increased intracellular H₂O₂ levels and actin filament disorganization.[65]

In order to provide a defense mechanism against the attack of ROS, cells may exert nonenzymatic and enzymatic systems incorporating agents such as Gluthione S transferase (GSH), catalase, superoxide dismutase (SOD), and glutathione peroxidase, in order to prevent or minimize the toxic damage potentially elicited by ROS.[66,67] ROS acts by (1) Directly gene mutations, (2) Attack salivary proteins and oral mucosa—structural changes—penetration of various objects, (3) Inflammatory cell infiltration—more ROS—mutation of adjacent cells.[66]

ANE-induced unscheduled DNA synthesis (UDS) in gingival keratinocytes may be inhibited by vitamin C, glutathione, desferoxamine (iron chelator and free radical scavenger); while, banthocuproine (copper chelator), 1,10-phenanthroline (lipid permeable iron chelator), and specific reactive oxygen species scavengers such as dimethyl-sulfoxide, mannitol, dimethylthiourea, pyruvate, catalase, and SOD lacked these preventive effects. Higher concentrations of H₂O₂ inhibited the basal levels of UDS. Thus, it can be stated that these effects are associated with free radical reaction.[68]

However, the extracellular addition of GSH and cysteine has been shown to prevent the arecoline cytotoxicity to cultured OMF in vitro, although SOD and catalase lacked similar preventive effects. This indicates that the cytotoxicity of arecoline to cultured OMF is not mediated by the extracellular production of superoxide radicals and H₂O₂.[69]

**Cell damage**

Salivary AN-specific carcinogen 3NPA is known to form DNA protein cross-links and DNA single-strand breaks.[81] Different concentrations of extracts of AN induced dose-dependent UDS in Hep 2 cells. Aqueous and acetic acid extract induce relatively more UDS.[70] Arecoline lowers poly ADP ribosylation in most cellular proteins in
Swiss mice. These changes may be the earlier events for initiation of carcinogenesis.\[^{71}\] Arecoline induced cyclin β1, wee1, phosphorylated CDC 2 protein, and declined p21 expression in KB epithelial cells in gingival Gingival keratinocytes causes the reverse action and ultimately leads to arrest of late S, G2/M cycle. Thus, differential regulation of S and/or G2/M cell cycle-related proteins in the GK and KB cells play a crucial role in different stages of AN-mediated carcinogenesis.\[^{72}\] ANE is known to cause upregulation of Asb6, a coupling protein to the adapter protein with Pleckstrin homology and Src homology 2 adapter protein, which is involved in insulin signaling for glucose transportation which can be used as prognostic marker.\[^{73}\] Amount of substance needed for an event is shown in the Table 5.

### GENES/BIOMARKERS

DNA repair machineries play a pivotal role in maintaining genome integrity. Deregulation of DNA repair can result in genomic instability, which is a hallmark of cancer cells.\[^{74}\] p53 plays important role in cellular response to stress and is tumor suppressor gene, is the most frequent target (90% involve missense mutation in one allele) for genetic alterations in cancer, and involves in more than 50% of cancers. In Taiwanese, oral cancers infrequent p53

![Figure 1: Molecular events](image-url)

#### Table 5: Amount of substance needed for an event

| Event                                      | AN extract (μg/ml) | Arecoline (μM) |
|--------------------------------------------|--------------------|----------------|
| Inhibition of growth of oral keratinoblasts (%) | 100-800 (36-90)    | 20-120 (15-75) |
| Increased PgE2 and 6 keto PgK1α production | 200-400            |                |
| G2/M cycle arrest of KB and OMF cells, cell retraction and intracellular vacuole formation | >400 (only KB cells) | >0.2 |
| Decrease Glutathione levels               | 800-1200           | 0.4-1.2        |
| Hydrogen peroxide production              | 800-1200           | 0.1-1.2        |
| Hyperpolarization of ΔpH (increase rhodamine uptake) | 800-1200          | 0.4-1.2        |
| Little DNA fragments of KB cells          | 100-1200           | 0.1-1.2        |

**AN - Areca nut**
mutations have been reported and 80% of the etiology involves betel-quid which do not contain tobacco in Taiwanese formulations.[78] Some reports from India have also shown infrequent p53 mutation.[79] There is an alternative mechanism of p53 inactivation besides mutations. The mechanism may be either inactivation by abrogating specific DNA binding resulting in p53 sequestering or other genes related to oral cancer (p16/ pRb pathway, p21ras, cyclin D1, CD44v7-8, c -myc, N-myc, and Ki-ras).[79]

The role of tissue growth factor (TGF)-β in epithelial malignancy is complex, but it is becoming clear that in the early stages of carcinogenesis, the protein acts as a potent tumor suppressor, while later, TGF-β can function to advance tumor progression.[77] The observed methylation of the p16/MTS1 promoter regions for 54% of tongue squamous-cell carcinoma specimens obtained from BQ-chewers has recently been reported.[78]

ANE induces c-jun proto-oncogene mRNA levels and the effect is independent of glutathione. This may be the mechanism of carcinogenesis.[79,47] Patients with that have poor prognosis. Liu et al. demonstrated presence of safrole DNA adducts in peripheral blood lymphocytes. That can be traced to polymorphism of the CYP2E1 gene, alone and in combination with the GSTM1 and GSTT1-deletion polymorphisms. Thus, CYP2E1 plays important role for adduct formation.[80]

ANE has shown mutagenicity to S. typhi strains in in vitro studies.[9] It has also induced chromosomal aberrations, sister chromatid exchange, and micronucleated cells and decrease in sperm motility and tumor production in other organs in other in vitro studies.[9]

Arecoline can induce hyperphosphorylation of γ-H2AX which is a marker to examine DNA damage. Upon DNA damage, various molecular events result and ataxia telangiectasia mutated (ATM) kinase plays an important role. Arecoline induces γ-H2AX phosphorylation, triggers ATM-dependant signal pathway and G2/M cycle arrest, suppresses DNA repair, and inhibits expression and transactivation function of p53.[74]

Indian childhood cirrhosis; upregulation of lysyl oxidase is also seen: An enzyme associated with collagen synthesis and cross-linkage.[81] Upregulation of COX-2 and increased levels of proinflammatory cytokines and reduced levels of anti-fibrotic IFN-γ are also found. Genetic polymorphism is also found to be associated. Increased production of tissue inhibitors of matrix metalloproteinases protein is found in OSE.[83] Autoimmunity is also shown to be involved with that.

**CONCLUSION**

The pathogenesis of AN carcinogenesis is a complex multistep process involving various pathways and constituents. Carcinogenesis of tobacco is well known and reported in the literature, but no single study is found that has completely supported definite carcinogenesis pathway. Different in vivo and in vitro studies have shown different pathway of carcinogenesis, but when substance inhibiting that particular pathway was used that has not completely inhibited the cellular changes caused by AN substitute. On the other hand, when effects caused by a single AN agent were blocked, even then carcinogenesis was found. So, neither single agent is responsible nor single pathway can produce carcinogenesis and oral submucus fibrosis (OSMF) is related with the carcinogenesis and definite genetic mutations are found to be present.

**REFERENCES**

1. Gupta PC. Areca nut use in India. Indian J Med Sci 2007;61: 317-9.
2. Sharan RN. Association of betel nut with carcinogenesis: A review. Cancer J 1996;9:13-19.
3. Bobba R, Khan Y. Cancer in India – An Overview. GOR 2003;5: 93-6.
4. Control of oral cancer in developing countries. A WHO meeting. Bull World Health Organ 1984;62:817-30.
5. Ray CS, Gupta PC, Beyer JD. Research on tobacco in India (including betel quid and areca nut). An annotated bibliography of research on use, health effects, economics, and control efforts August, 2003.
6. State of California Environmental protection agency chemicals known to the state to cause cancer or reproductive toxicity February 3, 2006.
7. Trivedy CR, Craig G, Warnakulasuriya S. The oral health consequences of chewing Areca nut. Addict Biol 2002;7:115-25.
8. Murti PR, Bhonsle RB, Pindborg JJ, Daftary DK, Gupta PC, Mehta FS. Malignant transformation rate in oral submucus fibrosis over a 17-year period. Community dent. Oral Epidemiol 1985;13:340-1.
9. Nair U, Bartsch H, Nair J. Alert for an epidemic of oral cancer due to use of the betel quid substitutes gutkha and pan masala: A review of agents and causative mechanisms. Mutagenesis 2004;19:251-62.
10. International Agency for Research on Cancer: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 37, Tobacco Habits other
than Smoking; Betel-quid and Areca-nut Chewing; and some related Nitrosamines. Lyon: International Agency for Research on Cancer; 1984.

11. International Agency for Research on Cancer: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans Vol. 85, Betel-quid and Areca-nut Chewing and Some Areca-nut-derived Nitrosamines. Lyon: International Agency for Research on Cancer; 2003.

12. Rooban T, Joshua E, Rooban A, Kumar G, Govind. Health hazards of chewing arecanut and products containing arecanut. Calicut Med J 2005;3:3-6.

13. COC 2008 Statement on Betel Quid, Pan Masala and Areca Nut-Chewing COC/08/S2 – July 2008. Available from: http://www.iaccog.org.uk [Last accessed on 2008 July].

14. Sundqvist K, Graffstrom RC. Effects of areca nut on growth, differentiation and formation of DNA damage in cultured human buccal epithelial cells. Int J Cancer 1992;52:305-10.

15. Saraswathi TR, Sheeba T, Nalin Kumar S, Ranganathan K. Effect of glutathione on arecanut treated normal human buccal fibroblast culture. Indian J Dent Res 2006;17:104-10.

16. Van Wyk CV, Olivier A, De Miranda CM, Van der Bijl P, Grobler-Rabie AF. Observations on the effect of areca nut extracts on oral fibroblast proliferation. J Oral Pathol Med 1994;23:145-8.

17. Shanta V, Krishnamurthi S. A study of aetiological factors in oral squamous cell carcinoma. Br J Cancer 1959;13:381-8.

18. Chandra A. Different habits and their relation with cancer cheek. Chittaranjan cancer hospital, Calcutta. Natl Cancer Res Centre Bull 1982;1:33-6.

19. Hiramaya T. An epidemiological study of oral and pharyngeal cancer in central and south-east asia. Bull World Health Organ 1966;34:41-69.

20. Jafarey NA, Zaidi SH. carcinoma of the oral cavity and oropharynx in Karachi. An Apraisal. Trop Doct 1976;6:63-7.

21. Sankaranarayanan R, Duffy SW, Day NE, Nair MK, Padmakumary G. A case–control investigation of cancer of the oral tongue and the floor of the mouth in Southern India. Int J Cancer 1989;44:617-21.

22. Sankaranarayanan R, Mathew B, Jacob BJ, Thomas G, Siddiqui AR, et al. A population-based case–control investigation on cancers of the oral cavity in the Gujarat community in the UK. Addict Biol 2000;5:173-9.

23. Nandakumar A, Thimmaseettty KT, Sereramareddy NM, Venugopal TC, Rajanna, Vinutha AT, et al. A population-based case–control investigation on cancers of the oral cavity in Bangalore, India. Br J Cancer 1999;2000;62:847-51.

24. Ko YC, Huang YL, Lee CH, Chen MJ, Lin LM, Tsai CC. Betel quid chewing, cigarette smoking and alcohol consumption related to oral cancer in Taiwan. J Oral Pathol Med 1995;24:450-3.

25. Lu CT, Yen YY, Ho CS, Ko YC, Tsai CC, Hsieh CC, et al. A case control study of oral cancer in Chianghua County, Taiwan. J Oral Pathol Med 1996;25:245-8.

26. Dikshit RP, Kanhere S. Tobacco habits and risk of lung, oropharyngeal and oral cavity cancer: A population based case control study in Bhopal, India. Int J Epidemiol 2000;29:609-14.

27. Hashibe M, Mathew B, Kuruvilla B, Thomas G, Sankaranarayanan R, Parkin DM, et al. Chewing tobacco, alcohol and the risk of oropharynula. Cancer Epidemiol Biomarkers Prev 2000;9:639-45.

28. Merchant A, Hussain SS, Hosain M, Fikree FF, Pitiphat W, Siddiqui AR, et al. Pan without tobacco: An independent risk factor for oral cancer. Int J Cancer 2000;86:128-31.

29. Balaram P, Sridhar H, Rajkumar T, Vaccumella S, Herrero R, Nandakumar A, et al. Oral cancer in southern India: Influence of smoking, drinking, paan chewing and oral hygiene. Int J Cancer 2002;98:440-5.

30. Shen PC, Kuo C, Pan CC, Chou MY. Risk of oral cancer associated with human papillomavirus infection, betel quid chewing, and cigarette smoking in Taiwan-an integrated molecular and epidemiological study of 58 cases. J Oral Pathol Med 2002;31:137-22.

31. Znaor A, Brennan P, Gajalaxmi V, Mathew A, Shanta V, Vargese C, et al. Independent and combined effects of tobacco smoking, chewing and alcohol drinking on the risk of oral, pharyngeal and esophageal cancers in Indian men. Int J Cancer 2003;105:681-6.

32. Chitra S, Ashok L, Anand L, Srinivasan V, Jayanthi V. Risk factors for esophageal cancer in Cimbotare, southern India: A hospital-based case-control study. Indian J Gastroenterol 2004;23:19-21.

33. Thomas SJ, Bain CJ, Battistutta D, Ness AR, Piaissat D. Betel quid not containing tobacco and oral cancer: A report on a case-control study in Papua New Guinea and a meta-analysis of current evidence. Int J Cancer 2008;123:1871-6.

34. Zhang X, Reichart PA. A review of betel quid chewing, oral cancer and precancer in Mainland China. Oral Oncol 2007;43:424-30.

35. Thomas SJ, Harris R, Ness AR, Taulo J, Maclellan R, Howes N, et al. Betel quid not containing tobacco and oral leukoplakia: A report on a cross-sectional study in Papua New Guinea and a meta-analysis of current evidence. Int J Cancer 2008;123:1871-6.

36. Burton-Bradley BG. Betel chewing in retrospect. P N G Med J 1978;21:236-41.

37. Schneider E. Betel – A popular pleasure – giving substance in South Asia. Pharm Unis Zeit 1986;15:161-6.

38. Norton SA. Betel: Consumption and consequences. J Am Acad Dermatol 1998;38:81-8.

39. Chu NS. Effects of betel chewing on the central and autonomic nervous systems. J Biomed Sci 2001;8:229-36.

40. Chu NS. Neurological aspects of areca and betel chewing. Addict Biol 2002;7:111-4.

41. Cavte J. Psychoactive substances in the South seas: Betel, kava, and pituri. Aust N Z J Psychiatry 1985;19:83-7.

42. Pickwell SM, Schimpelfening S, Palinkas LA. ‘Betelmania’. Betel quid chewing by Cambodian women in the United States and its potential health effects. West J Med 1994;160:326-30.

43. Talonu NT. Observation on betel-nut use, habituation, addiction and carcinogenesis in Papua New Guineans. P N G Med J 1989;32:193-7.

44. Winstock AR, Trivedy CR, Warrnakuluriya KA, Peters TJ. A dependency syndrome related to areca nut use: Some medical and psychological aspects among areca nut users in the Gujrat community in the UK. Addict Biol 2005;5:173-9.

45. Kuruppuarachi KA, Williams SS. Betel use and schizophrenia. Br J Psychiatry 2003;182:455.

46. Jeng JH, Chang MC, Hahn LJ. Role of areca nut in betel quid-associated chemical carcinogenesis: Current awareness and future perspectives. Oral Oncol 2001;37:477-92.

47. Kuo R, Lin C, Kuo MY. Prognostic Role of c-Jun Activation in Patients with Areca Quid Chewing-related Oral Squamous Cell Carcinomas in Taiwan. J Formos Med Assoc 2006;105:229-34.

48. Cohen SM, Ellwein LB. Genetic errors, cell proliferation and carcinogenesis. Cancer Res 1991;51:6493-5.

49. Hursting SD, Slaga TJ, Fischer SM, DiGiovanni J, Phang JM. Mechanism-based cancer prevention approaches: Targets, examples and the use of transgenic mice. J Natl Cancer Inst 1999;91:219-25.

50. Jeng JH, Kuo ML, Hahn LJ, Kuo MY. Genotoxic and non-genotoxic effects of betel quid ingredients on oral mucosal fibroblasts in vitro. J Dent Res 1994;73:1043-9.

51. Sundqvist K, Graffstrom RC. Effects of areca nut on growth, differentiation and formation of DNA damage in cultured human buccal epithelial cells. Int J Cancer 1992;52:305-10.

52. Calmels S, Ohshima H, Henry Y, Bartsch H. Characterization of bacterial cytochrome cdt-nitrite reductase as One enzyme responsible for catalysis of nitrosation of secondary amines. Carcinogenesis 1996;17:533-6. 

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53. Nery R. The metabolic interconversion of arcoline and arcoline 1-oxide in the rat. Biochem J 1971;122:503-8.
54. Jeng JH, Hahn LJ, Lu FJ, Wang YJ, Kuo MY. Eugenol triggers different pathobiological effects on human oral mucosal fibroblasts. J Dent Res 1994;73:1050-5.
55. Amonkar AJ, Bagabushan M, D’Souza AV, Bhide SV. Hydroxychavicol: A new phenolic antimitagen from betel leaf. Food Chem Toxicol 1986;24:1321-4.
56. Amonkar AJ, Padma PR, Bhide SV. Protective effect of hydroxychavicol, a phenolic component of betel leaf, against the tobacco-specific carcinogens. Mutat Res 1989;210:249-53.
57. Shivapurkar NM, Bhide SV. Role of betel nut constituents on Sulfhydryl metabolism. Ind J Pharm 1978;10:257-64.
58. Singh A, Rao AR. Effect of arecanut on the black mustard (Brassica niger, L.)-modulated detoxification enzymes and sulfhydryl content in the liver of mice. Cancer Lett 1993;72:45-51.
59. Jeng JH, Wang YJ, Chiang BL, Lee PH, Chan CP, Ho YS, et al. Roles of keratinocyte inflammation in oral cancer regulating the prostaglandin E2, interleukin-6 and TNF-B1 production of oral epithelial cells by areca nut extract and arcoline. Carcinogenesis 2003;24:1301-15.
60. Lin SC, Lu SY, Lee SY, Lin CY, Chen CH, Chang KW. Areca (betel) nut extract activates mitogen-activated protein kinases and NF-kappab in oral keratinocytes. Int J Cancer 2005;116:526-35.
61. Jeng JH, Ho YS, Chan CP. Areca nut extract up-regulates prostaglandin production, cyclooxygenase-2 mRNA and protein expression of human oral keratinocytes. Carcinogenesis 2000;21:1365-70.
62. Er TK, Tsai EM, Tsai LY, Ko YC, Lee JN. In vitro effects of arecoline on sperm motility and cyclooxygenase-2 expression. J Toxicol Sci 2006;31:75-82.
63. Nair UJ, Obe G, Friesen M, Goldberg MT, Bartsch H. Role of lipid peroxidation in the generation of reactive oxygen species from betel-quid ingredients. Environ Health Perspect 1992;98:203-5.
64. Nair UJ, Nair J, Friesen MD, Bartsch H, Ohshima H. Ortho- and meta-tyrosine formation from phenylalanine in human saliva as a marker of hydroxyl radical generation during betel quid chewing. Carcinogenesis 1995;16:1195-8.
65. Lin CC, Chang MC, Chang HH, Wang TM, Tseng WY, Tai TF, et al. Areca nut-induced micronuclei and cytokinesis failure in Chinese hamster ovary cells is related to reactive oxygen species production and actin filament deregulation. Environ Mol Mutagen 2009;50:367-74.
66. Kehrer JP. Free radicals as mediators of tissue injury and disease. Crit Rev Toxicol 1993;23:21-48.
67. Amstad P, Cerutti P. Genetic modulation of the cellular antioxidant defense capacity. Environ Health Perspect 1990;88:77-82.
68. Chang MC, Ho YS, Lee JJ, Kok SH, Hahn LJ, Jeng JH. Prevention of the areca nut extract-induced unscheduled DNA synthesis of gingival keratinocytes by vitamin C and thiol compounds. Oral Oncol 2002;38:258-65.
69. Jeng JH, Kuo ML, Hahn LJ, Kuo MY. Genotoxic and non-genotoxic effects of betel quid ingredients on oral mucosal fibroblasts in vitro. J Dent Res 1994;73:1043-9.
70. Sharan RN, Wary KK. Study of unscheduled DNA synthesis following exposure of human cells to arecoline and extracts of betel nut in vitro. Mutat Res 1992;278:271-6.
71. Saikia JR, Schneeweiss FH, Sharan RN. Arecoline-induced changes of poly-ADP-ribosylation of cellular proteins and its influence on chromatin. Cancer Lett 1999;139:59-65.
72. Lee PH, Chang MC, Chang WH, Wang TM, Wang YJ, Hahn LJ, et al. Prolonged exposure to arecoline arrested human KB epithelial cell growth: Regulatory mechanisms of cell cycle and apoptosis. Toxicology 2006;220:81-9.
73. Hung KF, Lai KC, Liu TY, Liu CJ, Lee TC, Lo JF. Asb6 upregulation by Areca nut extracts is associated with betel quid-induced oral carcinogenesis. Oral Oncol 2009;45:543-8.
74. Tsai YS, Lee KW, Huang JL, Liu YS, Juo SH, Kuo WR, et al. Arecoline, a major alkaloid of areca nut, inhibits p53, represses DNA repair, and triggers DNA damage response in human epithelial cells. Toxicology 2008;249:230-7.
75. Yan Huaxin. Betel nut genotoxic stress. Of p53 stability. National Science Council - research report. Project number: NSC90-2314-B-039-022. Executive Unit: China Medical University School of Dentistry. Republic of China September 22, 1992.
76. Kannan K, Muniraj AK, Krishnamurthy J, Bhuvarahumurthy V, Mohanprasad BK, Panishankar KH, et al. Low incidence of p53 mutations in betel quid and tobacco chewing-associated oral squamous carcinoma from India. Int J Oncol 1999;15:1133-6.
77. Prime SS, Davies M, Pring M, Paterson IC. The role of TGF-beta in epithelial malignancy and its relevance to the pathogenesis of oral cancer (part II). Crit Rev Oral Biol Med 2004;15:337-47.
78. Lin SC, Chang KW, Chang CS, Liu TY, Tseng YS, Yang FS, et al. Alterations of p16/MTS1 gene in squamous cell carcinomas from Taiwan. J Oral Pathol Med 2000;29:159-66.
79. Ho TJ, Chiang CP, Hong CY, Kok SH, Kuo YS, Yen-Ping Kuo M. Induction of the c-jun protooncogene expression by areca nut extract and arcoline on oral mucosal fibroblasts. Oral Oncol 2000;36:432-6.
80. Liu TY, Chung YT, Wang PF, Chi CW, Hsieh LL. Saffrole-DNA adducts in human peripheral blood—an association with areca quid chewing and CYP2E1 polymorphisms. Mutat Res 2004;559:59-66.
81. Triedy CR, Warnakulasuri KA, Hazardey VK, Tavassoli M, Sommer P, Johnson NW. The upregulation of lysyl oxidase in oral submucous fibrosis and squamous cell carcinoma. J Oral Pathol Med 1999;28:146-51.
82. Tillesy WM, Klinikowski MF, Saku T, Peters TJ, Warnakulasuriya S. Oral submucous fibrosis: Review on aetiology and pathogenesis. Oral Oncol 2006;42:561-8.
83. Shieh DH, Chiang LC, Shieh TY. Augmented mRNA expression of tissue inhibitor of metalloproteinase 1 in buccal mucosal fibroblasts by arecoline and safrole as a possible pathogenesis for oral submucous fibrosis. Oral Oncol 2003;39:728-35.

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