

Genotoxic Effect of Local & Commercial Arecanut & Tobacco Products- A Review

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ABSTRACT

Oral cancer progression is a multistep process which includes genetic alterations induced by DNA damage representing the genetic, epigenetic and phenotypic changes which are the hallmarks of carcinogenesis. DNA damage could result either from various carcinogens accumulating from etiologic influences or due to genetic errors. Tobacco, alcohol, betel nut are most commonly classified carcinogens in oral cancer. In Asian culture betel nut is traditionally masticated either alone or as a quid along with a variety of ingredients such as betel leaf, slaked lime, catechu, different types of tobacco and various additives, perfumes, and stimulants. With the emergence of commercial *gutkha* about three decades ago, not only did the Indian market witness massive growth in the sales of smokeless tobacco and areca nut products, but also a huge worldwide export market developed. The packaging revolution has made these products portable, cheap and convenient, with the added advantage of a long shelf-life. There is an increasing trend of consuming these pre-packaged easily available pan masala, gutkha among younger generation. Hence the present review focuses on the genotoxic effect of local areca nut & smokeless tobacco preparation (betel quid) and pre-packaged arecanut and tobacco preparation.

Key Words: Genotoxicity, DNA, betel quid, pan masala, Gutkha.

INTRODUCTION

The human population is inevitably exposed to a number of chemical mutagens/ carcinogens either accidentally, occupationally or by life style. ^[1] Social and cultural practices of people have a great influence on their health. Chewing habits, smoking and consumption of alcohol are common social habits present globally. ^[2] According to the WHO (World Health Organization) assessment, oral cancer is the third most common malignancy in males and sixth most common malignancy in

females. ^[3] It is postulated that one out of every three patients diagnosed with oral cancer would die within 5 years of detection. Thus prevention of oral cancer is a desirable goal, and with its early diagnosis and treatment. ^[4]

The chewing habit associated with the use of areca nut and tobacco is more prevalent in South Asian countries and is spreading to Western countries among settled Asian migrant communities. ^[5] Chewing habit is socially acceptable among

all sections of society, including women and quite often, children. [6]

History of Arecanut use & its various forms

The arecanut palm, *Areca catechu* L. is the source of the masticatory nut, popularly known as arecanut. Arecanut is one of the most important commercial crops in the Southeast Asia. [5] It is called by different names such as 'betel nut' in English, *supari* in Hindi, *adike* or *betta* in Kannada, *adakka* in Malayalam, and *pakku* in Tamil. [6]

Areca nut is not a true nut, but rather a drupe. While fresh, the husk is green and nut inside is so soft that it can be easily cut with an average knife [7] Arecanut is normally harvested as unripe (yellow-green) or ripe (orange/red) fruit from the tropical palm, *Areca catechu*. It may be sun dried for several weeks, fibrous shells removed and the hard, dry nuts, commonly called 'supari' in India, which is very hard, and is cut into small pieces to make it easier to masticate. In contrast ripe, partly ripe or unripe areca fruits are freshly picked, fibrous shells removed and the relatively soft nuts are ready for mastication [8]

Chewing of areca nut is an ancient custom in India, several parts of south-east Asia, the south Pacific islands and Taiwan. The practice of areca nut chewing dates back several thousand years and is deeply related to the tradition and culture of the population. [6]

The use of 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. Several forms of areca nut such as green unripe; ripe but raw; baked roasted or boiled; fermented; or, processed with sweeteners are used according to individual preference. [9] Areca (betel) nut is regarded by many Indians as a fruit of divine origin. It is considered an auspicious ingredient in

Hinduism and is used along with betel leaf in religious ceremonies and when honouring individuals. [10]

The type of Areca nut grown and its consumption varies among regions. It can be chewed raw or processed by roasting, sun drying, soaking or boiling prior to chewing. Those who chew soaked or boiled nuts demonstrated lower incidence of mucosal changes than those who chew raw, sundried or roasted nuts. Marked reduction in active chemical constituents in the nuts namely arecoline and polyphenols were observed when nuts were subjected to soaking and boiling as compared to sundried or roasted nuts. Curing improves colour, taste and freshness of the nuts. Cured nuts are less astringent and are better to chew and taste. [11]

Chemical constituents of Arecanut

Areca nut primarily consists of alkaloids like arecoline, arecaidine, guvacine and guvacoline. The other constituents of the arecanut are carbohydrates, fats, proteins, crude fibre, polyphenols (flavonoids and tannins), and mineral matter. Polyphenols (flavonoids, tannins) constitute a large proportion of the dry weight of the nut and are responsible for the astringent taste of the nut. Areca nut also contains sodium, magnesium, calcium, vanadium, manganese and copper in trace amounts. [12]

Composition of Betel quid

The Betel Quid (BQ) is a mixture of areca nut (*Areca catechu*), catechu (*Acacia catechu*) and slaked lime (calcium oxide and calcium hydroxide) wrapped in a betel leaf (*Piper betel*). Condiments, sweetening agents and spices may be added according to individual preferences.

There is wide regional variation in the composition of the chew and individuals prepare the quid according to their own recipe. Tobacco is often added to quid by many who chew in Indian subcontinent and other south asian countries. [13]

Carcinogenicity & Genotoxicity of Local arecanut & tobacco products

Carcinogenicity

There is a strong epidemiological support for the association exists for the betel quids which contain tobacco and the risk of oral cancer. Several studies on animals also have demonstrated the carcinogenicity of betel quids. [14]

According to Hoffman et al several carcinogens are derived from tobacco but also from areca nut. [15] Another study by Nair et al showed chewing of tobacco with betel quid results in high exposure to carcinogenic tobacco specific nitrosamines(TSNA). The carcinogenic TSNAs N'-nitrosornicotine (NNN), 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N-nitrosoanabasine (NAB),as well as the volatile nitrosamines N-nitrosodimethylamine and N-nitrosodiethylamine, have been detected in the saliva of chewers of BQ with tobacco. [16]

Genotoxicity

The composition of betel quid differs geographically. Piper betel a constituents of betel quid contains high concentrations of hydroxychavicol and safrole, whereas arecoline, a major areca nut alkaloid, is considered to be the most important carcinogen in the areca nut. A study conducted by Lai ch et al has shown that Areca nut extract (ANE) is highly cytotoxic and genotoxic to cultured human oral mucosal epithelial cells and fibroblasts. [16] Exposure of human keratinocytes to ANE results in apoptosis, generation of reactive oxygen species, genetic damage and micronuclei formation. The same study has also found that 24-h treatment with ANE induces mutations at the hypoxanthine phosphoribisyltransferase (HPRT) locus in human keratinocytes. [17] Increased intracellular levels of reactive oxygen species and 8- hydroxyguanosine in cells exposed to ANE have also reported. Salivary concentration of arecoline during betel quid chewing has been detected to be in the millimolar concentration range.

Arecoline has also been shown to induce structural chromosomal aberration,

sister chromatid exchange and micronuclei formation in different cell types. [18] Studies in human oral cancer cells have shown that exposure to arecoline or ANE results in growth arrest in the late S and G2/M phases.(34) Moreover, it has been shown that arecoline induces a significant elevation of p21waf1 and a decline of cdc2 (cyclin dependent protein kinase) and Cyclin B1 in gingival keratinocytes. [19] Piper betel contains safrole which causes DNA adducts that plays an important role in oral carcinogenesis. Hydroxychavicol, a phenolic component of betel leaf, has been found in human saliva at a 4.6 mM concentration after betel quid chewing. Hydroxychavicol may induce the formation of single-strand DNA breaks and 8-hydroxydeoxyguanosine a marker of oxidative DNA damage in cultured cells. Moreover, COX-2 expression and PGE2 production have been shown to be significantly enhanced by hydroxychavicol in human normal oral keratinocytes. [20]

Commercial preparation of arecanut and tobacco (betel quid)

Betel leaf is perishable and the preparation of BQ is somewhat complex. Hence, over the past few decades, commercial BQ substitutes, a flavored and sweetened dry mixture of BN, catechu and slaked lime either with tobacco (*gutkha* or *khaini*) or without tobacco (*paan masala*), have become increasingly popular. ⁷ These products are packaged in small, attractive and inexpensive sachets, and are easily advertised and marketed, often claimed to be safe products. Use of *gutkha* often begins at a very young age. *Gutkha* contains large amounts of sweeteners to conceal the bitterness of tobacco, and children often consider it as a type of candy. Many people think *gutkha* to be harmless and as mere 'mouth freshener' *Gutkha* and *paan masala* are consumed by very young and old alike, particularly in India, and also among migrant populations. [21]

Carcinogenicity and Genotoxicity of Commercial arecanut and tobacco products.

The mutagenic and genotoxic potential of commercial arecanut and tobacco products and its main constituents are assessed through many studies. In vitro studies using aqueous extract of Pan Masala on Chinese hamster ovaries (CHO) have shown that there is elevation in sister chromatid exchange (SCE), chromosomal aberration (CA) and micronucleated cells (MN). **Mojidra Bn et al has conducted studies in male mice using aqueous suspension of Pan Masala have shown increased X-Y univalent frequency, sperm head abnormalities and significant delay in cell cycle progression. The increased production of superoxide ions led to increased lipid peroxidation, DNA fragmentation and triggered apoptotic cell death which was similar to tobacco containing products.** [22]

Of the several ingredients of Pan Masala, areca nut is a proven carcinogen and catechu, lime may have carcinogenic potential. PM mixture contains nitrosamines, polycyclic aromatic hydrocarbons, residual pesticides and toxic metals like lead, cadmium, nickel. Areca nut contains various alkaloids (arecoline, arecaidine, guvacine, guvacoline) which lead to formation of nitrosamines in the saliva. Two of these nitrosamines are accepted as carcinogenic in animal studies. Out of which 3-(methyl-N-nitrosamino) propionitrile (MNPN) is the most carcinogenic. [23]

There is enhanced nitrosation of amines present in areca nut in people with poor oral hygiene due to greater formation of nitrite and bacterial enzyme mediated reactions. Slaked lime or calcium hydroxide leads to the formation of reactive oxygen species (ROS) in presence of areca nut and causes oxidative damage to DNA. Catechu induces SCE and dominant lethal mutation. In combination with lime and areca nut, it also leads to formation of ROS. Heavy metals such as iron and copper that are

found in areca nut lead to increased production of ROS as they act as catalyst in the reaction. ROS oxidizes DNA bases, e.g., deoxyguanosine to yield 8-oxo-dG that promotes tumor formation in oral cavity. Tender areca nut causes more 8-oxo-dG formation in DNA than the ripe variant. [24]

An in vitro study conducted by Nair et al had shown that areca nut extract increased expression of COX-2 and prostaglandin production in human oral mucosa cells which aids in carcinogenesis. The frequency of CA, SCE and MN increases with the number of pouches consumed every day and age of the subject. People with increase CA have 2 times more chances of developing cancer. PM users do not spit the juice which leads to carcinogenic effects not only at oral cavity but at other sites including lung, liver, stomach and rarely in prostate, testis, skin. [15]

Methods of detection of DNA damage

Method of detecting the injure site is an important component of DNA damage study. A number of methods have been used to detect DNA damage.

Methods that are commonly used for the detection and quantification of DNA damage are Polymerase chain reaction (PCR), Comet assay, Halo assay, Terminal deoxyribonucleotidyl transferase mediated deoxyuridine triphosphate nick end labeling (TUNEL) assay, Gas chromatography-mass spectrometry (GS-MS), Fluorescence in situ hybridization (FISH), Annexin V labeling, Flow cytometry (FCM), Immunological assay, Radioimmunoassay (RIA), Immunohistochemical assay, Enzyme-linked immunosorbent assay (ELISA), Electrochemical methods and Buccal micronucleus cytome assay. [25]

Methods available for detecting DNA damage are with some or other limitations. So there is a need to combine the features of different detection methods and to develop a unique strategy that can localize damage in genome, the nature of damage and quantify damage and repair processes which will be helpful in

developing repair strategies and also provide better insight into the process of carcinogenesis and ageing.

CONCLUSION

Arecanut and the products derived from it are widely used as a masticatory substance among various communities, and in several countries across the world, as a socially endorsed habit. Over a long period, several additives got added to a simple BN preparation, thus, creating the BQ and encompassing chewing tobacco in the preparation. The addictive nature of BN and/or its additives that make BQ, are essentially responsible for its rampant usage among individuals. The popularity has led to industrial preparation of convenient substitutes of the BN/BQ in the form of *paan masala*, *gutkha*. Extensive studies by several workers over the years conclusively prove the role of BN, and its components in genotoxicity and carcinogenicity. These substances not only have general mutagenic, cytotoxic and genotoxic properties, but are also intricately involved in enzymatic, molecular and genetic mechanisms that result in the development of carcinogenesis at various sites, specifically in the oral cavity.

More research is required for understanding of the seemingly highly complex interactions of BN with the life process and its manifestation in Head & Neck Cancer, particularly Oral Cancer. Control over human consumption of BN and BQ without or with additives, including tobacco, or its convenient commercial substitutes, such as *gutkha* and *paan masala*, is proving to be difficult because the habit is not associated with any social stigma and taboo. Hence, strong multifaceted intervention is required to discourage or control the habit of BN/BQ mastication and also public awareness should be created regarding the harmful effects of these products among all sections of society, particularly among children.

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