CHAPTER 1
INTRODUCTION

The use of betel quid and areca nut as a masticator by human beings has been known since the 4th century A.D in different parts of the world and is widely practiced in South and South-East Asian countries, Africa, as well as migrant communities from these areas to western countries. It is estimated that over 600 million people consume areca nut in one form or another worldwide. It has been estimated that areca nut chewing is the fourth most popular habit worldwide, after the use of tobacco, alcohol, and caffeine. This practice dates back several thousand years and is deeply entrenched in the culture of the population. A number of progressive changes have occurred in workplaces, in the environment and in lifestyles during the past few decades. Lifestyle factors are estimated to be responsible for about 80% of human neoplasias (Doll, 1990; Doll and Peto, 1981). Therefore, there is an increasing effort worldwide to determine the impact of environmental, genetic and lifestyle factors on genomic stability in human populations.

Areca nut is the seed of the oriental palm Areca catechu L. that contains closely related alkaloids arecoline, arecaidine, guvacoline, and guvacine (Giri et al., 2006; IARC, 2004). Different ways of areca nut processing and consumptions has been reported from India, Taiwan and Southeast Asian countries. The betel quid (BQ) is a mixture of areca nut, catechu (Acacia catechu) and slaked lime
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(calcium oxide and calcium hydroxide) wrapped in a betel leaf (Piper betel). Condiments, sweetening agents and spices may be added according to individual preferences. BQ chewing was already a socially well accepted practice and the introduction of tobacco reinforced this practice. The dried tobacco leaves was given to Columbus as a ceremonial gift by Native Americans in 1492 led to the introduction of tobacco into the rest of the world. It arrived in India in the 16th century; a sample was presented to the emperor Akbar, who patronized smoking, rapidly spreading the habit in the sub-continent. An attempt to ban it in 1619 had little effect, as the revenues from tobacco were already considerable. In India, most habitual chewers of BQ add tobacco. In some countries, such as Papua New Guinea and China, tobacco is not added. Due to the complexity of the ingredients in BQ, little is known about the mechanisms of BQ-induced oral carcinogenesis.

BQ alone or in combination with smokeless tobacco and/or smoking has been considered as major risk factors for oral cancer and pre-cancerous lesions in habituates (IARC, 1985, 2004, 2007). In India, smokeless tobacco in the form of gudakhu, mishri, snuff, chewing tobacco, masher and mawa are consumed with or without areca nut. However, in the North-Eastern region of India, southern part of the state of Assam and the neighbouring states Meghalaya and Mizoram, a very unique preparation of smokeless tobacco known as “sadagura” is highly popular among the local population. Sadagura, a smokeless tobacco preparation, contains sun dried and roasted tobacco leaves along with very small amount of fenugreek seed and aniseed for flavor. It is chewed and swallowed mostly with betel quid unlike most other smokeless tobacco preparations. The stimulating effect coupled with strong flavors results in addiction among the users who
consume *sadagura* without being aware of its ill effects (Kausar *et al.*, 2009). Therefore, it is important to assess the effects of newer forms of tobacco preparations like *sadagura* alone or in combination with other such habits. The highest incidence of oral cancer in India is reported in Assam in the North-East region, where it is the second leading cancer among men and third among women. International Agency of Research on Cancer has evaluated and classified BQ without tobacco as a human carcinogen (IARC, 2003), yet unfortunately the annual global production of areca nut is continuously increasing.

Oral cancer is the fifth most common cancer worldwide (Parkin *et al.*, 1993). People who chew betel nut have a higher prevalence of periodontal diseases than those who do not (Panigrahi and Rao, 1996). Esophageal subepithelial fibrosis, an extension of oral submucosal fibrosis, was seen more frequently in patients who had consumed pan masala, gutkha, areca nut, tobacco or a combination of some or all of these, with or without betel leaf, for more than five year than in those consuming these products for a shorter period. Areca nut chewing manifests several pharmacological effects that are predominantly parasympathetic in nature, including euphoria, central nervous system stimulation, vertigo, salivation, tremor, and bradycardia, muscarinic effects that are thought to be largely attributable to the predominant alkaloid arecoline (Winstock *et al.*, 2000; Nieschulz and Schmersahl, 1968).

Several studies have reported a dependency syndrome associated with areca nut chewing that is said to produce relaxation, improved concentration, mild euphoria and enhanced postprandial satisfaction. A withdrawal syndrome has also been described, comprising mood swings, anxiety, irritability, and insomnia.
The severity of dependence was reported to be similar to that associated with amphetamine use. One dominant theory has been that various nitrosamines may be formed in the mouth from areca alkaloids and that these are causative of human oral cancer (Wenke et al., 1984, 1984a, b).

During chewing many carcinogens and mutagens are suspected to be released into the buccal cavity and play an important role in carcinogenesis. Many of the undesirable effects of betel nut have been attributed to Arecoline (1, 2, 5, 6-tetrahydro-1-methyl-3-pyridinecarboxylic acid methyl ester) is the major alkaloid of betel nut and plays major role in betel quid induced carcinogenesis. Arecoline being the major alkaloid is considered to be the main cause of cellular transformation. However the mechanism of action of arecoline in cellular transformation as well as the steps of its metabolism remains obscure.

Despite its social and toxicological importance, relatively little is known about the metabolism of arecoline. The metabolic profile and pathway involved in arecoline metabolism in mouse test system has been reported and eleven metabolites of arecoline in mouse urine was detected (Giri et al., 2006). The majority of arecoline metabolites are formed after the compounds first hydrolysis to arecaidine. The flavin monooxygenase induced arecoline N-oxide is one of the major metabolite formed in mouse besides ten other metabolites including arecaidine (Giri et al., 2007). No study has been undertaken to determine areca nut derived secondary metabolites.

In a recent report, mutagenic effect of arecoline was tested in Salmonella typhimurium TA 100 and TA 98 in the absence or presence of rat liver S9 preparation. A low mutagenic effect was observed in S9 positive fraction. In
addition, a dose dependent increase in the mutagenic effect was observed by N-oxide of arecoline (Lin et al., 2011). Thus, metabolic activation is considered to be a critical step in mutation and carcinogenesis.

Arecoline induces abnormal sperm heads in mice by damaging the germ cell nuclear DNA and also unscheduled DNA synthesis in early spermatid stages of the mouse (Sinha and Rao, 1985), displays genotoxic effects (Sharan, 1996). All these reports indicate the general toxic effects of arecoline in the recipient. The enhancing effects of dietary administration of areca nut on carcinogenesis in the liver and upper digestive tract were also observed (Tanaka et al., 1983). However, all these data are insufficient, contradictory or not available especially with arecoline metabolites.

Keeping in mind the large number of people around the world consuming areca nut as well as its importance in clinical use against Alzheimer’s disease, it is very important to understand the toxicological, carcinogenic potentials involved with such secondary metabolites of the major areca nut alkaloids. Although a few scattered reports have demonstrated that subtoxic concentrations of arecoline could affect immunological responses, studies indicate that different aspects of the immune response can be simultaneously reduced by systemic administration of the cholinergic agonist arecoline (Wen et al., 2006). Despite its metabolites formed in consumers, little is known about its genotoxicity or carcinogenic ability.

The general conclusion is that (i) there is sufficient evidence in experimental animals for carcinogenicity of areca nut (ii) there is limited evidence in experimental animals about the carcinogenicity of arecoline (iii) there is
insufficient evidence in experimental animals for the carcinogenicity of arecaidine or other metabolites (IARC, 1985). However, there is no report about the genotoxic and carcinogenic potentials of major metabolites of arecoline.

In view of the importance of the study in absence of any report on primary metabolites of arecoline, betel quid with or without tobacco chewing habit and the impact of arecoline-induced carcinogenesis in chewers, the basic objectives of the proposed study are as follows:

**OBJECTIVE OF THE STUDY:**

1. To evaluate the genotoxic potential of arecoline and metabolites *in vivo* in murine test system.

2. To evaluate the tumorigenic potential of arecoline and its metabolites *in vivo* in murine test system.

3. To compare the above results with whole betel quid with or without tobacco induced genotoxicity and tumorigenicity.

4. Biochemical analysis like lipid peroxidation level as other toxicological parameter for evaluation of cellular dysfunction due to oxidative stress in arecoline, its metabolites and betel quid exposed animals.