EPILOGUE

Cancer is a multistage process and has multifactorial etiology. Both, genetic as well as environmental risk factors affect the causation of the disease. The environmental factors embrace all aspects including occupational, accidental and lifestyle associated exposures to carcinogens. Population comparisons and evidence from experimental and epidemiological studies strongly suggest that as many as 80-90% of human cancers are determined environmentally, and are thus theoretically avoidable, more so, when the exposure to carcinogens is lifestyle/habit associated.

In India, oral cavity cancers are the most common cancers among males, while they are third commonest among females. They encompass nearly one third of the total cancer cases and cause forty percent of cancer deaths. Betel quid chewing has been implicated for the causation of the disease. There has been an irrefutable evidence about the use of tobacco and development of site specific cancers, and public opinion against its use is heightening. Conversely, inspite of the fact that areca nut constitutes the major amount of betel quid or other commonly chewed mixtures, the possible harmful effects of areca nut consumption have not received due consideration.

Considering the magnitude of oral cancers in India and the increasing number of people practising areca nut chewing in a variety of combinations, and also the lack of hard evidence against areca nut, we have attempted to study the effects of this masticatory in mammalian cell system as well as in individuals practising areca nut chewing. Since areca nut is mostly, if not always, consumed together with tobacco, studies were also directed towards analysing the possible potentiation of the effects on combining these two substances. Recently, a new mixture, pan masala, is being consumed abundantly. Areca nut constitutes about 70-80% of this mixture.

Keeping this in view, the genotoxic potentials of this complex mixture were also investigated.

*The **FIRST PART** envisaged the in vitro short term assays with (a) Aqueous extract of areca nut (AN ext.), (b) Arecoline, the major areca nut alkaloid and (c) Arecoline with Nicotine (A + N), a combination of the major alkaloids of areca nut and tobacco. Chinese hamster ovary (CHO) cells were employed to analyse the effects on cell viability, cell division, micronucleus frequency (MNC), chromosome aberration (CA) frequency, sister chromatid exchange (SCE) frequency, cellular kinetics and DNA synthesis. The results revealed:*

* *AN ext. as well as arecoline exhibited a dose and duration related cytotoxic effects.*

* *Arecoline treatments resulted in a decrease in the mitotic index, with accumulation of cells in metaphase, leading to a speculation that arecoline might be causing interference with the functioning of the mitotic spindle.*

* *The capacity of arecoline to function as a clastogen was deciphered by the increase in MNC and CA frequencies with concurrent increase in the concentrations. AN ext. also induced a dose correlated increase in CAs, with a high frequency of chromatid aberrations, indicating the effects on late S or G_2 phase of the cell cycle.*

* *SCE assay proved to be extremely sensitive in assessing the genotoxic potentials of AN ext. and arecoline. The elevations in SCE frequencies, on treatments with these substances, were highly significant statistically.*

* *The average generation time of the CHO cells was found to be adversely affected by AN ext. and also by arecoline.*

* *Inhibition of DNA synthesis was observed as a consequence of treatments with AN ext. as well as with arecoline, which exhibited correlation with the doses and durations employed.*

* *Considering the content of arecoline in areca nut, and the observed genotoxic effects of AN ext., it was evident that the extract was more genotoxic compared to equivalent concentration of arecoline, suggesting that in addition to arecoline, the extract contains other water extractable component(s), potentiating the ultimate effects.*

* *As evidenced by the studies combining arecoline and nicotine, apart from being cytotoxic, clastogenic and inhibitory to DNA synthesis in its own merit, areca nut can cause more severe damage when coupled with tobacco.*

The ***SECOND PART*** *covered the investigations on the possible genotoxic effects of areca nut, assessed directly in humans. Two different modes of areca nut consumption were considered, (1) Chewing areca nut alone and (2) Chewing areca nut with tobacco. A battery of three cytogenetic endpoints, including SCEs and CAs in peripheral blood lymphocytes (PBLs) and frequency of MNC in exfoliated buccal mucosa cells, was employed in individuals categorized in four different groups, viz. Controls, Normal chewers, Chewers with oral submucous fibrosis (OSMF) and Chewers suffering from oral cancer. The findings indicated:*

* *Statistically significant increase in the frequency of SCEs and CAs in the lymphocytes and MNC frequency in the exfoliated buccal mucosa cells of individuals consuming areca nut, signifies the in vivo genotoxic effects of areca nut on the target as well as nontarget tissues.*

* *The mean values observed for all the three endpoints were comparable among the individuals chewing areca nut alone and those consuming it with tobacco.*

Theoretically, one would expect higher values among those chewing areca nut with tobacco, however, comparable values in both the groups can be explained on the basis of differences in chewing patterns. When areca nut is chewed together with tobacco, chewers generally spit out the saliva, whereas, when it is chewed alone, it is always consumed in toto.

* *Areca nut (betel quid without tobacco) chewing has been causally associated with oral and oesophageal cancers, however, contemplating the genotoxic effects observed in PBLs in the present study, it can be presumed that areca nut might increase the risk of cancers even at sites other than oral cavity.*

* *The simultaneous application of the three cytogenetic endpoints increased the sensitivity of genotoxicity studies which points towards the benefits of using a battery of cytogenetic endpoints in monitoring habit associated conditions.*

* *Looking to the in vivo damage, henceforth, in population monitoring with cytogenetic parameters, the habit of chewing only areca nut also should be considered as one of the confounding factors.*

*The **THIRD PART** detailed our studies on pan masala without tobacco. Seeking to determine the genotoxic potentials of this relatively new product, one is immediately confronted with the fact that areca nut constitutes almost 75% of this complex mixture. With our data regarding areca nut on hand, the probable annulment or augmentation of its genotoxic potentials in a complex mixture were further analysed. Investigations were designed to study the in vitro effects as well as in individuals with a habit of consuming this mixture. The observations manifested:*

* *Aqueous extract of pan masala (PM ext.) was cytotoxic to CHO cells.*

* *A statistically significant and dose dependent increase in CAs and SCEs was caused by PM ext. treatments, revealing its genotoxic potentials.*

* *Treatments with PM ext. inhibited DNA synthesis in CHO cells and retarded the proliferation rates.*

* *Elevations in CA and SCE frequencies in PBLs and MNC in exfoliated buccal mucosa cells were observed in individuals consuming pan masala, thus confirming the in vitro genotoxic effects of pan masalas.*

* *Combining these results one can safely summarize that pan masala, per se, are genotoxic.*

On the whole, the data generated in the present study, would provide scientific basis to grow the much needed and overdue public awareness against areca nut, and would also help in making more reasoned decisions towards abstaining from the habit of consuming this widely accepted masticatory, erroneously considered safe.