

Epilogue

Cancer poses an alarming situation all over the world. The magnitude of the disease may increase with a rise in life expectancy of the population and excellent control over communicable diseases. At the same time, striking variations have also been observed regarding the incidence of different cancers in different countries. Genetic variations can account for only a small proportion of these differences. Generally, it has been interpreted as reflection of differences in exposure to environmental risk factors. Human population is inevitably exposed to a large number of chemical mutagens/carcinogens either occupationally, accidentally or by life style. Population comparisons and evidence from experimental and epidemiological studies have strongly suggested that about 80-90 % of human cancers are determined by these environmental factors.

Tobacco consumption is the most important and avoidable factor of human morbidity and mortality worldwide. In the United States, tobacco smoking is common and around 30 % of all deaths from cancer may be attributed to this habit. In India, oral use of smokeless tobacco is more common and the habit related oral cavity cancers present a major health problem. They account for almost 40 % of the cancer deaths. A strong association, between various tobacco habits and the cancers of different sites in oral cavity (ICD 140-145), has been confirmed by numerous epidemiological studies.

Various types of chewing tobacco and their extracts have

been tested by oral administration in mice, by topical application to the oral mucosa of mice, rats and hamsters, and by subcutaneous administration, skin application etc. However, all of these studies suffered from certain deficiencies. As a consequence, International Agency for Research on Cancer (1985) has concluded that the evidence to evaluate the carcinogenicity of chewing tobacco or snuff to experimental animals is inadequate. Hence, to begin with, it is important to have laboratory research to evaluate the carcinogenic potential of these forms of tobacco consumptions. There is high probability that compound which exhibits genotoxic activity will also exhibit carcinogenic activity (Tennant et al., 1987). Hence, genetic toxicology studies have major application in determining the exposure to genotoxic carcinogens of tobacco. It has also been suggested that in genetic toxicology, the *in vitro* cytogenetic assays should take a central role in a test battery (Natarajan and Obe, 1982). Therefore, an attempt was made to evaluate the possible genotoxic potential of smokeless tobacco with the help of different cytogenetic endpoints.

The FIRST PART presented the findings of *in vitro* short term assays with (i) Aqueous extract of unprocessed tobacco (Nicotiana tabacum - 'Deshi' tobacco), (ii) Nicotine (N), the major tobacco alkaloid and (iii) Nicotine plus Arecoline (N+A), a combination of the major alkaloids of tobacco and areca nut, respectively. CHO cells were employed to study the effects on Cell viability, Mitotic index (M.I.),

Micronucleated cell (MNC) frequency, Chromosome aberration (CA) frequency, Sister chromatid exchange (SCE) frequency and DNA synthesis.

It can be inferred from the results of *in vitro* studies that:

- (i) nicotine, in addition to being addictive, is genotoxic as well.
- (ii) T-ext. is cytotoxic/clastogenic/genotoxic to CHO cells.
- (iii) In addition to nicotine, tobacco contains other water extractable constituent(s), which potentiate the genotoxic effect of nicotine.
- (iv) Combined application of nicotine and arecoline results into more severe effects than nicotine alone.

Nevertheless, the extrapolation of data on genotoxicity/-carcinogenicity, generated from *in vitro* short term assays and laboratory animals, to the heterogeneous human population is often complicated. Hence, the **SECOND PART** emphasized the possible genotoxic effects of smokeless tobacco consumption (sun-dried and unprocessed) on cells collected from human beings consuming it in different forms. Three different modes of tobacco consumption were considered: (i) Rubbing powdered tobacco (dry snuff) on teeth and gums, (ii) Chewing tobacco with lime, and (iii) Chewing tobacco with areca nut and lime. A battery of three assays, viz. frequency of micronucleated cells (MNC) in exfoliated buccal mucosa and CA and SCE frequencies in PBLs, was employed in individuals categorized in

three groups, namely (i) Controls (ii) Normal chewers and (iii) Chewers suffering from oral diseases (pre-malignant or malignant).

The observations clearly suggested that:

- (i) Frequency of MNC in exfoliated buccal mucosa and CA & SCE frequencies in PBLs are the efficient indicators of genomic damage caused by the habit on the target as well as nontarget tissues, respectively.
- (ii) A comparable mean frequencies of all the three cytogenetic endpoints, among the normal individuals consuming either snuff, tobacco with lime or tobacco with areca nut and lime, suggested that oral uses of smokeless tobacco in different forms are equally harmful.
- (iii) The significantly low level of exposure to tobacco-areca nut mutagens/carcinogens, as evidenced by lower pack-years, among the oral submucous fibrosis patients, indicate that factors other than tobacco-areca nut genotoxins might be playing a role in causation of the disease.
- (iv) Analysis of oral smokeless tobacco habits as a risk factor, during epidemiologic studies related to the cancers at sites other than oral cavity, may furnish information about the role of smokeless tobacco (oral use) in the causation of cancer at distant sites.

The **THIRD PART** dealt with the studies on 'pan masala with

tobacco' (PM-T). The product is a complex mixture of several ingredients like zarda, areca nut, catechu, lime, spices and unspecified flavouring agents. For evaluating possible genotoxic effects of a mixture containing several compounds with doubtful mutagenic potential, an aqueous extract (PM-T-ext.) was selected for experiments on *in vitro* mammalian system. Using cytogenetic endpoints such as MNC frequency in exfoliated buccal mucosa and CA & SCE frequencies in PBLs, the *in vivo* effects of chewing 'tobacco containing pan masala', of the brand that was used for the *in vitro* analysis, were studied among PM-T consumers.

The findings of *in vitro* assays revealed that:

- (i) PM-T-ext. is cytotoxic/clastogenic/genotoxic to CHO cells.
- (ii) PM-T-ext. contains water soluble constituents other than tobacco which add to the genotoxicity of unprocessed tobacco. Areca nut, being the major constituent of PM-T, might be the factor responsible for the additional damage.

A significantly higher frequency of MNC in exfoliated buccal mucosa as well as CA and SCE frequencies in PBLs of individuals consuming PM-T compared to controls, further confirmed the findings of *in vitro* analysis. Since a long latent period may exist between an effective exposure to a carcinogen and manifestation of the disease, and since the epidemiological studies are very slow in establishing a

correlation, the present findings should serve to enlighten the PM-T consumers regarding their habit associated health risks.

In conclusion, the findings of the *in vitro* assays have been confirmed by the cytogenetic studies in exfoliated buccal mucosa cells and PBLs of the smokeless tobacco consuming individuals. For the first time, the analysis of three different cytogenetic endpoints have conclusively demonstrated the genotoxic effects of tobacco chewing on target as well as nontarget tissues of same individual. The findings proved that oral use of smokeless tobacco is not a safer alternative to tobacco smoking and support the presumption that all forms of tobacco consumption, including PM-T, are equally carcinogenic.