SUMMARY
Betel nut (BN) or areca nut is the hard, edible, endosperm of the palm *Areca catechu* Linn (palmaceae) which grows throughout south and south east Asia and in several pacific ocean Islands. The North-East Indian variety of BN is raw, wet and unprocessed (RBN, locally known as *Kwai* by the Khasi tribe of the North-Eastern region of India) consumed with betel leaf and slaked lime. The constituents of this nut show higher alkaloids, polyphenols and tannins as compared to the dry one. There are strong indications for a casual association between BN or quid chewing habit and oral mucosal diseases such as leukoplakia, oral submucous fibrosis and oral cancer. BN-extract (BNE) can induce DNA strand breaks, sister chromatid exchanges (SCEs) and micronuclei in various kinds of cells. The frequency of lymphocytic SCE was elevated in BN chewers and oral cancer patients in comparison to non-chewers.

Reduced glutathione (GSH), a tripeptide containing cysteine, is an important thiol compound present in cells. It plays an important role in regulation of cellular proliferation and cellular defense against radiation and various toxic effects of xenobiotics but not against radiomimetic drugs like bleomycin (BLM). Arecoline (ARC, an alkaloid of betel nut) induced chromosomal aberrations (CAs) in mice is enhanced by buthionine sulfoximine (BSO), a glutathione synthesis inhibitor and the genotoxic effect of ARC was reduced when it was administered with N-acetyl-L-cysteine (NAC). Therefore, the present study was undertaken to determine the genotoxic effect of RBNE with respect to endogenous GSH level. The disturbances in the antioxidant systems might be useful indicators of the susceptibility of subjects to free radical damage. Intracellular GSH status appears to be sensitive indicator of the cells’ overall health and of its ability to resist toxic challenge. Two types of extract were chosen for this study- Aqueous extract of betel nut (AEBN) and Acetic acid extract of betel nut (AAEBN).

Tumor suppressor gene p53 mutations are the most common genetic abnormalities found in human cancers, especially in the development of head and neck cancer. Elevated levels of p53 protein have been observed not only in
oral squamous cell carcinoma but also in oral dysplastic lesions, suggesting that p53 alteration is an early event in oral carcinogenesis. These findings suggest that inactivation of p53 protein may precede over tumour development in oral tumorigenesis and thus it may serve as an intermediate biomarker for risk assessment. Another important purpose of this study was to investigate the extent of DNA damage, delay in cell kinetics and p53 expression in Kwai chewers in the tribal population of Meghalaya state of North-eastern region of India and its correlation with endogenous GSH level. We have made an attempt to see the level of p53 protein in mouse in vivo after RBNE treatment.

The development of head and neck cancer may depend upon the interaction between host susceptibility factors and environmental carcinogens. Studies by using the mutagen sensitive assay have demonstrated that head and neck cancer patients may be abnormally sensitive to BLM induced chromosomal damage as compared with age and sex matched healthy controls. The assay makes use of peripheral blood lymphocytes in order to test for BLM induced chromosomal damage through the generation of free radical oxygen, and thus is reflective of one measure of tobacco / BN induced damage. The basis of this mutagen sensitivity may reflect an underlying DNA repair deficiency or factors which control susceptibility to initial clastogenic influences. Risk assessments in these studies suggest an interaction between carcinogenic exposure and mutagen sensitive measures, risk estimates being higher in those individuals who both consume Kwai and express sensitivity to free radical damage in vitro. So in addition to these, an attempt has been made for mutagen sensitivity assay of these chewers since such assay may reflect either an underlying DNA repair deficiency or factors which control susceptibility to initial clastogenic influences. Western blot analysis to determine the p53 protein levels in BN chewers and non-chewers and mutagen sensitivity assay in them were also performed.
The important aspect of this investigation is that the genotoxic effect of BN was studied in the non-target tissue (HPBLs) and it is known that genetic damage in lymphocytes reflects similar damage in cells undergoing carcinogenesis. From this study the following are the major observations that were made:

> The RBNE is genotoxic and the endogenous glutathione (GSH) level could influence its effect. The depletion of GSH did not have much influence on CAs but SCEs and delay in cell proliferation were significantly enhanced. The level of p53 protein appreciably increased after treatment with RNBE for longer duration.

> The population study carried out in RBN chewers showed that the amount of DNA-lesions was increased in Heavy chewers (HC) than Moderate chewers (MC) and Non chewers (NC). CAs also showed a positive tendency of increase in HC. Significant delay in cell cycle progression was seen in HC and the GSH level also significantly reduced in HC. Such increased DNA-damage could arrest the cells at G1 checkpoint presumably allowing time for DNA-repair. This speculation is consolidated after observing an increased level of p53 protein in HC than NC.

> From the mutagen sensitivity assay performed using bleomycin (BLM), it was observed that the frequency of CAs significantly enhanced in HC than NC. It is thus evident that HC are more susceptible to free radical damage or to damage by other toxicant. This reflects either an underlying DNA repair deficiency in BN chewers or factors which control susceptibility to initial clastogenic influences. Thus, from the present investigation, it is clear that continuous and chronic exposure to BN causes more DNA damage and delay in cell cycle. The depletion of cellular GSH may render the cells susceptible to potential further attack by
other BN components. Such continuous exposure may attack the already present DNA damaged cells or cells which has just repaired and thereby activate the repair machinery for further action. This repeated repair process may lead to error-prone repair and ultimately leading to altered gene expression. Thus the endogenous GSH level and p53 protein expression serve as important biomarkers of DNA damage in RBN chewers and may be useful to assess and control the high-risk population of long-term health outcomes associated with exposure to xenobiotics.