purchased from the local market. An aqueous extract of the product was prepared as follows: *pan masala* was finely powdered with the help of a blender and 25g was added to 75 ml of distilled water and mixed thoroughly to give a smooth paste. This was allowed to stand overnight at 4-8°C. It was next stirred for 4 h and the extract was collected by centrifugation. This extraction procedure was repeated twice more, adding 75 ml distilled water to the same residue. All three extracts were pooled. The pooled extract, representing extract of 25 g *pan masala* ni 225 ml distilled water, was sterilised by filtration through a 0.22 μ millipore filter and stored at —70°C in small aliquotes. The extract contained 1.21 g per cent water soluble material by dry weight.

For the primary evaluation of genotoxicity of an agent, short term genotoxicity assays and *in vitro* mammalian test systems, constitute a set of rapid, reliable and economic criteria[6]. Cytogenetic end points, such as SCE and CA frequencies are widely preferred parameters for their sensitivity and quantitative preciseness[7].

CHO cells were maintained in growth medium containing minimum essential medium (MEM) with Earle's salts and non-essential amino acids (Centron Research Lab., Bombay, India), 20 per cent new-born calf serum (Sera Lab., UK), 100 units/ml of penicillin and 100 μg/ml of streptomycin sulphate.

After about 42 to 45 h of subcultivation, the cultures were treated with 3 different doses of aqueous extract of *pan masala* for 3 h. The treated and the control cultures were washed twice with prewarmed calcium and magnesium free phosphate buffered saline (CMF-PBS) and fresh growth medium containing 10 μg/ml of 5-bromo deoxy uridine (BrdU, Sigma Chem. Co., USA) was added. Other culture conditions, culture termination and slide preparation was carried out as reported earlier[8]. The staining and the SCE scoring procedures remained the same as described previously[9]. CA scoring recommended by WHO was followed[6]. AGT (h) was calculated according to the method of Tice and Ivett[10]. Briefly, a minimum 100 metaphases were scanned for their staining pattern and were accordingly classified into M1, M2 and M3 stages of cell cycle. AGT was calculated by following formula,

$$\text{AGT (h)} = \frac{\text{Hours since onset of BrdU exposure}}{\text{Proliferation index (PRI)}}$$

$$\text{where PRI} = \frac{1\ \text{M1} + 2\times\text{M2} + 3\times\text{M3}}{100}$$

The statistical significance of the results was evaluated by employing Student's 't' test.

Results & Discussion

The SCE/cell values in untreated control cultures ranged from 3.0 to 11.0, giving a mean value of 6.76 (Table 1). For the cultures treated with 10 μl/ml extract the mean value of 9.64 resulted from SCE/cell values ranging between 4.0 to 15.0. Treatment with 20 μl/ml of extract resulted in SCE/cell value between 6.0 to 16.0 providing a mean of 11.0 and a mean value of 14.16 for the dose of 50 μl/ml extract resulted from SCE/cell values ranging between 8.0 to 20.0. The SCE elevations for all the three samples treated with aqueous extract of *pan masala* were dose dependent and highly significant statistically ($P<0.001$).

Cellular kinetics, expressed as AGT, indicated that there was a definite retardation

SCE frequencies, indicating the genotoxic properties of *pan masala.*

As estimated by us for the brand studied, *pan masala* contain 75-80 per cent betel nut by weight. An aqueous as well as dimethyl sulphoxide (DMSO) extract of betel nut have been shown to produce tumours in experimental animals[2,3]. Saliva of betel nut chewers has also been reported to induce SCEs in CHO cells[11]. We have observed an increase in SCE and CA frequencies on treating CHO cells with a similarly prepared aqueous extract of betel nut as well as elevated levels of SCEs in peripheral blood lymphocytes of betel nut chewers (unpublished data). Recently, Giri *et al*[4] have reported clastogenic effects of catechu, which is another constituent of *pan masala.* The available evidence on the genotoxic properties of betel nut and catechu thus explain the elevated SCE and CA frequencies observed in the present study.

The results of this report, preliminary as they are, do warrant a restriction to the indiscriminate use and sale of this widely accepted product, *pan masala,* until further in depth *in vitro* and *in vivo* studies are accomplished.

Acknowledgment

The authors are grateful to Dr T.B. Patel, the Director and Dr D.B. Balar, Head, Department of Cancer Biology, for providing facilities for this study and the National Institute of Virology, Pune, for providing CHO cell line.

References

1. Panigrahi, G.B. and Rao, A.R. Study of the genotoxicity of the total aqueous extract of betel nut and its tannin. *Carcinogenesis* 7 (1986) 37.
2. Ranadive, K.J., Gothoskar, S.V., Rao, A.R., Tezabwalla, B.U. and Ambaye, R.Y. Experimental studies on betel nut and tobacco carcinogenicity. *Int J Cancer* **17** (1976) 469.
3. Bhide, S.V., Shivapurkar, N.M., Gothoskar, S.V. and Ranadive, K.J. Carcinogenicity of betal quid ingredients : Feeding mice with aqueous extracts and the polyphenol fraction of betel nut. *Br J Cancer* **40** (1979) 922.
4. Giri, A.K., Banerjee, T.S., Talukdar, G. and Sharma, A. Induction of sister chromatid exchange and dominant lethal mutation by *Katha* (catechu) in male mice. *Cancer Lett* **36** (1987) 189.
5. Nagbhushan, M., Amonkar,A.J., Nair, U.J., Santhanam, U., Ammigan, N., D'Souza, A.V. and Bhide, S.V. Catechin as an antimutagen : its mode of action. *J Cancer Res Clin Oncol* **114** (1988) 177.
6. *Guide to short term tests for detecting mutagenic and carcinogenic chemicals.* Environmental health Criteria 51 (World Health Organization, Geneva) 1985 p 57
7. Hsu, T.C. In : *Cytogenetic assays of environmental mutagens*, T.C. Hsu, Ed. (Allanheld, Osmun and Co., Publishers, Inc. Totowa, N.J.) 1982 p 1.
8. Adhvaryu, S.G., Trivedi, A.H., Jani, K.H., Vyas, R.C. and Dave, B.J. Genotoxic effects of ketamine on CHO cells. *Arch Toxicol* **59** (1986) 124.
9. Adhvaryu, S.G., Vyas, R.C., Dave, B.J., Trivedi, A.H. and Parikh, B.N. Spontaneous and induced sister chromatid exchange frequencies and cell cycle progression in lymphocytes of patients with carcinoma of the uterine cervix. *Cancer Genet Cytogenet* **14** (1985) 67.
10. Tice, R.R. and Ivett, J.L. Cytogenetic analysis of bone marrow damage. In : *Toxicology of the blood and bone marrow,* R.D. Irons, Ed. (Raven Press, New York) 1985 p 119.
11. Stich, H.F. and Stich W. Chromosome-damaging activity of saliva of betel nut and tobacco chewers. *Cancer Lett* **15** (1982) 193.

Reprint requests : Dr S.G. Adhvaryu, Senior Scientific Officer, Cell Biology Division
Gujarat Cancer and Research Institute, Ahmedabad 380016