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An *in vitro* assessment of the genotoxic potential of *pan masalas*

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An aqueous extract of *pan masala* was tested for its *in vitro* effects on Chinese hamster ovary (CHO) cells by utilizing parameters like sister chromatid exchange (SCE), cellular kinetics and chromosome aberration (CA) assay. The cytogenetic damage brought about by the extract was dose dependent. The increase in SCE values was highly significant ($P<0.001$) for all the three concentrations tested. The treatment delayed the cell cycle progression. Frequencies of chromosome aberrations were elevated for all the concentrations utilized, however, a significant rise was obtained only at the highest concentration of 50 μl/ml.

In recent years, a new food product (*pan masala*) has captured the market not only in India but in other parts of the world. The agressive manner in which this product is being advertised through the media, has promoted a high degree of social acceptance. Unlike betel (areca) nut and tobacco chewing, which is generally avoided by women and children, use of *pan masala* is considered to be harmless. As printed on the packings, *pan masala* is stated to be a combination of ingredients like betel nut, catechu, lime, cardamom and unspecified flavouring agents. Recently, genotoxic effects of betel nut[1-3] and catechu[4] have been reported. At the same time, catechu has also been reported to possess antimutagenic properties[5]. When all such suspected substances are combined in a complex mixture, such as in *pan masala*, the possibility exists that either the genotoxic potentials of the constituents may get nullified or synergised resulting in more severe genotoxicity. Normally, most tobacco chewers spit out the juice, whereas, *pan masala* is consumed in toto. Therefore, in addition to the local effects on the oral mucosa and oesophageal lining, these substances are likely to have systemic effects as well. We undertook a study on the genotoxic potential of *pan masala in vitro.*

In the present communication, we report our findings on the effects of various doses of an aqueous extract of *pan masala* on sister chromatid exchanges (SCE), chromosome aberration (CA), frequencies and cellular kinetics, calculated in terms of average generation time (AGT), in Chinese hamster ovary (CHO) cells.

Material & Methods

A widely consumed brand of *pan masala*, without tobacco as an ingredient, was

in the proliferation index and hence the time taken by the cells to complete the cell cycle increased by 6.51 h for the CHO cells treated with 50 μl of the extract.

The details of various types of chromosome aberrations observed are seen in Table II. There was a dose dependent increase in the number of aberrations produced, however, the increase was statistically significant only for the cultures treated with the highest dose of the extract *i.e.*, 50 μl/ml growth medium. Exchanges and double minutes were not observed in the cells treated with any concentrations.

According to the method employed in the present study, a 10 μl of aqueous extract contains the soluble constituents of 1.11 mg of *pan masala.* The small pouch of *pan masala* contains about 4.0 to 5.0 g of the material. By interviewing 25 consumers of either sex, we could work out the average human consumption in the range of 6.0 to 8.0 g *i.e.*, 6000 to 8000 mg/day. The end effects would be the result of a cumulative effect of the product consumed. In the present study, cells treated with the extract equivalent to as little as 1.11 mg of *pan masala* and for a brief period of just 3 h, also produced statistically significant elevations in

Table I. Effects of aqueous extract of *pan masala* on SCE frequencies and cellular kinetics in CHO cells

Concentration/ml	SCE/cell± SE	M1	M2	M3	AGT (h)
Control	6.76±0.432	02	70	28	21.24
10 μl	9.64±0.557*	03	71	26	21.53
20 μl	11 0 ±0.540*	08	85	07	24.12
50 μl	14.16±0.715*	28	71	01	27.75

*$P<0.001$. AGT, average generation time; SCE, sister chromatid exchanges; CHO, Chinese hamster ovary

Table II. Details of chromosome aberrations in CHO cells induced by aqueous extract of *pan masala*

Concentration/ml	Aberrant meta. (%)	Types of aberrations					Aberr/cell± SE
		G	B	A	R	D	
Control	7	5	—	2	—	—	0.07+0.026
10 μl	11	8	1	2	—	—	0.11±0.031
20 μl	13	13	—	1	—	—	0.14±0.038
50 μl	18	12	4	3	1	1	0.21±0.048*

*P <0.02; <0.05 (excluding gaps). G, gap; B, break; A, acentric fragments; R, ring formation; D, dicentric