Studies on Cellular Damage by Extracts of Betel Leaves Used for Chewing

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Betel chewing is a common habit in countries like India and Ceylon. Like cigarette smoking it has become a habit among the poor as well as rich sections of the people. The ingredients chewed usually consist of betel leaf (Piper betle Linn.) smeared with lime and wrapped around a few pieces of arecanut (Areca catechu Linn.) with or without tobacco (Nicotiana tabaccum Linn.). Persons with chewing habit resort to this up to about 20 times a day.

Mature leaves of the betel vine contain volatile oil (eugenol), an unsaturated aromatic phenol and terpenes, potassium nitrate and small quantities of sugar, starch and tannin (Schonland and Brandshaw 1969).

Tennekoon and Bartlet, (1969), Pindborg et al. (1969), Stephen and Uragoda (1970) have reported that buccal carcinoma and oropharyngeal cancers are common in countries like Ceylon and India where betel chewing is a habit. However, there are no reports on cellular damage produced by betel leaves. This paper presents results of studies conducted on chromosomal and cytoplasmic damage produced by extracts of betel leaves on root tip cells of onion.

Materials and methods

Fresh, green betel leaves (Piper betle Linn.) used for chewing, were selected for these studies. Ten leaves each weighing 3 grams were crushed in a mortar and 20 cc of the extract thus obtained was diluted with distilled water to obtain desired concentrations. Concentrations above 20% were found to be toxic to cells and hence lower concentrations (10%, 5%, 2.5%, 1% and 0.5%) were used. Growing bulbs of onion with roots 1–2 cm. long were kept in the extracts for 6 hours and 24 hours. Root tips taken from the bulbs before treatment, served as controls. The roots after treatment were fixed in a mixture of absolute alcohol-acetic acid (3:1), hydrolysed in 1N HCl at 60°C for 8–10 minutes and squashed in hematoxylin. Photographs were taken from fresh preparations.

Observations

The untreated roots of onion showed normal divisions and none of the abnormalities described below was seen in the control materials.

Agglomeration of chromosomes was a characteristic feature of the treated...
Figs. 1–6. All figures ×850. 1–3, metaphases showing agglomeration of chromosomes. 4, metaphase showing chromatid break (arrow) and stickiness of chromatin. 5, metaphase showing uncondensed chromosomes. 6, metaphase showing non-synchronization in condensation of the chromosomes.

Figs. 7–12. All figures except figures 9, 10 and 11, ×850. Figs. 9, 10 and 11, ×400. 7, anaphase showing arrest of movement of the daughter chromosomes. 8, anaphase in cell showing agglomeration of chromosomes. 9, group of cells showing bizarre forms of nuclei. 10, group of cells; large and small with varying amounts of cytoplasm. 11, cells showing eccentric nuclei with extruded chromatin. 12, nucleus with irregular outline showing protrusions to the exterior.
cells. In such cells the chromosomes appear as fused or bridged together (Figs. 1–3). In such cells anaphase separation is completely hindered (Fig. 8). Rarely, fragments were also observed in cells showing agglomeration of chromosomes (Fig. 3). However, the frequency of cells showing fragments was very low (Table 1). In treatments using very low concentrations of the extract (1% and 0.5%) breakage of chromatids and stickiness of chromosomes were observed as in Fig. 4. In many of the treated cells, the chromosomes do not get condensed as prophase advances and remain so even at metaphase (Fig. 5). When the chromatids separate, the daughter chromosomes do not move to opposite poles in regular orderly fashion, but remain entangled together as in Fig. 7 and form restitution nuclei. As a result the chromatin content increases. Non-synchronization in condensation of chromosomes at the metaphase stage could also be observed occasionally as in Fig. 6.

Differences in nuclear patterns, size and shape were observed in cells treated for longer durations of time (Fig. 9). Occasionally, cells showing abnormally large nuclei were seen among the normal ones as in Fig. 10. In such cells no corresponding increase in cytoplasm has been noted. Nuclei are seen eccentrically located in many cells. In such cells where the nuclei lie near the periphery of the cell, extrusion of chromatin to the exterior of the cell has been found to occur as in Fig. 11. The nuclear border in many cells is irregular with protrusions to the periphery (Fig. 12).

### Table 1. Distribution of abnormalities at metaphase in root tip cells of onion treated with extracts of betel leaves

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total cells analysed</th>
<th>% of cells showing agglomeration of chromosomes</th>
<th>% of cells showing fragments</th>
<th>Average fragments per cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>350</td>
<td>0</td>
<td>0.57</td>
<td>0.0057</td>
</tr>
<tr>
<td>0.5% – 6 hours</td>
<td>304</td>
<td>29.27</td>
<td>0.98</td>
<td>0.0197</td>
</tr>
<tr>
<td>1%</td>
<td>237</td>
<td>28.69</td>
<td>3.37</td>
<td>0.0928</td>
</tr>
<tr>
<td>2.5%</td>
<td>326</td>
<td>33.43</td>
<td>1.22</td>
<td>0.0306</td>
</tr>
<tr>
<td>5%</td>
<td>364</td>
<td>40.38</td>
<td>2.47</td>
<td>0.0494</td>
</tr>
<tr>
<td>10%</td>
<td>279</td>
<td>43.51</td>
<td>1.43</td>
<td>0.0358</td>
</tr>
<tr>
<td>20%</td>
<td>218</td>
<td>46.33</td>
<td>0.91</td>
<td>0.0183</td>
</tr>
<tr>
<td>1% – 24 hours</td>
<td>327</td>
<td>35.77</td>
<td>1.83</td>
<td>0.0397</td>
</tr>
<tr>
<td>2.5%</td>
<td>341</td>
<td>34.89</td>
<td>2.05</td>
<td>0.0351</td>
</tr>
<tr>
<td>5%</td>
<td>273</td>
<td>57.50</td>
<td>1.46</td>
<td>0.0146</td>
</tr>
<tr>
<td>10%</td>
<td>207</td>
<td>57.49</td>
<td>0.48</td>
<td>0.0241</td>
</tr>
</tbody>
</table>

### Discussion

It is well known that habitual betel chewing causes dulling of the sensibility of the buccal mucous membrane, recession of gums and atrophy of the alveolar processes. Tennekoon and Bartlet (1969) have pointed out that about 14% of the heavy chewers in Ceylon, who have continued the habit for over 20 years, show changes in oral mucosa which can be regarded as pre-cancerous. Stephen and Uragoda (1970) in their studies on the occurrence of oesophagal carcinoma in
Ceylon and its relationship to betel chewing have pointed out that a large proportion of patients having both oral and oesophageal carcinoma are betel chewers. Tussawalla et al. (1968) have pointed out that the high incidence of buccal cavity and pharyngeal cancers in Greater Bombay and indeed throughout India is associated with the habit of betel chewing, poor oral hygiene, malnutrition etc.

The results of the present study show that extracts of betel leaves produce agglomerated chromosomes and as may be observed from Table 1, in some treatments up to 58% of the cells show agglomeration. “Agglomerated mitotics and bridged metaphases” were reported previously in Vicia faba by the application of ultrasound at medically relevant powers and intensities (Cataldo et al. 1973). This study has also shown that the presence of cells with large nuclei with no corresponding increase in cytoplasm, bizarre forms of nuclei, cells with nuclei which are eccentrically placed etc. are characteristic features of the treated cells.

It is interesting to note that in persons with regular chewing habit, the chewed betel leaves remain in contact with the buccal mucosa for varying periods of time. Persons with betel chewing habit do this many times a day thus exposing the buccal mucosa to the betel extract continuously. It is true that the ingredients of the betel leaf is diluted to a certain extent by saliva. The present study has however shown that even low concentrations of the betel leaf extract produced agglomeration of chromosomes and other abnormalities in root tip cells thus indicating that betel chewing can lead to drastic irrepairable changes in cells of the buccal mucosa.

Acknowledgement

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Summary

This paper presents results of investigations on cellular changes produced by extracts of betel leaves (Piper betle Linn.) on root tip cells of onion. Betel leaves together with lime, a few pieces of arecanut and dried tobacco leaves are chewed as ‘paan’ by many people in countries like India and Ceylon.

The specific effect of the extract on mitoses was the production of agglomeration of chromosomes and bridged metaphases. Chromosome fragments were rarely observed. Differences in condensation of the chromosomes, bizarre forms of nuclei, cells with large nuclei with no corresponding increase in cytoplasm etc. were also observed in the treated cells. The present study has provided for the first time informations regarding cellular damage produced by extracts of betel leaves.

References