Clastogenic effects of dietary supplement—*Spirulina* alga, and some medicinal plant products from *Boswellia serrata*, *Withania somnifera* on mice

Sarbani Ghoshal, Madhumita J Mukhopadhyay & Anita Mukherjee*
Centre of Advanced Study in Cell and Chromosome Research, Department of Botany, University of Calcutta, 35, Ballygunge Circular Road, Kolkata 700 019, India

Fax: 91-033-466-8892

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Pretreatment of aqueous extracts of *Spirulina* (Spirulina), *Aswagandha* (*Withania*) and *Nopane* (*Boswellia*) on colchicine induced chromosome damage showed weakness of clastogenic activity in Swiss albino mice. None of the treatments increased significantly the number of chromosome aberrations.

Herbal products which are sold as dietary supplements and not drugs, are virtually unregulated. Hundreds of botanical extracts now sold worldwide have been tested and found wanting or have never been adequately tested for their effectiveness or safety especially if used for more than few weeks. Many herbal medicines are marketed under different trade names. *Spirulina* is widely marketed under the name 'Zyrulina', *Withania* as 'Ashwagandha' and *Boswellia* as 'Nopane'.

*Spirulina*, an unicellular filamentous blue green alga has been consumed by man since ancient times. It has anti-oxidant properties and high nutritive contents. The roots of *Withania somnifera*, referred to as Indian - ginseng, has effect in general debility as well as it is an adiaptogenic. The oleogum resin of *Boswellia serrata* has analgesic, anti-inflammatory and anti-tumour properties. Its natural ingredients are known to alleviate common problems in arthritis also.

In view of the widespread use of these herbal medicines, the aim of the present study is to find out the clastogenicity of 'Zyrulina', 'Ashwagandha' and 'Nopane' on mice *in vivo*.

Healthy laboratory bred Swiss albino mice (33), 8-10 weeks old, weighing 25-30g maintained on commercial pellet diet (Lipton India Ltd) and water provided *ad libitum* were kept under standard laboratory conditions 25±2°C, 60±5% RH and 12 hr light/dark photoperiod.

*Correspondent author
For ZY, AG, and NP, 16.66, 60, and 10.50 mg/kg respectively were administered as the lowest dose. Selection of doses were based on those doses used for humans as a single recommended dose taking into consideration the average weight of Indian male is 60 kg. Other doses were gradual increase of the lowest dose.

The slides were coded to eliminate observer bias, 50 well-scattered metaphase plates were scanned per animal per treatment set. For calculation of total percentage of damaged cells (% DC) all cells having at least one aberration (barring gaps) were included. For the count of breaks per cell (CA/cell), chromosome and chromatid breaks and chromosomal rearrangements including dicentrics and rings were counted as one break, irrespective of the number of breakage events involved.

The Cochran-Armitage trend was carried out to statistically analyse whether a dose-related increase in aberrations was obtained with the three herbal compounds. Trend test involves linear regression for detecting weak effects at low doses.

Table 1 shows the chromosomal aberrations induced by ZY, AG and NP. The types of aberrations observed were mainly chromatid breaks. The frequency of damaged cells increased marginally with the increase of dosage. The Cochran-Armitage trend test gave non-significant values suggesting that the compounds failed to induce any dose-response effect.

The positive control compound CP gave a significantly higher percentage of damaged cells and breakage events as compared to the others.

Plant products, particularly dietary supplements, are being increasingly popularised for therapy against a wide range of ailments including stress relief, immune enhancement, memory boost etc. In recent times Spirulina, Withania, Boswellia have been formulated and marketed under different trade names as they are cost effective, readily available and widely used in traditional medicine\(^1\). Reports on the pharmacology and toxicological studies of these formulations are scarce. In animal experiments for acute, subchronic and chronic toxicity, mutagenicity and teratogenicity; the effect of Spirulina consumption in rats did not cause any adverse effects\(^2\). The active principles in ‘Nopane’-boswellie acids isolated from gum resin of Boswellia serrata were found to have anti-tumour activity in human leukemia HL-60 cells\(^6\). Withaferin A—a steroidal lactone from Withania somnifera inhibited tumour growth and increased survival of mouse Ehrlich ascites carcinoma\(^14\). Moreover, withaferin had radio-sensitizing effects on mouse tumours and was less cytotoxic than cyclophosphamide\(^15\). Literature search is deplete of information on genotoxic potential of these herbal drugs.

In the present investigation ZY, NP and AG have been taken which are complex mixtures of a number

<table>
<thead>
<tr>
<th>Compound and doses (mg/kg, body wt)</th>
<th>Chromosome aberrations/150 cells</th>
<th>DC% (mean±SD)</th>
<th>CA/cell (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>5</td>
<td>1.33±0.94</td>
<td>0.01±0.000</td>
</tr>
<tr>
<td>ZY 16.66</td>
<td>2</td>
<td>2.00±0.00</td>
<td>0.02±0.00</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.67±0.94</td>
<td>0.03±0.00</td>
</tr>
<tr>
<td>66.66</td>
<td>6</td>
<td>4.00±0.00</td>
<td>0.04±0.00</td>
</tr>
<tr>
<td>Trend test Z value</td>
<td></td>
<td>1.08±0</td>
<td></td>
</tr>
<tr>
<td>NP 10.50</td>
<td>3</td>
<td>2.66±0.94</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3.33±0.94</td>
<td>0.03±0.01</td>
</tr>
<tr>
<td>42.00</td>
<td>4</td>
<td>4.00±1.63</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>Z value</td>
<td></td>
<td>1.44±0</td>
<td></td>
</tr>
<tr>
<td>AG 60</td>
<td>4</td>
<td>3.33±2.49</td>
<td>0.03±0.02</td>
</tr>
<tr>
<td>120</td>
<td>5</td>
<td>4.67±0.94</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>240</td>
<td>3</td>
<td>5.33±0.94</td>
<td>0.05±0.01</td>
</tr>
<tr>
<td>Z value</td>
<td></td>
<td>1.07±0</td>
<td></td>
</tr>
<tr>
<td>CP 20</td>
<td>13</td>
<td>28.67±8.32</td>
<td>0.50±0.16</td>
</tr>
</tbody>
</table>

CdG, CrG = Chromatid and chromosome gaps, CdB, CrB = Chromatid and chromosome breaks, RR = Rearrangements include dicentrics and rings, DC = Damaged cells, CA = Chromosomal aberration, Mean= mean of 3 animals, 50 cells/animal, SD = Standard Deviation of the mean; *Z value significant at 0.05; ns = not significant at 0.05

ZY = Spirulina, NP = Boswellia, AG = Withania
of chemical compounds. The results show that within the restrictions of the protocol, the clastogenicity is marginal and is not statistically significant for all the three preparations. Thus the degree of clastogenicity exhibited by these compounds here may be regarded as the effect of different constituents present in the individual herbal preparations. Plants contain a variety of mutagens, desmutagens or antimutagens to modulate the clastogenic/mutagenic/carcinogenic rates. Therefore, thorough investigation on the constituent of plant crude drugs is required before more plants/plant products are released as over-the-counter-medicine.

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References