Effect of Salajeet treatment on biochemical and cytological changes induced by cyclophosphamide in mice

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SUMMARY. Salajeet was found not to increase the incidence of micronucleated polychromatic erythrocytes (PCE) in the bone marrow cells of mice as compared to the control. A mild reduction in RNA contents followed by slight decrease in PCE/NCE (normochromatic erythrocytes) ratio was noted in Salajeet-treated groups. Cyclophosphamide (CP) treatment caused reduction of DNA, RNA and protein contents of liver and showed significant cytotoxicity and mutagenicity. Salajeet treatment reduced significantly the increase in micronucleated PCE caused by CP without altering its cytotoxicity.

Key words: Salajeet; mutagenicity.

Salajeet (also called Shilajit) is an organic exudation from steep rocks of the Himalayan belt and is a versatile remedy commonly used for rehabilitation of muscles, bones and nerves. It is also used to treat many geriatric complaints including arthritis, diabetes and allergic manifestations.1-3

Salajeet was found to contain several common phenolic constituents comprising biphenyl carboxylates, mono- and di-oxygenated dibenzyl-a-pyrene and anacardic acids.4-8 The nature of humic substances in Salajeet are reported to be influenced by vegetation, nature of rocks and other natural factors.7 Recent studies verified antirheumatic, antiulcer and mast cell protecting effects of Salajeet.7-10 However, so far no reports are available on the mutagenicity and antioxidant properties of this popular drug. The present study was designed to investigate the cytological and biochemical effects of Salajeet and its response to the changes induced by a known mutagen cyclophosphamide (CP) in mice.

EXPERIMENTAL

Plant material. Salajeet was collected during summer (1991) from mountains of district Zobab of Balochistan province in Pakistan and stored at 4°C. The fresh aqueous suspension was prepared in distilled water before administration.

Animals. Male mice (SWR, home bred), aged 6-7 weeks and weighing 25-60 g were used. A total of 40 animals were randomly assigned to different control and treated groups (5 animals in each group) which were maintained under standard conditions of humidity, temperature and light (12 h light, 12 h dark). The animals were fed with purina chow diet and had free access to water.

Treatment. The highest dose of Salajeet used in the present study (500 mg/kg) was earlier reported to be pharmacologically active.11 Fresh aqueous suspension of Salajeet was prepared and administered orally (gavage) to the animals for seven days. The experimental

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groups of mice consisted of: (1) Untreated control (distilled water); (2) Salajeet 125 mg/kg/day; (3) Salajeet 250 mg/kg/day; (4) Salajeet 500 mg/kg/day; (5) CP 100 mg/kg; (6) Salajeet 125 mg pre-treatment (7 days) + CP 100 mg/kg; (7) Salajeet 250 mg pre-treatment (7 days) + CP 100 mg/kg; (8) Salajeet 500 mg pre-treatment (7 days) + CP 100 mg/kg. CP was injected intraperitoneally 30 h before sacrifice. To the animals in groups 6, 7 and 8, CP was injected simultaneously with the last dose of Salajeet. In each case animals were killed 30 h after the last treatment and the bone marrow cells were collected in foetal calf serum. After centrifugation the cells were spread on slides and air-dried. Coded slides were fixed in methanol and stained with May-Gruenwald solution followed by Giemsa staining. The polychromatic erythrocytes (PCE, 1000 per mouse) were screened for micronuclei and the mitodepression was obtained by PCE/NCE (normochromatic erythrocytes) ratio as described.12

Biochemical procedures. The levels of proteins and nucleic acids in liver were determined according to the following procedure. The liver from the same animals were quickly excised, frozen in liquid nitrogen and stored at -20°C until analyzed for total proteins and nucleic acids (DNA, RNA). Total proteins were determined by the method of Lowry et al.13 The method described by Bregman14 was used to determine the levels of nucleic acids. Tissues were homogenized and the homogenate was suspended in ice cold trichloroacetic acid (TCA). After centrifugation, the pellet was extracted with ethanol. DNA was determined by treating the nucleic acid extract with diphenylamine and reading the intensity of blue colour at 600 nm. For quantification of RNA, the nucleic acid extract was treated with orcinol and the green colour was read at 660 nm. The quantification of nucleic acids was done by using standard curves.

Statistical analysis. The statistical analysis of the data was done by using analysis of variance (Newman-Keuls' test).

RESULTS AND DISCUSSION

The results of our study are presented in Table 1 and 2. Salajeet treatment (groups 2, 3, 4) was found not to increase the incidence of micronucleated PCE in the bone marrow cells of mice as compared to the control; rather a slight reduction in the micronucleated PCE was observed in these groups not statistically significant. These findings confirmed Salajeet to be devoid of clastogenic activity.

Salajeet treatment was found to cause mild reduction in PCE/NCE ratio as compared to the control. The results of biochemical studies revealed an increase in the levels of proteins and DNA. The increase was statistically significant in the levels of DNA after Salajeet treatment at the highest dose (500 mg/kg). There was dose dependent reduction in liver RNA contents of Salajeet-treated animals. Although these changes were statistically insignificant, they were consistent enough to conclude that the mild cytotoxicity shown by Salajeet may be due to its RNA reducting property, known to be responsible for cytotoxicity.15 The chemical constituents isolated from Salajeet have not been investigated for such biochemical effects; however, the phenolic constituents of this natural drug may be held responsible for the observed cytotoxicity because several phenolic compounds possess such potential.16-19

CP treatment (group 5) caused a highly significant mitodepression and highly significant increase in the incidence of micronucleated PCE as compared to controls. In CP-treated animals a significant reduction in protein and a highly significant reduction in DNA and RNA contents of liver was observed. These cytological and biochemical changes are in agreement with earlier reports and may be attributed to the established cytotoxic and mutagenic properties of CP.12,20

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<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Polychromatric erythrocytes screened</th>
<th>Micronucleated polychromatric erythrocytes (%)</th>
<th>Normochromatric erythrocytes screened</th>
<th>PCE/NCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control (dist. water)</td>
<td>5578</td>
<td>0.28 ± 0.09</td>
<td>5225</td>
<td>1.04 ± 0.02</td>
</tr>
<tr>
<td>2. Salajeet 125 os</td>
<td>4986</td>
<td>0.19 ± 0.07</td>
<td>5920</td>
<td>0.90 ± 0.07</td>
</tr>
<tr>
<td>3. Salajeet 250 os</td>
<td>5409</td>
<td>0.18 ± 0.03</td>
<td>6116</td>
<td>0.90 ± 0.09</td>
</tr>
<tr>
<td>4. Salajeet 500 os</td>
<td>5080</td>
<td>0.20 ± 0.14</td>
<td>5565</td>
<td>0.89 ± 0.08</td>
</tr>
<tr>
<td>5. Cyclophosphamide (CP) 100 i.p.</td>
<td>5430</td>
<td>5.20 ± 0.45*</td>
<td>10013</td>
<td>0.56 ± 0.02*</td>
</tr>
<tr>
<td>6. Salajeet 125 os + CP 100 i.p.</td>
<td>4761</td>
<td>3.50 ± 0.39*</td>
<td>6842</td>
<td>0.71 ± 0.09</td>
</tr>
<tr>
<td>7. Salajeet 250 os + CP 100 i.p.</td>
<td>6603</td>
<td>2.55 ± 0.40*</td>
<td>10886</td>
<td>0.62 ± 0.06</td>
</tr>
<tr>
<td>8. Salajeet 500 os + CP 100 i.p.</td>
<td>4931</td>
<td>2.50 ± 0.45*</td>
<td>8846</td>
<td>0.61 ± 0.09</td>
</tr>
</tbody>
</table>

*p < 0.05; Analysis of variance (Newman-Keul's test).
Five animals were used in each group.
Groups 2, 3, 4 and 5 were statistically compared with group 1.
Groups 6, 7 and 8 were statistically compared with group 5.

Table 1 - Effect of Salajeet on the frequency of micronuclei in bone marrow cells of mice induced by cyclophosphamide (mean ± S.E.).

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Proteins mg/100 mg</th>
<th>RNA ug/100 mg</th>
<th>DNA ug/100 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control (dist. water)</td>
<td>15.32 ± 0.11</td>
<td>816.40 ± 26.00</td>
<td>211.57 ± 9.91</td>
</tr>
<tr>
<td>2. Salajeet 125 os</td>
<td>15.45 ± 0.22</td>
<td>780.60 ± 26.72</td>
<td>202.40 ± 5.87</td>
</tr>
<tr>
<td>3. Salajeet 250 os</td>
<td>15.59 ± 0.10</td>
<td>771.33 ± 24.03</td>
<td>218.80 ± 6.80</td>
</tr>
<tr>
<td>4. Salajeet 500 os</td>
<td>15.73 ± 0.18</td>
<td>747.00 ± 22.00</td>
<td>237.20 ± 6.96</td>
</tr>
<tr>
<td>5. Cyclophosphamide (CP) 100 ip</td>
<td>14.98 ± 0.06</td>
<td>652.00 ± 20.19</td>
<td>157.60 ± 7.57</td>
</tr>
<tr>
<td>6. Salajeet 125 os + CP 100 i.p.</td>
<td>15.06 ± 0.13</td>
<td>667.20 ± 19.12</td>
<td>171.00 ± 4.71</td>
</tr>
<tr>
<td>7. Salajeet 250 os + CP 100 i.p.</td>
<td>15.11 ± 0.08</td>
<td>685.80 ± 17.05</td>
<td>185.00 ± 3.84</td>
</tr>
<tr>
<td>8. Salajeet 500 os + CP 100 i.p.</td>
<td>15.13 ± 0.09</td>
<td>680.05 ± 17.28</td>
<td>208.83 ± 5.67</td>
</tr>
</tbody>
</table>

*p < 0.05; Analysis of variance (Newman-Keuls’ test)
Five animals were used in each group.
Groups 2, 3, 4 and 5 were statistically compared with group 1.
Groups 6, 7 and 8 were statistically compared with group 5.

Table 2 - Effect of Salajeet on biochemical changes induced by cyclophosphamide in mice (mean ± S.E.).
Salajeeet and CP combined treatment (groups 6, 7, 8) confirmed that Salajeeet reduced significantly the increase in micronucleated PCE caused by CP in a dose dependent manner without altering the cytotoxicity of CP. These findings are supported by the results of our biochemical studies (Table 2) where a dose dependent increase in the level of DNA was noticed and the effect on RNA and protein levels was insignificant. These results showing Salajeeet to selectively inhibit the clastogenicity induced by CP without interfering with its cytotoxicity indicate the possibilities of different constituents of Salajeeet operating via independent routes. This possibility is supported by earlier reports where DNA damage and cytotoxicity by some cytotoxic drugs has been found to proceed by different mechanisms. Halliwell and Gutteridge demonstrated that genetic damage induced by some cytotoxic drugs is mediated by binding to DNA and interfering with replication and/or transcription or causing strand breakage and generating free radicals where Triton and Yee reported the cytotoxic activity to be a simple interaction of cytotoxic drugs at the cell surface.

The results of our present study add support to the safe use of Salajeeet. Further studies are warranted to explore the possible utilization of Salajeeet in combination with cytotoxic drugs to reduce the mutagenic potential of such drugs.

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REFERENCES