Effects of concomitant administration of Processed Shilajit (PS, 0.1 and 1 mg/kg, i.p.), in Swiss mice were evaluated on the development of tolerance to morphine induced analgesia in the hot plate test. Chronic administration of morphine (10 mg/kg, i.p., b.i.d.) to mice over a duration of 10 days resulted in the development of tolerance to the analgesic effect of morphine. Concomitant administration of PS with morphine, from day 6 to day 10, resulted in a significant inhibition of the development of tolerance to morphine (10 mg/kg, i.p.) induced analgesia. Processed Shilajit per se, in the doses used, did not elicit any significant analgesia in mice; nor did the chronic concomitant administration of Processed Shilajit alter the morphine-induced analgesia. These findings with Processed Shilajit indicate its potential as a prospective modifier of analgesic tolerance to morphine. Copyright © 2001 John Wiley & Sons, Ltd.

Keywords: Processed Shilajit; morphine-tolerance; analgesia.
Table 1. The AUCs of morphine induced analgesia on day 11: Showing the effect of Processed Shilajit (PS) on the development of analgesic tolerance to morphine

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>AUC, (mean ± s.e.m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOR naïve + VEH</td>
<td>18</td>
<td>10659.9 ± 389.0</td>
</tr>
<tr>
<td>MOR tolerant + VEH</td>
<td>6</td>
<td>2067.0 ± 321.0*</td>
</tr>
<tr>
<td>MOR naïve + PS0.1</td>
<td>6</td>
<td>9615.8 ± 638.9b</td>
</tr>
<tr>
<td>MOR tolerant + PS0.1</td>
<td>6</td>
<td>5262.8 ± 577.9*</td>
</tr>
<tr>
<td>MOR naïve + PS1</td>
<td>6</td>
<td>9400.0 ± 593.1b</td>
</tr>
<tr>
<td>MOR tolerant + PS 1</td>
<td>6</td>
<td>7408.8 ± 895.6*</td>
</tr>
</tbody>
</table>

* p < 0.05 vs MOR naïve + VEH.
* p > 0.05 vs MOR naïve + VEH.
* p < 0.05 vs MOR tolerant + VEH.

The analgesic effect was measured by the hot plate method (Eddy & Leimbach, 1953). This consisted of a hot plate maintained at 51°C ± 1°C. The reaction times, of the mice to the thermal stimulus were determined at various times up to 240 min after an injection of morphine (10 mg/kg, i.p.). A cut-off time of 60 sec was fixed to prevent injury to the paws of the mice.

The reaction times were converted to percent analgesic effect using the formula given below:

\[
\text{Percent analgesia} = \frac{\text{Reaction} - \text{Basal} - \text{Cut-off} - \text{Basal}}{\text{Basal}} \times 100
\]

To determine the effect of PS on the development of tolerance to the analgesic effect of morphine, PS (two dose levels; 0.1 and 1 mg/kg, i.p.) was concomitantly administered to mice once a day from day 6 to day 10 of the treatment schedule. Each dose of PS was co-administered to the mice receiving chronic doses of morphine or vehicle. For example, two groups of mice on chronic morphine started receiving their respective PS doses from day 6 onwards once a day; and similarly, two groups of mice receiving chronic vehicle started receiving their respective PS doses following the same schedule. Finally, on day 11, all mice were tested for the analgesic response of morphine (10 mg/kg, i.p.).

**Statistics.** The area under the time-response curve was calculated for each mouse, and represented as mean ± standard error of the mean. The responses in different groups were analysed by Student’s t-test. Values of p < 0.05 were considered significant.

**RESULTS AND DISCUSSION**

The repeated administration of morphine (10 mg/kg, i.p., b.i.d.) for 10 days to mice resulted in the development of tolerance to the analgesic effect of morphine. The AUC in the chronic morphine-treated mice was reduced to nearly one-fifth when compared to the AUC in the morphine-naive mice, confirming the development of tolerance (Table 1).

Concomitant administration of PS, (0.1 and 1 mg/kg, i.p.; referred to as PS0.1 and PS1 in the text respectively), resulted in protection against the development of tolerance to the analgesic effect of morphine as evidenced by the time-analgesic response curves of the PS treated mice compared to the morphine-tolerant mice (Figure 1). The AUC for the PS0.1 and PS1-treated mice was significantly different from the AUC for the morphine-tolerant mice (Table 1). The mice treated with the higher dose of PS (PS1 mg/kg) elicited a larger response as compared with the mice treated with lower dose of PS (PS0.1). The protective effect of PS, therefore, appeared to be dose-dependent. However, PS per se (0.1 and 1 mg/kg) elicited no analgesic response in mice. Finally, chronic concomitant administration of PS had no influence on morphine-induced analgesic response in morphine-naive mice (Table 1).

Among the natural products modifying the development of tolerance to opioids, Panax ginseng extract (200mg/kg), given in combination with morphine to rats, has been reported to antagonize the acute analgesic, hyperthermic and cataleptic action of morphine. The analgesic and the hypothermic responses to ginseng were not reversed by naltrexone, indicating a nonopioid receptor involvement in its actions (Ramarao and Bhargava, 1990). Ginseng extract also inhibited the development of tolerance to analgesic and hyperthermic actions of morphine in rat (Bhargava and Ramarao, 1991) and in mice (Kim et al., 1987). However, the effect of the individual ginseng saponins on this phenomenon remains to be investigated.

There are many research studies supporting the hypothesis that there are bi-directional circuits between the immune system and the central nervous system (Bhargava, 1991; Jankovic, 1985). Alteration in the immune function of subjects taking centrally acting drugs has also been studied. It is reported that abusers of
morphine have depressed immune functions (Bhargava, 1994). There are studies reporting inhibition of tolerance and physical dependence on morphine mediated possibly through nonspecific immunosuppression (Meisheri and Isom, 1978). The beneficial effects of *Withania somnifera* extract on morphine tolerance, reported earlier (Ramarao et al., 1995) have also pointed to a possibility of the immune axis being involved in the phenomenon. In this perspective, it is very important to note that the reported immunomodulatory property of Processed Shilajit could play a role in the inhibition of development of analgesic tolerance to morphine. Therefore it appears that Processed Shilajit could have a great potential as a prospective inhibitor of analgesic tolerance to morphine.

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