Anti-inflammatory and analgesic activities of *Dashana Samskara Churna* and its paste form

K P P Peiris¹, B K Ashok², R Manjusha³* and B Ravishankar²

¹Department of Shalakya, ²Pharmacology Laboratory, Institute for Post Graduate Teaching & Research in Ayurveda, Gujarat Ayurved University, Jamnagar-361 008, Gujarat, India

Received 3 September 2010; Accepted 4 May 2011

Anti-inflammatory and analgesic activities of *Dashana Samskara Churna* and its paste form were examined in different animal models. Anti-inflammatory activity was evaluated in acute, sub-acute and chronic inflammatory models and compared with Phenylbutazone, Diclofenac sodium and Dexamethasone standards, respectively. Analgesic activity was evaluated in formalin induced paw licking (chemically induced pain) and tail flick method (thermally induced pain) where Indomethacin and Pentazocine were used as standard drugs, respectively. Test formulation in the form of paste inhibited the carrageenan induced paw oedema (P<0.05), formalin induced paw licking (P<0.01) and increased the Tail Flick Latency (P<0.05) in comparison to control rat. Test formulation in the form of Churna did not inhibit the paw oedema induced by phlogistic agents, however it exhibited significant analgesic activity by decreasing formalin induced paw licking response (P<0.05). The results suggest that the paste form of *Dashana Samskara Churna* has significant analgesic and anti-inflammatory potential as reflected by the parameters investigated, while Churna form has only analgesic activity. Thus, it can be concluded that *Dashana Samskara Churna* in the form of paste will be the most appropriate modality in treatment of gingivitis.

Keywords: Analgesic, Anti-inflammatory, Carrageenan, *Dashana Samskara Churna*, Dental problems, Gingivitis.

IPC code; Int. cl. (2011.01) — A61K 36/00, A61P 1/02, A61P 25/04, A61P 29/00

Introduction

Gingivitis which is known as *Shitada* in Ayurveda, is an inflammation of the marginal gingiva, which occurs widely in most populations affecting both children and adults. The high general prevalence of gingivitis is 50% and this may be due to deficient oral health care¹,². Therapy to treat gingivitis is aimed primarily at reduction of etiologic factors to reduce or eliminate inflammation, thereby allowing gingival tissues to heal. Considerable research efforts have focused on systemic application of host modulating agents such as non-steroidal anti-inflammatory drugs³,⁴.

*Dashana Samskara Churna* a polyherbal formulation is well known *Pratrisarana* (A kind of local application) drug mentioned in different classical texts and also in Ayurvedic formulary of India as remedy for all types of dental problems including gingivitis². This formulation contains drugs like *Shunti* (*Zingiber officinale* Roxc.), *Haritaki* (*Terminalia chebula* Retz.), *Musta* (*Cyperus rotundus* Linn.), *Khadirra* (*Acacia catechu* Willd.), *Karpura* (*Cinnamomum camphora* Nees.), *Puga* (*Areca catechu* Linn.), *Maricha* (*Piper nigrum* Linn.), *Lavanga* (*Syzygium aromaticum* Linn.), *Twak* (*Cinnamomum zeylanicum* Breyn.) and *Khataki* (*Chalkon* powder)⁵. Almost all drugs of this formulation are reported to have anti-inflammatory and antibacterial activities⁶,⁷ which is the prime therapeutic intervention in the treatment of gingivitis.

Considering some of inferences of this formulation like less palatability to patients especially children, complaining from patients due to inconvenient form, more chances of microbial contamination, short shelf-life, this formulation was converted in to paste form which is the most suitable and convenient for treatment in dental application. Thus, prepared *Dashana Samskara* paste (DSP) was subjected to comparative anti-inflammatory and analgesic activities with *Dashana Samskara Churna* (DSC).

Materials and Methods

Test drugs

The raw materials (Table 1) of the test formulation were collected from pharmacy attached to the institute and were subjected to pharmacognostical studies in
Table 1 — Formulation composition of Dashana Samskara Churna

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Botanical name and family</th>
<th>Part/Quantity* used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shanti</td>
<td>Zingiber officinale Rosc. (Zingiberaceae)</td>
<td>Rhizome</td>
</tr>
<tr>
<td>Haritaki</td>
<td>Terminalia chebula Retz. (Combretaceae)</td>
<td>Fruit</td>
</tr>
<tr>
<td>Mantha</td>
<td>Cyparrhus rotundus Linn. (Cyperaceae)</td>
<td>Rhizome</td>
</tr>
<tr>
<td>Khadira</td>
<td>Acorus calamus Willd. (Lilaceae)</td>
<td>Bark</td>
</tr>
<tr>
<td>Karpura</td>
<td>Cinnamomum camphora Nees. (Lauraceae)</td>
<td>Exudate</td>
</tr>
<tr>
<td>Puga</td>
<td>Areca catechu Linn. (Palmae)</td>
<td>Seed</td>
</tr>
<tr>
<td>Maricha</td>
<td>Piper nigrum Linn. (Piperaceae)</td>
<td>Fruit</td>
</tr>
<tr>
<td>Lavangaa</td>
<td>Syzygium aromaticum Linn. (Myrtaceae)</td>
<td>Flower bud</td>
</tr>
<tr>
<td>Twak</td>
<td>Cinnamomum zeylanicum Brey. (Lauraceae)</td>
<td>Flower bud</td>
</tr>
<tr>
<td>Khatika</td>
<td>Chalk</td>
<td>--</td>
</tr>
</tbody>
</table>

*All ingredients were taken 1 part except chalk which was used 5 parts.

order to evaluate the authenticity. From the raw materials, the test formulation Dashana Samskara Churna was prepared by following the classical guidelines. In the pharmacy attached to the institute. Further formulation was also converted to paste form by adopting standard protocol.17,18

Experimental animals
Wistar strain rats of either sex weighing 200 ± 20 g and Swiss albino mice of either sex weighing 24 ± 4 g were procured from the animal house attached to our institute (Registration No.548/2002/CPCSEA). They were housed in large spacious polypropylene cages and fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries and tap water given ad libitum. The animals were acclimatized for at least one week in lab conditions before the commencement of experiment in standard laboratory conditions 12 ± 1 hour day and 12 ± 01 hour night rhythm, maintained at 25 ± 3°C and 40 to 60% humidity. Before the test, the animals were fasted for at least 12 hours. Institutional animal ethics committee had approved the experimental protocol (Approval number: IAEC 05/09-10Ph.D.09) and the care of animals was taken as per the CPCSEA guidelines.

Dose selection
The clinical dose of Dashana Samskara Churna is 2g/day as per the classical texts. The dose fixation for the experimental animals was done on the basis of body surface area ratio by referring to the standard table of Paget and Barnes (1964)19. On this basis the rat dose was found to be 180 mg/kg and for mouse 260 mg/kg. The test drug was suspended in distilled water with suitable concentration depending up on body weight of animals and administered orally with the help of gastric catheter sleeve to syringe. The drugs were administered to overnight fasted animals.

Anti-inflammatory activity

Carrageenan induced rat paw oedema

The Wistar strain albino rats of either sex were weighed and randomly divided in to four groups of six each. First group received distilled water and served as control group. The second and third groups received test drugs DSC and DSP, respectively. Fourth group was administered with standard anti-inflammatory drug phenylbutazone (Wilson Laboratories, Mumbai) in the dose of 100 mg/kg. The vehicle and test drugs were administered to the respective groups for five consecutive days; whereas standard drug was given only once i.e. one hour before the carrageenan injection.

Initially left hind paw volumes up to the tibio-tarsal articulation were recorded prior to carrageenan injection by using plethysmograph. The plethysmograph employed, consisted of 10 ml glass vessel (25 x 65 mm) fixed to 2 ml glass syringe through pressure tubing. About 4 ml of mercury was filled in the syringe and the mercury level was adjusted to zero mark on the micropipette. The space between the zero mark and the fixed mark on the glass vessel was filled with water and few drops of teepol. The initial level of fluid was adjusted and set at zero. The paw was immersed in water exactly up to the tibio-tarsal articulation. The increased level of water in the glass vessel was adjusted to the prefixed mark by releasing the pressure of the connected syringe. The level where water and mercury interface in the micropipette was recorded as paw volume.

On fifth day one hour after drug administration oedema was produced by injecting 0.1 ml freshly prepared 1% carrageenan in sterile saline solution to the sub-plantar aponeurosis of the left hind limb. The rats were administered tap water in the dose of 2 ml per 100 g body weight to ensure uniform hydration and hence to minimize variations in oedema formation. Paw volume was recorded three hour after carrageenan injection. Results were expressed as an increase in paw volume compared to baseline (oedema free).
volume in comparison to the initial paw volumes and also in comparison with control group.

Formaldehyde induced paw oedema

The test conditions and groupings were similar to carrageenan induced paw oedema as mentioned above, except the standard anti-inflammatory drug used (Diclofenac sodium-5mg/kg Novartis India Limited). Pedal inflammation was induced by injecting 0.1 ml of 3% formaldehyde solution below the plantar aponeurosis of the right hind paw of the rats. The paw volume was recorded immediately prior to compound administration (0 h) and then at 24 and 48 h after formaldehyde injection. Results were expressed as an increase in paw volume in comparison to the initial paw volumes and also in comparison with control group.

Cotton pellet-induced granuloma formation

The method used by Meir (1950) was adopted. The selected rats were anaesthetized with ether. Dorsum was shaved and swabbed with 70% (v/v) alcohol. Midline incision of 1 cm was made in the intrascapular region. A small tunnel was made on either side of the incision with the help of small blunt forceps. One sterile cotton pellet weighing 100 mg was inserted per tunnel and closed the incision with interrupted sutures after expelling the air from the tunnel. Thus cotton pellet inserted rats were randomly divided into 4 groups of six rats each. Group 1 was treated with distilled water and considered as control group. Groups II and III were administered with the test formulations DSC and DSP, respectively for 7 consecutive days starting from the day of implantation. The fourth group was taken as standard and administered with the standard drug Dexamethasone (0.1 mg/kg, orally-Cadila healthcare limited) daily for seven consecutive days. On the eighth day, the animals were anaesthetized again; the cotton pellets were removed surgically and made free from extraneous tissues. The pellets were incubated at 37°C for 24 h and dried at 60°C to constant weight. The increment in the dry weight of the pellets was regarded as a measure of granuloma formation.

Analgesic activity

Tail flick test

Tail flick test was used to measure the latency of the response as described by Watkins et al (1961). Mice were placed on the tail flick unit so that a constant heat intensity was applied to the lower third of the animal’s tail. When the animal flicked its tail in response to the noxious stimulus both the heat source and timer were stopped. A cut off time of 10 seconds was set to avoid tail damage. Thus, basal reaction time of each mouse to radiant heat was recorded and those having TFL (tail flick latency) less than 10 seconds were selected. The selected mice were randomly divided in to four groups of six each. Control animals (First group) received similar volume of vehicle as test drug. Mice in group II and III were pre-treated with test drugs DSC and DSP, respectively. To group IV standard analgesic drug Pentazocine (20 mg/kg i.p.-Ranbaxy laboratories) was administered. The vehicles and test drugs were administered to the respective groups for five consecutive days, whereas standard drug was given only once i.e., one hour prior to experiment. The TFL was recorded at 30, 60, 120, 180 and 240 minutes.

Formalin induced hind paw licking

Animal grouping and test drug administration are similar to carrageenan induced paw oedema model. Indomethacin (10 mg/kg orally-Cipla) was used as standard drug. Pain was induced by injecting 0.1 ml of 3% formalin in distilled water in subplantar region of right hind paw and the duration of paw licking as an index of nociception was counted in periods of 0 to 10 min (Early phase) and 20 to 30 min (Late phase).

Statistical analysis

Data are expressed as mean ± SEM. Statistical evaluation was carried out by one-way analysis of variance (ANOVA followed by Dunnett’s multiple t test) and also by unpaired Student t test. Statistical significance is expressed as *P<0.05, **P<0.01, and ***P<0.001.

Results

Effect on carrageenan induced paw edema

The result shows (Table 2) that DSP at the dose of 180 mg/kg has significant reduction in the carrageenan induced paw oedema (P<0.05) when compared to control. Standard anti-inflammatory drug treated group has also shown significant (P<0.001) reduction in paw oedema.

Effect on formalin induced paw edema

Test drug both in the form of churna and paste did not suppress the formalin induced paw oedema at both 24 and 48 h intervals. Diclofenac sodium
suppressed the formalin induced paw oedema in significant manner at both 24 and 48 h comparison to control group (Table 3).

**Effect on weight of granulation tissue**

The effect of DSC and DSP on cotton pellet granuloma has been given in Table 4. The results showed a considered protection in granuloma by markedly reducing the dry weight of the cotton pellet in DSP, however the observed decrease is found to be statistically non-significant due to variation in data. The dexanethasone treated group has shown significant reduction ($P<0.05$) in dry weight of granuloma in comparison to control group.

**Effect on formalin induced paw licking response**

Table 5 shows the results of the formalin induced paw licking response. Test drug in both the forms significantly decreased the paw licking response at both early phase and late phase. The observed effect is more significant in paste treated group in comparison to *churna* treated group. However, the observed effect is comparatively less to Indomethacin treated group.

**Effect on tail flick response**

Mice pre-treated with Pentazocine (20 mg/kg) significantly increased TFL (P<0.01) after 30 min and non-significantly at 60 min onwards (Table 6). DSC failed to alter TFL significantly at all time intervals, whereas significant increase in TFL was observed in DSP treated group at 60, 120 and 240 min in comparison to control group.

**Discussion**

Gingivitis is a reversible disease and the therapy should be aimed primarily at reduction of inflammation, thereby allowing gingival tissues to heal. Appropriate supportive periodontal maintenance that includes personal and professional care is important in preventing re-initiation of inflammation

In this regard *Dashana Samskara Churna* is very helpful as it contains sufficient ingredients which may take overall care of the dental as well as oral hygiene i.e., chalk provides the mild abrasive and polishing effect, clove oil acts as analgesic and aromatic ingredients like *Cinnamomum* alay the bad breath. Tannin content of *Terminalia* and *Acacia* provides anti-bacterial activity. Thus, anti-inflammatory and analgesic activities of this formulation was screened by adopting different experimental models for assessment of *Dashana Samskara Churna* used in the health care system for the management of gingivitis.

Carrageenan induced inflammation in rats is one of the most suitable acute model to screen anti-inflammatory agents. The development of carrageenan induced oedema is bi-phasic, the first phase is attributed to the release of histamine, 5-hydroxytryptamine and kinins, while, the second phase is release of prostaglandins

Among the two forms of test drug DSP produced a considerable suppression of oedema formation against carrageenan induced paw oedema in rats. The observed effect may be due to inhibition of phlogistic mediators, antagonizing their interaction with their respective receptors or it may be due to general mechanism like increasing the membrane stability in the cell.

The formalin-induced inflammation in the rats foot may be conveniently divided into two parts, the first involving 5-hydroxytryptamine as mediator and the second mediator which is unrelated to

---

<table>
<thead>
<tr>
<th>Treatment</th>
<th>3 hours</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>64.36 ± 06.28</td>
<td>—</td>
</tr>
<tr>
<td>DSC (180mg/kg,po)</td>
<td>62.50 ± 03.63</td>
<td>02.88 ↓</td>
</tr>
<tr>
<td>DSP (180mg/kg,po)</td>
<td>42.31 ± 04.88**</td>
<td>34.26 ↓</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>14.94 ± 04.03***</td>
<td>76.78 ↓</td>
</tr>
<tr>
<td>(100mg/kg,po)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data: Mean ± SEM; ↓ - Decrease $P<0.05$, $**P<0.001$.

*One-way ANOVA-F value 25.898; P<0.001: DMTT-P<0.05 for DSP and PRZ vs normal control.

---

<table>
<thead>
<tr>
<th>Treatment</th>
<th>24 hours</th>
<th>Percentage inhibition</th>
<th>48 hours</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>39.50 ± 03.07</td>
<td>—</td>
<td>24.31 ± 02.87</td>
<td>—</td>
</tr>
<tr>
<td>DSC (180mg/kg,po)</td>
<td>43.60 ± 04.34</td>
<td>10.38 ↓</td>
<td>24.17 ± 03.17</td>
<td>—</td>
</tr>
<tr>
<td>DSP (180mg/kg,po)</td>
<td>42.12 ± 04.47</td>
<td>06.64 ↓</td>
<td>21.28 ± 02.87</td>
<td>12.46 ↓</td>
</tr>
<tr>
<td>Diclofenac sodium (5mg/kg, po)</td>
<td>22.76 ± 02.11***</td>
<td>42.38 ↓</td>
<td>16.67 ± 01.79**</td>
<td>31.43 ↓</td>
</tr>
</tbody>
</table>

Data: Mean ± SEM; ↓ - Decrease $P<0.05$, $**P<0.001$.

*One-way ANOVA-F value 6.651; P=0.04: DMTT-P<0.05 for Diclo vs normal control.
5-hydroxytryptamine. Test drug in both the forms did not inhibit the formalin induced paw at both time intervals.

Granulomatous tissue formation is related to the chronic inflammatory process which is an indication for the proliferative phases of inflammation. Inflammation involves proliferation of macrophages, neutrophils and fibroblasts, which are basic sources of granuloma formation thus this method is widely used to evaluate the transudative and proliferative components of the chronic inflammation. The wet weight of the cotton pellets correlates with the transudate and the dry weight of the pellets correlates with the amount of the granulomatous tissue. DSP has remarkably decreased the weight of granulation tissue and dexamethasone which was used as standard anti-inflammatory agent in this model significantly decreased the weight of granulation tissue. This may indicate ability of the test compounds in reducing the synthesis of proteins, collagen and infiltration of macrophages.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight of granulation tissue (mg/100 g body wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>140.50 ± 15.97</td>
</tr>
<tr>
<td>DSC (180 mg/kg, po)</td>
<td>127.33 ± 10.30</td>
</tr>
<tr>
<td>DSP (180 mg/kg, po)</td>
<td>103.67 ± 18.30</td>
</tr>
<tr>
<td>Dexamethasone (0.1 mg/kg, po)</td>
<td>98.30 ± 0.23*</td>
</tr>
</tbody>
</table>

Data: Mean ± SEM; ↓ - Decrease *P<0.05

When formalin is injected subcutaneously into the paw, it produces intense pain reaction. The effect is seen in two phases. The initial phase lasts for 0-10 min of formaldehyde injection; it is supposed to be mediated through modulation of neuropeptides. The second phase, which is observed 20-30 min of formaldehyde injection, is supposed to be mediated through release of inflammatory mediators like prostaglandin, etc. Both the forms of test drug significantly decreased the paw licking episodes at both the phases so as the Indomethacin which is a non-selective cyclooxygenase inhibitor.

Tail flick model which is thermal induced nociception indicates narcotic involvement which is sensitive to opioid µ receptors. The ability of the DSP to prolong the reaction latency to thermally induced pain in mice further suggests central analgesic activity. The effect observed with pentazocine was short lived. This indicates that the DSP exhibit analgesic effect by central action. The mechanism through which this effect is brought about may be due to modulation of opioid receptors or by release of endogenous analgesic factors like encephalin and endorphin.

**Conclusion**

In conclusion, Dashana Samskara Churna in the form of paste has very good anti-inflammatory potential and also has antinociceptive action while in Churna form it has only antinociceptive activity. Thus, paste form will be most appropriate and the

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0-10 min</th>
<th>20-30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.00 ± 0.93</td>
<td>---</td>
</tr>
<tr>
<td>DSC (180 mg/kg, po)</td>
<td>11.67 ± 0.49**</td>
<td>27.06 ↓</td>
</tr>
<tr>
<td>DSP (180 mg/kg, po)</td>
<td>10.33 ± 0.26**</td>
<td>35.43 ↓</td>
</tr>
<tr>
<td>Indomethacin (10 mg/kg, po)</td>
<td>08.33 ± 0.67***</td>
<td>48.00 ↓</td>
</tr>
</tbody>
</table>

Data: Mean ± SEM; ↓ - Decrease *P<0.05, **P<0.01, ***P<0.001

* One-way ANOVA; F value 13.403; P<0.001; DMRT; P<0.05 for DSC, DSP and Indomethacin vs normal control.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial TFL (sec.)</th>
<th>TFL after drug administration (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
<td>60 min</td>
</tr>
<tr>
<td>Control</td>
<td>04.04 ± 0.01</td>
<td>03.28 ± 0.062</td>
</tr>
<tr>
<td>DSC (260 mg/kg, po)</td>
<td>04.07 ± 0.33</td>
<td>03.98 ± 0.022</td>
</tr>
<tr>
<td>DSP (260 mg/kg, po)</td>
<td>04.86 ± 0.037</td>
<td>05.08 ± 0.057</td>
</tr>
<tr>
<td>Pentazocine (50 mg/kg, ip)</td>
<td>2.10 ± 0.20</td>
<td>06.83 ± 1.08**</td>
</tr>
</tbody>
</table>

Data: Mean ± SEM; *P<0.05, **P<0.01 (unpaired t test)
new appreciation of the role of inflammation in gingivitis provides a mechanistic framework for understanding the clinical benefits.

References


18. Anonymous, Manufacture of Beauty Products, Published by Small Business Publications, Board of Consultants and Engineers, Delhi, 2005, pp. 82-84.


