RESEARCH ARTICLE

Anti-inflammatory and Analgesic Activity of an Ayurvedic Liniment Formulation

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ABSTRACT

Aim: The objective of these studies was to assess the anti-inflammatory and analgesic activity claim of an Ayurvedic liniment (ZRL) formulation prepared basis the wisdom of classical formulations.

Materials and methods: Carrageenan-induced paw edema was used to investigate the anti-inflammatory activity. Analgesic activity was examined using hot plate method and tail flick method. The efficacy of the topically applied ZRL formulation was evaluated vis-à-vis control.

Results: The percentage of reduction of paw volume after ZRL treatment and standard diclofenac Gel were 49.18 ± 2.12 and 81.79 ± 1.24 respectively. In Eddy's hot plate model, after a latency period of 2 hours following topical application of the ZRL formulation, there was a significant reduction (66%) to painful sensation compare to control. In tail flick method, 89% reduction to painful sensation was observed with respect to pretreatment.

Conclusion: The present study provided a scientific justification for pain relief and management of inflammation, by showing its peripheral and central acting anti-nociceptive and anti-inflammatory properties.

Clinical significance: The present in vivo anti-inflammatory and analgesic activity of ZRL formulation showed its potential in the management of pain and inflammation associated with musculoskeletal disorders. The taila formulation is in clinical use for management of pain associated with musculoskeletal and neurological disorders.


Source of support: Nil
Conflict of interest: None

INTRODUCTION

Inflammation is a protective response of the human body against different stimulation, which may generate a series of the complex reaction. These events involve series of inflammatory mediators such as eicosanoids and other inflammatory mediators at inflammation site which are responsible for many connected disorders. Non-steroidal anti-inflammatory drugs (NSAIDs) are the drug of choice for inflammation and its associated symptoms. However, these drugs over a period of usage showed unwanted effects, i.e., gastro-intestinal irritations and ulcers, kidney failure and Liver toxicity. These shortcomings associated with NSAIDs can be avoided or lessened by replacing them with comparatively safer and effective plant-based drugs. Complementary and alternative medicine (CAM) is gaining popularity in term of health and wellness therapy worldwide. It significantly reduces the cost of private and public health. Plant parts and its derived products are major sources of CAM. Moreover, the inadequacies of treatment options for prolonged inflammatory conditions like arthritis has led to the innovation of new medicinal agents from botanical sources.

Ayurvedic oils are extensively used for the treatment of joint diseases (Vatatyadhi, diseases essentially caused by Vata). The Ayurvedic oil formulation such as Mahamash taila, Vishgarbha taila, Narayan taila, and Gandhapuro taila (oil of Gaultheria) are being used in the treatment of musculoskeletal pain and inflammatory condition such as arthritis. The main ingredient of Mahamash oil is Urad or black lentil along with Til taila (Sesame oil), Dashmool, Ashwagandha etc. Mahamash Tail is used as massage in treating various conditions such as tremors in hands, sciatica, muscular wasting of forearm, brachialgia.

Vishgarbha taila is used in various conditions like sciatica, stiffness and tightness/stiffness in limbs. Narayan taila...
is used since ages in various conditions such as neck rigidity/torticollis, lockjaw, muscle wasting.\textsuperscript{6} \textit{Gandhapuro Taila} is useful in conditions such as rheumatism, sciatica, acute pain of nervine origin.\textsuperscript{8} The combination of these classical oils is expected to have an inherent benefit in various musculoskeletal symptoms associated with rheumatological and neurological conditions.

The objective of these tests was to verify the anti-inflammatory and analgesic activity claim of a combination of these four well known Ayurvedic classical preparations together in a liniment dosage form.

**MATERIALS AND METHODS**

**Chemical and Reagents**

**Preparation of Ayurvedic Liniment**

The Zandu Rhumasyl Liniment coded as ZRL was prepared by mixing of four classical taila (oils) preparations namely Narayan taila, Mahamash taila, Vishgarbha taila and Gandhapuro taila (Oil of Gaultheria) each in equal quantity (Table 1). The samples of ZRL for the studies were obtained from the Healthcare Research and Development Centre, Emami Ltd, 13, BT Road, Belgharia, Kolkata, West Bengal with batch no EJ0006 and Manufacturing date 04/2015.

**Animal**

Wistar male rats (200 to 250 g) of were used for anti-inflammatory studies. Swiss albino mice of either sex (47 ± 2 days) and a body mass index (BMI) between 20 and 38 g were for pain models. The animals were obtained from the animal house of NSHM College Animal House, NSHM Knowledge campus, Kolkata, India. The rodents were kept for seven days in the pharmacology laboratory for acclimatization before initiating the experiments. The animals were grouped as eight per cage under standard environmental settings (12:12 h light: dark cycle at 25 ± 2°C); were fed with standardized pellet diet and water ad libitum. They were starved for 12 hours before experiments. The study was approved by the Institutional Animal Ethics Committee (CPCSEA Reg No. 1458/PO/E/11/CPCSEA), and the experiments were conducted following the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Chennai, India.

**Treatment Groups**

Wistar rats of male sex were randomly assigned into three groups of five animals each for a single set of experiment. Group 1 animals were served as control and were treated with normal saline. Group 2 animals were treated on the plantar area of the hind paw with standard Volini Gel (Diclofenac sodium; Sun Pharmaceutical Industries Ltd.). The third test group (Group 3) was treated with ZRL formulation. During the course of an experiment, all animals were closely observed for apparent behavioral abnormalities.

**Carrageenan-induced Paw Edema**

Carrageenan (CA) induced rat paw edema was performed by the method of Winter et al.\textsuperscript{9} Edema was induced in the plantar region of the right hind paw of rats by subcutaneous injection of 0.1 mL of freshly prepared carrageenan (1%) aqueous suspension in normal saline. The test and standard groups of rats received topical application of ZRL formulation and diclofenac (Volini Gel) respectively. The control group received vehicle (distilled water). Paw edema was induced by injecting carrageenan 60 minutes after the topical application. Plethysmometer was used to measure the paw volume at 0 hour (Vo before carrageenan injection) and hourly intervals till 5th hour (Vt). The change between Vt and Vo was taken as the edema value. The inhibition percentage was calculated as per the formula:

\[
\text{Percentage of Inhibition} = \left(1 - \frac{V_t}{V_o}\right) \times 100
\]

**Tail Flick Method**

Groups 1 and 3 served as control, standard and ZRL treated respectively. After 1 hour, the tail tip up to 5 cm was dipped into hot water being maintained at 58°C. The response time was documented as the sudden withdrawal of the tail from the hot water. Across the groups, a cut off time of 10 seconds was maintained to avoid damage to the tail. The time taken for the tail flick was recorded to assess response to the stimulus.\textsuperscript{10}

**Thermally-induced Pain in Mice (Eddy’s Hot Plate Model)**

Mice were placed for 10 minutes each day gently on a plate maintained at 25 ± 1°C for two consecutive days. On 3rd day, each mouse was kept quiet on a hot plate at 55 ± 1°C for a maximum period of 25 seconds. The baseline data for licking of forepaws and shaking or jumping

**Table 1:** Composition of Ayurvedic liniment (ZRL) each 10 mL contains

<table>
<thead>
<tr>
<th>Name</th>
<th>Quantity (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maha mash taila</td>
<td>2.5</td>
</tr>
<tr>
<td>Vishgarbha taila</td>
<td>2.5</td>
</tr>
<tr>
<td>Narayan taila</td>
<td>2.5</td>
</tr>
<tr>
<td>Gandhapuro Taila</td>
<td>2.5</td>
</tr>
</tbody>
</table>
off the plate was recorded as a nociceptive response. The animals were grouped into three with five animals each. Groups 1 and 2 served as control and standard respectively. Group 3 was treated topically with ZRL formulation, respectively. Control mice were treated with normal saline. After 30 minutes, the latency time in each mouse was noted to determine the analgesic response. Complete analgesia reflected a latency of 25 second. The mean latency of nociceptive responses for each treatment group was calculated. The percentage of analgesia was calculated as per the formula:

$$\text{Percent analgesia} = \frac{(\alpha - \bar{\alpha}) \times 100}{\alpha}$$

Where $\alpha$ denotes after treatment reaction time and $\bar{\alpha}$ as baseline recording.

Statistics
Data were given as mean ± standard deviation (SD). Dataset was analyzed using t-test where the control group was compared with the sample treated group. Significance was fixed at $p < 0.05$, $p < 0.01$, $p < 0.001$.

RESULTS

Anti-inflammatory Activity
There was a significant reduction in the paw volume thickness after 3 hours with ZRL treatment for control. The percentage of reduction of paw volume after ZRL treatment and standard Volini Gel were $49.18 \pm 2.92$ and $81.79 \pm 1.24$ respectively (Fig. 1).

Antinociceptive Activity

Tail Flick Test
After a latency period of one hour of ZRL topical application, there was a significant reduction to pain sensation. The anti-nociceptive property of the ZRL formulation was intense at 2 hours (Fig. 2).

DISCUSSION
Zandu Rhumasyl is prepared with four oils (Taila) i.e. Narayan taila, Vishgarbha taila, Mahamash taila and Gandhaphuro taila. Narayan taila, Mahamash taila and Vishgarbha taila are being referred in Ayurveda texts for management of Vataavyadhi (Neurological disorders). They have properties like Vedana sthapaka (analgesic), sosa (Wasting S) and sarvangagraham (stiffness and tightness in all limbs). Gandhaphuro taila having properties of Uttejak (Stimulant) and Vatahar, and is useful as vednasthapak (analgesic) and in Arthritis (Aamavata). These Tailas have also been scientifically studied to have potent analgesic and anti-inflammatory properties. Moreover, these oils...
are prepared by various combinations of herbs which also possess analgesic, anti-inflammatory, anodyne and emollient properties.\textsuperscript{5-8} They help in relieving pain and swelling and improve the mobility of joints, relax the muscles and improve the overall quality of life. Table 2 summarized the background of selection of four oils based on its Ayurvedic usefulness in the treatment of musculoskeletal disorders related to rheumatological and neurological origin. As per Ayurvedic text, tail alleviates \textit{Vata} and does not aggravate \textit{Kapha}. It promotes body strength and provides firmness of muscles. Taila assimilates the properties of other drugs added to it during the \textit{paka} of \textit{Sneha} without losing its properties (\textit{Charka Samhita Sutrasthan 13/15}).

Carrageenan-generated paw edema is a recognized animal study design for acute inflammation. This design provided a noteworthy measurement for assessment of anti-edematogenic compounds working through a mechanism by interfering with the inflammatory mediators.\textsuperscript{11} Carrageenan, an inflammatory agent, does not have any systemic activity.\textsuperscript{12} Moreover, numerous scientists have established that acute inflammation occurred due to carrageenan injection has biphasic effects. In the first phase, histamine and serotonin have been stimulated to release in the systemic circulation, the later phase of inflammation, prostaglandins mainly responsible for modulating the edema.\textsuperscript{13,14} Experimental evidence indicated that during tenderness the prostaglandins increases the inflammatory exudates. The treatment of NSAIDs significantly reduces this inflammatory condition.\textsuperscript{15} The series of evidence through various experiments revealed that ZRL formulation was effective in reducing this acute inflammation indicating its anti-inflammatory potential. However, the anti-inflammatory mechanisms of ZRL formulation are not yet established. Our observation suggested that ZRL formulation reduced the edema formation in the late phase showing the effectiveness of ZRL formulation possible inhibition of prostaglandins.

Analgesic activity of medicines occurs through several mechanisms. ZRL was studied in different mouse models for its anti-nociceptive property. The thermal method is considered for centrally acting analgesics, e.g., opioid-like substances, mainly to understand the centrally acting anti-nociceptive mechanism at spinal and supraspinal levels.\textsuperscript{16} The tail immersion/tail-flick response acts by spinal reflex and is considered as a specific screening tool for centrally acting analgesics.\textsuperscript{17} Our results showed that ZRL formulation considerably delays the reaction time in tail flick test. The results are consistent with the interpretation that its analgesic property might have a central origin. These results showed that ZRL possessed analgesic activity.

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|l|l|}
\hline
\textbf{Therapeutic area / Activity} & \textbf{Gandhapuro} & \textbf{Narayan Tail} & \textbf{Mahamash Tail} & \textbf{Vishgarbha Tail} \\
\hline
\textbf{Stimulant} & Uttejak & -- & -- & -- \\
\hline
\textbf{Neurological Disorder (Vatahar/ Vatadosahar)} & Vatahar/ Vatadosahar & -- & -- & -- \\
\textbf{Analgesic} & Vedanastapak & -- & -- & -- \\
\textbf{Rheumatological disorder} & Amavata, Vata- rakta (Gout) & -- & -- & -- \\
\textbf{Neuralgia} & Gridhasi (Sciatica), Nadishula (Acute pain of nervine origin) & -- & Gridhasi (Sciatica), Avabahuk (Brachialgia), Visvachi (Brachial Neuralgia) & -- \\
\textbf{Paralysis} & -- & Pangu (Paraplagia)/ Pakshaghath (Paralysis) & Ardita (Facial Paralysis) & Pangu (Paraplagia)/ Pakshaghath (Paralysis) \\
\textbf{Muscle wasting} & -- & Ekang Sosha (Wasting of Limb) & Bahu Sosha (Muscular wasting of forearm) & -- \\
\textbf{Muscle stiffness} & Manya stambha (Neck R rigidity), Hanu stambha (Lock Jaw) & Sarbanghara (Stiffness and tightness in all limbs), Katigraha (Stiffness of lumbosacral region), manyastambha (Neck rigidity), Hanu stambha (Lock Jaw) & -- & -- \\
\hline
\end{tabular}
\caption{Background of selection of four oils based on its Ayurvedic usefulness}
\end{table}
CONCLUSION

Ayurvedic liniment formulation possesses significant anti-inflammatory potential and analgesic activity. The present study provided a scientific justification for pain relief and management of inflammation. The Ayurvedic liniment may be useful in relief of musculoskeletal pain, inflammation, and stiffness.

CLINICAL SIGNIFICANCE

The present experimental evidences on the marketed ZRL formulation have reconfirmed the traditional claims of its known ingredients mentioned in the Ayurveda texts. The taila formulation has shown anti-inflammatory and analgesic activity and thereby authenticating its clinical use for management of pain associated with musculoskeletal and neurological disorders.

REFERENCES

हिन्दी सारांश
आयुर्वैदिक लेप औषधियोग की संक्रमण रोगी और पीड़ाहर गतिविधि

सारांश: इन अथवाने का जेतपथ शास्त्रीय औषधियोग का प्रत्यय पर आधारित आयुर्वैदिक लेप औषधियोग की संक्रमण रोगी और पीड़ाहर गतिविधि का मुख्यांकन करना था।

सामग्री और पद्धति: संक्रमक रोगी गतिविधि की जीवन करने के लिए कारागिन इंडियन पाव एडेना का प्रयोग किया गया। पीड़ाहर गतिविधि की जीवन होटेल पद्धति और टेल मिलक पद्धति से की गई। विषयानुसार प्रयुक्त आयुर्वैदिक लेप औषधियोग की प्रभावकारिता का मुख्यांकन नियंत्रण के साथ किया गया।

परिणाम: पांव अपवजन का जेआर सूट उपचार एवं मानक डिकोलामेस जेल के पश्चात बनी का प्रतिशत क्रमम: 49.18±2.12 और 81.79±1.24 था।

एक से होट प्लेट मॉडल मे, जेआर उपचार के 2 घंटे तक सम्पूर्ण उपचार के पश्चात दर्द से संबंधित अवस्था में मिश्रित क्रमम की तुलना मे महत्वपूर्ण बनी (66%) पाई गई। तेल मिलक सिफत, पूर्ण उपचार के साथ दर्द से संबंधित अवस्था मे 88% क्रमम पाई गई।

निष्कर्ष: तत्काल अथवा ने दर्द निवारण एवं संक्रमण अवस्था के प्रभाव में, अधीनस्थ एवं एंटी नॉसिलेदिव तथा गैर-संक्रमक गुणों के प्रति वैज्ञानिक पुष्टिकरण का प्रभावित किया है।

चिकित्सीय गतिविधि: चिकित्सक इन विकास ने मक्कलोस्केलेटल विकार से संबंधित पीड़ा और संक्रमण के प्रभाव में जेआर औषधियोग की संक्रमण रोगी और पीड़ाहर गतिविधि को हमला को दर्शाया। तेल औषधियोग का चिकित्सीय प्रभाव मक्कलोस्केलेटल और न्यूट्रोलाइजिक विकार से संबंधित पीड़ा के प्रभाव में किया गया है।

मुख्य शाखा: आयुर्वैदिक, संक्रमण रोगी, पीड़ाहर, तेल, तेल।