



# 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  $49.18 \pm 2.12$  and  $81.79 \pm 1.24$  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.

**Keyword:** Ayurveda, Anti-inflammatory, Analgesic, Liniment, Taila.

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## 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.<sup>1</sup> 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.<sup>2</sup> These shortcomings associated with NSAIDs can be avoided or lessened by replacing them with comparatively safer and effective plant-based drugs.<sup>3</sup> 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.<sup>4</sup>

Ayurvedic oils are extensively used for the treatment of joint diseases (*Vatavyadhi*, diseases essentially caused by *Vata*).<sup>5</sup> 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.<sup>6</sup> *Vishgarbha taila* is used in various conditions like sciatica, stiffness and tightness/stiffness in limbs.<sup>7</sup> *Narayan taila*

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is used since ages in various conditions such as neck rigidity/torticollis, lockjaw, muscle wasting.<sup>6</sup> *Gandhapuro Taila* is useful in conditions such as rheumatism, sciatica, acute pain of nervine origin.<sup>8</sup> 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 Rhumasyil 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 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.<sup>9</sup> 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 ( $V_0$  before carrageenan injection) and hourly intervals till 5th hour ( $V_t$ ). The change between  $V_t$  and  $V_0$  was taken as the edema value. The inhibition percentage was calculated as per the formula:

$$\text{Percentage of Inhibition} = [1 - (V_t/V_0)] \times 100$$

#### Tail Fick 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.<sup>10</sup>

#### 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

Name	Quantity (mL)
Maha mash taila	2.5
Vishgarbha taila	2.5
Narayan taila	2.5
Gandhapuro Taila	2.5

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.<sup>11</sup> The percentage of analgesia was calculated as per the formula:

$$\text{Percent analgesia} = (\alpha - \delta) \times 100 / \alpha$$

Where  $\alpha$  denotes after treatment reaction time and  $\delta$  as baseline recording.

### Statistics

Data were given as mean  $\pm$  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

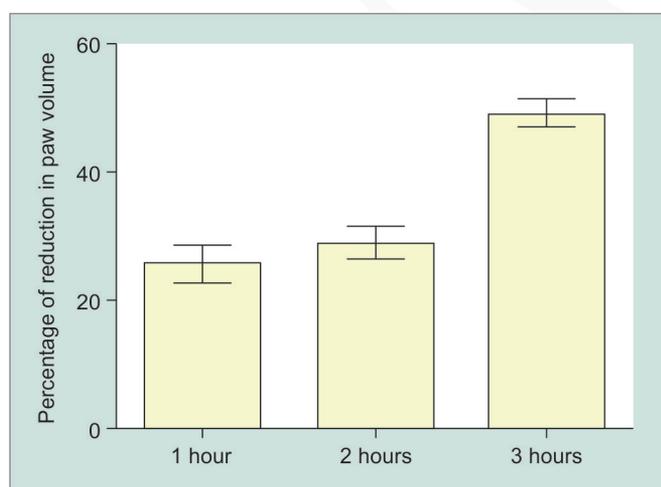
### Anti-inflammatory Activity

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).

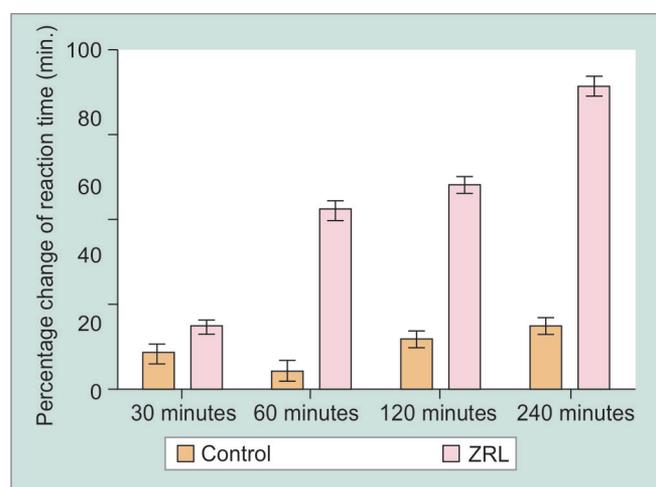
### Antinociceptive Activity

#### 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).



**Fig. 1:** Effect of ZRL on carageenan induced edema in wistar rats. Results are given as mean  $\pm$  SD of five animals

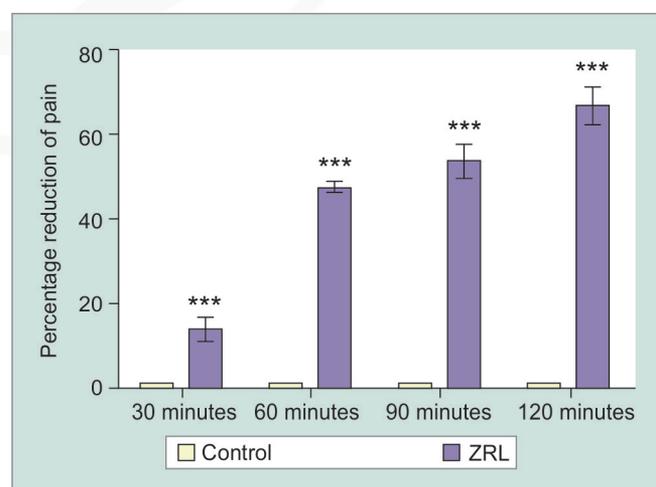


**Fig. 2:** Effect of ZRL formulation on tail flick response in wistar rats. Results are given as mean  $\pm$  SD of five animals in each group. Control group compared with rest of the treated group. Significance at  $***p < 0.001$

The results of the hotplate test revealed that the reaction time for the mice was significantly increased after treatment with ZRL (Fig 3).

### DISCUSSION

Zandu Rhumasyil is prepared with four oils (*Taila*) i.e. *Narayan taila*, *Vishgarbha taila*, *Mahamash taila* and *Gandhapuro taila*. *Narayan taila*, *Mahamash taila* and *Vishgarbha taila* are being referred in Ayurveda texts for management of *Vatavyadhi* (Neurological disorders). They have properties like *Vedana shapaka* (analgesic), *sosa* (Wasting s) and *sarvangagrahanam* (stiffness and tightness in all limbs). *Gandhapuro taila* having properties of *Uttejak* (Stimulant) and *Vatahar*, and is useful as *vednashapak* (analgesic) and in Arthritis (*Amavata*). These Tailas have also been scientifically studied to have potent analgesic and anti-inflammatory properties. Moreover, these oils



**Fig. 3:** Effect of ZRL formulation on Eddy's hot plate model in mice. Results are given as mean  $\pm$  SD of five animals in each group. Control group compared with rest of the treated group. Significance at  $***p < 0.001$

are prepared by various combinations of herbs which also possess analgesic, anti-inflammatory, anodyne and emollient properties.<sup>5-8</sup> 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 *Vata* and does not aggravate *Kapha*. It promotes body strength and provides firmness of muscles. Taila assimilates the properties of other drugs added to it during the *paka* of *Sneha* without losing its properties (*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.<sup>11</sup> Carrageenan, an inflammatory agent, does not have any systemic activity.<sup>12</sup> 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.<sup>13,14</sup> Experimental evidence indi-

cated that during tenderness the prostaglandins increases the inflammatory exudates. The treatment of NSAIDs significantly reduces this inflammatory condition.<sup>15</sup> 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.<sup>16</sup> The tail immersion/tail-flick response acts by spinal reflex and is considered as a specific screening tool for centrally acting analgesics.<sup>17</sup> 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.

**Table 2:** Background of selection of four oils based on its Ayurvedic usefulness

Therapeutic area / Activity	Gandhapuro <sup>8</sup>	Narayan Tail <sup>18</sup>	Mahamash Tail <sup>19</sup>	Vishgarbha Tail <sup>7</sup>
Stimulant	Uttejaka	--	--	--
Neurological Disorder (Vatahar/Vatadosahar)	Vatahar/Vatadosahar	Vatahar/Vatadosahar, Kampa (Tremor)	Vatadosahar, Kampa (Tremor)	Vatahar/Vatadosahar, Shirakampa (Terror of head)
Analgesic	Vedanastapak	--	--	--
Rheumatological disorder	Amavata, Vatarakta (Gout)	--	--	--
Neuralgia	Gridhasi (Sciatica), Nadishula (Acute pain of nerve origin)	--	Gridhasi (Sciatica), Avabahuk (Brachialgia), Visvachi (Brachial Neuralgia)	--
Paralysis	--	Pangu (Paraplegia)/ Pakshaghat (Paralysis)	Ardita (Facial Paralysis)	Pangu (Paraplegia)/ Pakshaghat (Paralysis)
Muscle wasting	--	Ekan Soshha (Wasting of Limb)	Bahu Soshha (Muscular wasting of forearm)	--
Muscle stiffness	--	Manya stambha (Neck Rigidity), Hanu stambha (Lock Jaw)	--	Sarbanghara (Stiffness and tightness in all limbs), Katigraha (Stiffness of lumbosacral region), manyastambha (Neck rigidity), Hanu stambha (Lock Jaw)

## 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.

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## हिन्दी सारांश

### आयुर्वेदिक लेप औषधयोग की संक्रमण रोधी और पीड़ाहर गतिविधि

**सारांश:** इन अध्ययनों का उद्देश्य शास्त्रीय औषधयोगों की प्रज्ञता पर आधारित आयुर्वेदिक लेप औषधयोग की संक्रमण रोधी और पीड़ाहर गतिविधि का मूल्यांकन करना था।

**सामग्री और पद्धति:** संक्रामक रोधी गतिविधि की जाँच करने के लिए कारागिन्न इंड्यूज्ड पाव एडेमा का प्रयोग किया गया। पीड़ाहर गतिविधि की जाँच हॉट प्लेट पद्धति और टेल फिलक पद्धति से की गई। विषयानुसार प्रयुक्त आयुर्वेदिक लेप औषधयोग की प्रभावकारिता का मूल्यांकन नियंत्रण के साथ किया गया।

**परिणाम:** पॉव आयतन का जेआरएल उपचार एवं मानक डिकोलफेनेक जेल के पश्चात कमी का प्रतिशत क्रमशः  $49.18 \pm 2.12$  और  $81.79 \pm 1.24$  था। एडी के हॉट प्लेट मॉडल में, जेआरएल योग के 2 घंटे तक समुचित उपयोग के पश्चात दर्द से संबंधित अवस्था में नियंत्रण की तुलना में महत्वपूर्ण कमी (66%) पाई गई। तेल फिलक विधि में, पूर्व उपचार के साथ दर्द से संबंधित अवस्था में 89% कमी पाई गई।

**निष्कर्ष:** वर्तमान अध्ययन ने दर्द निवारण एवं संक्रमण अवस्था के प्रबंधन में, अधीनस्थ एवं एंटी नोसिसेप्टिव तथा गैर-संक्रामक गुणों के प्रति वैज्ञानिक पुष्टिकरण को प्रमाणित किया है।

**चिकित्सीय महत्व:** वर्तमान इन विवो ने मस्कुलोस्केलेटल विकार से संबंधित पीड़ा और संक्रमण के प्रबंधन में जेआरएल औषधयोग की संक्रमण रोधी और पीड़ाहर गतिविधि की क्षमता को दर्शाया। तैल औषधयोग का चिकित्सीय प्रयोग मस्कुलोस्केलेटल और न्यूरोलाजिकल विकार से संबंधित पीड़ा के प्रबंधन में किया गया है।

**मुख्य शब्द:** आयुर्वेद, संक्रमण रोधी, पीड़ाहार, लेप, तैल।

