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RESEARCH ARTICLE

Anti-inflammatory and Analgesic Activity of an Ayurvedic Liniment (ZRL) 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 ayurvedic liniment (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 h 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 pre-treatment.

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, *Taila*, Liniment, Anti-inflammatory, Analgesic

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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.[1] 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.[2] These shortcomings associated with NSAIDs can be avoided or lessened by replacing them with comparatively safer and effective plant-based drugs.[3] 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.[4]

Ayurvedic oils are extensively used for the treatment of joint diseases (*Vatavyadhi*, diseases essentially caused by *Vata*.[5] 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.[6] *Vishgarbha Taila* is used in various conditions like sciatica, stiffness and tightness/stiffness in limbs.[7] *Narayan Taila* is used since ages in various conditions such as neck rigidity/torticollis, lockjaw, muscle wasting.[6] *Gandhapuro*

Taila is useful in conditions such as rheumatism, sciatica, acute pain of nervine origin.[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 (ZRL)

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 (200–250 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 purpos e 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) w as 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. (1962).[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:

Percentage of Inhibition= [1-(Vt/Vo)] ×100

Tail flick method

Groups 1 and 3 and 3 served as control, standard and ZRL treated respectively. After one 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.[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 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 min, the latency time in each mouse was noted to determine the analgesic response. Complete analgesia reflected a latency of 25 sec. The mean latency of nociceptive responses for each treatment group was calculated.[11] The percentage of analgesia was calculated as per the formula:

Percent analgesia = (α∂)×100/α

Where αdenotes after treatment reaction time and ∂ as baseline recording.

Statistics

Data are 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 ± 2.92 and 81.79 ± 1.24, respectively (Fig. 1).

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

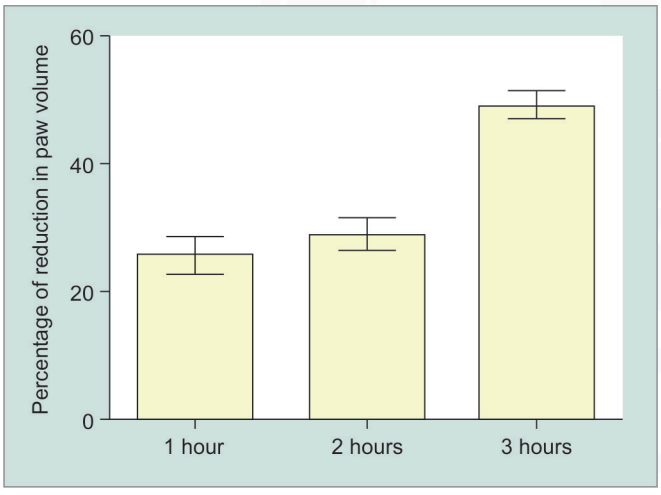

Fig. 1: Effect of ZRL on carageenan induced edema in wistar rats. Results are given as mean ± SD of five animals.

Antinociceptive activity

Tail Flick Test

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

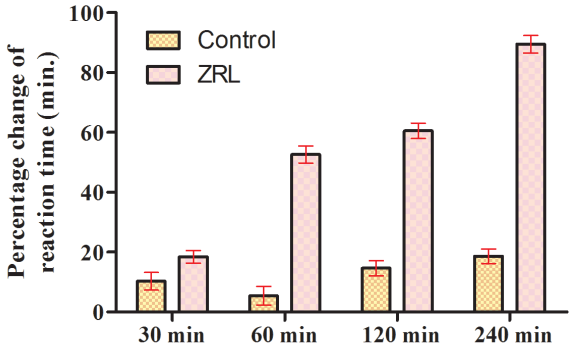

Fig. 2: Effect of ZRL formulation on tail flick response in wistar rats. Results are given as mean ± SD of five animals in each group. Control group compared with rest of the treated group. Significance at ***P < 0.001

Eddy's Hot Plate Test

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

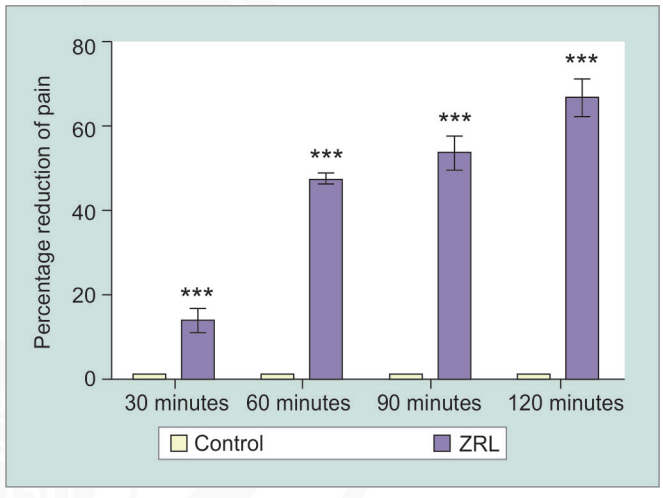

Fig. 3: Effect of ZRL formulation on Eddy's hot plate model in mice. Results are given as mean ± SD of five animals in each group. Control group compared with rest of the treated group. Significance at ***p < 0.001.

DISCUSSION

Zandu Rhumasyl 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 sthapaka* (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 *vednasthapak* (analgesic) and in Arthritis (Amavata). 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. [5-8] They help in relieving pain and swelling and improve the mobility of joins, relax the muscles and improve the overall Quality of life. Table 2 summarized the background of selection of four oils bas ed 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 mediato rs.[11] Carrageenan, an inflammatory agent, does not have any systemic activity.[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.[13,14] Experimental evidence indicated that during tenderness the prostaglandins increases the inflammatory exudates. The treatment of NSAIDs significantly reduces this inflammatory condition.[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.[16] The tail immersion/tail-flick response acts by spinal reflex and is considered as a specific screening tool for centrally acting analgesics.[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.

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

Therapeutic area / Activity	*Gandhapuro*[8]	*Narayan Tail*[18]	*Mahamash Tail*[19]	*Vishgarbha Tail*[7]
Stimulant	Uttejak	--	--	--
Neurological Disorder (Vatahar/ Vatadosahar)	Vatahar/ Vatadosahar	Vatahar/ Vatadosahar, Kampa (Tremor)	Vatadosahar, Kampa (Tremor)	Vatahar/Vatadosahar, Shirakampa (Termor of head)
Analgesic	Vedanastapak	--	--	--
Rheumatological disorder	Amavata, Vata-Rakta (Gout)	--	--	--
Neuralgia	Gridhasi (Sciatica), Nadishula (Acute pain of nervine origin)	--	Gridhasi (Sciatica), Avabahuk (Brachialgia), Visvachi (Brachial Neuralgia)	--
Paralysis	--	Pangu (Paraplagia)/ Pakshaghat (Paralysis)	Ardita (Facial Paralysis)	Pangu (Paraplagia)/ Pakshaghat (Paralysis)
Muscle Wasting	--	Ekang Sosha (Wasting of Limb)	Bahu Sosha (Muscular wasting of forearm)	--
Muscle Stiffness		Manya stambha (Neck Rigidity), Hanu stambha (Lock Jaw)		Sarbanghara (Stiffness and tightness in all limbs), Katigraha (Stiffness of lumbosaccral 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 Ayurvedic Liniment (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 musculo-skeletal and neurological disorders.

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