

Original Article

EVALUATION OF ANTI-INFLAMMATORY (*IN VIVO*) ACTIVITY OF ARIFLEX LINIMENT IN COMPARISON WITH DICLOFENAC GEL IN CARRAGEENAN INDUCED RAT PAW EDEMA MODEL

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ABSTRACT

Objective: The present study was conducted to evaluate anti-inflammatory activity of Ariflex liniment (conceptualized and developed by Ari Healthcare Pvt. Ltd) in comparison with Diclofenac gel in carrageenan induced rat paw edema model.

Methods: Wistar rats of either sex weighing 150-180 g were taken and divided into 3 groups with 6 animals in each group i.e. Group 1 (Controlled Group), Group 2 (Diclofenac gel) and Group 3 (Ariflex liniment). The study drugs were topically applied 30 min prior to carrageenan injection. After 30 min 1% w/v of 0.05 ml carrageenan was injected subcutaneously in the paw. The paw was marked with ink at the level of lateral malleolus and immersed in mercury up to the lateral malleolus mark. The paw volume was measured plethysmographically, immediately after injection i.e. on 0 min, and then on 30 min, 1h, 2h, 3h, 4h and 5hr after injection.

Results: Diclofenac gel sodium treated group showed significant inhibition ($p < 0.01$) of paw edema at 30 min, 1, 2, 3, 4 and 5th hrs as compared to control group. Ariflex Liniment showed significant inhibition ($p < 0.05$) of paw edema at 30 min, 1, 2, 3, and 4th hrs as compared to the control group. Group treated with Ariflex Liniment did not show any significant decrease in paw edema volume at 5th hrs when compared to the control group.

Conclusion: Ariflex Liniment possesses anti-inflammatory activity.

Keywords: Ariflex liniment, Diclofenac gel, Anti-inflammatory activity, Carrageenan induced rat paw edema model

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INTRODUCTION

Rheumatoid arthritis is one of the common autoimmune diseases. It is a chronic progressive inflammatory disorder affecting the synovial joints and typically producing symmetrical arthritis. If the inflammation goes unchecked it can damage bones and cartilage of the joint leading to deformity and disability. Several treatments are available for RA including prolonged use of NSAIDs (Non-steroidal anti-inflammatory Drugs), DMARDs (Disease-Modifying anti-rheumatic drugs), TNF inhibitors (tumor Necrosis factor inhibitors) and joint surgeries in severe cases. Though these medications are better treatment options for RA management, the side effects of these medications such as osteoporosis, gastric ulcers, nephrotoxicity etc. make them unsuitable for long-term use. And people tend to search for alternative medicinal drugs for the management of pain and inflammation of joints [1].

OA is the most common form of arthritis and is estimated to be the eighth leading cause of disability in the world [2]. It is more common in women than men. In India, OA is the most frequent joint disease with a prevalence of 17% to 60.6% (Sharma MK *et al.* 2007) [3]. Also, it is second most common rheumatological problem in India [4].

Osteoarthritis (OA) is a chronic degenerative joint disease characterized by loss of or injury to articular cartilage, sub-chondral thickening, hypertrophy of bone and alterations of the synovial membrane and joint capsule [4]. In OA, bone rubbing causes pain, swelling and restricted range of motion at the affected joint. The joint may also lose its normal shape. In the normal adult, articular cartilage consists of a delicate system of cells and matrix proteins, which have the function of creating a viscoelastic tissue with high biomechanical stability and low friction. Articular cartilage remains stable, if the process of degeneration and regeneration of cells and matrix proteins occurs in equilibrium. Chondrocytes are the cartilage cells, which produce and maintain the cartilaginous matrix, which consists mainly of collagen and proteoglycans. The alteration of chondrocyte transplantation and degeneration of cartilage due to various triggering factors causes OA [5].

Presently very few underlying factors are known to cause OA. But, some common factors such as age, sex, obesity, genetics, bone density, smoking, local factors including trauma are main contributors in the pathogenesis of OA. OA with no known cause is termed as primary OA and it is mostly related to aging. Secondary OA results as a result of another disease or condition. The above-mentioned factors initiate alterations in the equilibrium of cartilage formation and enhance degenerative cascade thus cause OA [4, 5].

Generally, OA is managed by symptomatic treatment methods such as use of pain and inflammation medications. NSAIDs (Diclofenac, Aceclofenac), and acetaminophen are considered to be the first-line therapy in the management of OA. Selective COX II inhibitors are widely recommended by virtue of their efficacy and relatively lower adverse effects. Also, the symptomatic slow-acting drugs for OA (SYSADOA) such as diacerein, hyaluronic acid (HA), chondroitin sulfate are useful in OA management [6]. In OA; intra-articular corticosteroid injections are believed to be most effective in patients with evidence of inflammation, effusion, or both. Various other therapies such as transcutaneous nerve stimulation, thermal modalities, acupuncture, and surgery (including joint replacement) have also been used to treat OA. Currently, though pharmacological, mechanical and surgical interventions are used, there is no known cure for OA. Also, above mentioned treatment options lead to many side effects and drawbacks. Thus, physicians and patients tend to move towards the use of alternative treatment methods [6-8].

In the view of this situation, Ari Healthcare Pvt. Ltd. has conceptualized and developed Ariflex Liniment. It is a polyherbal formulation used topically for the effective management of Arthritis. Maha Vishagarbha Taila (*Ayurvedic Classical Formulation*) [9], Gandhapura Taila (*Gaultheria fragrantissima*) [10], Tailaparnah Taila (*Eucalyptus globulus*) [11], Katuveera Arka (*Capsicum annum*) [12], Menthol (*Mentha arvensis*) [13], Karpooa (*Borneo camphor*) [14] and Linseed Oil (*Linum usitatissimum*) [15] are used in Ariflex Liniment. It is recommended for use in Joint pain and inflammation, Muscle strain and Muscle sprain.

The present study was conducted to evaluate anti-inflammatory (*in vivo*) potential of Ariflex Liniment in comparison with Diclofenac gel in carrageenan induced rat paw edema.

MATERIALS AND METHODS

Study site

The study was conducted at Padmashree Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune-411018. The material used for the study is given in table 1.

Ethical consideration and approval

Institutional Animal Ethics Committee (IAEC) has approved the study in the meeting held on 31st Dec 2014. The protocol no. approved was DYPIPSR/IAEC/14-15/P-03.

Anti-inflammatory activity of Ariflex liniment against carrageenan induced paw edema in rats

Wistar rats of either sex weighing 150-180 g were taken and divided into 3 groups with 6 animals in each group. Group 1 (Control Group) animals were starved overnight. Group 2 animals were topically applied with Diclofenac gel as Standard drug and Group 3 Animals were topically applied with Ariflex liniment as test drug. The test and standard drugs were topically applied 30 min prior to carrageenan injection. After 30 min 1% w/v of 0.05 ml carrageenan was injected subcutaneously in the paw. The paw was marked with ink at the level of lateral malleolus and immersed in mercury up to lateral malleolus mark. The paw volume was measured plethysmographically, immediately after injection i.e. on 0 min, and then on 30 min, 1h, 2h, 3h, 4h and 5hr after injection.

Table 1: Materials used for the study

Material	Make
Carrageenan	Irish moss
Diclofenac Gel	Novartis
Ariflex liniment (AHPL/AYTOP/0314)	Ari Healthcare Pvt. Ltd., Pune

Table 2: Animals used for the study

S. No.	Species	Age in weeks	Weight/Size in gm	Gender	Numbers to be used
1	Wister Rat	6-8	150-180	Male	40

RESULTS

The development of the edema in the rats after injection of Carrageenan has been described as biphasic event. In the first phase histamine, serotonin and kinins are released and in second phase mostly prostaglandins are released. Diclofenac gel sodium (Standard) treated

group showed significant inhibition ($p < 0.01$) of paw edema at 30 min, 1, 2, 3, 4 and 5th h as compared to control group. Groups treated with Ariflex Liniment showed significant inhibition ($p < 0.05$) of paw edema at 30 min, 1, 2, 3, and 4th hr as compared to control group. Groups treated with Ariflex Liniment did not show any significant decrease in paw edema volume at 5th h when compared to control group.

Table 3: Anti-inflammatory activity of Ariflex Liniment using carrageenan induced rat paw edema model

Group	Paw edema volume (ml) (mean±SEM)						
	0 min	30 min	1 h	2 h	3 h	4h	5h
Control	3.08±0.19	1.62±0.05	2.22±0.24	2.92±0.26	3.32±0.25	2.48±0.25	2.28±0.2
STD (Diclofenac Gel)	2.35±0.05	0.578±0.11**	0.87±0.26**	1.108±0.14**	1.064±0.29**	0.6±0.29**	1.11±0.39**
Ariflex Liniment	2.56±0.12	1.22±0.23*	1.46±0.07*	1.36±0.07*	2.54±0.08*	1.56±0.06*	1.6±0.11 ^{ns}

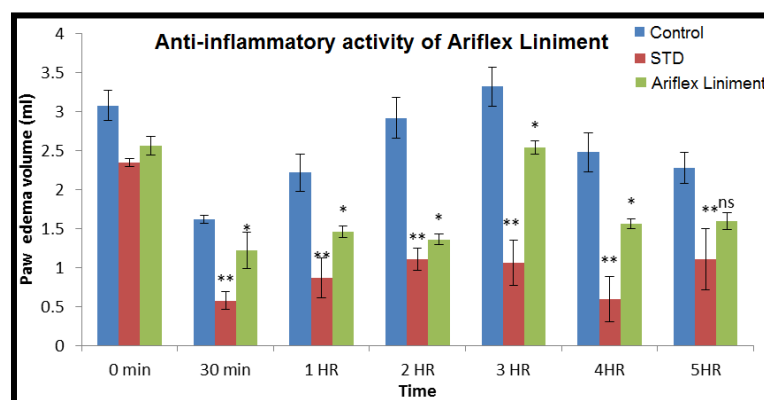


Fig. 1: Anti-inflammatory activity of Ariflex Liniment with standard and control, Data is expressed as mean±SEM of n=5 observations. All treatments were compared with control (ANOVA followed by Dunnett's test) ** $p < 0.01$, * $p < 0.05$

DISCUSSION

Osteoarthritis (OA) is a chronic degenerative joint disease characterized by loss of or injury to articular cartilage, sub-chondral thickening, hypertrophy of bone and alterations of the synovial membrane and joint capsule.

Various NSAIDs, analgesics, paracetamol, COX-2 inhibitors that reduce pain and inflammation in OA and other drugs like Chondroitin sulfate, diacerein, hyaluronic acid are generally used for the treatment of OA. Intra-articular steroid injection provides excellent symptomatic relief but fails to provide permanent relief. Various other therapies such as transcutaneous nerve stimulation,

thermal modalities, acupuncture, and surgery (including joint replacement) have also been used to treat OA. Though pharmacological, mechanical and surgical interventions are used, there is no known cure for OA. Also, above mentioned treatment options lead to many side effects and drawbacks like nephrotoxicity with Diclofenac and hepatotoxicity with paracetamol, osteoporosis with corticosteroids on their long term irrational use. Hence patients tend to search safer and effective alternative therapeutic options [1].

Keeping in mind the need of the time to have drugs with minimum side effects, Ari Healthcare Pvt. Ltd. has conceptualized and developed Ariflex Liniment for the treatment of Arthritis.

In the present study evaluation of anti-inflammatory activity (*in vivo*) of Ariflex liniment in comparison with Diclofenac Gel in carrageenan induced rat paw edema model was done. It was observed from the results of the present study that Ariflex liniment possesses anti-inflammatory activity in rat paw edema model. The action of Ariflex liniment started immediately. At the end of 30 min inflammation was reduced by almost 53% compared to 75% of diclofenac gel. Anti-inflammatory effect of the Ariflex liniment sustained till the end of 5 h. The anti-inflammatory activity of Ariflex liniment was found to be close to that of Diclofenac gel used as standard.

Ariflex Liniment contains Maha Vishagarbha Taila (*Ayurvedic Classical Formulation*) [9], Gandhapura Taila (*Gaultheria fragrantissima*) [10], Tailaparnah Taila (*Eucalyptus globulus*) [11], Katuveera Arka (*Capsicum annum*) [12], Menthol (*Mentha arvensis*) [13], Karpoora (*Borneo camphor*) [14] and Linseed Oil as base (*Linum usitatissimum*) [15].

Maha Vishagarbha Taila is medicated oil composed of more than 50 different herbs. In Ayurveda, its use is recommended in various *Vatavyadhis* (musculoskeletal disorders). Its topical application over affected area is said to relieve the stiffness (*Sarvangagraha*) [9].

Gandhapura Taila (*Gaultheria fragrantissima*) is widely used topically in the management of various types of arthritis [16]. Methyl salicylate and its glycoside derivatives obtained from this species are reported to possess remarkable anti-inflammatory and analgesic properties [10]. Gandhapura Taila is standardized to 95% methyl salicylate.

Tailaparnah Taila (*Eucalyptus globulus*) is a volatile oil obtained from *Eucalyptus globulus*, traditionally used in the treatment of upper respiratory tract infections, wounds, fever and rheumatism. Anti-inflammatory and analgesic activities of Eucalyptus oil are reported in various studies [11].

Katuveera Arka is obtained from *Capsicum annum*. The active ingredient, Capsaicin is supposed to be responsible for its pharmacological activities. Capsaicin binds to specific receptors/nociceptors in the skin, which initially causes a state of neuronal excitation and a period of increased local sensitivity. This results in burning sensation, stinging, and itching associated with cutaneous vasodilation. These manifestations are attributed to the stimulation of afferent C-fibers and the release of substance P. Soon after, a refractory period occurs with reduced sensitivity. Depletion of substance P and degeneration of peripheral nerve fibres occurs on repeated application and reduced sensitivity persists. Its effectiveness in treating pain associated with Osteoarthritis and Rheumatoid arthritis is documented [17].

Menthol is obtained from the oil of plant *Mentha arvensis*. Methanolic extracts of this plant are reported to possess significant anti-inflammatory and anti-arthritis activities in experimental studies [18].

Menthol is a topical vasodilator that enhances the absorption of other topical skin medications. It is said to stimulate the cold receptors on the skin and dilate the blood vessels causing cooling and analgesic effects. Menthol in low concentration causes cooling sensation and the same when applied topically in high concentration causes local anesthesia and pain relief. It is also reported to reduce histamine induced irritation and itching in some studies [19].

Karpoora is obtained from the plant *Borneo camphora*. Camphor is a known agonist of TRPV2, TRPA1 and TRPV1 quickly deactivating TRP channels. This results in long-term pain relief. It is a relatively

weak sensory irritant having a modest excitatory effect on thermo-sensitive (and perhaps nociceptive) cutaneous fibers [14].

Studies have reported that combination of menthol with camphor enhances skin penetration of Methyl salicylate and inhibits both *in vivo* and *in vitro* synthesis of Methyl salicylate to salicylic acid [14].

Linseed oil is obtained from dried ripe seeds of *Linum usitatissimum*. It is a rich source of linolenic acid which is proved to possess noticeable anti-inflammatory activity. Anti-inflammatory, analgesic and anti-oxidant properties of this edible oil are confirmed in various studies. Its topical use has been approved in various dermatological conditions [15].

It is evident from the above discussion that almost all ingredients of Ariflex liniment possess anti-inflammatory activity hence it can be concluded that anti-inflammatory effect of Ariflex liniment could be the synergistic action of these herbs.

CONCLUSION

It can be concluded that Ariflex liniment possess significant anti-inflammatory effect which is close to that of Diclofenac gel. Thus Ariflex liniment can be used as an effective topical anti-inflammatory agent in various types of arthritis, joint pain and inflammation, muscle strain and muscle sprain.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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