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Original Research Article

Evaluation of in vivo anti-inflammatory activity of Ariflex tablet in comparison with diclofenac and aceclofenac tablet in carrageenan induced rat paw edema model

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ABSTRACT

Background: Osteoarthritis is a major cause of pain and locomotor disability worldwide. Though various pharmacological, mechanical and surgical interventions are used, there is no known cure for OA. The present study was conducted to evaluate anti-inflammatory activity of Ariflex tablet (conceptualized and developed by Ari Healthcare Pvt. Ltd.) in comparison with diclofenac and aceclofenac tablet in carrageenan induced rat paw edema model.
Methods: Wistar rats of either sex weighing 150-180 g were taken and divided into 4 groups with 6 animals in each group i.e. group 1 (control group), group 2 (diclofenac tablet), group 3 (aceclofenac tablet) and group 4 (ariflex tablet). The study drugs were orally administered with feeding needle, 30 minutes prior to carrageenan injection. After 30 min 1% w/v of 0.05 ml carrageenan was injected subcutaneously in the rat 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 after injection at 30 minutes, 1 hour, 2 hour, 3 hour, 4 hour and eventually at 5 hour.
Results: All the test formulations possess statistically significant ($p<0.05$) anti-inflammatory activity as compared to control group. The maximum percentage inhibition for Ariflex tablet was 96.97% at the end of 5 hours. When compared to control group, statistically significant reduction of paw edema was observed. The anti-inflammatory activity of Ariflex tablet from 2 hours onwards is comparable to that of diclofenac tablet and aceclofenac tablet.
Conclusions: Ariflex tablet possesses significant anti-inflammatory activity.

Keywords: Ariflex tablet, Diclofenac tablet and aceclofenac tablet, Anti-inflammatory, Carrageenan induced rat paw edema model

INTRODUCTION

Osteoarthritis (OA) is made up from two words such as, ‘osteon’ meaning bone and ‘arthron’ meaning joint with the suffix‘-itis’ for inflammation. OA is the most common form of arthritis. It is more common in women than men. OA is estimated to be the eighth leading cause of disability in world.[1,2] In India, OA is second most common rheumatological problem with prevalence rate of 17% to 60.6% among the joint disorders.[3] 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.[3] 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 cartilage cells, which produce and maintain cartilaginous matrix. Cartilaginous matrix consists of mainly of collagen and proteoglycans. Alteration of chondrocyte transplantation and degeneration of cartilage due to various triggering factors causes OA.[4]

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. It is mostly related to aging. Secondary OA results subsequent to another disease or condition. The above-mentioned factors initiate alterations in the equilibrium of cartilage formation and enhance degenerative cascade thus cause OA.[3,4] Generally, OA is managed by symptomatic treatment methods such as use of pain killer and anti-inflammatory medications. Acetaminophen is considered to be the first line therapy in the management of OA. NSAIDs (selective and non-selective COX-2 inhibitors) are also commonly used for OA. Also, the symptomatic slow acting drugs for OA (SYSADOA) such as diacerein, hyaluronic acid (HA), chondroitin sulfate are useful in OA management.[5] 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 on long term usage. Thus, physicians and patients tend to move towards the use of alternative treatment methods.[5-7]

Sandhigata vata described under vata vyadhi in ayurveda can be correlated to osteoarthritis. According to ayurveda, sandhi means joints and vata has been considered the most important dosha (humor) among the three doshas. Thus, sandhigata vata means vitiated vata residing at sandhi. In sandhigata vata, vitiated vata in joints causes severe pain, dryness and obstructed joint movements. In ayurveda, various local as well as oral treatment modalities have been used in the management of sandhigata vata. Various types of hot fomentations have been advocated as effective treatment measures. Local therapy includes massage with medicated oils such as maha narayan taila, narayan taila, etc. followed by hot fomentation, whereas the oral therapy mainly includes the use of rasnadi guggulu, Maha Yograj guggulu, trayodashanga guggulu, etc. Several plants such as shallaki, ashvagandha, guggulu, rasna, nirgundi, eranda, guduchi, shunthi, etc. are also effectively used to treat OA.[8-10]

Keeping in mind the basic concept of ayurveda, Ari Healthcare Pvt. Ltd., has developed Ariflex tablet for effective management of various types of arthritis. Ariflex tablet contains shallaki extract (*Boswellia serrata*), guggulu extract (*Commiphora mukul*), rasna extract (*Pluchea lanceolata*), ashvagandha extract (*Withania somnifera*), nirgundi extract (*Vitex negundo*), guduchi extract (*Tinospora cordifolia*), eranda extract (*Ricinus communis*) and shunthi extract (*Zingiber officinale*).[11-18]

Aim

In the present study, an attempt has been made to evaluate *in vivo* anti-inflammatory activity of Ariflex tablet in comparison with diclofenac sodium and aceclofenac tablets by using carrageenan induced rat paw edema model.

METHODS

This was a prospective *in-vivo* (animal) study conducted at Padmashree Dr. D. Y. Patil institute of pharmaceutical sciences and research, Pimpri, Pune, Maharashtra. The study was conducted over a period of three months (January 2015 to March 2015). Male Wistar rats of 6-8 weeks age and having weight between 150-180 g were used in the study. The details are given in (Table 1).

Table 1: Animals used for the study.

Species	Age (weeks)	Weight/size (gm)	Gender	N
Wistar rat	6-8	150-180	Male	40

All animals were obtained from National institute of biosciences (NIB) Pune. Animals were housed in standard laboratory conditions of temperature and 12 hours light and 12 hours dark cycle with free access to standard pellet diet and water. All laboratory animal handling and experimental procedures were performed in accordance with the CPCSEA guidelines (198/99/CPCSEA) and study protocol. The brief of study material used for the study is given in (Table 2).

Table 2: Materials used for the study.

Material	Make
Carrageenan	Irish moss
Diclofenac tablet	Novartis
Aceclofenac tablet	Novartis
Ariflex tablet	Ari Healthcare Pvt. Ltd. Pune

Study drugs used in the study were diclofenac sodium tablet, aceclofenac tablet and Ariflex tablet. Drugs including diclofenac sodium and aceclofenac were purchased from market; whereas Ariflex tablet was

supplied by Ari Healthcare Pvt. Ltd. Pune. The composition of Ariflex tablet is mentioned in (Table 3).

Table 3: Composition of ariflex tablet; contents of each film coated tablet.

Ingredients	Botanical name	Quantity (mg)
Shallaki extract	*Boswellia serrata*	110
Guggulu extract	*Commiphora mukul*	100
Rasna extract	*Pluchea lanceolata*	65
Ashwagandha extract	*Withania somnifera*	65
Nirgundi extract	*Vitex negundo*	60
Guduchi extract	*Tinospora cordifolia*	55
Eranda extract	*Ricinus communis*	50
Shunthi extract	*Zingiber officinale*	20

During the study procedure, Wistar rats of either sex weighing 150-180 g were taken and divided into 4 groups with 6 animals in each group. Group 1 (control group) animals were starved overnight. Group 2 animals were orally administered with diclofenac tablet as standard drug. Group 3 animals were orally administered with aceclofenac tablet as standard drug and group 4 animals were orally administered with Ariflex tablet as test drug. The test and standard drugs were orally administered with feeding needle, 30 minutes prior to carrageenan injection. After 30 min 1% w/v of 0.05 ml carrageenan was injected subcutaneously in the rat 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 after injection at 30 minutes, 1 hour, 2 hour, 3 hour, 4hour and eventually at 5 hour. All the results were carefully recorded in Microsoft excel 2010 and then statistical analysis of data was carried out using the statistical package for social sciences (SPSS) software. One-way ANOVA test followed by Dunnett's test were used to compare the effect of drugs on different groups.

RESULTS

Anti-inflammatory activity of Ariflex tablet assessed using Carrageenan induced rat paw edema model

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. Till the end of 5 hours, no reduction in edema was observed in control group.

Diclofenac sodium and aceclofenac (standard) treated group showed significant inhibition ($p<0.01$) of paw edema at 30 min, 1, 2, 3, 4 and 5 hours. The action of both the drugs started at 30 minutes and lasted till 5hours. The maximum percentage inhibition of rat paw edema for diclofenac group was 96.39% at 5 hours, whereas the maximum percentage inhibition of rat paw edema for aceclofenac group was 97.38% at 5 hours.When compared to control group, statistically significant ($p<0.05$) reduction of paw edema was observed in diclofenac and aceclofenac groups.

In the group treated with Ariflex tablet, increase in rat paw edema was observed at the end of 30 minutes. The change was statistically insignificant ($p>0.05$). At the end of 1 hour, slight reduction in paw edema was observed when compared to 30 minutes reading, but when compared to 0 minute reading, no change in paw edema was observed. Statistically significant inhibition ($p<0.01$) of paw edema was observed at 2, 3, 4 and 5 hours. The maximum percentage inhibition was 96.97% at the end of 5 hours. When compared to control group, statistically significant reduction ($p<0.01$) of paw edema was observed. The details are given in (Table 4 and Figure 1).

DISCUSSION

The present study was conducted to evaluate in vivo anti-inflammatory activity of Ariflex tablet in comparison with diclofenac tablet and aceclofenac tablet in carrageenan induced rat paw edema model.[19-34] It was observed that there is a significant correlation between the efficacy of test drugs in animals models and clinical effectiveness of the ingredients of drugs. From the results of the study, it was observed that action of diclofenac and aceclofenac started immediately after administration, which was evident from significant reduction in rat paw edema at the end of 30 minutes. The statistically significant reduction in rat paw edema in diclofenac and aceclofenac group continued till the end of 5 hours. On the contrary, Ariflex tablet was not able to reduce rat paw edema till the end of 2 hours. Since Ariflex tablet is polyherbal formulation, it might have taken time to reach the targeted site i.e. rat paw edema. But at the end of 2 hours, statistically significant reduction in rat paw edema was observed. The statistically significant reduction in rat paw edema was continued till the end of 5 hours. The anti-inflammatory activity of Ariflex tablet from 2 hours onwards was comparable to that of diclofenac and aceclofenac.

Various ingredients of ariflex tablet such as ashvagandha (*Withania somnifera*), eranda (*Ricinus communis*), guduchi (*Tinospora cordifolia*), shunthi (*Zingiber officinale*), shallaki (*Boswellia serrata*), rasna (*Pluchea lanceolata*), nirgundi (*Vitex negundo*), guggulu (*Commiphora mukul*) inhibit COX enzyme and thereby reduce inflammation. Ingredient such as shallaki (*Boswellia serrata*) work on lypooxygenase pathway and inhibit production of leukotrienes.[12-18,23-26] Guggulsterone, which is a marker compound found in (guggulu) commiphora mukul inhibits NF-kB activation

and decreases the expression of inflammatory cascade in arthritis.[27,28] *P. lanceolata* probably decreases the COX and TNF-alpha enzymes production thus exerts anti-inflammatory activity.[29]

Table 4: Carrageenan induced rat paw edema volume.

Group	Paw edema volume (ml) (Mean±SEM)						
	0 min	**30 min**	**1 hr**	**2 hr**	**3 hr**	**4 hr**	**5 hr**
Control	2.37±0.19	2.522±0.09	3.426±0.11	3.99±0.11	4.43±0.06	4.15±0.08	3.09±0.06
% inhibition		-6.33%	-44.56%	-68.35%	-86.92%	-75.11%	-30.37%
Diclofenac tablet	2.436±0.25	1.83±0.15**	1.464±0.2**	1.084±0.39**	0.422±0.02**	0.178±0.02**	0.088±0.013**
% inhibition		24.88%	39.90%	55.50%	82.68%	92.69%	96.39%
Aceclofenac tablet	2.518±0.21	1.69±0.22**	1.28±0.16**	0.974±0.14**	0.44±0.03**	0.154±0.07**	0.066±0.03**
% inhibition		32.88%	49.17%	61.32%	82.53%	93.88%	97.38%
Ariflex tablet	2.376±0.17	2.51±0.26ns	2.376±0.28	1.244±0.19**	0.274±0.14**	0.166±0.05**	0.072±0.029**
% inhibition		-5.63%	0%	47.64%	88.47%	93.01%	96.97%

*p<0.05, **p<0.01, hr: hours, min: minutes.

W. somnifera has shown direct chondroprotective activity in vitro. *W. Somnifera* inhibits production of pro-inflammatory cytokines such as Interleukins (IL-1beta, etc.) and TNF-alpha in healthy individuals as well as rheumatoid arthritis patients. Also, in an animal study, W. somnifera has shown to have anti-inflammatory effects via immunomodulation and immunosuppression.[30-31] *Vitex negundo* and *Tinospora cordifolia* have reported to possess anti-osteoporotic activity.[15-17, 33-34]

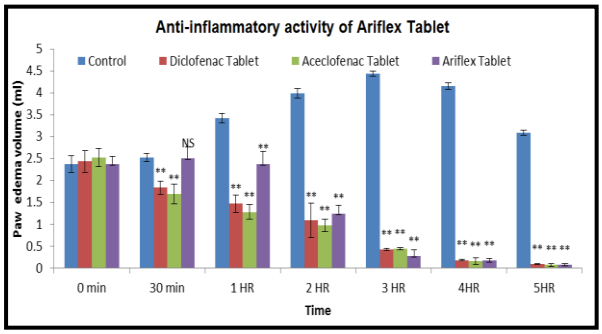

Figure 1: Anti-inflammatory activity of ariflex tablet; Data is expressed as Mean±SEM of n=5 observations. All treatments were compared with control (ANOVA followed by Dunnett's test) *p<0.05, **p<0.01

Gingerol from shunthi (*Zingiber officinale*) is inhibitor of COX enzyme. In another research study, it has been observed that the analgesic and anti-inflammatory activities of Ginger are due to its ameliorative effects could be related to inhibition of leukotrienes and PG synthesis.[25-26] From the above discussion, it has been observed that the ingredients of Ariflex tablet possess anti-inflammatory, analgesic, antipyretic, chondro-protective, anti-osteoporotic, immunomodulator, anti-oxidant, rejuvenating and anti-stress activities. These multiple activities of the ingredients present in the formulation help to reduce inflammation and pain in arthritis of varied etiology. Besides this, the ingredients also help to protect articular cartilage from damage and help to reduce osteoporosis. The observed significant anti-inflammatory activity of Ariflex tablet could be the result of synergetic activities of various anti-inflammatory herbs present in the formulation.

CONCLUSION

It can be concluded that Ariflex tablet possesses significant anti-inflammatory activity. Ariflex tablet can be used in the management of osteoarthritis, rheumatoid arthritis, gouty arthritis, lumbago, sciatica and spondylitis.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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