Anti-inflammatory effects of Dashmula, an Ayurvedic preparation, versus Diclofenac in animal models

1Ravi Shekher Singh, 2Mushtaq Ahmad, 3Zahoor Ahmad Wafai, 4Vikas Seth*, 2Vijay Vasant Moghe and 2Prerna Upadhyaya

1P.D.M. Medical College, Amravati
2Mahatma Gandhi Medical College, Jaipur
3S.K.I.M.S, Soura, Srinagar (J&K) State
4Hind Institute of Medical Sciences, Barabanki, Lucknow

ABSTRACT
Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly employed drugs for the treatment of inflammatory conditions but the adverse effect profile is the limitations in their use. Dashamula, a combination of roots of ten plants, is a standard Ayurvedic remedy for inflammatory conditions. However, studies to evaluate its role in the inflammation process and comparison with standard NSAIDs are lacking. Albino Wistar rats of either sex were used to test the anti-inflammatory effects; carrageenin induced rat hind paw edema for acute inflammation and cotton pellet implantation for chronic inflammation. Animals (n=48) were allocated to two groups of 24 animals each. Each group was further subdivided into four subgroups, receiving either saline as control or Dashamula low dose or Dashamula high dose or Diclofenac sodium. Statistical analysis was done by using Student’s ‘t’ test. Highly significant (p<0.001) paw edema reduction in acute inflammation test and decrease in final weight of the cotton pellet in chronic inflammation test was seen in groups treated with Diclofenac sodium and significant (p<0.05) difference in Dashamula low dose in both the test models as compared to control. However, this difference was statistically insignificant between Dashamula high dose and Diclofenac sodium in both the test models. Therefore, it is concluded that the anti-inflammatory activity of Dashamula is comparable to that of Diclofenac sodium and Dashamula can be a possible alternative to NSAIDs.

Keywords: Anti-inflammatory effect, Dashamula, Diclofenac Sodium, NSAIDs.
INTRODUCTION

Inflammation is defined as the reaction of vascularised living tissue to injury [1]. The inflammatory response is closely intertwined with the process of repair. Inflammation serves to destroy, dilute or wall off the injurious agent, but in turn it sets into motion a series of events that as far as possible heal and reconstitute the damaged tissue. Repair begins during the early phases of inflammation but reaches completion usually after the injurious influence has been neutralized. Inflammation, however, if runs unchecked, leads to onset of vasomotor rhinorrhoea, rheumatoid arthritis, hypersensitivity reactions, fetal renal disease and atherosclerosis [2]. Many drugs including steroidal and non-steroidal anti-inflammatory drugs are employed to control the inflammatory reaction and pain associated with it. Out of the anti-inflammatory drugs, the non-steroidal anti-inflammatory drugs (NSAIDs) are preferred and commonly used in clinical practice. These drugs have wide range of chemical nature and share, the common mechanism of action, pharmacological actions and adverse effect profiles. Although these drugs are effective in controlling signs of inflammation, numbers of adverse effects encountered are the biggest limitations to their use. Because of the side effect profile of NSAIDs, patients are inclined to choose the alternative system of treatment. Dashamula, a combination of roots of ten plants, is standard Ayurvedic remedy for inflammatory disease [3, 4]. It is an age old herbal preparation commonly used by Vaidyas (Ayurvedic practitioners) in their practice to reduce inflammation. However, studies to evaluate its role in the inflammation process and comparison with standard NSAIDs are lacking.

Hence, the present study was planned to evaluate the anti-inflammatory efficacy of Dashamula, and compare it with a standard NSAID, Diclofenac Sodium, in chronic and acute inflammation models in experimental animals.

EXPERIMENTAL SECTION

Animals
Healthy albino Wistar rats (3 months old) of either sex weighing 150-200g were used. Animals were housed within the departmental animal house and the room temperature was maintained at 27 degree Celsius. Animals were fed with standard pellet diet (Lipton India laboratories, Bangalore) and water ad libitum and were starved over night before the day of experiment. The protocol was approved by the Institutional Animal Ethics Committee.

Investigational drugs and dosage preparation
Tablet Diclofenac sodium 50mg (Torrent Labs Pvt. Ltd- “Torrent House” Near Dinesh Hal, off Asram Road, Ahmedabad) was purchased from the hospital pharmacy counter and Dashamula from a standard Ayurvedic shop (Mankarika Aushadhalaya, 1015, Sadasivepeth, Pune-30). Dashamula is a collection of ten ingredients viz. Aegle marmelos (Bilwa), Premnain tegrifolia (Agnimantha), Oroxyllum indicum (Shyonaka), Stereospermum suaveolens (Patla), Gmelina arborea (Kashmiri), Desmodium indicum (Shaliparni), Urari alagopoides (Prishniparni), Solanum indicum (Brahati), Solanum xanthocarpum (Kantkari) and Tribulus terrestris (Gokshura). These were procured separately in coarse powder form. The identification of the first three ingredients of Dashamula viz. Aegle marmelos (Bilva), Premnain tegrifolia (Agnimantha) and Oroxyllum indicum (Shyonaka) was done at Poona College of Pharmacy,
Pune, and rest of the ingredients were identified at Bharti Vidyapeeth Ayurvedic College, Pune. The appropriate body weight adjusted doses of test drugs as extrapolated from doses to be 1.44 ml and 2.88 ml/200g rat as low and high doses for Dashamula and 2.70 mg/200g rat for diclofenac sodium were used. Formulation for Diclofenac sodium was made as suspension prepared in gum acacia 2% w/v uniformly mixed. The formulations were fed to the animals through gastric tube (9mm) for rat. Saline 1.44ml/200g was used concomitantly as control in all the groups.

Method of extraction of Dashamula [5]: One gram of each ingredient of the Dashamula was taken and mixed properly in grinder by adding 160ml of water to make a 10g/160ml of Dashamula mixture. The mixture so prepared was boiled till it reduced to 1/8th of initial volume i.e. 20ml and filtered. The decoction hence produced was tested for anti-inflammatory activity in rats for acute and chronic inflammation in rat hind paw edema and cotton pellet implantation in animal models. Fresh decoction of the Dashamula was made every time for use in experimental animal groups.

Experimental protocol
Animals (n=48) were allocated to two main groups (GI, GII) of 24 animals each. Depending upon the treatment design each group was further divided into four sub groups of 6 animals each receiving saline as control (Glc, GIIc), Dashamula low dose (GIdld, GIIIdld), Dashamula high dose (GIdhd, GIIIdhd) and diclofenac sodium (GIds, GIIIds) as test drugs respectively.

Assessment of acute anti-inflammatory activity by rat hind paw edema [6, 7]
The paw volume measured before carrageenin injection was considered as initial paw volume. Two hours after per oral administration of control or investigational drugs, all the rats (n=24) in GI received 0.2ml of 1% carrageenin solution subcutaneously into the planter portion of right hind paw by using tuberculin syringe to produce chemically induced inflammation. After carrageenin injection, paw volume was measured hourly for three hours by UGO basile plethysmometer. The increase in paw volume was calculated by deducting the initial paw volume observed at different time intervals.

Assessment of chronic anti-inflammatory activity by cotton pellet implantation [6, 8]
Cotton pellet weighing 50±1 were sterilized and animals were anaesthetized with ether. The skin of the back was shaved and disinfected with 70% ethanol. A mid line incision under aseptic precaution was made in the lumber area and a subcutaneous tunnel was made on the either side over the scapular region with blunt forceps. A cotton pellet was inserted in each tunnel and switched with sterile catgut. Treatment was continued for seven days postoperatively. On 8th day, the animals were first anesthetized and then sacrificed. The cotton pellets were removed with granuloma tissue. The pellets were dried in oven; any increase in weight of the pellet was calculated and subtracted from the initial weight. The final weight was recorded and compared among treatment groups.

Statistical analysis
The results of both the experimental models viz.rat hind paw edema and cotton pellet implantation methods were expressed as mean ± SEM. Statistical analysis for both the methods
was done by using Student’s ‘t’ test. A probability value of less than 0.05 (P< 0.05) was considered to be statistically significant.

RESULTS AND DISCUSSION

Inflammation is a common clinical condition and many drugs are available for its treatment in modern medicine. All the drugs have a limitation of their wide range of adverse effects. These unpleasant effects of the drugs available, including NSAIDs in modern medicine drives the research for search of anti-inflammatory agents from Indian medicinal plants [9]. Many traditional medicinal preparations have been scientifically evaluated for anti-inflammatory effects and are practiced in alternative system of medicine [10-14]. Dashamula, one such an ayurvedic preparation is used by Ayurvedic physicans to treat “Samrambh” a condition having signs and symptoms resembling that of inflammation. According to Ayurvedic literature, Dashamula has not only analgesic and anti-inflammatory effects but also antipyretic effect [15, 16]. Dashamula, a combination of roots of ten plants, is the standard Ayurvedic remedy for the treatment of inflammatory diseases [3, 4]. Therapeutically, it is either used alone or as formulation with other medicinal preparations in Ayurvedic clinical practice.

Figure 1: Effect of saline as control (C), Dashamula low dose (Dld), Dashamula high dose (Dhd), and Diclofenac sodium (Dic.) on Carrageenin-induced hind paw edema in rats. Each point represents mean ± SEM (n= 6). *P<0.05 and **P<0.001, compared with control treatment

In all the preparations, Dashamula is used in the form of decoction. Hence, in this study, Dashamula decoction was used as well. For evaluation of anti-inflammatory effect, one of the non-steroidal anti-inflammatory drug is used for comparison in experimental and clinical settings [17-21]. Diclofenac was choosen for comparison in this study as it is one of the most commonly employed NSAID clinically. Dashamula is supposed to be very effective in reducing all the symptoms and signs of inflammation, so it was decided to screen its role in both acute and chronic inflammation in experimental animal models. Out of models available, commonly used models, the carrageenin-induced rat hind paw edema and cotton pellet granuloma methods were
used for assessing acute and chronic inflammation respectively. The outcomes of Dashamula, in experimental models for its role in acute and chronic inflammation were degree of anti-inflammatory effect and time course of action in acute study.

On assessing the acute anti-inflammatory activity, the group treated with Dashamula, high dose and diclofenac sodium have shown highly significant (p<0.001) reduction in paw edema when compared to control group (Figure 1).

While the group treated with Dashamula low dose showed significant (p<0.01) difference as compared to control. However, the difference in degree of reduction in paw edema achieved by Dashamula high dose and Diclofenac sodium was statistically insignificant.

Therefore, Dashamula in high dose is equally as effective as standard Diclofenac Sodium. Interestingly, while assessing the chronic anti-inflammatory effect, the groups treated with Dashamula high dose and Diclofenac sodium showed highly significant (p<0.001) and Dashamula low dose significant (p<0.01) reduction in granuloma formation when compared to control group (Figure 2).

It is important to note that the degree of reduction in granuloma formation achieved by Dashamula high dose was comparatively greater than Diclofenac sodium, however, this difference was statistically insignificant. Though the studies evaluating the anti-inflammatory effect of Dashamula preparation are not available, but few studies on individual ingredients of Dashamula, stating the anti-inflammatory are available and the results of this study are in support of the findings of these studies [22-24]. Comparing the time course of action of Dashamula high dose and diclofenac sodium in acute and chronic experimental models, both Dashamula high
dose and diclofenac are superimposed on each other. It implies that the time taken to produce the observed effect was absolutely equal and same in both the preparations.

Hence, this study reveals that Dashamula in low as well as high dose is an effective anti-inflammatory preparation as is evident from the results of both the experimental animal models. However, Dashamula in high dose is equieffective to Diclofenac sodium in reducing inflammation.

**CONCLUSION**

Dashamula was found to be an effective anti-inflammatory agent comparable to Diclofenac sodium both in acute and chronic animal models. It can be a possible alternative to NSAIDs. Further clinical and experimental studies are required to explore this possibility.

**REFERENCES**