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Original Article

Experimental Research Section

Analgesic Effects of Dashamula, an Ayurvedic Preparation, versus Diclofenac Sodium in Animal Models

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ABSTRACT

Background and Objectives: Non-steroidal anti-inflammatory drugs (NSAIDs) are drugs which are most commonly used to control pain. But the adverse effects that they produce are the limitations in their use. Many medicinal preparations are commonly used for the treatment of pain in alternative medicine. One such commonly used Ayurvedic preparation is Dashamula, a combination of the roots of ten plants, a standard Ayurvedic remedy for the treatment of pain. However, studies which have evaluated its role as an analgesic in comparison with NSAIDs are not available.

Materials and Methods: Healthy albino mice of either sex, which weighed 30–50 g, were used for the anti-nociceptive test: the writhing test. The animals (n=24) were allocated to four groups (GI, GII, GIII and GIV) of six mice each, which received either saline as a control or a low dose of Dashamula or a high dose of Dashamula or Diclofenac sodium. The statistical analysis was done by using the Student's 't'-test. A probability value of less than 0.05 ($P< 0.05$) was considered to be statistically significant.

Results: A highly significant ($p<0.001$) reduction in the number of writhes was noted in the groups which were treated with high dose Dashamula and Diclofenac sodium and there was a significant ($p<0.01$) difference in the number of writhes in the group which was treated with low dose Dashamula as compared to that in the controls. Although the degree of analgesia was greater in the group which was treated with high dose Dashamula as compared to the group which was treated with Diclofenac sodium, the difference was not statistically significant ($p>0.05$). The analgesic action of high dose Dashamula and Diclofenac sodium started within 30 min, with a complete abolition of the writhes from 90 min onwards. The time course of action in the group which was treated with high dose Dashamula was quicker and faster than in the Diclofenac sodium group, however, this difference was statistically insignificant ($p>0.05$).

Conclusions: The analgesic activity of Dashamula, both in terms of the degree of analgesia and the time course of action was comparable to that of Diclofenac sodium, a standard NSAID. Hence, Dashamula could be a possible alternative to NSAIDs. Further studies, experimental and clinical, are needed to explore this possibility.

Key Words: Analgesic effect, Dashamula, Diclofenac sodium, Writhing test, NSAIDs

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are the mainstay of treatment for a variety of painful conditions [1]. Various NSAIDs and their fixed dose combinations are routinely employed in the clinical practice to control pain. Though these drugs are effective in controlling pain, their wide range of adverse effects are the biggest limitations in their use. Because of the side effect profile of NSAIDs, the patients are inclined to choose an alternative system of treatment. Many studies have compared the alternative medicinal preparations and the classical nonsteroidal anti-inflammatory agents for their analgesic effect in clinical and experimental settings [2-4]. A number of alternative medicinal preparations are commonly employed in Ayurveda to control pain. Dashamula, a combination of the roots of ten plants, is a standard Ayurvedic remedy for the treatment of pain [5,6]. It is an age old herbal preparation which is commonly used by the Vaidyas (Ayurvedic practitioners) in their practice, to reduce pain. However, studies which have evaluated its role in controlling pain both in experimental and clinical settings, are not available.

Hence, the present study was planned to evaluate the analgesic efficacy of Dashamula, and to compare it with a standard NSAID, Diclofenac sodium, in animal models.

MATERIALS AND METHODS

Animals

Healthy albino mice of either sex (6 to 7 weeks old), which weighed 30–50 g, were used for the anti-nociceptive test: the writhing test. The animals were housed within the departmental animal house and the room temperature was maintained at 27 degree Celsius. The animals were fed with the standard pellet diet (Lipton India laboratories, Bangalore) and water ad libitum and they were starved overnight before the day of the experiment. The study protocol was approved by the Institutional Animal Ethics Committee.

Investigational Drugs and Dosage Preparation

A tablet formulation of Diclofenac sodium, 50 mg, (Torrent Labs Pvt. Ltd- "Torrent House" Near Dinesh Hall, off Asram Road, Ahmedabad) was purchased from the hospital pharmacy counter and Dashamula was purchased from a standard Ayurvedic

shop (Mankarika Aushadhalaya, 1015, Sadasivepeth, Pune-30). Dashamula is a collection of ten ingredients viz. Aegle marmelos (Bilva), Premnain tegrifolia (Agnimantha), Oroxylum indicum (Shyonaka), Stereospermum suaveolens (Patla), Gmelina arborea (Kashmiri), Desmodium indicum (Shaliparni), Urari alagopoides (Prishniparni), Solanum indicum (Brahati), Solanum xanthocarpum (Kantkari) and Tribulus terrestris (Gokshura). Each ingredient was procured separately in coarse powder form. The identification of the first three ingredients of Dashamula viz. Aegle marmelos (Bilva), Premnain tegrifolia (Agnimantha) and Oroxylum indicum (Shyonaka) was done at Poona College of Pharmacy, Pune, and the rest of the ingredients were identified at Bharti Vidyapeeth Ayurvedic College, Pune. The appropriate body weight adjusted doses of the test drugs were extrapolated from the doses to be 0.28 ml and 0.41 ml/20 gm mice as low and high doses for Dashamula and 0.39 mg/20 gm mice for Diclofenac sodium were used [7]. The formulation for Diclofenac sodium was made as a 2% w/v uniformly mixed suspension which was prepared in gum acacia. The formulations were fed to the animals through a 2–3 cm polythene tubing which was sleeved on an 18–20 gauge blunted hypodermic needle. Saline 0.28ml/ 20gm mice was used concomitantly as a control in all the groups.

Method of the extraction of Dashamula [8]: One gram of each ingredient of Dashamula was taken and mixed properly in a grinder by adding 160 ml of water to make 10 gm/160 ml of the Dashamula mixture. The mixture which was so prepared was boiled till it was reduced to 1/8th of its initial volume i.e. 20ml and it was filtered. The decoction which was hence produced was tested for its anti-nociceptive activity in mice. A fresh decoction of Dashamula was made every time for use in the experimental animal groups.

Experimental Protocol

The animals (n=24) were allocated to four groups (GI, GII, GIII and GIV) of six mice each. Depending upon the treatment design, each group received saline as a control (GIc) and Dashamula low dose (GIIdld), Dashamula high dose (GIIIdhd) and Diclofenac Sodium (GIVds) as the test drugs respectively.

Assessment of the Analgesic Activity by Acetic Acid-Induced Writhing in Mice [9,10]

Calculated, weight adjusted, doses of the test drugs, i.e. Dashamula low dose 0.28 ml/20 gm, Dashamula high dose 0.41 ml/20 gm, Diclofenac 0.39 mg/20 gm and saline 0.28 ml/20 gm mice as the control were given p.o. to the mice 2 hours prior to their anti-nociceptive tests. The anti-nociceptive effect was tested by counting the number of writhes, by calculating the average number of writhes which were recorded every 30 min up to 240 min for each test group after the injection of 1% (1 ml/100 g) acetic acid i.p [11].

The anti-nociceptive activity was calculated as the percent maximum possible effect (% MPE)

$$\% \text{ MPE} = \frac{\text{Mean writhes GIc} - \text{Mean writhes test}}{\text{Mean writhes GIc}} \times 100$$

Test groups = GIIdld, GIIIdhd, GIVds

Statistical Analysis

The results were expressed as mean ± SEM. The statistical analysis was done by using the Student's 't'-test. A probability value of less than 0.05 ($P< 0.05$) was considered to be statistically significant.

RESULTS

Degree of Analgesia which was Produced by Dashamula and Diclofenac Sodium

The groups which were treated with Dashamula high dose and Diclofenac sodium showed a highly significant ($p<0.001$) reduction in the number of writhes and the group which was treated with Dashamula low dose showed a significant difference ($p<0.01$) as compared to the control [Table/Fig-1]. By comparing the degree of analgesia, as indicated by a reduction in the number of writhes, no significant difference was observed between the treatment groups i.e Dld Vs. Dhd and Dld Vs. Ds ($p> 0.05$). Though the degree of analgesia was greater in a group which was treated with Dashamula high dose as compared to a group treated with Diclofenac sodium, this difference was statistically insignificant ($p>0.05$).

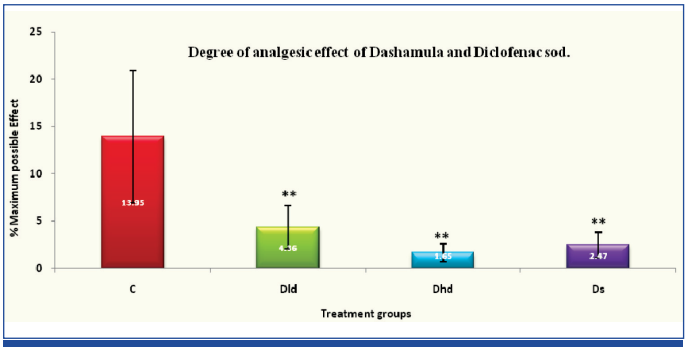
Time Course of the Analgesic Action of Dashamula and Diclofenac Sodium

The drug treated groups showed a significant difference ($p<0.001$) in the time course of the analgesic action, as was indicated by the percentage of reduction in the number of writhes after different time intervals as compared to the controls [Table/Fig-2]. On studying the time course of action of the test drugs, the analgesic action of Dashamula high dose was found to have started within 30 min (less than 60% reduction in the number of writhes), with the complete abolition of the writhes from 90 min onwards. As against this, Diclofenac sodium showed less than 40% reduction in the number of writhes within 60 min, though the complete abolition of the writhes from 90 min onwards was the same as it was for Dashamula high dose. However, this difference in the time course of action of the drug treatment was statistically insignificant ($p<0.05$). The time course of analgesic action was also within 30 min in the group which was treated with Dashamula low dose, though the complete abolition of the writhes was observed 120 min onwards.

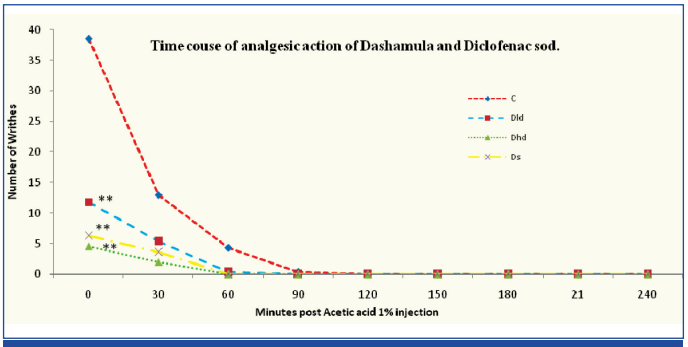
DISCUSSION

Many treatment strategies are utilized to alleviate pain in clinical as well as post surgical conditions. Non-steroidal anti-inflammatory drugs (NSAIDs), either alone or in fixed dose combinations, are the drugs which are commonly used for analgesia in modern medicine. As their side effect profile has always been a focus of concern and limitation to their use, many alternative agents have been tried in the clinical practice to alleviate pain as well. One such commonly used Ayurvedic preparation in Ayurveda is Dashamula, a combination of the roots of ten plants, for the treatment of pain and inflammation [5,6]. Apart from its use with other Ayurvedic preparations for the treatment of different conditions, it is commonly used with milk to relieve pain in the Ayurvedic practice [6]. Studies which have evaluated its role as an analgesic and have compared it with standard NSAIDs in modern medicine are not available. Hence, this study was undertaken to evaluate the analgesic efficacy of Dashamula and to compare it with Dilofenac sodium in animal models. The comparison was done in terms of the degree of analgesia and the time course of action of the drugs.

As far as the degree of analgesia was concerned, all the test groups showed a significant difference in the reduction in the



[Table/Fig-1]: Effect of saline as control (C), Dashamula low dose (Dld), Dashamula high dose (Dhd), and Diclofenac sodium (Ds) on acetic acid induced writhing in mice. Each point represents mean ± SEM (*n* = 6). **$P<0.001$, compared with control treatment.



[Table/Fig-2]: Effect of saline as control (C), Dashamula low dose (Dld), Dashamula high dose (Dhd), and Diclofenac sodium (Ds) on acetic acid induced writhing in mice. Each point represents mean ± SEM (*n* = 6). **$P<0.001$, compared with control treatment.

number of writhes after 1% acetic acid injection as compared to the controls [Table/Fig-1]. A comparatively greater degree of analgesia was observed in a group which was treated with Dashamula high dose as compared to a group which was treated with Diclofenac sodium; however, this difference was statistically insignificant (p>0.05). This indicated that the degree of analgesia which was achieved in both the groups which were treated with Dashamula high dose and Diclofenac sodium was comparable. Though studies which have compared the Dashamula preparation with NSAIDs for the evaluation of analgesia are lacking, studies which have evaluated the analgesic effect of either of the active ingredients of Dashamula are available. Studies on Aegle marmelos (Bilva), an active ingredient of Dashamula, have shown significant analgesic properties [12-14]. Oroxylum indicum (Shyonaka) and Stereospermum Suaveolens (Patla), the other active ingredients of Dashamula, have also shown significant analgesia [15-17]. Hence, the results of our study are not only in accordance to the findings of these studies in stating the analgesic effect of Dashamula, but they also support the Ayurvedic texts and other studies which claim Dashamula as a remedy for pain [6,18,19]. On assessing the time course of action of the test drugs, all the treatment groups were found to show a significant difference as compared to the controls as well [Table/Fig-2]. The reduction in the number of writhes, though it started within 30 min with complete abolition in 90 minutes in the treatment groups which were treated with Dashamula high dose and Diclofenac sodium, the percentage in the reduction in the number of writhes was quicker and greater in the group which was treated with Dashamula high dose. However, this difference was not statistically significant [Table/Fig-2]. This also indicated that the onset and the duration of the analgesic action of Dashamula high dose and Diclofenac sodium in both the groups were the same. Therefore, on assessing the overall results in terms of the degree of analgesia and the time of the course of action, both the Dashamula high dose and Diclofenac were found to be superimposed on each other.

Hence, this study revealed Dashamula's activity both in terms of the degree of analgesia and the time course of action to be comparable to Diclofenac sodium. Thus, Dashamula can be a possible alternative to the non-steroidal anti-inflammatory agents (NSAIDs).

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