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RESEARCH ARTICLE

EVALUATION OF ANALGESIC ACTIVITY OF DASHAMOOLARISHTHA FORMULATION BY USING EXPERIMENTAL MODELS OF NOCICEPTION

Joshi SS, Bhalerao PP, Gajbhiye SV*

Seth GS Medical College & KEM Hospital, Parel, Mumbai

ABSTRACT

Antimicrobial resistance is not only increasing the healthcare costs, but also the severity and death rates from certain In this study analgesic activity of Dashamoolarishtha (Ayurvedic multi-ingredient formulation) were evaluated by using three models of centrally induced nociception that is hot plate & tail clip model in mice and tail immersion model in rats. Two doses of Dashamoolarishtha low (1.56ml/kg in mice & 1.08 ml/kg in rats) & high (3.12 ml/kg in mice & 2.16 ml/kg in rats) were used and pentazocine served as a positive control. Results of the present study has shown that Dashamoolarishtha in the dose of 3.2ml/kg showed significant increase in reaction time as compared to control in hot plate model. In tail clip model Dashamoolarishtha low dose & high dose showed statistical significant antinociceptive effect as compared to control. In tail immersion model also Dashamoolarishtha in both doses showed statistically significant reduction in pain. Thus to conclude Dashamoolarishtha showed analgesic activity in various central models of nociception suggesting a promising source of ayurvedic medication for pain.

Keywords: Dashamoolarishtha, antinociceptive, hot plate & tail clip model, tail immersion model

INTRODUCTION

Pain is a pervasive public health problem, and analgesic drugs play a central role in its treatment. Historically, the most widely used analgesics have included μ-opioid agonists such as morphine, anti-inflammatory steroids such as cortisone, and nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin. Although these drugs are useful across a wide range of conditions, they have undesirable side effects which often limit their use. [1,2] Therefore the search for better analgesic agent is ongoing.

Medicinal plants play an important role in folk medicine, and different plant species have been used in the treatment of many diseases. Detailed studies are necessary to prove their biological activity as herbal medicines derived from the plant extracts are being increasingly utilized to treat a wide variety of clinical diseases. Dashamoolarishtha, an Ayurvedic multi-ingredient formulation is used as an analgesic, anti-arthritic agent and in rheumatism.[3] Dashamoolarishtha , a classical Ayurvedic preparation contains ten plants - Aegle marmelos, Oroxylum indicum, Stereospermum suaveolens , Premna integrifolia , Gmelina arborea , Solanum xanthocarpum , Solanum indicum , Desmodium gangeticum , Uraria picta and Tribulus terrestris . In the Ayurvedic system of medicine it is used as analgesic, antiarthritic, against cough, rheumatism, etc. [3] Many of these ingredients have been evaluated in experimental models of inflammation and pain and have shown to possess anti-inflammatory and analgesic activities for

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e.g., Oroxylum indicum, [4] Desmodium gangeticum, Premna integrifolia L. and Gmelina arborea, [5] Aegle Marmelos, [6,7,8] T. terrestris, [9] Solanum xanthocarpum,[10] Premna Integrifolia [11]. However, surprisingly the widely prescribed Dashamoolarishta has never been evaluated in any of the experimental and clinical study.

This prompted us to investigate the effects of pharmacological activities of Dashamoolarishtha in experimental models of pain. The purpose of the present study is to evaluate the possible antinociceptive activity of Dashamoolarishtha in several animals models of nociception.

MATERIAL AND METHODS

Ethics: Permission of the Institutional Animal Ethics Committee was obtained prior to the commencement of the study. The study was conducted according to CPCSEA guidelines.

Experimental animals:

The study was carried out in mice of either sex weighing between 20-25 g and rats of either sex weighing between 170-250 g. Mice were kept in an air conditioned rooms with temperature 22±3°C, humidity 30-70 %. A 12-h light–dark cycle was maintained throughout the experimental protocol. Mice were housed 8 per cage and rats 4 per cage during acclimatization and treatment. The cages were of stainless steel top grill having facilities for food and water ad libitum.

Drugs: Dashamoolarishtha was obtained from Sandu Brothers in powder form. The certificate of analysis was given from the same company. The doses used in the study were extrapolated for animals from the lowest and highest dose recommended in the Ayurvedic texts for human.[12]

Experimental groups:

Group comprised of 6 animals each
Group 1: Vehicle control (Distilled Water)
Group 2: Pentazocine
Group 3: Dashamoolarishtha – [Low Dose]
Group 4: Dashamoolarishtha – [High Dose]

Serial number	Study drugs	Dose in Mice (mg/kg)	Dose in Rat (mg/kg)
1	Control	Distilled water	
2	Pentazocine	7.8	5.4
3	Dashamoolarishtha (Low dose)	1.56	1.08
4	Dashamoolarishtha (High dose)	3.12	2.16

Experimental Procedure:

1) Hot plate method[13] - Groups of Swiss mice of either sex weighing between 20-25 g were used. Mice were placed on a hot plate maintained at 55±0.5°C. The reaction time was taken as the interval from the instant animal reached the hot plate until the moment animal licked its feet or jumped out. A screening was done and only those mice which react in 5s were selected to avoid thermal injury. The latency is recorded before and after 20, 60 and 90 min following oral administration of the test compounds and the standard drug. The mice was not placed on hot plate for more than 15 seconds to avoid thermal damage to the paw.

2) Tail clip method in mice[14]- Group of Swiss mice of either sex weighing between 20-25 g were used. An artery clip is applied to the base of the tail (approx 1 cm from the body) to induce pain. The animal quickly responds to this noxious stimulus by biting the clip or the tail near the location of the clip. The animals which did not show efforts to dislodge the clip within 15 s were not used for the experiments. The time between onset of stimulation and the response is measured. The tail clip was applied at 30 min after drug administration and the basal reaction time was noted.

3) Tail immersion test[15]- Wistar rats of either sex weighing between 170-250 g

were used. They were placed into individual cylindrical rat holders leaving the tail hanging out freely. The animals were allowed to get acclimatized to rat holders for 30 min before testing. The lower 5 cm portion of the tail was marked. This part of the tail was immersed in a cup of freshly filled water at 55°C. The reaction time was recorded and the animals having reaction time of more than 15 seconds were not used for the experiment. The reaction time is determined before and periodically at 30, 60 and 120 min after the oral administration of test substance.

Statistical Analysis: All values are expressed as mean ± SEM. One way ANOVA was followed by Tukey's test. Statistically significant results with values of $p< 0.05$ were considered.

RESULTS

Hot plate method – As depicted in table 3.1 Dashamoolarishtha high dose showed statistically significant reaction time at 20 min as compared to control. Dashamoolarishtha low dose did not show statistical significance as compared to control at any time point. Pentazocine, a kappa receptor agonist exerted a significant analgesic effect at all time points compared to control. The analgesic effect of Dashamoolarishtha high dose when compared to pentazocine, was comparable except at 90 min.

Tail clip method –

As depicted in table 3.2 Dashamoolarishtha at both doses showed statistically significant difference in reaction time ($P<0.001$) as compared with control (Distilled water) at 30 min. The analgesic effect of pentazocine was statistically significant as compared to Dashamoolarishtha low dose.

Tail Immersion method

As depicted in table 3.3 Dashamoolarishtha low dose showed statistically significant values at 30 min and at 60 min as compared to control. Dashamoolarishtha high dose showed statistically significant values at 30,60 & 120 min. Pentazocine, exerted a significant analgesic effect at all time points compared to control. The analgesic effect of pentazocine was statistically significant as compared to Dashamoolarishtha low dose at 60 & 120 min.

Table 1- Effect of Dashamoolarishtha on the reaction time in Hot plate method.

Group	0 min	20 min	60 min	90 min
DW	4.46±0.72	4.8±1.54	5.93±2.48	5.49±1.66
PZ	4.49±0.53	13.94±1.1**	13.42±1.26*	12.22±1.25**
D1	4.42±0.65	6.26±4.47$^{@@}$	7.71±4.92$^{@}$	4.85±2.94$^{@@@}$
D2	3.74±1.08	10.95±3.4*	10.47±3.5	6.71±2.71$^{@}$

Values are expressed as Mean±S.D, n=6 .
** p<0.001, * p<0.01 (as compared to control)
$^{@@@}$ p<0.001, $^{@@}$ p<0.01, $^{@}$ p<0.05 (as compared to Pentazocine)
[DW= Distilled water, PZ= Pentazocine, D1= low dose Dashamoolarishtha, D2= High dose Dashamoolarishtha]

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Table 2- Effect of Dashamoolarishtha on the reaction time in Tail clip method.

Group	0 min	30 min
DW	3.78 ± 1.22	3.49 ± 1.14
PZ	3.16 ± 1.33	12.62 ± 2.37**
D1	3.95 ± 0.53	6.8 ± 2.03*@
D2	3.81 ± 1.14	10.03 ±2.09**

Values expressed are Mean±S.D. (n=6).
** p<0.001, * p<0.05 (as compared to control)
@ p<0.05 (as compared to Pentazocine)
[DW= Distilled water, PZ= Pentazocine, D1= low dose Dashamoolarishtha, D2= High dose Dashamoolarishtha]

Table 3- Effect of Dashamoolarishtha on the reaction time in Tail Immersion method.

Group	0 min	30 min	1 hour	2 hour
DW	3.49±0.47	3.1±0.83	3.76±0.57	3.88±0.71.66
PZ	3.14±1.52	8.44±1.98***	7.52±1.15***	5.75±0.79**
D1	2.86±0.88	6.48±0.62**	5.71±0.76*@	4.29±0.86@
D2	3.16±0.97	7.84±1.43***	6.95±1.21***	5.2±0.69*

Values expressed as Mean±S.D. (n=6).
*** p<0.001, ** p<0.01, * p<0.05 (as compared to control)
@ p<0.05 (as compared to Pentazocine)
[DW= Distilled water, PZ= Pentazocine, D1= low dose Dashamoolarishtha, D2= High dose Dashamoolarishtha]

DISCUSSION

The results of the present study demonstrates that dashamoolarishta has central analgesic effect as shown by reduction in reaction time by high dose dashamoolarishta in all three models of nociception hot plate method, tail clip & tail immersion method.

There are various studies reported validating claims put forth in Ayurveda regarding use of Dashamoolarishtha in practice. Dashamoolarishta, an Ayurvedic multi-ingredient formulation is used as an analgesic, antiarthritic, cough and rheumatism. [3] This formulation contains roots of ten different plants. These could be serving different roles like active principles, adjuvant, carrier agent and stabilizer. [12] However, in most cases of herbal drugs the claims have not been substantiated with evidence for its use in the present indication. Hence the present study was conducted wherein two doses of Dashamoolarishta were selected. The doses were extrapolated from the human dose used in the clinical practice. A study by Parekar et.al. [16], has shown anti-inflammatory activity of Dashamoolarishtha by using cotton pellet induced granuloma and carrageenan induced rat paw edema models of inflammation. This shows that Dashamoolarishtha also has peripheral action by inhibiting the inflammatory process The study also showed that Dashamoolarishtha has peripheral analgesic activity in acetic acid induced writhing model. Hence it was decided to evaluate the central analgesic activity of Dashamoolarishtha by using 3 different central models for pain i.e. hot plate, tail clip and tail immersion in hot water. Pentazocine is a synthetic opiate with partial agonist/antagonist activity. As it has central analgesic activity with little or no abuse potential, it was selected as a positive control. [17]

These models differ in their ability to respond to nociceptive stimuli through different neuronal pathways. The hot plate and tail-clip test is useful in elucidating centrally mediated antinociceptive responses, which mediates painful stimuli above the spinal cord level.[18] Whereas tail immersion represents painful stimuli at spinal level. Thermal nociceptive tests are more sensitive to μ opioid agonists & non thermal nociceptive tests to κ opioid agonists. [19] The results from present study show that there may be involvement of both μ and κ opioid receptors in the analgesic activity of dashamoolarishta . Also Dashamoolarishtha high dose effectively reversed reaction time in all the three pain models.

Thus to conclude Dashamoolarishtha showed analgesic activity in various central models of nociception suggesting a promising source of ayurvedic medication for pain. Further studies at molecular level to evaluate the mechanism of action are required. Also studies aimed at isolation and characterization of active components will be worthwhile.

REFERNCES

1) Bjarnason I, Hayllar J, MacPherson AJ, Russell AS. Side effects of nonsteroidal anti-inflammatory drugs on the small and large intestine in humans. Gastroenterology 1993;104(6):1832-1847
2) Stanbury RM, Graham EM. Systemic corticosteroid therapy—side effects and their management. British Journal of Ophthalmology 1998;82:704-708.
3) Jabbar S, Khan MT, Choudhuri MS, Sil BK. Bioactivity Studies of the Individual Ingredients of the Dashamularishta. Pakistan J Pharmac Sciences 2004; 17(1): 9-17.
4) Ahad A, Ganai AA, Sareer O, Najm MZ, Kausar MA, Mohd M, Siddiqui WA. Therapeutic potential of oroxylum indicum: a review. 2012; 10:163– 172.
5) Singh A, Malhotra S, Subban R. Anti-inflammatory and analgesic agents from Indian medicinal plants. International

journal of integrative biology. 2008; 3(1): 57-72.
6) Khare CP(ed.) (2007). Indian Medicinal Plants. An Illustrated Dictionary. Springer. New Delhi. P.21.
7) Trivedi HP, Pathak NL, Gavaniya MG, Patel AK, Trivedi HD, Panchal NM. Aegle marmelos suppresses inflammation and cartilage destruction in collagen-induced arthritic rat. International Journal of Pharmaceutical Research and Development. 2011;3(4):38 – 45.
8) Sharma GN, Dubey SK, Sharma P, Sati N. Medicinal Values of Bael (Aegle marmelos) (L.) Corr.: A Review. International Journal of Pharmaceutical and Clinical Research 2011; 2(1):12-22.
9) Akram M, Asif H.M., Akhtar N, Shah PA, Uzair M, Shaheen G et.al. Tribulus terrestris Linn.: A review article. Journal of Medicinal Plants Research.2011; 5(16): 3601-3605.
10) Sahu RK, Singh A, Gupta R, Roy A, Dwivedi J. Development and evaluation of antipyretic and antinociceptive activity of polyherbal formulation containing some indigenous medicinal plants. International Journal of Pharmacology 2012; 8: 271-276.
11) Karmarkar UK, Pramanik S, Sadhu SK, Shill MC, Biswas SK. Assessment of analgesic and antibacterial activity of premna integrifolia linn.(family: verbenaceae) leaves. International Journal of Pharmaceutical Sciences and Research 2011; 2(6):1430-1435.
12) Gogte VM (2000). Ayurvedic Pharmacology and therapeutic uses of medicinal plants In: Ramkrishnan S. (editor) Dravyagunavidnyan: Bharatiya Vidya Bhavan, Mumbai; 287-8.
13) Eddy NB, Liembach D. Synthetic analgesics II:Dithienylbuttenyl and dithiennylbulyl-amines. J Pharmacol Exp Ther 1957; 107: 385–393.
14) C Bianchi., G Franceschini. British Journal of Pharmacology 1954 ; 9, 3: 280-284.
15) Turner RA. (1971) Screening Methods in Pharmacology Academic Press. 100-113.
16) Parekar RR, Dash KK, Marathe PA, Apte AA, Rege NN. Evaluation of anti-inflammatory activity of root bark of *Clerodendrum phlomidis* in experimental models of inflammation. International journal of applied biology and pharmaceutical technology 2012;3(3): 54-60.
17) Armstrong SC, Wynn GH, Sandson NB. Pharmacokinetic drug interactions of synthetic opiate analgesics. Psychosomatics. 2009;50(2):169–176.
18) Flores JA, Banoua FE, Beatriz Gala´n-Rodrı´guez, Emilio Fernandez-Espejo. Opiate anti-nociception is attenuated following lesion of large dopamine neurons of the periaqueductal grey: critical role for D1 (not D2) dopamine receptors. Pain. 2004;110(1-2):205-14
19) Furst S, Gyires K, Knoll J. Analgesic profile of rimazolium as compared to different classes of pain killers. Arzneimittel-Forschung 1988;38(4):552–7.