**Original article**

# Evaluation of analgesic activity of Dashamoola formulation by using experimental models of pain

**Pramod P. Bhalerao[1], Rajendra B. Pawade[2], Shirish Joshi[3]**

[1]Assistant Professor, [2]Associate Professor, Department of Pharmacology, Rural Medical College of Pravara Institute of Medical Sciences (DU), Loni, Maharashtra, India

[3]Professor, Department of Pharmacology, Seth G. S. Medical College & KEM Hospital, Mumbai

Correspondence author: Dr. Pramod P. Bhalerao

**Abstract:**

**Objectives:** The study was done to evaluate the analgesic activity of Dashamoola formulation by using Hot plate model in mice, Tail clip model in mice, Tail immersion model in rats.

**Methods:** The experimental study was carried out in mice of either sex weighing between 20-25 g and rats of either sex weighing between 170-250g. According to Ayurveda, Dashamoolarishtha is administered on full stomach. The animals were fed by Dashamoolarishtha only on full stomach. The doses of which were computed from the doses documented in the Ayurvedic and standard textbooks. Commercially available preparation of Dashamoolarishta was used in group compared with vehicle control (Distilled Water), Pentazocine, Dashamoolarishtha 1(D1) [Low Dose], Dashamoolarishtha 2(D2) [High Dose] by using Hot plate method, Haffner's tail clip method in mice and Tail immersion test.

**Results:** The present study has shown that Dashamoolarishta an Ayurvedic multi-ingredient formulation can be used as an analgesic.

**Conclusion:** Administration of Dashamoolarishtha significantly showed analgesic activity in 3 different models of pain which are centrally induced. This study demonstrates that Dashamoolarishtha in low and high dose was found to normalize pain.

**Keywords:** Dashamoolarishtha, Pentazocine, NSAIDs

## Introduction:

Pain has been defined by the International Association for the Study of Pain (1979) as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage."[1] Zimmermann (1986) re-interpreted the IASP definition of pain so that it could be applied to animals: "an aversive sensory experience caused by actual or potential injury that elicits progressive motor and vegetative reactions, results in learned avoidance behavior, and may modify species specific behavior, including social behavior."[2] Pain is an unpleasant sensation localized to a part of body. It is often described in terms of a penetrating or tissue-destructive process (stabbing, burning, twisting, tearing, squeezing) and/or of a bodily or emotional reaction (e.g., terrifying, nauseating, and sickening). Furthermore, any pain of moderate or high intensity is accompanied by anxiety and a urge to escape or terminate the feeling. These properties illustrate the duality of pain: it is both sensation and emotion. When acute, pain is characteristically associated with behavioral arousal and a stress response consisting of

increased blood pressure, heart rate, pupil diameter and plasma cortisol levels. In addition, local muscle contraction (limb flexion, abdominal wall rigidity) is often presented.[3] 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 are not uniformly effective, and undesirable side effects often limit their use. Consequently, one long-standing focus of drug discovery has been the search for novel analgesics.[1]

Chronic painful conditions (like gout, rheumatoid arthritis etc) are a few of the oldest known diseases of mankind affecting majority of population. No substantial progress has been made in achieving permanent cure of these conditions. Although a number of potent synthetic drugs are available they afford only symptomatic relief and their toxic effects pose limitation to continuous use. Therefore the search for better analgesic and anti-inflammatory agent is ongoing. More and more research is being focused on developing drugs from indigenous medicinal plants. 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 and provide necessary information about their therapeutic use. Herbal medicines derived from the plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available.

A bibliographic survey showed that there are fewer reports on the analgesic activity of Dashamoolarishtha. Dashamoolarishtha, an Ayurvedic multi-ingredient formulation is used as an analgesic, anti-arthritic and in rheumatism.[4] This prompted us to investigate the effects of pharmacological activities of Dashamoolarishtha in experimental models of pain. The purpose of the present study was to evaluate the possible antinociceptive activity of Dashamoolarishtha in several animals' models of nociception.

**Objectives:**

The objective of the study was to evaluate the analgesic activity of Dashamoola formulation by using -Hot plate, Tail clip model in mice and Tail immersion model in rats.

**Materials 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.

**Randomization**: Randomly selected at the time of delivery.

**Animal Identification**: By cage number and individual marking on tail.

**Husbandry conditions**: Air conditioned with 12-15 filtered fresh air changes per hour, temperature 22±3 $^{o}$ C, humidity 30-70 %. A 12-h light–dark cycle was maintained throughout the experimental protocol.

**Accommodation**: Experimental animals- mice 8 per cage and rats 4 per cage were housed during acclimatization and treatment (approx. sized cage 1.290 X W 220 X 1+ 140 mm). The cages were of



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stainless steel top grill having facilities for Rodent food and water (Aqua guard pure water) ad libitum.

**Acclimatization**: Seven days prior to start of the treatment.

**Animals required for study**: 24 mice and 24 rats.

According to Ayurveda, Dashamoolarishtha is administered on full stomach. The animals were fed by Dashamoolarishtha only on full stomach.

The samples of Dashamoolarishtha were obtained from Sandu Brothers. The certificate of analysis was approved from the same company.

The doses of which were computed from the doses documented in the Ayurvedic and standard textbooks.[5]

Vehicle control: Distilled water was administered for an equivalent period.

Commercially available preparation of Dasha-moolarishta was used.

**Study groups -**

**The experimental groups in each model were as follows:**

(n = 6 animals in each group)

**Group 1**: Vehicle control (Distilled Water)

**Group 2**: Pentazocine

**Group 3**: Dashamoolarishtha 1(D1) [Low Dose]

**Group 4**: Dashamoolarishtha 2 (D2) [High Dose]

**Study drugs and their doses in mice and rat**

| Sr. no. | Study Drugs | Dose in mice | Dose in rat |
|---|---|---|---|
| 1 | Distilled water (ml/kg) - Vehicle | 10 | 10 |
| 2 | Pentazocine (mg/kg) – Positive Control | 7.8 | 5.4 |
| 3 | Dashamoolarishta 1 (ml/kg) | 1.56 | 1.08 |
| 4 | Dashamoolarishtha 2 (ml/kg) | 3.12 | 2.16 |

**1) Hot plate method[6]**

**Experimental procedure:**

Groups of Swiss mice of either sex weighing between 20-25 g were used for each dose. The commercially available Eddy's hot plate consists of an electrically heated surface. The temperature is controlled at 55-56°C.

The animals are placed on a hot plate and the time until either licking or jumping occurs is recorded by a stop-watch. Only those mice which react in 5s are selected. 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 should not to be placed on hot plate for more than 15 s (cut off time) to avoid damage to the paw.



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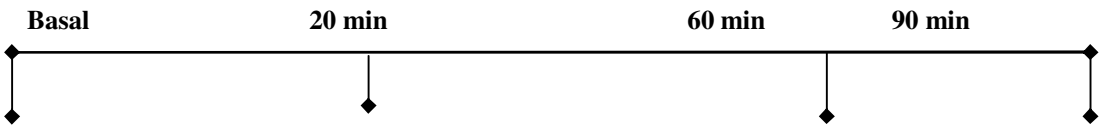
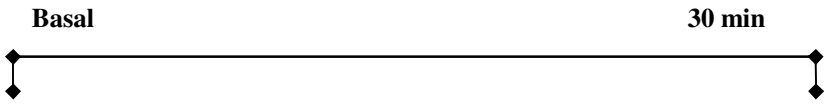



**2) Haffner's tail clip method in mice[6]**

**Experimental procedure:**

Group of Swiss mice of either sex weighing between 20-25 g were used. The test compounds are administered orally to mice. The drug is administered 30 min prior to testing.

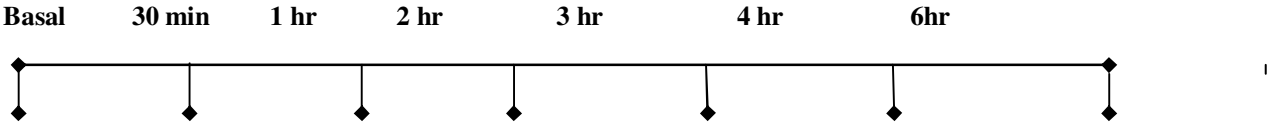
An artery clip is applied to the root of the tail (approx 1 cm from the body) to induce pain. The animal quickly responds to this noxious stimulus by biting the clip or the tail near the location of the clip. The time between onset of stimulation and the response is measured by stopwatch.




**3) Tail immersion test [6]**

**Experimental procedure:**

Wistar rats of either sex weighing between 170-250 g were used. They are placed into individual cylindrical rat holders leaving the tail hanging out freely. The animals are allowed to get acclimatized to rat holders for 30 min before testing. The lower 5 cm portion of the tail is marked. This part of the tail is immersed in a cup of freshly filled water at 55°C. The reaction time is recorded using a stop-watch. After each determination the tail is carefully dried. The reaction time is determined before and periodically 0.5, 1, 2, 3, 4 and 6 hr after the oral administration of test substance. The cut off time of immersion is 15 s.




**Results:**

Results of the present study have shown that Dashamoolarishta an Ayurvedic multi-ingredient formulation can be used as an analgesic. Dashamoolarishtha high dose (D2) showed statistically significant values at 20 min ($p< 0.01$) as compared to control (Distilled water) in hot plate Model. The findings were repeated in Tail clip Model where Dashamoolarishtha low dose (D1) showed statistical significant values ($p<0.05$) as compared with control (Distilled water) at 30 min. Dashamoolarishtha high dose (D2) showed statistically significant values ($P<0.001$) as compared with control (Distilled water) at 30 min. In tail Immersion Model also the same thing happened in which Dashamoolarishtha low dose (D1) showed statistically significant values at 30 min ($p<0.01$) and at 60 min ($p<0.05$) as compared to control. Dashamoolarishtha high dose (D2) showed statistically significant values at 30 min ($p<0.001$), at 1 hr ($p<0.001$) and at 2 hrs ($p<0.05$) as comp control.

Table 1

| 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# |

**Effect of Dashamoolarishtha on the reaction time in Hot plate method**

Values are expressd as Mean±S.D, n=6

One way ANOVA followed by Tukey-Kramer Multiple Comparisons Test

**= p<0.001, *= p<0.01 ( as compared to control)

@@@= p<0.001, @@ = p<0.01, @= p<0.05 (PZ Vs D1)

# = p<0.05 (PZ Vs D2)

[DW= Distilled water, PZ= Pentazocine, D1= Dashamoolarishtha 1, D2= Dashamoolarishtha 2]

**Hot Plate Model**

- Hot plate test was done and readings at four time periods were done i.e. basal, 20, 60 and 60 min. Dashamoolarishtha high dose (D2) showed statistically significant values at 20 min (p< 0.01) as compared to control (Distilled water).
- Dashamoolarishtha low dose (D1) did not show any statistical significance as compared to control at all time periods.
- Similarly Dashamoolarishtha was also compared with pentazocine. Pentazocine showed statistically significant values at 20 min (p< 0.01), at 60 min (p<0.05) and at 90 min (p<0.0001) as compared to Dashamoolarishtha low dose (D1). Pentazocine showed statistically significant values at 90 min (p<0.05) as compared to Dashamoolarishtha high dose (D2).
- Dashamoolarishtha high dose (D2) did not showed any statistically significant values as compared to Dashamoolarishtha low dose (D1) at all time periods. (As shown in Table-1)

**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 |

Cont.....

| | | |
|---|---|---|
| PZ | 3.16 ± 1.33 | 12.62 ± 2.37** |
| D1 | 3.95 ± 0.53 | 6.8 ± 2.03*, @ |
| D 2 | 3.81 ± 1.14 | 10.03 ± 2.09**, £ |

Values expressd as Mean±S.D. (n=6).

One way ANOVA followed by Tukey-Kramer Multiple Comparisons Test.

**= p<0.001, *= p<0.05 (as compared to control)

@ = p<0.001 (PZ VS D1)

£=p<0.05 (D2 Vs D1)

[DW= Distilled water, PZ= Pentazocine, D1= Dashamoolarishtha 1, D2= Dashamoolarishtha 2]

**Tail clip Model**

- In this test, basal readings were taken and were compared with the readings 30 min after giving the drug. Basal values were comparable.
- Dashamoolarishtha low dose (D1) showed statistical significant values (p<0.05) as compared with control (Distilled water) at 30 min.
- Dashamoolarishtha high dose (D2) showed statistically significant values (P<0.001) as compared with control (Distilled water) at 30 min.
- Pentazocine showed statistically significant values as compared to Dashamoolarishtha low dose (p<0.001) at 30 min.
- Pentazocine did not show any statistical significant values as compared to Dashamoolarishtha high dose (D2) at 30 min.
- Dashamoolarishtha high dose (D2) showed statistically significant values as compared with Dashamoolarishtha low dose (D1) (p<0.05) at 30 min. (As shown in Table -2)

**Table 3 : Effect of Dashamoolarishtha on the reaction time in Tail immersion method**

| Group | 0 min | 30 min | 1 hr. | 2 hr. |
|---|---|---|---|---|
| DW | 3.49±0.47 | 3.1±0.83 | 3.76±0.57 | 3.88±0.71 |
| 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 expressd as Mean±S.D. (n=6).

One way ANOVA followed by Tukey-Kramer Multiple Comparisons Test.

***=p<0.001, **=p<0.01, *=p<0.05 (as compared to control)

@ = p<0.05 (PZ Vs D1)

[DW= Distilled water, PZ= Pentazocine, D1= Dashamoolarishtha 1, D2= Dashamoolarishtha 2]

**Tail Immersion Model**

- In this test after taking Basal readings, the readings were taken at 30 min, 1 hr, 2 hr, 3 hr, 4 hr and 6 hrs after giving drug.
- Dashamoolarishtha low dose (D1) showed statistically significant values at 30 min (p<0.01) and at 60 min (p<0.05) as compared to control.
- Dashamoolarishtha high dose (D2) showed statistically significant values at 30 min (p<0.001), at 1 hr (p<0.001) and at 2 hrs (p<0.05).
- Pentazocine showed statistically significant values at 1 hr (p<0.05) and at 2 hr (p<0.05) as compared to Dashamoolarishtha low dose (D1). All the values were comparable at 3 hr, 4 hr and 6 hr. (As shown in Table -3)

**Discussion:**

Complementary and Alternative Medicines (CAMs) include 'spirituality faith healing - prayers, physiotherapy, numerous relaxation therapy, chiropractic, acupuncture, massage therapy, dietary or nutraceutical supplements, Homeopathy, Unani, herbs/ herbal formulations and Ayurvedic medicines'.[7] Crude drugs and finished herbal products are often used as a part of CAMs and are found marketed as herbal medicines or dietary supplements for their claimed therapeutic effects. However, in most cases the claims have not been substantiated and only a few herbal medicines have been subjected to double-blind, randomized, placebo-controlled clinical trials. It is important to be aware that most herbal medicines fall outside the regulatory framework which exempts them from safety and efficacy requirements and regulation. Hence, evidence is generally lacking on their safety, efficacy or standards of manufacture and control. Acute painful conditions include injury, surgery, illness, trauma, or painful medical procedures.

The pain generally lasts for a short period and usually disappears after treatment of the underlying cause or after adequate time for healing. However, acute

may lead to chronic pain problems, which exist beyond an expected time for healing.[8] Therefore the search for better analgesic and anti-inflammatory agent is ongoing. More and more research is being focused on developing drugs from indigenous medicinal plants. The major class of analgesics used in the management of moderate to severe pain is NSAIDs and Opioids. They are associated with many side effects and provide only symptomatic relief. Common side effects of NSAIDs include gastrointestinal toxicity, stomach ulcers, and gastric bleeding. Inhibition of platelet cyclooxygenase can result in decreased hemostasis as well as surgical bleeding. Renal dysfunction can occur with prolonged and excessive use of NSAIDs. Other adverse effects of NSAIDs include hepatic dysfunction or necrosis, asthma, vasomotor rhinitis, angioneurotic edema, urticaria, laryngeal edema, and even cardiovascular collapse. Cardiovascular risks of NSAIDs, especially COX-2 inhibitors, have become a major focus of attention over the past several years. Common side effects of opioids include constipation, sedation, nausea, vomiting, and respiratory depression due to overdose. Occasionally, opioids may cause myoclonus, seizures, hallucinations, confusion, sexual dysfunction, sleep disturbances, and pruritus. Tolerance and physical dependence should be expected with long-term opioid treatment.[8]

Dashamoolarishta, an Ayurvedic multi-ingredient formulation is used as an analgesic, antiarthritic, cough and rheumatism.[4] This formulation contains roots of ten different plants. These may be serving different roles like active principles, adjuvant, carrier agent and stabilizer.[9] Dashamoolarishtha is a classical Ayurvedic preparation. It 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 Ayurvedicsystem of medicine it is used as analgesic, antiarthritic, against cough, rheumatism, etc.[4] 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 e.g., Oroxylum indicum[10] Desmodium angeticum, Premna integrifolia L. and Gmelina arborea[11], Aegle Marmelos[12,13,14] T. terrestris[15] , Solanum xanthocarpum, Premna Integrifolia.[16] However, surprisingly the widely prescribed Dashamoolarishta has never been evaluated in any of the experimental and clinical study. Hence it was of interest in pharmacological profiling of this classical Ayurvedic formulation through the present study.

Two doses of Dashamoolarishta were selected (low dose and high dose) for the present study. The doses were extrapolated from the human dose used in the clinical practice. Studies by Parekar et al. has shown anti-inflammatory activity of Dashamoolarishtha by using cotton pellet induced granuloma and carrageenan induced rat paw edema models of inflammation.[7] This shows that Dashamoolarishtha has peripheral action by inhibiting the inflammatoryprocess. But it was not effective in acetic acid induced writhing model. The justification for the negative result was not provided. However in the same study, Dashamoolarishtha when combined with aspirin showed positive results i.e. reduction in number of writhes. The study showed that Dashamoolarishtha has peripheral analgesic activity probably by inhibiting the inflammatory process. Hence it was decided to evaluate the central ana activity of Dashamoolarishtha by using 3 di central pain models i.e. hot plate, tail clip and tail

immersion in hot water. Several methods are available for testing analgesic activity in animals, such as -

1) Haffner's tail clip method in mices
2) Tail flick or other radiant heat methods
3) Hot plate methods in mice or rats
4) Tail immersion tests
5) Electrical stimulation (stimulation of tail or tooth pulp ,grid shock)
6) Formalin test in rats
7) Writhing tests
8) Pain in inflamed tissue (Randall-Selitto-test).[6]

Of these tests, we selected 3 models of pain i.e. hot plate method, Haffner's tail clip method and tail immersion in hot water. The rationales of choosing these models are given below:

**Hot plate method-**

The hot plate test has been used by many investigators and has been found to be suitable for evaluation of centrally but not of peripherally acting analgesics. Mice as well as rats have been used. The method has the drawback that sedatives and muscle relaxants or psychotomimetics cause false positives, while mixed opiate agonists-antagonists provide unreliable results. The validity of the test has been shown even in the presence of substantial impairment of motor performance. Mixed opiate agonists-antagonists can be evaluated if the temperature of the hot plate is lowered to 49.5 °C.

**Tail clip method**-

The test does not need any sophisticated equipment but a skilled, preferably "blind", observer. Peripheral analgesics of the salicylate type are not detected by this test. Tail-clip test is useful in elucidating centrally mediated antinociceptive responses, which focuses mainly on changes above the spinal cord level.[17]

**Tail immersion test**-

The test is useful to differentiate central opioid like analgesics from peripheral analgesics.

Thus, all the 3 models are used for testing central analgesic activity.

**Mechanical stimulation**-

The application of a noxious mechanical stimulus can be progressive or coarse. Responses produced by noxious mechanical stimuli are graded in relation to the intensity and/or duration of the stimulus, from reflexes up through vocalizations ultimately to complex motor behaviors. The stimulus is stopped as soon as a response is obtained. The mechanical stimulus has the disadvantage of activating low-threshold mechanoreceptors as well as nociceptors. Consequently, the stimulus is not specific. There are also technical difficulties in applying mechanical stimuli, especially in freely moving animals. In addition, when mechanical stimuli are truly nociceptive, they are likely to produce changes in the tissues (sensitization or actual lesions).[2]

**Pentazocine-**

Pentazocine was synthesized as part of a deliberate effort to develop an effective analgesic with little or no abuse potential. Pentazocine is a Synthetic Opiate with Partial agonist/antagonist activity. The pattern of CNS effects produced by pentazocine generally is similar to that of the morphine-like opioids, including analgesia, sedation, and respiratory depression. The analgesic effects of pentazocine are due to agonistic actions at opioid receptors. As it has analgesic activity with little or no abuse potential, it was selected as a positive control.[18] Pentazocine has worked as a positive control in all the three models of pain proving its basis of use in certain types

It has been well established that their relationship between central pain and action of

opioids. Administration of Dashamoolarishtha significantly produced analgesia caused by different models of pain which are centrally induced. This study demonstrates that Dashamoolarishtha in low and high dose was found to normalize pain. However, analgesic activity of high dose was superior as compared to low dose in all the pain models. It can be postulated that Dashamoolarishtha showed analgesic activity due to analgesic property of its individual plants. Further isolation of active components and its molecular activity in pain model have to be evaluated for its possible clinical application.

**Conclusion :**

Pentazocine has worked as a positive control in all the three models of pain proving its basis of use in certain types of pain. It has been well established that their exist a relationship between central pain and action of opiods. Administration of Dasham-oolarishtha revealed analgesic activity in three different models of pain which are centrally induced. This study demonstrates that Dashamoolarishtha in low and high dose was found to normalize pain. However, analgesic activity was significantly seen at high dose as compared to low dose. It can be postulated that Dashamoolarishtha showed analgesic activity due to analgesic activity of its individual plants. Further isolation of active components and its molecular activity in pain model have to be evaluated for its possible clinical application.

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