Investigation of the central and peripheral analgesic and anti-inflammatory activity of Draksharishta an Indian Ayurvedic formulation

Abstract

Rationale: Draksharishta (DRK) is an Ayurvedic formulation approved by the “National formulary of Ayurvedic Medicine 2011”, of Bangladesh. It is widely available in the Bangladeshi market as an effective preparation to treat lumbago, sciatica and arthritic pain of joints. But there are very scientific evidences available to support their common uses. Objectives: Our present studies make an attempt toward identifying probable antinociceptive and anti-inflammatory effect and its mechanisms of DRK. Findings: DRK, at three doses, (10 mL/kg, 20 mL/kg, and 40 mL/kg) showed no involvement of the CNS in antinociceptive activity of the test drug. Both Carrageenan-induced paw edema and acetic acid writhing tests gave significant results ($P < 0.05$), indicating possible peripheral analgesic and anti-inflammatory action. Formalin-induced paw-licking test showed that DRK had significant effect in suppressing inflammatory pain ($P < 0.05$) but not neurogenic pain. Conclusions: Hence our study shows anti-inflammatory and peripheral analgesic action for DRK.

Key words: Anti-inflammatory, anti-nociceptive, central analgesic, Draksharishta, peripheral analgesic

Introduction

World Health Organization (WHO) has stated that up to 80% of the population in many Asian and African countries depend on traditional and complimentary drugs to meet their medical necessities.[1] It is also an extremely attractive business for many drug vendors which often results in misleading claims being made and confusion in the mind of consumers. Persistent continuation of a regimen with one of these drugs which do not have any pharmacological activity, in reality, would seriously aggravate the morbidity of the patients. For these reasons and others, there has been a demand for ensuring the safety and efficacy of some of these traditional/herbal medicines.[2] Under the status quo, these products are often sold under hyperbolic and outrageous claims without much scientific evidences.[3] In this paper, we analyzed the analgesic and anti-inflammatory property of Draksharishta (DRK), a commonly available herbal product licensed under the Directorate General of Drug Administration (DGDA) of Bangladesh.

Pain has been defined by The International Association for the Study of Pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage.[4] This process enables an individual to take protective measures, by providing with rapid awareness about threatening or potentially threatening injury.[5] However, if the painful sensation remains after removal of the detectable stimulus, it calls for a regimen for pain management.[6]

DRK is included in the Bangladesh National Formulary of Ayurvedic Medicine 2011 (2nd Ed.).[7] It is primarily indicated in rheumatoid arthritis, lumbago (low-back pain) and sciatica (pain which may arise from compression and/or irritation of one of five spinal nerve roots which give rise to each sciatic nerve).[7] We have used the following in vivo animal models to test the analgesic and anti-inflammatory effect of the test drug: Hot Plate test, Tail Immersion test, Formalin-induced Paw licking, Carrageenan-induced Paw Edema test, Acetic Acid Writhing test.

Access this article online

Website: www.jbclinpharm.org

DOI: 10.4103/0976-0105.105335

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Materials and Methods

Reagents used
All reagents and chemicals that were used in the experiments were of analytical grade. DRK was procured from University Ayurvedic Research Centre, Jahangirnagar University, Savar, Bangladesh. Pharmaceutical grade Tramadol, and Diclofenac Sodium were collected from Square Pharmaceuticals Bangladesh Ltd. All other reagents were procured from Sigma Aldrich (USA) unless mentioned otherwise.

Dose and route of administration
0.9% NaCl was administered to the animals per oral (p.o.) at a volume that would not cause any additional psychological or physiological stress to the animals. For experimental purpose 10 mL/kg, 20 mL/kg, and 40 mL/kg doses of DRK were used. The drug proved to be nonlethal at all doses in previous toxicity screening studies.

Maintenance and use of test animals
Healthy Swiss Albino mice (5-6 weeks old, of both sex) weighing 20-25 g and Sprague-Dawley rats, weighing 130-160 g, were procured from Jahangir Nagar University Animal House. The test subjects were provided with standard rat pellet diet and filtered drinking water ad libitum. This study was approved by an ethics committee of North South University which gave its consent in absolute accordance with the recommendations of the International Association for the study of Pain.[18]

Grouping and drug administration
The animals were randomly divided into several groups of 8 mice/rats for the planned analgesic and anti-inflammatory tests. Control groups were treated p.o. with 0.9% NaCl. Positive controls were treated with Tramadol and Diclofenac Sodium. Treatment groups were treated with three doses (10 mL/kg, 20 mL/kg, and 40 mL/kg) of DRK.

Determination of CNS modulation in analgesic activity

**Hot plate test**
The Hot plate test was performed on the test subjects in a constant temperature water bath at 50 ± 0.2°C. The reaction time, i.e., the amount of time it takes the animal to withdraw its tail was measured. DRK (10 mL/kg, 20 mL/kg, and 40 mL/kg p.o.), Tramadol (10 mg/kg p.o.), and 0.9% NaCl (p.o.) were administered to treatment groups. Naloxone (5 mg/kg i.p.) was administered with DRK (10 mL/kg, 20 mL/kg, and 40 mL/kg p.o.) and Tramadol to four different groups, other than the treatment groups.

**Tail immersion test**
The tail immersion test was performed according to the procedures used by Yang et al.,[10] with minor modifications. Briefly, the lower two-third of mouse’s tail was immersed in a constant temperature water bath at 50 ± 0.2°C. The reaction time, i.e., the amount of time it takes the animal to withdraw its tail was measured. DRK (10 mL/kg, 20 mL/kg, and 40 mL/kg p.o.), Tramadol (10 mg/kg p.o.), and 0.9% NaCl (p.o.) were administered to treatment groups. Naloxone (5 mg/kg i.p.) was administered with DRK (10 mL/kg, 20 mL/kg, and 40 mL/kg p.o.) and Tramadol to four different groups, other than the treatment groups.

**Dissociation between CNS and peripheral analgesic activity**

**Acetic-acid induced writhing test**
The test was carried out using a modified method from the procedure previously described.[11] DRK at three doses (10 mL/kg, 20 mL/kg, and 40 mL/kg p.o.) were administered to treatment groups. Positive control group was administered with Diclofenac sodium (10 mg/kg p.o.) and 0.9% NaCl p.o. was administered to the control group. Forty-five minutes after drug treatment, the mice were given 0.7% v/v acetic acid (0.15 mL/10 mL i.p.) to induce writhing.

**Carrageenan-induced paw edema test**
Carrageenan-induced paw edema test was carried out by following the method described previously.[13] Male and female Sprague-Dawley rats were used. The control rats received 0.9% NaCl p.o. and the experimental rats received DRK (10 mL/kg, 20 mL/kg, and 40 mL/kg p.o.). Thirty minutes later, the rats were given a subcutaneous injection of 0.05 mL of 1% solution of carrageenan.

**Statistical analysis**
Results were expressed as mean ± SEM (standard error of mean) of responses. All tests were done using SPSS Software Ver. 20. For Hot Plate test, Tail Immersion test, and Carrageenan-induced Rat Paw Edema test, Statistical significance was determined by Repeated Measures One-way Analysis of Variance (ANOVA) followed by post hoc Dunnett test. Later, Pair-wise comparison test along with Bonferroni correction were done. For Acetic-acid-induced writhing test
and Formalin test, Statistical significance was determined by One-way Analysis of Variance (ANOVA) followed by post hoc Dunnett test. Then Pair-wise comparison test along with Bonferroni correction were done. The P-values less than 0.05 were considered to be significant.

Result

Hot-plate test
In the Hot Plate Test, DRK treatment caused no significant increase in analgesia. In the presence of Naloxone, an antagonist of opioid receptor, the effect of Tramadol was reduced profoundly as shown in Table 1.

Tail immersion test
Table 2 shows that the analgesic effect of DRK was also not significant in Tail immersion test. DRK failed to induce any “tail flick antinociceptive” index.

Acetic acid-induced writhing test
Intraperitoneal injection of 0.7% acetic acid given to the control group caused 16.83 ± 0.87 writhes in a 5-minute interval. The treatment with DRK induced a significant decrease, with a 43.56% (P < 0.05) inhibition observed in the 20 mL/kg group and 84.16% (P < 0.01) in the 40 mL/kg group [Figure 1].

Carrageenan induced paw edema test
The injection of carrageenan at rat paw created an edema that increased gradually [Table 3]. DRK 20 mL/kg showed 20.80% and 23.10% reduction in the volume of the edematous paw at 4 h and 5 h after carrageenan injection, respectively. Whereas, DRK 40 mL/kg showed significant anti-inflammatory activity starting from 2 h after the injection of carrageenan to throughout the experiment time with a highest reduction of 36.20% (5 h after the carrageenan injection).

Formalin-induced paw-licking test
In the Formalin-induced paw-licking test, DRK treated mice groups except 10 mL/kg group showed significant activities in the later phase pain responses (20 mL/kg 65.06% and 40 mL/kg 79.66%) compared to that of the control group [Table 4]. All three doses (10 mL/kg, 20 mL/kg, and 40 mL/kg) of DRK failed to induce any significant analgesic activity at early phase of the experiment. In combination studies using Naloxone, an antagonist of opioid receptor, the analgesic activity of the Tramadol was diminished in both phases. The analgesic activity of Diclofenac Na was not diminished by the co-treatment with Naloxone. Cotreatment with naloxone also did not affect the analgesic activity of DRK in the later phase of the experiment, suggesting that there might be no involvement of opioid receptor in the analgesic activity of DRK.

Discussion
Two well-known models of thermal nociception, hot-plate test and tail immersion test were employed to double check on possible involvement of spinal, supraspinal pathways, and m-opiate receptor agonism in regulation (CNS modulation) of pain response by DRK. Our findings demonstrated no activity of in either model. Hence, probable involvement of the central nervous system, in this case, could be ruled out.

To reinforce the above findings, we employed the formalin induced paw-licking test. This test is capable of discerning...
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between nurogenic pain (early phase, acute, non-inflammatory and CNS modulated) and inflammatory (chronic and peripheral pain).[15-17] The neurogenic pain (first phase) is caused by direct chemical stimulation of nociceptive afferent fibers (predominantly C fibers) which can be suppressed by opiate like morphine.[18] The inflammatory pain (second phase) is caused by the release of inflammatory mediators like histamine, prostaglandins, bradykinin, serotonin in the peripheral tissues,[19] and from functional changes in the spinal dorsal horn.[20] Our results showed that DRK had no effect on neurogenic pain suppression (first phase) but had effective antinociceptive effect in the peripheral inflammatory (second phase) pain. Cotreatment with naloxone partially blocked the activity of Tramadol in both the phases while that of DRK and Diclofenac Sodium remained unaffected. Hence, we have definitive evidence to conclude that DRK has no CNS-modulated pain suppression activity; however, probably has significant peripheral analgesic and anti-inflammatory effect.

To further ascertain its anti-inflammatory activity, we performed the Acetic Acid-induced writhing test and carrageenan-induced paw edema test. Carrageenan-induced edema is commonly used as an experimental model for acute inflammation, and is proven to be biphasic.[21] The early phase (1-2 hours) of the carrageenan model is chiefly mediated by serotonin and histamine release and increased synthesis of prostaglandins in the damaged paw tissues. These induce inflammation and paw swelling. The later phase is sustained by prostaglandin release and is also mediated by bradykinin, leukotrienes, poly-morphonuclear cells, and prostaglandins produced by tissue macrophages.[22] DRK showed, in a dose-dependent manner, significant peripheral analgesic activity at the end of the early phase (2h) and throughout the later phase indicating its possible ability to hinder endogenous synthesis or release of inflammatory mediators such as prostaglandins, histamine, serotonin, bradykinin and leukotrienes.

The acetic acid induced writhing test was carried out to confirm the peripheral analgesic activity of DRK. The acetic acid used in this test increased the prostaglandin level (mainly

Table 3: Effect of DRK on anti-inflammatory responses in carrageenan-induced rat paw edema test

| Group | Dose | 0 min | 30 min | 1 h | 2h | 3h | 4h | 5h |
|-------|------|-------|--------|-----|----|----|----|----|
| Control | – | 0.80 ± 0.02 | 0.94 ± 0.09 | 1.02 ± 0.09 | 1.16 ± 0.11 | 1.20 ± 0.06 | 1.25 ± 0.09 | 1.30 ± 0.09 |
| DRK 10 ml/kg | 0.77 ± 0.05 | 0.98 ± 0.02 | 0.99 ± 0.03 | 1.05 ± 0.11 | 1.09 ± 0.10 | 1.12 ± 0.08 | 1.16 ± 0.02 |
| DRK 20 ml/kg | 0.79 ± 0.04 | 1.03 ± 0.06 | 0.93 ± 0.03 | 0.94 ± 0.11 | 0.96 ± 0.11 | 0.98 ± 0.09 | 1.00 ± 0.06 |
| DRK 40 ml/kg | 0.77 ± 0.02 | 0.96 ± 0.06 | 0.92 ± 0.10 | 0.90 ± 0.09 | 0.92 ± 0.14 | 0.90 ± 0.13 | 0.83 ± 0.04 |
| Diclofenac Na 10 mg/kg | 0.76 ± 0.05 | 0.94 ± 0.03 | 0.95 ± 0.21 | 0.90 ± 0.14 | 0.89 ± 0.11 | 0.85 ± 0.12 | 0.78 ± 0.09 |

Values are expressed as Mean ± S.E.M. (n = 8). Differences between groups are determined by One-Way Repeated Measures ANOVA followed by post hoc Dunnett test; and then pair-wise comparison tests were done with Bonferroni correction. *P < 0.05 compared to the control-treated group

Table 4: Effect of DRK on nociceptive response in the formalin test

| Treatment Group | Dose | Early phase | Later phase |
|-----------------|------|-------------|-------------|
|                 | Licking time (s) | Inhibition (%) | Licking time (s) | Inhibition (%) |
| Control | – | 84.50 ± 12.79 | – | 43.42 ± 13.50 |
| DRK 10 mL/kg | 71.50 ± 10.33 | 15.38 | 33.67 ± 2.64 | 22.46 |
| DRK 20 mL/kg | 67.33 ± 9.94 | 20.32 | 15.17 ± 0.91* | 65.06* |
| DRK 40 mL/kg | 69.50 ± 10.67 | 17.75 | 8.83 ± 1.11** | 79.66** |
| Tramadol 10 mg/kg | 4.17 ± 1.49** | 95.07** | 6.50 | 33.83 ± 0.83** |
| Diclofenac Na 10 mg/kg | 62.50 ± 8.65 | 26.04 | 5.50 | 87.33** |
| Cotreatment with naloxone | DRK + Naloxone 40 mL/kg | 79.00 ± 11.25 | 6.50 | 8.33 ± 0.83** |
| Tramadol + Naloxone 10 mg/kg | 67.83 ± 8.10 | 19.72 | 36.50 ± 4.18 | 15.94 |
| Diclofenac Na + Naloxone 10 mg/kg | 71.83 ± 5.87 | 15.00 | 4.83 ± 1.08** | 88.88** |

Values are expressed as Mean ± S.E.M. Differences between groups are determined by One-Way ANOVA followed by post hoc Dunnett test. *P < 0.05 and **P < 0.01 compared to the control-treated group
PGE₂) in the peritoneal fluid of the mice. Prostaglandins induce abdominal constriction by activating and sensitizing the peripheral chemo-sensitive nociceptors which are mostly responsible for causing inflammatory pain. In our study, DRK, significantly attenuated the writhing in mice in response to acetic acid administration (i.p.), although to a slightly lesser extent compared to the highly potent diclofenac sodium. Hence, the analgesic and anti-inflammatory action of DRK can be attributed to reduction of peripheral nociception by inhibition of prostaglandin release.

Conclusions

In summary, our present study has successfully elucidated the likely mechanism of antinociceptive and anti-inflammatory effect of Draksharishta. We have drawn a sound conclusion that DRK does not have any CNS-modulated effect in pain inhibition, based on three different in vivo models. Its peripheral analgesic activity has been also repeatedly confirmed by three in vivo models. Through this study, it is apparent that the mechanism of action of DRK is similar to that of the commonly used NSAIDs. Hence, its traditional use in arthritis, sciatica, and lumbago held the test of time, not by its mere placebo effect but by some potent analgesic and anti-inflammatory molecules hidden in this age-old Ayurvedic concoction. We believe further studies are required to elucidate the complete pharmacological profile of this potential analgesic preparation for safer and more effective use.

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