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ORIGINAL ARTICLE **OPEN ACCESS**

Rubia Cordifolia Augments Pain Perception Threshold in Paclitaxel-Induced Neuropathic Pain in Experimental Animal Models

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ABSTRACT
Neuropathic pain is triggered or produced by a nerve system lesion or dysfunction. Rubia cordifolia Linn is known to possess analgesic and anti-oxidant activity. The effect of an alcoholic extract of R. cordifolia roots and rhizomes (RC) on the development and perception of paclitaxel-induced neuropathic pain was studied using sub-acute anti-nociceptive activity and cold allodynia model in mice. A peripheral mononeuropathy was induced in Swiss albino mice using Paclitaxel (2 mg/kg, i.p.). Writhing was induced by Paclitaxel in combination with 0.6% v/v acetic acid (10 ml/kg, i.p.), heat hyperalgesia was induced by hot water maintained at 55 ± 0.5ºC and cold allodynia was induced using ice slab. Co-administration of AERC for 8 days resulted in significant reduction in nociception in above mentioned models indicating its usefulness in neuropathic pain.
Keywords: *Acetic acid induced writhing, hyperalgesia, cold allodynia, neuropathic pain, paclitaxel, Rubia cordifolia.*

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INTRODUCTION

Nociception involves the activation of nociceptors at free endings of primary sensory neurons, generation of action potential, and transmission of action potential to the dorsal horn, second-order neuron activation to transit the signal to the thalamus and parabrachial nucleus in the brain, and third-order neuron transmission of the signal to the cerebral cortex via spinothalamus.[1] Nerve pain includes various types of pain in that it is induced by a disease of the non-neural tissues. Conditions includes osteoarthritis and inflammatory pain are cases of non-neuropathic pain entities known as nociceptive pain. By definition, neuropathic pain can be caused by a profound neural lesion. Numerous diseases could be at responsible. Autoimmune disease (e.g., multiple sclerosis), metabolic diseases (e.g., diabetic neuropathy), infection (e.g., shingles and the sequel, postherpetic neuralgia), vascular disease (stroke), trauma, and cancer are just a few examples. The lesion causing pain must directly engage the nociceptive pathways, as per a guideline that seems to have no exceptions.[2] As a result, lesions, for example, pain is not generated by the medial lemniscal system (e.g., dorsal columns). Thousands of herbal and traditional compounds are being investigated as anti-cancer medications all around world, and *R. cordifolia* is one amongst them. It contains significant levels of anthraquinones, which are responsible for anti-tumor activity, especially in the roots. The pharmacological effect of the plant is due to the presence of large amounts of anthraquinones, especially in the roots. Cancer is a dreadful disease, and every practical solution for combating it is vital to public health. Using the rising corpus of knowledge gathered via scientific breakthroughs, an integrated strategy to cancer management is required. *R. cordifolia* has the potential to be a source of effective pharmacophore for the treatment of diseases such as cancer. As a result, cancer patients who have already been debilitated by the disease and are now plagued by drug-induced hazardous side effects have turned to complementary and alternative medicine in the hopes of a better cure.[3] *Rubia cordifolia* Linn. (Family: Rubiaceae) is known to show analgesic [4], antistress [5], antihyperglycemic [6], cardioprotective [7] anti-sickling, antioxidant and anti-inflammatory activities. [8]

MATERIAL AND METHODS

Animals

Adult Swiss albino Mice (20 ± 2 g) and Wistar rats were used for this study. The animals were housed at 24 ± 2°C and relative humidity 55 ± 5 with 12:12 h light and dark cycle. They had free access to food and water *ad libitum.* The animals were acclimatized for a period of seven days before the study. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of MGV's Pharmacy College, Nashik.

Drugs and treatment schedule

Paclitaxel (Torrent Pharmaceuticals, Thane), Aspirin (Research Lab, Mumbai) and Pentazocine (Fortwin, Ranbaxy) were used as reference standard for anti-nociceptive activity. All the chemicals were of analytical grade.

Animals

Male Swiss Albino mice (n=5) were used in this study.

Plant material and extraction

Roots and rhizomes of *R. cordifolia* were obtained from Aushadhi Bhavan, Ayurved Seva Sangh, Nashik, Maharashtra. The plant material was identified and authenticated by Prof. SC Pal, Head of Pharmacognosy Department of MVP Samaj's College of Pharmacy, Nashik.

Preparation of extract

The coarse powder (200 gm) of dried *R. cordifolia* was defatted with petroleum ether (60-80^{o}C) and then the marc was extracted with 1000 ml ethanol. It was subjected to Soxhlet apparatus. The extract was concentrated on water bath, stored in airtight container, in dry place.

Phytochemical Analysis

Phytochemical analysis of the extract was performed according to standard procedures.

ANTINOCICEPTIVE ACTIVITY

Acetic acid-induced writhing method

Alcoholic extract of *R. cordifolia* (RC) was administered for 8 days in a dose of 100, 200 and 400 mg/kg p.o. The antinociceptive activity was measured by counting the number of writhes due to Paclitaxel (2 mg/kg) and 0.6 percent v/v acetic acid (10 ml/kg, i.p.) administered before test in the next 20 minutes. The reference standard was aspirin (25 mg/kg, p.o.). Control group received Paclitaxel and acetic acid. [9]

Tail immersion method

Control group received paclitaxel (2 mg/kg) *per se*. RC (100, 200 and 400 mg/kg p.o) was administered for 8 days and 1 hr prior before subjecting tail of the animals in hot water. Animals received reference standard Pentazocine (17.5 mg/kg, i.p.) 30 min prior to observation. The distal 2-3 cm portion of mouse-tail was immersed in hot water maintained at 55 ± 0.5ºC. The time taken by the animal to withdraw the tail from hot water was noted as reaction time at 60 min. Paclitaxel was administered as an inducer immediately before reading.[10]

Cold Allodynia

Animals in control group received distilled water (5 ml/kg, p.o.). Paclitaxel (2 mg/kg, i.p.) *per se* was administered immediately before observations. Animals received AERC (100, 200, 400 mg/kg, p.o.) for 8 days. Pentazocine (17.5 mg/kg, i.p. for 21 days) was used as reference standard and administered 30 minutes before the experiment. The number of paw licking was measured at 60, 120, and 180-min interval after paclitaxel by keeping the animal on the ice slab. At the time of the reading, paclitaxel was used as an inducer.[11]

Statistical analysis

All data was expressed as mean ± SEM. One-way ANOVA was used in the statistical analysis, followed by Dunnett's test. *p<0.05, **p0.01, $^{\#}$p<0.05, $^{\#\#}$ p<0.01 were considered statistically significant differences.

RESULT AND DISCUSSION

Herbal medications have a wide range of therapeutic benefits and are used to treat a variety of diseases. Pain is associated with autoimmune disorders (e.g., multiple sclerosis), metabolic diseases (e.g., diabetic neuropathy), infection (e.g., shingles and its sequel, postherpetic neuralgia), vascular disease (stroke), trauma, and cancer.[12] According to a criterion that appears to have no exceptions, the lesion causing pain must directly impact the nociceptive pathways. For example, lesions of the medial lemniscal system (dorsal columns) do not induce pain.[13]

Pain is a natural reaction to a traumatic event that causes tissue damage, such as an injury, inflammation, or cancer.[14] Analgesics treat pain as a symptom rather than the underlying cause. The majority of analgesic medicines used today are synthetic in nature, and long-term use generates a variety of side and

toxic effects, including respiratory depression, constipation, kidney damage, physical dependence, and gastrointestinal irritation. Furthermore, aspirin use, both acute and chronic, causes anemia with leukocytosis.[15]

As a consequence, pharmacological treatments for acute and chronic pain should be substituted with organically derived medicines with less adverse effects. Standard analgesics are typically ineffective in treating neuropathic pain induced by chemotherapeutic agents.

Paclitaxel is a chemotherapeutic agent commonly used to treat ovarian, breast, lung, head, and neck cancers. It shows myelosuppression and peripheral sensory neurotoxicity as well-known side effects. These side effects frequently necessitate the use of suboptimal doses (dose-limiting therapy), if not complete treatment suspension. Paclitaxel-induced peripheral neuropathy is defined by sensory neuron degeneration, which manifests clinically as numbness, pain, and thermal hyperesthesia in the hands and feet.[16]

Preliminary phytochemical analysis of ethanolic extract of *R. cordifolia* revealed the presence of saponins, tannins, alkaloids, glycosides, flavonoids, and essential oils. The effects of an ethanolic extract of *R. cordifolia* roots and rhizomes on Paclitaxel-induced neuropathic pain in mice was observed in this work. Paclitaxel therapy produces long-lasting effects in mice.[17]

The purpose of study was to understand involvement of AERC in peripheral anti-nociceptive activity in acute pain. Different animal models were used to test the activity, which might respond to two different types of painful stimuli: chemically produced tissue injury and thermal stimulus.[18] Acetic acid raises peritoneal fluid levels. In part, a fluid containing prostaglandins such as PGE2 and PGF2, as well as serotonin and histamine, was implicated, which was a typical paradigm for screening peripheral analgesics.[19] The taxane family of medications includes a mitotic inhibitor paclitaxel used in cancer chemotherapy, and docetaxel, a derivative of paclitaxel. The structure of these diterpenes is based on the skeleton of taxanes or Abeaotaxanes, anti-microtubule agent, preventing depolymerization and stimulates microtubule assembly. Paclitaxel is an irritant, a substance that can irritate the vein through which it is administered. It is possible that the medicine will escape from the vein and cause tissue injury. Analgesia will be demonstrated by any drug that reduces the number of writhing, ideally by inhibiting prostaglandin synthesis, a peripheral mode of pain control.[20] The results demonstrate that RC suppressed acetic acid and paclitaxel-induced writhing considerably (Figure 1).

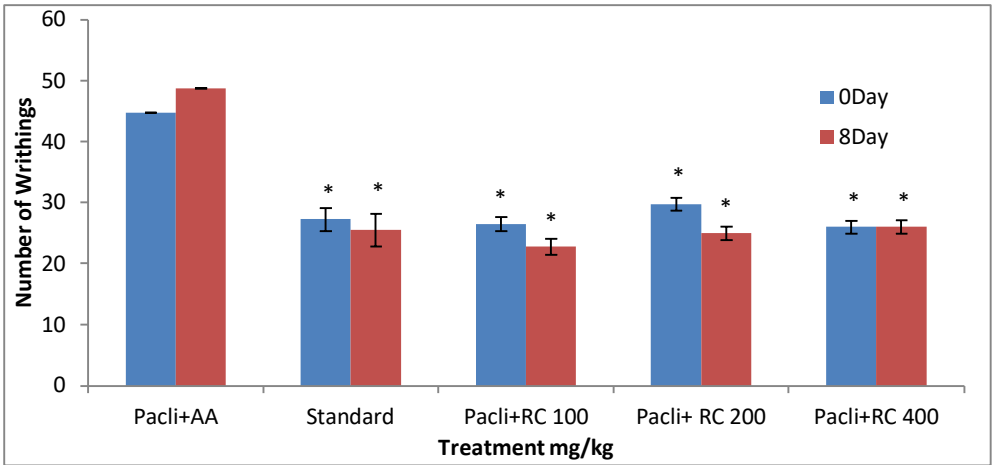

Figure 1: Effect of *Rubia cordifolia* on Acetic acid-induced writhing in mice.

The Tail immersion test was selected to understand involvement of *R. cordifolia* in central antinociceptive effect on paclitaxel-induced neuropathic pain in mice. This is the most popular nociception test, which uses a high-intensity phasic stimulus. An increase in reaction time to hot stimuli is regarded an essential metric for evaluating central analgesic activity in the tail immersion test. This test is commonly used to evaluate central antinociceptive activity. Because the tail immersion mediates spinal reflexes to nociceptive stimuli, this test has a tendency to respond to pain stimuli travelling across neural networks.[21]

Analgesic effects of opioids are mediated through supraspinal and spinal receptors.[22] The test is thought to be selective for opioid-like substances, which are centrally acting analgesics that bind to opioid receptors that are sensitive to them. In a tail immersion test, RC demonstrated analgesic effect mediated by opioid receptors. The prolonged delay in response time when the mice were exposed to a nociceptive stimulus in the tail immersion test suggests that RC has a central antinociceptive effect (Figure 2).[23]

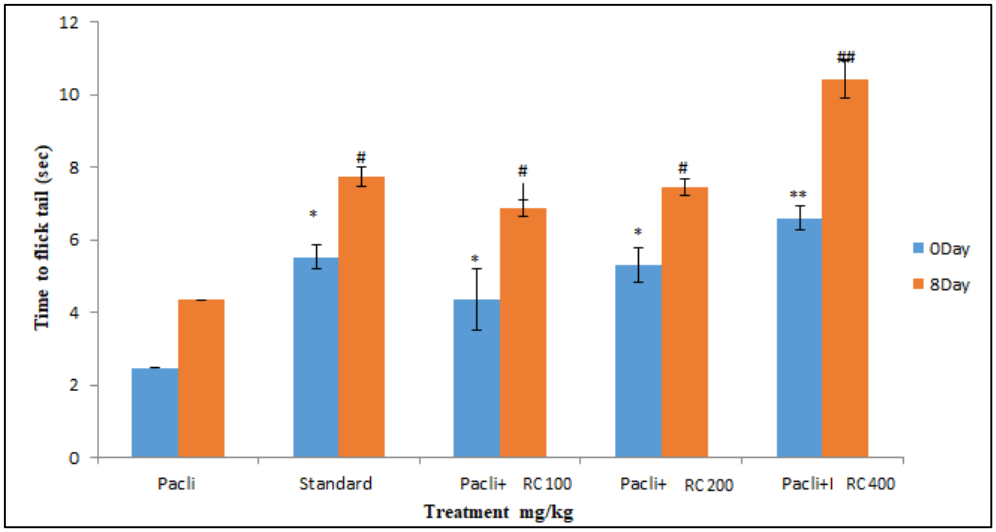

Figure 2: Effect of *Rubia cordifolia* on time to flick tail in Tail immersion method.

R. cordifolia showed analgesic efficacy in many animal models of analgesia and may have acted through a variety of mechanisms including both central and peripheral pathways. Analgesic effects have been observed for glycosides, alkaloids, and other bioactive substances. The tail immersion approach has been proven to be suitable for evaluating analgesics that act centrally but not peripherally. It is made comprised of responses to nociceptive stimuli organised at a supraspinal level and involves higher brain activities.[24]. The expression of paclitaxel-induced cold allodynia in mice was also reduced by acute systemic injection of RC. Cold allodynia caused by inputs from myelinated axons (mostly A-fibers). Findings suggest that RC-sensitive sodium channels play a significant role in the generation and maintenance of paclitaxel-induced neuropathic pain.[4] RC and pentazocine were given together to prevent the development of cold allodynia. The effects were seen following the administration of RC, and the number of paw licking was significantly reduced (Figure 3). Anti-convulsant that potentiate GABA transmission, reduce glutamate-mediated excitatory transmission, and block voltage-activated ion channels have adjunctive analgesic effects [25]. *R. cordifolia* has been shown to have anti-convulsant properties. GABA content in the brain was enhanced in a dose-dependent manner by an alcoholic extract of *R. cordifolia*. [26, 27] suggesting involvement of GABAergic transmission in analgesic activity.

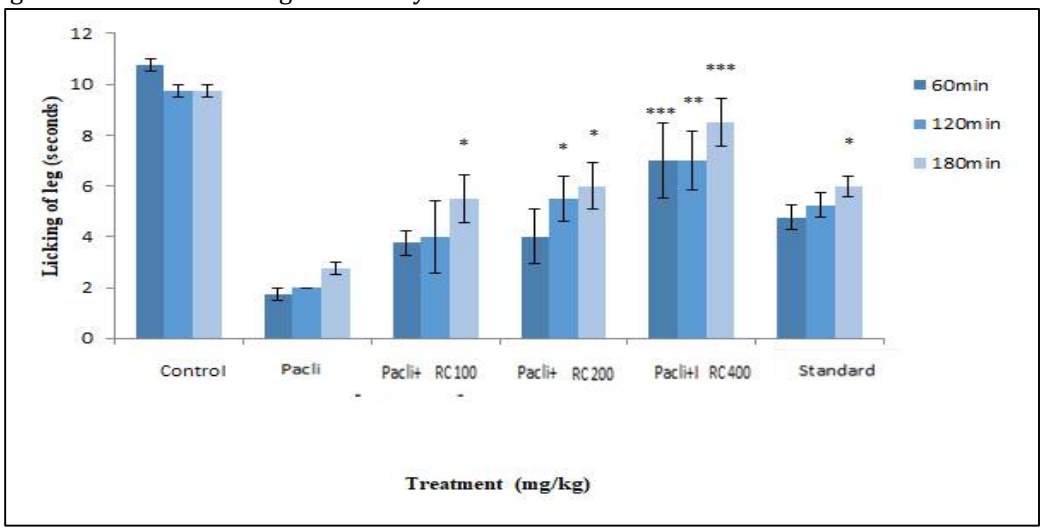

Figure 3: Effect of *Rubia cordifolia* on number of paw licking in Cold Allodynia method in mice.

CONCLUSION

This study concludes that the alcoholic extract of R. *Cordifolia* inhibits Paclitaxel-induced neuropathic pain, which could be owing to the involvement of GABA or an antioxidant mechanism.

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