

To Assess *Azadirachta Indica*'s Neuroprotective Potential in an Animal Model of Paclitaxel-Induced Neuropathy

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Abstract:

Introduction: *Azadirachta indica* (family: Maleaceae), commonly known as Neem, is the most useful traditional medicinal plant in India. Each part of plant has some medicinal properties. Neuropathic pain caused by lesions in the peripheral or central nervous system, comes in many forms. Paclitaxel is an anticancer drug used widely in treatment of lung and breast cancer. But repeated administration of Paclitaxel is responsible for development of neuropathic pain.

Aim and objective: In this study, we investigated the effect of aqueous extract of leaves of *Azadirachta indica* (AEAI) in paclitaxel Induced neuropathic pain.

Material and methods: Effect of AEAJ (100, 200, and 400 mg/kg) for 8 days evaluated on acetic acid induced-writhing and latency for withdrawal of tail in tail immersion method. Effect of AEAJ (100, 200 and 400mg/kg, p.o) was studied on Paclitaxel-induced neuropathic pain after sciatic nerve ligation using cold allodynia and hot plate method.

Result: Oral administration of AEAJ (100, 200, and 400 mg/kg) for 8 days significantly reverses acetic acid induced-writhings and latency for withdrawal of tail in tail immersion method. Oral administration of AEAJ for 21 days significantly reverses pain in Paclitaxel treated Wistar rats after sciatic nerve ligation in cold allodynia and hot plate method.

Conclusion: The results of this study indicate usefulness of AEAJ in treatment of Paclitaxel induced neuropathic pain.

Keywords: hyperalgesia, cold allodynia, neuropathic pain, paclitaxel, sciatic nerve ligation

INTRODUCTION:

Neuropathic pain syndromes are chronic pain disorders caused as a direct consequence of lesion or by disease of the parts of the Nervous system that normally signal pain^[1]. Patient with neuropathic pain demonstrate distinct sensory symptoms that can coexist in various combinations. Responses to physical examination of neuropathic pain can be graded as normal, decreased or increased to determine whether negative or positive sensory phenomena are involved. The stimulus-evoked (positive) pain types are classified as Dysesthetic, Hyperalgesic or Allodynic and according to the dynamic or static character of the stimulus^[2].

Paclitaxel is widely used anticancer agent for the treatment of lung, liver, ovarian, prostate, colon and breast cancer. Paclitaxel is known for two main side effects, myelosuppression, and peripheral

sensory neurotoxicity. In rodents, administration of paclitaxel causes neuropathic pain that is comprised of thermal hyperalgesia and mechanical allodynia, which can be recovered by various analgesic agent ^[3].

Azadirachta indica Linn. (Meliaceae) also known as “Neem” is an important medicinal plant. It possesses antitumor ^[4], analgesic ^[5], antipyretic ^[6], antibacterial-antifungal^[7], antioxidant, antidiabetic ^[8], anti-inflammatory ^[9] activities.

A. indica is known to possess analgesic ^[5] and anticancer ^[4] activities. Nimbidine and nimbolide from this plant shows in vitro antioxidant activity ^[10]. Limonoids from this plant are under clinical trial for analgesic and anticancer activity. Hence, in this study, we evaluated the effect of aqueous extract of leaves of *A. indica* on paclitaxel induced neuropathic pain in sciatic nerve ligated experimental animals.

Material and Method

Animals

Adult albino Wistar rats (100-150 g) of either sex were used for this study. The animals were housed at $24 \pm 2^\circ\text{C}$ 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 (Approval No: MGV/PC/CPCSEA/XXX/01/2016/05).

Drugs and treatment schedule

Paclitaxel was obtained from Emcure Pharmaceuticals Ltd, Pune, India. Pentazocine (Fortwin, Ranbaxy) was used as reference drug for anti-nociceptive activity. All the chemicals were of analytical grade and chemicals required for sensitive biochemical assays were obtained from Merck.

Plant material

Leaves of *A. indica* were obtained from local market, Nashik, Maharashtra. The plant material was identified and authenticated by Pharmacognosy department of MGV's Pharmacy College, Nashik.

Preparation of extract

The coarse dried powder of leaves of *A. indica* (200 g) was soaked overnight in distilled water. The supernatant fluid was allowed to evaporate in glass petridish. When completely dry, the powder was collected. The aqueous extract of *A. indica* (AEAI) (yield: 7.75% w/w) was stored in airtight container in a dry place ^[11].

Phytochemical analysis

Standard phytochemical screening tests were carried out for various constituents of the AEA. It revealed presence of flavonoids, tannins, phenols, steroids, and fats. ^[12].

Sciatic Nerve Surgery

Male Wistar rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.; supplemented as necessary). The common sciatic nerve was exposed at the level of the middle of the thigh by blunt dissection through biceps femoris. Proximal to the sciatics trifurcation about 7 mm of nerve was freed of adhering tissue and 4 ligatures (4.0 chromic gut) were tied loosely around it with about 1 mm spacing. The length of nerve thus affected was 4-5 mm long. Great care was taken to tie the ligatures such that

the diameter of the nerve was seen to be just barely constricted when viewed with 40X magnification. The desired degree of constriction retarded, but did not arrest, circulation through the superficial epineurial vasculature and sometimes produced a small, brief twitch in the muscle surrounding the exposure. The incision was closed in layers ^[13]. These animals were used to study effect of AEAI on paclitaxel induced neuropathic pain in cold allodynia and hot plate method.

Treatment schedule

Male Wistar rats (n=5) were divided into six groups, first group received distilled water (5 ml/kg, p.o.). Group 2 received Paclitaxel (2 mg/kg, i.p.) for 21 days. Group 3, 4 and 5 received AEAI (100, 200, 400 mg/kg) respectively for 21 days along with paclitaxel (2 mg/kg, i.p.) for 21 days. Group 6 received Pentazocine (17.5 mg/kg, i.p.) and Paclitaxel (2 mg/kg, i.p.) for 21 days.

Cold Allodynia

One day after sciatic nerve ligation animals received AEAI (100, 200 and 400 mg/kg, p.o.) and Pentazocine (17.5 mg/kg, i.p.). One hour after administration of AEAI and 30 minutes after administration of Pentazocine, Paclitaxel was administered as an inducer. Number of paw licking was measured on 7th, 10th and 21st day at 0, 60, 120 and 180 min interval after paclitaxel by keeping the animal on the ice slab ^[14].

Hot plate method

One day after sciatic nerve ligation animals received AEAI (100, 200 and 400 mg/kg, p.o.) and Pentazocine (17.5 mg/kg, i.p.). One hour after administration of AEAI and 30 minutes after administration of Pentazocine, Paclitaxel was administered as an inducer. Animals were placed individually on hot plate maintained at a temperature of $55 \pm 0.5^{\circ}\text{C}$. The latency to lick the paw was the reaction time. Hyperalgesia was assessed by measuring the latency to lick the paw on 7th, 10th and 21st day at 0, 60, 120 and 180 min after administration of paclitaxel. The cut off time was set at 12 sec to avoid damage to the skin ^[14].

Statistical analysis:

All the data was shown as mean \pm SEM. Statistical Analysis was performed with one-way ANOVA followed by Dunnett's test. Differences of $\#p < 0.05$ and $*p < 0.05$ were considered statistically significant.

Discussion

Neuropathic pain is pain caused by damage or disease affecting the Somatosensory Nervous system ^[15]. The Neuropathic pain definition is that the lesion in the nervous system has to be within somatosensory system. It means that the lesion in the cerebellum ^[16].

The new treatment for the Neuropathic pain is not satisfactory more than the two-third of neuropathic pain patient are obtain insufficient pain relief and there is a poor response is likely related to our failure to target relevant pain generating mechanism in the individual patient ^[17].

The lack of structural abnormalities in that called dysfunctional state such as fibromyalgia, interstitial cystitis, Vulvodynia etc. which prevent us from finding a relationship between structure and function, which is important in study of pain. If anyone patient found with these disorder to represent cases of

small-fibre neuropathies then those patient would easily fall under the umbrella of neuropathic pain [18, 19].

Paclitaxel is a widely-used anticancer agent for treatment of ovarian, breast, lung, head, and neck cancer. There are two well documented side effects of this treatment: Myelosuppression and peripheral sensory neurotoxicity. These side effects often necessitate the use of suboptimal doses (dose-limiting therapy), or even a complete suspension of treatment. In patients, paclitaxel-induced peripheral neuropathy is characterized by degeneration of sensory axons and is clinically manifested as numbness, pain, and thermo hyperesthesia in hands and feet [20].

The aqueous extract of *A.indica* obtained by soaking leaves overnight with distilled water was subjected to preliminary phytochemical analysis which revealed the presence of saponins, tannins, phenols fats, flavonoids and essential oils. In the present study, we investigated the influence of an aqueous extract of leaves of *A.indica* on Paclitaxel-induced neuropathic pain in sciatic nerve ligated rats. The study was attributed to study the possible central anti-nociceptive activity of AEAI in neuropathic pain.

Studies show that treatment with paclitaxel (2mg/kg) produced neuropathic pain by its repeated dose in rats by sciatic nerve surgery [21]. Present study demonstrated that numbers of paw lickings were increased due to repeated administration of paclitaxel dose but the number was found to be revised due to administration of AEAI .Significantly decreased paw licking latency in cold allodynia method as compared to Paclitaxel at 0, 60, 120 and 180 min. (fig.1, 2and 3).

Present study demonstrated that numbers of paw withdrawal latency were increased due to repeated administration of paclitaxel dose but the number was found to be revised due to administration of AEAI. AEAI Significantly decreased withdrawal latency of paw in hot plate method as compared to Paclitaxel at 0, 60, 120 and 180 min.(fig 4, 5 and 6).

AEAI produced a significant analgesia in paclitaxel induced neuropathic pain induced by sciatic nerve surgery.

Hence, this study concludes that AEAI exhibits significant inhibition of paclitaxel-induced neuropathic pain in sciatic nerve ligated animals.

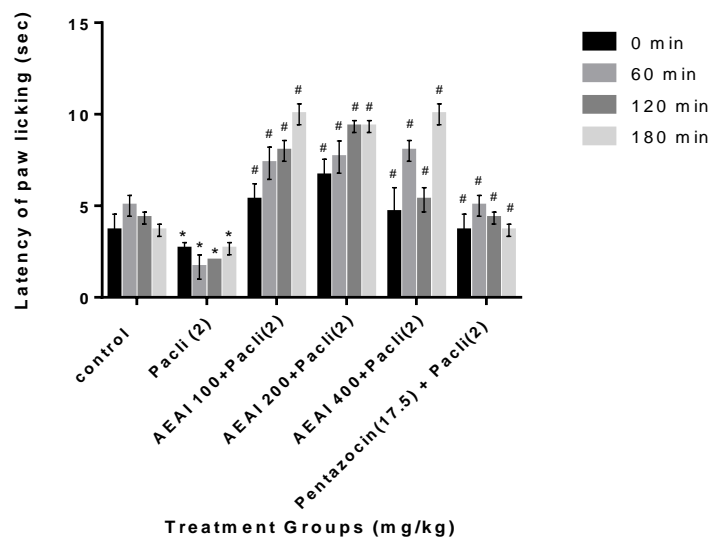


Fig 1: Effect of aqueous extract of *Azadirachta indica* (AEAI) on paclitaxel (Pacli)-induced neuropathic pain in cold allodynia method on 7th day.

n=5. The observations are mean ± SEM. * p < 0.05 compared to control. #p < 0.05 compared to Paclitaxel (one-way ANOVA followed by Dunnett’s test).

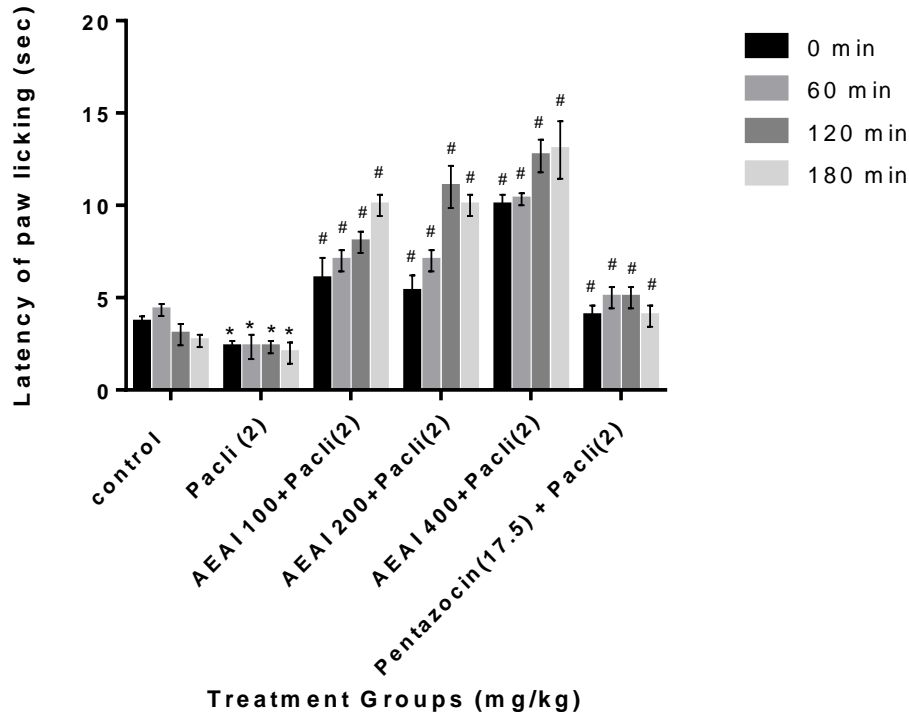


Fig 2: Effect of aqueous extract of *Azadirachta indica* (AEAI) on paclitaxel (Pacli)-induced neuropathic pain in cold allodynia method on 10th day.

n=5. The observations are mean ± SEM. * p < 0.05 compared to control. #p < 0.05 compared to Paclitaxel (one-way ANOVA followed by Dunnett’s test).

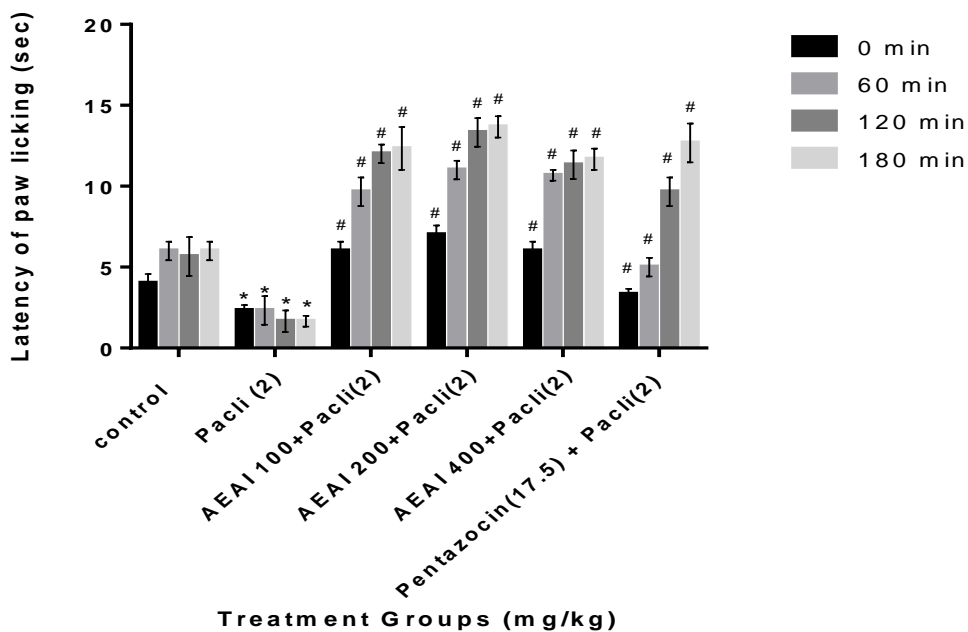


Fig 3: Effect of aqueous extract of *Azadirachta indica* (AEAI) on paclitaxel (Pacli)-induced neuropathic pain in cold allodynia method on 21th day.

n=5. The observations are mean ± SEM. * p < 0.05 compared to control. #p < 0.05 compared to Paclitaxel (one-way ANOVA followed by Dunnett’s test).

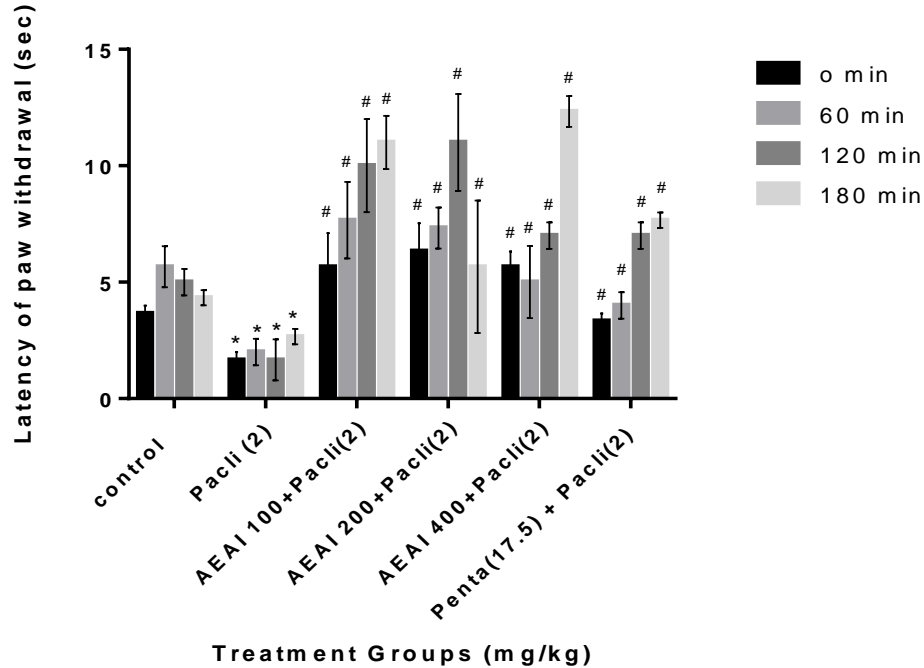


Fig 4: Effect of aqueous extract of *Azadirachta indica* (AEA1) on paclitaxel (Pacli)-induced neuropathic pain in Hot plate method on 7th day.

n=5. The observations are mean ± SEM. * p < 0.05 compared to control. #p < 0.05 compared to Paclitaxel (one-way ANOVA followed by Dunnett’s test).

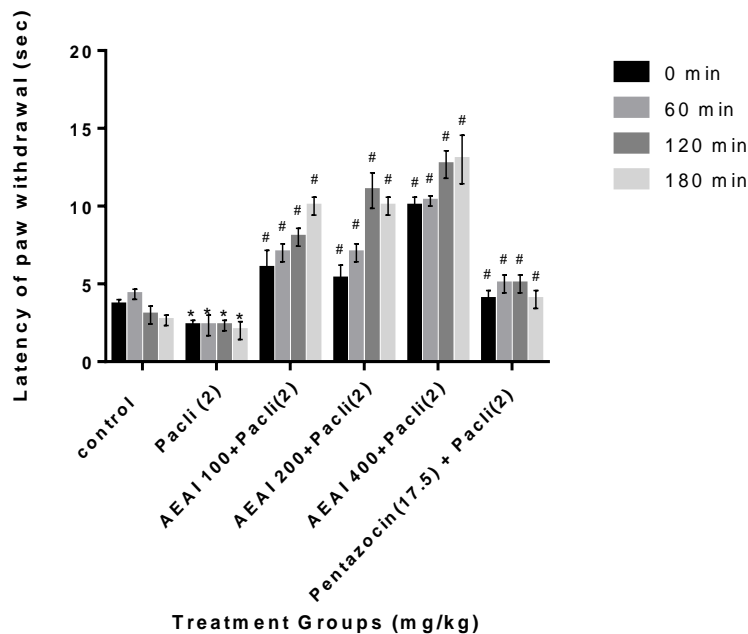


Fig 5: Effect of aqueous extract of *Azadirachta indica* (AEA1) on paclitaxel (Pacli)-induced neuropathic pain in Hot plate method on 10th day.

n=5. The observations are mean ± SEM. * p < 0.05 compared to control. #p < 0.05 compared to Paclitaxel (one-way ANOVA followed by Dunnett’s test).

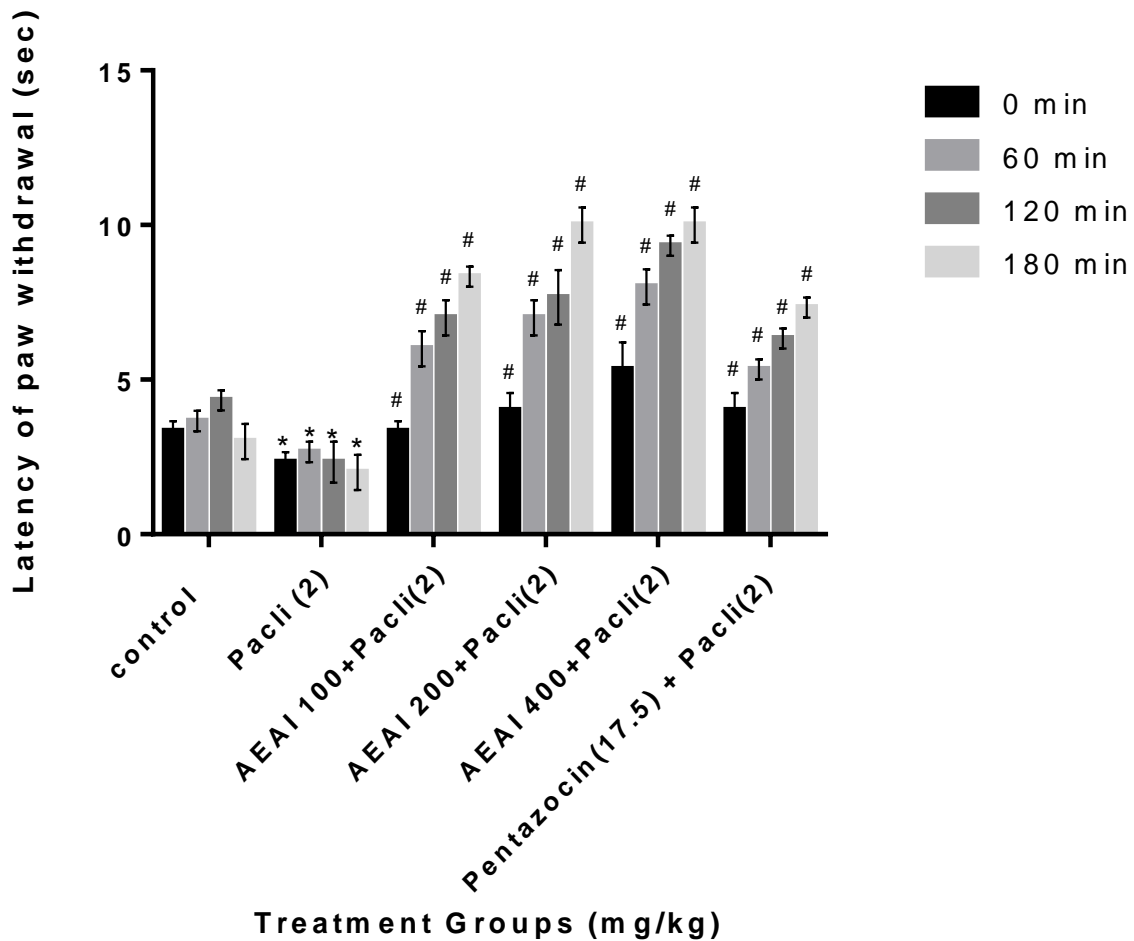


Fig 6: Effect of aqueous extract of *Azadirachta indica* (AEAI) on paclitaxel (Pacli)-induced neuropathic pain in Hot plate method on 21th day.

n=5. The observations are mean ± SEM. * p < 0.05 compared to control. #p < 0.05 compared to Paclitaxel (one-way ANOVA followed by Dunnett’s test).

Conclusion

The study concludes that aqueous extract of *Azadirachta indica* (AEAI) significantly inhibited paclitaxel induced neuropathic pain after sciatic nerve ligation. Combination of *Azadirachta indica* with Paclitaxel may help to reduce side effects and dose of paclitaxel.

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