IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

# Evaluation of analgesic activity of ethanolic extract of leaves of Nerium oleander Linn. albino rats

***Author1: Mr. Pratik Khandappa Kore, ** Author 2: Mr. Laxman IrannaNimbale,**

*****Author 3: Mr. Mangesh S Khillare**

**Assistant Professor, Department of Pharmacology, Tatyaraoji More College of Pharmacy, Omerga, Maharashtra,

***Assistant Professor, Department of Pharmaceutics, Tatyaraoji More College of Pharmacy, Omerga, Maharashtra

**Abstarct:**

Pain is defined by international association for the study of pain (IASP) as an unpleasantsensory and emotional experience associated with actual or potential tissue damage,or describedin terms of such damage. Pain mechanisms serve as a natural protective function of organismsagainst noxious stimuli by changing the physiology and behaviour to reduce or avoid further damage, and promote recovery. Analgesic activity was evaluated by hot plate method and cold plate method and parameters analysed were paw licking and jumping. In hot plate and cold plate method the highest significant activity was showed at 4h, 6h and 8h time intervals as compared to control.

**Conclusion:** The ethanolic extract of leaves of *Nerium oleander* Linn. was showed significant diuretic and analgesic activity.

**Keywords:** Diclofenac sodium, analgesic activity, EENO, allodynia.

**Introduction:**Pain is defined by international association for the study of pain (IASP) as an unpleasantsensory and emotional experience associated with actual or potential tissue damage,or describedin terms of such damage. Pain mechanisms serve as a natural protective function of organismsagainst noxious stimuli by changing the physiology and behavior to reduce or avoid further damage, and promote recovery. People with a loss of pain function appear to have recurrent injuries such as burns, repeat fractures, and self-injuries[1]. Many of them do not survive childhood because without feeling pain they cannot learn self-awareness necessity to avoid danger.

Analgesics are medications that relieve or reduce pain without causing loss of consciousness.They are sometimes referred to as pain killer.[2,3] There are three classes of analgesics; they are: opioids, non-opioids and adjuvant analgesics.

**Conventional Management of Pain:** Non-steroidal anti-inflammatory drugs (NSAIDs) belong to an important class of therapeutic agents that are prescribed all over the world for treatment of orthopaedics conditions such as fractures, soft-tissue injuries and osteoarthritis[4]. Aspirin and arachidonic acid compete for the active binding site in the COX enzyme. This competition result in displacement of arachidonic acid from binding to the active site leading to inhibition of prostaglandins synthesis.[5] Generally NSAID's exhibit three modes of inhibiting COX.

i. Competitive reversible inhibition that competes with arachidonic acid for binding to the COX site for example ibuprofen and piroxicam.

ii. Competitive, reversible inhibitors, time dependent that bind COX active site in the early phase to form a reversible enzyme inhibitor complex for example diclofenac and flurbiprofen.

iii. Competitive, time independent irreversible inhibitors that form an enzyme inhibitor complex for example aspirin.

**Herbal Management of Pain:** Throughout history man has used different formsof therapy to relief pain. Morphine for example was isolated from a medicinal plant Papaver somniferium[6]. The search of herbal plants with analgesic activities, used as pain relievers should be viewed as a successful search for new pain relieving drugs[7]. Considering that most of the anti-inflammatory, analgesic, anti-malarial and anti-pyretic synthetic drugs such as aspirin, morphine, artemisinin, atrophine and chloroquine were derived from the plant products[8].

**Figure: Leaves of *Nerium Oleander L.***

## Classification of pain:

There are several variables for the classification of pain but no international classification system has been unanimously adopted. The most commonly used system for pain classification is four and they are 1) the pathophysiological mechanism of pain, 2) the duration of pain, 3) the etiology of pain and 4) the anatomic location of pain[9,3].

### Pathophysiological classification of pain:

Pathophysiological classification of pain has divided pain into two major types; nociceptive and neuropathic (and uncommonly; physiologic pain). Clinical distinction between these pains is useful because the treatment approaches are different[3].

### Nociceptivepain:

Nociceptive pain is defined as noxious perception resulting from cellular damage following surgical, traumatic or disease-related injuries. It arises when tissue injury activates specific pain receptors called nociceptors, which are sensitive to noxious stimuli. Nociceptors can respond to heat, cold, vibration, stretch stimuli and chemical substances released from tissues in response to oxygen derivation, tissue disruption or inflammation[4].

### Neuropathicpain:

Neuropathic pain is defined as ―pain initiated or caused by a pathologic lesion or dysfunction‖ in peripheral nerves and CNS. It is caused by structural damage and nerve cell dysfunction[10]. It is often intense and unrelenting and resistant to relief by available therapies[4]. It is usually constant and described as burning, electrical, lancinating, and shooting. Disease states associated with classic neuropathic symptoms include infection (e.g. herpes zoster), metabolic derangements (e.g. diabetic neuropathy), toxicity (e.g.chemotherapy), and Walleri and generation secondary to trauma or nerve compression[4].

## Methods and Materials:

### Collection of plant material:

The leaves of *Nerium oleander* Linn were collected from the local area of Kalaburagi (Karnataka) in the month of August. The fresh leaves were authenticated by Dr. N G Patil, Professor and HOD, Botany Department, H.K.E.Society's Smt. Veeramma Gangasiri College for Women, Kalaburagi.

The plant leaves were dried in shade at room temperature, powder and stored in air tight container for studies.

### Preparation of ethanolic extracts:

*Nerium oleander* L. leaves powder (100gm) was defatted with 150ml of petroleum ether and extracted with ethanol

by continuous hot percolation method using soxhlet apparatus at 40˚ C to obtain the ethanolic extract of the plant. The filtrate of the extract was concentrated and dried at under temperature of 30˚C.

## ➢ Hot plate method:

In this method, heat is used as a source of pain. Animals was placed on a hot plate maintained at constant temperature and the reaction of animals, such as paw licking or jumping response was taken as the end point. The Swiss albino mice weighing (18-22g) were used. They are divided in to 4 groups each group contain 6 animals.

**Group-I:** Control (They were administered with normal saline)

**Group-II:**Standard drug Diclofenac sodium (75mg/kg p.o.)

**Group-III:** They were administered with Lower dose of extract of EENO leaves (100mg/kg p.o.)

**Group-IV:** They were administered with Higher dose of extract of EENO leaves (200mg/kg p.o.)

Animals were placed on the hot plate, which consists of electrically heated surface. Temperature ofhot plate was maintained at 55±1$^0$C. Responses such as jumping, withdrawal of the paws and licking of the paws were seen. The time period (latency period) when animals are placed and until responses occur is recorded by a stopwatch. Test compounds were administered respective treatments to respective groups orally or latency period were recorded after 0, 1, 2, 4, 6, 8h. These values were compared with the values before administration of test drug.

## ➢ Cold plate method:

The Swiss albino mice were weighing between 18-22g. were used divided into 4 groups each group contain 6 animals.

**Group I:** Control (They were administered with normal saline)

**Group II:** Standard drug Diclofenac sodium (75mg/kg po)

**Group III:** Were administered with Lower dose of EENO leaves (100mg/kg p.o.)

**Group IV:** Were administered with Higher dose of EENO leaves (200mg/kg p.o.)

**Result:**

- **Hot plate method:** The EENO (200mg/kg) treated group showed significant (P<0.01) response at 2h, 4h, 6h, time interval when compared to control group (Table-1 and Figure-1). Both standard and test group showed Analgesic effect when compared to control group.

**Table 1: Analgesic effect of EENO in Hot plate method at different time intervals**

All the values are mean ± SEM (N=6) ns= Not significant, One way Analysis of Variance (ANOVA) followed by multiple comparison Tukey's test was applied.
***p<0.001,**p<0.01, * p<0.05 v/s control group.

| Groups | Treatment | Mean reaction time in seconds ± SEM | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0h | 1h | 2h | 4h | 6h | 8h |
| I | Control | 5.33±0.33 | 5.0±0.36 | 5.83±0.30 | 5.5±0.42 | 5.66±0.33 | 5.83±0.30 |
| II | Diclofenac sodium(75mg/kg) | 6.83±0.30 | 6.50±.0.76 | 8.0±0.73 ** | 8.16±0.47 ** | 8.16±0.47 * | 7.33±0.88 |
| III | Test-1(100mg/kg) | 4.33±0.49 | 4.83±0.60 | 6.0±0.36 * | 7.66±0.61 *** | 6.50±0.42 * | 5.0±0.25 |
| IV | Test-2(200mg/kg) | 3.83±0.40 * | 5.50±0.34 ** | 5.83±0.47 *** | 8.83±0.54 **** | 7.83±0.30 *** | 6.83±0.30 **** |

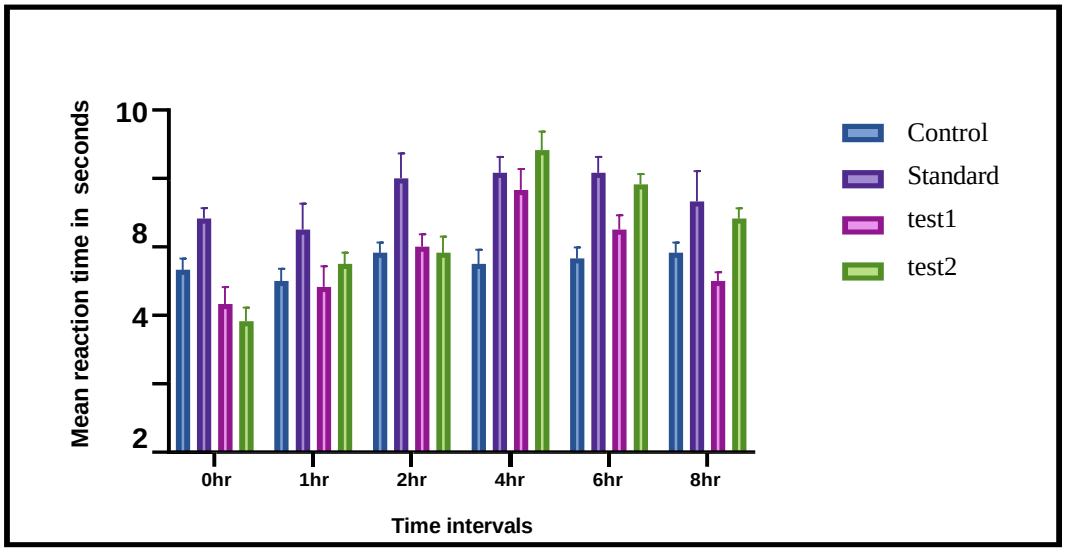


**Fig No.1: Analgesic effect of EENO in Hot plate method at different time intervals**

**Cold plate method:** The EENO (200mg/kg) treated group showed significant (P<0.01) response at 2h, 4h, 6h time interval when compared to control group (Table-2 and Figure-2). Both standard and test group showed Analgesic effect when compared to control group.

**Table2:Analgesic effect of EENO in cold plate method at different time intervals**

All the values are mean ± SEM (N=6) ns= Not significant, One way Analysis of Variance (ANOVA) followed by multiple comparison Tukey's test was applied
***p<0.001, **p<0.01, *p<0.05 v/s control group

| Groups | Treatments | Mean reaction time in seconds ± SEM | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0h | 1h | 2h | 4 h | 6h | 8h |
| I | Control | 3.83±0.40 | 4.33±0.49 | 4.83±0.47 | 5.00±0.36* | 5.66±0.42 | 4.66±0.33 |
| II | Diclofenac sodium (75mg/kg) | 4.16±0.30 ** | 6.16±0.60 **** | 7.16±0.30 *** | 7.66±0.42 **** | 7.50±0.42 **** | 4.33±0.33 |
| III | Test-1 (100mg/kg) | 4.16±0.47 | 4.16±0.30 | 5.5±0.42 | 6.83±0.47 *** | 4.83±0.30 | 3.83±0.30 |
| IV | Test-2 (200mg/kg) | 3.33±0.21 | 4.0±0.25 | 6.33±0.21** ** | 7.16±0.47 **** | 7.50±0.42** ** | 3.83±0.30 |

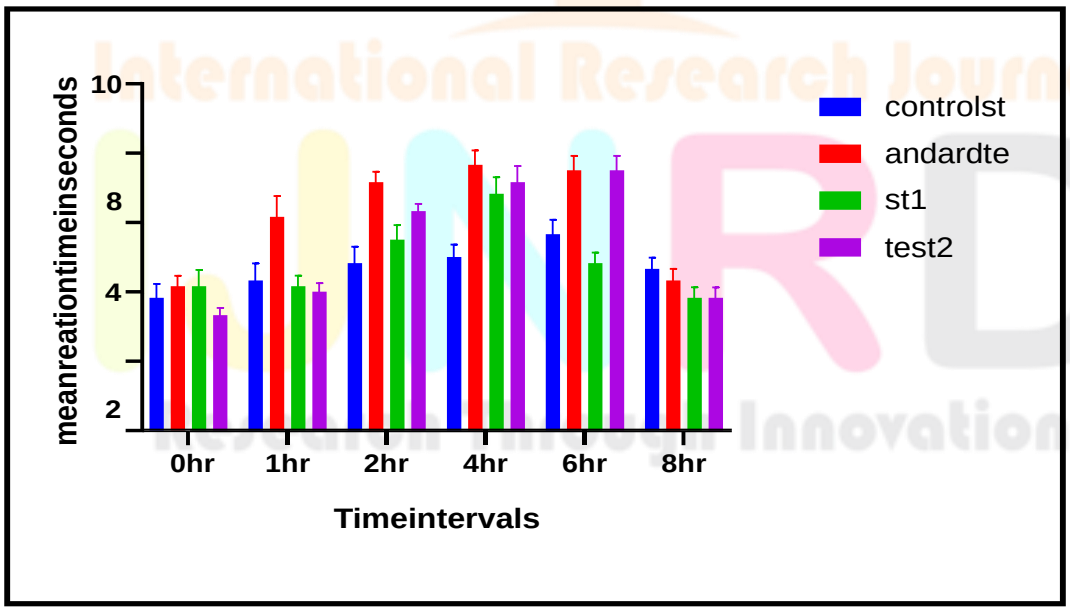


**Fig No. 2: Analgesic effect of EENO in cold plate method at different time intervals**

**Discussion:**

Hot plate test is useful for the evaluation of centrally acting analgesics and these analgesics are universally used to elevate the pain threshold of mice towards heat[11]. The advantages ofhot plate test is objective, quantifiable and pain can be administered frequently without causing inflammation and by this test.

Drugs are used clinically to treat pain were studied. The results showed that, compared with the control group, the pain thresholds in the diclofenac sodium-treated and EENO-treated groups were significantly increased at different time intervals $P < 0.05$, especially at 4h $P < 0.01$.This result suggested that EENO obviously prolong the time standing on hot plate and improve the heat-resisting ability of the mice, revealing that EENO had effective analgesic activity. Since it was showed that the EENO had potent analgesic action.

**Conclusion:**

Analgesic activity was evaluated by different methods namely, hot plate method and cold plate method. The EENO showed potent analgesic activity at different time intervals.

**List of references:**


1. Ma A, Turner A. A life without pain: congenital insensitivity to pain due to compound heterozygous SCN9A mutation. J Paediatr Child Health 2012;48:285286.
2. Fields HL and Basbaum AI. Central nervous system mechanisms of pain modulation. InWall P. D. and Melzack, R. (eds.) Textbook of Pain. London: Churchill. 2000;309–330.
3. World Health Organization. WHO guidelines on the pharmacological treatment of persisting pain in children with medical illnesses. ISBN 9789241548120.2012.
4. Boursinos LA, Karachalios T, Poultisides L, and malizos TK. Do steroids, conventional non-steroidal anti-inflammatory drugs and selective Cox-2 inhibitors adverse affect fracture healing. Journal of Musculo skeletal Neuronal Interact.2009;9: 44-52.
5. Rao P N and Knaus E E. Evaluation of Non-steroidial anti-inflammatory drugs cyclo-oxygenase inhibition and beyond. Journal of Pharmaceutical Sciences. 2008;11: 81-110.
6. De Sousa D P. Analgesic-like Activity of Essential Oils Constituents Molecules. Phytopharmacology. 2011;16:223-225.
7. Elisa betskey E A, Ahmador R R, Nunse D S, and Carvallo A T. Analgesic activity of Phshotua colorata Muell. Journal of Ethnopharmacology. 1995;48:77–83.
8. Gupta M, Mazumder UK, Gomathi P, and Selvan VT. Anti- inflammatory activity of extract of Vernonia amygdalina. Complementary and Alternative Medicine.2006;6:36-43.
9. Vadivelu N, Whitney CJ and Sinatra R S. Pain Pathways and Acute Pain Processing. In: Sinatra R. S., de Leon-Cassasola O.A., Ginsberg B. and Viscusi E.2009.
10. Inotai A, Hanko B, and Meszaro A. Trends in the non-steroidal anti- inflammatory drug market in six central-eastern European countries based on retail information. Pharmacoepidemiology and Drug Safety.2010;19:183–190.
11. Hiruma-Lima C. A, Gracioso J. S, Bighetti E. J. B, Germonsen Robineou L, and Souza Brito A. R. M, “The juice of ´ fresh leaves of Boerhaa via diffusa L. (Nyctaginaceae) markedly reduces pain in

mice," Journal of Ethnopharmacology, vol. 71, no. 1-2, pp. 267–274, 2000.

12. Wang Y. F, Shao S. H, Xu P, Yang X. Q, and Qian L. S, "Catechin-enriched green tea extract as a safe and effective agent for antimicrobial and anti- inflammatory treatment,"AfricanJournal of Pharmacyand Pharmacology, vol. 5,no. 12, pp.1452–1461, 2011.