Journal of
Pharmacology & Clinical Research
ISSN: 2473-5574

Research Article
Volume 3 Issue 1 – May 2017
DOI: 10.19080/JPCR.2017.03.555603

J of Pharmacol & Clin Res

Antinociceptive Activity of Ethanolic Neem (*Azadirachta Indica*) Leaf Extract (Enle) Through Opioid Pathway in Adult Rats

***Meenakshi Maurya**

Dept of Pharmacology, Sarojini Naidu Medical College, India

Submission: April 24, 2017; **Published:** May 30, 2017

***Corresponding author:** Meenakshi Maurya, Dept of Pharmacology, Sarojini Naidu Medical College, Agra, Uttar Pradesh, India, Email: meenakshimaurya23@gmail.com

Abstract

Aims: To evaluate antinociceptive activity of ethanolic neem leaf extract (ENLE) and determine involvement of opioidergic pathway in its effect.

Methods and Material: The antinociceptive activity of ENLE was studied using hot plate method. Two different doses of ENLE (50mg/kg and 100 mg/kg, p.o.) were administered in adult rats. Morphine (1 mg/kg, s.c.) was used as reference drug. To determine opioidergic pathway, pre-treated rats with naloxone (1mg/kg, i.p.) were used.

Statistical analysis used: Statistical analysis was done by one way ANOVA followed by Least significant difference test.

Results: ENLE shows antinociceptive effect in dose dependent manner as compared to control ($p<0.01$). When compared with morphine, morphine shows significant difference in its activity ($p<0.05$ and $p<0.01$) as compared with ENLE. Effects were reduced in naloxone pre-treated rats.

Conclusion: ENLE exhibited antinociceptive activity through central mechanism as compared with control. Involvement of opioidergic pathway is suggested.

Keywords: Ethanolic neem leaf extract; Hot plate test; Anti nociceptive activity

Key message: *Azadirachta Indica* is traditional plant and is being used for research due to its various therapeutic properties. Its antinociceptive potential is being investigated previously and was found to be present. In this study, higher doses of its ethanolic extract was studied for antinociceptive activity and pathway of its activity is investigated which was not done before

Introduction

Pain is an unpleasant though very important protective mechanism [1]. It is usually associated with actual or potential tissue damage and is primary reason for seeking medical attention [2]. Opioids are one of the most efficacious analgesics for moderate to severe pain [3]. But they possess strong addictive potential and various other side effects like respiratory depression, drowsiness etc [4]. Therefore, analgesic drugs which lack these side effects as an alternative are need of time [1].

Azadirachta Indica (neem) is used in Indian culture in traditional medicine and earlier studies have showed that it has various active substances with medicinal properties [5]. It has been reported as hypoglycemic, hypolipidemic, anti-inflammatory ,apoptotic and antineurotoxic agent [6-8]. So natural products can be explored as an important source of new chemical substance with therapeutic application [9]. Fewer studies has been done to explore its antinociceptive potential [6,10,11]. To further confirm this property of ethanolic neem leaf extract (ENLE) through opioidergic system, this study was planned.

Subjects and Methods

Plant material

The fresh matured leaves of A. indica were collected locally from their natural habitat in month of august –september 2015.

The leaves were identified by a pharmacognosy expert and voucher specimen was retained in museum of the department for further reference.

Preparation of plant extract and reference drugs

The ethanolic extract was prepared by procedure described by Chattopadhyay (1998). A. indica leaves were shade dried and powdered. Powder was mixed with 70% ethyl alcohol and left at room temperature for 36 hrs. It was stirred intermittently and filtered. The filtrate was concentrated under reduced pressure (bath temp 50º C) and dried in vaccum dessicator. The residue obtained was stored in storage vial and refrigerated until used for experiment. The doses of ENLE of 50 mg/kg and 100 mg/kg were prepared by suspending extracts in normal saline. The reference drug used in this study was morphine (1mg/kg, s.c.) and naloxone (1mg/kg, i.p.). All reference drugs were prepared by dissolving in normal saline.

Experimental Animals

In this study, adult rats weighing 150-250g of either sex were used. The animals were kept under standard condition at 24±2 ºC with 12 h light and 12h day cycle. Food and water were available ad libitum. The study was approved by Institutional Animal Ethics Committee.

Evaluation of antinociceptive activity

Hot plate test: The adult rats were taken randomly in test and reference group. In this test, morphine and naloxone were used as reference drugs. The animals were placed on heated surface. The paws of rats are very sensitive to hot surface at 55±1ºC. A cut off period of 15s was set to avoid any damage to paw. The responses were withdrawal of paw, licking of paw or jumping. The reaction time was noted with help of stop watch and measured every 15min at interval of 0, 15, 30, 45, 60, 75 and 90 min.

Rats were divided into four groups of six animals each. In Group I, animals received normal saline (10ml/kg, s.c.) as negative control while Group II was treated with morphine (1mg/kg, s.c.) served as positive control. In test group (Group III and Group IV) rats were treated with two different doses of ENLE (50mg/kg and 100 mg/kg, p.o.) respectively. To study the effect of naloxone pre-treatment, rats were divided into five groups of six animals each. Group I received naloxone (1 mg/kg, i.p.) alone. Group II and Group III received naloxone (1 mg/kg, i.p.) 30 min prior to control (10ml/kg,s.c.) and morphine (1 mg/kg, s.c.) respectively. Group IV and Group V received naloxone (1 mg/kg,i.p.) 30 min prior to ENLE (50 mg/kg and 100 mg/kg ,p.o.) respectively.

Drugs

Normal saline and morphine sulphate were used s.c. while naloxone was used i.p. ENLE was dissolved in normal saline to desired concentration and used p.o. The dose of drugs used were selected on basis of previous studies [5,6].

Statistical Analysis

Statistical analysis was done by using One way analysis of variance (ANOVA) followed by Least significant difference test for multiple comparison to assess the significant differences between the groups. All the values were expressed as mean ± SEM. Value of $p<0.05$ and $p<0.01$ were considered significant.

Results

Effect of ENLE on hot plate test

Table 1: Effect of Ethanolic Neem Leaf Extract (ENLE) on reaction time in rats in Hot plate method.

Treatment	Dose (mg/kg)	Latency of reaction time(sec)						
		0 min	15 min	30 min	45 min	60 min	75 min	90 min
Control	10 ml/kg , s.c.	3.91±0.04	3.93±0.05	3.83±0.07	3.73±0.00	4.14±0.17	3.93±0.19	3.74±0.23
Morphine	1, s.c.	4.08±0.07	7.37±0.25*#c	9.75±0.16*#c	9.94±0.24*#	11.94±0.37*#b	13.13±0.16*#c	13.43±0.28*#b
ENLE	50 , p.o.	3.77±0.07	4.12±0.19	6.84±0.35*	8.84±0.49*	10.21±0.33*	10.98±0.19*	12.00±0.31*
ENLE	100 , p.o.	3.80±0.23	4.57±0.17a	7.95±0.24*	9.70±0.26*	10.87±0.25*	11.45±0.17*	12.47±0.25*

n=6. The observations are mean±SEM.

*p<0.01 compared to control; ap<0.05 compared to control; #p<0.01 compared to ENLE (50mg/kg).

bp<0.05 compared to ENLE(50mg/kg). cp<0.01 compared to ENLE(100mg/kg).

The results of hot plate test are reported in (Table 1 & 2). Both dose of ENLE (50 mg/kg and 100 mg/kg) shows dose dependent increase in mean latency time ($p<0.01$) as compared to control. Morphine (1mg/kg) shows significant increase ($p<0.01$) in mean latency time throughout whole observation period when compared with control and ENLE (50 mg/kg). It also shows significant difference in mean latency time($p<0.01$ and $p< 0.05$) when compared to ENLE (100mg/kg) (Table 1).

Effect of Naloxone pre treatment

After naloxone pre treatment, both morphine and ENLE (50 mg/kg and 100 mg/kg) shows no significant difference in increase in mean latency time as both were antagonized by naloxone (Table 2).

How to cite this article: Meenakshi M. Antinociceptive Activity of Ethanolic Neem (*Azadirachta Indica*) Leaf Extract (Enle) Through Opioid Pathway in Adult Rats. J of Pharmacol & Clin Res. 2017; 3(1): 555603. DOI: 10.19080/JPCR.2017.02.555603.

Table 2: Effect of Naloxone pre treatment on reaction time in rats in hot plate method.

Treatment	Dose (mg/kg)	Latency of reaction time (sec)						
		0 min	15 min	30 min	45 min	60 min	75 min	90 min
Naloxone	1, i.p.	3.57±0.10	3.72±0.15	3.84±0.20	3.77±0.12	3.76±0.07	3.86±0.05	3.89±0.05
Naloxone+control	1, i.p. ; 10ml/kg s.c.	3.58±0.09	3.53±0.03	3.64±0.08	3.75±0.10	3.60±0.03	3.60±0.05	3.62±0.04
Naloxone+morphine	1, i.p. ; 1, s.c.	3.45±004	3.45±0.00	3.49±0.04	3.57±0.03	3.60±0.02	3.60±0.03	3.58±0.02
Naloxone +ENLE	1, i.p.; 50, p.o.	3.63±0.05	3.63±0.04	3.54±0.04	3.64±0.04	3.61±0.03	3.62±0.05	3.52±0.07
Naloxone +ENLE	1, i.p. ;100, s.c.	3.66±0.06	3.64±0.07	3.69±0.06	3.72±0.08	3.75±0.07	3.78±0.07	3.83±0.07

n=6; The observations are mean±SEM.

Discussion

The dose administered in this study i.e. 50 mg/kg and 100 mg/kg is based on previous studies [5,6]. The dose used in this study is several times lower then oral LD50 for ENLE, which is around 4.75g/kg in acute toxicity test [7]. The hot plate test is one of the suitable tests for determining the difference between centrally and peripherally acting analgesics. In this test, high intensity phasic stimuli are given. The pain induced in hot plate method is very specific for centrally mediated activity [4]. Thus this test is very useful in elucidating centrally mediated antinociceptive response which concentrates on changes above the level of spinal cord [12]. The results obtained in this study shows that ENLE has dose dependent antinociceptive activity and this activity is probably centrally mediated as depicted through hot plate test. The hot plate test is also selective for opioid like compounds [13]. So, it can be concluded that ENLE shows its effect through opioid pathway. To determine the involvement of opioid receptors, pre treatment with naloxone was done. Naloxone is non selective antagonist at μ, κ and δ opioid receptors [14]. In naloxone pre treatment, the antinociceptive activity of ENLE was inhibited which suggests that it acts by blocking opioid receptors. The antinociceptive activity of ENLE may be due to presence of bioactive constituents in it. Thus, the findings of this study will be helpful for further phytochemical and pharmacodynamic investigations to explore its antinociceptive potential further.

Conclusion

The results obtained from this study suggest that there is presence of antinociceptive activity in ENLE which may be centrally mediated through opioid pathway. Further studies would be helpful to explore this potential for benefit of human being.

References

1. Bhattacharya A, Agrawal D, Sahu PK, Kumar S, Mishra SS, et al. (2014) Analgesic effect of ethanolic leaf extract of *Moringa oleifera* on albino mice. Indian J Pain 28: 89-94.
2. Kamilla L, Ramanathan S, Sasidharan S, Mansor SM (2014) Evaluation of antinociceptive effect of methanolic leaf and root extracts of *Clitoria ternatea Linn*. in rats. Indian J Pharmacol 46: 515-520.
3. Ozdemir E, Gursoy S, Bagcivan I, Durmus N, Altun A (2012) Zimelidine attenuates the development of tolerance to morphine induced nociception. Indian J Pharmacol 44: 215-218.
4. Kothari S, Kushwah A, Kothari D (2013) Involvement of opioid and monoaminergic pain pathways in *Aegle marmelos* induced analgesia in mice. Indian J Pharmacol 45: 371-375.
5. Maragathavalli S, Brindha S, Kaviyarasi NS, B Annadurai, Gangwar SK (2012) Antimicrobial activity in leaf extract of Neem (*Azadirachta Indica Linn*.). Int J of Sci and Nature 3(1): 110-113.
6. Patel JP, Hemavathi KG, Bhatt JD (2006) Study of antinociceptive effect of neem leaf extract and its interaction in mice. Indian J Pharmacol 37: 37-45.
7. R Subapriya, V Bhuvaneswari, S Nagini (2005) Ethanolic Neem (*Azadirachta indica*) Leaf Extract induces apoptosis in the Hamster buccal pouch carcinogenesis model by modulation of Bcl-2, Bim, Caspase B and Caspase 3. Asian Pac J Cancer Prev 6: 515-520.
8. Moneim AA (2014) *Azadirachta indica* attenuates cisplatin-induced neurotoxicity in rats. Indian J Pharmacol 46: 316-321.
9. Farouk L, Laroubi A, Aboufatima R, Benharref A, Chait A (2008) Evaluation of the analgesic effect of alkaloid extract of *Peganum harmala L.* Possible mechanisms involved. J Ethopharmacol 15: 449-454.
10. Khosla P, Bhanwra S, Singh J, Srivastava RK (2000) Antinociceptive activity of *A. indica* (neem) in rats. Indian J Pharmacol 32: 372-374.
11. Khanna N, Goswami M, Sen P, Ray A (1995) Antinociceptive action of *A. indica* (Neem) in mice: Possible mechanisms involved. Ind J Exp Biol 33: 848-850.
12. Debasis Mishra, Goutam Gosh, P Sudhir Kumar, Prasanna Kumar Panda (2011) An Experimental study of analgesic activity os selective COX-2 inhibitors with conventional NSAIDs. Asian J of Pharm and Cli Research 4(1): 78-81.
13. Patil RA, Langade PM, Dighade PB, Haray YA (2012) Antinociceptive activity of acute and chronic administration of *Murraya Koenigii L.* leaves in experimental models. Indian J Pharmacol 44: 15-19.
14. Rose JB (2008) Analgesic medications for acute pain and pain management in children. In GA Walco, KR Goldschneider (Eds.). Pain in children: A practical guide for primary care. Humana Press, Totwa, China, 73-86.

How to cite this article: Meenakshi M. Antinociceptive Activity of Ethanolic Neem (*Azadirachta Indica*) Leaf Extract (Enle) Through Opioid Pathway in Adult Rats. J of Pharmacol & Clin Res. 2017; 3(1): 555603. DOI: 10.19080/JPCR.2017.02.555603.

DOI: 10.19080/JPCR.2017.03.555603

How to cite this article: Meenakshi M. Antinociceptive Activity of Ethanolic Neem (*Azadirachta Indica*) Leaf Extract (Enle) Through Opioid Pathway in Adult Rats. J of Pharmacol & Clin Res. 2017; 3(1): 555603. DOI: 10.19080/JPCR.2017.02.555603.