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ANALGESIC EFFECT OF AZADIRACHTA INDICA (NEEM) LEAF EXTRACT ON ALBINO RATS

Ayon Bhattacharya*[1], Divya Agrawal[2], Priti Das[3], Bandana Rath[4], Sanjay Kumar[5], Shantilata Patnaik[6]

*[1]Tutor, Dept. of Pharmacology, IMS & SUM Hospital, SOA University, India
[2]Assistant Professor, Dept. of Anatomy, IMS and SUM Hospital, SOA University, India
[3]Associate Professor, Dept. of Pharmacology, MKCG Medical College, India
[4]Associate Professor, Dept. of Pharmacology, MKCG Medical College, India
[5]Professor, Dept. of Pharmacology, IMS & SUM Hospital, SOA University, India
[6]Professor, Dept. of Pharmacology, IMS & SUM Hospital, SOA University, India

*ayonbhattacharya@yahoo.in

Abstract

Neem (Azadirachta indica) is an evergreen, fast growing ancient tree, popularly known as 'Wonder tree'. Neem has a multitude of medicinal properties and active ingredients like azadirachtin, nimbidin, flavonoids, triterpenoids which contribute for its various pharmacological actions. The analgesic activity using the Neem Seed Oil (NSO) has already been done but not the Neem Leaf Extract (NLE). Hence the present study is done to evaluate the analgesic effect of NLE on albino rats. It is a randomized control study. The animals were randomly divided into 6 groups each group consisting of 10 rats ; Group I: Control (distilled water 0.5ml/rat); Group II: Standard (Morphine 1mg/kg i.p); Group III,IV,V,VI (NLE 62.5 ,125,250, 500 mg/kg body weight i.p respectively). The analgesic effect of Neem Leaf Extract(NLE) was assessed by the experimental pain model of tail flick response to thermal stimulation. The results were statistically analyzed by applying the chi-square test (χ^2) (with yates' correction).NLE in all doses enhanced the Tail flick Latency (TFL) and showed a dose dependent increase in effect. The Neem Leaf Extract (NLE) exhibited analgesic activity showing its central analgesic action.

Key words : *Azadirachta indica*, NLE, Analgesiometer, Analgesic

Introduction

Since time immemorial man is in search of remedies for pain. Pain is the commonest symptom that brings a patient to the hospital. Traditional medicine provides an alternative through which this quest can be fulfilled.

Neem (Indian lilac, *Azadirachta Indica*), is a fast growing evergreen tree, is a native of Indian subcontinent, Africa, America. It is known for over 4000 years now and is called 'arishtha' in Sanskrit meaning 'perfect, complete ,imperishable', reliever of sickness, hence it has been acclaimed as 'Sarbarogaribarini'[1]. This 'wonder tree' Neem also exhibits

antibacterial, antiviral, antioxidant, antimutagenic, i mmunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antim alarial, antifungal, and anticarcinogenic properties[2]. Phytochemical analysis done previously revealed the presence of alkaloids, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids , azadirachtin, and nimbidin which is claimed to possess analgesic and anti-inflammatory properties[3],[4].

NSAID's and opioids although rampantly used nowadays, is limited by its own side effects . The analgesic activity using the Neem Seed Oil (NSO) has already been done but not the Neem Leaf Extract (NLE) [5]. Hence the present study is done to evaluate the analgesic effect of NLE on albino rats.

Material and Methods

Materials

Collection of plant material:

Neem leaf extract was obtained from (Indian herbs research supply Co. Ltd., Saharanpur, India).

Chemicals:

Morphine (Morphitroy, Troikaa Pharmaceuticals Ltd,Gujrat,India) , Acetic acid (Fischer inorganic and Aromatic Ltd, Chennai, India).

Animals:

Healthy albino rats of either sex, weighing between 150 -200 grams were selected for the study. These animals were housed in the animal room of the Department of Pharmacology, V.S.S. Medical College, Burla and were exposed to natural temperature and humidity.

Methods

It is a randomized control study. The animals were randomly divided into 6 groups each group consisting of 10 rats ; Group I: Control (distilled water 0.5ml/rat); Group II: Standard (Morphine 1mg/kg i.p); Group III,IV,V,VI (NLE 62.5 ,125,250, 500 mg/kg body weight i.p respectively).

Morphine sulphate was used as reference standard drug for this study and normal saline was used as its vehicle. Neem leaf extract was dissolved in distilled water. The standard drug (Morphine) as well as the test drugs (NLE) were given intraperitoneally with all aseptic measures. The volume of all intraperitoneal injection was kept constant within 0.5 ml.

The analgesic effect of NLE was assessed by the experimental pain model of tail flick response to thermal stimulation. The thermal stimulation was given by analgesiometer (Techno Lucknow, India).

The analgesiometer is a closed instrument with a nichrome wire at the top. This nichrome wire is attached between two points. When the analgesiometer is switched-on, the nichrome wire becomes red hot and gives radiant heat. There is a meter for the adjustment of the current supplied to the nichrome wire. The rat's tail (2 cm from tip) was kept 3 mm above the nichrome wire so that it received the radiant heat only. When the rat feels the radiant heat, it flicks off its tail. The time taken for the tail flick to occur was measured as tail flick latency (TFL) [6]**.**The heat intensity of nichrome wire was adjusted such that the rats had a basal TFL of 3-5 seconds. A cut off time of 10 seconds was taken to prevent injury to the tail. The TFL in each animal was measured before and after drug administration . TFL was recorded at 15 min, 30 min, 45 min, 60 min, 90 min, 120min, 150 min and 180 minutes after drug administration. NLE, in doses of 62.5 mg, 125 mg, 250 mg and 500 mg/kg body weight were given intraperitoneally to different groups of rats.

Results

The results were statistically analyzed by applying the chi-square test (χ^2) (with yates' correction).

Morphine showed significant analgesic effect from 30 minutes to 60 minutes after administration. Peak effect was observed at 45 minutes. The control group did not show any significant change in basal TFL (Table 1) NLE in all doses enhanced the TFL and showed a dose dependent increase in effect. The TFL started increasing from 15 minutes of NLE administration till 90 minutes. TFL decreased thereafter to reach the basal value at 180 minutes. NLE at 250 mg/kg. body weight showed significant increase in TFL from 60 minutes to 90 minutes of its administration.

With 500 mg/kg body weight NLE, the TFL increased significantly from 45 minutes to 90 minutes. NLE in doses of 250 mg/kg and 500 mg/kg revealed maximum percentage of response (70 %) at 60 minutes of drug administration (Table 2). The effect of NLE and standard drug morphine on TFL at varying time intervals are depicted in figure1. Bar diagram with error bars in figure 2 shows the pattern in TFL at 60min. The percentage inhibition of TFL of NLE was maximum at 45min and 60min shown in figure 3.

Discussion

Here the tail flick model is used to screen the central analgesic action of the extract. Pain induced by thermal stimulus of the analgesiometer is specific for testing centrally mediated analgesic activity. Opioid agents (Morphine) acts via supraspinal (μ_1 ,κ_3, σ_2, δ_1) and spinal (μ_2,κ_1,δ_2) receptors[7],[4]. NLE showed anti-nociceptive activity by increasing the tail flick latency. Thus we can postulate that NLE could act on the periaqueductal gray matter to release endogenous peptides (endorphins or encephalins)[8]. These endogenous peptides thereafter descend the spinal cord and inhibit the pain impulse transmission at the synapse in the dorsal horn[9],[10]. The possible mechanism of NLE leaves could be due to its action on the central opioid receptors or through release of endogenous opioid peptides [11],[4].

Conclusion

NLE produces significant analgesic activity at doses of 250 mg and 500 mg/kg body weight. However, further experimental studies on different analgesic models, isolation of active constituents responsible for the analgesic activity and elucidating the exact mechanism of action are needed to establish the analgesic potential of NLE.

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Table 1. Effect of NLE on tail flick latency (TFL) at various time intervals

Drugs	Mean basal TFL (in seconds)	Mean T.F.L. ± SEM in second						
		15 minute	30 minute	45 minute	60 minute	90 minute	120 minute	180 minute
Distilled water 0.5 ml/rat	3.9 ± 0.23	3.9 ± 0.28	4.0 ± 0.1	4.0 ± 0.21	4.1 ± 0.28	4.1 ± 0.24	4.0 ± 0.21	4.2 ± 0.29
Morphine 1mg/kg	3.8 ± 0.28	7.1±0.41	9.2 ± 0.41**	10.2± 0.34**	10.3±0.4**	9.9±0.7**	9.1±0.2**	4.2±0.2
NLE 62.5 mg/kg body weight	3.7 ± 0.26	4.4 ± 0.27	4.6 ± 0.22	5.8 ± 0.55	6.3 ± 0.72	6.7 ± 0.8	5.7 ± 0.67	3.9 ± 0.23
NLE 125 mg/kg body weight	3.7 ± 0.26	5.4 ± 0.37	6.5 ± 0.58	7.3 ± 0.56*	8.7 ± 0.42*	9.0 ± 0.29**	7.8 ± 0.42*	4.0 ± 0.21
NLE 250 mg/kg body weight	3.9 ± 0.28	5.6 ± 0.37	6.8 ± 0.47	8.3 ± 0.52*	9.4 ±0.34**	9.4 ±0.27**	8.0 ± 0.26*	4.2 ± 0.29
NLE 500 mg/kg body weight	4.1 ± 0.28	6.9 ± 0.43	8.5 ± 0.45*	9.4 ± 0.34**	9.6 ± 0.22**	9.5 ± 0.22**	8.3 ± 0.52*	4.1 ± 0.24

n =10, * p < 0.05, **p < 0.01

Table 2: Percentage inhibition of NLE on TFL response at varying time intervals

Drugs	No. of animals in each group	No. and % of animals showing TFL ≥ 10 seconds													
		15 minute		30 minute		45 minute		60 minute		90 minute		120 minute		180 minute	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
NLE 62.5 mg/kg body weight	10	0	0	0	0	0	0	0	0	1	10	0	0	0	0
Morphine 1mg/kg	10	1	0	5[a]	50	9[d]	90	5[a]	50	1	10	0	0	0	0
NLE 125 mg/kg body weight	10	0	0	1	10	2	20	4	40	4	40	2	20	0	0
NLE 250 mg/kg body weight	10	0	0	0	0	3	30	7[c]	70	6[b]	60	0	0	0	0
NLE 500 mg/kg body weight	10	0	0	3	30	7[c]	70	7[c]	70	6[b]	60	3	30	0	0

a ⇒ p < 0.05, b ⇒ p = 0.02, c ⇒ p < 0.01 d ⇒ p = 0.001

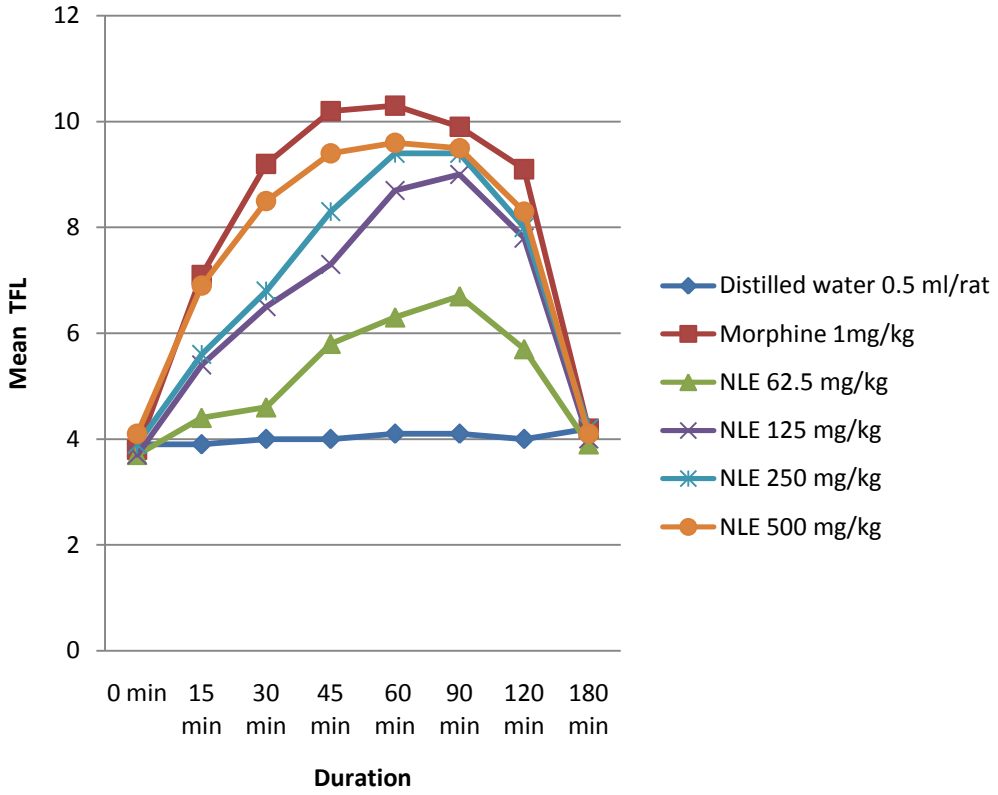

Figure 1: Line diagram showing the effect of NLE and Morphine on mean TFL (Tail flick latency) at various intervals.

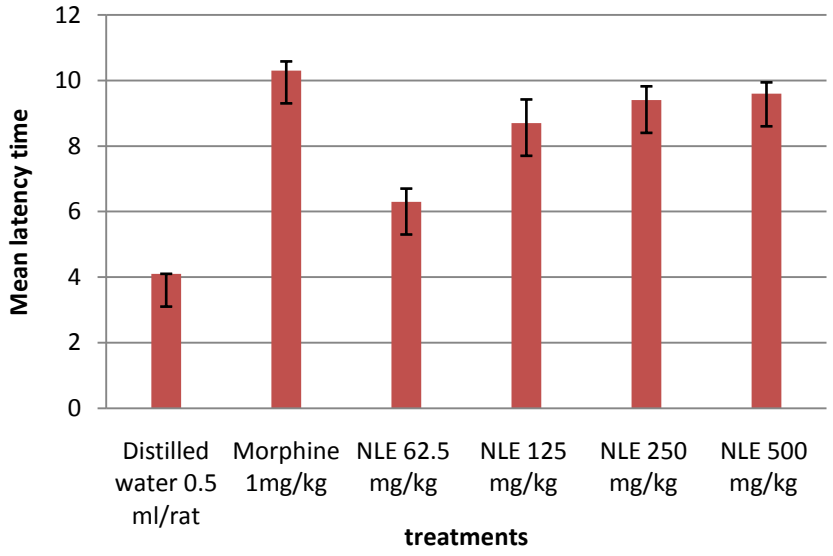

Figure 2: Bar Diagram with error bars showing the Effect of NLE and Morphine on mean TFL at 60 min

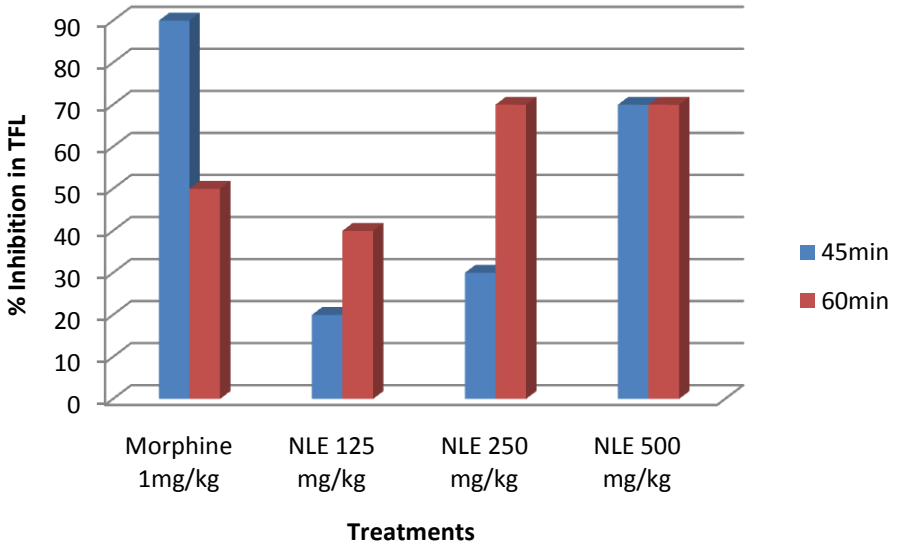

Figure 3 : Bar diagram showing % inhibition of NLE and Morphine on mean TFL response at 45min and 60 min.