

**ANTINOCICEPTIVE ACTIVITY OF FREEZE DRIED POWDERED MORINDA CITRIFOLIA L. FRUIT****HARIOM SINGH, SASWATA BANERJEE, SAUMEN KARAN, TAPAN KUMAR CHATTERJEE\***Division of Pharmacology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700032, India.  
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**ABSTRACT**

**Objective:** The main objective of the study was to establish the antinociceptive action of Freeze dried powdered Noni against Swiss albino mice with the help of various in vivo analgesic models.

**Method:** Antinociceptive effect of powdered Freeze Dried Noni at doses 250 and 500mg/kg b.w was established in Swiss albino mice through acetic acid induced writhing test, radiant heat model, tail immersion model and formalin induced pain model. Diclofenac (10mg/kg) and Pethidine (10mg/kg) were taken as reference for peripheral and central action. Naloxone (2mg/kg) was used to confirm the central action of Noni.

**Result:** It was found that the oral administration of Noni causes significant ( $p < 0.01$ ) reduction in writhes with Diclofenac as standard. Significant ( $p < 0.01$ ) and dose dependent increase in the reaction time in radiant heat and tail immersion model revealed the central antinociceptive effect of Noni. In Formalin induced pain model, Noni (500mg/kg) showed significant inhibition of paw biting and licking events in both the phases of pain. Inhibition of analgesic activity on i.p administration of Naloxone in tail flick test of Noni and Pethidine confirmed the central action of Noni.

**Conclusion:** Thus it was revealed that Noni has significant analgesic activity and act through both mechanisms central as well as peripheral.

**Keywords:** Freeze dried Noni, Antinociceptive, Diclofenac, Pethidine, Naloxone

**INTRODUCTION**

In recent years, medicinal plants emerged as an important source of new chemical substances with potential therapeutic effects. On the basis of the bioactive phytochemical constituents, various parts of the plant such as root, leaves, fruit and bark are used for medicinal purposes [1]. Approximately 25% of the all modern drugs that are used in the treatment of various disorders are derived somehow directly or indirectly from the higher plants [2]. Chronic inflammatory disorders and analgesia are one of the world's major health problems since past [3]. About one-third of the world's population suffers from persistent pain which costs approximately \$100 billion per year in health care [4]. Pain, an unpleasant sensation, generally occurs by external and internal noxious stimuli [5]. In case of tissue injury, visceral distension, and in many other factors pain occurs because of the stimulation of the peripheral nociceptors. In such cases, pain sensation is supposed to be a normal physiological response mediated by healthy nervous system (Fields and Martin, 2008) [6]. There are two types of pain i.e. fast pain and slow pain. Fast pain occur within 0.1 second after the application of noxious stimulus, and slow pain gets initiated after 1 second or more and then gradually increases up to seconds and sometimes even up to minutes [7]. It involves the localized reaction inside the body against the injurious agent caused by release of mediators such as prostaglandins, leukotrienes, bradykinin, histamine, interleukin etc. from the tissue to kill those irritant substances [8]. NSAID'S and opiates are the drugs most widely used across the world to treat pain and inflammation associated with various disorders, but the main drawback of these drugs is occurrence of gastric lesions or ulcers, tolerance and dependence on chronic administration. This gives rise to the need of agents which lacks the above side effects and also possesses potent analgesic and anti-inflammatory activity. Study of plants that were used traditionally as pain-killer seems to be good tool for the development of novel analgesic drugs [9,10]. *Morinda citrifolia* L. which is commonly known as Noni, belonging to Rubiaceae family, a tropical medicinal plant found in South Pacific Asia and other tropical regions of the world, Noni found its use in traditional medicine by Polynesians over 2000 years (Solomon 1999) [11]. Studies reveal the presence of scopoletin, terpenoids, flavanoids, octanoic acid, alkaloids and anthraquinones and various other chemical constituents [12,13]. Traditionally, it has been used as antibacterial, antiviral, analgesic, hypotensive, anti-inflammatory, antitumor, antifungal and immune enhancing agents [14]. Taking into account the traditional use of Noni as pain reliever and search

of medicinal plants having no adverse effect like that of NSAIDS and opioids, attempt has been taken to evaluate the antinociceptive effect of Freeze Dried Powdered *Morinda citrifolia*.

**MATERIALS AND METHODS****Plant materials**

Powdered Freeze Dried Noni (Salveo Lifesciences Ltd., Delhi, India) was taken in the present study for the evaluation of antinociceptive activity.

**Experimental animals**

Swiss albino mice (22±2 gm) were used in the present study, obtained from the Indian Institute of Chemical Biology (IICB), Kolkata, India. Standard laboratory conditions, temperature around 25±2 °C, 12 hour dark/light cycle and relative humidity 55-60 % was maintained throughout the experiment. Animals were provided with the standard laboratory diet (Hindustan Uniliver Limited, Kolkata) and water *ad libitum*. All the explained procedures were carried out as per the Jadavpur University Animal Ethics Committee guidelines (147/1999/CPCSEA).

**Reagents and chemicals**

All reagents and chemicals used were of analytical grade. Formalin (Rankem Fine Chem Limited, New Delhi India), Acetic acid (Merck Ltd, Mumbai, India), Pethidine (Pethitroy-100, Troikka Pharmaceutical, Gujarat, India), Diclofenac sodium (Novartis India Ltd. Mumbai, India) Naloxone HCl (Nalox, Samarth Life Science Pvt. Ltd, Mumbai, India), Sodium chloride (Merck Ltd, Mumbai, India).

**Acute toxicity study**

The LD50 value of Freeze dried Noni in Swiss albino mice was found to be >2 g/kg b.w by following the procedure according to OECD guidelines 425 (OECD 2008) [15]. Thus, it was cleared that the drug was non toxic to the animals. So, the effective doses selected for the evaluation of analgesic effect were 250mg/kg and 500mg/kg b.w.

**Analgesic activity****Writhing activity by acetic acid (writhing model)**

In this method, mice were divided into 4 groups with six animal in each group, group 1 as normal control received only vehicle (0.9% w/v NaCl), group 2 received Diclofenac (10mg/kg b.w) orally, and

group 3 and 4 were orally administered with the Freeze dried Noni at doses of 250 and 500mg/kg b.w 30 min before the administration of 0.8% Acetic acid intraperitoneally [16]. Abdominal constriction (writhes) per animal was counted over a period of 20 min [17,18] just 5 min after the i.p administration of nociceptive agent. Index of analgesia was referred to as the percentage protection against abdominal constriction [19].

It is calculated as:

$$\frac{\text{No. of writhing in control group} - \text{No. of writhing in treated group} \times 100}{\text{No of writhing in control group}}$$

#### Radiant heat model (central analgesic activity)

The prescreened animals with reaction time 2-3 sec [20] were taken for the study, divided into four groups with six mice in each group. Central analgesia was confirmed by tail flick method by using analgesiometer. Group 1 received normal saline (0.9%w/v), group 2 received Pethidine (10mg/kg b.w) as standard intraperitoneally, and group 3 and 4 were administered orally with Noni at doses of 250 and 500 mg/kg b.w. The intensity of current in naked nichrome wire was maintained at 6 amps and distance between the radiant heat source and tail was maintained at 1.5 cm. The site of application in tail was taken 2.5 cm from tip of the tail [20] and the cut off time was maintained at 10 sec to avoid the tissue damage [21]. After 30 min of all drug treatment except pethidine (after 15 min) reading was taken. Confirmation of central antinociceptive action of Noni was evaluated by using antinociceptive antagonist.

For the evaluation of central antinociceptive effect of Noni, naloxone (2mg/kg b.w) a strong  $\mu$ -receptor blocker was administered intraperitoneally. Freeze dried Noni at doses 250mg/kg and 500mg/kg were orally administered and tail flick response was taken at 15 min, 30 min, 60 min, and 90 min respectively. Pethidine (10mg/kg) i.p. was used as standard [22].

#### Tail immersion model

Mice were divided into the four groups. Group 1 received normal saline, group 2 received Pethidine (10mg/kg b.w) as standard intraperitoneally, and group 3 and 4 were administered orally with Noni at doses of 250 and 500 mg/kg b.w. In above model, the lower region of the tail up to 5 cm was immersed in a beaker of water maintained at temperature of  $55 \pm 0.5$  °C [Janssen et al., 1963] [23]. Reaction time was the time taken by mice to withdraw its tail and

cut off time taken as maximum of 10 sec to avoid tissue damage [24]. After 30 min of all drug treatment except Pethidine (after 15 min) reading was taken.

#### Formalin test

The method used was similar to that described previously (Shibata et al., 1989) [25]. Mice were divided into five groups Group 1 i.e. normal control group received normal saline, group 2 received diclofenac (10mg/kg), group 3 and 4 received 250 and 500mg/kg, b.w of Noni and group 5 received Pethidine (10mg/kg b.w). After 30 min of all drug treatment except pethidine (after 15 min), 20 microlitres of 1% formalin was injected subcutaneously into the right hind paw of mice and responses were taken. The time (in seconds) during which the mice licks and bites the injected paw was taken as an indicator of pain response. Response by drug was measured in both phases for 0-5 min (first phase) and 15-30 min (second phase) after formalin injection [26].

The percentage inhibition of pain response was calculated based on the following formulae [27].

$$\frac{(\text{control mean} - \text{treated mean}) \times 100}{\text{control}}$$

#### Statistical analysis

The results obtained were expressed as Mean  $\pm$  SEM (standard error of mean). Graph pad prism 5.0 was used for statistical analysis by using One way ANOVA (analysis of variance) followed by Dunnett's post-hoc test. The unpaired t-test was used to compare between 2 groups.

## RESULTS

#### Acetic acid induced writhing

Oral administration of freeze dried powdered Noni at doses (250 and 500mg/kg b.w) leads to significant ( $P < 0.01$ ) and dose dependent inhibition of writhing reflex (50.29% and 59% respectively) when compared to control group which confers the antinociceptive activity (Table 1). In first 15 minutes, number of writhes showed by the control group was  $50.50 \pm 3.89$  and that of Noni at 250 and 500mg/kg b.w were  $25.10 \pm 1.96$  and  $20.70 \pm 3.54$ . Percentage inhibition of pain of orally administered Diclofenac (10mg/kg b.w) was found to be 79.60% with respect to the control group.

**Table 1: Effect of NONI and Diclofenac on acetic acid induced writhing response in mice.**

Treatment Groups	Dose(mg/kg)	Number of writhing	Inhibition (%)
Control	10	50.50 $\pm$ 3.89	--
Diclofenac	10	10.30 $\pm$ 1.35*	79.60
NONI	250	25.10 $\pm$ 1.96*	50.29
NONI	500	20.70 $\pm$ 3.54*	59.00

Animals (n=6) were administered with Noni orally and one way ANOVA followed by Dunnett's test was done. Values were expressed in Mean  $\pm$  SEM, \*p<0.01 was considered as significant.

#### Radiant heat model (central analgesic activity)

Oral administration of Noni showed dose dependent and significant ( $P < 0.01$ ) increase in the reaction time (time taken by mice to flick the tail) at doses (250 and 500mg/kg b.w), which confers the centrally acting antinociceptive action of Noni. Increase in reaction time was found to be significant after 30mins and effective up to 60

mins and thereafter it shows a reduction in reaction time at 90 min (Table2).

#### Effect of opioid antagonist

Naloxone (2mg/kg b.w) showed significant reduction in reaction time of Pethidine as well as of Freeze dried Noni (Table 3), revealing the centrally acting property of the drug.

**Table 2: Effect of NONI on reaction time in mice by tail flick test**

Treatment Groups	Dose (mg/kg)	Reaction Time(sec)				
		Basal Time	15mins	30mins	60mins	90mins
Control	10	2.86 $\pm$ 0.14	2.78 $\pm$ 0.09	2.75 $\pm$ 0.13	2.66 $\pm$ 0.04	2.60 $\pm$ 0.14
Pethidine	10	2.83 $\pm$ 0.21	8.21 $\pm$ 0.33*	8.35 $\pm$ 0.32*	8.50 $\pm$ 0.29*	8.31 $\pm$ 0.28*
NONI	250	2.68 $\pm$ 0.09	5.75 $\pm$ 0.49*	5.62 $\pm$ 0.28*	5.85 $\pm$ 0.21*	5.51 $\pm$ 0.36*
NONI	500	2.93 $\pm$ 0.12	6.10 $\pm$ 0.43*	6.58 $\pm$ 0.44*	6.90 $\pm$ 0.29*	6.86 $\pm$ 0.20*

Drug was administered orally, n=6 animals were taken and one way ANOVA followed by Dunnett's test was done. Datas obtained were expressed in Mean  $\pm$  SEM, \*p<0.01 was considered as significant.

Table 3: Effect of NONI and Naloxone in mice by tail flick test

Treatment Groups	Dose (mg/kg)	Reaction Time(sec)				
		Basal Time	15mins	30mins	60mins	90mins
Naloxone	2	3.25±0.33	3.35±0.30	4.58±0.57	4.43±0.47	3.46±0.36
Naloxone +	2	2.86±0.21	3.75±0.21	3.66±0.16	3.56±0.20	3.65±0.16
Pethidine	10					
Naloxone +	2	2.71±0.09	3.68±0.24	4.18±0.20	3.56±0.20	4.43±0.23
NONI	250					
Naloxone +	2	3.21±0.25	3.70±0.15	4.18±0.20	4.18±0.27	4.21±0.33
NONI	500					

Naloxone and Pethidine were given intraperitoneally in (n=6) animals. One way ANOVA followed by Dunnett's test was done. Datas obtained were expressed in mean ± SEM.

Table 4: Effect of NONI in mice by tail immersion test

Treatment Groups	Dose (mg/kg)	Reaction Time(sec)				
		Basal Time	15mins	30mins	60mins	90mins
Control	10	1.66±0.21	2.00±0.36	2.16±0.30	2.08±0.28	2.11±0.28
Pethidine	10	2.33±0.42	6.21±0.84*	6.38±0.65*	6.05±0.48*	5.73±0.45*
NONI	250	1.83±0.30	3.38±0.32*	3.66±0.91*	3.70±0.73*	3.55±0.80*
NONI	500	1.83±0.30	4.41±0.41*	4.83±0.70*	4.95±0.68*	4.81±0.73*

Noni was given by oral route in (n=6) animals and one way ANOVA followed by Dunnett's test was done. Data obtained were expressed in Mean ± SEM, \*p<0.01 was considered as significant.

Table 5: Effect of NONI and Diclofenac on mice in formalin induced pain model.

Treatment Groups	Dose (mg/kg)	Licking of hind paw (sec)			
		(0-5mins)	Inhibition%	(15-20mins)	Inhibition%
Control	10	94.33±1.30	--	96.00±0.96	--
Diclofenac	10	95.00±2.16		27.00±2.08*	71.87
Pethidine	10	24.33±2.06*	74.20	18.50±1.76*	80.72
NONI	250	86.33±2.97	8.48	50.83±2.24*	47.05
NONI	500	83.33±2.96**	11.66	41.83±3.26*	56.42

Drug was given orally in six animals and one way ANOVA followed by Dunnett's test was done. Data obtained were expressed in Mean ± SEM, \*\*P<0.05 and \*P<0.01 was considered as significant.

#### Tail immersion model

Powdered freeze dried Noni administered orally at different doses (250 and 500mg/kg b.w) showed significant (P<0.01) and dose dependent inhibition in tail flick response. Antinociceptive effect of drug was found to be significant after 30 min and get reduced after 90 min. (Table 4)

#### Formalin induced pain model

Inhibition of Formalin induced pain was observed on oral administration of Noni at doses of 250 and 500mg/kg b.w, Dose of 500mg/kg b.w showed significant (P<0.05) inhibition (74.20%) in early phase (0-5min) of pain. In the late phase both the doses showed significant (P<0.01) inhibition (47.05 and 56.42%) in the licking and biting of paw i.e. pain response. Diclofenac (10 mg/kg b.w) showed significant (P<0.01) inhibition in the second phase of pain response, and Pethidine (10mg/kg b.w) showed significant (P<0.01) in both phases of pain (Table5).

#### DISCUSSION

The above study reveals the antinociceptive activity of Noni which was evident by the use of acetic acid induced writhing model (peripheral acting), radiant heat model (centrally acting), tail immersion model (centrally acting), and formalin induced pain model (peripheral and central acting both).

The acetic acid induced writhing is one of the simplest and reliable methods for evaluation or screening of analgesic drug [28]. Intraperitoneal administration of acetic acid produced abdominal constriction thereby causing hind limb extension because of the nociceptor sensitization due to increased production of

prostaglandins (PGE2 and PGF2α) in peritoneal fluid of albino mice [29,30]. Freeze dried Noni causes significant reduction in the pain response as compared to acetic acid control group, but less than the standard drug (diclofenac sodium). Inhibition of pain response or less number of abdominal contractions reveals that drug act somewhat through inhibiting the prostaglandin synthesis. The acetic acid model provides the evidence that Noni relieves pain through peripheral mechanism.

The radiant heat model of the tail flick test involves spinal reflex mechanism, and used for the screening of central analgesic agents (Jensen and Yaksh, 1986; Le Bars et al., 2001) [31]. The drug shows increase in the pain threshold or increase in reaction time when compared to normal control. Pethidine was used as standard. Naloxone is an opioid antagonist and blocks all three opioid receptor (μ,δ,κ) that are involved in central analgesia [32]. Blocking of opioid receptor by naloxone abolishes the antinociceptive effect of Noni and pethidine showing that the drug also acts centrally via opioid receptor.

In the tail flick response of tail immersion, spinally mediated reflex is mediated by a supraspinal inhibitory mechanism [32,33,34]. Inhibition of pain response or increase in pain threshold was observed on Noni administration.

In the formalin induced pain model, central and peripheral both types of pain were induced. Biphasic pain measured for 0-5 min (neurogenic i.e. first phase) and 15-30 min (inflammatory origin i.e. second phase) after formalin injection [26]. Pain in first phase was because of direct activation of nociceptor and is not responsive to anti inflammatory drugs. Whereas the second phase is based on peripheral pain mechanism and the chemical mediators released

from the damaged cells which acts on nociceptor and induces pain. Noni and pethidine showed inhibition of pain responses in both the phases whereas diclofenac showed inhibition in second phase.

#### CONCLUSION

The above study reveals that the Powdered Freeze dried Noni relieves pain by peripheral and central mechanism both without any gastric side effects. However further study is going on for finding the exact mechanism by which it relieves pain.

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