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# Anti-Inflammatory Effect of Neem Leaf Extract (NLE) On Albino Rats.

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**ABSTRACT**

Neem (*Azadirachta indica*) is a divine tree known since prehistoric times and utilized by mankind in various diseases**.** Based on the evidence of its anti-inflammatory principles and rich medicinal values the present study has been taken up to evaluate the anti-inflammatory action using the Neem Leaf Extract (NLE).The present study is a randomised control study. The study was divided into 6 groups each group consisting of 6 rats: Group I: Control (distilled water 0.5ml/rat); Group II: Standard (Aspirin 200mg/kg body weight orally ); Group III,IV,V,VI (NLE 62.5 ,125,250, 500 mg/kg body weight intraperitoneally respectively). The experimental model used in this study was the Carrageenan induced rat paw oedema. Results were statistically analyzed by applying unpaired't' test. All values of $p< 0.05$ were taken as significant. NLE with 62.5 mg/kg body weight did not show any significant reduction in oedema, but NLE in dose of 125 mg/kg weight showed significant reduction of oedema at 3$^{rd}$ and 4$^{th}$ hour.NLE with 250 and 500 mg/kg body weight inhibited the oedema by 52.32 % and 63.01 %, respectively at 4$^{th}$ hour of Carrageenan injection.Thus the present study demonstrated the anti-inflammatory action of NLE which increased with the progressive increase with dose.

**Keywords:** NLE, Carrageenan, Anti-inflammatory

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## INTRODUCTION

Neem is a divine tree, and considered to be a gift of nature to mankind. It has been known since prehistoric times and utilized by mankind in various diseases. The latin name of neem, is Azadirachta indica, originally derived from Persians. It is aptly described as a village dispensary [1]. It is about 25 meters high and drought resistant [2]. The phytochemical analysis showed bioactive compounds like alkaloids, lavonoids, triterpenoids, azadirachtin, nimbinin which possess anti-inflammatory property [3]. Neem leaf also has immunomodulatory, analgesic, anti-inflammatory, anti-pyretic, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, anticarcinogenic properties [4-6]. Hence the present study is to evaluate the anti-inflammatory action of Neem Leaf Extract (NLE).

## MATERIALS AND METHODS

### Material

### Collection of plant extract

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

### Chemicals

Aspirin (Burgoyne Burbidges and Co,India), Carrageenan (Sd fine-Chem Ltd,India). Other solvents and chemicals used were of analytical grade.

### Animals

Healthy albino rats irrespective of sex, weighing between 150 -200 grams were selected for the study. These animals were obtained from the central animal house of V.S.S. Medical College, Burla under the Department of Pharmacology. The animals were exposed to 12hrs of dark light cycle and kept under natural temperature and humidity. The animals once selected for the study were separated in cages and were given food and water ad libitum. The animals were acclimatized to laboratory conditions for 7 days prior to the experiments.

### Method

### Carrageenan induced rat paw edema

The present study is a randomised control study. The study was divided into 6 groups each group consisting of 6 rats: Group I: Control (distilled water 0.5ml/rat); Group II: Standard ( Aspirin 200mg/kg body weight orally ); Group III,IV,V,VI (NLE 62.5 ,125,250, 500 mg/kg body weight i.p respectively). Total volume of the oral dose kept constant at 1 ml/rat and for all intra-peritoneal injections the volume was kept constant at 0.5 ml/rat.

The inflammation in the form of hind paw edema was produced by injection 0.1 ml of 1 % suspension of carrageenan in normal saline below the planter aponeurosis of right hind paw of rats and simultaneously 0.1 ml of normal saline was injected below the planter aponeurosis of left hind paw of each rat included in the control group [7]. A mark was made on both the hind limbs just beyond the tibio-tarsal joint. Volume of paw edema was measured by water displacement method and the displaced water was collected in the microburette. A glass tube with side outlet was used for this purpose. The outlet was fixed inside the microburette of 2 ml capacity with micro-graduations. The glass tube and the microburette were fixed on different stands. The whole glass apparatus was washed with chromic acid so that water does not stick to its sides. When the hind limb was dipped inside the tube up to the given mark, water over flowed from the side outlet to microburette. Reading in the microburette was read to find out the volume of water displaced. The volume of displaced water was equal to the volume of paw. Standard and the test drugs were given 1 hour before carrageenan injection. Volumes of both the hind paws were measured before and 1, 2, 3, 4, 6, 12 and 24 hours after carrageenan injection. The efficacy of the drug was tested on its ability to inhibit paw edema.

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NLE was given intra-peritoneally in the doses of 62.5 mg, 125 mg, 250 mg and 500 mg/kg body weight to the different groups of rats.

The percentage inhibition of paw edema was calculated by the formula [8]

$$\Rightarrow \frac{Vc - Vt}{Vc} \times 100$$

Where: Vc = Mean volume of paw edema in the control group of animals
Vt = Mean volume of paw edema in the drug treated group of animals.

## RESULTS

Results were statistically analyzed by applying unpaired't' test. All values of $p < 0.05$ were taken as significant. It is evident that there is a gradual increase in paw volume after carrageenan injection and it reached maximum at 4th hour after which it declined gradually (Table 1). Aspirin 200 mg/kg inhibited the development of edema significantly from 1st hour onwards. It showed maximum reduction (72.05 %) in paw edema at 4th hour (Table 2). NLE with 62.5 mg/kg body weight did not show any significant reduction in edema but NLE in dose of 125 mg/kg weight showed significant reduction of edema at 3rd and 4th hour. Whereas NLE with 250 mg and 500 mg/kg body weight showed significant inhibition in edema from 2nd hour to 6th hour of carrageenan injection. However, distilled water had no effect in inhibition of edema. NLE at the dose of 125 mg/kg body weight showed 27 % inhibition of edema at 3rd hour and 26 % at 4th hour of carrageenan injection. However at 4th hour of carrageenan injection, NLE with 250 and 500 mg/kg body weight inhibited the edema by 52.32 % and 63.01 %, respectively (Figure 2). Figure 1 shows the gradual decrease in paw edema with progressive increase in doses NLE.

**Table 1: Effect of Neem Leaf Extract (NLE) on carrageenan induced hind paw edema**

| Treatments | Mean volume of edema in ml ± SEM at different hours of carrageenan injection | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 hours | 1 hours | 2 hours | 3 hours | 4 hours | 6 hours | 24 hours |
| Distilled water 0.5 ml/rat | 0.1 | 0.39 ± 0.05 | 0.72 ± 0.05 | 1.16 ± 0.06 | 1.25±0.07 | 1.0±0.08 | 0.38±0.048 |
| Aspirin 200mg/kg | 0.1 | 0.25 ± 0.03a | 0.32 ± 0.028d | 0.37 ± 0.02d | 0.34 ± 0.02d | 0.32 ± 0.02d | 0.29 ± 0.00a |
| NLE 62.5 mg/kg bw | 0.1 | 0.37 ± 0.06 | 0.72 ± 0.05 | 1.15 ± 0.06 | 1.25± 0.07 | 0.98±0.08 | 0.28±0.06 |
| NLE 125 mg/kg bw. | 0.1 | 0.35 ± 0.043 | 0.62 ± 0.08 | 0.85 ± 0.11 a | 0.9±0.086c | 0.73±0.081 | 0.38±0.048 |
| NLE 250 mg/kg bw | 0.1 | 0.35 ± 0.05 | 0.45 ± 0.062b | 0.57 ± 0.1 d | 0.58± 0.11d | 0.45±0.07d | 0.25±0.043 |
| NLE 500 mg/kg bw | 0.1 | 0.32 ± 0.031 | 0.42 ± 0.017 c | 0.5 ± 0.026 d | 0.45± 0.022 | 0.32±0.031d | 0.22±0.03c |

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

**Table 2: Percentage inhibition of edema at various doses of NLE at different hours**

| Treatments | 1 hours | 2 hours | 3 hours | 4 hours | 6 hours | 24 hours |
|---|---|---|---|---|---|---|
| Aspirin 200mg/kg | 35.5 | 57.33 | 68.37 | 72.05 | 66.31 | 17.14 |
| NLE 62.5 mg/kg | 7.5 | 4.00 | 1.70 | 2.70 | 3.10 | 2.00 |
| NLE 125 mg/kg | 12.5 | 17.33 | 27.00 | 26.00 | 23.00 | 8.50 |
| NLE 250 mg/kg | 12.5 | 40.00 | 51.28 | 52.32 | 52.63 | 28.57 |
| NLE 500 mg/kg | 20.0 | 44.00 | 57.26 | 63.01 | 66.31 | 37.14 |

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**Figure 1: Line Diagram showing the effect of NLE and Aspirin on Carrageenan induced hind paw edema.**

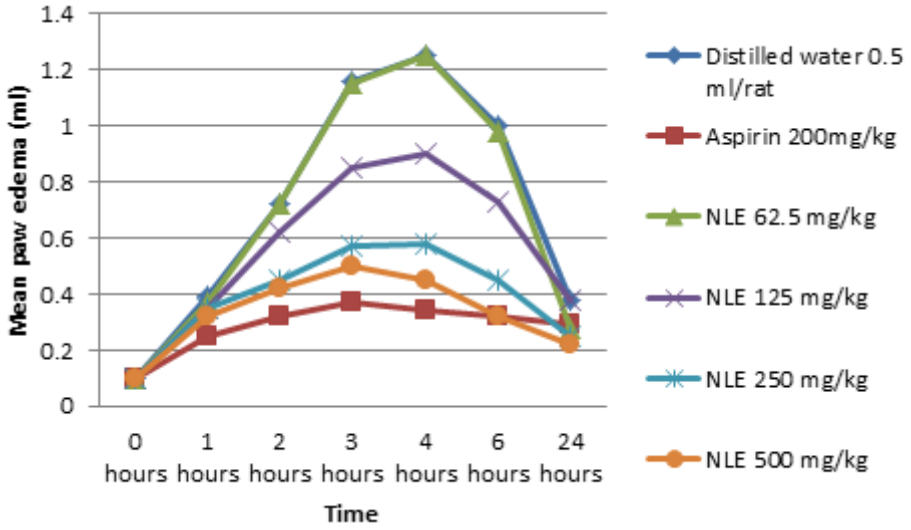
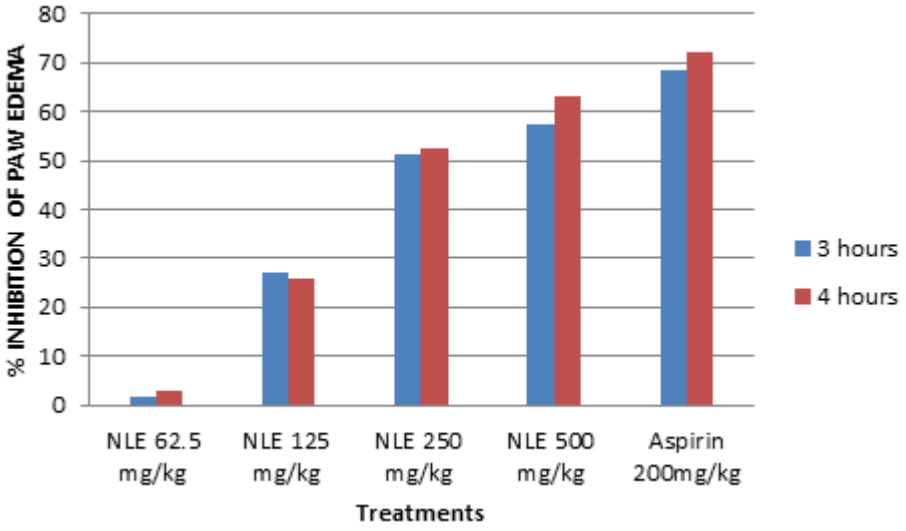



**Figure 2: Bar Diagram showing the percentage (%) inhibition of paw edema of NLE and Aspirin on Carrageenan induced hind paw edema at 3rd and 4th hour.**




## DISCUSSION

Inflammation is the complex protective biological response of living tissues to sublethal stimulus.

Inflammation is studied using two models acute and chronic. Here we are concerned with the acute model which is designed to test drugs that modulate erythema, changes in vascular permeability, leukocyte migration and chemotaxis, phagocytosis [9]. The paw edema induced by carrageenan in rats is biphasic,the first phase (0 -2 hrs) due to release of 5-HT, histamine, bradykinin from mast cells, in between is plateau phase( 3hrs) maintained by kinins [10], second phase (4hrs) produced by prostaglandins ,protease and lysosomes [11]. Aspirin is a non-selective COX inhibitor used here as standard showed significant reduction of paw edema volume compared with control. The anti-inflammatory activity of NLE may be postulated to its COX inhibitory (Prostaglandin synthesis inhibition) activity in a dose dependent manner. Bioactive compounds like nimbinin, nimbin, nimbidinin, nimbolide, nimbidic, flavonoids, glycosides could be responsible for the anti-inflammatory activity [12].

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## CONCLUSION

The Neem Leaf extract showed potential anti-inflammatory activity. Further studies are required for the isolation of chemical constituents and the mechanism of action involved in the anti-inflammatory action of this plant.

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