International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

Analgesic, Anti-inflammatory and Anti-Pyretic Effects of *Azadirachta indica* (Neem) Leaf Extract in Albino Rats

Sanjay Kumar[1], Udaya Kumar Vandana[2], Divya Agrawal[3], Jagadish Hansa[4#]

[1]Department of Pharmacology, IMS & SUM Hospital, Siksha O Anusandhan University, Kalinganagar, Odisha, India

[2]Department of Biotechnology, Assam University, Silchar, Assam

[3]Department of Anatomy, IMS & SUM Hospital, Siksha O Anusandhan University, Kalinganagar, Odisha, India

[4]Department of Surgical Oncology, IMS & SUM Hospital, Siksha O Anusandhan University, Kalinganagar, Odisha, India

Abstract: ***Neem (Azadirachta indica), popularly known as 'Wonder tree' is an evergreen tropical tree, which has been reported to have beneficial effects on various disease pathology from the ancient time. Such health benefits are the sum of molecular actions of the natural ingredients present in it. Among all ingredients azadirachtin, nimbidin, flavonoids, triterpenoids are notable for their beneficial effects against disease pathology. Neem has been reported to have anti-allergenic, anti-dermatic, anti-feedent, anti-fungal, anti-inflammatory, anti-pyorrhoeic, anti-scabic mode of actions. Besides these, neem is also beneficial for cardiac, diuretic, insecticidal, larvicidal, nematicidal, spermicidal and other biological activities. Though, here in the present study, our focus was only in three parameters i.e., whether neem leaf extracts (NLE) can contribute to anti-analgesic, anti-inflammatory and antipyretic action in albino rats, which is a novel one. Our experimental observations revealed that NLE have analgesic, anti-inflammatory and antipyretic effects in Albino rats.***

Keywords: Neem, Neem leaf extract, Biological activity

1. Introduction

Since time immemorial man is in search of remedies for pain, fever and inflammation [1, 2], which are the commonest of human sufferings. Analgesics, anti-inflammatory and antipyretic drugs are frequently used as a remedy [3, 4]. Traditional plant medicines are used globally for different inflammatory disorders [5, 6]. One such plant, which is believed to possess antiseptic, anti-helminthic, insecticidal, antiseptic, anti-diabetic and anti-hypertensive properties, is neem (Indian lilac, *Azadirachta indica*) [7, 8], a native of Indian subcontinent is a highly esteemed tree for the people in the region [1, 9]. *Azadirachta indica* has now been universally accepted as the wonder tree. Currently, neem has attained a place of pride in the international scientific research and literature with the upsurge of interest among the Indian scientific community for which it caught the fascination of the eminent international scientists [9, 10].

Products and preparations of almost every part of *A. indica*, like leaves, flowers, bark and seeds are used for different ailments [11, 12]. The leaves of *A. indica* are used as antidiabetic, antiseptic, wound healing agent, curative agent for skin diseases, anti-ulcer drug and as an anti-inflammatory agent [13–16]. Neem seed oil has been found to be a potential source for the naturally occurring insecticide azadirachtin [17]. The oil has stimulant, alternative, anti-rheumatic and skin disease curing properties [13–16]. Some influential works have shown that, the neem seed oil is relatively non-toxic in nature [18].

Till now different steroidal and non-steroidal anti-inflammatory drugs are used clinically to treat pain, inflammation and fever [19]. But these drugs are not free from side effects like gastric irritation and on chronic use non-steroid anti-inflammatory drugs cause peptic ulcer as well as upper gastrointestinal tract bleedings [20–22]. For these reasons man is always in search of a better anti-inflammatory drug with minimum side effects. So the present work has been undertaken to study the effect of NLE and on experimental rat models of pain, inflammation and fever.

2. Methodology

2.1 Materials

Healthy albino rats of either sex, weighing between 150-200 grams were selected for the study. These animals were housed in the animal room and were exposed to natural temperature and humidity. The animals once selected for the study were separated in cages and were given food and water *ad libitum*. Food in these animals consisted of soaked grams, bread and milk prepared from skimmed milk powder. During the experiment the animals were kept in a fasting state and later on they were separated so that they were not used subsequently. The apparatus and drugs used in various studies are as follows: Analgesiometer (Techno), Tuberculin Syringe; Morphine Sulphate (Morphitroy, Troikaa Pharmaceuticals Limited, Gujarat, India), Neem Leaf extract (Indian herbs research supply Co. Ltd., Saharanpur, India.), Glass jar with micro burette (Borosil, India), Tuberculin Syringes, Feeding tube, Carrageenin (Sd fine-Chem Ltd, India) Aspirin (Burgoyne Burbidges and Co, India), neem leaf extract (NLE), Clinical Thermometer, Dried brewer's

yeast (HiMedia Laboratories Private Limited, Mumbai, India), Paracetamol (Dr. Reddy's Laboratory, Hyderabad)

2.2 Methods

Analgesic Study: For the study of analgesic property, albino rats were randomly divided into different groups. Ten rats were taken in each group (n=10). Morphine sulphate was used as reference standard drug for this study and normal saline was used as vehicle. The test drugs used was NLE, which was dissolved in distilled water. The standard drug morphine as well as test drugs NLE were given intraperitoneally with all aseptic measures. The volume of all intraperitoneal injection was kept constant within 0.5ml. The analgesic effect of the two drugs was assessed by the experimental pain model of tail flick response to thermal stimulation. The thermal stimulation was given by analgesiometer. 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.

Rat's tail (2cm from tip) was kept 3mm 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). 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 15min, 30min, 45min, 60min, 90min, 120min and 180min after drug administration. NLE, in doses of 62.5mg, 125mg, 250mg and 500mg/ kg body weight were given intraperitoneally to different groups of rats. The results were statistically analyzed by applying the chi-square test.

Anti-inflammatory study: The anti-inflammatory property of Neem leaf extract was tested on the model of acute inflammation. Acute inflammation in the form of hind paw edema was produced according to previously described method [23] by injecting 0.1 ml of 1% suspension of carrageenin in normal saline below the plantar aponeurosis of right hind paw of rats simultaneously 0.1 ml of normal saline was injected below the plantar aponeurosis of left hind paw of each rat included in the control group. '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 micro-burette. A glass tube with side outlet was used for this purpose. The outlet was fixed inside the micro-burette of 2 ml capacity with micro-graduations. The glass tube and the micro-burette 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 micro-burette. Reading in the micro-burette was read to find out the volume of water displaced. The volume of displaces water was equal to the volume of paw. Standard and the test drugs were given 1 hour before carrageenin injection. Volumes of both the hind paws were measured before and 1, 2, 3, 4, 6, 12 and 24 hours after carrageenin injection. The efficacy of the drug was tested on its ability to inhibit paw edema. Initially two groups of rats were taken for the study of effect of Aspirin, which was chosen as the reference standard drug. Aspirin was made into suspension with 5% Gum acacia. To the first group only 5% Gum acacia suspension (1ml/rat) and to the second group, suspension of aspirin (200mg/kg bw.) was given orally with the help of a feeding tube. Total volume of the oral dose kept constant at 1ml/rat.

NLE was given intraperitoneally in the doses of 62.5mg, 125mg, 250mg, and 500mg/kg body weight to the different groups of rats. For all intraperitoneal injections the volume was kept constant at 0.5ml/rat. Results were statistically analyzed by applying unpaired't' test. All values of $p< 0.05$ were taken as significant. The percentage of inhibition of paw edema was calculated by the formula used by previously described method [24].

Vc-Vt X 100 Vc

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.

Anti-pyretic study: In this study the albino rats were randomly divided into groups of six. Prior to the experiment the rats were kept in separate cages for 7 days in the laboratory to make them acquainted with room temperature and humidity. Rectal temperatures of these animals were recorded at 9:00 am and 9:00 pm daily to see whether any diurnal variation of temperature exists. This process was continued for 7 days. The animals were given food and water ad libitum. Those animals that had a constant rectal temperature or a variation of less than 10c were included in the study. Rectal temperatures were recorded with the help of a clinical thermometer.

The antipyretic study was done according to a previously described method [25] by using the brewer's yeast induced pyrexia model in rats. Fever was induced by injecting 20ml/kg body wt of 20% suspension of brewer's yeast subcutaneously below the nape of neck. During the period of experiment the rats were not given solid food and only given water ad libitum. In our set up the rats developed fever after 10 hours of yeast injection. Only those animals which developed fever were taken for further study and rest were rejected. Both the standard and test drugs were given intraperitoneally after development of initial pyrexia and the volume of injection were kept constant at 0.5ml/rat. Paracetamol 100mg/kg body wt. was taken as standard drug for comparison. NLE was given in the doses of 62.5mg, 125mg, 250mg, and 500mg/kg body wt. intraperitoneally to different sets of rats. The rectal temperatures were recorded 15 min, 30 min, 1 hour, 2 hour, 3 hour, 4 hour, 5 hour, 6 hour and 12 hour after the drug treatment. Results were statistically analyzed by using unpaired't' test.

3. Results & Discussion

Analgesic properties of the test drugs: NLE was studied on the experimental model of tail flick response to thermal stimulation in albino rats (Davies et al., 1946). Morphine sulphate 1mg/kg body weight was taken as reference standard drug for analgesic study [26]. A control study was also made with normal saline which was taken as vehicle for morphine. Similar study was also undertaken with distilled water which served as negative control for NLE. The results obtained are tabulated below (Table A1). In order to establish the relationship between drug difference and respective responses chi-square test has been employed here. The data of responses at 45 minutes (Table A1) have been summarized in 2x2 contingency (Table A2).

For this study, we selected the tail flick response model which was originally postulated by Davis et al., [28]. Tail flick response to thermal stimulus was measured as tail flick latency (TFL) on analgesiometer (Techno). This model of pain was selected because it involves central mechanism, causes minimal injury to animals and is reproducible. Morphine sulphate 1 mg/kg body weight showed significant analgesic response from 30 min. to 60 min. of its administration being maximum at 45 min (90%). Then this effect declined and reached to basal TFL by 180 min. (Table A1). Carta et al in 1990 had also obtained similar response in TFL with morphine [26]. We observed that NLE in the dose of 250 mg/kg body weight showed significant rise in TFL from 60 minutes to 90 minutes. With 500 mg/kg body Weight. TFL increased significantly from 45 minutes to 90 minutes and in the dose of 62.5 mg and 125mg/kg body weight, it produced no significant effect (Table A2 & Table A3). These findings corroborate with the findings of Khosla et al., [29].

The maximum percentage of response (70%) was observed with NLE in the doses of 250mg and 500 mg/kg body weight at 60 minutes (Table A3). Khosla et al had also found the peak analgesic effect with 500 mg/kg body weight of NLE P.O. at 60 minutes of drug administration. At 60 min Morphine 1mg/kg body weight showed 50% response whereas NLE with 250mg and 500mg/kg body weight showed 70% response (Table A1 and Table A3).

From the results of our study it may be deduced that NLE possessed significant analgesic activities at the doses of 250mg/kg, 500mg/kg and 1ml/kg and 2ml/kg body weight respectively. There is paucity of data regarding the analgesic effect of NLE. Some workers had showed that the analgesic effect of NLE might involve opioid as well non-opioid mechanisms.

From the results as shown in the above table it is indicated that the response differ significantly with the difference of the category of drug (Normal saline and Morphine sulphate) administers to the subjects of experiment. Morphine showed higher percentage (90%) of response than normal saline (0%).

Anti-inflammatory study: For the anti-inflammatory study, Albino rats of either sex were divided randomly into group of six. In this study we took the carrageenin induced hind paw edema as the model of acute inflammation [23]. This model was selected for its simplicity. There was minimal injury to animals and facts can be easily presented. In the anti-inflammatory study the peak volume of carrageenin induced hind paw edema was reached at 4th hour, corresponding to the report of Winter et al., [23] (Table B1). 0.1ml of 1% suspension of carrageenin was injected in the plantar aponeurosis of right hind paw. 0.1ml of Normal saline was injected in left hind paw of control group. The paw volumes were measured by water displacement method. The volume of paw edema was calculated to be the difference between the final paw volume and the initial paw volume. Aspirin was taken as the reference standard drug and 5% Gum acacia was taken as the suspending agent for aspirin. Distilled water served as a negative control of NLE. Percentage inhibition of edema is calculated as follows, (Table B1).

Mean volume of paw edema of control group at 1st hour = 0.4ml

Mean volume of edema of aspirin treated group at 1st hour = 0.258 ml (Table B2).

Percentage inhibition of paw edema:

$$\frac{Vc-Vt}{Vc} \times 100 = \frac{0.4-0.258}{0.4} \times 100 = 35.5\%$$

Aspirin in the dose of 200mg/kg inhibited the development of edema significantly from the very first hour. At 4th hour Aspirin produced 72.05% inhibition of rat paw edema (Fig 1, Table B2). Winter et al found 26% of inhibition of paw edema at a dose of 33.3mg/kg of Aspirin [23]. However Okapanyi et al reported 27.5% inhibition of paw edema with doses of 50mg/kg body weight of Aspirin at 6th hour of carrageenin injection [27]. NLE in the dose of 125 mg/kg body weight showed significant reduction in edema at 3rd and 4th hour of carrageenin injection (Table B3). However in both the doses of 250mg and 500 mg/kg body weight NLE reduced the carrageenin induced edema significantly from 2nd to 6th hour.

Chattopadhyay et al, studied on 5-HT induced inflammation and reported 28.8%, 46.8%, 68.4% inhibition of edema with doses of 0.5gm/kg, 1gm/kg and 2mg/kg body weight of NLE respectively [28]. With the same doses of NLE they also studied on PGE1 induced inflammation model and found 38.5%, 60.5% and77.0% inhibition of edema respectively.

In the present study the percentage inhibition of edema at 4th hour was found to be 2.7%, 26%, 52.32% and 63.01% with the doses of 62.5, 125mg, 250mg, 500mg/kg body weight of NLE respectively given intraperitoneally (Fig 1, Table B4). Okpanyi et al (1981) found the percentage inhibition of edema with NLE 400mg/kg and NLE 800 mg/kg body weight to be 20.6% and 31.8%. In the present study NLE produced dose dependent inhibition of carrageenin induced paw edema [27]. A similar dose dependent anti-inflammatory effect of nimbidin was also observed by Pillai et al 1981 at the doses of 20mg, 40mg, and 80mg/kg body weight nimbidin is a compound derived from Neem Seed oil (Pillai et al 1981), where aspirin showed 63.01% inhibition of paw edema at 4th hour.

Anti-pyretic study: Anti-pyretic study was performed by the method elaborated by Loux et al was selected in which

pyrexia was induced by injecting brewer's yeast suspension subcutaneously to Albino rats [25]. The Brewer's yeast induced pyrexia model was selected because of its simplicity. The reference standard drug was paracetamol in the dose of 100mg/kg body wt. given intraperitoneally. The test drugs taken were NLE in the dose of 62.5mg, 125mg, 250mg and 500 mg/kg body weight. In our set up the pyrexia developed after 10 hours of brewer's yeast injection temperature came down to normal 12 hour after the initial pyrexia (Table C1). Paracetamol showed significant antipyretic effect from 15 min of drug administrator till the period of observation (Fig 2, Table C2). 20% suspension of brewer's yeast in Normal saline was injected sub-cutaneously at a dose of 20ml/kg body weight below the nappe of neck of rats. Fever developed after 10 hours of injection which was referred to as initial pyrexia. Drugs were given after development of initial pyrexia and temperatures were recorded thereafter. Normal saline, the vehicle of brewer's yeast, did not change the basal temperature. Paracetamol was taken as the reference standard drug and a study was also made with distilled water which was taken as vehicle for paracetamol as well as NLE (Fig 2).

NLE in the dose of 125mg/kg body wt. showed significant anti pyretic effect only 4rth hour of Initial pyrexia (Fig2, Table C3). Our observation closely corroborate with the results obtained by Okpanyi et al, with NLE in the dose of 400mg/kg and 800 mg/kg body weight orally in rabbits [27].

On one hand paracetamol 100mg/kg body wt brought the temperature to basal value at 1 hour of its administration but NLE in the dose of 250mg/kg body weight could bring the temperature to basal value only at 6th hour of its administration (Fig2, Table C2 and C3). NLE in the dose of 500 mg/kg body weight brought the temperature to basal value at 4th hour (Fig 2, Table C3).

4. Conclusion

NLE produces significant analgesic, at doses of 250mg and 500mg/kg body weight. But significant anti-inflammatory and antipyretic effects were obtained at doses of 125mg, 250mg and 500mg/kg body weight these action were in dose related manner. These observations revealed that *A. indica* extract is a mixture of several substances and fractionation of the extract could yield an active analgesic, anti-inflammatory and antipyretic substances comparable potency to any established analgesic, anti-inflammatory and antipyretic agent.

5. Acknowledgement

We are thankful to the director of the institution to provide such platform to do the work.

6. Conflict of Interest

Nothing to declare

References

[1] Kumar VS, Navaratnam V. Neem (*Azadirachta indica*): Prehistory to contemporary medicinal uses to humankind. Asian Pac J Trop Biomed 2013;3:505–14. doi:10.1016/S2221-1691(13)60105-7.
[2] Zuskin E, Lipozencić J, Pucarin-Cvetković J, Mustajbegović J, Schachter N, Mucić-Pucić B, et al. Ancient medicine-a review. Acta Dermatovenerologica Croat ADC / Hrvat Dermatolosko Drus 2008;16:149–57.
[3] Aboelsoud N. Herbal medicine in ancient Egypt. J Med Plants Res 2010;4:82–6. doi:10.5897/JMPR09.013.
[4] Kamboj V. Herbal medicine. Curr Sci 2000;78:35–9.
[5] Cohen M. Rosehip: Evidence based herbal medicine for inflammation and arthritis. Aust Fam Physician 2012;41:495–8.
[6] Mobasheri A. Intersection of inflammation and herbal medicine in the treatment of osteoarthritis. Curr Rheumatol Rep 2012;14:604–16. doi:10.1007/s11926-012-0288-9.
[7] Subapriya R, Nagini S. Medicinal properties of neem leaves: a review. Curr Med Chem Anticancer Agents 2005;5:149–146. doi:10.2174/1568011053174828.
[8] Schumacher M, Cerella C, Reuter S, Dicato M, Diederich M. Anti-inflammatory, pro-apoptotic, and anti-proliferative effects of a methanolic neem (*Azadirachta indica*) leaf extract are mediated via modulation of the nuclear factor-κB pathway. Genes Nutr 2011;6:149–60. doi:10.1007/s12263-010-0194-6.
[9] Karmakar A, Karmakar S, Mukherjee S. Biodiesel production from neem towards feedstock diversification: Indian perspective. Renew Sustain Energy Rev 2012;16:1050–60. doi:10.1016/j.rser.2011.10.001.
[10] Kaushik N, Gurudev Singh B, Tomar UK, Naik SN, Vir S, Bisla SS, et al. Regional and habitat variability in azadirachtin content of Indian neem (*Azadirachta indica* A. Jusieu). Curr Sci 2007;92:1400–6.
[11] Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. Biological activities and medicinal properties of neem (*Azadirachta indica*). Curr Sci 2002;82:1336–45.
[12] Jayaraj S. Neem - the wonder tree of India. Chang Villages 1989;8:195–9 LA – English.
[13] Mukherjee PK, Maiti K, Mukherjee K, Houghton PJ. Leads from Indian medicinal plants with hypoglycemic potentials. J Ethnopharmacol 2006;106:1–28. doi:10.1016/j.jep.2006.03.021.
[14] Patil R, Patil R, Ahirwar B, Ahirwar D. Current status of Indian medicinal plants with antidiabetic potential: A review. Asian Pac J Trop Biomed 2011;1. doi:10.1016/S2221-1691(11)60175-5.
[15] Aqil F, Ahmad I, Mehmood Z. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Turkish J Biol 2006;30:177–83.
[16] Kumar VP, Chauhan NS, Padh H, Rajani M. Search for antibacterial and antifungal agents from selected Indian medicinal plants. J Ethnopharmacol 2006;107:182–8. doi:10.1016/j.jep.2006.03.013.
[17] Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH. Natural plant chemicals: sources of industrial and

medicinal materials. Science 1985;228:1154–60. doi:10.1126/science.3890182.
[18] Boeke SJ, Boersma MG, Alink GM, Van Loon JJA, Van Huis A, Dicke M, et al. Safety evaluation of neem (*Azadirachta indica*) derived pesticides. J Ethnopharmacol 2004;94:25–41. doi:10.1016/j.jep.2004.05.011.
[19] Bateman DN. Non-steroidal anti-inflammatory drugs. Medicine (Baltimore) 2012;40:140. doi:10.1016/j.mpmed.2011.12.027.
[20] Wilson JC, Murray LJ, Hughes CM, Black A, Anderson LA. Non-steroidal anti-inflammatory drug and aspirin use and the risk of head and neck cancer. Br J Cancer 2013;108:1178–81. doi:10.1038/bjc.2013.73.
[21] Schmidt M, Christiansen CF, Mehnert F, Rothman KJ, Sørensen HT. Non-steroidal anti-inflammatory drug use and risk of atrial fibrillation or flutter: population based case-control study. BMJ 2011;343:d3450. doi:10.1136/bmj.d3450.
[22] Chan FKL, Graham DY. Review article: prevention of non-steroidal anti-inflammatory drug gastrointestinal complications--review and recommendations based on risk assessment. Aliment Pharmacol Ther 2004;19:1051–61. doi:10.1111/j.1365-2036.2004.01935.x.
[23] Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs. Proc Soc Exp Biol Med 1962;111:544–7.
[24] Pillai NR and Santhakumari G. Planta Med. 1981;43; 59-63.
[25] Loux JJ, DePalma PD, Eby RZ, Yankell SL. Antipyretic testing of commercial aspirin formulations in rats. J Pharm Sci 1977;66:674–6.
[26] Carta F, Bianchi M, Argenton S, Cervi D, Marolla G, Tamburini M, et al. Effect of nifedipine on morphine-induced analgesia. Anesth Analg 1990;70:493–8. doi:10.1213/00000539-199005000-00005.
[27] Okpanyi SN EG. Anti-inflammatory, and antipyretic activities of *Azadirachta indica.* Planta Medica. Planta Med 1981;41:34–49. doi:DOI: 10.1055/s-2007-971670.
[28] Chattopadhyay R, Bandyopadhyay M. Effect of *Azadirachta indica* leaf extract on serum lipid profile changes in normal and streptozotocin induced diabetic rats. African J Biomed Res 2006;8:101–4. doi:10.4314/ajbr.v8i2.35769.
[29] Khosla P, Bhanwra S, Singh J, Seth S, Srivastava RK. A study of hypoglycaemic effects of *Azadirachta indica* (Neem) in normaland alloxan diabetic rabbits. Indian J Physiol Pharmacol 2000;44:69–74.

Figures Legends

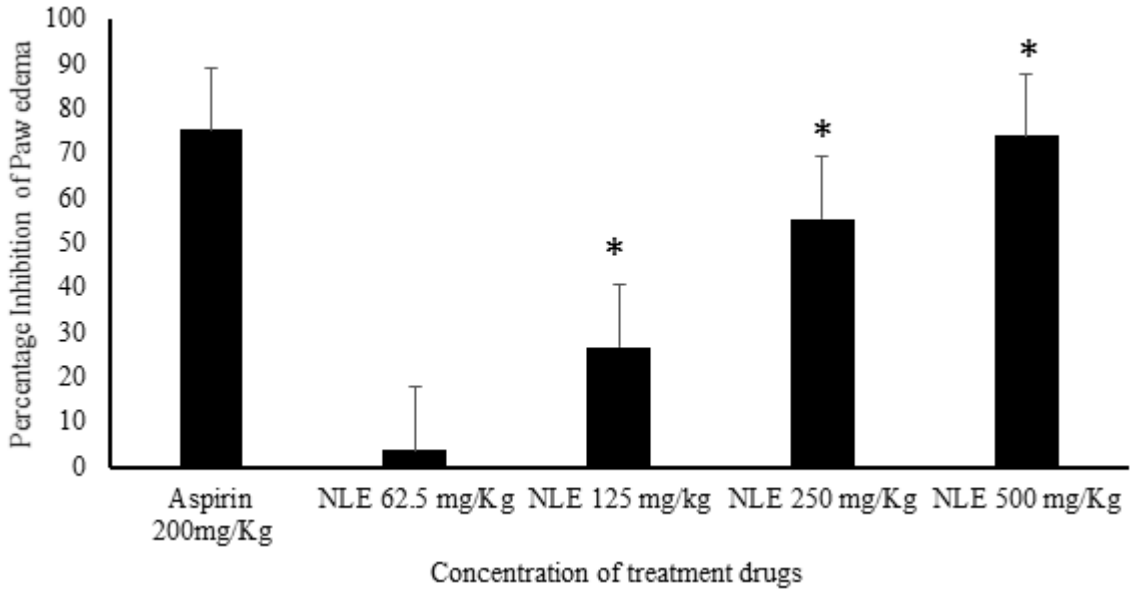

Figure 1: Percentage inhibition of paw edema of Aspirin and NLE at 4rth hour of carrageenin injection

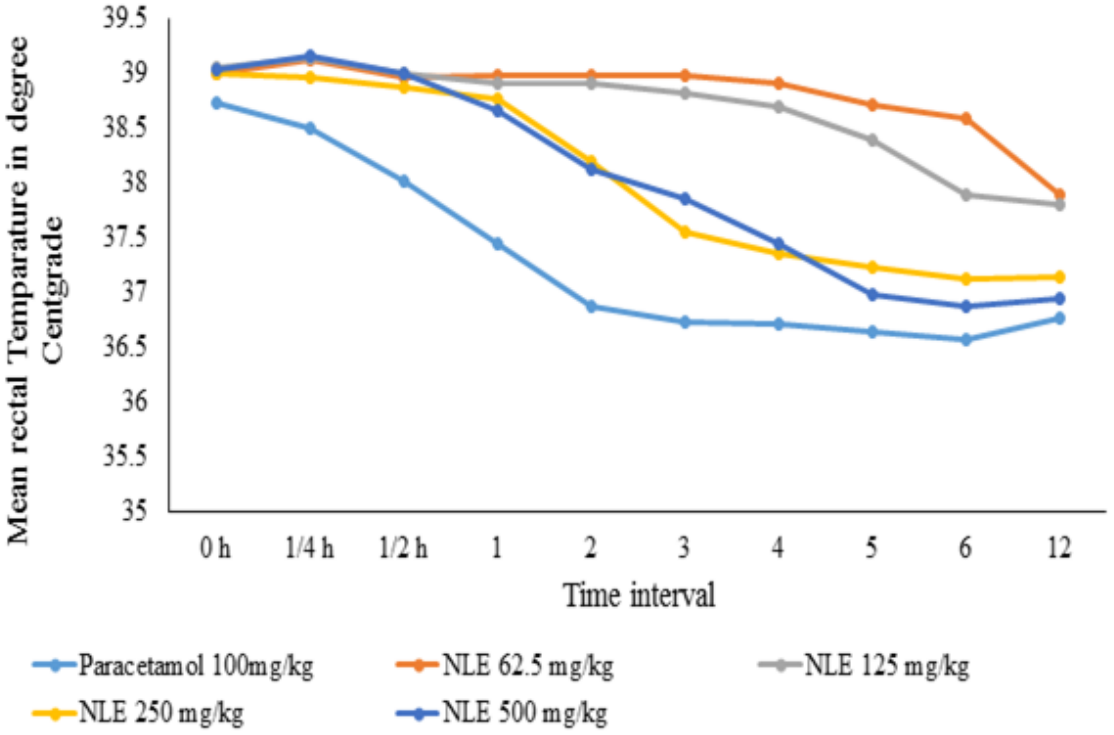

Figure 2: Effect of neem leaf extract & paracetamol on yeast induced pyrexia

Table Legends:

Table A1
Table A2

Effect of Morphine on TFL response (TFL in seconds)								
DRUGS	Mean basal TFL (in seconds)	Mean T.F.L. ± SEM in second						
		15minute	30minute	45minute	60minute	90minute	120 Minute	180 minute
Distilled water 0.5ml/rat	3.9±0.23	3.9±0.28	4.0±0.21	4.0±0.21	4.1±0.28	4.1±0.24	4.0±0.21	4.2±0.29
NLE 62.5mg./kg. body wt.	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 125mg./kg. body wt.	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 250mg./kg. body wt.	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±2.6	4.2±0.29
NLE 500mg./kg. body wt.	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								
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. There is no change in TFL with distilled water injection.								

Table A3

Effect ofNLE on TFL response at varying time intervals															
DRUGS	No. of animals in each group	No. & % of animals showing TFL ≥ 10 seconds													
		15minute		30minute		45minute		60minute		90minute		120 Minute		180 minute	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
NLE 62.5mg./kg. body wt.	10	0	0	0	0	0	0	0	0	1	10	0	0	0	0
NLE 125mg./kg. body wt.	10	0	0	1	10	2	20	4	40	4	40	2	20	0	0
NLE 250mg./kg. body wt.	10	0	0	0	0	3	30	c 7	70	b 6	60	0	0	0	0
NLE 500mg./kg. body wt.	10	0	0	3	30	c 7	70	c 7	70	b 6	60	3	30	0	0
	a=> p<0.05			b=> p<0.02				b=> p<0.01				d=> p<0.001			

NLE in dose of 250mg/kg body wt. showed significant increase in TFL from 60 minutes at 90 minutes of its administration. With 500 mg/kg body wt., the TFL increased significantly from 45 minutes to 90 minutes. NLE in doses mg/kg & 500mg/kg revealed maximum. percentage of responses (70%) at 60min of drug administration.

Table: B1

Volume of paw edema in ml at different hours of carrageenin injection															
No. of rats	Initial paw volume	0 HR		1 HR		2 HR		3 HR		4 HR		6HR		24 HR	
		Final paw volume	Volume of edema	Final paw volume	Volume of edema	Final paw volume	Volume of edema	Final paw volume	Volume of edema	Final paw volume	Volume of edema	Final paw volume	Volume of edema	Final paw volume	Volume of edema
1	1	1.1	0.1	1.5	0.5	2	1	2.4	1.4	2.3	1.3	2	1	1.4	0.4
2	1.9	1	0.1	1.3	0.4	1.5	0.6	1.8	0.9	2	1.1	1.8	0.9	1.3	0.4
3	0.8	0.9	0.1	1	0.2	1.3	0.5	1.9	1.1	1.9	1.1	1.6	0.8	1.1	0.3
4	1.1	1.2	0.1	1.6	0.5	1.8	0.7	2.4	1.3	2.4	1.3	2.3	1.2	1.4	0.3
5	1	1.1	0.1	1.3	0.3	1.9	0.9	2.2	1.2	2.2	1.2	1.8	0.8	1.3	0.3
6	0.8	0.9	0.1	1.3	0.5	1.6	0.8	1.9	1.1	2.1	1.3	1.8	1	1.2	0.4
Mean			0.1		0.4		0.75		1.17		1.22		0.95		0.35
SEM			0		0.051		0.08		0.07		0.04		0.062		0.022
It is evident that there is a gradual increase in paw volume after carrageenin injection and it reached maximum at 4th hour after which it declined gradually.															

Table B2

No. of Rats	Volume of paw edema in ml in different hours of carrageenin injection						
	0 hrs	1 hrs	2 hrs	3 hrs	4 hrs	6 hrs	24 hrs
1	0.1	0.25	0.3	0.36	0.35	0.32	0.3
2	0.1	0.3	0.35	0.39	0.32	0.3	0.29
3	0.1	0.36	0.41	0.46	0.44	0.42	0.28
4	0.1	0.29	0.37	0.42	0.38	0.35	0.3
5	0.1	0.17	0.22	0.26	0.24	0.24	0.29
6	0.1	0.18	0.28	0.32	0.3	0.29	0.3
Mean	0.1	a 0.258	d 0.32	d 0.37	d 0.34	d 0.32	a 0.293
SEM	0	0.03	0.028	0.029	0.028	0.024	0.003
% inhibition of edema	0	35.5	57.33	68.37	72.05	66.31	17.14
	a=> p<0.05		b=> p<0.02		c=> p<0.01		d=> p<0.001
Aspirin 200mg/kg ihibited the development of edema significantly from 1st hour onwards. It showed maximum reduction (72.05%) in paw edema at 4th hour.							

Table B3

Effect of neem leaf extract (NLE) on carrageenin induced hind paw edema							
DRUGS	Mean volume of edema in ml ± SEM at different hours of carrageenin injection						
	0 hour	1 Hour	2 hour	3 hour	4 hour	6 hour	24 hour
0.1% of carrageenin (control)	0.1	0.4±0.051	0.75±0.08	1.17±0.07	1.22±0.04	0.95±0.062	0.35±0.022
Distilled water 0.5ml/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
NLE 62.5mg./kg. body wt.	0.1	0.37±0.06	0.72±0.05	0.72±0.05	1.25±0.07	0.98±0.08	0.28±0.06
NLE 125mg./kg. body wt.	0.1	0.35±0.043	0.62±0.08	a 0.85±0.11	c 0.9±0.086	0.73±0.081	0.38±0.048
NLE 250mg./kg. body wt.	0.1	0.35±0.05	b 0.45±0.062	d 0.57±0.1	d 0.58±0.11	d 0.45±0.07	0.25±0.0043
NLE 500mg./kg. body wt.	0.1	0.32±0.031	c 0.42±0.017	d 0.5±0.026	d 0.45±0.022	d 0.32±0.031	c 0.22±0.023
	a=> p<0.05		b=> p<0.02		c=> p<0.01		d=> p<0.001
NLE with 62.5 mg/kg body wt did not show any significant reduction in edema but NLE in dose of 125 mg/kg body wt showed significantreduction of edema at 3rd and 4rth hour. Whereas NLE with 250mg and 500mg/kg body wt showed significant inhibition in edema from 2nd hour to 6th hour of carrageenin injection. However distilled water had no effect in inhibition of edema.							

Table B4

DRUGS	Mean volume of edema in ml ± SEM at different hours of carrageenin injection						
	1 Hour	2 hour	3 hour	4 hour	6 hour	24 hour	
NLE 62.5mg./kg. body wt.	7.5	4	1.7	2.7	3.1	2	
NLE 125mg./kg. body wt.	12.5	17.33	27	26	23	8.5	
NLE 250mg./kg. body wt.	12.5	40	51.28	52.32	52.63	28.57	
NLE 500mg./kg. body wt.	20	44	57.26	63.01	66.31	37.14	
NLE at the dose of 125 mg/kg body weight showed 27% inhibition of edema at 3rd hour and 26% at 4rth hour of carrageenin injection. However at 4th hour of carrageenin injection, NLE with 250 and 500 mg/kg body weight inhibited the edema by 52.32% and 63.01% respectively.							

Table C1

Effect of yeast (20ml/kg) on temperature pattern												
No. of rats	Rectal temeprature In degree centigrade (°C)											
	Basal Temp	Initial pyrexia (10 hours after yeast)	Temperature at different hrs. after development of pyrexia									
			1/4	1/2	1	2	3	4	5	6	12	24
1	36.8	39.2	39.2	39.4	39.6	39	38.8	39.1	38.7	38.7	37.7	36.8
2	37	39.4	39.6	39.4	39.2	39.1	39.4	38.8	38.8	38.7	38	37.2
3	37.4	39.6	39.6	39.4	39.4	39.2	39	38.9	38.4	38.4	37.5	37.4
4	37.2	39.1	39.2	39	39	39	39.2	38.8	38.6	38.5	37.8	36.9
5	37.1	38.9	39.1	38.9	38.8	38.9	38.9	39	38.8	38.7	38	37
6	36.9	39.4	38.9	38.9	38.9	39.2	39	38.9	38.4	38.4	37.7	37.3
Mean	37.07	39.27	39.27	39.17	39.15	39.07	39.05	38.9	38.62	38.6	37.8	37.1
SEM	0.09	0.103	0.115	0.106	0.13	0.05	0.09	0.048	0.075	0.062	0.08	0.097
From the Above table iy is evident that, the yeast induced pyrexia at 10th hr. of it's injection and the pyrexia sustained for 12 hours. Then gradually the temperature reached basal level at about 24 hour of initial pyrexia.												

Table C2

Effect of paracetamol 100mg/kg body weight on yeast induced pyrexia										
No. of rats	Rectal temeprature In °C after drug administration									
	Basal Temp	Initial pyrexia (10 hours after yeast)	Temperature at different hrs. of drug administration								
			1/4	1/2	1	2	3	4	5	6	12
1	37	39	38.4	37.8	36.7	36.6	36.6	37	37.1	36.9	37.2
2	36.9	38.9	38.4	37.6	37.3	37	37.2	37	37	37	37
3	37.1	39.2	39	38.2	37.1	37	37.1	37	36.8	37	37
4	37.1	39	38.8	37.8	36.9	36.5	36.9	36.8	36.9	37	37.1
5	36.5	38.7	38	38.3	37	36.8	36.8	36.8	36.5	36.5	37
6	36.9	38.8	38.4	38	37.5	37.4	37.2	37	36.9	36.9	37
Mean	36.92	38.93	c 38.5	d 37.95	d 37.08	d 36.9	d 36.9	d 36.9	d 36.86	d 36.88	d 37.05
SEM	0.091	0.072	0.144	0.109	0.41	0.133	0.099	0.042	0.085	0.08	0.034
	a=> p<0.05		b=> p<0.02		c=> p<0.01		d=> p<0.001				
Table C2 vs C1 shows that paracetamol produced significant antipyretic effect from 15 minutes of its administration till the observation period. Paracetamol brought the temperature down to normal from 1st hour of drug administration.											

Table C3

Effect of paracetamol 100mg/kg body weight on yeast induced pyrexia											
Drugs	Mean rectal temperature in °C ± SEM										
	Basal Temperature	Initial Pyrexia	Temperature at different hrs of drug administration								
			1/4	1/2	1	2	3	4	5	6	12
Control	37.07± 0.088	39.27± 0.103	39.27± 0.115	39.17± 0.106	39.15± 0.13	39.07± 0.05	39.05± 0.089	38.8± 0.048	38.62± 0.075	38.6± 0.062	37.8± 0.08
Distilled water 0.5ml/rat	36.92± 0.091	39± 0.078	39.15± 0.13	39.27± 0.115	39.15± 0.13	39.12± 0.05	39.02± 0.05	39± 0.078	38.98± 0.09	38.62± 0.09	37.58± 0.06
NLE 62.5mg/kg bw.	36.92± 0.048	39.02± 0.05	39.12± 0.05	39.2± 0.045	39.2± 0.052	39.1± 0.06	39.1± 0.082	38.98± 0.09	38.77± 0.081	38.62± 0.09	37.73± 0.13

NLE 125mg/kg bw.	36.95± 0.077	39.07± 0.042	39.18± 0.031	39.1± 0.037	39.05± 0.022	38.95± 0.034	38.83± 0.049	a 38.7±0.063	c 38.3± 0.052	d 37.95± 0.062	37.58± 0.06
NLE 250mg/kg bw.	36.9± 0.064	39± 0.078	38.98± 0.098	38.93± 0.089	a 38.75± 0.096	c 38.53± 0.109	d 38.18± 0.102	d 37.72± 0.095	d 37.22± 0.084	d 37.05± 0.067	d 37.07± 0.05
NLE 500mg/kg bw.	36.92± 0.031	39.02± 0.060	39.3± 0.021	38.92± 0.04	d 38.38± 0.054	d 37.95± 0.043	d 37.43± 0.072	d 37.02± 0.048	d 36.65± 0.067	d 36.57± 0.05	d 36.83± 0.05
	a=> p<0.05		b=> p<0.02		c=> p<0.01		d=> p<0.001				
NLE 62.5mg/kg body weight vs control group showed that there is no significant fall in temperature whereas NLE 125mg/kg body weight decreased the temperature significantly from 4rth hour of its injection. With 250 mg and 500 mg/kg body weight, NLE caused significant fall in temperature from 1st hour onwards. distilled water didn't show any antipyretic effect on yeast-induced pyrexia.											