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Antipyretic Effect of *Azadirachta indica* Leaf Extract (Neem Leaf Extract) on Albino Rats.

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ABSTRACT

Evaluation of antipyretic activity of Neem Leaf Extract (NLE) by using Brewer's yeast induced pyrexia model. In this study the albino rats were randomly divided into groups of six each group consisting of 6 rats. Group I: Control (distilled water 0.5ml/rat); Group II: Standard (Paracetamol 100mg/kg intraperitoneally); Group III, IV, V, VI (NLE 62.5, 125, 250, 500 mg/kg body weight intraperitoneally respectively). It is a randomized controlled experimental study. Fever was induced by injecting 20 ml/kg body weight of 20 % suspension of brewer's yeast subcutaneously below the nape of neck. Those animals that had a constant rectal temperature or a variation of less than 1^{o}C were included in the study. Drugs were given after development of pyrexia and temperatures recorded. Paracetamol at 100mg/kg orally was taken as the standard drug. NLE 62.5 mg/kg body weight showed no significant fall in temperature, whereas NLE 125 mg/kg body weight decreased the temperature significantly from 4^{th} hour of its injection along with NLE 250 mg and 500 mg/kg body weight. NLE caused significant fall in temperature from 1^{st} hour onwards. Distilled water did not show any antipyretic effect on Yeast-induced pyrexia. Thus NLE exhibited significant ($p<0.05$) antipyretic activity with the progressive increase in dose.

Keywords: NLE, Antipyretic, Brewer's yeast.

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INTRODUCTION

Azadirachta indica (Neem) is an indigenous plant widely available in India and other tropical and subtropical areas of the world. Different parts of the plant e.g., the bark, leaves, branches, flowers, fruits seeds, oil and gum are reported to have pharmacological properties. It is aptly described as a village dispensary. Various parts of the tree and their preparations are used in traditional medicine for the treatment of inflammatory based disorders [1,2].

Though various preparations of the tree are used in traditional medicine for the treatment of a variety of ailments, very few reports are available regarding the antinociceptive, anti-inflammatory and antipyretic effects of *Azadirachta indica* leaf extract. The chemical mediators like prostaglandins, serotonin, histamine, bradykinin etc. are involved in the mediation of pain, inflammation and fever.

Pyrexia or fever is defined as the elevation of body temperature. It is a natural defence mechanism to tissue damage, inflammation, malignancy, or graft rejection. Due to poor hygiene practices and malnutrition, children from developing countries suffer from infections, which primarily manifests as fever. These fevers cause pain (myalgia), leading to morbidity and mortality [3].

Till now different steroidal and non steroidal anti-inflammatory drugs are used clinically to treat pain, inflammation and fever. But these drugs are not free from side effects. Non steroidal anti-inflammatory drugs cause peptic ulcer as well as upper gastrointestinal tract bleeding [4, 5]. All the currently available drugs such as NSAIDs are subject to their own side effects such as gastric erosions. Therefore, man has been on hunt since ages for suitable alternatives to NSAIDs [5]. So the present work has been undertaken to study the effect of Neem leaf extract (NLE) on fever.

MATERIAL AND METHODS

Materials

Collection of Plant Materials

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

Chemicals

Paracetamol (Dr.Reddy's laboratory, Hyderabad)) , Yeast Extract Powder (HiMedia Laboratories Pvt Ltd, Mumbai, India),was purchased from the following sources.

Animals

Wistar strain of Albino rats weighing between 100-200 g of either sex were obtained from central animal house V.S.S. Medical College, Burla under the Department of Pharmacology. 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 $1^{o}C$ were included in the study. Rectal temperatures were recorded with the help of a clinical thermometer. CPCSEA and Good Laboratory Practice Guidelines were stringently followed for the experiments. No animals were sacrificed at the end of the study.

Methods

In this study the albino rats were randomly divided into groups of six each group consisting of 6 rats. Group I: Control (distilled water 0.5ml/rat); Group II: Standard (Paracetamol 100mg/kg intraperitoneally); Group III, IV, V, VI (NLE 62.5, 125, 250, 500 mg/kg body weight intraperitoneally respectively). Total volume of the oral dose kept constant at 1 ml/rat. It is a randomized control study.

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Brewer's Yeast Induced Pyrexia Model

The antipyretic study was done by using the brewer's yeast induced pyrexia model in rats [6]. Fever was induced by injecting 20 ml/kg body weight 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 intra-peritoneally after development of initial pyrexia and the volume of injection was kept constant at 0.5 ml/rat. Paracetamol 100 mg/kg body weight was taken as standard drug for comparison. NLE was given in the doses of 62.5 mg, 125 mg, 250 mg and 500 mg/kg body weight intra-peritoneally 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

Results were statistically analyzed by using unpaired 't' test. The level of significance was taken at $p<0.05$. Paracetamol produced significant antipyretic effect from 15 minutes of its administration throughout the observation period. Paracetamol brought the temperature down to normal from 1st hour of drug administration (Table 1). NLE 62.5 mg/kg body weight Vs control group showed that there is no significant fall in temperature whereas NLE 125 mg/kg body weight decreased the temperature significantly from 4th hour of its injection With 250 mg and 500 mg/kg body weight (Figure 1). NLE caused significant fall in temperature from 1st hour onwards. Distilled water did not show any antipyretic effect on Yeast-induced pyrexia. Figure 2 shows the comparative line diagram of paracetamol and NLE 500 mg/kg. In figure 3, the radar diagram portrays paracetamol to be the best drug in the control of temperature over the period of 12 hours followed by NLE 500mg/kg in this research.

Table 1: Effect of Neem Leaf Extract (NLE) and Paracetamol on yeast induced pyrexia

Drugs	Mean rectal temperature in °C ± SEM										
	BBT.	Initial pyrexia (10 hours after yeast)	Temperature at different hours of drug administration								
			¼	½	1	2	3	4	5	6	12
Distilled water 0.5 ml/rat	36.92 ± 0.09	39.0 ± 0.07	39.15± 0.13	39.27 ± 0.11	39.15 ± 0.13	39.12 ± 0.05	39.02 ± 01.05	39.0 ± 0.07	38.98± 0.09	38.62± 0.09	37.58 ± 0.06
Paracetamol 100mg/kg	36.92 ± 0.09	38.93 ± 0.07	38.5 ± 0.14[c]	37.95 ± 0.10[d]	37.08 ± 0.41[d]	36.9 ± 0.13[d]	36.9 ± 0.09[d]	36.9 ± 0.04[d]	36.86 ± 0.08[d]	36.88 ± 0.08[d]	37.05 ± 0.03[d]
NLE 62.5 mg/kg	36.92 ± 0.04	39.02 ± 0.05	39.12± 0.05	39.2 ± 0.04	39.2 ± 0.05	39.1 ± 0.06	39.1 ± 0.08	38.98± 0.09	38.77± 0.08	38.62± 0.09	37.73± 0.13
NLE 125 mg/kg	36.95 ± 0.07	39.07 ± 0.04	39.18± 0.03	39.14 ± 0.03	39.05 ± 0.02	38.95 ± 0.03	38.83 ± 0.04	38.7 ± 0.06[a]	38.3 ± 0.05[c]	37.95 ± 0.06[d]	37.58 ± 0.06
NLE 250 mg/kg	36.9 ± 0.06	39.0 ± 0.07	38.98± 0.09	38.93 ± 0.08	38.75 ± 0.09[a]	38.53 ± 0.10[c]	38.18 ± 0.10[d]	37.72 ± 0.09[d]	37.22± 0.08[d]	37.05± 0.06[d]	37.07± 0.05[d]
NLE 500 mg/kg	36.92 ± 0.03	39.02 ± 0.06	39.13± 0.02	38.92 ± 0.04	38.38 ± 0.05[d]	37.95 ± 0.04[d]	37.43 ± 0.07[d]	37.02± 0.04[d]	36.65 ± 0.06[d]	36.57 ± 0.05[d]	36.83 ± 0.05[d]

a ⇒ $p < 0.05$, b ⇒ $p = 0.02$, c ⇒ $p < 0.01$ d ⇒ $p = 0.001$
BBT- Basal Body Temperature

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Figure 1: Line diagram showing the effect of Neem Leaf Extract (NLE) on yeast induced pyrexia model at various time intervals.

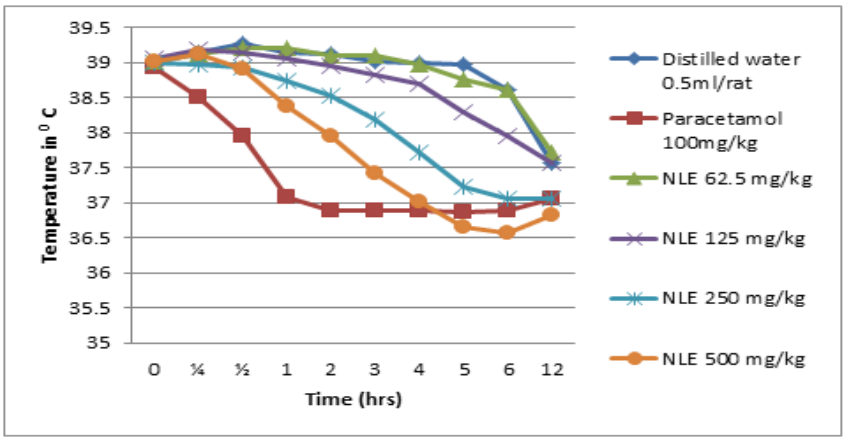


Figure 2: Line diagram showing the effect of Neem Leaf Extract (NLE) 500mg/kg and Paracetamol on yeast induced pyrexia model

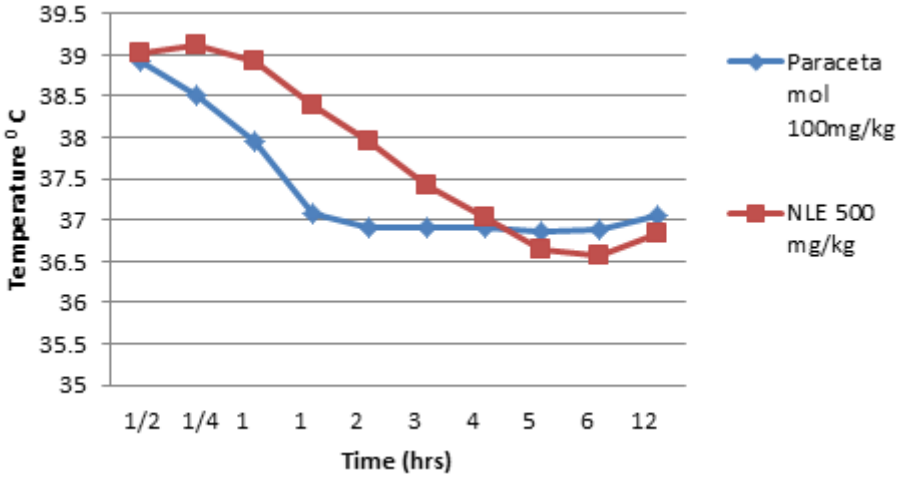
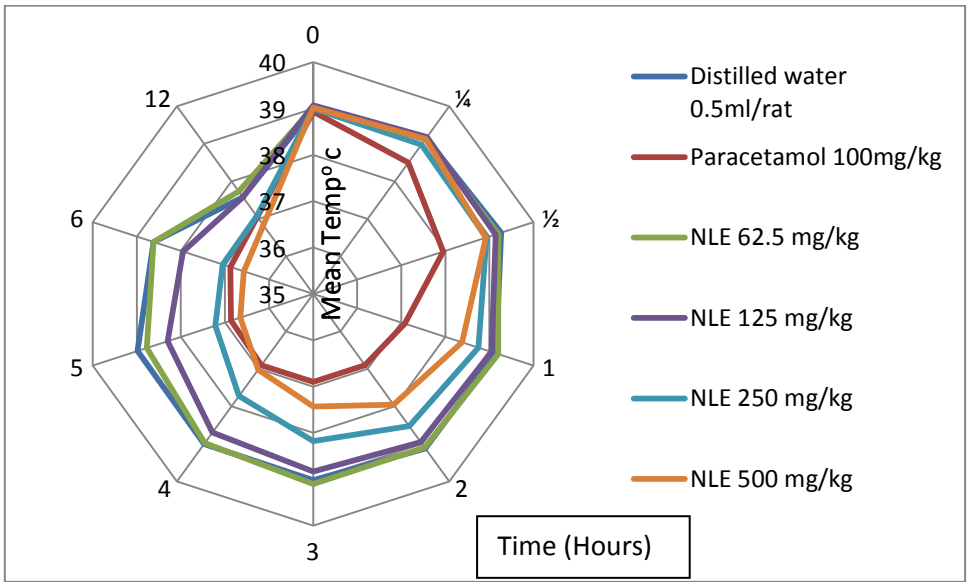


Figure 3: Radar diagram showing the effect of Neem Leaf Extract (NLE) and Paracetamol on yeast induced pyrexia models



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DISCUSSION

Brewer's yeast used in this experiment is an exogenous pyrogen. It is a lipopolysaccharide which is the cell wall component of gram negative bacteria. Brewer's yeast binds to the immunological protein called the Lipopolysaccharide Binding Protein (LBP)[7].This binding results in the synthesis and release of various endogenous cytokine factors like IL-1, IL-6, TNFα. These endogenous cytokines easily cross the blood brain barrier and act on the preoptic/anterior hypothalamus,thus activating the arachidonic acid pathway thus resulting in the synthesis and release of prostaglandin E2[8]. PGE2 produced from (COX)-2 cause the rise in body temperature [9].

The anti-pyretic activity of NLE could be attributed to its cyclooxygenase inhibitory activity. Thus the present study postulates that NLE could reduce pyrexia by reducing the concentration of PGE2 in hypothalamus or by interrupting the steps that connect the peripheral inflammation with the central production of PGE2 or both [10].

CONCLUSION

Thus Neem Leaf Extract (NLE) exhibited significant antipyretic action at 125, 250, 500 mg/kg. Exploring the exact mechanism of action of NLE could not be done in this study and left for other enthusiastic researchers to take forward.

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