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Study of Effect of Azadirachta *Indica* Seed Oil (Neem Seed Oil) on A Model of Pyrexia in Albino rats

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ABSTRACT

To investigate the antipyretic activity of Neem Seed Oil(NSO) using Brewer's yeast induced pyrexia model. In this study the albino rats were randomly divided into groups of 6, each group consisting of 10 rats. Group I: Control (distilled water 0.5ml/rat); Group II: Standard (Paracetamol 100mg/kg intraperitoneally); Group III, IV, V, VI (0.125 ml, 0.25 ml, 0.5 ml and 1 ml/kg body weight intraperitoneally respectively). 20 % suspension of brewer's yeast was injected subcutaneously below the nape of neck to induce fever. Drugs were given after development of pyrexia and temperatures recorded.Animals showing a rise in body temperature to at least 39^0C were taken in the study, a total of 36 rats were taken with a minimum of 6 in each group. NSO at doses of 0.125 and 0.25 ml/kg body weight did not show any significant antipyretic effect. NSO in the dose of 0.5 ml/kg and 1ml/kg body weight showed significant ($p<0.05$) reduction in temperature from 2^{nd} to 6^{th} hour of its administration. Thus NSO exhibited significant ($p<0.05$) antipyretic activity at 0.5 and 1 ml/kg doses respectively.

Keywords: NSO, Antipyretic, Brewer's yeast.

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INTRODUCTION

Azadirachta indica is popularly known as Neem, belonging to the family mahogany. Neem trees grow up to 30m tall and 2.5m in girths. It is known by various names like 'arishtha',' Indian Liliac', Sarbarogaribarini',' A tree for solving global problems'[1-3]. The tree is known from prehistoric times and mentioned in ancient manuscripts like Charak Samhita and Susruta Samhita[2]. Neem has a multitude of medicinal properties like antiallergenic, antidermatic, antifeedent, antifungal, anti-inflammatory, antipyorrhoeic, antiscabic, cardiac, diuretic, insecticidal, larvicidal, nematicidal, spermicidal and lots more to mention [2,4]. Apart from the rich medicinal value the people of India have always valued and treasured neem tree for centuries and even today for its teeth cleansing action,skin disorders, astonic, and as insect repellants [5]. Hence it has been declared as a green treasure. The phytochemical analysis of neem seed revealed the presence oftriterpenes, flavonoids, tannins, saponins, nimbidin, sodium nimbidate, gallic acid, catechin, polysaccharides [6, 7]. Neem seed oil (NSO) which is taken for this studyhasantifertility, hypoglycaemic, antifungal, anti-inflammatory, antiarthritic, antiulcer, antibacterial properties [1].

Pyrexia is defined as the elevation of body temperature. We all are aware that pyrexia is caused by infections, malignancy and other disease states [8]. In developing countries like India due to malnutrition and improper hygiene practises, fever has been a cause of morbidity and mortality [9]. It is a natural defence mechanism of our body solely to create a hostile environment for the offending organism and damaged tissues. Most of the antipyretic drugs we use today act by inhibiting (COX)-2 and thus lowering the prostaglandin synthesis and body temperature. However these synthetic agents inhibit (COX)-2 irreversibly with high selectivity at the cost of damage to the liver, kidneys and the brain cortex [10]. High fever is also a potential threat to the body in terms of the high catabolism rate, dehydration etc [11]. This dilemma has caused researchers to turn to natural agents, which have lower selectivity to (COX)-2 and lower incidence of side effects [10]. The present study has been undertaken keeping in mind the gravity of the situation, to unfold the antipyretic effect of Neem Seed Oil (NSO), a natural agent , based on its rich traditional, pharmacological and ethnopharmacological importance.

MATERIAL AND METHODS

Materials

Collection of Plant Materials

Neem Seed Oilwas procured 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).

Animals

Wistar Albino rats weighing between 100-200g irrespective of sex were obtained from central animal house, IMS and SUM Hospital, Bhubaneswar, under the Department of Pharmacology. Albino rats chosen for the experiment were isolated and kept in separate cages. They were acclimatized for 7 days in the laboratory before the experiments. The animals were kept at ambient temperature of $22 \pm 1^{o}C$, 12hr light and dark cycle allowed. Rectal temperatures of these animals were recorded twice daily at 9.00 AM and 9.00 PM for 7 days and seen for any diurnal variation of temperature. Food was given as recommended by the veterinary officer, clean drinking water given *ad libitum*. Onlythose animals with a variation of temperature less than $1^{o}C$ were included in the study. Clinical thermometer was used to record the rectal temperatures.No animals were sacrificed at the end of the study.

Methods

It is a randomized control experimental study. The study was divided into 6 groups, each group comprising of 10 rats, taking only those rats showing the rectal temperature varying to less than $1^{0}C$. Albino

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rats showing a rise in body temperature to at least 39^0Cafter 10 hours of Brewer's yeast injection were included in the study. A total of 36 rats were taken for the experiment with a minimum of 6 in each group.Group I: Control (distilled water 0.5ml/rat); Group II: Standard (Paracetamol 100mg/kg intraperitoneally); Group III, IV, V, VI (NSO 0.125 ml, 0.25 ml, 0.5 ml and 1 ml/kg body weight intraperitoneally respectively). For all intraperitoneal injections the volume was kept constant at 0.5 ml/rat.

Brewer's Yeast Induced Pyrexia Model

Brewer's yeast induced pyrexia model, known as a classical model of antipyresis testing was used in this study [12,13].Wistar strain of albino rats of either sex weighing between of 100-200 gram were taken for the study. The animals were fasted for 18 h prior to commencement of the experiment, but water was provided *ad libitum*. An initial rectal temperature was recorded using a rectal thermometer to a depth of 1.5 cm in the rectum of rats. Animals with a body temperature between 36^0C to 38^0C were included in the study. A 20% Brewer's yeast in 0.9% w/v saline was injected subcutaneously below the nape of neck in the dose of 10ml/kg thereafter. The injection site was massaged to ensure the spread of suspension below the skin. Room temperature was maintained at 22-24^0 C. After the yeast injection food was immediately withdrawn.

After 10 hrs post challenge the rise in rectal temperature was recorded. Animals which showed a rise in body temperature to at least 39^0C were included in the study, allowing a minimal of 6 rats in each group, total of 36 rats. The animals received the standard (paracetamol 100 mg/kg) or the test compounds ((NSO 0.125 ml, 0.25 ml, 0.5 ml and 1 ml/kg body weight) by intraperitoneal administration and the rectal temperature was recorded at at different time intervals of 15 min, 30 min, 1 hour, 2 hour, 3 hour, 4 hour, 5 hour, 6 hour and 12 hour after the drug treatment.

RESULTS

Statistical analysis was done using unpaired t- test. Table 1 clearly depicts the effect of NSO at different doses and paracetamol,over the temperature pattern at different time intervals. Paracetamol used as the standard drug here revealed significant ($p<0.001$) antipyretic activity right from 15min onwards up to 12 hours. It took paracetamol to bring down the body temperature to the basal body temperature in just 1 hour time. NSO at doses of 0.125ml/kg and 0.25ml/kg did not show any antipyretic activity. NSO in the dose of 0.5 ml/kg body weight showed significant ($p<0.05$) reduction in temperature from 2^{nd} to 6^{th} hour of its administration, whereas with 1 ml/kg body weight, NSO lowered the temperature significantly ($p<0.001$)from 2^{nd} hour onwards. NSO at 1ml/kg lowered the body temperature to basal body temperature in 4hours. Figure 1 illustrates the temperature pattern shown by all the six groups by means of line diagram. In figure 2, we can appreciate the trend of temperature reduction pattern at different time intervals shown by paracetamol and NSO 1ml/kg. Here we can note that paracetamol showed a sharp decline in temperature from 1/2hr till 2 hours and thereafter maintaining a near constant temperature. NSO at 1ml/kg showed an initial erratic pattern by a rise in body temperature at 1/2hr and thereafter a gradual decline in body temperature upto 5 hour. However the impact of paracetamol outweighed the pattern of NSO 1ml/kg in terms of the temperature reduction pattern.

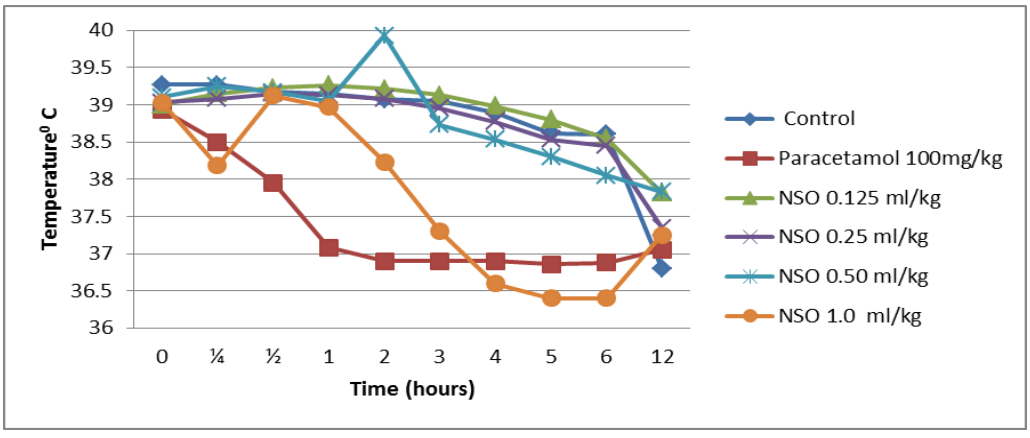

Figure 1: Line diagram showing the effect of Neem Seed Oil (NSO) on yeast induced pyrexia model.

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Table 1: Effect of Neem Seed Oil (NSO) and Paracetamol on yeast induced pyrexia in ^{0}C.

Drugs	Rectal temperature after drug administration(Mean±SE)										
	BBT	Initial pyrexia (10 hours after yeast)	Temperature at different hours of drug administration								
			¼	½	1	2	3	4	5	6	12
Yeast induced pyrexia (Control)	37.07 ± 0.08	39.27 ± 0.10	39.27 ± 0.11	39.17 ± 0.10	39.15 ± 0.13	39.07 ± 0.05	39.05± 0.08	38.9 ± 0.04	38.62± 0.07	38.6 ± 0.06	36.8 ± 0.08
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]
NSO 0.125 ml/kg	36.98 ± 0.05	39.0 ± 0.06	39.15 ± 0.07	39.23 ± 0.06	39.26 ± 0.05	39.21 ± 0.08	39.13± 0.07	38.98 ± 0.07	38.8 ± 0.07	38.55 ± 0.08	37.82 ± 0.09
NSO 0.25 ml/kg	37.03 ± 0.04	39.03 ± 0.05	39.08 ± 0.04	39.15 ± 0.03	39.13 ± 0.05	39.08 ± 0.03	38.95± 0.03	38.77 ± 0.05	38.53± 0.03	38.45 ± 0.04	37.35± 0.41
NSO 0.50 ml/kg	36.98 ± 0.04	39.1 ± 0.05	39.25 ± 0.03	39.17 ± 0.02	39.05 ± 0.03	39.93± 0.03[a]	38.73± 0.05[b]	38.53± 0.07[c]	38.3 ± 0.05[c]	38.05± 0.04[d]	37.83 ± 0.16
NSO 1.0 ml/kg	36.98 ± 0.05	39.03 ± 0.07	38.18 ± 0.06	39.12 ± 0.06	38.97 ± 0.04	38.23± 0.08[d]	37.3 ± 0.15[d]	36.6 ± 0.25[d]	36.4± 0.28[d]	36.4 ± 0.27[d]	37.25± 0.10[b]

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

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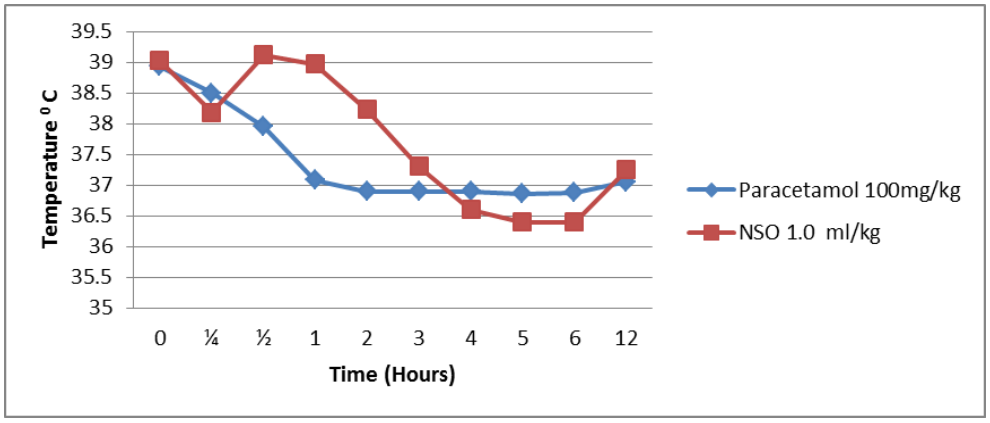

Figure 2: Line diagram showing the effect of Neem Seed Oil (NSO) 1ml/kg and Paracetamol on yeast induced pyrexia model

DISCUSSION

Fever is caused by several endogenous pyrogens such as interleukins(IL-1β,IL-6,IL-8), tumor necrosis factor-α, macrophage protein-1 and prostaglandins. Tumor necrosis factor-α, phospholipase A2 are some of the mediators to stimulate prostaglandin synthesis [14]. Brewer's yeasthere is a lipopolysaccharide which induces peripheral inflammatory mediators from mononuclear macrophages like TNF-α, IL-1,IL-2 etc[15],[16]. These endogenous cytokines generated in the periphery cross the blood brain barrier and act on the preoptic/anterior hypothalamus, causing the release of prostaglandin E_2, thus raising body temperature [7, 17].An effective febrifuge like paracetamol acts by blocking the effect of these pyrogens in the temperature sensitive neurons in the preoptic region of the hypothalamus to cylooxygenase formation of prostaglandin E_2 [18].

NSO here could act by either inhibiting PGE_2 in the hypothalamus by inhibiting (COX)-2 or by inhibiting the peripherally generated inflammatory mediators like TNFα or by both mechanisms. However this needs to be confirmed by further research.

The phytochemical ingredients like triterpenes, flavonoids, tannins, saponins, nimbidin, sodium nimbidate could contribute to the antipyretic activity of NSO [6, 7].

Isolation of individual phytochemical ingredients will yield fruitful results as neem contains various bioactive compounds; so further studies need to be conducted in the isolation of phytoconstituents by column chromatographic techniques. This can be followed by the relevant toxicity studies [19].

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 [20].

CONCLUSION

Thus from the results of the study we can conclude that NSO has antipyretic activity at 0.5 and 1ml/kg body weight doses. However further studies need to be undertaken to understand the mechanism of action of NSO. The isolation of phytoconstituents by column chromatographic techniques, followed by their characterization through different analytical techniques (UV-Vis, FTIR, NMR,Mass Spectroscopy), and thereafter studying their biological activities and toxicity profile can also be taken up by enthusiastic researchers.

REFERENCES

[1] Sharma P, Tomar L, Bachwani M, Bansal V. Int Res J Pharm 2011;2:97-102.
[2] Biswas K, Chattopadhyay I, Banerjee KR, Bandyapadhyay U. Curr Sci 2002; 82:1336-1345.

[3] Shail KC, Tulsidas GS, Meenakshi T, Shilpa P, Anima T, Ajay KP. Neem (Azadirachta indica L) leaf extract deteriorates oocyte quality by inducing ROS- mediated apoptosis in mammals. Springer Plus. 2014; 3:464.
[4] Dinda A, Das D, Ghosh G, Kumar S. Pharmacologyonline 3. 2011; 477-484.
[5] Sergio MV, Alberto AI, Luis MR, Humberto VD, Jesus BF. Neem Tree Morphology and Oil content. Issues in new crops and new uses.2007:126-128.
[6] Imam H, Hussain A, Ajij A. Int Res J Biol Sci 2012;1:76-79.
[7] Debjit B, Chiranjib, Jitender Y, Tripathi KK, Sampath K. J Chem Pharm Res 2010; 2:62-72.
[8] Chattopadhyay D, Arunachalam G, Ghosh L, Rajendren K, Mandal AB, Bhattacharya SK. J Pharm Pharm Sci 2005; 8:558-564.
[9] Santra S, Naik M, Behera R, Agrawal D, Kumar S, Patnaik S. Res J Pharma Biol Chem Sci 2014; 5:669-673.
[10] Thirumal M, Surya S, Kishore G, Vadivelan R, Anand K. Der Pharmacia Lettre 2013;5:315-323.
[11] Veugelers PJ, Kaldor JM, Strathdee SA, Page-Shafer KA, Schechter RA, Countinho IP et al. J Inf Dis 1997;176:112-117.
[12] Loux JJ, Depalma PD, Yankell SL. Toxicol Appl Phermacol 1972; 22:672-5.
[13] Bhattacharya A, Manas RN, Agrawal D, Rath K, Kumar S, Mishra S S. J Young Pharm 2014; 6:20-24.
[14] Rititid W, Ruangsang P, Reanmongkol W, Wongnawa M. J Sci Technol 2007;29:1519-1526.
[15] Ukwuani AN, Abubakar MG, Warra SH et al. Int J Pharm Res 2012; 4(4).48-51.
[16] Bhattacharya A, Behera R, Agrawal D, Sahu PK, Kumar S, Mishra SS. Tanta Med J 2014; 42:74-8.
[17] Saper CB, Breder CD. The New England J Med330.1880-1886.
[18] Li S, Dou W, Tang Y et al. Prostaglandin Other Lipid Mediators 2008; 85:89-99.
[19] Naik MR, Bhattacharya A, Behera R, Agrawal D, Dehury S, Kumar S. J Health Res Rev 2014;1:66-9.
[20] Kumar S, Agrawal D, Patnaik J, Patnaik S. Int J Pharma Bio Sci 2012; 3:222-5.