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Study of biomedicine properties of neem seed oil (*Azadiracta indica*) on albino rats

KEYWORDS: Neem seed oil, analgesia, pyrexia, inflammation

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ABSTRACT *From the ancient time nature gifted mankind with her natural medicine for all kind of sufferings. Pain, fever and irritation clinically termed as analgesia, pyrexia and inflammations, which are the most prevalent sufferings that human suffers from any physiological alteration due to pathogen attack that tends towards severe pathogenesis. Products of 'Neem' a natural wonder drug has been shown to be effective against various health complications. Present study evaluates the efficacy of neem seed oil (NSO) on albino rat to analyze its effect on said physiological alterations. Result of which has showed that NSO is having anti-analgesic, anti-pyretic and anti-inflammatory properties, which helps to recover with no side effects. Hence it is suggestive that, NSO could be the center of drug formulation for most common human suffering like: analgesia, pyrexia, and inflammation.*

INTRODUCTION:

Plant medicines are used throughout the globe for different inflammation induced pathologies (Paulsen, 2010; Thomas et al., 2008). Neem is believed to possess antiseptic, anti-helminthic, insecticidal, anti-diabetic and anti-hypertensive properties (Darshan and Doreswamy, 2004; McManus et al., 2002). Besides, being a native of Indian subcontinent, neem (Indian lilac, *Azadirachta indica*) a highly apprised tree for the people throughout the globe for its 'wonder-ness' (Biswas et al., 2002; Brahmachari, 2004; Mishra and Dave, 2013). In traditional Indian Ayurvedic practice, since thousands of years, neem has been known for its promising disease curing properties. Each part of the neem tree has some medicinal property and can also be used as household pesticide (Biswas et al., 2002; Brahmachari, 2004). The extract from bark, leaves, fruits and root have been used to control leprosy, intestinal helminthiasis and respiratory disorders in children (Deng et al., 2013; Gahukar, 2000; Hashmat et al., 2012; Murugan et al., 2011). The bark extract is also used as tonic, astringent and useful in relieving fever, thirst, nausea, vomiting and skin diseases (Biswas et al., 2002; Schumacher et al., 2011). Bark, leaves, fruits and flowers of neem constitute many essential compounds like flavonoids, flavone glycosides, dihydrochalcones, tannins etc (Brahmachari, 2004; Subapriya and Nagini, 2005). European "Materia Medica" have acknowledged neem tree as "Panacea of all Disease" (Subapriya and Nagini, 2005). In India it is recognized with different names like 'Divine Tree', due to its innumerable traditional and Ayurvedic uses including treatment of fever, leprosy, malaria, tuberculosis, chicken pox, various skin problems, dental disorders etc (Hashmat et al., 2012; Pankaj et al., 2011). 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 (Arora and Kaur, 2007; Kumar et al., 2015). Besides its medicinal uses, neem seed oil is a potential source for the naturally occurring insecticide azadirachtin (Mordue (Luntz) and Blackwell, 1993; Veitch et al., 2008). Boeke et al., have reported that the neem seed oil is relatively non-toxic in nature (Boeke et al., 2004). Various steroidal and non-steroidal anti-inflammatory drugs used clinically to treat pain, inflammation and fever are not free from side effects (Barry, 2010; Wilson et al., 2013). On chronic use non-steroid anti-inflammatory drugs cause peptic ulcer as well as upper gastrointestinal tract bleedings (Malfertheiner et al., 2009). Neem can provide a convenient alternative to the present day drugs causing almost little or no side effect. Depending upon available literature on beneficial attributes from neem, the present study focused whether neem seed oil contains such anti-inflammatory, anti-analgesic and anti-pyretic agent properties to the experimental animal.

MATHEDOLOGY (300 words)

Healthy albino rats of either sex, weighing between 150-200 grams were selected for the study and were exposed to natural temperature and humidity. During the experiment the animals were kept in a fasting state and later on they were separated so that they were not used subsequently.

For the study of analgesic property albino rats were randomly divided into different groups. Ten rats were taken in each group. Morphin sulphate was used as reference standard drug for this study and normal saline was used as its vehicle. Neem seed oil (NSO) was used directly as test drug. The standard drug morphine as well as test drugs of NSO were given intraperitoneally with all aseptic measures. The analgesic effect of the two drugs was assessed by the experimental pain model of tail flick response to thermal stimulation. NSO in the doses of 0.25 ml, 0.5 ml, 1 ml and 2ml/kg body weight were given intraperitoneally to different groups of rats.

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The anti-inflammatory property of Neem seed oil was tested on the model of acute inflammation. Acute inflammation in the form of hind paw edema was produced by injecting 0.1 ml of 1% suspension of carrageenan in normal saline below the plantar aponeurosis of right hind paw of rats (winter CA, et al 1962). 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. 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. NSO was directly used for intraperitoneal injections in doses of 0.25 ml, 0.5 ml and 2ml/kg body weight for different groups of rats. For all intra-peritoneal injections the volume was kept constant at 0.5ml/rat.

In antipyretic study the albino rats were randomly divided into groups of six. 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. Those animals that had a constant rectal temperature or a variation of less than 1^0c were included in the study. Rectal temperatures were recorded with the help of a clinicial thermometer. The antipyretic study was done by using the brewer's yeast induced pyrexia model in rats (loux et al 1972). Fever was induced by injecting 20ml/kg body weight of 20% suspension of brewer's yeast subcutaneously below the nape of neck. 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. The standard and test drug was given intraperitoneally after development of initial pyrexia and the volume of injection was kept constant at 0.5ml/rat. Paracetamol 100mg/kg body weight was taken as standard drug for comparison.

RESULTS

Analgesic properties of the test drugs

Morphine showed significant analgesic effect from 30 minutes to 60 minutes after administration. Peak effect was observed at 45 minutes. Normal saline did not show any significant change in basal T.F.L. As evident from the table-1, NSO in the doses of 0.5ml, 1ml and 2ml/kg body weight showed a continued rise in TFL from 15 minutes to 45 minutes of drug administration and thereafter the TFL declined and reached the basal value at 180 minutes (Table-1). NSO in the doses of 1ml and 2ml/kg body weight showed significant rise in TFL (only at 45 minutes) of administration. The percentage of animals showing TFL ≥ 10 sec were 60% and 80% respectively. NSO in the doses of 0.25ml and 0.5ml/kg body weight did not increase the TFL significantly.

DRUGS	Basal TFL	Mean TFL ± SEM in second						
		15 minute	30 minute	45 minute	60 minute	90 minute	120 Minute	180 minute
NSO 0.25 ml/kg	3.9 ± 0.28	4.1 ± 0.27	4.1 ± 0.28	4.2 ± 0.33	4.2 ± 0.33	4.1 ± 0.31	4.0 ± 0.29	4.0 ± 0.3
NSO 0.5 ml/kg	4.3 ± 0.26	5.9 ± 0.31	7.7 ± 0.37	8.2 ± 0.33	7.5 ± 0.37	5.6 ± 0.27	4.9 ± 0.23	4.5 ± 0.22
NSO 1 ml/kg	4.2 ± 0.2	6.9 ± 0.32	8.5 ± 0.37	9.3 ± 0.3	8.2 ± 0.53	7.5 ± 0.43	5.2 ± 0.29	4.5 ± 0.22
NSO 2 ml/kg	3.9 ± 0.23	6.5 ± 0.5	8.8 ± 0.44	9.8 ± 0.133	9.0 ± 0.33	7.5 ± 0.45	5.3 ± 0.33	4.2 ± 0.2
n=10								

Table-1: Effect of NSO on TFL response at varying time intervals

Inflammatory properties of the test drugs

NSO in the dose of 0.25ml/kg body weight did not show any significant anti-inflammatory activity. However NSO1ml/kg showed significant inhibition of edema at 3rd & 4th hour of carrageenan injection whereas NSO 2ml/kg body weight showed significant reduction of edema from 1 to 6 hours of carrageenan injection. NSO is the dose of 0.25ml/kg body weight inhibited the edema significantly only at 4th hour of carrageenan injection (Table-2).

Effect of NSO on carrageenan induced hind paw edema							
DRUGS	Mean volume of edema in ml ± SEM at different hours of carrageenan injection						
	0 hour	1 Hour	2 hour	3 hour	4 hour	6 hour	24 hour
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
NSO 0.25 ml/kg bw	0.1	0.43±0.07	0.77±0.067	1.12±0.1	1.27±0.08	0.92±0.07	0.33±0.05
NSO 0.5 ml/kg bw	0.1	0.35±0.06	0.62±0.05	1.01±0.08	a 1.02±0.08	0.85±0.11	0.32±0.05
NSO 1 ml/kg bw	0.1	0.3±0.026	0.6±0.026	c 0.95±0.22	c 1.0±0.052	0.77±0.042	0.317±0.048
NSO 2 ml/kg bw	0.1	a 0.25±0.022	b 0.416±0.087	d 0.55±0.077	d 0.57±0.056	d 0.48±0.048	0.3±0.045
a=> p<0.05, b=> p<0.02, c=> p<0.01, d=> p<0.001							

Table-2: Effect of NSO on carrageenan induced hind paw edema

Pyretic properties of the test drugs

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. NSO in the dose of 0.5ml/kg body weight showed significant reduction in temperature from 2nd to 6th of its administration, whereas, with 1ml/kg body weight the temperature significantly reduced from 2nd hour onwards (Figure-1). NSO in the doses of 0.125ml and 0.25ml/kg body weight did not show any significant antipyretic effect.

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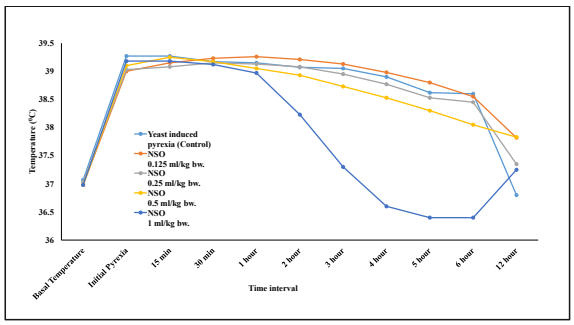

Figure-1: Effect of NSO on yeast induced pyrexia.

DISCUSSION (500 words)

Through 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* seed oil. The chemical mediators like prostaglandins, secrotinin, histamine, bradykinin etc. are involved in the mediation of pain, inflammation and fever. The major part of this study is to get the information related to biomedicine properties of *A. indicia* on albino mice in three aspects like analgesic study, inflammatory study and antipyretic study. Tail flick response model was selected for analgesic study because it involves central mechanism, causes minimal injury to animals and is reproducible. In this present study morphine sulphate of 1mg/kg body weight showed maximum analgesic response after its administration at 45 min, the response started from 30 min, significant analgesic response was from 30 min to 60 min as similar like NSO dose. The result corroborates the data obtained by Carta in similar fashion of TFL with morphine (Carta et al., 1990). The effect of analgesic in albino declined and reached to basal TFL at 180 min (Table-1). These finding is also supported by Khosla et al. as they observed maximum peak of analgesic effect at 60 min of Oral administration in 2ml/kg body weight (Khosla et al., 2000). All these supported data deduced that the NSO doses of 250mg/kg, 500mg/kg and 1ml/kg and 2ml/kg body weight possessed significant analgesic activities.

For the anti-inflammatory study, the carrageenin induced hind paw edema was as the model of acute inflammation (Winter et al 1962). Winter et al found 26% of inhibition of paw edema at a dose of 33.3mg/kg of Aspirin. However Okapanyi et al reported 27.5% inhibition of paw edema with doses of 50mg/kg body weight of Aspirin at 6th hour of carrageenan injection (Okapanyi et al., 1981). Inflammation occurs in various phases: The first phase of vascular permeability, exudation of plasma, release of mediators; the second phase is bymigration of leukocytes; and the final phase is by granuloma formation. With Neem seed oil in the dose of 0.5ml/kg body weight we observed significant reduction in paw edema was noted at 3rd and 4th hour and with 2ml/kg body weight. Pillai et al was also observed similar dose-dependent anti-inflammatory effect of nimbidin at the doses of 20 mg, 40 mg, and 80 mg/kg body weight (Pillai et al., 1981). Similar study of anti-inflammatory effect of neem leaf oil studied by Jagadeesh et al at 1, 2, 4, and 8 ml/kg body weight on albino rats, which showed 45.2%, 50%, and 48.2% reduction of paw edema at 1, 2, and 4 ml/kg, respectively (Jagadeesh et al., 2014). In the present study, we found a lucid impact of NSO at 0.5, 1, and 2 ml/kg in comparison to control (Table-2). NSO at 0.25 ml/kg did not show an appreciable difference with respect to control. NSO in the dose of 2 ml/kg body weight showed maximum (53.14%) inhibition of edema at 4th hour of carrageenan injection. The NSO act of edema suppression was maximum at 3rd and 4th hour viz. inhibiting prostaglandin-like substances.

For pyrexia study Brewer's yeast suspension subcutaneous to Albino rat's method was selected for its simplicity (Loux et al., 1977). The several substances and fractionation of the neem seed oil extract made powerful active agent for antipyretic study. The test drugs of NSO were given in different doses of 0.125ml, 0.25ml, 0.5ml and 1ml/kg body weight respectively and compare with reference standard drug of paracetamol of 100mg/kg body weight. In this present study set up of brewer's yeast injection was administered for pyrexia development, occurrence of pyrexia developed after 10 hrs of administration and then the temperature came down to normal at 12 hrs (Figure-1). As paracetamol was used for antipyretic study it showed a significant effect from 15 min of drug administration. The peak came down to normal after 1 hour of observation. At the other side, NSO didn't show any effect over the temperature at the doses of 0.125 ml in 0.25ml/kg body weight. The significant effect came to the dose of 0.5ml/kg body weight and reduction of temperature started from 2nd hour to 6th hour of initial pyrexia. On one hand paracetamol 100mg/kg body weight brought the temperature to basal value at 1 hour of its administration but NSO in the dose of 1ml/kg body weight reduced the pyrexia to basal value after 3rd hour of its intra-peritoneal administration. These showed the competency of neem seed oil in all three aspects of biomedicine properties.

CONCLUSION

The best of this study is the strength in cost-effectiveness. The observations from the result revealed that NSO have analgesic, anti-inflammatory and antipyretic effects. However further experimental studies in different models are needed to establish the analgesic, anti-inflammatory and antipyretic potentialities of these agents. The prospects of this study can be comprehensive to untie the effects of *A. indica* on abiding dose schedules.

Conflict of interest: All authors agreed, there is none to declare.

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