

Evaluation of Anti-Inflammatory, Analgesic and Antipyretic Effects of *Azadirachta indica* Leaf Extract on Fever-Induced Albino Rats (Wistar)

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Abstract: The present study was carried out to investigate the anti-inflammatory, antipyretic and analgesic effect of the crude ethanol extract of *Azadirachta indica* leaves on experimental rat model at three different dose levels- 100, 200 and 300 mg/kg, respectively. Hot plate test were used to assess analgesic activity, formalin induced inflammation was used for anti-inflammatory study and baker's yeast was used to induce pyrexia. Acute toxicity test was also performed in rats after administration of the extract orally at high dose level (4 g/kg). In addition, ethanol extract obtained from *Azadirachta indica* leaves at different doses and different periods of study showed significant effect ($p < 0.05$) compared to control. For analgesic study, the extract at 100 mg/kg showed a slow but time dependent effect, at 200 mg/kg, its effect was noticed in all the periods although still time dependent and at 300 mg/kg, the effect was significant in all the periods and long-lasting at the final minutes (90 min) with values expressed in mean \pm SEM of 14.0 \pm 1.41 which was significant ($*p < 0.05$) compared to control and all other groups. The anti-inflammatory study of the ethanolic extract of *Azadirachta indica* showed a time and dose dependent effect at different periods. It's effect was noticed in all doses but was most significant ($**p < 0.05$) in group 4 which was given 300 mg/kg of the extract with a value of 40.6 \pm 8.80 expressed in mean \pm SEM compared to control and all other groups. The extract at all dose showed significant effect ($*p < 0.05$) over control. Its effect was time and dose-dependent. However, the extract attenuated the pain, fever and inflammation induced in the rats at 100, 200 and 300 mg/kg, respectively dose levels but its significant protective effect was noticed at higher doses than low doses and at a longer period of time. In acute toxicity study, no mortality was observed at 4 g/kg dose level.

Key words: Albino Wistar rats, analgesic, anti-inflammatory, anti-pyretic, *Azadirachta indica*, baker' yeast, fever

INTRODUCTION

The neem tree (*Azadirachta indica* A. juss) is a tropical evergreen tree. Natural to Indian sub-continent (Roxburgh, 1874). All parts of neem tree-its leaves, flower, seeds, fruits, roots and bark are highly important in Ayurvedic medicine and has been used extensively in homeopathic medicine and has become a cynosure of modern medicine (Singh *et al.*, 1996).

The presence of flavonoids, tannins, alkaloids and tetranor triterpenes, including nimbin, nimbinin, nimbidinin, nimbolide and nimbidic acid in the leaves extract of this plant (Basak and Chakroborty, 1968) may be responsible for the anti-inflammatory, analgesic and antipyretic property demonstrated by *Azadirachta indica*, as these photochemical are well-known for their ability to

inhibit inflammation, pain and fever (Biswas *et al.*, 2002; Ferrandiz and Alcaraz, 1991; Brousseau, 1989). This effect might be due to inhibition of the synthesis of prostaglandin E2 which is described as key mediator of fever (Dascombe, 1985).

Oil from the leaves, seeds and bark possesses a wide spectrum of antibacterial action against Gram-negative and Gram-positive microorganisms, including *M. tuberculosis* and streptomycin resistant strains (Chopra *et al.*, 1956). *In vitro*, it inhibits *Vibrio cholerae*, *Klebsiella pneumoniae*, *M. tuberculosis* and *M. pyrogenes* (Satyavati *et al.*, 1976). Antimicrobial effects of neem extract have been demonstrated against *Streptococcus mutans* and *S. faecalis* (Almas, 1999).

NIM-76, a new vaginal contraceptive from neem oil showed inhibitory effect on the growth of various

pathogens, including bacteria, fungi and virus. Recently, the antibacterial activity of neem seed oil was assessed *in vitro* against 14 strains of pathogenic bacteria (Baswa *et al.*, 2001). Persistence on the use of this herb in the amelioration of various ailments such as fever and pain and inflammation predominantly in Africa necessitates a thorough scientific evaluation of the claims to ascertain its potent curative properties in inflammation, pyrexia and pain and that formed the core objectives of this study.

Animals and diet: Thirty five albino wistar rats weighing between 159-254 g were used. These animals were procured from animal house in University of Nigeria Nsukka and were housed at the animal house of the faculty of basic medical science Madonna University. The animals were caged at room temperature ($28\pm 5^{\circ}\text{C}$) with relative humidity of ($55\pm 5\%$) in a standard wire gauzed cage throughout the study and were given free access to food and water during the period of acclimatization which was up to 4 weeks.

Plant: Fresh leaves of *Azadirachta indica* were collected from the surrounding of Madonna university, Elele campus, Rivers State. The plant was adequately identified by a botanist.

Preparation of extract:

Preparation of ethanolic extract of *azadirachta indica* leaves: Fresh leaves of *Azadirachta indica* were collected and dried adequately; the dried leaves were manually grinded using manual grinder into a coarse form. The grinded leaves were stirred in 750 mL of ethanol and allowed to stay for 48 h on the mechanical shaker. After the 48 h, the mixture was sieved and dried using rotary evaporator and was further concentrated to dryness at 50°C in an electric oven which finally, a blackish green coloured residue was obtained after which it was stored in a refrigerator at 4°C until the time of use.

The extraction was done in physiology department laboratory, Madonna University.

Administration of extract: The plant extract was administered orally at different doses.

Experimental design: A total of 35 rats were used. The rats were divided into five groups based on their body weight and a total of 5 rats per group. They served as control, preventive and reference groups.

Grouping of animals: The rats were grouped into 5 groups after being weighed according to their related weights. The 5 groups were made of one control, three preventive and one comparative group. The remaining 10 rats were used for the acute toxicity study

Group one (control group): The control group weighed 159.06 kg and received distilled water but no extract

Group two (preventive group): These are the first preventive group of this study weighing 178.8 kg and were given 100 mg/kg of the extract.

Group three (preventive group): The second preventive group weighing 196.7 kg and received 200 mg/kg of the extract.

Group four (preventive group): Third preventive group weighing 214.5 kg and received 300 mg/kg of extract.

Group five (reference group): The rats used here weighed 254.1 kg and were given 200 mg/kg of aspirin for the analgesic and anti-inflammation and antipyretic test.

METHODOLOGY

This research study was undertaken at the animal research laboratory of the department of human Physiology, College of Health Sciences, Madonna University, Elele town, near Port Harcourt, Rivers State, Nigeria, in August, 2010

Acute toxicity study: Ethanol extract of *A. indica* leaves was administered orally at the rate of 4 g/kg per body weight on ten Wistar albino rats (weighing 150-250g). Five male and five female rats were closely observed for 24 h for any mortality and next ten days

For any delayed toxic effect. Their food consumption and growth rate were also examined twice daily up to ten days.

Assay for analgesic activity: The analgesic experiment was carried out using the hot plate. The hot plate was conducted on the experimental rats according to the method described by Woolfe and Mac Donald (1944). The preventive group one were given a dose of 100 mg/kg and the third and fourth were given 200 mg/kg each. The reference group was given 100 mg/kg of aspirin while the control was not administered with the extract. The rats were placed on the hotplate which was maintained at constant temperature of $55\pm 0.5^{\circ}\text{C}$. The stop clock was used to check the latency of the nociceptive response as soon as the rats were placed on the hotplate which was showed by the rats licking their paws and jumping out of the hot plate. The stop clock started ticking as the rat reached the hotplate till the first licking of paws and trying to jump out.

This was done at interval of 30, 60 and 90 min, respectively after the extract was administered. Finally, the average retention time of the preventive groups which were given the extract were compared with that of control which received no extract and the group which was given aspirin.

Assay of antipyretic activity: The antipyretic method was performed according to the method described by Adams *et al.* (1968). The initial normal temperature of the rats was taken after which pyrexia was induced through subcutaneous injection behind the ear with aqueous suspension of baker's yeast. About 15 g of baker's yeast was weighed and put in a beaker containing normal saline (0.9%).

The temperatures of the rats were taken before pyrexia was induced with a clinical thermometer rectally. After 18 h of inducing pyrexia, their temperature was taken again to certify a significant rise in temperature. The extract was administered to the preventive group one, two and three at different doses of 100, 200 and 300 mg/kg, respectively after the certification of the presence of fever. The reference group received 200 mg/kg of aspirin while the control group received distilled water. After the extract and the standard drug (Aspirin) were administered, the temperature of the rats were taken at intervals of 30 up to 120 min and then recorded.

Assay of anti-inflammatory activity: The method used was that of the modified form of (Shibata *et al.*, 1989). (2.5 mL) of formalin was dissolved in 97.5 mL of normal saline to get 2.5% of formalin solution. Twenty μ L of the solution was administered subcutaneously at the right forelimb of the rats, 30 min after administering the extract orally to the rats with group one receiving 0.4 mL of water, group 2 received 100 mg/kg of extract, group 3 received 200 mg/kg of extract and group 4 received 300 mg/kg and group five 200 mg/kg of aspirin, respectively. Immediately after administering the 20 μ L of 2.5% formalin, the number of times the rats licked the injected right forelimb were counted at interval 25 min. The results were recorded up to 80 min.

Statistical analysis: All statistical analysis were performed using Statistical Package for Social Sciences (SPSS Version 15.0). The results were analyzed using the one-way Analysis of Variance (ANOVA) with a statistically significant difference at $p < 0.05$. Tukey's Multiple Comparison was used to test for statistically significant differences between control and experimental groups. The result are presented as mean \pm Standard Error of Mean.

RESULTS

The results obtained were presented in tables below. The experiments were done to evaluate the anti-inflammatory, antipyretic and analgesic effect of *Azadirachta indica* leaves.

Effect of ethanolic extract of azadirachta indica leaves on formalin-induced anti-inflammatory study: The extract showed a time-dependent and dose dependent

effect. The higher the dose and time interval, the more significant effect of the extract.

The extract action was time dependent and dose dependent. Its effect was noticed more at a longer time interval.

The extract showed a time-dependent and dose-dependent effect. Its action was gradual and long-lasting.

DISCUSSION

This study was done to evaluate the anti-inflammatory, analgesic and antipyretic effects of *Azadirachta indica* leaves in albino wistar rats.

The result in Table 1 showed that there was significant ($*p < 0.05$) anti-inflammatory effect of the extract in group 2, 3 and 4 at 100, 200 and 300 mg/kg, respectively over control. The reference group (group 5) which was given aspirin (100 mg/kg) showed significant effect over control. The result also revealed that group 3 and 4 showed more significant effect than group 5 which the effect of group 4 was most significant ($**p < 0.05$) with a final mean of 40.6 ± 8.80 at 75-80 min.

Table 2 presented the antipyretic effect of *Azadirachta indica* leaves in fever induced albino wistar rats which showed a significant effect ($*p < 0.05$) in all groups over control but in different periods. In groups 3 and 4 which were given 200 and 300 mg/kg of the extract respectively, showed significant effect ($*p < 0.05$) in all periods over control. The presence of flavonoids, tannins, alkaloids and tetranor triterpenes, including nimbin, nimbinin, nimbidinin, nimbolide and nimbidic acid in the leaves extract of *Azadirachta indica* (Basak, 1968) may be responsible for the anti-inflammatory, analgesic and antipyretic property demonstrated by *Azadirachta indica*, as these photochemical are well-known for their ability to inhibit inflammation, pain and fever (Biswas *et al.*, 2002; Ferrandiz and Alcaraz, 1991; Brasseur, 1989). Group 5 which was given 200 mg/kg of aspirin also showed significant effect ($*p < 0.05$) over control. This effect might be due to inhibition of the synthesis of prostaglandin E2 which is described as key mediator of fever (Dascombe, 1985).

Table 3 showed the analgesic effect of *Azadirachta indica* leaves. The result indicated that there was significant effect noticed in all groups over control at different periods of this study. At 30 min after the administration of extract, the rats stayed in the hotplate up to 10.8 ± 1.20 sec but there was a slight fall at 60 min with a value of 9.8 ± 1.10 sec and at 90 min 14.0 ± 1.41 sec in group 4 (300 mg/kg) which was significant ($*p < 0.05$) compared to control which showed 5.6 ± 0.81 , 6.6 ± 1.00 and 3.8 ± 0.40 (all in seconds), respectively. The reference group (group 5) which was given standard drug aspirin was very significant ($*p < 0.05$) at 30 and 60 min period

Table 1: Showing the anti-inflammatory effect of *Azadirachta indica* leaves extract on albino wistar rats

Group	Treatment	Dosage	Licking response following treatment			
			0-5 min (mean±SEM)	25-30 min (mean±SEM)	50-55 min (mean±SEM)	75-80 min (mean±SEM)
Group 1 (control)	Distilled water	0.4 mL	101.20±11.04	102.80±8.50	102.2±9.82	108.6±9.50
Group 2	Extract	100 mg/kg	84.4±10.64	73.8±10.50	71.4±10.34*	70.4±9.60*
Group 3	Extract	200 mg/kg	78.0±14.10	56.6±11.03*	48.2±11.10*	43.4±4.41*
Group 4	Extract	300 mg/kg	75.4±8.43	52.0±5.50*	48.0±4.30*	40.6±8.80**
Group 5 (reference)	Aspirin	200 mg/kg	94.2±11.23	82.0±6.61	78.6±8.83*7	4.8±8.50*

All values expressed as mean±SEM; *: p<0.05 significantly different from control

Table 2: Showing the antipyretic effect of *Azadirachta indica* leaves on pyrexia induced in albino rats

Groups	Temp. before inducing fever (mean±SEM)	Temp. 18 h after fever induction (mean±SEM)	Treatment	Dosage	Average rectal temperature following treatment			
					18½ h (mean±SEM)	19 h (mean±SEM)	19½ h (mean±SEM)	20 h (mean±SEM)
Group 1 (control)	36.7±0.09	38.1±0.07	Distilled water	0.4 mL	38.5±0.40	38.3±0.30	38.7±0.15	38.4±0.25
Group 2	36.6±0.05	38.0±0.11	Extract	100 mg/kg	37.9±0.11	37.6±0.21	37.3±0.23*	36.9±0.24*
Group 3	36.7±0.10	38.1±0.11	Extract	200 mg/kg	37.2±0.20*	37.1±0.24*	36.9±0.22*	36.7±0.17*
Group 4	36.9±0.01	38.5±0.10	Extract	300 mg/kg	37.6±0.29*	37.2±0.20*	36.9±0.22*	36.6±0.12*
Group 5 (reference group)	36.5±0.14	38.4±0.22	Aspirin	200 mg/kg	37.7±0.22*	36.9±0.22*	36.8±0.12*	36.7±0.10*

All values expressed as mean±SEM; *: p<0.05 Significant compared to control

Table 3: Showing the analgesic effect of *azadirachta indica* leaves in albino wistar rats

Groups	Treatment	Dosage	Reaction time in the hot plate following treatment		
			After 30 min (mean±SEM)	After 60 min (mean±SEM)	After 90 min (mean±SEM)
Group 1(control)	Distilled water	0.4 mL	5.6±0.81	6.6±1.00	3.8±0.40
Group 2	Extract	100 mg/kg	7.2±1.40	9.6±1.33	10.4±1.81*
Group 3	Extract	200 mg/kg	6.6±0.70	9.0±0.71	13.4±1.78*
Group 4	Extract	300 mg/kg	10.8±1.20*	9.8±1.10	14.0±1.41*
Group 5 (reference)	Aspirin	200 mg/kg	16.0±1.41*	11.2±1.00*	9.6±0.92

All values are expressed as mean±SEM; *: p<0.05 very significant compared to control

control and the preventive groups but fell at 90 min periods. Moreover, recent research findings revealed that nimbidin significantly suppresses some of the functions of macrophages and neutrophils, prostaglandin E₂ (PGE₂) production relevant to the analgesic response (Kaur *et al.*, 2004). So, it is not unreasonable to speculate that nimbidin and related compounds like nimbidinin, nimbolide of *Azadirachta indica* leaf extract are probably responsible for its analgesic activity.

CONCLUSION

The ethanol extract of the leaves of *Azadirachta indica* showed a significant anti-inflammatory, analgesic and antipyretic effects in experimental rat models and could be an alternative source to treat inflammation, pain and fever. From the result tables above, for analgesic, antipyretic and anti-inflammatory study, it showed a time dependent and dose dependent effect. However, further studies are necessary to elucidate the mechanism behind this effect. This report may serve as an investigative model for further studies.

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