University Journal of Pre and Para Clinical Sciences

ISSN 2455–2879 2021, Vol.7(1)

Evaluation of Antipyretic Effect of Azadirachcta indica Leaf Extract on Fever-Induced Albino Rats (Wistar)

VIJAYALAKSHMI M
Department of Pharmacology, MADURAI MEDICAL COLLEGE AND HOSPITAL

Abstract : OBJECTIVES - To evaluate Antipyretic Effect of Azadirachcta indica Leaf Extract on Fever-Induced Albino Rats (Wistar). MATERIALS AND METHODS - 30 adult male albino rats weighing 200-250 gm were selected and allocated into five roups of 6 animals each. The control group received normal feed and water, standard group received T.Aspirin and test group received ethanolic extract of Azadirachcta indica Leaf . Rectal temperature of the rats were taken before inducing pyrexia. Pyrexia induced by subcutaneous injection of aqueous suspension of backers yeast behind the ear. After 18 hrs of inducing pyrexia, rectal temperature recorded again. The extract was administered to group3,4 ,5 at graded doses of 100mg, 200mg, 300mgkg after the certification of fever. The temperature of the rats were taken at 60mts, 90mts120mts and then recorded. The findings were recorded, tabulated and analyzed with suitable statistical method. RESULTS - Azadirachcta indica Leaf Extract has significant antipyretic effect. The results were comparable to that produced by standard drug aspirin.CONCLUSION - Azadiraccta indica Leaves has antipyretic effect which was comparable to aspirin.

Keyword : Antipyretic, Backers yeast, Azadirachcta indica.

Introduction

Fever may be due to infection or as a result of tissue damage, inflammation, graft rejection or other disease states. Antipyretics are agents, which reduce the elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained. In fever this set point elevates and a drug like paracetamol does not influence body temperature when it is elevated by the factors such as exercise or increase in ambient temperature. Yeast induced fever is called pathogenic fever. Its etiology includes production of prostaglandins, which set the thermoregulatory center at a lower temperature. In general, NSAIDS produce their antipyretic action through the inhibition of prostaglandin synthetase within the hypothalamus. Medicinal plants are integral part of human health system from the dawn of civilization. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs. *Azadirachta indica* (neem tree) is a tropical evergreen tree. Natural to Indian sub-continents1. 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 medicine2. The presence of flavonoids, tannins, alkaloids and tetranor triterpenes, including nimbin, nimbinin, nimbidinin, nimbolide and nimbidic acid in the leaves extract of this plant3 may be responsible for the antipyretic property as these photochemical are well-known for their ability to inhibit inflammation, pain and fever4 might be due to inhibition of the synthesis of prostaglandin E25.

Aims and objectives:

The study was aimed to evaluate anti- pyretic effect of ethanolic extract of *Azadirachta indica* leaves. The study was undertaken after obtaining approval from Institutional Animal Ethics committee. Ref.No: Roc No 12677/E1/5/2012.

Materials and Methods:

Plant material

The leaves of *Azadirachta indica* were collected from the Madurai medical college campus, Tamil nadu. The leaves were identified and authenticated by Professor of Botony, American college, Madurai.

Preparation of extract

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 hour, the mixture was sieved and dried using rotary evaporator and was further concentrated to dryness at 50ºC in an electric oven. Finally, a blackish green coloured residue was obtained after which it was stored in a refrigerator at 4ºC until the time of use.

Selection of animals, caring and handling:

A total of 30 inbred healthy adult albino male wistar rats (200-250gm), in the central animal house of Madurai Medical College, Madurai were selected for the study. They were housed under controlled conditions of temperature of 22±2º C, relative humidity of 30-70% and 12 h light -12 h dark cycle. Animals were fed with sterile commercial pelleted rat chow supplied by Hindustan Lever Ltd. (Mumbai, India) and had free access to water ad libitum. Animals were kept under fasting for overnight and weighed before the experiment. The animals were acclimatized to laboratory conditions for 7 days before commencement of experiment.

Study design

The rats were randomly allocated into five groups of six animal each for testing anti pyretic activity. Group I (control) received water and food .GroupII (standard) received Tab.Aspirin. Group III, IV, V received ethanolic extract of *Azadirachta indica* leaves 100mg/kg, 200mg/kg, 300mg/kg orally.

Determination of the drug dosage and dosing schedule

Doses were selected and determined according to the previous acute toxicity studies of ethanolic extract of *Azadirachta indica* leaves7. Three different doses were selected 100mg/kg, 200mg/kg, 300mg/kg for anti pyretic activity.

Methods

The antipyretic method was performed according to the method described by Adams *et al*6. 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 test group III, IV and V at different doses of 100, 200 and 300 mg/kg, respectively after the certification of the presence of fever. The standard 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 60 up to 120 min and then recorded.

Statistical analysis

The statistical analysis was carried by one way ANOVA followed by Games-Howell post-hoc test. P values < 0.05 (95% confidence limit) was considered statistically significant.

Results

"A one-way between subjects ANOVA was conducted to compare the effect of antipyretic activity of crude ethanol extract of *Azadirachta indica* leaves administered to group at different doses of 100mg, 200mg, and 300mg/kg after the certification of fever. The study group received aspirin and the control group received plain water. The temperature of the rats will be taken at one hour, two hour &three hours and then recorded. There was a significant effect of antipyretic activity of crude ethanol extract of *Azadirachta indica* leaves administered to group at different doses of 100mg, 200mg, 300mg/kg, study and control at the p<.05 level for the four conditions [F(4, 25) = 76.510, p = 0.001] after one hour.

		Sum of Squares	df	Mean Square	F	Sig.
1 hour	Between Groups	4.999	4	1.250	76.510	.001
	Within Groups	0.408	25	.016		
	Total	5.407	29			

After two hours there was a significant effect of antipyretic activity of crude ethanol extract of *Azadirachta indica* leaves administered to group at different doses of 100mg, 200mg, 300mg/kg, study and control at the p<.05 level for the four conditions [F(4, 25) = 101.430, p = 0.001].

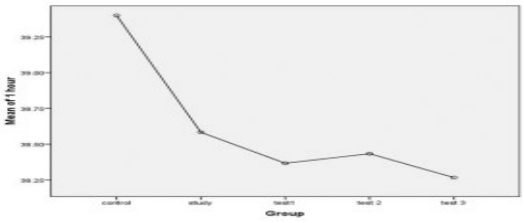
		Sum of Squares	df	Mean Square	F	Sig.
2 hour	Between Groups	9.602	4	2.400	101.430	.001
	Within Groups	.592	25	.024		
	Total	10.194	29			

After three hours also there was a significant effect of antipyretic activity of crude ethanol extract of *Azadirachta indica* leaves administered to groups at different doses of 100mg, 200mg, 300mg/kg, study and control at the p<.05 level for the four conditions [F(4, 25) = 474.345, p = 0.001].

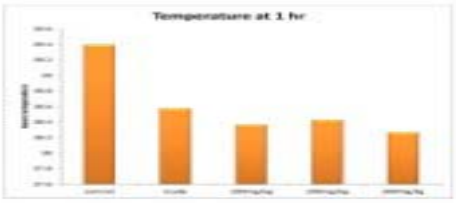
		Sum of Squares	df	Mean Square	F	Sig.
3 hour	Between Groups	18.341	4	4.585	474.345	.001
	Within Groups	.242	25	.010		
	Total	18.583	29	18.583		

The significance value for homogeneity of variances is > .05 at one hour. so the variances of the group are statistically significant. But at two and three hours the significance value for homogeneity of variances is < .05, so the variances of the group are significantly different. However the Welch, Brown-Forsythe options display alternative version of the F statistic which shows statistically significant at .05 level The Tukey test relies on homogeneity of variance, so we ignore these results. The Games-Howell post-hoc test does not rely on homogeneity of variance (this is why we used two different post-hoc tests) and so can be used. "Post hoc comparisons using the Games-Howell test indicated that after one hour the mean temperature was significantly reduced when the crude ethanol extract of *Azadirachta indica* leaves administered at dose of 300mg/kg (M = 38.27, SD =0.082) than the control (M = 39.40, SD = 0.110) and the mean temperature is also significantly reduced when the crude ethanol extract of *Azadirachta indica* leaves administered at dose of 200mg/kg (M = 38.43, SD =0.103) and100mg (M = 38.37, SD =0.150) and for study group(M = 38.58, SD =0.172)when compared to the control group."

1 hour	n	Mean	SD	Mean difference	Std. Error	sig	95% CI	
							Lower Bound	Upper Bound
control	6	39.40	0.110	-	-	-	-	-
study	6	38.58	0.172	0.81667*	0.08333	0.001	0.5327	1.1006
Extract 100mg/kg	6	38.37	0.150	1.03333*	0.07601	0.001	0.7786	1.2881
Extract 200mg/kg	6	38.43	0.103	0.96667*	0.06146	0.001	0.7642	1.1691
Extract 300mg/kg	6	38.27	0.082	1.13333*	0.05578	0.001	0.9468	1.3198

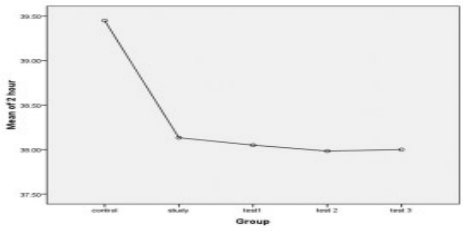


"Taken together, the rectal temperatures of the rats were recorded after one hour these results suggest that crude ethanol extract of *Azadirachta indica* leaves administered at higher dose (300mg/kg) reduces the temperature drastically. Similarly the temperature is also reduced to certain extent when administered with moderate dose (200mg/kg) and also for mild dose (100mg/kg) but not to the normal level before study."

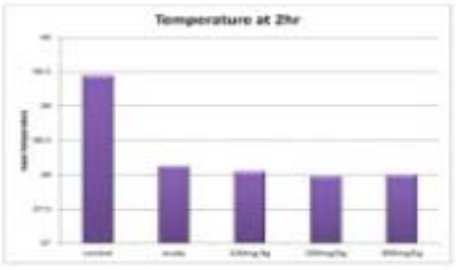


"Post hoc comparisons using the Games-Howell test indicated that after two hours the mean temperature was significantly reduced when the crude ethanol extract of *Azadirachta indica* leaves administered at dose 300mg/kg (M = 38.00, SD =0.110) than the control (M = 39.45, SD = 0.187) and the mean temperature is also significantly reduced when the crude ethanol extract of *Azadirachta indica* leaves administered at dose 200mg/kg (M = 37.98, SD =0.232) and100mg (M = 38.05, SD =0.122) and for study group (M = 38.133, SD =0.052)when compared to the control group."

2 hour	n	Mean	SD	Mean difference	Std. Error	sig	95% CI	
							Lower Bound	Upper Bound
control	6	39.45	0.187	-	-	-	-	-
study	6	38.13	0.052	1.31667*	0.07923	0.001	1.0153	1.6181
Extract 100mg/kg	6	38.05	0.122	1.40000*	0.09129	0.001	1.0901	1.7099
Extract 200mg/kg	6	37.98	0.232	1.46667*	0.12156	0.001	1.0632	1.8702
Extract 300mg/kg	6	38.00	0.110	1.45000*	0.08851	0.001	1.1449	1.7551

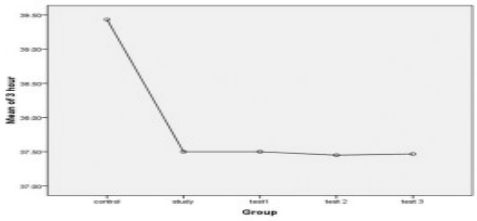


"The rectal temperatures of the rats were recorded after two hours; these results suggest that crude ethanol extract of *Azadirachta indica* leaves administered at higher dose (300mg/kg) reduces the temperature to certain extent. But the temperature is reduced drastically when administered with moderate dose (200mg/kg) when compared to higher dose (300mg/kg). Similarly the temperature is also reduced to some extent mild dose (100mg/kg) but not to the normal level before study."

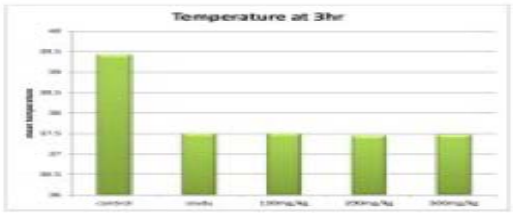


"Post hoc comparisons using the Games-Howell test indicated that after two hour the mean temperature was significantly reduced when the crude ethanol extract of *Azadirachta indica* leaves administered at dose 300mg/kg (M = 37.47, SD =0.082) than the control (M = 39.43, SD = 0.163) and the mean temperature is also significantly reduced when the crude ethanol extract of *Azadirachta indica* leaves administered at dose 200mg/kg (M = 37.45, SD =0.055) and100mg (M = 37.50, SD =0.089) and for study group (M = 37.50, SD =0.063)when compared to the control group.

3 hour	n	Mean	SD	Mean difference	Std. Error	sig	95% CI	
							Lower Bound	Upper Bound
control	6	39.43	0.163	-	-	-	-	-
study	6	37.50	0.063	1.93333*	0.07149	0.001	1.6715	2.1952
Extract 100mg/kg	6	37.50	0.089	1.93333*	0.07601	0.001	1.6687	2.1980
Extract 200mg/kg	6	37.45	0.055	1.98333*	0.07032	0.001	1.7212	2.2455
Extract 300mg/kg	6	37.47	0.082	1.96667*	0.07454	0.001	1.7036	2.2298



"After three hours the results of the rectal temperatures of the rats suggest that crude ethanol extract of *Azadirachta indica* leaves administered at higher dose (300mg/kg) reduces the temperature to below normal level. Similarly the temperature is also reduced to below normal level when administered with moderate dose (200mg/kg) and the temperature is reduced to normal level when administered with mild dose (100mg/kg)."



In yeast induced pyrexia model, the standard and test drug produced significant reduction in body temperature as compared to the control. Here the test compound showed significant anti pyretic effect as compared to the control.

Discussion

The extract markedly decreased the rectal temperature of pyretic rats. This postulation is supported by the antipyretic effect of the extract, evidenced by its impact on the pathogenic fever induced by the administration of a yeast injection. Its etiology includes the production of prostaglandins in central nervous system which is the final common pathway responsible for fever induction. In general, NSAIDS produce their antipyretic action through the inhibition of prostaglandin synthetase within the hypothalamus8 .Therefore, it appears that the flavonoids content of *Azadirachta indica* may also be responsible for its

antipyretic activity by inhibiting prostaglandin synthesis in hypothalamus. In addition, the flavonoids are known to inhibit prostaglandin synthetase9. O.J. Olorunfemi, D.C. Nworah, J.N. Egwurugwu and V.O. Hart from Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Rivers State, Nigeria showed that ethanol extract of the leaves of *Azadirachta indica* showed significant antipyretic effects in experimental rat models and could be an alternative source to treat fever7.

CONCLUSION

The present study concludes that the ethanolic extract of *Azadirachta indica* leaves has antipyretic activity in rats. However, this is a preliminary study and further study needs to be carried out for knowing the possible mechanism of actions and isolation of active principle(s) responsible for such activity.

REFERENCES

1.Roxburgh, W., 1874. Description of Indian Plants. Today and Tomorrow's Printers and Publishers, New Delhi, India.

2.Singh, R.P., M.S. Chari, A.K. Raheja and W. Kraus, 1996. Neem and Sternberg E.M., Neuralimmune interaction in health and disease. J. Clin. Invest., 100: 2641-2647. .

3.Basak, S.P. and D.P. Chakroborty, 1968. Chemical investigation of A. indica leaf (Melia azadirachta). J. India Chem. soc., 45: 466-467

4. Biswas, K., K. Chattopadhyay, R.K. Banerjee and U.Bandyopadhyay, 2002. Biological activities and medicinal properties of neem (*Azadirachta indica*).Curr. Sci., 82: 1336-1345.

5.Dascombe, M.J., 1985. The pharmacology of fever. Prog. Neuro Boil, 25: 327-373.

6.Adams, S.S., P. Hebborn and J.S. Nicholas, 1968. Some aspect of pharmacology of Ibufenac a non steroidal anti inflammatory agent. J. Pharm., 20: 305-312.

7.O.J. Olorunfemi et al. Evaluation of Anti-Inflammatory, Analgesic and Antipyretic Effects of *Azadrichcta indica* Leaf Extract on Fever-Induced Albino Rats (Wistar). British Journal of Pharmacology and Toxicology 3(2): 49-53, 2012

8. Chakraborty A, Devi BRK, Sanjebam R, Khumbong S, Thokchom IS. Preliminary studies on local anaesthetic and antipyretic activities of *Spilanthes acmella* Murr. In experimental animal models. *Indian J Pharmacol*, 2010; 42(5): 277-79.

9. Shukla P, Shukla P, Mishra SB, Gopalakrishna B. Screening of anti-inflammatory and antipyretic activity of *Vitex Leucoxylon* Linn. *Indian J of Pharmacol*, 2010;42(6): 409-11.