ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF AZADIRACHTA INDICA LEAVES ON EXPERIMENTAL ANIMAL MODELS

Geetamoni Dutta¹, Chinmoyee Deori², Swarnamoni Das³

¹Assistant Professor, Department of Pharmacology, Assam Medical College and Hospital, Dibrugarh, Assam.

²Associate Professor, Department of Pharmacology, Assam Medical College and Hospital, Dibrugarh, Assam.

³Professor, Department of Pharmacology, Assam Medical College and Hospital, Dibrugarh, Assam.

ABSTRACT

BACKGROUND

To study the anti-inflammatory action of ethanolic extract of *Azadirachta indica* (EEAI) leaves on experimental animal models (both acute and chronic).

MATERIALS AND METHODS

The extract of *Azadirachta indica* leaves was prepared by percolation method using 95% ethanol. Acute oral toxicity test of the extract was performed as per OECD 423 guidelines. Acute inflammation was studied by carrageenan-induced rat paw oedema method and paw volumes were measured plethysmometrically at one hour interval for 4 hours. Activity against chronic inflammation was studied by Freund's complete adjuvant induced arthritis method. Paw volumes were measured on 1st day, 5th day (injected paws) and on 21st day (non-injected paw). Aspirin 100 mg/kg was taken as the standard drug. A control group is maintained in both the models.

RESULTS

The results were analysed by ANOVA followed by Dunnett's multiple comparison test. EEAI in doses of 200, 400 and 800 mg/kg produced dose-dependent and significant ($p < 0.05$) reduction of paw oedema in carrageenan-induced acute inflammation in comparison to control. EEAI also found effective in chronic arthritis model in dose dependent manner.

CONCLUSION

The present study indicates that EEAI has significant anti-inflammatory activity in both the models.

KEYWORDS

Azadirachta Indica, Carrageenan, Adjuvant Induced Arthritis, Anti-Inflammatory Activity.

HOW TO CITE THIS ARTICLE: Dutta G, Deori C, Das S. Anti-inflammatory activity of ethanolic extract of *Azadirachta indica* leaves on experimental animal models. *J. Evolution Med. Dent. Sci.* 2016;5(85):6335-6338, DOI: 10.14260/Jemds/2016/1431

BACKGROUND

Inflammation is a reaction in vascularised tissue to an infection, irritation or foreign substances which cause cell injury.^[1]

The processes of inflammation consists of, (i) Dilatation and increased capillary permeability, (ii) Emigration of leucocytes and cellular infiltration, (iii) Proliferation of fibroblasts and synthesis of new connective tissues.²

Medicinal plants play a key role in human health care. *Azadirachta indica* (Family Meliaceae; Assamese, Bengali - Neem) is an evergreen indigenous plant widely available in India and Burma. Different parts of this plant have been traditionally used to treat skin diseases, ulcers, helminthiasis, diabetes, rheumatism and general weakness.^[3] Active constituents isolated from the plant are the alkaloids, sterols, diterpenes, polyphenols, flavonoids, sesquiterpene lactones, etc.^[4]

Various extracts of neem leaves have been reported to possess anti-microbial, anti-hyperglycaemic,⁵ analgesic,⁶ antipyretic⁷ and hepatoprotective activity.⁸ There is not much information regarding the anti-inflammatory activity of its leaf extract. Therefore, the present study was undertaken to evaluate anti-inflammatory activity of *Azadirachta indica* alcoholic leaf extract on carrageenan-induced rat paw oedema and adjuvant induced chronic arthritis in rat.

MATERIALS AND METHODS

Plant Material

The study was carried out in the Department of Pharmacology, Assam Medical College and Hospital, Dibrugarh. Fresh matured leaves of *A. indica* were collected from the college campus and authenticated by Prof. L. R. Saikia, Department of Life Sciences, Dibrugarh University, Assam (Voucher Specimen No. DULSc 449).

Plant Extract

The leaves were cleaned, shade dried and powdered by electrical grinder; 300 gms of powdered leaves were extracted by percolation at room temperature with 95% ethanol.⁹ The extract thus obtained was concentrated by using controlled temperature (bath temperature 40–50°C) and finally dried in a vacuum desiccator. The yield at the end of extraction was 15 gm. The extract of *A. indica* was administered orally as a suspension in 3% gum acacia to the rats.

Financial or Other, Competing Interest: None.

Submission 17-09-2016, Peer Review 11-10-2016,

Acceptance 17-10-2016, Published 24-10-2016.

Corresponding Author:

Dr. Geetamoni Dutta,

Assistant Professor,

Department of Pharmacology,

Assam Medical College & Hospital,

Dibrugarh-786002, Assam.

E-mail: duttageeta30@gmail.com

DOI: 10.14260/Jemds/2016/1431

Animals

Healthy albino rats of Wistar strain of either sex weighing 150-200 gm each were procured from the Central Animal House, Assam Medical College and Hospital, Dibrugarh, Assam. The study was conducted in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiment on Animals) guidelines and the study was approved by the Institutional Animal Ethics Committee (Registration No. -634/02/a/CPCSEA). Rats were kept on standard laboratory diet and water ad libitum.

Acute Oral Toxicity Study

Acute toxicity test was performed for the ethanolic extract of *Azadirachta indica* following OECD 423 guidelines.¹⁰ As no mortality was recorded up to maximum dose of 2000 mg/kg. Hence, 1/10th of the maximum dose, i.e. 200 mg/kg is selected as minimum dose for the study.

Anti-Inflammatory Studies

For each experiment, the animals were divided into 5 groups with 6 animals in each group.

- Group A (control) received 3% gum acacia 10 mL/kg p.o.
- Group B1 (Test) received EEAI leaf extract 200 mg/kg p.o.
- Group B2 (Test) received EEAI leaf extract 400 mg/kg p.o.
- Group B3 (Test) received EEAI leaf extract 800 mg/kg p.o.
- Group C (standard) received aspirin 100 mg/kg p.o.

Anti-Inflammatory Study Against Acute Inflammation

Acute inflammation was produced by subplantar injection of 0.1 mL of freshly prepared 1% carrageenan suspension in normal saline in the right hind paw of the rats.¹¹ Paw volume was measured plethysmometrically as described by Chattopadhyay et al at '0' hour and then at the end of 1st, 2nd, 3rd and 4th after carrageenan injection.¹⁰ The volume of oedema was recorded as the difference between the paw volume at '0' hour and at the end of each hour. The drugs were administered orally in the respective groups one hour before carrageenan injection. The percentage inhibition of the rat paw oedema was calculated after each hour of carrageenan injection up to 4 hours by the following formula.¹²

$$\% \text{ inhibition} = \frac{(\text{Control mean} - \text{treated mean})}{\text{Control mean}} \times 100$$

The data were statistically analysed using ANOVA (One-Way analysis of variance) followed by Dunnett's multiple comparison test. P values < 0.05 were considered significant.

Study of Anti-Inflammatory Action in Chronic Inflammation

The anti-inflammatory activity of EEAI against chronic inflammation was tested by Freund's adjuvant-induced arthritis method in rats.¹³ On 1st day, 0.1 mL of complete Freund's adjuvant is injected into the subplantar region of the right hind paw of the rats. Treatment with the test and standard drugs to the respective groups was started on the same day and continued for 12 days. The paw volume on both sides was measured plethysmographically on the day of injection. The body weight of the rats was also recorded on the first day. On 5th day, the volume of the injected paw was measured again indicating the primary lesion and the effect of the therapeutic agent on this phase. The severity of the induced adjuvant disease was followed by measurement of the volume in non-injected paw (secondary lesions) with a plethysmometer. Purposely, from day 13 to 21, the animals were not administered with the test drug or the standard. On day 21, the non-injected paw volume and the body weight were determined again^[13] and the polyarthritis severity was graded on a scale of 0-4: 0 = no swelling; 1 = isolated phalanx joint involvement; 2 = involvement of phalanx joint and digits; 3 = involvement of the entire region down to the ankle; 4 = involvement of entire paw including ankle. The maximum joint score was 12 including 3 secondary arthritis paws for each rat.^[14]

1. For primary lesions: The percentage inhibition of paw volume of the injected right paw over control was measured at day 5.
2. For secondary lesions: The percentage inhibition of paw volume of non-injected left paw over control was measured at day 21.

An Arthritis index was calculated as the sum of the scores as indicated above for each animal.^[13]

Statistical Analysis was done using one-way ANOVA followed by Dunnett's multiple comparison test. P value < 0.05 was considered as significant.

Group	Drug	Dose, Oral, Single Dose	Mean Increase in Paw Oedema (Mean ± SEM) in mL			
			1 st hr.	2 nd hr.	3 rd hr.	4 th hr.
A (CONTROL)	3% gum acacia	10 mL/kg	0.36 ± 0.04 (-)	0.43 ± 0.05 (-)	0.46 ± 0.05 (-)	0.49 ± 0.05 (-)
B1	EEAI	200 mg/kg	0.29 ± 0.01 (13.6%)	0.32 ± 0.01 ^a (20.1%)	0.34 ± 0.02 ^b (20.9%)	0.35 ± 0.02 ^a (22.6%)
B2	EEAI	400 mg/kg	0.23 ± 0.01 ^a (33.2%)	0.26 ± 0.01 ^a (36.3%)	0.23 ± 0.01 ^a (47.3%)	0.21 ± 0.01 ^a (53.5%)
B3	EEAI	800 mg/kg	0.22 ± 0.01 ^a (37.9%)	0.24 ± 0.01 ^a (41.9%)	0.21 ± 0.01 ^a (52.9%)	0.20 ± 0.01 ^a (58.1%)
C (STANDARD)	Aspirin	100 mg/kg	0.20 ± 0.01 ^a (40.6%)	0.22 ± 0.01 ^a (44.7%)	0.18 ± 0.01 ^a (59.6%)	0.16 ± 0.01 ^a (65.8%)
One-Way ANOVA		F	12.5	12.5	22.22	35.94
		Df	4, 25	4, 25	4, 25	4, 25
		ap	< 0.05	< 0.05	< 0.05	< 0.05

Table 1. Anti-Inflammatory Effect of Ethanolic Extract of *Azadirachta indica* on Carrageenan Induced Rat Paw Oedema at the End of 1st, 2nd, 3rd and 4th hour

n=6 rats in each group. Figures in the parentheses indicate the percentage inhibition of paw oedema. (-) indicates no inhibition. ^a p < 0.05 when compared to control. One-Way ANOVA followed by Dunnett's multiple comparison test is done.

Group	Drug	Dose	Mean Increase in Paw Volume (mean±SEM) in mL (% Inhibition in Parentheses) Injected Non-Injected Paw		Weight Change on 21 st Day (% Change in Parentheses)	Arthritis Index
			on 5 th day	On 21 st day		
A	Control (3% gum acacia)	10 mL/kg	0.91±0.02 (-)	0.52±0.02 (-)	-28±0.32	7.8±0.24
B1	EEAI	200 mg/kg	0.71±0.03 ^a (22%)	0.20±0.02 ^a (62.14%)	-15±0.21 ^a (46.42%)	5.9±0.16 ^a
B2	EEAI	400 mg/kg	0.64±0.12 ^a (29.80%)	0.15±0.04 ^a (71.15%)	-10±0.3 ^a (64.28)	5.1±0.12 ^a
B3	EEAI	800 mg/kg	0.58±0.02 ^a (36.2%)	0.12±0.02 ^a (76.92%)	-7±0.31 ^a (75%)	4.5±0.14 ^a
C	ASPIRIN	100 mg/kg	0.56±0.02 ^a (39.18)	0.11±0.02 ^a (80.67%)	-5±0.14 ^a (82.14%)	3.2±0.10 ^a
One-Way ANOVA		F df P ^a	182.8 4,25 < 0.05	82.36 4,25 < 0.05	66.80 4,25 < 0.05	96.32 4,25 < 0.05

Table 2. Anti-Inflammatory Activity of Ethanolic Extract of Azadirachta Indica on Chronic Inflammation by Adjuvant-Induced Arthritis in Rat

n = 6 rats in each group. (-) indicates no inhibition. ^ap < 0.05 when compared to control. One-Way ANOVA followed by Dunnett's multiple comparison test is done.

DISCUSSION

Carrageenan-induced hind paw oedema in rats is the standard model of acute inflammation. It is a biphasic response. The first phase is mediated through release of histamine, serotonin and kinins, whereas the second phase is related to the release of prostaglandin-like substances which peak at 3 hours.¹⁵ The results of the present study suggests that EEAI in doses of 200, 400 and 800 mg/kg significantly suppressed carrageenan-induced paw oedema in rats when compared to control. Maximum anti-inflammatory activity was observed at 3rd and 4th hours of carrageenan injection. The probable cause of anti-inflammatory action EEAI in acute inflammation might be due to the inhibition of some of the mediator release within 3 hours of carrageenan injection.

Freund's complete adjuvant-induced arthritis is a widely used model of chronic inflammation. This model is widely utilised because of the strong correlation between the efficacy of therapeutic agents used in this model and in rheumatic arthritis in human. EEAI in all doses significantly reduced adjuvant induced paw oedema. The leaves of Azadirachta indica are rich in flavonoids. Flavonoids have demonstrated significant anti-inflammatory activity.¹⁶ Hence, anti-inflammatory action of EEAI might be due to the action of flavonoids.

Adjuvant induced arthritis is characterised by increased production of proinflammatory cytokines such as tumour necrosis factor- α (TNF- α) and interleukin-1 (IL-1) associated with loss of body weight.¹⁷ These cytokines influence the hormones that regulate metabolism and also act directly on the target organs like muscle, liver, gut and brain.¹⁸ Thus, arthritis induced reduction in body weight can be prevented by EEAI and it might be due to inhibition of TNF- α and interleukin-1.

CONCLUSION

The leaves of Azadirachta indica has showed significant anti-inflammatory activity against both acute and chronic

inflammation models at all the tested doses establishing its traditional use in acute inflammatory conditions and rheumatism. Hence, further studies are required to establish and elaborate its molecular mechanism and proper clinical utility.

ACKNOWLEDGEMENTS

We are thankful to Prof. L. R. Saikia, Department of Life Sciences, Dibrugarh University for helping us with taxonomical identification of the plant. We are also thankful to the faculty and laboratory staff members of Pharmacology Department of Assam Medical College and Hospital, Dibrugarh for their support and encouragement.

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