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Research Article

Evaluation of Anti-Inflammatory and Analgesic Activity of Azadirachta Indica (leaf) Extract in Chemical and Thermal Induced Pain Models in Rats

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ABSTRACT

The present study carried out to evaluate the Anti-inflammatory and Analgesic activity of Azadirachta indica (leaf extract) by using chemical and thermal induced pain models in albino rats. Anti-inflammatory activity was measured by Carrageenan induced paw edema volumes at 0,1,2,3 hours using mercury plethysmometer which served as chemical induced pain model and the Analgesic activity was evaluated by using Eddy's Hot plate induced hyperalgesia which served as thermal induced pain, where the animal was placed on the hot plate and the reaction time to (lick the paw/ jump out) from the hot plate was observed, for every 30min from 0 to 3hrs. Azadirachta indica (500mg/kg BW) and Celecoxib (100mg/kg BW) was administered per oral. Animals treated with A. indica (500mg/kg) and Celecoxib (100mg/kg) has shown maximum inhibition of paw edema by 0.99±0.06 at 3 hrs and 0.79±0.05 at 2hrs respectively, where the percent of inhibition was16.27% and 34.83%, ($p<0.001$) which revealed that A.indica has a potent anti-inflammatory activity. In the Hot plate induced hyperalgesia peak analgesic action was observed at 5.26±0.13 at 2 hrs in Azadirachta indica and 5.04 ± 0.33 at 1.5 hrs in Celecoxib treated rats. The analgesic activity and anti inflammatory activity of of A.indica was significant effect over to Saline and lower when compared with comparator drug Celecoxib and it was statistically significant ($p<0.001$). The results indicate that the aqueous extract of Azadirachta indica (leaf) extract revealed significant Anti-Inflammatory and Analgesic activity in Thermal and Chemical induced Pain Models.

Key words: Anti-inflammatory, Analgesic, Azadirachta indica, Carrageenan, Celecoxib

INTRODUCTION

Inflammation which is defined as a response to an injurious stimulus , which can be evoked by wide variety of noxious agents like physical injuries, infections , foreign bodies and Cardinal signs of inflammation include edema, hyperalgesia, pain and erythema. Histamine and Bradykinin are the primary mediators of edema formation during inflammatory process. The presence of vasodilator prostaglandins at the site of inflammation can potentiate the effects of histamine and bradykinin to produce edema by increasing flow to the inflamed region.[1] Nociception (pain) being the one of the most common problem where the human beings suffer a lot in their day to day life ,where the individual seeks for medical help. Hyperalgesia is an increased pain response to a noxious stimulus, which is caused by sensitization of peripheral nociceptors or by sensitization of central neurons that carry nociceptive information either by an irritant, by inflammation or by disease.

The majority of the drugs that are used for the pain management and inflammatory conditions have more or less adverse and lethal effects.[2] Consequently the medicines that are derived from plant origin were used to treat different diseased conditions since primitive time, devoid of any adverse effects. It is therefore essential that more efforts should be made to develop new drugs of plant origin, which will be useful to treat with less adverse, toxic effects and at a lower cost.

Azadirachta indica is the most useful, time-honored medicinal plant in India, which has been used in Ayurvedic medicine for more than 4000 years due to its medicinal properties. It is a tropical evergreen tree in its native Indian subcontinent and distributed widely all over the world. It is known to possess an anti-inflammatory activity in-addition to diverse therapeutic properties. Almost all the parts, such as fruits, seeds, leaves, bark and roots contain compounds with proven antiseptic, anti-viral, antipyretic, antiulcer and antifungal uses. It is now considered as a precious source for development of medicines against various diseases.[3,4]

For the reason that it possesses diverse medicinal properties like anti inflammatory action, which helps in relieving inflammatory pain predominantly in geriatric age group and analgesic property that helps in treating acute pain, it can be used for treating both acute and chronic models of pain.

The present study is carried out to evaluate the Anti-inflammatory and Analgesic activity of aqueous extract of

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Table 1: Anti-Inflammatory activity of A.indica in Carrageenan induced paw edema

Drugs	Paw edema Volume (ml) at hours				% of Inhibition at Hours			T Max
	0 Hrs	1 Hr	2 Hr	3hr	1 Hr	2 Hr	3 Hr	
Saline	0.26±0.04	0.53±0.09	0.89±0.11	1.20±0.10	--	--	--	
A.Indica	0.31±0.03	0.43±0.04	0.58±0.04	0.79±0.05*	--	10.11	16.27	3
Celecoxib	0.30±0.02	0.59±0.02	0.80±0.05	0.99±0.06*	18.86	34.83	34.16	2

Table 2: Effects of A.indica in hot plate induced Hyperalgesia

Time in hours	Reaction Time in seconds			Percentage of Inhibition	
	A.indica	Celecoxib	Saline	A.indica	Celecoxib
0	1.64±0.08	1.74±0.34	1.38±0.33	19.13	26.39
0.25	2.04±0.29	2.64±0.10	1.35±0.33	51.3	95.67
0.5	2.35±0.26	3.47±0.23	1.34±0.32	75.56	159.6
1	4.63±0.26	4.53±0.77	1.32±0.32	242.07	249.75
1.5	4.88±0.12	5.04±0.33*	1.27±0.00	298.16*	296.45
2	5.26±0.13*	4.02±0.30	1.26±0.34	218.65	317.72*
2.5	5.07±0.06	3.38±0.17	1.28±0.31	163.93	295.56
3	5.02±0.30	2.62±0.27	1.27±0.30	106.31	285.02

Azadirachta indica by chemical and thermal induced pain models and to compare it with Celecoxib.

OBJECTIVES

- To evaluate Anti-inflammatory and Analgesic activity of Azadirachta indica leaf extract in thermal and chemical induced pain models in Albino Rats
- To compare the Anti-inflammatory and Analgesic activity of A.indica with Celecoxib

MATERIALS AND METHODS

Drugs: Celecoxib (Cipla, Uttarakhand); Carrageenan (Sigma, Mumbai), Gum Acacia (Indian Research Products, Chennai) were used Preparation of A.indica (A.Indica) Leaf Extract: - (NLE):

One kg freshly collected, shade dried, powdered leaves of Azadirachta indica were ground in 2 liters of distilled water and allowed to soak overnight. The suspension was centrifuged at 5000 rpm for 20 min and filtered through a Whatman No. 1 filter paper. The supernatant fluid was allowed to evaporate in glass Petri-dishes. When completely dry, the powder was collected by scraping and was stored. Stock solution was prepared by suspending 5gm of NLE in 50 ml of distilled water. The dose administered was 500mg/kg, per orally (p.o.)[5]

Animals: Institutional Animal Ethics Committee's (IAEC) consent was obtained prior to this study protocol. The animals (Wister strain albino rats) weighing 150-200gms were bred in Central animal house, Narayana Medical College for the animal experiments. The animals were maintained under standard laboratory conditions (light period of 12hrs/day and temperature 250C ± 10C with free access to food and water ad libitum).

Grouping of Animals: The analgesic and anti-inflammatory activity of the compounds were studied using two different models viz. Carrageenan induced rat paw edema, Hot-Plate induced hyperalgesia. The animals were divided in to three, each group containing 6 animals.

Group 1: Animals were treated with Saline which served as Control;

Group 2: Animals treated with A.indica;

Group 3: Animals treated with Celecoxib.

Methods

Carrageenan induced Rat paw Edema: Acute inflammation was produced by sub plantar injection of 0.1ml of freshly prepared 1% (w/v) suspension of Carrageenan in normal saline in all the animals. The drugs were administered orally one hour before Carrageenan injection. The paw volumes were measured at 0,1,2,3 hrs after Carrageenan injection using Plethysmometer by noting the displacement of mercury when the paw is dipped in mercury column up to a predetermined mark on the paw. A mark was made on the leg at lateral malleolus to facilitate uniform dipping and recording of paw volumes.[6]

Hot-Plate induced hyperalgesia: In this method the rats were placed on the hot plate and the latency of nociceptive response such as licking and jumping were measured at 0, 0.25, 0.50, 01, 1.5, 2.0, 2.5, 3.0 hrs. The percentage of change in the reaction time was calculated using the formula (100 X RTt -RTc/ RTc) where, 'RTc' represents reaction times of saline at a time point &'RTt' represents reaction time of test drugs at a time point. All the drugs were administered orally 1hr prior to the commencement of estimation of reaction time. The cut off time was considered as 15 sec to prevent the injury to the animal.[6]

STATISTICAL ANALYSIS

Statistical analysis was performed using Microsoft Excel and Sigma Graph pad prism version-4 USA. Data was described as Mean ± SD and Percentages. Negative sign (-) indicates decrease and positive (+) sign indicates increase respectively. ANOVA followed by Tukeys multiple comparison tests was used for analysis multiple group comparisons. For all inferential statistical tests a two tailed P value of 0.05 was considered significant

RESULTS

Carrageenan- induced Rat Paw Edema: The aqueous extract of A. indica leaf (500mg/kg, p.o) has significantly ($P < 0.001$), inhibited the Carrageenan induced rat paw

edema. A Maximum inhibition of rat paw edema was shown by A.Indica, and Celecoxib was 0.99±0.06 at 3 hrs and 0.79±0.05 at 2hrs after carrageenan injection and the percentage of inhibition was 16.27% and 34.83% when compared with Control group. A statistically significant difference was observed between A.Indica vs. Saline ($P < 0.001$), Celecoxib vs. Saline ($P < 0.001$) and A.Indica vs. Celecoxib ($P < 0.001$). (Table-1)

Hot Plate Induced Hyperalgesia: The animals treated with aqueous extract of A. indica leaf (500mg/kg, p.o) has shown significant ($P < 0.001$), rise in reaction time i.e., time to Peak analgesic action which was described as time to maximum inhibition of pain was 5.26±0.13 and 5.04 ± 0.33 at 2.0 and 1.5 hours with A.Indica and Celecoxib respectively. On evaluation of the magnitude of inhibition measured at 2 hrs after the start of experiment, statistically significant differences was observed between A.Indica vs. Saline ($P < 0.001$), Celecoxib vs. Saline ($P < 0.001$) and A.Indica vs. Celecoxib ($P < 0.001$). (Table-2).

DISCUSSION

Carrageenan induced paw edema is a suitable test for evaluating anti-inflammatory properties of natural drugs, because of its sensitivity in detecting orally active anti-inflammatory agents particularly in the acute phase of inflammation.[7] Using this model, Chattopadhyay et al., (1993)conducted an experiment with Azadirachta indica leaf extract and reported that its anti-inflammatory effect might be due to inhibiting the action of 5-HT and PGE or due to stabilization of lysosomal membranes and 1 Anti proliferative effect.[8] The presence of flavonoids , tannins, alkaloids and tetranortriterpenes, (nimbin, nimbinin, nimbidinin, nimbolide and nimbidic acid) in the leaf extract of this plant might be responsible for such an antiinflammatory, analgesic and antipyretic properties of A. indica.[9,10,11,12]Vinger.et al., (1969) and Crunkhon and Meacock (1971) had attributed the first hour response to carrageenan administration was due to release of histamine and serotonin and later response to the release of prostaglandins, protease, and lysosome.[13,14] In our study, the anti inflammatory response was observed only after the first hour when treated with A.indica and Celecoxib, suggesting the anti-inflammatory mechanism might possibly be due to inhibitory action of prostaglandins. It is unlikely that the anti-proliferative effects with single dose might have contributed for such an observation.

In our study, we demonstrated significant anti-inflammatory activity ($P< 0.001$) with 500mg/kg of aqueous extract of A. indica leaf. Gupta Sonika et.al, 2010, reported significant decrease in paw volumes ($P<0.01$) even at a smaller dose of 200 mg/kg of A. Indica,[15] in contrast to an earlier study conducted by Mahabub-Uz-Zaman, et.al, (2009) who demonstrated inhibition ($P<0.05$) of paw volume with 1 g/kg dose of ethanol extract of Azadirachta indica leaves. However in that study, 500 mg/kg and 100 mg/kg doses failed to illustrate any significant inhibition as compared to controls.[16] Variations in the magnitude of inhibition with A.Indica were observed across studies. It is well established that magnitude of responses may vary between species, strains, age, sex, diet and nourishment, in-addition to type of extract, dose and bioavailability of medications.

We evaluated the anti-nociceptive activity of A. indica by using Eddy's hotplate induced hyperalgesia model. In our study we observed a significant increase in jump-off time as compared to baseline with all the A.Indica and Celecoxib. Both A.Indica and Celecoxib increased the jump-off time significantly as compared to baseline. When our study results were compared with an earlier report by Mahabub-Uz-Zaman, et.al, (2009) neither the ethanol extract of A. indica (1g/kg, 500 mg/kg and 100 mg/kg) nor Diclofenac sodium (40 mg/kg) showed any significant protective effect on heat induced pain in mice.[16] The possible explanations for such a variation across studies includes, thermal painful stimuli are known to be selective to centrally, but not peripherally acting analgesic drugs[17].

CONCLUSION

The current study demonstrates significant Anti-inflammatory and Analgesic activity of Azadirachta indica in Thermal and Chemical induced pain models. Both the experimental procedures were compared with control, and the active comparator Celecoxib. In the Carrageenan induced paw edema, the magnitude of the Anti-inflammatory activity of A.indica was less when compared to Celecoxib. The present study also serves as an experimental basis for the further research in the development of drugs derived from natural plant sources.

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