

Anti Inflammatory Effect of Azadirachta Indica (Neem) In Albino Rats-An Experimental Study

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ABSTRACT:

Introduction: Inflammation is body's attempt at self protection characterized by five cardinal signs; it may be acute or chronic. NSAID(non steroidal anti-inflammatory drugs are the commonly used to reduce the inflammation. traditional Indian system of medicine mentions neem(Azadirachta Indica)to have many medicinal properties. so the present study was carried out to access the anti-inflammatory effect of neem.

Materials and methods: Albino rats were used; they were divided into three groups. Control group treated with normal saline, standard treated with Indomethacin and test drug used was neem oil. For acute inflammation; Carregennan induced rat paw edema inhibition method and for sub acute inflammation: cotton pellet granulation method. Ulcer index of Indomethacin and test compound were also studied.

Results: it is found that neem oil showed significant anti-inflammatory effect in both acute as well as chronic inflammation, it was also found to have low ulcerogenic potential compared to Indomethacin, hence can be safely used as a potent anti-inflammatory agent

KEYWORDS: Neem (Azadirachta-Indica), Inflammation, Indomethacin, Carregennan, Cotton pellet

I. INTRODUCTION:

Inflammation is the body's attempt at self-protection; to remove harmful stimuli, damaged cells, irritants, or pathogens - and begin the healing process. The traditional names for signs of inflammation come from Latin: Dolor (pain), Calor (heat), Rubor (redness), Tumor (swelling), Functio laesa (loss of function). The first four (classical signs) were described by Celsus (ca. 30 BC–38 AD), while *loss of function* was added later by Galen.¹ The process of acute inflammation is initiated by cells already present in all tissues, mainly resident macrophages, dendritic cells, histiocytes, Kupffer cells and mastocytes.^{1,2} Inflammation is of two types acute and chronic. Acute inflammation - starts rapidly (rapid onset) and quickly becomes severe. Signs and symptoms are only present for a few days, but in some cases may persist for a few weeks. Examples: acute bronchitis, infected ingrown toenail, sore throat from a cold or flu, a scratch/cut on the skin, exercise (especially intense training), acute appendicitis, acute dermatitis, acute tonsillitis, acute infective meningitis, acute sinusitis, or a blow.^{2,3} Chronic inflammation - last for several months and even years. The important causes are an autoimmune response to a self antigen. A chronic irritant of low intensity that persists. Examples of diseases and conditions with chronic inflammation include: asthma, chronic peptic ulcer, tuberculosis, rheumatoid arthritis, chronic periodontitis, ulcerative colitis and Crohn's disease, chronic sinusitis, and chronic active hepatitis.^{3,4} The drugs that reduce the inflammatory process are called as anti-inflammatory drugs. NSAID (non steroidal anti-inflammatory drugs) is the most commonly used anti-inflammatory drugs. They mainly bring about the action by inhibiting the (COX) cyclooxygenase enzyme.⁵

Classification of anti-inflammatory agents^{5,6}:

1. Non-steroidal anti-inflammatory drugs:

a. Salicylates: Aspirin, Methyl salicylate

b. Propionic Acid Derivatives: Ibuprofen, Naproxen, Fenoprofen, Ketoprofen, Flurbiprofen, Oxaprozin

c. Acetic Acid Derivatives: Indomethacin, Sulindac, Etodolac

d. Oxicam Derivatives: Piroxicam, Meloxicam

e. Fenamates: Mefenamic Acid, Meclofenamate

f. Other Agents: Diclofenac, Ketorolac, Tolmetin, Nabumetone, Diflunisal

2. COX-2 Selective NSAIDs: Celecoxib, Lumiracoxib, Etoricoxib

3. Drugs for Arthritis: a. Drugs for anti-cytokine therapy Etanercept, Infliximab, Adalimumab, Anakinra b. Methotrexate .

C. Leflunomide d. Chloroquine and hydroxychloroquine e. d-Penicillamine f. Gold salts

4. Drugs used to treat Gout: Colchicine, Allopurinol, Uricosuric agents such as Probenecid and Sulfinpyrazone

NSAIDs aside from aspirin, both newer selective COX-2 inhibitors and traditional anti-inflammatory agents, increase the risk of myocardial infarction and stroke.⁷ In people with heart failure,

NSAIDs increase mortality risk by approximately 1.2-1.3 for naproxen and ibuprofen, 1.7 for rofecoxib and celecoxib, and 2.1 for diclofenac. A 2005 study linked long term (over 3 months) use of NSAIDs, including ibuprofen, with a 1.4 times (140%) increased risk of erectile dysfunction. NSAIDs cause a dual assault on the GI tract: the acidic molecules directly irritate the gastric mucosa, and inhibition of COX-1 and COX-2 reduces the level of protective prostaglandins. Common gastrointestinal ADRs include:^[9] Nausea/Vomiting, Dyspepsia, Gastric ulceration/bleeding, diarrhea.⁷ Azadirachta Indica, commonly known as neem, has attracted worldwide prominence in recent years, owing to its wide range of medicinal properties. Neem has been extensively used in Ayurvedic, Unani and Homoeopathic medicine and has become a cynosure of modern Medicine.⁸ All parts of the neem tree- leaves, flowers, seeds, fruits, roots and bark have been used traditionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders.^{8,9} The medicinal utilities have been described especially for neem leaf. Neem leaf and its constituents have anti-inflammatory, antihyperglycaemic, antioxidant, antimutagenic and anticarcinogenic properties¹⁰. So the present study was taken up to explore the anti-inflammatory properties in albino rats.

II. MATERIALS AND METHODS:

Chemicals: carragennan, carboxy methyl cellulose, Indomethacin, gum acacia, neem oil and ether.

Equipments: plethysmograph, ryles (infant feeding) tube, wooden mouth gag, syringes, dissection set, skin suturing set, magnifying lens, mortar and pestle, sterile cotton pellets and grass pith, Gum acacia: is used as vehicle for the administration of indomethacin. The drug are administered orally, through the infant feeding tube, as fine suspension in 5% gum acacia. Ether: is used to anaesthise the rats during implantation and removal of cotton pellets and while sacrificing the rats for postmortem examination. Indomethacin: has a prominent anti-inflammatory, analgesic and antipyretic property similar to those of salicylates.⁵ Neem: is found to exert an analgesic and anti-inflammatory activity¹¹ in the albino rats. The test material showed significant anti-inflammatory effect against 5HT and PGE1 induced inflammation.⁹ Though the exact mechanism is not known, there is always scope for further studies. Albino rats weighing 100 to 200gms between 21 days to 4 months of age, with no visible disease.

III. METHODS:

For acute inflammation: carragennan induced rat paw edema inhibition method.

For subacute inflammation: cotton pellet granulation method.

Carragennan induced rat paw edema inhibition method^{12,13}: wistar albino rats were divided into 3 groups each containing 6 rats. Inflammation was produced by injecting 0.1 ml of carragennan suspension in normal saline to right hand paw. One hour after drug administration, 0.1ml of 1% carragennan in 0.5 carboxymethyl cellulose was injected into the sub plantar region of one of the hind paws. a mark was made on the leg at the malleous to facilitate uniform dipping at subsequent readings. The paw edema volume was measured with the help of plethysmograph by mercury displacement method at zero hour (immediately after injecting carragennan). The same procedure was repeated at 1, 2, 3 & 4 hours. The percentage inhibition of oedema in various treated groups was then calculated by using the formula.

First group: control group receiving no drug

Second group: standard control was administered Indomethacin in a dose of 20mg/kg body weight.

Third group: was given the test compound in the dose of 1,2,4,8ml/kg bodyweight intraperitoneally 1 hour prior to carragennan injection. paw volumes will be measured using plethysmograph in each group at 0 and 3 hours after carragennan injection.

$$\% \text{ edema inhibition} = \frac{V_c - V_t}{V_c} \times 100$$

Where V_t is the mean edema volume in the drug treated group

V_c is the mean edema volume in control group.

Plethysmograph¹²: fabrication of this instrument consists of vertical glass cylinder A which is 8 cms in length and 14 mm inside diameter. This cylinder is attached to another glass tube B of 3mm diameter by means of a glass tube which is horizontal in position. This system enables mercury to stand at nearly same levels. The diameter of the horizontal connecting glass tube is same as that of inside tube B, the cylinder A is also connected with the graduated pipette by means of another connecting glass tube of 3 mm diameter. The pipettes capacity is 2ml and divided into 200 divisions. The zero mark is so adjusted that mercury in these three vertical glass cylinders and side tubes stand at same level. The upper end of the pipette is connected to an H shaped stopper. The upper way has got a stopper SI and the side limb is connected to a syringe interfered by another stopper S2. after putting mercury upto the levels should be so adjusted that they lie at the same horizontal line.

The zero on the pipette corresponds to the level of mercury in it, at the initial setting. Operation of the apparatus: at the beginning the mercury level was adjusted to zero mark by the help of syringe X, stopper S1 was closed and S2 was kept open.

The hind paw of the rat was made to dip in the mercury of cylinder A. as a result the levels of mercury go little higher in all the tubes as well as in cylinder A. keeping the paw inside the cylinder the level of mercury was brought down to the initial level by drawing the piston of syringe X. This results in elevation of mercury level I the pipette and lowering of mercury levels in the cylinder A and tube B. After the levels in the cylinder A and side tube B were brought to initial levels the stopper S2 was closed and the paw was taken out of cylinder A. Elevated mercury from zero in the pipette gives the volume of the paw. This procedure needs two operators; one to dip the rat paw and other to adjust the mercury levels. The whole apparatus was supported with the help of a fixing stand Z during the procedure. Cotton pellet granuloma method^{14,15}: rats were divided into 5 groups consisting 6 rats. Under light ether anesthesia the hair in the axillary and groin region were cut and sterile cotton pellets of 15 mg each were implanted in the subcutaneous tissue on either sides of axilla and sterile grass pith(25x2mm) in the groin region. Wounds were then sutured and animals were caged individually after recovery from anesthesia. The rats then received treatments as described. The schedule of drug administration was started on the day of implantation and repeated every 24 hours, regularly for 7 days, any change in food intake, motor activity and diarrhea if any were noted. On the 8th day, the rats were sacrificed and cotton pellets and grass pith removed. The pellets freed from tissue, were dried overnight at 60 C to their dry weight. Net granuloma weight was calculated by subtracting initial weight of cotton pellet (15mg) from weight noted. The grass pith was served in 10% formalin for histopathological studies. Percentage of granuloma inhibition in various treated groups was calculated using the formula;

% inhibition= $\frac{Wc-Wt}{Wc} \times 100$ where

Wc-mean dry weight of pellet granuloma for control group

Wt- mean dry weight of pellet granuloma of test group.

Stomachs were cut open along the greater curvature and washed with normal saline. Gastric mucosa was examined for the erosions, hemorrhagic spots, ulcers and perforations, if any with the help of magnifying glass. To determine the severity of the ulcers arbitrary scoring system as described by Gupta M B etal, 1985 was followed.

a) Denuded epithelium==10 b) petechial and frank hemorrhages=20; one or two ulcers=30; multiple ulcers=40; perforated ulcers=50

IV. RESULTS:

Table – 1: CARRAGENAN INDUCED RAT PAW OEDEMA INHIBITION TEST

Group	Test compound					
	Control	Indomethacin	A=IML	B= 2 ML	C=4ml	D=SML
1	0.3	0.1	0.1	0.2	0.1	0.1
2	0.4	0.2	0.3	0.1	0.2	0.3
3	0.3	0.1	0.2	0.3	0.1	0.1
4	0.4	0.2	0.3	0.4	0.2	0.2
5	0.4	0.3	0.2	0.1	0.3	0.1
6	0.3	0.1	0.1	0.2	0.2	0.1
Mean	0.37	0.17	0.20	0.22	0.18	0.19
SD	0.08	0.08	0.09	0.12	0.08	0.08
SE	00.3	0.03	0.04	0.05	0.03	0.03
P-Value		<01	<05	NS	<01	<05
% oedema inhibition		54.5	45.5	40.9	50.0	48.2

ANOVA, F = 4.39, P<.01, significant,

Studentized range test: LSD: 0.16 P.05

0.19 P<.01

SD: Standards deviation, SE: standard error

% edema inhibition = $\frac{Vc-Vt}{Vc} \times 100$

Where, Vc : Mean oedema volume for the control group.

Vt : Mean oedema volume for the test group

Dosages: Indomethacin 20 mg / kg b.w.

Text compounds A= 1 ml, B= 2ml, C= 4ml, D= 8ml.

Group	Control					Indomethacin					Test Compound																			
	Wt. of the cotton pellet					pellet					A= 1 ml					B= 2ml					C=4 ml					D= 8ml				
Rat No	I	II	III	IV	M	I	II	III	IV	M	I	II	III	IV	M	I	II	III	IV	M	I	II	III	IV	M	I	II	III	IV	M
1	21	16	17	15	17.3	9	7	8	6	7.5	6	7	8	7	7.0	12	10	9	6	9.3	8	10	6	8	8.0	9	9	6	9	8.3
2	22	17	18	19	19.0	6	9	5	7	6.8	8	9	9	8	8.5	8	9	10	8	8.8	8	9	7	8	8.0	8	9	7	8	8
3	21	16	17	18	18.0	9	8	6	6	7.3	7	8	9	9	8.3	9	10	12	8	9.8	9	8	9	10	9.0	9	7	11	7	8.5
4	22	17	19	20	19.7	8	7	6	7	7.0	6	10	8	8	8.0	9	6	7	5	6.8	8	9	7	8	8.0	8	9	8	9	8.5
5	20	19	18	16	18.3	9	6	7	7	7.3	7	9	9	9	8.8	8	6	8	5	6.8	7	12	6	8	8.3	7	9	12	6	8.5
6	22	18	16	14	17.5	10	6	7	8	7.8	10	8	7	7	9.0	9	8	8	6	7.8	9	8	7	5	7.3	6	8	6	8	7
Mean					18.3					7.25					8.25					8.17					8.03					8.13
SD					0.91					0.35					0.7					1.28					0.56					0.58
SE					0.37					0.14					0.28					0.52					0.22					0.23

One way ANOVA, F=170.4, P<.001, Highly significant, SD: Standard deviation, SE: Standard error P<.01 when compared with control. No significant different between other groups

LSD = 1.39 P< .05
 = 1.70 P<.01
 % Inhibition = $Wc - Wt \times 100$

Wc : Mean dry weight of the pellet granuloma for the control group. Wt : Mean dry weight of the pellet granuloma for the test group.

Table -3 : ULCER INDEX OF INDOMETHACIN AND TEST COMPUND

Groups	Ulcer incidence		p - value	
	Rats with ulcer	%	With control	with Indomethacin
Controls	2/6	33.3		<0.05
Indomethacin	6/6	100	<0.05, S	
Test compound A	3/6	50	NS	NS
B	3/6	50	NS	NS
C	4/6	66.7	NS	NS
D	4/6	66.7	NS	NS

Fischer's exact test

P<0.05 Significant

P> 0.05 Non significant

Dosage : Indomethacin 20 mg / kg b.w

Text compounds A= 1 ml B=2ml, C= 4ml, D= 8 ml

V. DISCUSSION:

It is evident from table 1 that the test compound in the following dosage (1, 2, 4 ml) exhibit significant anti-inflammatory effect (45.2%, 50% and 48.2%) very much comparable to that of control in carragennan induced rat paw edema inhibition test. This goes in accordance to the observation of Kumar et al. The activity of test compound in the dose of 2ml/kg is not significant when compared to control. Table 2 indicates the chronic anti-inflammatory effect of the efficacy of test compound. It has got significant effect ($P < 0.01$) when compared to the control. This goes in accordance to Rath Bhabegral et al. Table 3 shows the ulcerogenic effect of test compound was not significant .when compared to control the standard drug Indomethacin shows significant ulcerogenic effect when compared to test and control compound. Mechanism of action: the exact mechanism of action is still unclear. In the study by Chattopadhyaya et al showed that the test compound extract produces dose dependent inhibition of the inflammatory edema produced by 5HT and PGE, but its extract failed to suppress histamine and bradykinin induced edema. These observations support the conclusion that test compound has anti-inflammatory action probably by its anti 5HT and anti-PGE activity. Between the two edemogens A.indica exhibited greater action against PGE. Therefore A.indica resembles Indomethacin like drugs.

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