Comparative study of medicinal plant (azadirachta indica) with diclofenac on anti-inflammatory activity

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ABSTRACT: The inflammation is a biologically action on living tissue homeostasis. Acute inflammation causes by bacterial infection, tissue necrosis and sign of inflammation are heat (calor), pain (dolor), redness (rubor), swelling (tumour) and loss of function (functio laesa). The inflammation produces by prostaglandins, serotonin, and cytokines. NSAIDs are used in treatment of inflammation. The Azadirachta indica also produce anti inflammatory activity. The ethanolic extract (100mg/kg) of leave of neem plant are showed anti inflammatory activity in albino rats. The Diclofenac(5mg/kg) standard drug used in the experimental region. The phlogistic agent (0.1 ml of 1.0% carrageenan) was used for induce inflammation in subplanter region of hind paw in wistar rats. The Diclofenac showed best anti inflammatory activity as compared as to ethanolic leaves extract of neem. The diclofenac drug is more effective as compared to neem leave extract.

Keywords: NSAIDs, Inflammation, redness, tissue necrosis, prostaglandins.

I. INTRODUCTION

Inflammation is a biological reaction to a disrupted tissue homeostasis.¹ Inflammation is the body's attempt at self-protection; to remove harmful stimuli, damaged cells, and irritants. 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; while loss of function was added later by Galen.² Inflammation is of two types acute and chronic. Acute inflammation-starts rapidly (rapid onset) and quickly becomes severe. The process of acute inflammation is initiated by cells already present in all tissues and histiocytes, macrophages, dendritic cells, Kupffer cells and mastocytes.²³ It is a tissue-destroying process that involves the enlistment of blood-Plasma products. This migration is facilitated by alterations in the local vasculature that lead to vasodilate, enlarged vascular permeability, and increased blood flow. Inflammation may have beneficial effects such as the destruction of invading micro-organisms and the walling-off of an abscess cavity to prevent spread of infection.⁴ At the site of injury, proinflammatory cytokines are released from damaged tissue. Activation of complement cascade forms complement products that act as chemotactic agents for the recruitment of neutrophils.⁵ Complement anaphylatoxins induce local mast cells degranulation with release of histamine, causing vasodilation and smooth muscle contraction.⁶ Leukocytes, kallikrein and bradykinin move out through blood vessels causing swelling. Bradykinin was binds to nearby capillaries cells and stimulates the production of prostaglandins which then binds to free nerve endings making them to start pain impulse.⁷ The principal causes of acute inflammation are Microbial infections, Hypersensitivity reactions, Physical agents, e.g. trauma, ionizing irradiation, heat, cold, Chemicals agent e.g. corrosives, acids, alkalis, reducing agents, bacterial toxins, Tissue necrosis, e.g. ischemic infarction.⁸ A chronic irritant of low intensity that persists. Examples of chronic inflammation includes: chronic peptic ulcer, rheumatoid arthritis, tuberculosis, chronic periodontitis, asthma, ulcerative colitis and chronic sinusitis Crohn's disease, chronic active hepatitis.⁹ The drugs that reduce the inflammatory process are called as anti-inflammatory drugs. NSAIDs (non steroidal anti-inflammatory drugs) are the most commonly used anti-inflammatory drugs. They mainly bring about the action by inhibiting the (COX) cyclooxygenase enzyme.¹⁰

II. MATERIALS AND METHODS

Experimental rats: Wister rats were obtained from the experimental animal house. The rats were divided randomly into 4 groups of 5 rats each. Each rat with body weight between 150g-250g was selected and housed separately (one rat, one cage). The animals were maintained on standard pellet diet and tap water. They were maintained in standard room condition, relatively humidity and 12 hour light dark cycle. The rat cages were cleaned changed bedding every day. Animals were weighed on the particular days of experiment. All animals are closely observed for any infection.

Animal ethics: Institutional Animal Ethics Committee (IAEC) conducting the laboratory experimental animals were maintained well in an animal house. Jaipur College of Pharmacy approved by Animal Ethics Committee. It’s registration no. (931/PO/ac/06/CPCSEA).
Comparative Study Of Medicinal

Plant material: *Azadirachta Indica* was collected from locally from botanical department of Rajasthan University (jaipur, Rajasthan). The *Azadirachta indica* was sent to botanical department of Rajasthan University for species authentication. The specimen number is RUBL211440.

Plant extraction: Mature fresh leaves of *Azadirachta indica* were washed first in sterilized distilled water to remove dirt and other impurities, followed by washing in mercuric chloride solution (0.1 %) and again washed in sterilized distilled water. Sterile leaf was weighed and transferred on to a sterile mortar and pestle to make crude crushing of the materia. 120 grams of powdered plant material was exhaustively extracted for 2 h with 200 ml of ethanol solvents at room temperature (20°C-25°C) in soxhlet apparatus. The extracts obtained were filtered and evaporated under reduced pressure. The extracts was dissolved in the dimethyl-sulphoxide solvent to make the final concentrations which were kept in refrigerator at -20°C for future used. Then filtered was dried at 500°C. The dried extract was used for the study.

Preparation of drug formulation: Diclofenac sodium was used as standard for comparing Anti-inflammatory activity of *Azadirachta indica* leaf extract in Carrageenan induce paw edema animal model. Leaves extract of neem (100mg/kg b.w.) was prepared and also prepared of 0.1ml of 1.0 ml of carrageenan solution in normal saline.

Procedure Acute toxicity study: In the initial phase, *Wistar* rat were divided into 3 groups of three and treated with the ethanol leaves extract of the plant at doses of 10, 50, 100, 200 and 500mg extract/ kg body weight intraperitoneally and were then observed for 24 hours for signs of toxicity. In the final phase, rat were divided into 4 groups of one mouse each and treated with the ethanol extract at doses of 600, 800, 1000 mg and 1200 mg/ kg body weight. The median lethal dose (LD50) was calculated from the second phase. The median lethal dose (LD50) in wistar rat was calculated to be 1200 mg/kg body weight.

Observations: Animals were observed at regular time intervals at least once at every 30 minutes of initial after dosing, for the first 24 hrs. In all the cases no death was observed within first 24 hrs. Additional observations like behavioral changes change in skin, fur, eyes, mucous membranes, respiratory, circulatory. Attention was also given to observation of tremors and convulsions and other pharmacotoxic signs included: salivation, diarrhoea, piloerection, increased motor activity, itching, sedation, and restlessness.

Anti-inflammatory Study: Increases in the rat hind paw diameter induced by subplanter injection of a phlogistic agent were used as the measure of acute inflammation. The phlogistic agent employed in this study was 0.1 ml of 1.0% carrageenan. The rats were randomly divided into four groups (n=6). After thirty minutes the induced paw edema by the injection of 0.1 ml of 1.0% Carrageenan and thirty minutes before the groups were treated i.p as follows group 1: normal saline, group 2: (5mg/kg of Diclofenac), group 3: Ethanolic Extract(100mg/kg b. w.) and group 4: received Ethanolic Extract+Diclofenac. The hind paw Inflammation was induced by injecting 0.1 ml of 1.0% carrageenan into the subplanter surface of the left hind paw. Paw diameter (ml) was measured by Phlethysmometer at 0min., 30min., 60min., 90min. and 120min. after 0.1 ml of 1.0% carrageenan injection. Paw diameter after administration of the phlogistic agent was measured using scale equipment.

Inhibition of edema (%) = Mean paw diameter (control) – Mean paw diameter (treated) x100/ Mean paw diameter (control)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean Paw volume measured before and after drug administration(s) by mercury Plethysmometer(ml)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Initial paw vol.</td>
</tr>
<tr>
<td>G-I</td>
<td>1.20±0.16</td>
</tr>
<tr>
<td>G-II</td>
<td>1.03±0.08</td>
</tr>
<tr>
<td>G-III</td>
<td>1.17±0.13</td>
</tr>
<tr>
<td>G-IV</td>
<td>1.02±0.20</td>
</tr>
</tbody>
</table>

Values are mean ± SD of six samples in each group.

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III. CONCLUSION

Anti-inflammatory activity of Leaves of *Azadirachta indica* was evaluated by subplanter region of injection of carrageenan in animals. The standard drug Diclofenac (5mg/kg) showed significant and the dose dependent of decrease of paw edema as compared to control group of animals. The diclofenac showed better anti-inflammatory activity as compared to test group of ethanolic leave extract of *Azadirachta indica*.

REFERENCES

[8]. A brief cause of acute inflammation rajesh prajapat