Research Article

Anti-Inflammatory Activity Of Achyranthes Aspera Leaf On Carrageenan Induced Paw Edema In Albino Rat.

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ABSTRACT

This study investigated the anti-inflammatory potential of the alcoholic extract of Achyranthes aspera Linn. (Amaranthaceae) in Wistar albino rats after oral administration (200, and 400mg/kg). This was done using the carrageenan-induced paw edema method (acute inflammatory model). The alcoholic extract showed significant suppressed granuloma formation. Collectively, these data demonstrate promising anti-inflammatory activity against both acute and chronic inflammation. It was discovered that none of the dosages of the extract of Achyranthes aspera used for acute oral toxicity was non poisonous. Even at the doses used, A. aspera extract did not cause any mortalities. Carrageenan subplantar injection in rats resulted in a time-dependent increase in paw thickness. At 0, 3, 5, and 24 hr after the injection of carrageenan, the thickness was measured. In the groups who received a vehicle injection, this rise started to be noticeable at 1 hours and peaked at 3 hours. However, treatment with 200 mg/kg and 400 mg/kg of extract and a prescribed dose of Diclofenac (10mg/kg) decreased the inflammation caused by carrageenan considerably (p≤0.05) after 24 hour in all phases of the trial.

Keywords:
anti-inflammatory activity, Achyranthes aspera, Diclofenac, ethanolic extract, Carrageenan model
INTRODUCTION:

Inflammation:
A living, vascularized tissue local reaction to external and endogenous stimuli is inflammation. The words come from the Latin word "inflammare," which means to burn up. Fundamentally, redness serves two purposes: to contain tissue damage with to localise and eradicate the cause. As a result, inflammation is a natural reactions to damage. Inflammation must not be viewed as an infection in and of itself, but slightly as a beneficial reaction to either aggression or certain diseases.\(^1\)

Classification:
Inflammation are classify depend on duration of abrasion and appearance into acute and chronic inflammation. As per the classification: acute and chronic.
A. Acute inflammation: Acute inflammation can persist for minutes, hours, or even a few days, is an acute and early reaction to an inflammatory substance.\(^2\)
B. Chronic inflammation: It is distinguished by fluid and plasma protein exudation, as well as the migration of mostly neutrophilic, leucocytes to the place of damage.\(^3\)

Inflammation symptoms:
1. Redness:
Redness is develops in cellulitis and is caused by tiny blood vessels in injured tissue dilating.
2. Increase in blood flow
The increase in blood flow (hyperemia) brought on by localized vascular dilatation, which causes heat (calor).\(^4\)
3. Swelling:
Swelling is results from fluid buildup in the extra vascular space and is, in turn, brought on by an increase in vascular permeability.\(^5\)
4. Pain:
Pain is caused in part by pus being pressed into an abscess cavity and in part by tissues being stretched and damaged as a result of inflammatory edoema. Bradykinins, prostaglandins, and serotonin are a few molecules associated with acute inflammation that be also known to cause pain.\(^6\)
5. Loss of function:
Pain inhibits to the inflamed region, and significant swelling may also render the tissue physically immobile.\(^7\)
Chemical mediators of inflammation:
The manifestations of inflammation are caused by chemical mediators. Following is the order of inflammation: cell damage chemical agent’s acute inflammation, which includes cellular and vascular processes. Mediators' sources: It is possible for cells or plasma to produce the chemical mediators of inflammation[8-9]

<table>
<thead>
<tr>
<th>Cellular mediator</th>
<th>Cells of origin</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>Mast cells, basophiles</td>
<td>Vascular leakage &amp; platelets</td>
</tr>
<tr>
<td>Serotonin</td>
<td>Platelets</td>
<td>Vascular leakage</td>
</tr>
<tr>
<td>Lysosomal ezmyme</td>
<td>Neutrophiles</td>
<td>Tissue destruction</td>
</tr>
<tr>
<td>Prostaglandin</td>
<td>Leukocytes</td>
<td>ache, fever</td>
</tr>
<tr>
<td>Leucotriene</td>
<td>Leukocytes</td>
<td>LB4</td>
</tr>
<tr>
<td>Chemo-attractant</td>
<td>Lc4, Lc4d4, &amp; Le4</td>
<td>Broncho and vasoconstriction</td>
</tr>
<tr>
<td>Platelet activity factor</td>
<td>All leukocytes</td>
<td>Vasoconstriction</td>
</tr>
<tr>
<td>Nitric oxides</td>
<td>Macrophages</td>
<td>Leucocytes activation</td>
</tr>
<tr>
<td>Cytokines</td>
<td>Lymphocytes</td>
<td>Leucocytes activation</td>
</tr>
</tbody>
</table>

MATERIALS AND METHOD:
Selection and Collection of plant materials:
The plant was select for the experiment on the base of its usual, seelection and collect from R. K. Pharmacy College, Kashipur, Surai, Sاثion, Azamgarh, Uttar Pradesh.

Authentication of plant:
The herbarium file of plant profile was submitted in BHU Varanasi for authentication.

Preparation of extract:
Leaf of plant parts that had been powdered (500g) were defatted through water before being fully extractedthrough ethanol by using a soxhlet equipment at (40-50) °C for 72 hours. On Whatman No. 1 filter paper, the extract was filtered. To get a powdered material, the solvent was entirely evaporated under decreased pressure in a rotator vacuum evaporator. Vacuum desiccators were used to preserve the concentrated extract for later use.

Methods for phytochemical screening tests:
The occurrence of alkaloid, glycoside, flavonoid, tannin, anthraquinone, saponin, volatile oil, cynogenic glycoside, coumarins, sterols, and/or triterpenoids was determined using introduction phytochemical screening of the ethanolic extracts.

Test of alkaloids:
The Mayer test on a steam bath, 0.5 gm of extracts was mix with 5.0 ml of 1% HCL before the mixture was filtered. A little drop of Mayer's reagent was added to 1 ml of filtrated. Alkaloid is present when a precipitate is white or creamy white.
The Wagner test on a steam bath, 0.5 gm of extracts was mix with 5.0 ml of 1% HCL before being filtered. A few drops of Wagner's reagent were added to 1 ml of filtrate. Precipitate that is dark brown or black suggests the presence of alkaloids.
The Hager test On a steam bath, 0.5 gm of extracts was mix with 5.0 ml of 1% HCL before being filtered. A a small number of drop of Hager's reagent was added to 1 ml of filtered water. The occurrence of alkaloids is indicated by yellow crystalline precipitate.

Glycosides test:
A tiny quantity of an alcoholic extracts of the clean or dried materials were dissolved in 1.0ml of waters for the general test of glycosides. Aqueous NaOH solution was added in a few drops. Glycosides are present because of the colour yellow.

Cardiac glycosides test:
The legal test The sodium nitroprusside solution (0.5%) was used to dissolve 0.1gm of an alcoholic plant extract. NaOH (0.2N) solution was added to the mixture to make it alkaline. Due to the lactone ring,
pink to red colour suggests the presence of cardiac glycosides.

**Test of Baljet** A small amount of an alcoholic plant material extract was mixed with a drops of Baljet's reagents. The five-member lactone ring on C-17 of the aglycone in cardiac glycosides caused the colour to be yellow-orange.

**Test of flavonoids:**

**General test** a modest amount of the plant material's alcoholic extract was consumed. Conc. HCL was added in small amounts. The presence of flavonoids is indicated by the rapid development of red colour.

**Specific test** a test tube was filled with around 0.5 ml of the sample's alcoholic extract. There was also a little ribbon of magnesium or zinc added. Then 5.0–10.0 drop of HCL solution was added. Several minutes were spent boiling the solution. Flavones, flavanols, and flavanones are indicated by the progression of orange to red, red to crimson, and crimson to magenta, respectively.

**Test of terpenoids:**

**The Test Salkowski** In a test tube, 0.1 gramme of plant extracts were used. Following 1.0 ml of chloroform, add 1.0 ml of conc. Sulfuric acid (H2SO4) was poured into the test tube from the side. If terpnoids are present, a red colour is formed in the chloroform layer.

**Test for tannins:**

Check for tannins FeCl₃ test A sample of dry powder weighing about 0.5 gm was cooked in 20.0 ml of water in a test tubes before being filtered. A a small amount of drop of 0.10% ferric chloride were added, and colour of the mixture was checked for brownish green or a blue-black color. The Phlobatannins testsin a test tube; 0.1gm of plant extract was added. It was subsequently heated in 1% aqueous HCL. Red precipitate deposition was considered proof of the presence of tannins.

**Test of steroid:**

**The Test Libermann-Burchard** in a test tube, a tiny amount (0.1gm) of plant extract was added and dissolved in 1ml of chloroform. 1 ml of strong sulfuric acids (H2SO4) was added after 2.0 ml of AA. The formation of a greenish hue showed the presence of steroids.

**Saponin test:**

0.5 gramme of an alcoholic extracts were located in a test tube, and 5.0 ml of distilled water was added before shaking. The presence of saponin was determined by the stable foam formation.[105]

**Experimental animals:**

Wistar albino rats of both sex (wt. 120–180g) was used in the study. The animals were kept in a usual cycle of light and darkness. They were given the typical pellet meal and infinite contact to water. The week before the trial, the animals were used to their surroundings. After receiving permission from the Institutional Animal Ethical Committee (IAEC), the tests were conducted. The experiment was performed following animals ethics guidelines of “institutional animal’s ethics committee” (approval no-1384/PO/Re/S/10/CPCSEA)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Group</th>
<th>No of animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Group I ( Normal saline )</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>Group II ( Negative control) Induced</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>Group III (Test I) 200mg/kg</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>Group IV ( Test II ) 400mg/kg</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>Group V (Positive control) Standard</td>
<td>6</td>
</tr>
</tbody>
</table>

**Table 6.1 Grouping of experiment animals**

**Inflammatory activity in vivo:**

**Acute inflammatory studies:**

The ability of *A. aspera* extracts to reduce inflammation was assessed using the carrageenan-induced paw edema technique. Wistar albino rat of both sex were placed into five groups for the research (n=6). 1 hr before to the sub plantar injection of 0.10
ml of 1.0% w/v solution of the carrageenan in usual saline in the right hand paw of the rat. Leaf extracts of *A. aspera* (200mg/kg and 400mg/kg) and Diclofenac (10.0 mg/kg) were given orally. At 0, 3, 5, and 24 hours following the injection, the plethysmographs were used to measure the paw's volume. Percent inhibition was computed using the mean paw volume. The rats were divided into five groups as follows:

- **Group I: Control – Normal saline**
- **Group II: Negative Control - Carrageenan (0.1ml of 1.0% w/v)**
- **Group III: Test I – Carrageenan + Alcoholic leaf extract (200mg/kg)**
- **Group IV: Test II – Carrageenan + Alcoholic leaf extract (400mg/kg)**
- **Group V: Positive control (Standard) – Carrageenan + Diclofenac (10.0mg/kg)**

**RESULTS AND DISCUSSION:**

**Preliminary phytochemical investigation:**
1. A receipt specimen no. Amaranth.2023/01 was deposited in the herbarium of BHU Varanasi for prospect indication. The plant was authenticated by professor N K Dubey, department of botany, BHU.
2. The recovered fractions were finally evaporated to give yields of 12.33% and ethanol. According to the preliminary qualitative analysis of *A. aspera*, alkaloids, flavonoids, glycosides, saponin, sterols, tannins, and terpenes are present. *A. aspera*’s ethanolic extract was discovered to have an 11.5% weight-to-weight phenolic content, indicating the presence of a variety of phenolic compounds including polyphenols, flavonoids, and phenolic acid. The quantity of flavonones in the ethanol extract of *A. aspera* was found to be 6.66% w/w from the calibration curve of naringenin and the concentration of flavonols in the extract of *A. aspera* was found to be 4.88% w/w from the calibration curve of quercetin. The total flavonoid content, calculated as the average of the results from these two techniques, was discovered to be 8.34% w/w.

**Table: The preliminary phytochemical analysis findings are shown in below table.**

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Name of test</th>
<th>Observation</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Mayer’s test</td>
<td>Creamy white PPT</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Wagner’s test</td>
<td>Deep down PPT</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Hager’s test</td>
<td>Yellow crystalline PPT</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>General test</td>
<td>Yellow test</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>Legal’s test</td>
<td>Pink to red colour</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Baljet’test</td>
<td>Yellow to orange</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Salkoski’s test</td>
<td>A redish brown colour</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Phlobatannins test</td>
<td>Red PPT</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>General test</td>
<td>Rose pink in the aqueous layer</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Specific test</td>
<td>Orange to red</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>Liberman-burchard’s test</td>
<td>No PPT</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>Feel3 test</td>
<td>Brownish green colour</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>Frothing test</td>
<td>Formation of stable foam</td>
<td>+</td>
</tr>
</tbody>
</table>

**Effect of ethnolic extract of achyranthes aspera on Carrageenan-induced paw edema:**
It was discovered that none of the dosages of the extract of *Achyranthes aspera* used for acute oral toxicity was non poisonous. Even at the doses used, *A. aspera* extract did not cause any mortalities. Carrageenan subplantar injection in rats resulted in a time-dependent increase in paw thickness. At 0, 3, 5, and 24 hr after the injection of carrageenan, the thickness was measured. In the groups who received a vehicle injection, this rise started to be noticeable at 3 hours and peaked at 5 hours. However,
treatment with 200 mg/kg and 400 mg/kg of extract and a prescribed dose of Diclofenac (10mg/kg) decreased the inflammation caused by carrageenan considerably (p≤0.05) after 24 hour in all phases of the trial.

Table: Anti inflammatory activity of A. aspera extracts on carrageenan induced rat paw edema.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Rat paw volume measured at different time intervals (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hr</td>
</tr>
<tr>
<td>Group I (Normal group)</td>
<td>0.74 ± 0.03*</td>
</tr>
<tr>
<td>Group II (Negative control)</td>
<td>0.75 ± 0.04</td>
</tr>
<tr>
<td>Group III (Test I)</td>
<td>0.74 ± 0.06*</td>
</tr>
<tr>
<td>Group IV (Test II)</td>
<td>0.73 ± 0.05*</td>
</tr>
<tr>
<td>Group V (Positive control)</td>
<td>0.74 ± 0.07*</td>
</tr>
</tbody>
</table>
One of the most accurate test methods for excluding anti-inflammatory substances is the inhibition of carrageenan-induced inflammation in rats. Histamine, 5-HT, and kinin release are responsible for the first phase of carrageenan-induced edema, while prostaglandin release is responsible for the second phase. It was discovered that the triterpenoid saponin, was present in the alcoholic extracts of the leaves. Other triterpenoid, saponins have been shown to be beneficial in treating inflammatory disorders. Alkaloids and saponins are present in the extracts of *A. aspera* according to phytochemical screens. In summary, the studies have shown that the alcoholic extract of *A. aspera's* leaf and may have anti-inflammatory properties.

**CONCLUSION:**
The findings of the present investigation showed that *achyranthes aspera* L. leaf extract had strong anti-inflammatory effect. Its therapeutic properties are greatly enhanced by the presence of numerous kinds of phyto compounds, such as phenols, flavonoids, saponins, alkaloids, etc., which may be one of the causes for its use in treating a variety of disease. The goal of the current study was to scientifically back up the claims made by various tribes regarding the healing powers of *A. aspera* leaves on skin conditions as well as inflammation. It also offers a starting point for additional research into the herb's potential as a medication condition.

**ACKNOWLEDGEMENT:** The author would like to thanks Dr. Abhay Pratap Yadav for permitting permission to use the institute facilities to carry out my research work. Our sincerely thanks to my guide Miss. Sivanki Verma for her guidance and care throughout the work.

**REFERENCE**
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