TO STUDY THE ANTI-INFLAMMATORY ACTIVITY OF CRUDE DRUG FORMULATION AGAINST HAEMORRHOIDES ON RATS

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Abstract:
Inflammation is a tissue–reaction to infection, injury, irritation or foreign substance. It is a part of the host defense mechanism but when it becomes uncontrolled it is a hopeless condition. There are several tissue factors or mechanisms that are known to be involved in the inflammatory reactions such as histamine, bradykinin and prostaglandins. The development of non-steroidal anti-inflammatory agents in recent years has contributed a lot in not only overcoming the human sufferings such as arthritis but also has helped in understanding the tissue mechanisms of inflammation. The inflammatory reaction is readily produced in rats in the form of paw oedema with the help of irritants substances such as carraggeenan, formalin etc. The animal model for anti-inflammatory activity used here is of formalin induced acute inflammation. Bark of Berberis aristata, Leaves and bark of Cassia fistula, Leaves of Cynodon dactylon, Fruits of Emblica officinalis, Leaves of Tamarindus indica, Fruits of Terminalia chebula and Terminalia belerica, Inflorescence of Sphaeranthus indicus, Bark and Leaves of Syzigium cumini, Bark of Holarrhena antidysentrica and Fruits of Mesua ferrea were the plant materials used in the preparation of crude drug formulation against haemorrhoides. Inflammation is one of the major contributors to haemorrhoides hence the effect of anti-inflammatory activity of the crude drug formulation was an essential aspect in the study. The conclusion of our study with this animal model was thus that the Dose 3 (200 mg/kg) of the Crude anti-haemorrhoidal formulation was found to be the most effective dose for the anti-inflammatory activity.

Keywords: Anti-inflammatory, formalin, acute inflammation, haemorrhoides, etc.

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Introduction:

Inflammation is a local response of living mammalian tissues to injury due to any agent. It is a body defense reaction in order to eliminate or limit the spread of injurious agents as well as to remove the consequent necrosed cells and tissue and it is manifestation of the body’s response to tissue damage and infection. Thus it is a protective and defensive mechanism of body. The result of each inflammatory reaction may be beneficial (defense the body against agents deranging its homeostasis) or harmful causing damage to the surrounding tissues (Reynold, et. al., 1993). The Non-Steroidal Anti-inflammatory Drugs (NSAIDs) used in the inflammatory conditions do not cure and remove the underlying cause of the disease but they only modify the inflammatory response to the disease. Though there are standard drugs like Aspirin, Indomethacin, Phenylbutazone, etc., these drugs are not entirely free of side effects and have their own limitation (Reynold, et. al., 1993). Thus there is still a need to develop newer and safer anti-inflammatory drugs. Herbal medicines used in Ayurveda remain the major source of health care for the world’s population. World Health Organization (WHO) has recognized herbal medicine as an essential building block for primary health care of vast countries like India (Suralkar, et. al., 2012).

Inflammation is protective in some situations. If untreated it can lead to serious complications (Suralkar, et. al., 2008). In the 1st century A.D. the Roman writer Celsus named the four cardinal signs of inflammation as: Rubor –Redness; Tumor –Swelling or edema; Color –Heat; Dolor –Pain; 5th sign functio laesa –loss of function was later added by Virchow (Harsh, M., 2002). The main symptoms of the body are increased body temperature and pain. Some causes of an inflammatory reaction are infection invasion and multiplication with in tissue by various bacteria, fungi, viruses and protozoa, which in many instances, cause damage by release of toxins that directly destroy host cells. Trauma penetrating injury, blunt trauma, thermal injury, chemical injury and as a result of the loss of blood supply, mechanical causes, autoimmune diseases (Suralkar, et. al., 2008). Inflammation can be classified as acute inflammation- is of short duration caused by increase in vascular permeability resulting in exudation of fluids from the blood into the interstitial space (Suralkar, et. al., 2008).

Chronic inflammation- is of longer duration and occurs either after the causative agent of acute inflammation persists for long time, or the stimulus is such that it induces chronic inflammation from the beginning. In this stage granuloma formation and tissue repair will occur. In some instances, the term sub acute inflammation is used for the state of inflammation between acute and chronic; this stage involves the infiltration of leukocytes from the blood into tissues. If inflammation is not controlled and the body continually fights this battle, symptoms of chronic inflammation can show itself as arthritis, colitis, chronic fatigue, sinusitis, cataracts, chronic pain, hair loss, heart disease, stroke, Alzheimer’s and other ailments and conditions. Inflammation is a complex process and ROS play an important role in the pathogenesis of inflammatory diseases (Conner, et. al., 1996). Thus antioxidants which can scavenge ROS are expected to improve these disorders.

Principle:

The inflammatory reaction is readily produced in rats in the form of paw oedema with the help of irritants substances such as carraggeenan, formalin, bradykinin, histamine, 5-hydroxytryptamine, mustard or egg white when injected in the dorsum of the foot of rats, they produce acute paw oedema within a few minutes of the injection. The animal...
model used for anti-inflammatory activity is formalin induced acute inflammation.

**Materials and Methods:**

**Plethysmometer:**

It is a simple apparatus containing mercury. The mercury displacement due to dipping of the paw can be directly read from scale attached to the mercury column or adjusting the mercury level in the arm B to the original level by moving arm B up/down and noting the volume required to bring the level in both the arms equal.

A microcontrolled water Plethysmometer (UGO Basile) can be used to accurately measure rat/mouse paw swelling (oedema). It consists of Persplex cell filled with 0.05% w/v sodium chloride solution. To reduce surface tension (adhesion of water to the skin of the paw) a surfactant provided by the manufacturer is added to the water reservoir. The cell is available in different diameters into which the paw is dipped. A transducer records small differences in water level caused by volume displacement the digital read-out shows the exact volume of the paw.

One ml syringe with 26 no. fine needle was used for injecting formalin in the plantar region.

**Animals**

Albino rats weighing between 200 – 250 g of either sex were used for the study. They were housed in the Animal House of the college (Sharad Powar College of Pharmacy) under the standard environmental conditions of temperature (25 ± 2°C), Humidity (55 ± 10%) and Light (12 : 12 hrs. Light/Dark cycle; lights on at 07 : 00 hrs). Rats were supplied with standard pellet diet (Goldmuhar Brand Rat Feed supplied by Lipton, India Ltd.) and tap water ad libitum. The animals were handled and acclimatized to laboratory conditions 24 hours before conducting the experiments. All the experiments were conducted between 09:00 and 18:00 hours. The parental administrations were given by disposable syringe and strict aseptic conditions were followed during the administration. The institutional animal ethics committee has approved the experimental protocols and was performed in accordance with the guidelines for the care and use of laboratory animals as adopted and promulgated by institutional animal ethical committee. (SPCP/2013/653-1 by CPCSEA).

**Preparation/Plants used for the Formulation:**

1 g crude drug contains *Berberis aristata* (90 mg), *Cassia fistula* (100 mg), *Cynodon dactylon* (80 mg), *Embellica officinalis* (120 mg), *Tamarindus indica* (80 mg), *Terminalia chebula* (100 mg), *Terminalia belerica* (100 mg), *Sphaeranthus indicus* (110 mg), *Syzygium cumini* (100 mg), *Holarrhena antidysentrica* (50 mg) and *Mesua ferrea* (70 mg). The above formulation may be very useful in Haemorrhoids/Piles. It was formulated on the basis of available Literature, local healers, doctors and most effective herbal formulations. It can make the patient rid of different problems associated with piles like Itching, Inflammation and Bleeding, Constipation and Acidity may also be relieved as the formulation possess the laxative properties.

**Requirements :**

Plethysmometer, as mentioned above, 1 ml syringe with 26 number fine needle, Insulin syringe, albino rats, Crude drug formulation Doses 1, 2 and 3, Indomethacin, 2 % Formalin Solution etc.

**Procedure :**

Before proceeding towards the animal experiments, it was necessary to undertake the safety and efficiency of the formulation. Thus, the acute toxicity studies were conducted.

**Acute toxicity studies :**

Acute oral toxicity studies were carried out according to OECD guidelines 423. The animals of both sexes were
selected by random sampling technique and divided into 5 groups of 3 animals each. A single oral dose (200, 400mg, 600mg, 800mg and 1000mg/kg) of each extract was administered orally at the dose level up to 1000mg/kg body weight. The animal groups were observed for appearance of toxic symptoms including behavioral changes, locomotion, muscle spasm, loss of righting reflex, tremor, convulsions and mortality for 24 hrs and further supervised for a period of 14 days for occurrence of toxic symptoms and mortality. However, from the first day till the 14th one, there were no such adverse symptoms as mentioned above. There was no change in their behavior or their living skills also, infact they remained unaffected completely. However, a bit of sluggishness was observed at higher doses.

Preparation of Formalin-Induced Rats for Screening of Anti-Inflammatory Activity of Crude Drug Formulation:
Albino rats of either sex weighing between 200 – 250 g were used in a group of 6 animals. Rats were numbered and a mark on lower right paw of each rat was made just beyond tibio – tarsal junction, so that every time the paw was dipped in the column up to the fixed mark to ensure constant paw volume. Initial paw volume was noted by displacement method. Care was taken to ensure that the rats are not diseased. They were kept on overnight fasting a day before the experiments were carried out. The test preparations (Crude drug formulation) were administered orally at a dose of 50, 100 and 200 mg/kg body weight. Simple water was given to the control group. After an hour, 0.1 ml of 2% Formalin was injected into the plantar tissue of the right hind paw with aseptic syringe and immediately paw volume was measured by dipping the paw up to the mark in the cell. The paw volume was again recorded after 15, 30, 60 and 120 minutes respectively. The same experiment was done using Indomethacin at a dose 10 mg/kg body weight through the intra-peritoneal route.

The crude drug formulation was tested for its anti-inflammatory activity and its effect was observed for its efficacy against haemorrhoids. Before observing the effect of crude drug formulation on human beings, it was necessary to test its safety and efficacy on animals. Hence, these animal trials were undertaken to get the results confirmed.

The determination of Carraggeenan induced at raw paw oedema, anti– inflammatory model first proposed by Winter, et. al., (1962) is the most frequently carried out test for screening of anti-inflammatory activity in biochemical laboratory among the in-vivo methods used. However, Carraggeenan can also be replaced with Formalin and we have performed the experiment using Formalin instead of Carraggeenan. The oedema which develops in raw paw after formalin injection is a biphasic event. The initial phase is attributed to the release of histamine and serotonin and the second phase to the prostaglandin-like compound.

Experimental Design :

Treatment Groups
The animals were divided into different groups and each group consists of six animals. The suspension of all dosages and Indomethacin as a standard were prepared using water.

Group 1 (Control Group) : Rats were treated with normal saline solution.

Group 2 (Standard Group) : Rats were treated with suspension of Indomethacin orally (10 mg/kg/day) through the intra-peritoneal route.

Group 3 (Dose 1 Group) : Rats were treated with suspension of Dose 1 orally (50 mg/kg/day) of the anti-haemorrhoidal crude drug formulation.

Group 4 (Dose 2 Group) : Rats were treated with suspension of Dose 2 orally (100 mg/kg/day) of the formulation.
**Group 5 (Dose 3 Group)**: Rats were treated with suspension of Dose 3 orally (200 mg/kg/day) of the formulation.

The activity of the drug is expressed as % inhibition of Oedema.

\[
\% \text{ Inhibition} = (1 - \frac{V_t}{V_c}) \times 100
\]

Where, \(V_t\) : Paw Volume of Test Sample.

\(V_c\) : Paw Volume of Control Sample.

**Observations**:

Table 1: Anti-inflammatory effect of Crude Drug Formulation and Indomethacin on Formalin induced rat paw oedema.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Paw Volume (ml) as measured by Hg Displacement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 Min.</td>
</tr>
<tr>
<td>1.</td>
<td>Control</td>
<td>-</td>
<td>0.68±0.02</td>
</tr>
<tr>
<td>2.</td>
<td>Standard</td>
<td>10</td>
<td>0.95±0.04</td>
</tr>
<tr>
<td>3.</td>
<td>Dose 1</td>
<td>50</td>
<td>0.78±0.05</td>
</tr>
<tr>
<td>4.</td>
<td>Dose 2</td>
<td>100</td>
<td>0.86±0.02</td>
</tr>
<tr>
<td>5.</td>
<td>Dose 3</td>
<td>200</td>
<td>0.92±0.02</td>
</tr>
</tbody>
</table>

Values are expressed as Mean Values ± Standard Error at N = 6

Table 2: Evaluation of Percent Inhibition of Oedema for Anti-inflammatory Activity in Albino Rats

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Percent Inhibition of Oedema</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 Min.</td>
</tr>
<tr>
<td>1.</td>
<td>Control</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Standard</td>
<td>10</td>
<td>39.70</td>
</tr>
<tr>
<td>3.</td>
<td>Dose 1</td>
<td>50</td>
<td>11.96</td>
</tr>
<tr>
<td>4.</td>
<td>Dose 2</td>
<td>100</td>
<td>26.47</td>
</tr>
<tr>
<td>5.</td>
<td>Dose 3</td>
<td>200</td>
<td>35.29</td>
</tr>
</tbody>
</table>

Values are expressed as Mean Values ± Standard Error at N = 6, (p < 0.5).
Results and Discussion:
The effect of the Anti-Haemorrhoidal Crude Drug Formulation on formalin induced rat paw oedema is shown in Table 1. The control animals progressively exhibited increasing paw volume in response to formalin injection during the study. In case of treated animals also, the paw volume increased due to oedema created by the formalin but then decreases later due to anti-inflammatory activity of the Standard (Indomethacin) or different Doses of crude anti-haemorrhoidal formulation. The reduction of the paw volume was faster from 0 min. to 120 min. in case of Standard (Indomethacin) followed by the Dose 3 of formulation. This trend was followed at each time interval i.e. Percent inhibition of oedema was higher in case of Standard (Indomethacin) at 15 min., 30 min., 60 min. and 120 min. followed by Dose 3 of the formulation. The maximum percent inhibition of oedema was observed in the Standard (Indomethacin) whereas nearly same activity was evident in Dose 3 (200mg/kg). The oral administration of Dose 1 (50 mg/kg) and Dose 2 (100 mg/kg) did not produce any significant effect but Dose 3 of 200 mg/kg of the Crude Drug Formulation produced a significant (p< 0.05) inhibition of the rat paw oedema. The maximum paw oedema percentage inhibition of 59.18 % and 51.02 % was found in Indomethacin and Dose 3 of the Anti-haemorrhoidal crude drug formulation respectively. However, in case of Dose 1 (50 mg/kg) and Dose 2 (100 mg/kg) the percent inhibition was observed to be 24.48% and 38.77% respectively. Hence, Dose 3 of the Anti-haemorrhoidal formulation was the most effective one.

Conclusion:
As the percent inhibition of oedema by Dose 3 (200mg/kg) was found nearer to that of Standard (Indomethacin), it could be concluded that it is more effective for its anti-inflammatory activity as compared to other two doses.

Formation of Oedema after Formalin Induction:
Digital Plethysmometer

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References: