Development and evaluation of polyherbal formulation for anti-inflammatory activity

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ABSTRACT

Inflammation is an important feature of great number of diseases. It is a response of the tissue to an injury, infection, irritation or foreign substance. It is a part of host defence, but when the response becomes too great it may be far worse than the disease itself and in extreme condition, it may be fatal. There is an increasing demand for the medicinal plants in developing countries like India. Attention need to be given to assess the medicinal value of such plants to explore the potential drugs out of it. Medicinal plants constitute important components of flora and are widely distributed in different region of India. The aim of present study was to assess the development of Polyherbal formulation i.e. F1 & F2 from ethanolic leaves extract of the plants Azadirachta indica A. Juss and Momardica charantia Linn and evaluation of anti-inflammatory activity of prepared Polyherbal formulation. The formulation F1 and F2 showed the significant anti-inflammatory activity comparable to the standard drug Indomethacin against carrageenan induced rat paw edema method. The formulation F1 is significantly reduced the inflammation induced by carrageenan as compared to F2 using standard drug Indomethacin. The F1 reduced the inflammation by 48.19% compared to the control treated group.

Keyword- Anti-inflammatory, Azadirachta indica, Momardica charantia, carrageenan, Indomethacin.

INTRODUCTION

Inflammation is the condition associated with many of the disease states and this review elaborate the medicinal plants, their parts used in the effective management of Inflammation and its associated conditions. Inflammation is an important feature of great number of diseases. It is a response of the tissue to an injury, infection, irritation or foreign substance. It is a part of host defence, but when the response becomes too great it may be far worse than the disease itself and in extreme conditions, it may be fatal. Anti-inflammatory drugs are considered important because of their wide therapeutic potential and their utility in a number of diseases such as arthritis, lupus erythematosus, pemphigus and rheumatic fever and in a number of other disorders associated with pain, pyrexia and inflammation. Now-a-days, the synthetic drugs are although dominating the market but the element of toxicity that these drugs entail, cannot be ruled out.
Many anti-inflammatory drugs (both NSAIDs and corticosteroids) have been developed but their safety profile studies have shown that none of them is clearly safe. They show wide ranges of adverse effects. Due to adverse reactions of synthetic and chemical medicines being observed round the globe, herbal medicines have made a comeback to improve our basic health needs. Many plants and herbs such as ginger, turmeric, olive oil, have been shown to exhibit potent anti-inflammatory effect.2

While searching for anti-inflammatory drug in natural product, highly encouraging result was obtained in our laboratory with Polyherbal formulation of Momordica charantia and Azadirachta indica A. Juss (neem). Neem has been found to possess several types of chemicals that could be exploited for the pest management. Neem seeds mostly contain the complex tetranorterpenoid lactones azadirachtin, Nimbin, nimbidin, salanin and nimbinol B out of which azadirachtin is the most active component. The leaves also contains azadirachtin, meliantrol, salanin, β-sitosterol, stimasterol and flavonoides. The fresh stem bark yielded the bitter principles, nimbin, 0.04%; nimbinin, 0.002%; and nimbidin, 0.4%. Another terpenic constituent, identical with Sugiol is reported to be present in the stem bark3.

Leaves of Momordica charantia Linn. Family Cucurbitaceae are effective in bilious affections as Emetic and purgative. Leaves are administered internally in leprosy, piles, jaundice. It is active as galactoguge; it is also applied round the eye orbit for night blindness. Leaf juice is rubbed to soles in burning of the feet, and used in liver complaint of children’s. In Cambodia and in Gold coast, leaves are also considered to be antipyretic4.

MATERIALS AND METHODS

Plant Material
The Momordica charantia Linn. And Azadirachta indica A. Juss Leaves for the proposed study were collected from area of Wanadongari, Dist.Nagpur and were authenticated by department of botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur. The freshly collected leaves of Momordica charantia Linn. And Azadirachta indica A. Juss were shade dried and then powdered to coarse size. About 1 kg of leaves powder of Momordica charantia Linn. And Azadirachta indica A. Juss was subjected to extraction with (ethanol 95%). After extraction, the solvent was distilled off and the extracts were concentrated on water bath. Then prepare the formulation and formulations were evaluated for hepatoprotective activity.

Preparation of Polyherbal formulation
The quantity of ethanolic extracts of leaves required for formulating herbal drug formulation (Table 1) are calculated on the basis of human dose of powder form and percentage practical yield of respective crude drugs. Three formulations are prepared using 2% w/v gum tragacanth as suspending agent and considered as Lower dose and higher dose formulation2,5.

Test Animal
The experimental protocol was submitted and approved by Institutional Ethical Committee, Wister albino rats (150-200 g) of approximate same age were employed in this investigation. The animals were fed with standard pellet diet and water and ad libitum. They were housed under standard conditions of temperature 22°C (± 3°C) humidity 35 % to 60 %, and light (12:12 hr light/dark cycle) in polypropylene mice cage. The animals received the drug treatments by oral gavages tube.
Chemicals
Carrageenan was obtained as a gift sample from German Remedies Ltd., Mumbai for research, and Indomethacin from Merck, India. The other chemicals and reagents used were of analytical grade.

Acute toxicity studies
Acute toxicity studies were carried out on Wister albino rats according to method proposed by Ghosh. The prepared formulation were subjected to toxicity study and were found to be safe up to daily dose of 4000 mg/kg of body wt. in rats of either sex with no toxic reaction being observed.

Anti-inflammatory Activity
Carrageenan Induced Rat Paw Edema Method
Healthy inbred Wister albino rats of either sex, (150-200 g) were selected and housed in polypropylene cages at a well-ventilated, temperature-controlled (30±1°C) animal room with food and water ad libitum. Animals were periodically weighed before and after experiments. Animals were divided in five groups of 6 animals each. The control group receives vehicle orally, while other groups receives standard drug and test drug respectively. The animals were treated with drugs by oral route and subsequently one hour after treatment, 0.1ml of 1% suspension of carrageenan in normal saline was injected to the sub planter region of left hind paw to induce edema. The paw volume was measured initially at 1, 2, 3 and 5 hours after carrageenan injection using plathismometer. The difference between the initial and subsequent reading gave the actual edema volume which was compared with control. The difference of average values between treated animals and control group is calculated for each time interval and evaluated statistically. The percent inhibition is calculated using the formula as follows- % inhibition of Edema = \( \frac{[C-T]}{C} \times 100 \), Where, C-Control Paw Edema, T-Test Paw Edema group’s respectively.

Data analysis
Data obtained from this study were expressed as mean ± SEM. Statistical analysis was performed using Student’s t test. P-values less than 0.05 were considered statistically significant.
RESULTS AND DISCUSSION

Table 1: Quantity of plant extracts used for preparing herbal formulations F1 and F2

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Extract Name</th>
<th>Quantity of Extract mg/kg (F1)</th>
<th>Quantity of Extract mg/kg (F2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Momordica charantia Linn</td>
<td>300</td>
<td>1000</td>
</tr>
<tr>
<td>2</td>
<td>Azadirachta indica A. Juss</td>
<td>400</td>
<td>700</td>
</tr>
</tbody>
</table>

Table No. 2: The Effect of Formulations on Carrageenan induced paw edema in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 Hr</th>
<th>1 Hr</th>
<th>2 Hr</th>
<th>3 Hr</th>
<th>5 Hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.16 ± 0.045</td>
<td>4.34 ± 0.05</td>
<td>4.69 ± 0.14</td>
<td>4.52 ± 0.11</td>
<td>4.42 ± 0.10</td>
</tr>
<tr>
<td>Standard (10 mg/kg) (Indomethacin)</td>
<td>4.01 ± 1.10</td>
<td>3.22 ± 1.01</td>
<td>2.76 ± 0.12</td>
<td>2.55 ± 0.10</td>
<td>1.99 ± 0.12</td>
</tr>
<tr>
<td>F1</td>
<td>4.66 ± 0.28</td>
<td>3.62 ± 0.17</td>
<td>3.31 ± 0.10</td>
<td>2.96 ± 0.15</td>
<td>2.29 ± 1.14</td>
</tr>
<tr>
<td>F2</td>
<td>4.55 ± 0.06</td>
<td>3.73 ± 0.07</td>
<td>3.34 ± 0.10</td>
<td>3.11 ± 1.12</td>
<td>2.41 ± 1.07</td>
</tr>
</tbody>
</table>

Table No. 3: The Effect of Formulations in percent paw edema volume of inhibition (% inhibition in Hrs)

<table>
<thead>
<tr>
<th>Groups</th>
<th>% Inhibition in Hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 Hr</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Standard (10 mg/kg) (Indomethacin)</td>
<td>25.80</td>
</tr>
<tr>
<td>F1</td>
<td>16.58</td>
</tr>
<tr>
<td>F2</td>
<td>13.76</td>
</tr>
</tbody>
</table>

DISCUSSION

The present study has been undertaken to demonstrate the anti-inflammatory effect of Polyherbal formulation of neem leaf extract and M. charantia Linn to compare the anti-inflammatory effect of formulation with that of Indomethacin. Indomethacin is very potent well known anti-inflammatory. Another agent is widely used well known herbal agent neem. In this study the leaves of Momordica charantia Linn and Azadirachta indica A. Juss were collected and authenticated raw plant material used in the activity. The authenticated leaves of the Momordica charantia Linn. and Azadirachta indica A. Juss. The freshly collected leaves of Momordica
charantia Linn. and Azadirachta indica A. Juss were shade dried and then powdered to coarse size. About 1 kg of leaves powder of Momordica charantia Linn. And Azadirachta indica A. Juss was subjected to extraction with (ethanol 95%). After extraction, the solvent was distilled off and the extracts were concentrated on water bath and we get the desired yield of the drug. Then prepare the formulation and formulation at the dose of Lower dose (F1) and higher dose (F2) and they were evaluated for anti-inflammatory activity. The prepared formulation were subjected to toxicity study and were found to be safe up to daily dose of 4000 mg/kg of body wt. in rats of either sex with no toxic reaction being observed.

Polyherbal formulation of ethanolic leaves extracts of A. indica A Juss and Momordica charantia Linn possesses potent anti-inflammatory activity at formulation F1, as it inhibits maximum edema at 5 hrs, which was comparable to that of standard Indomethacin due to the dose dependency. Since, serotonin, histamine and prostaglandins are the major mediators of inflammation, anti inflammatory effect of A. indica and M. Charantia extract could be due to inhibition of either their synthesis or release possibly due to inhibition of the enzyme cycloxygenase leading to inhibition of prostaglandin synthesis at third stage of inflammation. Based on the results of the present study, it can be concluded that formulation F1 showed significant anti inflammatory activity than F2 in rats.

CONCLUSION

In conclusion, we can confirm that the formulation F1 showed the potent anti-inflammatory activity as compared to the formulation F2 in rats.

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