ESTABLISHMENT OF A MECHANISM OF POLYHERBAL FORMULATION FOR ANTI-RHEUMATISM

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ABSTRACT

Objective: A marketed product Dr. Ortho has been used by many patients and is gaining a positive feedback from the patients. A study is designed to establish its mechanism of action on various anti-rheumatic models in experimental animals. No studies have been investigated on this product for its anti-rheumatic activity.

Methods: Dr. Ortho (55 mg/kg, p. o.) was administered during adjuvant-induced arthritis, phlogistic agents (Histamine, bradykinin, and serotonin) induced paw edema, Acetic acid induced writhing test in mice, and Eddy’s hot plate. Histopathology (T. S. of knee joint) and various hematological parameters (WBC’s, RBC’s, and ESR count) were observed during the study.

Results: Dr. Ortho decreases arthritis induced inflammation by antagonizing or inhibiting the inflammatory mediators, i.e., histamine, 5-hydroxy tryptamine and bradykinin, (20% (P <0.05) of carrageenan, 10.01%, (P <0.05) of histamine, 8% (P <0.05) of 5-hydroxy tryptamine induced paw volume). Dr. Ortho acts as both peripheral and central analgesic. Histopathology findings revealed that there is a reduction in the neutrophil infiltration, pannus formation, and bone. Hematological parameters show that Dr. Ortho decreases the elevated level of the WBC’s count.

Conclusion: The present study revealed some facts regarding the mechanism of Dr. Ortho in reducing arthritis induced inflammation. However study may be done further to illustrate its complete mechanism.

Keywords: Ortho, Adjuvant induced arthritis, Carrageenan, Histamine.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic, chronic inflammatory disease affecting many tissues, but principally attacking the joints to produce a non-suppurative proliferative synovitis that frequently progresses to destroy articular cartilage and underlying bone with resulting disabling arthritis.

RA is a very common condition, with a prevalence of approximately 1% it is three to five times more common in women than in men. The peak incidence is in the second to fourth decades of life, but no age is immune [1]. The disease affects systematically many extrarticular tissues, includes skin, blood vessels, heart, lungs and muscles [2]. The commonly used drugs such as nonsteroidal anti-inflammatory drugs (NSAIDS) and biologic (e.g. antitumour necrosis-α antibody) are effective in alleviating the symptoms of the disease. However, the prolonged use of these drugs is associated with severe adverse reactions. In addition, these drugs are expensive, and not all patients respond well to them. In view of these limitations, it is essential to continue the search for safer and less expensive alternatives to the conventionally used drugs In fact; herbal plants are being used widely as medicine around decades for the treatment of arthritis disease. Herbal medicine constitutes important resources for the treatment of various ailments [3]. Natural plant products represent a promising group of therapeutic agents for arthritis. However, one of the major concerns in seriously considering these products for therapeutic purposes is that the mechanisms of action of many of them are poorly defined, if at all [4,5].

Alternate medicines like Ayurveda, Unani, Chinese, etc. are more and more used for the treatment of diseases, illness prevention and maintenance of health. An ayurvedic polyherbal formulation named Dr. Orthocapsules, SBS biotech, Ambala city, Haryana (India) containing extracts of different herbal medicinal plants-Boswellia Serrata (Shallaki), Commiphora Mukul (guggul), Phutche Lanceolata (Rasna), Trigonella foemnugraecum (Methi), Zingiber Officinale (ginger), Withania Somnifera (Ashwagandha powder), Purified Asphatam (shilaajet) and Styrchnos Nux-vomica. The constituents of this capsule are used in folk medicine for the treatment of inflammation and pain associated with arthritis. Therefore, the present study was done to determine the mechanism of Dr. Ortho capsule.

MATERIALS AND METHODS

Animals
Adult wistar albino rats (180-230 g) of either sex or Swiss albino mice (25-30 g) were used for pharmacological activities. For both experiments, the animals were kept in polypropylene cages (3 per cage) at 25 ± 2 °C with relative humidity 45-55% under 12h light and dark cycles. All the animals were acclimatized to laboratory condition for a week before use. They were fed with standard animal feed and water ad libitum. The Institutional Animal Ethics Committee approved the experimental protocols, Reg no. 837/AC/04/CPCSEA.

Drugs and chemicals
The Dr. Ortho capsule was obtained from SBS Biotech, Himachal Pradesh (manufacturing unit). Indomethacin from Sigma-Aldrich. Complete Freund’s Reagent, histamine, 5-hydroxy tryptamine, bradykinin, carrageenan from Sigma Aldrich. AST, ALT and TP kits for estimation were obtained from Transasia bio-medicals limited, Mumbai (India).

Anti-arthritis activity-adjuvant induced arthritis in rats [6]
Albino rats weighing 150-200 g were divided into four groups of six animals. Controls animals were administered the vehicle. Group 2 (Arthritic control) animals were injected with CFA 0.1 ml to subplantar region of right hind paw. For Group 3 and Group 4 animals, respectively. Dr. Orthocapsule (55 mg/kg, p. o.) and indomethacin (10 mg/kg, p. o.) were administered twice a day, from
Male Albino Wistar rats weighing between 150-200 g were divided into twelve groups of six rats each. Animals of Group 1 receive Acetic acid induced writhing test in mice [12]. Analgesic activity Three groups were made, one group treated as a control and other groups were administered with poly herbal formulation (55 mg/kg p. o.) followed by 5-HT [0.1 ml (1 mg/ml)], bradykinin [0.1 ml (20 µg/ml)] 60 min thereafter and carrageenan [0.1 ml (1%), histamine [0.1 ml (1 mg/ml)] 30 min thereafter. All animals of the group were administered with indomethacin (10 mg/kg, p. o.) followed by respective phlogistic agents. The following was the groups prepared:

- **Group 1:** Control
- **Group 2:** Dr. Ortho (55 mg/kg, p. o.)
- **Group 3:** Dr. Ortho (10 mg/kg, p. o.)

**Anti-inflammatory activity—phlogistic agents induced paw edema in rats [10, 11]**

Three groups of six mice each of either sex with an initial weight of 18 to 22 g are used for each dose. The hot plate, which is commercially available, consists of an electrically heated surface. The temperature is controlled for 55° to 56° C. This can be a copper plate or a heated glass surface. The animals are placed on the hot plate and the time until either licking or jumping occurs is recorded with a stopwatch. The latency is recorded before and after 20, 60 and 90 min following oral or subcutaneous administration of the standard or the test compound [14]. The three groups are as follows:

- **Group 1:** Control
- **Group 2:** Tramadol (25 mg kg⁻¹, p. o.), as standard
- **Group 3:** Dr. Ortho (55 mg/kg, p. o.)

**Histopathology**

After euthanasia on day 21, the hind paws amputated above the knee joint were fixed in 7.4% formalin solution. The sections of articulation of the tarsal joints were stained with haematoxylin and eosin and were examined microscopically for mononuclear infiltration, pannus formation, and bone destruction [15].

**Statistical analysis**

All the results were expressed as means±SEM and analysis were carried out by one-way ANOVA. Post-hock analysis was done by Tukey’s multiple comparison tests to estimate the significance of the difference between various individual groups. P<0.05 was considered as significant.

**Evaluation of parameters**

Body weight changes were measured by an electronic weighing balance. Haematological parameters like RBC, Hb (hemoglobin), ESR, and WBC was measured by Autoanalyzer [16].

**RESULTS**

**Anti-arthritic activity—adjuvant induced arthritis in rats**

**Paw volume**

Dr. Ortho decreased the paw volume on day 4, 7, 14, and 21 respectively as shown in table 1.

**Lysosomal enzymes**

Dr. Ortho decreased (but not significant) the lysosomal enzyme ALT, AST and total protein compared with arthritic rats, as shown in the fig. 2.

**Histopathological study**

Section of the synovial joint of a normal control rat shows intact articular hyaline cartilage (fig. 3(A)), subchondrial bone layer and articular hyaline cartilage with intact subchondrial bone layer and partially effaced synovial layer. The distorted articular cartilage, i.e., bone erosion is replaced by mixed inflammatory cells comprising predominantly neutrophil and some lymphocytes. The synovial sub epithelium shows fibro-collagenous stroma with vascular spaces (fig. 3(A)). Section of the synovial joint of Dr. Ortho treated shows intact articular hyaline cartilage (fig. 3(C)), subchondrial bone layer and adjacent synovial layer. The synovial layer shows synovial lining cells within a normal range. The synovial sub epithelium shows fibro-collagenous stroma with vascular spaces (fig. 3(B)).
Table 1: Effect of Dr. Ortho on paw edema

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Paw volume (ml) (mean±SEM)</th>
<th>0th day</th>
<th>4th day</th>
<th>7th day</th>
<th>14th day</th>
<th>21st day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>1.23±0.01</td>
<td>1.37±0.034</td>
<td>1.17±0.086</td>
<td>1.26±0.0140</td>
<td>2.10±0.034</td>
</tr>
<tr>
<td>CFA</td>
<td></td>
<td>1.1±0.037</td>
<td>2.77±0.054**</td>
<td>3.2±0.041***</td>
<td>2.9±0.037**</td>
<td>2.5±0.044**</td>
</tr>
<tr>
<td>Dr. Ortho</td>
<td></td>
<td>1.2±0.031</td>
<td>2.5±0.043</td>
<td>2.5±0.023</td>
<td>2.10±0.037</td>
<td>1.70±0.033</td>
</tr>
<tr>
<td>Indomethacin</td>
<td></td>
<td>1.21±0.022</td>
<td>2.5±0.013</td>
<td>2.5±0.023</td>
<td>2.10±0.019</td>
<td>1.70±0.080</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM n=6; one way ANOVA followed by Turkey’s test, ***p<0.001 v/s control, **p<0.01 and *p<0.05 v/s control.

Anti-inflammatory activity—phlogistic agents induced paw edema in rats

Dr. Ortho significantly reduced the Carrageenan (fig. 4), Histamine (fig. 5), 5-HT (fig. 6), Bradykinin (fig. 7) induced paw edema respectively.

Analgesic activity

Acetic acid induced writhing test in mice

Dr. Ortho when compared to control animals, significantly (P<0.01) decreased the number of acetic acid induced writhing episodes and
the percentage protection was 41.2%, while the reference drug, Diclofenac sodium showed 66.4% protection.

Eddy’s hot plate

Fig. 8 shows the time interval of the anti nociception produced by Dr. Ortho. The effect reached a peak significantly at 120 min after administration and then gradually decreased.

Fig. 6: Effect of Dr. Ortho on 5-HT induced paw edema in rat
Values are expressed as mean±SEM. n=6; One-way ANOVA followed by TURKEY’s multiple comparison test. ‘P<0.01 vs arthritic control, ‘P<0.05 vs arthritic control

Fig. 7: Effect of Dr. ortho on bradykinin-induced paw edema in rat
Values are expressed as mean±SEM. n=6; One-way ANOVA followed by TURKEY’s multiple comparison test. ‘P<0.05 vs arthritic control

Body weight changes

Dr. Ortho (55 mg/kg) inhibits the loss of body weight observed during arthritic condition in a disease control group. The results are shown in table 2.

Hematological changes

Dr. Ortho and indomethacin-treated groups significantly decreased the total WBC count while ESR count regains its normal value. The results are shown in table 3.

DISCUSSION

We found that Dr. Ortho inhibited 20% (P <0.05) of Carrageenan-induced paw volume at 30 min, 10.01%, (P <0.05) of Histamine induced paw volume at 30 min respectively, 8%, 7% (P<0.05) of 5-HT induced paw volume at 30 min and 120 min. 3% (P<0.01) of Bradykinin-induced paw volume at 20 min. respectively. These results show that, though not very potent, Dr. Ortho decreased the inflammation by antagonizing or inhibiting the inflammatory mediators i.e., Histamine, 5-HT and Bradykinin. Analgesic activity of Dr. Ortho was illustrated through, acetic acid-induced prostaglandins mediated pain, where percentage protection was found to be 45.1% (P<0.001). Analgesic activity of Dr. Ortho using Eddy’s hot plate was found to be maximum of 120 min. This indicates that Dr. Ortho acts as both peripheral and central analgesic.

Pannus formation is one of the major events which lead to cartilage destruction and bone erosion in rheumatoid arthritis. The pannus formed due to CFA was reduced by Dr. Ortho. It is supported by...
histological studies of knee joints [18]. fig. 3 (A) is TS of knee joint of control rat. Severe neutrophil infiltration, pannus formation and bone erosion are seen in the knee joint of arthritic control rat as shown in fig. 3 (B). On treatment with Dr. Ortho, there is a reduction in the neutrophil infiltration, pannus formation and bone as shown in fig. 3 (C), respectively. Loss of body weight occurs in arthritis due to deficient absorption of nutrients through the intestine and distress caused by the severity of arthritis [19]. Dr. Ortho decreased the elevated level of WBC, thus leading to a reduction in mononuclear infiltration and hence reduced pannus formation.

CONCLUSION

The ayurvedic product is purely based on ancient Indian data for the treatment of pain coupled with arthritis. The study was taken up with an aim to explicate the mechanism of the anti-arthritic action of the mentioned polyherbal product.

In summary, the anti-rheumatic mechanism of Dr. Ortho is due to-
I. Inhibition of inflammation by inhibiting inflammatory mediators, i.e., Histamine, 5-HT, and Bradykinin.
II. Protection against prostaglandins mediated pain in mice, by inhibiting cyclo-oxygenase synthesis.
III. Reduction in pannus formation by inhibiting mononuclear infiltration.

Therefore, the data clearly indicate the antiarthritic action of a polyherbal formulation.

CONFLICT OF INTERESTS

All the authors declare no conflict of interest

REFERENCES