Screening of In-Vivo Analgesic Activity of Siddha herbal Preparation Vajjiravalli Chooranam

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Abstract: The Vajjiravalli chooranam is traditionally used in siddha system for various skin diseases and pain management and osteo problems. The Vajjiravalli chooranam was evaluated for its analgesic activity on Swiss albino mice according to acetic acid induced writhing method. Writhing method is the most common test for evaluating the analgesic efficacy of drugs in rodents. The Vajjiravalli chooranam was used 500mg/kg given orally to the animals. The end of the study result shows that the Vajjiravalli chooranam have 44.48% inhibition activity.

Kew word: Vajjiravalli chooranam, Siddha medicine, Analgesic.

I. Introduction

The International Association for the Study of pain defines, “Pain is an unpleasant sensory or emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Pain is always subjective. Each individual learns the application of the word through experiences related to injury in early life. It is unquestionably a sensation in a part of the body, but it is also unpleasant, and therefore also an emotional experience. Many people report pain in the absence of tissue damage or any likely pathophysiological cause; usually this happens for psychological reasons. There is no way to distinguish their experience from that due to tissue damage, if we take this subjective report”. The presently available pharmacological treatments in the market not only causing economical exploitation, but also associated with severe adverse effects. Siddha medicine in India has proven track record of 5000 years and forms part of the National Health Service, offered alongside conventional medicine [1-3]. Herbal medicines yielding about 25% of currently used crude drugs with another 25% derived from chemically altered natural products [4-5]. However, to develop a proper medication which will be ecofriendly and having very less side effects that can be used for prophylactic and therapeutic purpose to control many diseases is still a big challenge to a scientific community [6]. The purpose of the present study was, therefore, to evaluate the analgesic and anti-inflammatory effects of the Vajjiravalli chooranam using different acute and chronic models of pain and inflammation in mice and rats [7]. The Vajjiravalli chooranam was also studied for its acute and sub-acute toxicity effects in rodents.

II. Materials and Methods

Stock solution preparation:
The powdered form of Vajjiravalli chooranam was filtered through cheese cloth and was mixed uniformly in 2% CMC solution to achieve 50mg/ml as main stock solution and used in this study.

Drugs and Chemicals:
All drugs were administrated orally half an hour before the onset of pain stimulus in different models of nociception in albino mice. Diclofenac sodium (25mg/kg) in distilled water, Vajjiravalli chooranam (500mg/kg) in 2 % CMC.

Animals:
Swiss mice (25—35 g), were housed at 22±2°C under a 12-h light/12 h dark cycle and were acclimatized to the laboratory for at least 1 h prior to testing. The animals were acclimatized for one week under laboratory conditions. Healthy Swiss albino mice of either sex starved overnight were divided into 3 groups. The number of animals (6 per group of treatment) and intensities of noxious stimuli used were the minimum necessary to demonstrate consistent effects of the drug treatments. The experimental protocol was initially...
approved from the Institutional animal ethics committee and then experimental studies were undergone according to their rules and regulations. The animals were housed under standard environmental conditions and had free access to standard pellet diet and water ad libidum.

**Study of analgesic activity**

The *Vajjiravalli chooranam* was evaluated for its analgesic activity on Swiss albino mice according to acetic acid induced writhing method. The mice were randomly divided into different groups depending on the number of samples and doses to be applied and consisted of 6 mice in each group. All the animals were individually weighed and the dose of the *Vajjiravalli chooranam* and control material adjusted accordingly. The animals were kept in the laboratory atmosphere for at least one week for acclimatization prior to any experiment. The test samples were prepared as suspension in saline water with 2% CMC as suspending agent. Diclofenac sodium (25mg/kg body weight) was used as positive control in this experiment [8]. Glacial acetic acid was administered intra-peritoneally to the experimental animals to create pain sensation. As a result, the animals squirm their body at regular intervals out of pain. This squirm or contraction of the body is termed as writhing. Any substance that has got analgesic activity is supposed to reduce the number of writhing of animals within a given time and with respect to the control groups. At zero hour, test samples (at doses of 500mg/kg body weight), and negative control were administered orally by means of a long needle with a ball-shaped end. After thirty minutes, glacial acetic acid (0.7% at a dose of 0.1 ml/10 g body weight) was administered intra-peritoneally to each of the animals of all the groups. The thirty minutes interval was given to ensure the proper absorption of the administered samples. Five minutes after the administration of acetic acid, the number of writhing were counted for twenty minutes for each mouse [9-11].

The animals did not always accomplish full writhing, because the animals started to give writhing sometimes but they did not complete it. This incomplete writhing was considered as half writhing. Accordingly, half of the writhing was taken to convert all writhing to full writhing or real writhing [12].

**Statistical analysis**

The data was expressed as Mean ± SEM (standard error of mean ). Analysis of variance (ANOVA) followed by post hoc and dunnet-t-test was used to statistically analyzed data P value less than 0.05 (P<0.05) were considered as significant.

**III. Results and Discussion**

Mortality in the acute oral toxicity test was not seen in the limit test up to dose 5000mg/kg. One-tenth of the upper bound dose was considered as therapeutic dose for further experiments. The *Vajjiravalli chooranam* was evaluated for its analgesic activity on Swiss albino mice according to acetic acid induced writhing method. Writhing method is the most common test for evaluating the analgesic efficacy of drugs in rodents. The abdominal constriction response induced by glacial acetic acid is a sensitive procedure to establish peripherally acting analgesics. The mouse writhing assay is a useful test to evaluate analgesic agents.

Acetic acid causes algesia by liberating endogenous substances including serotonin, histamine, PGs, bradykinin and substance P which stimulate pain nerve endings. Local peritoneal receptors are postulated to be partly involved in the abdominal constriction (writhing) response. The method has been associated with prostanoids in general, i.e. increased levels of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products by some researchers. Therefore, the VVC might inhibit the synthesis and/or release of these endogenous substances and this response is thought to involve local peritoneal receptors. The number of writhing observed during a 20 min period in control group was 35.64 ± 3.25. Diclofenac significantly reduced the number of writhes (P<0.05). It is also observed that animals in test group showed delayed onset of writhes (after 8-9 min) as compared to other groups in which onset of writhes was within 5 min (Table 1 & Fig1 ).

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>No of Writhings (mean±SEM)</th>
<th>% of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2% CMC + 2% Acetic acid</td>
<td>38.33±4.20</td>
<td>--</td>
</tr>
<tr>
<td>Test</td>
<td>VVC (300mg/kg) + 2% Acetic acid</td>
<td>21.26±2.49**</td>
<td>44.48</td>
</tr>
<tr>
<td>Standard</td>
<td>Diclofenac (45mg/kg) + 2% Acetic acid</td>
<td>8.43±1.43**</td>
<td>78.00</td>
</tr>
</tbody>
</table>

The writhing in control was taken as 100%. The mean writhing was calculated from three determinations in each group (n=6) of mice. **P<0.01 Vs Control
**IV. Conclusion**

Drugs which act mainly centrally, such as narcotic analgesics, inhibits both phases of pain in this model while peripherally acting drugs, such as Diclofenac, aspirin or indomethacin, only inhibit the late phase. The Vajjiravalli chooranam inhibited both phases of the acetic acid induced pain with more potent effects on the second phase. The Vajjiravalli chooranam significantly reduced chemical induced nociception in mice when compared with vehicle treated group. Hence it is evident that the Vajjiravalli chooranam at dose 500 mg/kg have promising effect in the management of pain.

**References**


**Fig 1:** Analgesic effect of Vajjiravalli chooranam

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac sodium 25mg/kg</td>
<td>100</td>
</tr>
<tr>
<td>VVC (500mg/kg)</td>
<td>40</td>
</tr>
</tbody>
</table>

**ANALGESIC ACTIVITY OF VAJJARA VALLI CHORANAM**