EVALUATION OF ANALGESIC ACTIVITY OF METHANOLIC EXTRACT OF
THE AERIAL PARTS OF ALYSICARPUS MONILIFER L.(DC.)

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ABSTRACT
In the present study, methanolic extract of aerial parts of Alysicarpus monilifer L.(DC.) (Papilionaceae) was screened for analgesic activity using Tail flick Hot plate method. The effect was assessed by noting response time as the time when there is sudden withdrawal of the tail from the heat source in tail flick method and in hot plate, time at which animals reacted to the pain stimulus either by paw licking or jump response, whichever appeared first. Methanolic extract of aerial parts of Alysicarpus monilifer L.(DC.) (200 mg/kg & 400 mg/kg) were administered orally. Analgesic effects were compared with standard drug Indomethacin (20mg/kg). The study revealed that Methanol extract in higher doses gives more analgesic activity as compared to that in low dose in both hot plate and tail flick methods.

Keywords: Alysicarpus monilifer, Hot plate method, Tail flick method, analgesic activity, methanolic extract, aerial parts.

INTRODUCTION
Alysicarpus monilifer L.(DC.) (Papilionaceae), commonly known as Samervo (Gujarati) or Juhi ghas (Hindi), is a turf forming legume and native to Africa and Asia. In India it is distributed throughout the plains- Madras, Jammu, Bombay, Punjab, Gujarat (except Kutch and Bulsar), Madhya Pradesh and Uttar Pradesh. It is a prostate, procumbent or decumbent perennial herb; stem of which is around 12- 60cm long, woody at the base. It is a branched; branches are terete clothed with covering trichomes. The herb is up to 50cm in length and hairy when young [1,2]. The aerial parts of the plant have been studied for the pharmacognostic as well as preliminary phytochemical aspects[3]. Alysicarpus monilifer has been used in indigenous system of medicine as anti-inflammatory and in stomach-ache [4], an antidote to snake bite [5,6]. It is also used in skin diseases and as a diuretic [7,8]. The leaves are used in fever [9] and jaundice [10]. In our earlier study we have reported the anti-inflammatory activity of the plant [11].
The International Association for the Study of Pain (IASP) defines pain as “an unpleasant sensory and emotional experience which we primarily associate with tissue damage or describe in terms of such damage, or both.” Pain may be classified as persistent or acute. Persistent pain may be broadly divided nociceptive, neuropathic, psychogenic, mixed, or idiopathic according to the pain symptoms. [12]

So far no systematic study has been reported for analgesic property of methanolic extract of aerial parts of Alysicarpus monilifer. In the present study an effort has been made to establish the scientific validity to the claims of analgesic property of Alysicarpus monilifer methanolic extract using Tail flick method and Hot plate method in rats.

MATERIAL AND METHODS:
Collection of plant material: Fresh and fully grown plants of Alysicarpus monilifer were collected from New Vallabh Vidyanagar (beyond G.I.D.C. phase IV) in the month of September and identified by Dr. Bhanu H. Kakrani, Lecturer, Dept. of Botany, Tolani College of Arts & Science, Adipur (Kutch) and its voucher specimen deposited (PHK/Am-1/1/ARGH-11) with Department of Pharmacognosy, A. R. College of pharmacy & G.H. Patel Institute of Pharmacy, Vallabh Vidyanagar.

Animals: Albino Wistar rats (250-400 gm each) of either sex kept under standard environmental conditions (Temperature 22±3°C, Humidity at 30-70%) in polypropylene cages. Standard pelleted feed & drinking water were provided ad libitum throughout the experimental period. The animals were acclimated to laboratory conditions one week prior to the initiation of experimental work. The protocol was approved by the Ethics committee & the CPCSEA under the no. CPCSEA/IAEC/ARCP/10-11/11.

Tail flick method of evaluation for analgesic activity [13,14]: The animals were divided into six groups of 6 animals each. Group I served as control. Group II served as standard and were injected Indomethacin (20 mg/kg) intraperitonially. Group III and IV were treated orally with methanolic extract of 200 and 400 mg/kg body weight respectively. After one hour, the tip of tail was kept at the radiant heat source. The response time was noted as the sudden withdrawal of the tail from the heat source. Cut off time of 10 seconds was maintained to avoid damage to the tail for all groups. The time required for flicking of the tail, was recorded, to assess response to noxious stimulus. Data were statistically analyzed by analysis of variance (ANOVA) with the level of significance set
at p< 0.05. Critical differences between means were evaluated by Dunnett’s multiple comparison test and Student’s t-test at p< 0.05.

Hot plate method of evaluation for analgesic activity\textsuperscript{[13,14]}: The animals were divided into six groups of 6 animals each. Group I served as control. Group II served as standard and were injected Indomethacin (20 mg/kg) intraperitonially. Group III and IV were treated orally with methanolic extract of 200 and 400 mg/kg body weight respectively. The animals were individually placed on the hot plate maintained at 55 ± 1°C, one hour after their respective treatments. The response time was noted as the time at which animals reacted to the pain stimulus either by paw licking or jump response, whichever appeared first. The cut off time for the reaction was 15 seconds to avoid damage to the paws. Data were statistically analyzed by analysis of variance (ANOVA) with the level of significance set at p< 0.05. Critical differences between means were evaluated by Dunnett’s multiple comparison test and Student’s t-test at p< 0.05.

RESULTS:

a) Tail Flick method:

<table>
<thead>
<tr>
<th>ANIMAL GROUP</th>
<th>DRUG DOSE</th>
<th>BASAL REACTION TIME (MEAN TIME IN SEC ± SEM)</th>
<th>AFTER 1 HOUR REACTION TIME (MEAN TIME IN SEC ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (I)</td>
<td>Normal saline</td>
<td>3.5 ± 0.22</td>
<td>3.5 ± 0.22</td>
</tr>
<tr>
<td>Standard-Indomethacin (II)</td>
<td>20 mg/kg</td>
<td>3.16 ± 0.31</td>
<td>8.0 ± 0.36</td>
</tr>
<tr>
<td>Low dose methanolic extract (III)</td>
<td>200 mg/kg</td>
<td>3.33 ± 0.21</td>
<td>4.5 ± 0.23</td>
</tr>
<tr>
<td>High dose methanolic extract (IV)</td>
<td>400 mg/kg</td>
<td>6.5 ± 0.23</td>
<td>6.5 ± 0.21</td>
</tr>
</tbody>
</table>

n=6 number of rats in each group done by one way analysis of variance followed by Dunnett’s test.
The result of effect of Alysicarpus monilifer on the tail flick method is presented in Table 1.

The reference drug ASA and the extract significantly (P<0.05) increased the latency period from 3.5± 0.22 seen in the control group to 8.0± 0.36 sec in group 2 (Indomethacin, 20 mg/kg) and 4.5 ± 0.23 and 6.5 ± 0.21 sec in the 200 and 400 mg/kg treated groups respectively (Fig. 1). The effect of the reference drug Indomethacin was also higher than the extract.
b) Hot plate method:

**TABLE 2: EFFECT OF METHANOLIC EXTRACT OF AERIAL PARTS OF THE PLANT USING HOT PLATE METHOD**

<table>
<thead>
<tr>
<th>ANIMAL GROUP</th>
<th>DRUG DOSE</th>
<th>BASAL REACTION TIME (MEAN TIME IN SEC ± SEM)</th>
<th>AFTER 1 HOUR REACTION TIME (MEAN TIME IN SEC ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (I)</td>
<td>Normal saline</td>
<td>5.16 ± 0.307</td>
<td>5.16 ± 0.307</td>
</tr>
<tr>
<td>Standard- Indomethacin (II)</td>
<td>20 mg/kg</td>
<td>4.0 ± 0.26</td>
<td>9.0 ± 0.365</td>
</tr>
<tr>
<td>Low dose methanolic extract (III)</td>
<td>200 mg/kg</td>
<td>4.66 ± 0.21</td>
<td>6.5 ± 0.224</td>
</tr>
<tr>
<td>High dose methanolic extract (IV)</td>
<td>400 mg/kg</td>
<td>4.5 ± 0.22</td>
<td>8.0 ± 0.258</td>
</tr>
</tbody>
</table>

n = 6 in each group number of rats in each group done by one way analysis of variance followed by Dunett’s test.

![Hot Plate Method](image)

* = p<0.05, significant as compared to control

**Figure 2**
Effect of methanolic extract of Alysicarpus monilifer on analgesic activity on rats using Hot plate method

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The results showed that there was no significant difference in the pain reaction time (PRT) before drug administration in all the mice. 60 minutes after drug administration, the PRT was significantly (P<0.05) increased by the extract and the reference drug in a dose dependent manner when compared to the normal saline treated group (Fig. 2). There was no significant difference between the control group and the group that received the least dose of the extract (200 mg/kg). In the experiment the reference drug (Indomethacin) was more effective than the extract in reducing the PRT.

DISCUSSION

It is possible to assume that certain noxious stimuli (thermal, mechanical of chemical) are painful and that reflex movements or behaviours resulting from such hence the use of the two anti-nociceptive models viz: Hot plate and Tail flick models for the study of the analgesic activities of A. monilifer. A. monilifer methanolic extract produced a dose dependent and significant (p<0.05) increase in pain threshold in the rats in these models that was comparable to the reference drug indomethacin. In these models, increase in stress tolerance capacity of the animals indicates the possible involvement of a higher centre. It is therefore thought that the analgesic effect of A. monillifer seen in this study may involve central activity.

CONCLUSION

From the present investigations, it may be concluded that methanolic extracts of aerial parts of Alysicarpus monilifer demonstrated promising analgesic activity, which has been carried out scientifically for the first time.

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