ANTI-NOCICEPTIVE, ANTI INFLAMMATORY AND ANTIARTHRITIC ACTIVITY OF MARSDENIA TENACISSIMA STEM EXTRACT

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ABSTRACT
The main purpose of the present investigation is to evaluate anti-nociceptive, anti inflammatory and antiarthritic activity of Marsdenia tenacissima stem extract in Wistar albino rats. Analgesic activity was studied in rats using hot plate and acetic acid models. The anti inflammatory activity of Marsdenia tenacissima ethanolic extract (MTEE) was evaluated by using carrageenan induced paw edema and cotton pellet induced granuloma methods. While its anti arthritic activity was investigated by using freud's adjuvant induced arthritis model. The both doses of MTEE was found to produce significant analgesic activity. The MTEE suppressed the development of paw edema induced by carrageenan in rats and cotton pellet granuloma weight was significantly reduced in animals. The arthritic animals treated with MTEE significantly reduced the arthritic edema in a dose dependant manner .The results of the present study demonstrates that analgesic, anti inflammatory and antiarthritic activity of Marsdenia tenacissima stem extract in experimental animal models.

Key words: Marsdenia tenacissima, Anti Inflammatory, Antiarthritic, Anti-Nociceptive, Carrageenan Induced Paw Edema, Cotton Pellet Granuloma, Freud's Adjuvant Induced Arthritis.

INTRODUCTION
Inflammation is a body defense mechanism to harmful stimuli, Although it is a beneficial response, prolonged inflammation can be lead to many disease states like vascular changes, myopathies, atherosclerorosis and rheumatoid arthritis (Cotran et al., 1998). Arthritis is an chronic auto immune disorder affects the musculoskeletal system and leads to significant pain, inflammation, joint destruction, and functional decline. Arthritis is the leading cause of disability and second most frequently reported chronic condition in the united states (Yelin E et al., 2004, Benson V et al., 1995). Unfortunately conventional or synthetic drugs used in the treatment of arthritis are inadequate and sometimes can have serious side effects (Becker, Michael A,2005). Hence, the research for ideal drugs still continues and has been extended to herbal drugs . Heral medicines are in great demand in the developed as well as developing countries for primary health care because of their wide biological and medicinal activities, higher safety margins and lesser costs (Agus ZS et al., 1971). Marsdenia tenacissima (Asclepiadaceae) commonly called as Rajmahal Hemp, Devils tongue or Bush banana is one such medicinal plant possesses several pharmacological properties, where, few are already reported and few are yet to be investigated.

It is a large twining shrub possess characters such as a creeper; exudes milky juice or latex; possesses strong fibers (Vaidya B, 1982). It mainly found in the Himalayas from Kumaon to Assam up to an altitude of 1500 m. and extending southwards to the Deccan Peninsula. The medicinal values of the plant and its parts indicate that it isuseful in abdominal pain due to worms,
in fever, heart diseases, skin diseases etc. Commonly the plant is used primarily as an appetizer, in vomiting, indigestion, colic pain, fever etc (Khare CP, 2003).

To best of our knowledge, no other biochemical investigations had been carried out on anti-nociceptive, anti inflammatory and antiarthritic activity of *Marsdenia tenacissima* stem extract. Hence the present study was designed to investigate its activities in experimental animal models.

**MATERIAL AND METHODS**

**Plant Material**

The stems of the plant were collected in the month of March, 2013 from Kuber impex limited M.P. The plant was identified and authenticated. A herbarium specimen of the plant was preserved in the department of Pharmacognosy of our institute for further reference.

**Preparation of Extracts**

The stems of the plant were shade dried, powdered mechanically and subjected to soxhlet extraction successively with ethanol. After extraction the filtrate was concentrated on a rotary evaporator under vacuum at 20°C till a residual mass was obtained. Ten grams of the dried extract was triturated with 9 ml of vehicle. Vehicle was then added gradually until the final concentration of the extract was 10%.

From the literature review, it was found that the active constituents (alkaloids, glycosides, carbohydrates and phyto sterols) present in the *Marsdenia tenacissima* are soluble in ethanol. Hence, the present study used ethanolic extract.

**Experimental Animals**

Healthy Wistar albino rats at 8 weeks of age were purchased from Ghosh Enterprises, Kolkata. They were housed in a room, maintained at approximately 25 ± 2 °C, the photo period was 12 hrs light and 12hrs dark cycle. For feeding, the rodent laboratory diet used which is supplied from Ryan biotechnology Pvt ltd. Hyderabad. The study was performed following approval from Institutional Animal Ethical Committee (IAEC) of Talla Padmavathi College of Pharmacy, Warangal. (1505/PO/a/11/CPCSEA). Ethical norms were strictly followed during all experimental procedures.

**Acute Toxicity Studies**

Acute toxicity studies for ethanolic extract of *Marsdenia tenacissima* stem was determined as per the OECD guideline no.423 (acute toxic class method). Studies were carried out, using groups of three Wistar albino rats; by administering doses of 50-2000 mg/kg body weight, p.o., and the control group received normal saline, and observed all the animals closely for 24 hrs for any mortality (OECD, 2001).

**Anti nociceptive activity**

*Hot plate method*

This model was used to evaluate the central analgesic activity of MTEE. The rats were divided into four groups, each containing six animals.

- **Group I:** The animals of this group served as control received 0.1ml normal saline p.o.
- **Group II:** The animals received extract of *Marsdenia tenacissima* 100mg/kg.
- **Group III:** The animals received extract of *Marsdenia tenacissima* 200mg/kg.
- **Group IV:** The animals received pentazocin 10mg/kg.

The temperature of the hot plate was maintained at 55 ± 0.5°C. 30 min after drug administration the reaction time of each rat was recorded at 0, 30, 60, 90, and 120 min with a cut-off time of 30 sec.

**Acetic acid induced writhing test**

This test compound and model was used to evaluate the peripheral analgesic activity of MTEE. Animals were dosed with the test and reference compounds (diclofenac sodium 50mg/kg p.o) prior to the experiment. The writhing was induced by intraperitoneal injection of acetic acid (0.2 ml of 0.6 %). Extension of hind limbs and contraction of abdominal musculature is considered as writhing response. The number of writhes were counted within a span of 30 minutes (Collier HO et al., 1968).

**Carrageenan induced paw edema**

All animals were treated with extract and reference drug (Indomethacin 10mg/kg) before the administration of carrageenan. Acute inflammation in rats was induced according to Winter et al; 1962. A suspension of 0.1ml of 1% Carrageenan was injected into the sub plantar region of the left hind paw of each rat. The hind paw volume was measured after carrageenan injection at 0 and 3 hrs by using plethysmometer (Kavimani, S et al.,1996).

**Anti inflammatory activity**

*Cotton pellet induced granuloma method*

Sub acute Inflammation was induced according to Meier et al., 1950, by implanting sterilized cotton pellet granuloma in rats. Under ether anesthesia sterilized cotton pellets weighing 10 ± 1 mg were implanted near each axilla through subcutaneous incision. The animals were given MTEE and indomethacin for 7 consecutive days. On the 8th day animals were sacrificed by cervical dislocation and the pellets were carefully removed. Weight of the granuloma were calculated and compared with control.

**Antiarthritic activity**
Freud’s adjuvant induced arthritis
Anti inflammatory activity of MTEE has been studied in chronic inflammation by Freud’s adjuvant previously described by Newbound et al., 1963. Arthritis was induced with 0.1 ml of Complete Freund’s adjuvant injected into the subplantar region of the right hand paw. Drugs were administered to animals for 13 days. Volume of the paw was measured on alternate days till the duration of the experiment. percent change in the paw volume was calculated in comparison to the untreated control group. Aspirin (100mg/kg) was used as positive standard for comparison and authentication of the experiment.

Statistical analysis
The data are presented as mean ± SEM and analyzed by one-way ANOVA.

RESULTS
Acute toxicity
It was observed that MTEE was lethal to the rats at 2000mg/kg dose. Toxicity signs such as Tremors, behavioral changes, sedation and mortality were observed at 2000mg/kg. Hence 100mg/kg and 200mg/kg taken as effective dose for further study.

Analgesic Activity
Hot Plate method
The reaction time following the oral administration of different doses of MTEE is presented in table 1. It produced a significant increase in the mean reaction time throughout the observation period, compared to the control . At the dose of 200mg/kg body weight p.o showed a significant increase in the mean response time whih is closer to that of standard pentazocin.

Acetic Acid Induced Writhing Test
The MTEE (100, 200 mg/kg) dose significantly and dependently reduced the number of abdominal constriction induced by a solution of acetic acid 1%. But Marsdenia tenacissima at a dose of 200mg/kg shown maximum inhibition of 80.91%, which is closer to that of standard Diclofenac sodium.

Anti inflammatory activity
Carrageenan-Induced Paw Edema
In the carrageenan-induced oedema test, the difference in the paw volume and percentage inhibition of oedema by the ethanolic extract of Marsdenia tenacissia are shown in Table 3. The hind paw volume was measured after carragenan injection at 0 and 3 hrs. MTEE at the two doses (100mg/kg, 200mg/kg) used in the study significantly inhibited the carrageenan induced paw edema .At 100mg/kg dose there is 66% inhibition and at 200mg/kg there was 70% inhibition was observed.

Cotton Pellet Granuloma
The result shows that the extract at dose of 100 mg/kg and 200 mg/kg has significant protection in the cotton pellet induced granuloma in a dose dependant manner . The higher dose of the extract (200 mg/kg) exhibited inhibition of inflammation close to the inhibitory effect of indomethacin.

Antiarthritic activity
Freud’s adjuvant induced arthritis
Marsdenia tenacissima when tested against this chronic model of arthritis, showed marked inhibition in the injected paw swelling in a dose dependant manner. There were significant reductions in arthritic indices.

Table 1. Analgesic activity of MTEE by Eddy's hot plate method

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Response time (MEAN ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal saline</td>
<td>2.78 ± 0.2107</td>
</tr>
<tr>
<td>II</td>
<td>Marsdenia tenacissima 100mg/kg.</td>
<td>6.33 ± 0.3128</td>
</tr>
<tr>
<td>III</td>
<td>Marsdenia tenacissima 200mg/kg.</td>
<td>10.89 ± 0.3144</td>
</tr>
<tr>
<td>IV</td>
<td>Pentazocin 10mg/kg</td>
<td>12.76 ± 0.3456</td>
</tr>
</tbody>
</table>

One- way Analysis of Variance ANOVA: p value found to be 0.0001 is considered extremely significant. The data were expressed as mean ± S.E.M.; Tukey Kramer multiple comparison test: ***p<0.001, **p <0.01 (Extracts vs. control)

Table 2. Analgesic activity of MTEE by Acetic acid induced writhing

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No of wriths (Mean ± SEM)</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal saline</td>
<td>40 ± 9.10</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>Marsdenia tenacissima 100mg/kg.</td>
<td>15.4±3.84</td>
<td>58.64</td>
</tr>
<tr>
<td>III</td>
<td>Marsdenia tenacissima 200mg/kg.</td>
<td>6.4± 1.34**</td>
<td>80.91</td>
</tr>
<tr>
<td>IV</td>
<td>Diclofenac 50mg/kg</td>
<td>4.0 ± 2.0**</td>
<td>90.12</td>
</tr>
</tbody>
</table>

One- way Analysis of Variance ANOVA followed by Dunnett's test. The data is expressed as mean ± SEM. * p ≥ 0.05 * p ≤ 0.01 as compared to control.
Table 3. Effect of MTEE on carrageenan induced paw oedema

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Difference in paw volume at 3h (ml)</th>
<th>% inhibition of oedema</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal saline</td>
<td>0.70</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td><em>Marsdenia tenacissima</em> 100mg/kg.</td>
<td>0.30±0.10**</td>
<td>66.6</td>
</tr>
<tr>
<td>III</td>
<td><em>Marsdenia tenacissima</em> 200mg/kg.</td>
<td>0.25±0.03**</td>
<td>70.0</td>
</tr>
<tr>
<td>IV</td>
<td>Indomethacin 10mg/kg</td>
<td>0.23±0.05</td>
<td>78.3</td>
</tr>
</tbody>
</table>

Values are the mean ± s.d, n =6, **p ≤ 0.01 compared to control group. (Student’s t -test)

Table 4. Effect of MTEE on cotton pellet induced granuloma method

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Increase in the weight of pellet (mg)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal saline</td>
<td>0.18±0.01</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td><em>Marsdenia tenacissima</em> 100mg/kg.</td>
<td>0.16±0.02</td>
<td>14.3</td>
</tr>
<tr>
<td>III</td>
<td><em>Marsdenia tenacissima</em> 200mg/kg.</td>
<td>0.14±0.01</td>
<td>28.6</td>
</tr>
<tr>
<td>IV</td>
<td>Indomethacin 10mg/kg</td>
<td>0.13±0.01</td>
<td>35.7</td>
</tr>
</tbody>
</table>

Each weight value is the mean ± SEM of 5 rats. *P <0.05. **P <0.01, compared with control (Student’s t -test).

Graph 1. Anti inflammatory activity of MTEE on carrageenan induced paw oedema & cotton pellet induced granuloma method

Graph 2. Anti arthritic activity of MTEE in freud’s adjuvant induced arthritis

**DISCUSSION**

The present study demonstrates the analgesic, anti inflammatory and anti arthritic effects of *Marsdenia tenacissima*. The antinociceptive activity of the MTEE was investigated for both central and peripheral analgesic properties. The central analgesic effect was tested by using hot plate method, where as the peripheral analgesic effect was tested by acetic acid-induced writing in rats. Based on our observations, MTEE possesses antinociceptive activity against chemically and thermally induced nociception.
Carrageenan-induced edema model used as an experimental animal model for acute inflammation and is believed to be biphasic. The first phase is attributed to the release of histamine, serotonin and kinin and the second phase is related to the release of prostaglandin and bradykinins (Gupta M et al., 2006, Antônio M.A et al., 1998). Since the extract significantly inhibited paw edema induced by carrageenan in the second phase, this finding suggests a possible inhibition of cyclooxygenase synthesis by the extract.

The cotton pellet granuloma method has been widely employed to evaluate the transudative, exudative, and proliferative components of chronic inflammation, because the dried weight of the pellets correlates well with the amount of granulomatous tissue (Swingle K.F et al., 1972). The extract exhibited dose dependant action, suggesting that lesser doses are less effective in reducing leucocytes migration into areas of inflammation, since granuloma formation is due to leucocyte accumulation.

*Mycobacterium* induced arthritis in rats show many similarities with human arthritis (Katz L, Piliero SJ, 1969). *Marsdenia tenacissima* when tested against this chronic model of arthritis, showed marked inhibition in the injected paw swelling in a dose dependent manner.

**CONCLUSION**

The present study reveals that analgesic, anti-inflammatory and anti arthritic effects of *Marsdenia tenacissima* extract in experimental animal models. Further work is needed in this regard to determine the exact chemical constituent(s) and mechanism(s) of action of the constituent.

**REFERENCES**


