EVALUATION OF ANTI-INFLAMMATORY AND ANALGESIC POTENTIAL OF PETROLEUM ETHER EXTRACTS OF MALVASTRUM TRICUSPIDATUM STEM BARK ON ALBINO MICE

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ABSTRACT

In the present study, stem bark of plant Malvastrum tricuspidatum were selected for preliminary phytochemical investigation and pharmacological evaluation for analgesic & anti-inflammatory activity. Extraction of stem bark of M. tricuspidatum was carried out by defatting powder with petroleum ether followed by extraction with ethanol. As a model of inflammation, carrageenan induced mice paw edema, dextran induced mice paw edema, histamine induced mice paw edema, formalin induced paw edema and cotton pellet induced granuloma in rats were utilized in the present study. In conclusion, the studies have shown that the petroleum ether extract of Malvastrum tricuspidatum contains some active ingredients with the potential of being good anti-inflammatory and analgesic agents. The results of the study were subjected to one way analysis of variance followed by student t-test for multiple comparisons. Values with P < 0.05 were considered significant.

KEYWORDS: Analgesic activity, Anti-inflammatory activity, pharmacological evaluation.

INTRODUCTION

The drugs available in the modern system of medicine are the corticosteroids and immunosuppressive agents which bring about only symptomatic relief and in most cases have no influence on the disease process. Further, their use is associated with the risk of relapses and danger of side effects. Many synthetic drugs are available in the market but due to their side effects and their high cost, treatment of pain and inflammation disease not affordable by poor people.
Indigenous systems of medicine have a strong repository of plants that have been used traditionally to offer some sort of pain & inflammation protection. Less side effects, easy availability and highly economical factors makes the herbal drug better alternative of synthetic drugs.

It is considered worthwhile to investigate some indigenous plants which have reputation in Ayurveda and folk medicines. On the basis of literature survey it was found that *Malvastrum tricuspidatum* play important role to treat Pain & inflammation disease but still no scientific attempt has been made to investigate the analgesic and anti inflammatory activity of both the plants. So, the present work is an attempt to study the analgesic and anti inflammatory activity of *Malvastrum tricuspidatum* stem bark extract.

**MATERIALS AND METHODS**

*Malvastrum tricuspidatum* stem bark was collected from local area Alwar, Rajasthan and leaves of plant were collected from the natural habitats. Formic acid, Acetic acid, Acetone, Chloroform, Ethyl acetate, Diethylamine, Toluene, Ethanol and other chemical and solvents were of analytical grade/IP/equivalent gradeand procured from laboratory.

**Extraction procedure**

**Defattation of Malvastrum tricuspidatum stem bark (powdered)**

200g coarse powdered stem bark was defatted with 800 ml petroleum ether (60-80°C) using soxhlet apparatus. Extraction was continued until a drop of solvent from siphon tube, when evaporated on filter paper, did not leave a greasy spot (approximately 10-12 cycles). After the defattation, mark was taken out from extractor and spreaded as a bed on a clean paper and dried till evaporation of petroleum ether. Mark was kept for ethanolic extraction.

**Ethanolic extraction of M.tricuspidatumstem bark**

The dried mark obtained after defattation was packed in soxhlet apparatus and extracted with 800 ml ethyl alcohol in soxhlet apparatus. Extraction was continued until a drop of solvent from the siphon tube, when taken on TLC plate and sprayed with concentrated sulphuric acid, does not give a black spot. Dark brown extract thus obtained, was collected and solvent was evaporated under reduced pressure.
Evaluation of Anti-Inflammatory Activity

Drug dose
The doses considered for the experiment on mice for the anti-inflammatory and analgesic experiments were carried out in two different doses of 200 and 400 mg/kg body weight.

Preparation of test drug and standard drug
Test drug (petroleum ether extract of Malvastrum tricuspidatum) and standard drug (Indomethacin) were prepared as a suspension in distilled water using mortal and pestle.

Animal grouping
The animals were divided into four groups for anti-inflammatory and analgesic studies. Each group consisted of six animals of either sex. The groups were:

Group I: Negative control - Distilled water
Group II: Test drug- Petroleum ether extract of Malvastrum tricuspidatum - 200 mg/kg bodyweight (MLE-200)
Group III: Test drug- Petroleum ether extract of Malvastrum tricuspidatum - 400 mg/kg bodyweight (MLE-400)
Group IV: Positive control- Standard drug indomethacin- 2.5 mg/kg bodyweight (Indo-2.5)

Carrageenan induced mice paw edema
Acute inflammation was produced by sub plantar injection of 0.1 ml of 1% 0.1ml of 1% carrageenan in normal saline in the hind paw of rats 1 h after the administration of the test drug as well as positive and negative controls. The paw volume was measured at 1 h, 2 h and 3 h after carrageenan injection, using plethysmograph.

Dextran induced mice paw edema
The animals were treated in a manner similar to that of carrageenan induced paw edema model. 0.1 ml 1% dextran was used for the study. Paw volume was measured as mentioned in carrageenan induced paw edema model at 1 h, 2 h and 3 h.

Histamine induced mice paw edema
In this model paw edema of a mice was induced by subplantar injection of 0.1ml of 1% freshly prepared histamine in normal saline and the paw oedema was measured as mentioned in carrageenan induced paw edema model. The paw volume was measured at 0.5 h, 1 h, 2 h and 3 h.
Formalin induced paw edema
The test drug was administered once daily for seven consecutive days to all the groups. On seventh day, initial paw volume was measured before drug administration. After 1 h of drug administration, paw edema of the mice was induced by subplantar injection of 0.1 ml of 3% formalin solution in normal saline. Paw volumes were measured at 3 h, 24 h and 48 h after formalin injection as described earlier in carrageenan model.

Cotton pellet induced granuloma in rats
Cotton pellet induced granuloma formation in rats was performed for chronic anti-inflammatory study. This model represents the exudative and proliferative phases of inflammation. The cotton pellets weighing 100 mg were made by rolling of cotton piece and sterilizing. The rats were anaesthetized with ether; dorsum was shaved clear and swabbed with 70%(v/v) alcohol. Midline incision of 1 cm was made in the intrascapular region. A small tunnel was made on either side of the incision with the help of a small blunt forceps. Sterile cotton pellet (100 mg) was implanted in each tunnel. Air was removed from the tunnel and then incision was closed with sutures. The test drugs were administered for 7 consecutive days starting from the day of implantation. The rats were sacrificed on the 8th day, cotton pellets were removed and cleaned of extraneous tissue and dried by placing them in a hot air oven overnight at 80°C and then weighed. The difference between the initial weight and the final weight of the pellet after drying was taken as the granuloma tissue weight. The results were expressed as mg granulation tissue formed per 100 g body weight.

Evaluation of Analgesic Activity
Analgesic study was determined in two different models:

Acetic acid induced writhing in mice
Intraperitoneal injection of diluted solution of acetic acid is a well established animal model for tonic visceral pain in rodents. Swiss albino mice were used for acetic acid induced writhing test. Mice were divided in to four groups. Test drug or standard drug or distilled water was administered orally, 1 h prior to the injection of acetic acid. Writhing was induced by administrating of 3% petroleum ether extract solution of acetic acid (10ml/kg body weight) intraperitoneally. Immediately after the acetic acid injection, each animal was placed in a transparent observation cage and the number of writhes per mice was counted for 30 min. Writhing movement is accepted as contraction of the abdominal muscles accompanied by stretching of hind limbs. The percentage inhibition was calculated using the following ratio:
Formalin induced paw licking response in rats
The effect of petroleum ether extract of Malvastrum tricuspidatum upon formalin induced paw licking response was evaluated. The procedure is same as that followed for formalin induced hind paw edema, which is mentioned above. After the injection of formalin, the animals were kept under observation for half an hour. The amount of time spent licking the injected paw was noted, and was considered to be indicative of pain. The time taken for the onset of paw licking was initially measured. The first of the nociceptive responses normally peaked 5 min after formalin injection and the second phase 15-30 min after formalin injection, representing the neurogenic and inflammatory pain. Therefore, the frequency of paw licking was measured in five intervals at 0-5 min., 6-10 min., 11-15 min., 16-20 min. and 21-30 min.

RESULT AND DISCUSSION
The results of screening of anti-inflammatory activity of Malvastrum tricuspidatum in carrageenan induced rat paw edema. Malvastrum tricuspidatum was selected for further pharmacological studies. Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1-2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase (3 h) is associated with neutrophil originated free radicals, such as hydrogen peroxide, superoxide and hydroxyl radicals, as well as prostaglandin release.

The results of anti-inflammatory activity of petroleum ether extract of Malvastrum tricuspidatum in carrageenan induced paw edema is shown in Table 15. MLE-200 group showed potent anti-inflammatory activity at 1 h (26.41%), 2 h (32.36%) and 3 h (43.36%). MLE-400 group showed significant decrease in paw volume at 1 h (33.85%), 2 h (54.74%) and 3 h (59.69%). Indomethacin group showed significant decrease in paw volume at 1 h (67.45%), 2 h (60.62%) and 3 h (76.15%, P≤0.01). MLE-200 showed more anti-inflammatory activity than reference drug indomethacin at 1 h, while MLE-400 group showed more anti-inflammatory activity than reference drug indomethacin at 1 h and 2 h.
Thus, it can be concluded that petroleum ether extract of *Malvastrum tricuspidatum* has potent anti-inflammatory activity in carrageenan induced rat paw edema in early phase (1 h) and also in later phase (3 h).

It is well established that carrageenan and dextran induce rat paw oedema by different mechanisms. Dextran is a polysaccharide of high molecular weight that induces anaphylactic reaction after injection in rat’s extremities, which is characterized by extravasation and oedema formation, as a consequence of liberation of histamine and serotonin from mast cells.

The results of anti-inflammatory activity of petroleum ether extract of *Malvastrum tricuspidatum* in dextran induced paw edema is shown in Table 16. MLE-200 group and MLE-400 group showed significant anti-inflammatory activity in dextran induced paw edema model when compared with the control group. MLE-400 group showed significant decrease in paw volume at 1 h (41.26%), 2 h (57.70%) and 3 h (69.04%). Indomethacin group showed significant decrease in paw volume at 1 h (46.29%), 2 h (59.64%) and 3 h (71.04%). Petroleum ether extract of *Malvastrum tricuspidatum* showed anti-inflammatory activity in dose dependant manner. The results tend to suggest that the anti-inflammatory activity of the petroleum ether extract of *Malvastrum tricuspidatum* is possibly backed by its antihistamine or anti-serotonin activity.

The histamine is a basic amine related with inflammatory and allergic process causing, among several effects, both vasodilatation and increase of vascular permeability. The results of anti-inflammatory activity of petroleum ether extract of *Malvastrum tricuspidatum* in histamine induced paw edema is shown in Table 17. Anti-inflammatory activity of MLE-200 and MLE-400 groups was statistically significant at 1 h, 2 h and 3 has compared with the control group. MLE-400 group showed significant decrease in paw volume at 1 h (26.16%) and highly significant at 2 h (42.72%) and 3 h (46.42%). Reference drug indomethacin group showed highly significant decrease in paw volume at 1 h (57.08%), 2 h (66.45%) and 3 h (66.34%) as compared with the control group. Thus MLE-200 and MLE-400 groups did not show higher anti-inflammatory activity than reference drug indomethacin group.

The results of anti-inflammatory activity of petroleum ether extract of *Malvastrum tricuspidatum* formalin induced paw edema is shown in Table 18. It is well known that inhibition of formalin induced paw edema in rats is one of the most suitable test procedures to screen anti-arthritic and anti-inflammatory agents as it closely resembles human arthritis.
Thus formalin-induced paw edema is a model used for the evaluation of an agent with antiproliferative activity. Injection of formalin subcutaneously into hind paw of rats produces localized inflammation. The administration of MLE-200, MLE-400 and indomethacin daily for 7 days successfully inhibited edema induced by formalin (Table 18). MLE-200 group showed decrease in paw volume at 3 h (40.79%), 24 h (32.98%) and 48 h (36.96%). MLE-400 group showed decrease in paw volume at 3 h (44.96%), 24 h (38.50%) and at 48 h (39.67%). Indomethacin group showed decrease in paw volume at 3 h (56.98%), 24 h (42.64%) and 48 h (50.12%). MLE-200 and MLE-400 groups showed almost similar anti-inflammatory activity at 3 h, 24 h and 48 h. Thus, from the results, it can be concluded, that petroleum ether extract of *Malvastrum tricuspidatum* has higher anti-inflammatory activity in formalin induced paw edema test. MLE showed significant decrease in paw volume till 48 h with both doses, which suggests its long duration of action.

Cotton pellet granuloma test is a chronic inflammation model commonly used to evaluate the anti-proliferative activities of drugs. Tissue granulation, one of the distinctive features of chronic inflammation, which is composed of marked in filtration macrophages and neovascularization, was induced by subcutaneous implantation of biomaterials. The implanted material induces a host’s inflammatory response and modulates the release of inflammatory mediators which finally leads to tissue proliferation and granular formation.

The results of anti-inflammatory activity of petroleum ether extract of *Malvastrum tricuspidatum* in cotton pellet induced granuloma is shown in Table 19. In the present study, MLE-200 and MLE-400 groups showed dose dependent activity and markedly inhibited granuloma formation surrounding the pellets compared with the control group. MLE-200 group showed significant decrease in granuloma formation with 26.01% while MLE-400 group showed significant decrease in granuloma formation with 27.68% which was almost near to the reference drug indomethacin (35.97%) group. Thus, the results showed that petroleum ether extract of *Malvastrum tricuspidatum* in potent anti-inflammatory activity in chronic inflammatory model.

Petroleum ether extract of *Malvastrum tricuspidatum* in used to distinguish between the central and peripheral analgesic action by acetic acid induced writhing response in mice. This method is not only simple and reliable but also affords rapid evaluation of peripheral type of analgesic action. In this test the animals react with characteristic stretching behaviour, which is called writhing. The writhing response of the mouse to an intraperitoneal injection of
noxious chemical is used both peripherally and centrally acting analgesic activities. Intraperitoneal injection of diluted solutions of acetic acid is a well-established animal model for tonic visceral pain in rodents. Intraperitoneal injection of acetic acid induces a stereotypic response pattern in the form of abdominal contractions (lengthwise stretches of the torso with a concomitant concave arching of the back) that may persist beyond 6h after the administration, although most contractions occur within 30 min of the application of the irritant.

The results of analgesic activity of petroleum ether extract of *Malvastrum tricuspidatum* in acetic acid induced writhing test is shown in Table 20. MLE-200 and MLE-400 groups showed 16% and 35% inhibition significantly in abdominal writhes produced by acetic acid respectively as compared with the control group. Thus, MLE-200 and MLE-400 groups produced a significant and dose dependent inhibition of analgesic effect produced by acetic acid. Indomethacin group showed significant inhibition with 64% as compared with the control group. This probably means that the extract is able to reduce the receptor sensitivity to the acetic acid induced pain in a dose dependent manner.

The formalin test is a valid and reliable model of nociception, and it is sensitive to various MLEsses of analgesic drugs. The formalin test may be more useful as model of pain in which the first phase seems to be due to direct chemical activation on nociceptive afferent fibers, whereas the second phase is dependent of peripheral inflammation and changes in central processing.

The results of analgesic activity of petroleum ether extract of *Malvastrum tricuspidatum* in formalin induced paw licking are shown in Table 21. The onset time of paw licking was measured after formalin injection. MLE-200 and MLE-400 showed 9.52% and 19.04% increase in onset time respectively, while indomethacin group showed 25% increase in onset time as compared with the control group. Onset time of paw licking response of MLE-400 group was almost the same as that of the indomethacin group. MLE-400 and indomethacin groups showed statistically significant increase in paw licking response.

After formalin injection, the frequency of paw licking was measured 5 min, 10 min, 15 min, 20 min and 30 min. MLE-400 group showed 30% decrease in frequency between 0-5 min, 60.83% between 6-10 min, 64.13% between 11-15 min, 14.24% between 16-20 min and 13.93% between 21-30 min. Indomethacin group showed 23.01% decrease in frequency
between 0-5 min, 40.01% between 6-10 min, 48.62% between 11-15 min, 71.62% between 16-20 min. Statistically significant decrease in frequency between 0-5 min was seen in MLE-400 group and indomethacin group. Statistically significant decrease in frequency between 6-10 min was seen in MLE-400 group and indomethacin group.

Table 15: Anti-inflammatory activity of petroleum ether extract of *Malvastrum tricuspidatum* in carrageenan induced paw edema.

<table>
<thead>
<tr>
<th>Groups</th>
<th>After 1h Increase in Paw volume (%)</th>
<th>% Changes</th>
<th>After 2h Increase in Paw volume (%)</th>
<th>% Changes</th>
<th>After 3h Increase in Paw volume (%)</th>
<th>% Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>53.23± 1.87</td>
<td>-</td>
<td>42.21± 4.18</td>
<td>-</td>
<td>38.62± 4.69</td>
<td>-</td>
</tr>
<tr>
<td>MTA-200</td>
<td>39.03± 4.85*</td>
<td>26.41</td>
<td>28.32± 2.30**</td>
<td>33.85</td>
<td>15.61± 2.89**</td>
<td>60.62</td>
</tr>
<tr>
<td>MTA-400</td>
<td>36.85 ±2.96**</td>
<td>32.07</td>
<td>19.50± 2.50***</td>
<td>54.74</td>
<td>9.23± 2.66**</td>
<td>76.15</td>
</tr>
<tr>
<td>Indo-2.5</td>
<td>30.19± 2.53***</td>
<td>43.36</td>
<td>17.51± 3.44*</td>
<td>59.69</td>
<td>7.81± 3.11**</td>
<td>81.38</td>
</tr>
</tbody>
</table>

n=6, ns- non significant, *p<0.05- significant, **p<0.01-more significant v/s control, SEM= standard error mean, SD = standard deviation, n= number of animals MTA 200- *Malvastrum tricuspidatum* petroleum ether extract 200mg/kg b.wt, MTA 400- *Malvastrum tricuspidatum* petroleum ether extract 400mg/kg b.wt, Indo-2.5- Indomethacine 2.5 mg/kg b.wt.

![Figure 2: Anti-inflammatory activity of petroleum ether extract of Malvastrum tricuspidatum in carrageenan induced paw edema.](image)

Table 16: Anti-inflammatory activity of petroleum ether extract of *Malvastrum tricuspidatum* in dextran induced paw edema.

<table>
<thead>
<tr>
<th>Groups</th>
<th>After 1h Increase in Paw volume (%)</th>
<th>% Changes</th>
<th>After 2h Increase in Paw volume (%)</th>
<th>% Changes</th>
<th>After 3h Increase in Paw volume (%)</th>
<th>% Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>58.30± 1.27</td>
<td>-</td>
<td>46.20± 4.28</td>
<td>-</td>
<td>38.59± 6.69</td>
<td>-</td>
</tr>
</tbody>
</table>
n=6, ns- non significant, *p<0.05- significant, **p<0.01-more significant v/s control, SEM= standard error mean, SD = standard deviation,  n= number of animals

MTA 200- *Malvastrum tricuspidatum* petroleum ether extract 200mg/kg b.wt, MTA 400- *Malvastrum tricuspidatum* petroleum ether extract 400mg/kg b.wt, Indo-2.5- Indomethacine 2.5 mg/kg b.wt.

Figure 3: Anti-inflammatory activity of petroleum ether extract of *Malvastrum tricuspidatum* in dextran induced paw edema.

Table 17: Anti-inflammatory activity of petroleum ether extract of *Malvastrum tricuspidatum* in histamine induced paw edema.

<table>
<thead>
<tr>
<th>Groups</th>
<th>After 1h</th>
<th>After 2h</th>
<th>After 3h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Increase in Paw volume (%)</td>
<td>% Changes</td>
<td>Increase in Paw volume (%)</td>
</tr>
<tr>
<td>Control</td>
<td>58.42 ± 2.15</td>
<td>-</td>
<td>45.253 ± 1.76</td>
</tr>
<tr>
<td>MLE-200</td>
<td>43.24 ± 1.48**</td>
<td>25.99</td>
<td>28.72 ± 3.53*</td>
</tr>
<tr>
<td>MLE-400</td>
<td>43.14 ± 3.46***</td>
<td>26.16</td>
<td>25.92 ± 1.62***</td>
</tr>
<tr>
<td>Indo-2.5</td>
<td>25.07 ± 3.72***</td>
<td>57.08</td>
<td>15.19 ± 2.84***</td>
</tr>
</tbody>
</table>

n=6, ns- non significant, *p<0.05- significant, **p<0.01-more significant v/s control, SEM= standard error mean, SD = standard deviation,  n= number of animals
MTA 200- *Malvastrum tricuspidatum* petroleum ether extract 200mg/kg b.wt, MTA 400-
*Malvastrum tricuspidatum* petroleum ether extract 400mg/kg b.wt, Indo-2.5- Indomethacine 2.5 mg/kg b.wt.

![Figure 5: Anti-inflammatory activity of petroleum ether extract of *Malvastrum tricuspidatum* in histamine induced paw edema.](image)

Table 18: Anti-inflammatory activity of petroleum ether extract of *Malvastrum tricuspidatum* in formalin induced paw edema.

<table>
<thead>
<tr>
<th>Groups</th>
<th>After 1h Increase in Paw volume (%)</th>
<th>% Changes</th>
<th>After 2h Increase in Paw volume (%)</th>
<th>% Changes</th>
<th>After 3h Increase in Paw volume (%)</th>
<th>% Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50.05 ± 3.98</td>
<td>-</td>
<td>52.03 ± 4.98</td>
<td>-</td>
<td>38.57 ± 4.23</td>
<td>-</td>
</tr>
<tr>
<td>MLE-200</td>
<td>30.40 ± 2.76*</td>
<td>40.79</td>
<td>35.13 ± 2.12*</td>
<td>32.98</td>
<td>24.70 ± 4.31*</td>
<td>36.96</td>
</tr>
<tr>
<td>MLE-400</td>
<td>28.25 ± 4.76*</td>
<td>44.16</td>
<td>32.31 ± 3.45*</td>
<td>38.50</td>
<td>23.27 ± 4.71**</td>
<td>39.67</td>
</tr>
<tr>
<td>Indo-2.5</td>
<td>22.60 ± 3.73*</td>
<td>56.98</td>
<td>30.99 ± 2.52**</td>
<td>42.64</td>
<td>19.21 ± 3.89 **</td>
<td>50.12</td>
</tr>
</tbody>
</table>

n=6, ns- non significant, *p<0.05- significant, **p<0.01-more significant v/s control, SEM= standard error mean, SD = standard deviation,  n= number of animals

MTA 200- *Malvastrum tricuspidatum* petroleum ether extract 200mg/kg b.wt, MTA 400-
*Malvastrum tricuspidatum* petroleum ether extract 400mg/kg b.wt, Indo-2.5- Indomethacine 2.5 mg/kg b.wt.
Figure 5: Anti-inflammatory activity of petroleum ether extract of *Malvastrum tricuspidatum* in formalin induced paw edema.

Table 19: Anti-inflammatory activity of petroleum ether extract of *Malvastrum tricuspidatum* in cotton pellet induced granuloma formation.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pellet weight g/100g Body weight</th>
<th>% Change</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.151 ± 0.011</td>
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</tr>
<tr>
<td>MLE-200</td>
<td>0.118 ± 0.015*</td>
<td>27.10↓</td>
</tr>
<tr>
<td>MLE-400</td>
<td>0.114 ± 0.011*</td>
<td>29.62↓</td>
</tr>
<tr>
<td>Indo-2.5</td>
<td>0.101 ± 0.008**</td>
<td>35.71↓</td>
</tr>
</tbody>
</table>

n=6, ns- non significant, *p<0.05- significant, **p<0.01-more significant v/s control, SEM= standard error mean, SD = standard deviation, n= number of animals

MTA 200- *Malvastrum tricuspidatum* petroleum ether extract 200mg/kg b.wt, MTA 400-*Malvastrum tricuspidatum* petroleum ether extract 400mg/kg b.wt, Indo-2.5- Indomethacine 2.5 mg/kg b.wt.

Figure 6: Anti-inflammatory activity of petroleum ether extract of *Malvastrum tricuspidatum* in cotton pellet induced granuloma formation.
Table 20: Analgesic activity of petroleum ether extract of Malvastrum tricuspidatum in acetic acid writhing test in Swiss albino mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of writhing</th>
<th>% Inhibition</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>68.12±1.3</td>
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<tr>
<td>MLE-200</td>
<td>57.18±3.2**</td>
<td>16.18</td>
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<tr>
<td>MLE-400</td>
<td>44.25 ± 3.3**</td>
<td>35.29</td>
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<tr>
<td>Indo-2.5</td>
<td>24.13±3.2***</td>
<td>64.72</td>
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n=6, ns- non significant, *p<0.05- significant, **p<0.01-more significant v/s control, SEM= standard error mean, SD = standard deviation, n= number of animals

MTA 200- Malvastrum tricuspidatum petroleum ether extract 200mg/kg b.wt, MTA 400- Malvastrum tricuspidatum petroleum ether extract 400mg/kg b.wt, Indo-2.5- Indomethacine 2.5 mg/kg b.wt.

Figure 7: Analgesic activity of petroleum ether extract of Malvastrum tricuspidatum in acetic acid writhing test in Swiss albino mice.

Table 21: Analgesic activity of petroleum ether extract of Malvastrum tricuspidatum in formalin induced paw licking test in mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Onset time (Sec)</th>
<th>% Changes</th>
<th>5 Min</th>
<th>10 Min</th>
<th>15 Min</th>
<th>30 Min</th>
<th>Increase in Paw volume (%)</th>
<th>% Changes</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>42.05±2.13</td>
<td>-</td>
<td>20.60</td>
<td>5.33</td>
<td>6.17</td>
<td>20.17 ± 2.52</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>MLE-400</td>
<td>46.30 ± 9.52</td>
<td>16.35</td>
<td>20.04</td>
<td>3.6</td>
<td>3.33</td>
<td>57.24</td>
<td>17.67 ± 15.07</td>
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<tr>
<td></td>
<td>200</td>
<td>± 1.36</td>
<td></td>
<td>± 0.61</td>
<td></td>
<td>± 0.92</td>
<td></td>
<td>2.03</td>
</tr>
<tr>
<td>MLE-400</td>
<td>50.33 ± 2.56*</td>
<td>14.35 ± 2.13*</td>
<td>30</td>
<td>2.83 ± 0.42**</td>
<td>60.83</td>
<td>2.83 ± 1.18</td>
<td>64.13</td>
<td>11.50 ± 1.09</td>
</tr>
<tr>
<td>Indo-2.5</td>
<td>52.02 ± 1.14*</td>
<td>12.04 ± 1.34*</td>
<td>40.01</td>
<td>1.1 ± 0.78*</td>
<td>80.98</td>
<td>2.17 ± 0.97</td>
<td>71.62</td>
<td>8.04 ± 1.89</td>
</tr>
</tbody>
</table>

n=6, ns- non significant, *p<0.05- significant, **p<0.01-more significant v/s control, SEM= standard error mean, SD = standard deviation,  n= number of animals

MTA 200- *Malvastrum tricuspidatum* petroleum ether extract 200mg/kg b.wt, MTA 400- *Malvastrum tricuspidatum* petroleum ether extract 400mg/kg b.wt, Indo-2.5- Indomethacine 2.5 mg/kg b.wt.

**Figure 8:** Analgesic activity of petroleum ether extract of *Malvastrum tricuspidatum* in formalin induced paw licking test in mice.

**CONCLUSION**

These studies have shown that the petroleum ether extract of *Malvastrum tricuspidatum in contains some active ingredients with the potential of being good anti-inflammatory and analgesic agents. There is a need for detailed investigation of the mechanism of action of petroleum ether extract of *M. tricuspidatum in* based on which a possible therapy can be visualized.

**ACKNOWLEDGEMENTS**

Authors are profusely thankful to Alwar Pharmacy College, Alwar (Raj.), India staff for their constant and perennial support.
REFERENCES