IN VIVO ANALGESIC ACTIVITY OF METHANOLIC EXTRACT OF *DILLENIA INDICA* (L) LEAVES

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**Summary**

Traditionally different parts of *Dillenia indica* have been used for the relief of abdominal pain and the plant is a good source of flavonoids which are reported to possess the analgesic activity, hence the present study was aimed to screen analgesic activity of methanolic extract of leaves using in vivo animal models for pain. Central analgesic activity was assessed using hot plate, tail immersion, formalin induced nociception models while peripheral
analgesic activity using acetic acid induced writhing method. DIM at dose of 400 mg/kg showed a significant decrease in latency period 30 min and 60 min post dose, the findings are supported by tail flick latency period. DIM at 400mg/kg showed a significant inhibition of formalin induced liking behavior compared to control animals. Pain behavior which was presented as cumulative abdominal stretching response. DIM at all dose levels tested showed a significant inhibition of stretching response. The findings supports folkloric use on Dillenia indica in pain management.

**Keyword:** Analgeic, *Dillenia indica*, Flavonoids,

**Introduction**

Due to the adverse side effects, like gastric lesions, dyspepsia, nausea vomiting, renal insufficiency, nephropathy and hepatotoxicity associated with NSAIDs and tolerance and dependence induced by opiates, the use of these drugs as analgesic agents have not been successful in all the cases. Therefore, analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates. In search of newer analgesics, traditionally used plant based medicines have been paid great attention as herbals are cheap with little side effects, possess diverse pharmacological profile and are easily available. As per WHO still about 80% of the world population rely mainly on plant based drugs.\(^1\)

*Dillenia indica* L. belongs to the family Dilleniaceae is commonly known as karambel or karmal in Marathi, chalta in hindi and Ramphal in Nepal. Dillenia is a common evergreen tree that grows widely in tropical forests in western peninsula, bihar, assam, Bengal and southern india. It has been grown in gardens as an ornamental plant.
Different parts of the plant have a significant value in traditional system of medicine. It is used in the treatment of abdominal pain. The juice of fruit mixed with sugar and water, used as a cooling beverage in fever and in cough mixtures. The plant is also used as a laxative, tonic and has astringent properties. Phytochemical profiling of different parts revealed the presence of flavonoids such as kaempferol, quercetin, isorhamnetin, naringenin and lupeol type of triterpene such as betulin and betulinic acid. A far as pharmacological profile is concern the fruit of the plant showed antioxidant activity while alcoholic extract of leaves is reported to possess CNS depressant activity. Hence, considering the traditional use in abdominal pain and antioxidant potential mainly due to presence of phenolic constituents the work was proposed to study the possible analgesic activity of the leaves of *Dillenia indica* L. using in vivo models for central as well as peripheral analgesia.

**Material and Methods**

**Drugs and chemicals**

1. Indomethacin (Indocin®25 capsule), E. M. Pharmaceuticals Pvt. Ltd., Ankleshwar, was used as a reference standard for different studies.
2. Pentazocine (Riddof®, Pentazocine lactate injection I.P. 30mg/ml) of Neon Laboratories Ltd. Used as a reference/standard for evaluation of central analgesic activity

**Animals**

Wistar rats (200-220gm) and Swiss albino mice (20-25gm). Animals were housed in polypropylene cages (6 animals/cage) under hygienic conditions in the CPCSEA registered and approved animal house (CPCSEA/87/1999) of Institute of Chemical Technology, Matunga, Mumbai and fed with standard pelleted diet(Amrut India Ltd. Pune and Chakan oil mills, sangali) and water *ad libitum* Chakan. Light cycle of 12hr light/dark was strictly followed in the animal house.
The animals were observed for any infection or any metabolic diseases during the study period and respective physician was consulted in any critical conditions.

**Acute toxicity studies**

Oral acute toxicity study in rats and mice were carried out for methanolic extract of *Dillenia indica* in accordance with OECD guideline no.423 and LD50 of the extract was found to be 2000 mg/kg b.w. One tenth of this (i.e. 200 mg/kg b.w.) was selected as maximum dose for the evaluation of analgesic activity. One lower dose(100 mg/kg b.w) and one higher dose(400 mg/kg b.w) was selected for the evaluation.

**Plant Material**

*Dillenia indica* L. leaves were obtained in the month of September from Veermata Jijabai Bhosle Udyan Byculla, Mumbai, India and was authenticated. The leaves were shade dried and defatted with petroleum ether. The defatted material was extracted with 95% methanol and vaccume dried to get methanolic extract of *Dillenia indica* (DIM).

**Experimental Procedures**

**Phytochemical screening**

Methanolic extract of Dillenia indica (DIM) was subjected to different phytochemical tests for presence of phytoconstituents.

**Pharmacological screening**

For in vivo evaluation animals were divided into five groups (6 animal/ group). Group I- vehicle control which received distilled water, Group II - Positive control (Pentazocine/ indomethacin) and Group III-V as test groups.
**Hot plate method**

Experiment was carried out according to previously described method by Adzu et al.,\(^8\) was used with minor modifications. Positive control group received Pentazocine at 15mg/kg \textit{p.o.} and three treatment groups received DIM at 100mg/kg, 200mg/kg and 400mg/kg \textit{p.o.}

Mice were placed on hot plate maintained at 55±0.5\(^\circ\)C. The time elapsed until the occurrence of either hind paw licking or jump off the plate surface was recorded as the hot-plate latency. Mice with baseline latencies of more than 15 sec were excluded from the study. After the baseline determination of response latencies, hot-plate latencies were measured at 30min and 60min after treatment. The cut-off time was kept at 20 sec to avoid any damage to tissues. Prolongation of latency times of treatment groups were statistically compared with latencies of vehicle control group.

**Tail immersion method**

The method previously described by Asongalem et al.,\(^9\) was used with minor modifications. Positive control group received Pentazocine at 15mg/kg \textit{p.o.} and three treatment groups received DIM at 100mg/kg, 200mg/kg and 400mg/kg \textit{p.o.} Terminal 3cm part of rat tail was immersed in water bath maintained at 55 ±0.5\(^\circ\)C. The reaction time of rats to the thermal stimuli was recorded with stopwatch. Each animal served as its own control.

The reaction time of treatment groups was taken at intervals 0.5, 1, 2, 4 hr after a latency period of 30 min following treatment. The cut-off time, i.e. time of no response was kept at 15 sec. to avoid any injury to the tail.

The reaction time for each rat was recorded and statistically compared with vehicle control group.
Formalin induced nociception in mice
The formalin test identifies mainly centrally active drugs, whereas peripherally acting analgesics are almost ineffective. Therefore, the formalin test may allow dissociation between centrally and peripherally acting drugs.

The method previously described by Santos et al.,\textsuperscript{10} was used with minor modifications. Positive control group received Pentazocine at 15mg/kg \textit{p.o.} and three treatment groups received DIM at 100mg/kg, 200mg/kg and 400mg/kg \textit{p.o.} After 60 minutes of treatment animals were injected with 20 \textmu{l} of 5\% v/v formalin (in 0.9\% NaCl) subcutaneously into the right hind-paw. The duration of paw licking(s) as an index of painful response was determined at 0–15 min (early phase, neurogenic) and 15–60 min (late phase, inflammatory) after formalin injection.\textsuperscript{11}

Time spend by the animals in licking or biting the injected paw was measured in two phases, 0-15min and 15-60min post injection and results were statistically compared with vehicle control and percentage inhibition of paw licking was calculated as,

\[
\text{% Inhibition} = \frac{\text{mean licking time in control group} - \text{mean liking time in test group}}{\text{mean lining time in control group}} \times 100
\]

Acetic acid induced writhing in mice
Abdominal constriction induced by intraperitoneal injection of acetic acid was carried out according to previously described method by Elisabetsky et al.,\textsuperscript{12} with minor modifications. Positive control group received Indomethacin at 20mg/kg \textit{p.o.} and three treatment groups received DIM at 100mg/kg, 200mg/kg and 400mg/kg \textit{p.o.} One hour after treatment animals were injected with 0.1ml of 1\% v/v acetic acid. The number of constrictions of each animal was measured for a period of 20 min. after acetic acid injection and percentage of protection was calculated.

The total no. of writhes in all the treatment groups was recorded and statistically compared with vehicle control using. and percentage inhibition of writhings were calculated by
% Inhibition of writhes = (mean writhes in control group − mean writhes in test group/mean writhes in control group) X 100

**Statistical comparison**
Results are shown as mean ± SD for six animals in each group and results of test group were compared with control group using one way analysis of variance (ANOVA) followed by dunnetts test, P values< 0.01 are considered significant.

**Results**

**Phytochemical screening:**

<table>
<thead>
<tr>
<th>Extract</th>
<th>Steroids</th>
<th>Alkaloids</th>
<th>Flavonoids</th>
<th>Tannins</th>
<th>Glycosides</th>
<th>Saponins</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 1: Effect of DIM in hot plate test

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean Latency(seconds) before and after drug administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>13.83 ±1.60</td>
</tr>
<tr>
<td>Pentazocine 15mg/kg p.o.</td>
<td>11.25±1.37</td>
</tr>
<tr>
<td>DIM 100mg/kg p.o.</td>
<td>13.18±1.59</td>
</tr>
<tr>
<td>DIM 200mg/kg p.o.</td>
<td>10.42±1.26</td>
</tr>
<tr>
<td>DIM 400mg/kg p.o.</td>
<td>8.40±0.70</td>
</tr>
</tbody>
</table>

Values are Mean ± S.D (n=6) ** P<0.01, significant Vs vehicle control, # P>0.05 not significant
Table 2: Effect of DIM on tail flick latency in tail immersion method.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean Latency(sec) before and after drug administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>1.14±</td>
</tr>
<tr>
<td></td>
<td>0.22</td>
</tr>
<tr>
<td>Pentazocine 15mg/kg p.o.</td>
<td>1.18±</td>
</tr>
<tr>
<td></td>
<td>0.35</td>
</tr>
<tr>
<td>DIM 100mg/kg p.o.</td>
<td>1.45±</td>
</tr>
<tr>
<td></td>
<td>0.33</td>
</tr>
<tr>
<td>DIM 200mg/kg p.o.</td>
<td>1.20±</td>
</tr>
<tr>
<td></td>
<td>0.34</td>
</tr>
<tr>
<td>DIM 400mg/kg p.o.</td>
<td>0.66±</td>
</tr>
<tr>
<td></td>
<td>0.32</td>
</tr>
</tbody>
</table>

Values are Mean ± S.D (n=6) ** P<0.01, significant Vs vehicle control, # P>0.05 not significant

Table 3: Effect of DIM on Formalin Induced Nociception

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time (in seconds) spend in licking / biting the paw</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>91.29±28.35</td>
<td>-</td>
</tr>
<tr>
<td>Pentazocine 15mg/kg p.o.</td>
<td>39.70±7.22 **</td>
<td>56.51 %</td>
</tr>
<tr>
<td>DIM 100mg/kg p.o.</td>
<td>68.28±12.31</td>
<td>25.21 %</td>
</tr>
<tr>
<td>DIM 200mg/kg p.o.</td>
<td>61.40±7.07</td>
<td>32.74 %</td>
</tr>
<tr>
<td>DIM 400mg/kg p.o.</td>
<td>41.29±17.78**</td>
<td>54.77 %</td>
</tr>
</tbody>
</table>

Values are Mean ± S.D (n=6) ** P<0.01, significant Vs vehicle control
Table 4: Effect of DIM on acetic acid induced writhing

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total number of writhing</th>
<th>% inhibition of writhing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>109.20± 9.31</td>
<td>-</td>
</tr>
<tr>
<td>Indomethacin 20mg/kg p.o.</td>
<td>17.83± 5.71**</td>
<td>83.7</td>
</tr>
<tr>
<td>DIM 100mg/kg p.o.</td>
<td>47.50± 5.58**</td>
<td>56.5</td>
</tr>
<tr>
<td>DIM 200mg/kg p.o.</td>
<td>22.60±3.21**</td>
<td>79.3</td>
</tr>
<tr>
<td>DIM 400mg/kg p.o.</td>
<td>22.00±9.27**</td>
<td>79.9</td>
</tr>
</tbody>
</table>

Values are Mean ± S.D (n=6) ** P<0.01, significant Vs vehicle control,

**Analgesic effect**

Table 1 demonstrates the effect of DIM on hot plate latency period. DIM at dose of 200 mg/kg and 400 mg/kg showed a significant decrease in latency period 30 min and 60 min post dose. The tail flick latency period at 400mg/kg one hour post dose was significantly increased while lower doses 100 and 200 mg/kg do not show significant change (Table 2), DIM at 400mg/kg showed a significant % inhibition of formalin induced liking response compared to control animals. % inhibition at 100 and 200mg/kg was not significant. In pain behavior which was presented as cumulative abdominal stretching response. Treatment of animals with DIM (100, 200 and 400mg/kg b.w.) showed a significant inhibition of stretching response against control group.

**Discussion**

Analgesic activity was evaluated using different models for central as well as peripheral analgesia.

Hot plate test were predominantly a spinal reflex, and were considered to be selective for centrally acting analgesic compounds,
like pethidine... There are several advantages to select hot plate as a tool to evaluate central analgesic activity such as, the sensitivity to strong analgesics and limited tissue damage.\textsuperscript{13} The hot-plate test produces, at constant temperature, two kinds of behavioural response, which are paw licking and jumping. Both of these are considered to be supraspinally integrated responses.\textsuperscript{14} DIM at 200 and 400\,mg/kg showed significant prolongation of latency period 30 min and 60 min post dose. Findings from in the hot plate is supported by results obtained in tail immersion in which DIM at 400 mg/kg showed significant change in tail flick latency. Tail immersion method, which is used for evaluation of central analgesics,\textsuperscript{15} Study shows that mu (\(\mu\)), kappa (\(\kappa\)) and delta (\(\delta\)) receptors agonist showed dose dependant inhibition of hot plate and tail flick responses. The results obtained are in agreement with the same, hence we can hypothetise that the prolongation of reaction time due to extracts might be attributes to modulation of the receptors involved in pain modulation in the CNS. be due to modulation of these receptors.\textsuperscript{16}

Peripheral analgesic activity was evaluated using acetic acid induced abdominal stretching response. Results revealed that DIM at all dose levels showed significant inhibition of the stretching behaviour. The phlogistic agent, acetic acid induce pain as well as increase of vascular permeability \textsuperscript{13} this nociception model is considered as a non selective anti-nociceptive model because acetic acid indirectly induced the release of endogenous mediators such as bradykinins, substance P and PG which stimulates the nociceptors which are sensitive to non-steroidal anti-inflammatory drugs (NSAID'S).\textsuperscript{14,17} It was postulated that the abdominal constriction response is induced by local peritoneal receptors activation \textsuperscript{18} and involved prostanoids mediators. As a matter of fact, increased levels of PGE\textsubscript{2} and PGF\textsubscript{2} in peritoneal fluids (\textsuperscript{19} as well as lipooxygenase production were also reported.\textsuperscript{20} The results of the present study showed that Indomethacin, which inhibit cyclooxygenase, cause significant inhibition of acetic acid-induced constrictions. This is in accordance with previous reports indicating that this test is sensitive to non-
steroidal anti-inflammatory drugs (NSAID’S). Several studies have shown that compounds with significant mu (µ) and delta (δ) activity showed significant reduction on writhing response than hot plate and tail flick. This results supports the involvement of opioid receptors in pain management as seen in hot plate and tail immersion results. The analgesic actions of extracts may be mediated by inhibition of lipooxygenase and/or cyclo-oxygenase activity or by release of cytokines such as TNF-α, interleukin-1β and interleukin-8; by resident peritoneal macrophages and mast cells, or by direct modulation of mu (µ) and delta (δ) receptors and vanilloid receptors.

Then the extracts were screened using formalin induced nociception method, this test is very useful for evaluating the mechanism of nociception and analgesia. Formalin test is biphasic response with an early and a late phase involving different mechanisms of nociception. In the initial phase acute period (phase 1, duration of 10-15min), and after a short period of remission, phase 2 begins and consists of a longer period (1 hr) of sustained activity. Subcutaneous injection of formalin into the mice paw evokes an array of stereotyped behaviours. Among these behaviors, Flinching (consisting of an elevation and shrinking back of the injected paw) is a reliable parameter of pain behavior.

The initial response was initially attributed to a direct effect of formalin on the nociceptors whereas phase 2 was associated with the release of local endogenous mediators responsible for sensitization of primary and spinal sensory neurons and subsequent activation of the nociceptors. Central analgesic drugs, such as narcotics, inhibited equally in both phases, while peripherally acting drugs, such as steroids (hydrocortisone, dexamethasone) and NSAIDS (aspirin) suppressed mainly in the later phase this indicates that the late phase in formalin test depends on an inflammatory reaction in peripheral tissue. DIM showed significant analgesic activity in both phases suggest an involvement at both central and peripheral levels. This supports the results obtained in Hot plate, Tail immersion and writhing model.
Conclusion

The analgesic activity of methanolic extract of dillenia india leaves might be attributed to its potential to modulate release of inflammatory mediators responsible for pain also the presence of flavonoid constituents which are reported to possess intrinsic analgesic activity. To get detailed insight into the results obtained further studies involving isolated constituents from the leaves may prove to be a useful tool.

Acknowledgement

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References

2. The wealth of India- A dictionary of Indian Raw Materials and Industrial Products, Volume-III, D-E. 2003 National institute of Science communication and information research, CSIR, New Delhi, pp-64


