Evaluation of the analgesic activity of ethanolic extract of *Populus deltoides* leaves in mice

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**ABSTRACT**

The ethanolic leaves extract of *Populus deltoides* was tested for the presence of various phytoconstituents and designed to evaluate the analgesic activity in mice. The peripheral analgesic activity of ethanolic leaves extract of *P. deltoides* (250 and 500 mg/kg) was studied by using acetic acid stimulated writhing test and central analgesic activity of *P. deltoides* was studied by using hotplate process. The ethanolic leaves extract of *P. deltoides* professed the existence of a variety of chemical constituents like alkaloids, saponins, flavonoids, terpenes and steroids. Leaves extract of *P. deltoides* appreciably decreased the writhing actions in acetic acid-induced writhing test in mice and amplified the respond time in hotplate test. These results suggest that the extract may have NSAIDs like activity through the peripheral mechanism and central analgesic activities via opioid receptors. From our study, we endowed that leaves extract of *P. deltoides* has feasible to analgesic activity. This study reveals that it can be used in the management of pain and provide a scientific basis for its traditional use.

**INTRODUCTION**

Pain is the most frequent cause for consulting physician worldwide. It is one of the significant symptoms in various health situations, and can eloquently intrude an individual’s transcendence of life and general practices. Psychological factors like community prompting, hypnotic suggestion, enthusiasm, or perturbation can succinctly slacken pain’s amplitude or disaffection. Pain experiences may include acute and chronic. Chronic pain is decadence status and patient receiving palliative care. It is not solely physiological but further comprises spiritual, sentimental and social amplitude (Marskey and Bugduk, 2012). Various processes can evoke pain. They include nociception, peripheral sensitization, phenotypic switches, central sensitization, ectopic excitability and structural rearrangement is the mechanism that causes nociceptive pain and comprises the processes of transduction, conduction, transmission and perception (Woolf, 2004). Transduction is an alteration of a noxious stimulus including chemical, mechanical or thermal into electrical impulses in the peripheral terminals of nociceptor of free afferent nerve fibres. Conduction is the second phase of nociception, and the action potentials from the peripheral process conducted along axons to the central process of nociceptors in the central nervous system (Mannion et al., 1999) transmission is the synaptic transfer from presynaptic terminals to the postsynaptic terminals of neurotransmitter receptor (Urch, 2007) and modulation of input is an adaptive process from one neuron to another and involve excitatory of inhibitory mechanism (Kirkpatrick et al., 2015). Perception of pain is dependent on neural processing in the spinal cord.
and brain (Mordeniz, 2016).

*Populus deltoides* is a large tree belongs to family *Salicaceae*, rising to 20 to 40 meters height and with a body up to 1.8 meters diameter, the leaves are big, 4 to 10 cm long, 4 to 11 cm wide, triangular, 3 to 12 cm long petiole with a flattened base. The leaves are dark green, roughly toothed, the teeth are sinuate with glandular tipped, and the petiole is flat (Krishnkumar *et al.*, 2010). Many parts of *Populus deltoides* are used for their analgesic, antipyretic and anti-inflammatory activities and their efficacy is traditionally acclaimed.

**MATERIAL & METHODS**

*Populus deltoides* leaves were obtained from the village garden (Jaunpur District, Uttar Pradesh 222001). The plant was taxonomically identified by Dr K. Ravi Kumar, a Senior Botanist at FRLHT Yelahanka Banglore. A herbarium specimen was conserved in the college museum for future reference. The leaves were dried in the shade at room temperature, powdered and stored in an airtight container.

**Method of extraction**

The coarse powder was subjected for extraction with 70% ethanol by Soxhlet apparatus. The ethanolic extract was concentrated under vacuum and resulting dried extract kept in a desiccator until further use.

**Preliminary phytochemical studies**

Ethanolic leaves extract of the *Populus deltoides* was analyzed for the presence of their active constituents such as alkaloids, saponins, flavonoids, terpenes and steroids were conducted as per the standard procedure (Kulbant *et al.*, 2018; Yadav *et al.*, 2014).

**Test for steroid**

Libermann- Burchard Test

Ten mg of leaf extract was mixed in 1 ml of chloroform, to this 1 ml of acetic anhydride was poured following the pouring of 2 ml concentrated sulphuric acid.

The appearance of reddish-violet colouration reported the presence of steroids (Gul *et al.*, 2017).

**Test for triterpenoids**

Noller Test

To the extract, add few ml of chloroform and filter. To the filtrate, add tin powder and thionyl chloride and warmed gently.

The pink colour indicates the presence of terpenoids (Farooq *et al.*, 2014).

**Test for Alkaloids**

**Mayer’ Test**

One ml of alcoholic extract was put in a test tube and to this added 0.02 ml of dilute hydrochloric acid and 0.1 ml of Mayer’s reagent. A formation of yellowish buff colouration precipitate indicates the presence of alkaloid (Chandrashekar, 2011).

**Dragendroff’s Test**

2 ml alcoholic solution of extract was placed with 0.1 ml of hydrochloric acid and 0.1 ml of Dragendroff’s reagent in a test tube. An orange-brown coloured precipitate was formed, which suggest the presence of alkaloid (Chandrashekar, 2011).

**Test for Flavonoids**

Shinoda’s Test

The extract was mixed with ethanol and took a little quantity of magnesium; following this, added dropwise concentrated hydrochloric acid and heated. The formation of magenta colour exhibits the presence of flavonoids (Vimalkumar *et al.*, 2014).

**Test for Tannin**

Five ml of extract solution taken in a test tube, and 1 ml of 5% solution of ferric chloride was added. The formation of greenish-black colour shows the presence of tannin (Devmurari, 2010).

**Test for Saponin**

One ml extract solution was taken in a graduated cylinder, and distilled water was added and adjusted the volume 20 ml. The cylinder was shaken for 15 minutes. A formation of stable foam indicates the presence of saponins. In a test tube, 1 ml of extract is put with 1% lead acetate solution, the formation of white precipitate exhibit the presence of saponins (Devmurari, 2010).

**Determination of acute toxicity (LD₅₀)**

Acute toxicity test for *P. deltoides* ethanolic extract was conducted following standard method (OECD/OCDE No: 425). Ethanolic extract of *P. deltoides* was found to be safe up to 5000 mg/ kg body weight in albino mice. Hence in the present study, 500mg/kg was taken as an effective dose for the ethanolic extract of *P deltoides* for analgesic activity.

**Evaluation of the analgesic activity of alcoholic leaves extracts of *P. deltoids* by acetic acid-induced writhing in mice**

The analgesic activity of *P. deltoides* leaves extract was studied by using acetic acid-induced writhing syndrome in Swiss albino mice (Lalan,
The Institutional animal ethics committee approved the study protocol, Registration no. 1432/PO/a/11/CPCSEA.

The Swiss albino mice (20-30 g) were screened for writhing syndrome by injecting 1 ml/100 g of 0.06% v/v acetic acid intraperitoneally. The animals, which showed normal writhing, were selected for the studies. The experiment was carried out in four groups, and each was having six albino mice. Group I was given 0.1 ml of normal saline orally, as vehicle control. Group III and IV were given *P. deltoides* extract at a dosing of 250 and 500 mg/kg, p.o respectively as a solution of normal saline orally and group II has given aspirin 100 mg/kg as a positive control in a similar manner.

After 30 minutes of oral administration of the above drugs, 1 ml/100 gm of 0.06% of acetic acid was injected intraperitoneally to all mice. The number of writhing by an individual animal in various groups was counted and recorded for 20 min. The decrease in the number of writhing when compared to control was measured as confirmation for analgesia and which can be represented as a percentage inhibition of writhing (Rai et al., 2016).

\[
\text{Percentage Inhibition} = \frac{W_t(Control) - W_t(test\ group)}{W_t(Control)} \times 100
\]

Where, 
- *Wt* = Mean number of writhing

**Evaluation of the analgesic activity of ethanolic leaves extracts of *P. deltoides* by hot plate test in mice**

This is one of the most usual technique utilized for accessing the central analgesic potential of a drug. Heat is used to induce pain. Mice were grouped into four in a gathering of six each. Group I represented as control, group II represented as standard (Pentazocine 5 mg/kg, intraperitoneally), while III and IV groups administrated 250 and 500 mg/kg of *P. deltoides* extract separately. Following 0, 1, 2, 3, and 4 hrs, mice were independently put on hotplate which was warmed to 55°C ± 0.5°C for not more than 15 seconds to avoid any type of injury on the paws.

The response time taken to flick the rear paw or lick or bounce from the hotplate was estimated as the reaction time of an individual animal. An analgesic compound improves the response time. Per cent decline in response time was taken as an index of pain recognition at each interval (Ganeshpurkar and Rai, 2013).

**Statistical analysis**

The values were represented as means ± SEM. Statistical analysis was performed by graph and prism version 6; one-way analysis of variance (ANOVA) followed by Turkey multiple comparison tests. *P* values < 0.05 were considered as significant.

**RESULT AND DISCUSSION**

**Phytochemical studies**

The ethanolic extract of *P. deltoids* was exposed to a qualitative test to determine the presence of phyto-constituents.

Results were summarized in Table 1 and confirmed the presence of alkaloid, saponins, flavonoids and terpenes, which have demonstrated the analgesic activity on an acetic acid-induced writhing test. The extract did not contain steroids.

![Graph 1](image1.png)

**Figure 1: Effect of ethanolic leaves extract of *P. deltoides* by acetic acid induce writhing in mice**

![Graph 2](image2.png)

**Figure 2: Effect of ethanolic leaves extract of *P. deltoides* on hot plate test in rat**

**Effect of ethanolic leaves extracts of *P. deltoides* by acetic acid-induced writhing in mice.**

The results of acetic acid-induced writhing showed that *P. deltoides* leaves extract (250 and 500 mg/kg) significantly decreases the abdominal writhes count in a dose-dependent way with 67.67% and 69.06% protection while aspirin (100mg/kg) showed maximum 86.67% reduction in writhes count. The results were depicted in Table 2 and graphically represented in Figure 1.

The pain induction occurs by releasing endogenous substances as well as some other pain mediators.
**Table 1: Phytoconstituents present in 70% ethanolic leaves extract of *P. deltoides***

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Ethanolic leaves extract of <em>P. deltoides</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
</tr>
</tbody>
</table>

**NOTE:** + Presence, - Absence

**Table 2: Effect of ethanolic leaves extract of *P. deltoides* by acetic acid induced writhing in mice**

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Dose (mg/Kg)</th>
<th>No. of writhing/min</th>
<th>Percentage Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0-15 min</td>
<td>15-30 min</td>
</tr>
<tr>
<td>Control</td>
<td>Normal saline 5 ml/kg</td>
<td>24.5±1.78</td>
<td>43.1±2.48</td>
</tr>
<tr>
<td>Aspirin</td>
<td>100 mg / kg, p. o.</td>
<td>2.3±0.23</td>
<td>5.83±0.48*</td>
</tr>
<tr>
<td><em>P. deltoides</em></td>
<td>250 mg / kg, p. o.</td>
<td>9.0±0.37</td>
<td>14.3±0.90**</td>
</tr>
<tr>
<td><em>P. deltoides</em></td>
<td>500 mg / kg, p. o.</td>
<td>7.6±0.31</td>
<td>13.4±0.62**</td>
</tr>
</tbody>
</table>

Values represent the Reaction time mean ± S.E.M, (n = 6). *p < 0.05, different from control group

**Table 3: Effect of ethanolic leaves extract of *P. deltoides* on hot plate test in rat**

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Dose (mg/Kg)</th>
<th>Reaction time (sec) at different time intervals (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>Normal saline 5 ml/kg</td>
<td>5.27±0.45</td>
</tr>
<tr>
<td>Pentazocine</td>
<td>5 mg / kg, p. o.</td>
<td>7.92±0.63</td>
</tr>
<tr>
<td><em>P. deltoides</em></td>
<td>250 mg / kg, p. o.</td>
<td>6.95±0.46</td>
</tr>
<tr>
<td><em>P. deltoides</em></td>
<td>500 mg / kg, p. o.</td>
<td>7.75±1.26</td>
</tr>
</tbody>
</table>

Values represent the Reaction time mean ± S.E.M, (n = 6). *p < 0.05, different from control group

like arachidonic acid metabolites cyclooxygenase, Prostaglandin E2 and prostaglandin E2α in peritoneal fluid and also lipooxygenase which stimulates the nociceptive neurons to NSAIDs.

The acetic acid-induced writhing partly inhibits the lipooxygenase and cyclooxygenase peripherally and diminished prostaglandins production and also obstructing in the transduction mechanism of nociception. The phytochemicals of *P. deltoides* like alkaloids, flavonoids, terpenoids, saponins and tannins may be responsible for analgesic activity through a peripherally acting mechanism. Flavonoids also have reported for reduction of arachidonic acid production.

**Effect of ethanolic leaves extract of *P. deltoides* on hot plate test in mice**

*P. deltoides* significantly increases the thermal reaction time of paw withdrawal in mice in comparison to control. Pentazocine is centrally acting and efficiently amplified pain threshold. The extract slowdown the transmission of pain through C fibres to central nervous system and inhibit the licking response suggesting its antinociceptive effect. The
results were shown in Table 3 and graphically represented in Figure 2.

CONCLUSION

The present study showed that ethanolic leaves extract of P. deltoides possesses both central and peripheral analgesic activity. These results suggest that the extract may have NSAIDs like activity through the peripheral mechanism and central analgesic activities via opioid receptors. The result of the experiment indicates that extract is effective in alleviating peripheral and central pain in the acetic acid-induced writhing model the production of PGE2 and PGE2α in the peritoneal fluids. Therefore it is likely that the extract might suppress the formation of these substances or antagonizing their action for its analgesic activity. The peripheral mechanism of the extract may be via inhibition of cyclooxygenase or lipoxygenase and other inflammatory mediators while the central analgesic action may be via the inhibition of primary pain receptor.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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