ISOLATION AND PHARMACOLOGICAL STUDIES OF KARANJACHROMENE FROM THE SEEDS OF PONGAMIA PINNATA (L. PIERRE)

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ABSTRACT

Context: Isolation of chemical compound karanjachromene from the Seeds of Pongamia Pinnata and evaluation of its anti-inflammatory and analgesic activities.

Materials and methods: Karanjachromene has been successfully extracted from the seeds of Pongamia Pinnata using n-hexane, petroleum ether and alcohol with Soxhlet extraction. Anti-inflammatory and analgesic activities of the some were assessed administering in Swiss albino mice. The anti-inflammatory activity of the test compound was determined by mice paw edema inhibition method. The analgesic activity was determined by both acetic acid induced writhing and tail immersion methods.

Results: Karanjachromene at doses 25 mg/kg and 50 mg/kg shown 40.48% and 59.6% inhibition of paw edema respectively, at the end of 3 h standard drug diclofenac sodium produced 63.01% inhibition in paw volume at 10 mg/kg. The oral administration of test compound karanjachromene significantly inhibited writhing response induced by acetic acid in a dose dependent manner. Karanjachromene produced 29.64% and 42.14% inhibition of writhing at doses 25 mg/kg and 50 mg/kg respectively. Standard drug diclofenac sodium produced 56.47 % inhibition of writhing at 10 mg/kg.

Discussion and Conclusion: The administration of karanjachrome is potent to inhibit the paw edema starting from the 1st hour and during all phases of inflammation, which may be due to inhibition of different inflammatory mediators. The acetic acid induced writhing response could be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathways. The test compound inhibited both mechanisms of pain and inflammation and are more found active peripherally than centrally. Karanjachromene exhibited significant anti-inflammatory and analgesic agents

Key Words: Anti-inflammatory activity, Analgesic activity, Karanjachromene

INTRODUCTION

Pongamia pinnata belongs to the family Fabaceae. All parts of the plant viz, root, stem, leaves, flower, bark, seeds and its oil too are used in Ayurveda for the treatment of Anti-hyperglycemic (Badole SL, Bodhankar 2008, Badole SL, Bodhankar 2009), Antilipid peroxidase (Tamrakaretal., 2008; Ahmadetal., Punitha and Manoharan 2006a; Punitha and Manoharan 2006b), Antifungal and antibacterial (Siminet al., 2002, Amit et al., 2011), Antimicrobial (Koysomboon et al., 2004, Alam, 2004, Krishna and Grampurohit, 2006), antiviral (Elanchezhiyan, 1993), antidiarrheal activity (Brijesh et al., 2006), antiplasmodial (Simonsen et al., 2001), anticonvulson (Ashish and sunita, 2010; Ashish and sunita 2009), antidiabetic (Badole and Bodhankar, 2010), Antioxident (Sachin et al., 2011), anti-filarial (Uddin et al., 2003), Antiulcerant (Prabhaetal., 2009), antihyperammonic activity (Chopade et al., 2010, Dahanukumar et al., 2000), Cures leprosyand gonorrhea (Kirtikar and Basu, 1975), liver infections (Nadkarni, 1982) and the oil is used for scabies and rheumatism (Burkill, 1996, Bimla et al., 2003).

Many biologically active chemical compounds have been isolated from various parts of the plant. An aliphatic waxy mat-
ter kaempferol, pongamin, γ-sitosterol-glucoside, quercetin, neoglabin (A complex amino acids) resembling glabrin and galbrosaponin A furano-flavone (i.e., Penguin) (The Wealth of India 2003). Pongaglabol, a hydroxyfurano-flavone, and aurantiamide acetate, phenyl alanine dipeptide, have been isolated together with four furano-flavones (karanjin, lanche-olatin B, kanjone and pinnatin (Talapatra et al., 1980) have been isolated from flowers. Various chemical constituents are isolated from the bark of this plant include seven flavonoids viz., pongaflavone, karanjin, pongapin, pongachrome, 3,7-dimethoxy-3',4,7-tetramethoxy-flavone (Yin et al., 2004) two prenylated flavonoid derivatives viz., pongaflavonol and tunicatachalcone (Yin et al., 2006) cycloart-23-ene-3β, 25-diol (Badole et al., 2011) phenylpropanoids viz., pongapinone A and B (Kitagawa et al., 2008). Moreover, two hydroxy-chalcones – onganones I and II – have been isolated from the bark and characterized (Rastogi et al., 2011). Three furano flavonoids (Pongamosides A, B and C) and a flavonol, glucoside Pongamoside D, have been reported from the n-butanol-soluble fraction of the ethanolic extract P. pinnata fruit (Ahmad et al., 2004). The seeds contain traces of essential oil, complex amino acid termed glabrin, furano flavones, karanjin, kanjone, pongaglabrone, furano flavone (Rastogi et al., 2011; Li et al., 2006) and pyrano flavonoid called Karanjachromene (Naghmana et al., 2008). Furofla- vones viz. Keranjin, pongapin and pinnatin isolated from the seeds, leaves and bark (Chopra, 1969; Parmar et al., 1976) and roots also indicated the presence of protocatechuic, el-egiac, ferulic, gallic, gentisic, 4-hydroxybenzoic and 4-hy- droxycinnamic acids in bark, sorbic, ferulic, gallic, salicylic and p-coumaric acids in leaves; vanillic, gallic and tannic acids in seeds as the main phenolic acids (Sajid et al., 2012), flavonoids and its related compounds including flavones, furano-flavonoids, chromenoflavone, chromenocalchones, coumarins, flavone glycosides sterol, terpenes and modified phenylalanine dipeptides are found to be present (Khare, 2004). Since flavonoids are effective anti-inflammatory and analgesic compounds, and as per our knowledge there are no reports of anti-inflammatory and analgesic activities of Karanjachromene, hence we have carried out these experiments.

MATERIALS AND METHODS

Chemicals
All chemicals and reagents used to carry out the research work were analytical grade and were obtained from Hi-Media Mumbai, India.

Plant material
P. pinnata pods were collected from Gulbarga University campus in October 2010. This plant is as identified by using Flora of Gulbarga District (Seetharam et al., 2000) (Voucher No. HGUG-169). The voucher specimen is kept for the record in the Department of Botany Gulbarga University, Gulbarga.

Extraction and Isolation of karanjachromene
P. pinnata seeds were finely ground for an approximate particle size of 2 mm). The oil content of the seed was extracted with Soxhlet extractor with n-hexane for 20 h and maintaining the temperature at 60˚C. Oil recovered was stored at 4˚C in airtight container for further analysis. After 15 days of storage, granular particles were settled at the bottom of the container. These particles were separated and washed with n-hexane followed by petroleum ether repeatedly. These fine powdered particles were re-dissolved in double distilled alcohol; pointed yellowish crystals were formed at the bottom of the container within a week. This is most convenient and easiest method of isolation of this compound as compared to the previous methods of isolation.

Characterization
The characterization of the compound have been made by taking melting points, infrared spectra, 1H and 13C nuclear magnetic resonance (NMR) and mass spectral analysis.

Structure of Karanjachromene

Animal experiments

Experimental animal
Albino mice of either sex weighing 20-25 g were taken for experimental study. They were acclimated to animal house conditions fed with commercial pellets (Hindustan Lever Ltd., Bangalore, India), and tap water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee.

Determination of median lethal doses (LD50)
LD50 values were estimated by the acute toxicity test as described. The test compound is dissolved in 3% DMSO administered orally to different groups with increasing doses. Four animals were taken in each group. Mortality was deter-
mined after 24 h of treatment. The dose, at which the 50% mice survived, was considered as LD$_{50}$ value of the compound.

**Anti-inflammatory activity**

The anti-inflammatory activity (Winter et al., 1962) of the compound was determined using the carrageenan induced mice paw edema inhibition method employing 1.0% carrageenan solution as the phlogastic agent. The test compound was administered orally as suspensions in 3% DMSO, 30 min before the injection of phlogistic agent, at the dose level 25 and 50 mg/kg body weight. Diclofenac sodium was used as a standard at a dose level of 10 mg/kg body weight. 3% DMSO served as a control. Groups of four albino mice of either sex were used in each experiment. The volume of paw edema was measured with the help of plathysmograph by mercury displacement method at 0h (soon after injection of carrageenan). Then, the volume of paw edema was observed at 1, 2 and 3 h and the results are presented in the Table 1. The percentage inhibition of paw edema was calculated using the formula.

\[
\% \text{ Inhibition} = 1 - \frac{V_t}{V_c} \times 100
\]

Vt and Vc refer to the volumes of paw edema in treated and control group, respectively.

**Acetic acid induced writhing test for analgesic activity**

The analgesic activity of the test sample was studied (Ahmed et al., 2004) using acetic acid induced writhing model in mice. Swiss albino mice of either sex were divided into control, standard and different test groups contain four mice in each. The control group received 3% DMSO and standard group was treated with diclofenac sodium at a dose level of 10 mg/kg test sample and the vehicle were administered orally 30 min before intraperitonial administration of 0.6% acetic acid but diclofenac sodium was administered intraperitonially 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of the body referred to as writhing for the next 30 min the analgesic activity was expressed as percentage inhibition of writhing in mice. The results are given in Table 2.

**Tail immersion test for analgesic activity**

The procedure was based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice (Palanichamy and Nagarajan, 1990). The animals were treated as discussed above. From 1-2 cm of the tail of mice was immersed in warm water kept constant at (54±1°C) and the reaction time was the time taken from the mice to deflect their tails. A cutoff period of 5 sec was observed to avoid damage to their tail. Reaction time was recorded when animal picked up their tails from the hot water at 0, 30, 60 and 90 min after the administration of drugs. The results are shown in Table 3.

**Statistical analysis**

Data obtained from the experiments are expressed as Mean ± SEM. The difference between the control and the treatments in these experiments was tested for significance using one way ANOVA followed by a Dunnett’s t– test.

**RESULTS**

LD$_{50}$ value of karanjachromene was found to be 500 mg/kg body weight. Two doses of Karanjachrome 25 mg/kg and 50 mg/kg have been selected throughout the work. In the carrageenaninduced mouse paw edema test (Table 1) for acute inflammation, the test compound Karanjachromene at doses 25 mg/kg 50 mg/kg shown 40.48 and 59.6% inhibition of paw edema, respectively, at the end of 3 h standard drug diclofenac sodium produced 63.01% inhibition in paw volume at 10 mg/kg.

**Table 1: Anti-inflammatory activity of the Karanjachromene by carrageenan induced paw edema in mice.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose mg/kg (p.o.)</th>
<th>Paw edema volume (ml)</th>
<th>Percentage inhibition of edema volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>2 h</td>
<td>3 h</td>
</tr>
<tr>
<td></td>
<td>1 h</td>
<td>2 h</td>
<td>3 h</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>1.90± 0.058</td>
<td>2.017± 0.060</td>
</tr>
<tr>
<td>II</td>
<td>Standard (Diclofenac sodium) 10</td>
<td>1.019±0.058**</td>
<td>0.943±0.064**</td>
</tr>
<tr>
<td>III</td>
<td>Karanjachrome 25</td>
<td>1.472± 0.057**</td>
<td>1.364± 0.52**</td>
</tr>
<tr>
<td>IV</td>
<td>Karanjachrome 50</td>
<td>1.093± 0.62 **</td>
<td>0.985 ± 0.073**</td>
</tr>
</tbody>
</table>

Numbers of mice in each group were four. Results are shown as mean ±SEM. *p< 0.01 when compared to control group (one way ANOVA followed by Dunnett’s test).
Table 2 shows the effect of Karanjachromene on acetic acid-induced writhing in mice. The oral administration of test compound Karanjachromene significantly inhibited writhing response induced by acetic acid in a dose dependent manner. Karanjachromene produced 29.64% and 42.14% inhibition of writhing at doses 25 mg/kg and 50 mg/kg respectively. Standard drug diclofenac sodium produced 56.47% inhibition of writhing at 10 mg/kg the tail withdrawal reflex time following administration of the test compound was found to increase with increasing dose of the samples. The results were statistically significant and comparable to that of the reference standard drug morphine. The data are shown in Table 3.

Table 2: Effects of Karanjachromene on acetic acid induced writhing test in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose mg/kg (PO)</th>
<th>Frequency of writhing (mean ±SEM)</th>
<th>% inhibition of writhing</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>36± 1.880</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>Standard (Diclofenac sodium) 10</td>
<td>15.67 ± 0.989**</td>
<td>56.47</td>
</tr>
<tr>
<td>III</td>
<td>Karanjachrome 25</td>
<td>25.33 ±0.882**</td>
<td>29.64</td>
</tr>
<tr>
<td>IV</td>
<td>Karanjachrome 50</td>
<td>20.83 ±0.910**</td>
<td>42.14</td>
</tr>
</tbody>
</table>

Numbers of mice in each group were four. Results are shown as mean ±SEM. p < 0.01 when compared to control group (one way ANOVA followed by Dunnett’s test).

Table 3: Effects of Karanjachrome of tail immersion method of analgesic activity test in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose mg/kg (p.o.)</th>
<th>Mean latency period in seconds at a time of drug administration</th>
<th>% increase of the latency period with respect to control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
<td>30 min</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>2.38± 0.08</td>
<td>1.31± 0.10</td>
</tr>
<tr>
<td>II</td>
<td>Standard (Morphine) 10</td>
<td>2.05± 0.07</td>
<td>4.04± 0.15</td>
</tr>
<tr>
<td>III</td>
<td>Karanjachrome 25</td>
<td>1.43± 0.03</td>
<td>1.66± 0.03</td>
</tr>
<tr>
<td>IV</td>
<td>Karanjachrome 50</td>
<td>1.17± 0.14</td>
<td>1.76± 0.12</td>
</tr>
</tbody>
</table>

Numbers of mice in each group were four. Results are shown as mean ±SEM. p < 0.01 when compared to control group (one way ANOVA followed by Dunnett’s test).

**DISCUSSION**

The carrageenan induced paw edema is characterized by a biphasic event with the involvement of various inflammatory mediators. In the first phase (during 1st and 2nd hour after carrageenan injection), inflammatory mediators like serotonin and histamine play their role, while in the second phase (i.e., 3rd hour after carrageenan injection) bradykinins and prostaglandins are involved (Crunkhorn and Meacock, 1971). Our results revealed that administration of Karanjachromone is potent to inhibit the paw edema starting from the 1st h. and during all phases of inflammation, which may be due to inhibition of different inflammatory mediators.

Acetic acid-induced writhing model shows pain by enhancing localized inflammatory response. Such pain stimulus leads to the production of free arachidonic acid from phospholipids in the tissue. The acetic acid induced writhing response is a highly sensitive procedure to evaluate peripherally acting analgesics. The response could be mediated by peritoneal mast cells (Ronaldo et al., 2000) acid sensing ion channels (Voilley, 2004) and the prostaglandin pathways.

The tail immersion test is considered to be selective to examine compounds acting through opioid receptor, the test compound increased mean basal latency which indicates that they may act via centrally mediated analgesic mechanism (Dinesh Kumar, 2011). Narcotic analgesic inhibits both peripheral and central mechanisms of pain, while non-steroidal anti-inflammatory drugs inhibit only peripheral pain (Elisabetsky et al., 1995). Through the test compound inhibited both mechanisms of pain, these are more active in peripherally than centrally.
CONCLUSION

The experimental findings in this study suggest that the Karanjachromene possesses analgesic and anti-inflammatory activities, possibly mediated through central and peripheral mechanisms involving inhibition of release or the actions of vasoactive substances like prostaglandin and histamine, serotonin and kinins. The results obtained justify the use of the seed oil in traditional Indian medicine for the treatment of painful and inflammatory conditions. Further work is going on to elucidate the exact mechanism of action.

Conflict of interests

The authors report no conflicts of interest. The authors are merely responsible for the content and writing of the paper.

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