Pharmacological evaluation of the aqueous stem bark extract of *Bombax buonopozense* in the relief of pain and fever

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*Bombax buonopozense* is used in traditional medicine in Nigeria for the treatment of pains and feverish conditions. This study was orally carried out to establish the analgesic, anti-inflammatory and antipyretic properties of an aqueous stem bark extract of *Bombax buonopozense* in experimental animals. The analgesic activity was measured using the acetic acid-induced abdominal constriction and water tail immersion tests. The anti-inflammatory activity was assayed using the xylene and carrageenan-induced oedema tests, while the antipyretic activity was measured using the brewer’s yeast and 2, 4 dinitrophenol-induced pyrexia. The stem bark extract (50, 100 and 200 mg/kg) at all doses used, was found to have significant (P<0.05) analgesic, anti-inflammatory and anti-pyretic activities. Data shows that aqueous stem bark extract of *B. buonopozense* possesses constituents with therapeutic potential against pains and feverish conditions, thus supporting the use of the stem bark of this plant for similar ailments in traditional medicine.

**Key words:** *Bombax buonopozense*, stem bark, aqueous extract, pain, inflammation, fever.

**INTRODUCTION**

*Bombax buonopozense* P. Beauv which belong to the family bombaceae, is a large tropical tree which grows to 40 metres in height with large buttress roots which can spread 6 metres. The leaflets have entire margin and also large measuring between 8 to 23 cm in length and 3 to 8 cm in width. The undersides of the leaflets are glabrous or puberulous, and conical buds contain numerous seeds (Beentje and Sara, 2001).

*B. buonopozense* is widely distributed in African countries such as Nigeria, Ghana, Sierra Leone, Uganda.

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and Gabon. Common vernacular names in Nigeria include: Akpu (Igbo), Kurya (Hausa), Ogboro (Yoruba), and Vabga in Ghana. Different parts of the plant are used for different purposes (Dubost, 1984). The leaf, stem bark and root have been reported to possess antiplasmodial, anti-inflammatory, and anti-ulcer activities (Akuodor et al., 2011f; Akuodor et al., 2011g; Iwuanyanwu et al., 2012; Akuodor, 2011h; Nwagba et al., 2013). Earlier studies also have reported its nutritive and antimicrobial properties (Mann et al., 2003; Akuodor et al., 2011c; Mann et al., 2011). There is no doubt that Africa is blessed with abundant plants whose medicinal potentials are yet to be tapped. The interest in this plant was justified by its potential medicinal value against pains and feverish conditions. Therefore, the aim of this study was to investigate the analgesic, anti-inflammatory and antipyretic activities of the aqueous stem bark extract of *B. buonopozense* in experimental animals.

**MATERIALS AND METHODS**

**Plant collection and extraction**

The stem bark of *B. buonopozense* was collected in March, 2009 from a forest in Chaza village, Niger State, in Northcentral Nigeria. The plant material was identified and authenticated by a taxonomist in the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. A voucher specimen (NIPRD/H/6402) was deposited in the herbarium of the Department. Fresh stem bark parts of *B. buonopozense* were air-dried in the laboratory until a constant weight was obtained and pulverized. The powdered plant material was then macerated in distilled water for 24 h. The filtrate was dried on a water bath and a yield 18.75% was obtained. The solid brown extract was subsequently reconstituted in distilled water to obtain appropriate concentrations for the study.

**Experimental animals**

Albino mice (20 to 22 g) and wistar rats (180 to 220 g) of either sex used in this study were obtained from the Animal House of Faculty of Health Sciences, Ebonyi State University, Abakaliki, Nigeria. The animals were kept in hygienic, properly ventilated compartments and maintained under standard environmental conditions. Animals were sustained on standard rodent pellet and water *ad libitum*. The experimental procedures adopted in this study were in accordance with the United States National Academy of Sciences Guide for the Care and Use of Laboratory Animals (Committee for the Update of the Guide for the Care and Use of Laboratory Animals, National Academy of Sciences, 2011).

**Phytochemical screening**

Phytochemical screening of the aqueous stem bark extract of *B. buonopozense* was carried out in accordance with standard procedures (Ajayi et al., 2011; Kasolo et al., 2010; Inyang-Agha, 2006).

**Acute toxicity test**

The acute toxicity of *B. buonopozense* stem bark extract was tested to determine its safety adopting the guidelines of Organisation for Economic Co-operation and Development (OECD) (2010). The studies were done in two phases. Nine rats, randomized and divided into three were used in the first phase. The rats were orally administered with 100, 600 and 1000 mg/kg of the stem bark extract, respectively. The animals were observed for the first 4 h and 24 h for signs of toxicity and mortality. This was followed by the second phase in which 2000, 3000 and 5000 mg/kg of the extract was administered to the next three groups of three rats per cage. The signs of toxicity and mortality were observed for 24 h, 48 h and 72 h, respectively.

**Pharmacological studies**

**Analgesic assay**

**Abdominal constriction test:** Mice for this study were divided into five groups of six per cage. The different groups were orally treated with normal saline (20 ml/kg), *B. buonopozense* stem bark extract at doses of 50, 100, 200 mg/kg, acetylsalicylic acid (150 mg/kg). Thirty minutes later, mice were treated with 0.7% v/v acetic acid (10 ml/kg, i.p.). The number of writhes was then counted at 5 min interval for 30 min (Singh and Majumdar, 1995).

**Water tail immersion test:** Mice for this experiment were divided into five groups of six in each cage. The different groups of animals were orally treated with distilled water (20 ml/kg), *B. buonopozense* stem bark (50, 100, 200 mg/kg), and morphine (10 mg/kg). The initial reading was taken immediately before administration of test and standard drugs. Thirty minutes post drug administration, each mouse was restrained in a horizontal cylinder leaving the tail hanging freely and 4 cm portion of each mouse tail marked was then immersed in a water bath thermo-statically maintained at 50±1°C. However, the time taken for each mouse to remove its tail out of the water was recorded (Jansen and Jagenau, 1959; Akuodor et al., 2015). The tail flick latency was evaluated at 30, 60, 90 and 120 min.

**Anti-inflammatory test**

**Xylene-induced ear oedema in mice**

The mice used for this study were randomly divided into 5 groups of 6 in each cage. Mice in different groups were treated with normal saline (20 ml/kg, p.o.), *B. buonopozense* (50, 100 and 200 mg/kg, p.o.) and dexamethasone (4 mg/kg, p.o.). One hour post drug administration, oedema was induced in each mouse by applying 50 µl of xylene using a microliter pipette at the inner surface of the right ear. Thirty minutes after, the animals were sacrificed under light ether anaesthesia, and both ears were cut off to approximately equal size and weight. The mean difference between the right and left ear were determined for each group and recorded as an indication of inflammation (Junping et al., 2005).

**Carrageenan-induced rat paw oedema**

Rats for this experiment were divided into five groups of six in each cage and different groups were treated with normal saline (20 ml/kg, p.o.), *B. buonopozense* stem bark extract (50, 100, 200 mg/kg) and acetylsalicylic acid (150 mg/kg). One hour after administration, oedema was induced in rat by injection of 0.1 ml of freshly prepared carrageenan suspension (1%) in distilled water into the subplantar tissue of the right hind paw to all the groups (Gupta et al., 2005). The paw volumes were measured at 0.5, 1, 2, 3
Table 1. Effect of aqueous stem bark extract of *B. buonopozense* on acetic acid-induced writhing in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Writhing</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20 ml/kg</td>
<td>23.33±3.33</td>
<td>-</td>
</tr>
<tr>
<td>50</td>
<td>8.17±0.98</td>
<td>65*</td>
<td></td>
</tr>
<tr>
<td><em>B. buonopozense</em></td>
<td>100</td>
<td>4.67±0.33</td>
<td>80*</td>
</tr>
<tr>
<td>200</td>
<td>3.33±0.61</td>
<td>86*</td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>150</td>
<td>3.00±0.73</td>
<td>87*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; *Significant at p<0.05 when compared to control.

and 4 h respectively by using plethysmometer. The average swelling of paws in the groups of extract treated was compared with control group and the standard.

### Antipyretic assay

#### Yeast-induced pyrexia

Rats were randomly divided into five groups of six rats each. The basal rectal temperatures of the animals were recorded over a period of 30 min. Ten ml/kg of brewer’s yeast suspension (15 % in 0.5 % w/v methylcellulose) was injected subcutaneously into the rats to induce pyrexia. 24 h after yeast injection, the rectal temperatures of animals were taken and animals showing rise in temperature of less than 0.5°C were discarded. After the establishment of pyrexia, normal saline (20 ml/kg, p.o.); *B. buonopozense* extract (50, 100 and 200 mg/kg, p.o.), and acetylsalicylic acid (150 mg/kg, p.o.) were orally administered to rats in different groups. The rectal temperatures of animals were thereafter recorded at 1, 2, 3, and 4 h post-treatment (Agbaje et al., 2008; Akindele et al., 2012).

#### 2, 4-Dinitrophenol-induced pyrexia

Rats employed for the study were randomized into 5 groups of 6 in each cage. Their basal temperature was taken and 10 mg/kg of DNP prepared in normal saline was subcutaneously injected to each rat to induce pyrexia. Thirty min after administration of DNP (confirmation of pyrexia), the animals in different groups were orally treated with normal saline (20 ml/kg), *B. buonopozense* stem bark extract (50, 100 and 200 mg/kg), and acetylsalicylic acid (150 mg/kg). Rectal temperature of each rat was thereafter taken at 1 h interval for 4 h (Essien et al., 2015; Okokon and Nwafor, 2010).

### Statistical analysis

Results are expressed as mean ± SEM. The significant difference between mean was determined using one-way analysis of variance (ANOVA) to analyse results between groups. Statistical significance was established at P<0.05

### RESULTS

#### Phytochemical analysis

Phytochemical analysis of the aqueous stem back extract of *B. buonopozense* revealed the presence of alkaloids, terpenoids, flavonoids, tannins, saponins, reducing sugars and sterols.

#### Acute toxicity test

There was no mortality observed at any of the doses of aqueous stem extract of *B. buonopozense* examined. The animals were alive, healthy and active during the observation period.

The oral acute toxicity test of the leaf extract was estimated to be greater than 5000 mg/kg in rats. Thus, the experimental doses orally used (50,100 and 200 mg/kg) were within safe margin.

#### Acetic acid-induced writhing test

Table 1 shows the assessed analgesic profile of *B. buonopozense* aqueous stem bark extract using acetic acid induced abdominal constriction test in mice. The stem bark extract at all doses used, exhibited significant (P<0.05) analgesic activity that lasted until the end of the experiment. Interestingly, all doses of the stem bark extract had analgesic activity which is comparable to aspirin, the standard drug.

#### Tail immersion test

Table 2 shows the antinociceptive activity of the stem bark extract of *B. Buonopozense* assessed using the tail immersion test in mice. The stem bark extract at different post treatment times significantly (P<0.05) protected the animals from thermal stimuli. Morphine produced greater protection when compared to the extract at all post treatment times.

#### Xylene-induced ear oedema test

Table 3 shows the anti-inflammatory profile of the stem bark extract of *B. buonopozense* assessed using the
Table 2. Effect of aqueous stem bark extract of *B. buonopozense* on thermal stimuli response in the tail immersion test in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Control</td>
<td>20 ml/kg</td>
<td>7.17±0.87</td>
<td>8.17±0.40</td>
</tr>
<tr>
<td><em>B. buonopozense</em></td>
<td>50</td>
<td>7.67±0.49</td>
<td>10.0±0.45</td>
</tr>
<tr>
<td>Morphine</td>
<td>100</td>
<td>8.17±0.60</td>
<td>12.67±1.09</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>8.33±0.67</td>
<td>13.67±0.92</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM; *Significant at p<0.05 when compared to control.

Table 3. Effect of aqueous stem bark extract of *B. buonopozense* on xylene induced ear oedema in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Weight of right ear (g)</th>
<th>Weight of left ear (g)</th>
<th>Increase in ear weight (g)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20 ml/kg</td>
<td>0.073±0.00</td>
<td>0.031±0.00</td>
<td>0.042±0.00</td>
<td>-</td>
</tr>
<tr>
<td><em>B. buonopozense</em></td>
<td>50</td>
<td>0.050±0.00</td>
<td>0.029±0.00</td>
<td>0.021±0.00</td>
<td>50*</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.038±0.00</td>
<td>0.025±0.00</td>
<td>0.013±0.00</td>
<td>69*</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.034±0.00</td>
<td>0.023±0.00</td>
<td>0.011±0.00</td>
<td>74*</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>4</td>
<td>0.029±0.00</td>
<td>0.020±0.00</td>
<td>0.009±0.00</td>
<td>79*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SEM; *Significant at p<0.05 when compared to control.

Table 4. Effect of aqueous stem bark extract of *B. buonopozense* on carrageenan-induced paw oedema in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Paw oedema volume (ml) versus time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 h</td>
</tr>
<tr>
<td>Control</td>
<td>20 ml/kg</td>
<td>1.25±0.02</td>
</tr>
<tr>
<td><em>B. buonopozense</em></td>
<td>50</td>
<td>1.26±0.02</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1.24±0.02</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>1.22±0.02</td>
</tr>
<tr>
<td>Aspirin</td>
<td>150</td>
<td>1.19±0.03</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; *Significant at p<0.05 when compared to control.

Table 5 shows antipyretic profile of the stem bark extract of *B. buonopozense* assessed using brewer's yeast induced pyrexia in rats. The stem bark extract significantly (P<0.05) and dose dependently decreased the anal temperature of rats in all dose levels used in the test. The standard drug (aspirin), showed more activity than the extract.

Carrageenan-induced paw oedema test

Table 4 shows the anti-inflammatory activity of the aqueous stem bark extract of *B. buonopozense* against carrageenan induced paw oedema test in rats. The observed activity of the extract at both dose levels used were significant (P<0.05) dose dependent. However, data suggest more inhibitory activity with standard drug (Aspirin), than the stem bark extract.

Yeast-induced pyrexia test

Xylene induced ear oedema test in mice. The extract at dose levels used, exerted significant (P<0.05) and dose dependently reduced the oedema induced through topical application of xylene to the mouse ear. The standard drug, dexamethasone (4 mg/kg) exhibited greater effect than the stem bark extract (Table 3).
The observed abdominal constrictions in this study of standard drugs (aspirin) produced more activity than the extract. However, standard drug (aspirin) produced more activity than the extract.

DISCUSSION

This study on the stem bark extract of *Buonopozense* was conducted to establish potential pharmacological properties of the plant based on claims of its use in traditional medicine by Chaza community in Northcentral, Nigeria. The analgesic activity was tested using the abdominal constriction and tail immersion tests. The acetic acid induced writhing test is normally used to study the peripheral analgesic effects of drugs (Chang et al., 2011). The observed abdominal constrictions in this study are due to irritation of the peritoneal cavity induced by administration acetic acid (Vogel and Vogel, 1997). Prolonged irritation causes an increase in the levels of prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability (Ganesh et al., 2008; Gawade, 2012).

However, the constituents of the aqueous stem bark extract of *Buonopozense* reduced reasonably the duration of the writhing in each mouse and consequently the effects on arachidonate release and metabolism (Nuhu et al., 2007). This analgesic activity which was dose dependent may indicate the involvement of peripheral pathway. Moreso, the obtained results showed that the stem bark extract is effective in blocking the analgesic effect caused by the chemical stimulus in the writhing test.

To further confirm the analgesic action of the stem bark extract, tail immersion test was assessed. The activity of the extract in tail immersion confirmed its analgesic effect. It is well known that centrally acting analgesic agents elevate the pain threshold of mice towards heat and pressure (Akindele and Adeyemi, 2006). Significant activity in tail immersion test indicates involvement of central analgesic mechanism.

Table 6 shows the effect of the stem bark extract of *Buonopozense* on 2, 4 dinitrophenol induced pyrexia in rats. The extract was found to have significant (p<0.05) and dose dependent antipyretic activity when compared to control.

However, standard drug (aspirin) produced more activity than the extract.

### Table 5. Effect of aqueous stem bark extract of *B. buonopozense* on yeast induced pyrexia in rat (Rectal temperature °C).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>0 h</th>
<th>24 h</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
<th>5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20 ml/kg</td>
<td>35.39±0.06</td>
<td>37.75±0.04</td>
<td>37.88±0.03</td>
<td>37.69±0.03</td>
<td>37.51±0.03</td>
<td>37.32±0.02</td>
<td>36.72±0.02</td>
</tr>
<tr>
<td><em>B. buonopozense</em></td>
<td>50</td>
<td>35.27±0.03</td>
<td>37.32±0.06</td>
<td>36.99±0.11</td>
<td>36.45±0.03</td>
<td>36.18±0.01</td>
<td>35.54±0.04</td>
<td>35.24±0.02*</td>
</tr>
<tr>
<td><em>B. buonopozense</em></td>
<td>100</td>
<td>35.29±0.03</td>
<td>37.33±0.02</td>
<td>36.55±0.02</td>
<td>36.23±0.01</td>
<td>35.68±0.04</td>
<td>35.45±0.04</td>
<td>35.19±0.02*</td>
</tr>
<tr>
<td><em>B. buonopozense</em></td>
<td>200</td>
<td>35.25±0.02</td>
<td>37.35±0.03</td>
<td>36.49±0.05</td>
<td>36.24±0.04</td>
<td>35.55±0.03</td>
<td>35.32±0.02</td>
<td>35.17±0.01*</td>
</tr>
<tr>
<td>Aspirin</td>
<td>150</td>
<td>35.24±0.03</td>
<td>37.25±0.03</td>
<td>36.48±0.03</td>
<td>36.20±0.02</td>
<td>35.67±0.00</td>
<td>35.41±0.2</td>
<td>35.15±0.02*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; *significant at p<0.05 when compared to control.

### Table 6. Effect of aqueous stem bark extract of *B. buonopozense* on 2, 4 Dinitrophenol-induced pyrexia in rat (Rectal temperature °C).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>0 h</th>
<th>0.5 h</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
<th>5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20 ml/kg</td>
<td>35.37±0.05</td>
<td>37.52±0.04</td>
<td>37.80±0.02</td>
<td>37.63±0.02</td>
<td>37.42±0.02</td>
<td>37.29±0.02</td>
<td>36.69±0.03</td>
</tr>
<tr>
<td><em>B. buonopozense</em></td>
<td>50</td>
<td>35.29±0.02</td>
<td>37.33±0.05</td>
<td>36.86±0.09</td>
<td>36.40±0.03</td>
<td>36.14±0.04</td>
<td>35.49±0.02</td>
<td>35.24±0.01*</td>
</tr>
<tr>
<td><em>B. buonopozense</em></td>
<td>100</td>
<td>35.29±0.03</td>
<td>37.31±0.02</td>
<td>36.40±0.04</td>
<td>36.19±0.03</td>
<td>35.58±0.05</td>
<td>35.37±0.02</td>
<td>35.18±0.01*</td>
</tr>
<tr>
<td><em>B. buonopozense</em></td>
<td>200</td>
<td>35.28±0.04</td>
<td>37.29±0.03</td>
<td>36.51±0.04</td>
<td>36.23±0.02</td>
<td>35.54±0.05</td>
<td>35.32±0.04</td>
<td>35.13±0.02*</td>
</tr>
<tr>
<td>Aspirin</td>
<td>150</td>
<td>35.23±0.02</td>
<td>37.30±0.02</td>
<td>36.47±0.02</td>
<td>36.15±0.01</td>
<td>35.55±0.03</td>
<td>35.30±0.02</td>
<td>35.10±0.02*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; *significant at p<0.05 when compared to control.
agents (Zaninir et al., 1992). A similar oedema inhibition observed, suggest the possible action of the stem bark extract with PLA2 in its anti-inflammatory pathway.

The carrageenan induced rat paw oedema test which measures the ability of an agent to reduce local oedema induced in the rat paw by injection of an irritant agent such as carrageenan, has been widely used for screening for new anti-inflammatory drugs (Zakaria et al., 2008). The aqueous stem bark extract of B. buopozense caused marked inhibition at the early phase of inflammation indicating activity probably on histamine, serotonin and kinnins which are involved in the early stage of carrageenan induced oedema (Vane and Botting, 1987). The extract also reduced late phase of the oedema perhaps by inhibiting prostaglandin which is known to mediate the second phase of carrageenan induced inflammation (Necas and Bartosikova, 2013). The results obtained showed significant anti-inflammatory activity in the stem bark extract. This finding has scientifically confirmed the folklore use of B. buopozense in the treatment of ulcers in Nigeria (Nwagba et al., 2013).

The stem bark extract of B. buopozense was also found to possess antipyretic activity when assayed using the brewer’s yeast and 2, 4 dinitrophenol induced pyrexia tests. It is well known that pyretic activity involves stimulation of the region in the hypothalamus, which controls body temperature through prostaglandins synthesized within the central nervous system (Uzcátegui et al., 2004) and the blood brain barrier prevents drug substances from having access to the central nervous system (CNS). The ability to cross the blood brain barrier may be one of the factors contributing to the antipyretic activity, and could also explain the observed central analgesic activity of the aqueous stem bark extract of B. buopozense. This finding is also in agreement with our earlier study of the methanolic leaf extract of B. buonopozense (Akudor et al., 2011a).

The therapeutic importance of medicinal plants is often attributed to the combination of their active constituents. Different flavonoids isolated from medicinal plants have shown remarkable antipyretic, analgesic and anti-inflammatory activities (Sawadogo et al., 2006; Larkins and Wynn, 2004). The observed antipyretic, the analgesic and anti-inflammatory effects could be attributed to its flavonoids constituent, and this finding supported our recent observation on the stem bark extract of B. buopozense. The safety of the stem bark extract when taken orally is justified by the fact that oral administration of 5000 mg/kg in rats did not produce any mortality and visible toxic signs.

Conclusion

The present study indicates that B. buopozense stem bark extract has peripheral and central analgesic, anti-inflammatory and antipyretic activities, thus confirming its traditional use for the treatment of pain, inflammation and feverish conditions.

Conflict of Interest

The authors have not declared any conflict of interests.

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