Antinociceptive Activity of the Crude Methanolic Extract of *Pseudocedrela kotschyi* and Its Chloroform and n-butanol Fractions in Mice

**ABSTRACT**

*Pseudocedrela kotschyi* has been extensively used in traditional medicine for the treatment of rheumatism, malaria, dysentery and epilepsy. The antinociceptive effects of the crude methanolic extract, n-butanol and chloroform fractions of *P. kotschyi* were investigated in different experimental models in mice: (1) hot plate, (2) tail flick, (3) acetic acid induced writhing and (4) formalin induced test. In the writhing test 200 mg/kg of the crude extract significantly (P < 0.05) reduced the number of writhes and produced an 88.1% inhibition. The chloroform and n-butanol fractions produced 98.59 and 92.62% inhibition of writhes respectively. In the formalin induced test 100 and 200 mg/kg of the crude methanolic extract significantly (P < 0.01) produced late phase analgesia. Similarly, 200 mg/kg of the chloroform fraction significantly (P < 0.01) reduced paw licking behaviour in mice in both early and late phases of the experiment. However, at the doses tested, no significant activity was found in the hot plate and the tail flick test. The results suggest that *P. kotschyi* methanolic extract at 200 mg/kg dose is effective in non-steroidal anti-inflammatory drug type anti-nociception activities.

**KEYWORDS** *Pseudocedrela kotschyi*, antinociception, anti-inflammatory

**INTRODUCTION**

Nociception is defined as “the neural processes of encoding and processing noxious stimuli,” whereas, pain has been officially defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. It is always a warning signal and primarily protective in nature but often causes a lot of discomfort and lead to many adverse effects.

Analgesics are drugs used to treat or reduce pain and the classical analgesic drugs notably opiates and non-steroidal anti-inflammatory drugs have their origin in natural products but many synthetic compounds that act by the same mechanism have been developed and are associated with serious adverse effects such as ulceration, gastrointestinal bleeding, addictive potential, respiratory distress, drowsiness, nausea etc.

Owing to safety concerns associated with the use of synthetic anti-inflammatory and analgesic agent, the public prefer to take natural anti-inflammatory and analgesic treatments from edible materials such as fruits, spices, herbs and vegetables. Therefore, the development and utilization of more effective anti-inflammatory and analgesic agents of natural origin are desired.

*Pseudocedrela kotschyi* (meliaceae) is a medium-sized tree, sometimes up to 60 ft high. It is well distributed in Senegal, Congo basin, Uganda and Nigeria. The plant is known as *tuna* in Hausa and *Emi gbeji* in Yoruba. The plant is used traditionally in Ghana to treat leprosy and epilepsy, malaria and stomach aches. The roots and leaves are used to treat rheumatism and dysentery. In Northern Nigeria, the plant is used for treatment of insomnia, as chewing sticks for dental cleaning and in North Côte d’Ivoire, it is of value in the treatment of toothache and internal wound. Nephroprotective activities of ethanolic root extracts of the plant was reported by Ojewale et al. The stem and root barks of *P. kotschyi* contain essential oils, comprising exclusively sesquiterpenoids with very low antiradical and antioxidant activities.

**RESEARCH ARTICLE**


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of *P. kotschyi* contains a bitter non-nitrogenous principle, pseudocedrelin, which demonstrated potent piscidal activity. Limonoids, 7-deacetoxy-7-oxogedunin and pseudrelones A, B and C were isolated from the wood oil.

The n-butanol soluble portion of the ethanolic extract of the leaves of *P. kotschyi* has been shown to have anti-nociceptive and anti-inflammatory activities in mice and rats, respectively. It increased the depression or sedation time followed by sleep.

The present study was carried out to investigate the analgesic (antinociceptive) activity of the methanolic stem bark extract of *P. kotschyi* with the aim of establishing the pharmacological basis for the use of the plant for the management of pain in traditional medicine in north western part of Nigeria using standard in vivo analgesic models.

**MATERIALS AND METHODS**

**Collection of the plant materials**

*P. kotschyi* leaves and stem bark were collected from Zuru local government area of Kebbi state. It was identified as *Pseudocedrela kotschyi* by Dr. E.M. Mshelia of the Department of Pharmacognosy and Ethno pharmacy, Faculty of Pharmaceutical Sciences Usmanu Danfodiyo University Sokoto and a voucher specimen (PK509-10) was deposited in the herbarium of the department.

**Preparation of the crude extract**

The plant material was pulverised into fine powder with the aid of the grind mill. The powdered plant material (500 g) was placed into a thimble and extracted with 5 L of 95% methanol in a soxhlet extractor for 3 days. The extract was concentrated using Büchi RE121 rotary evaporator (Büchi Labortechnik AG, Switzerland) and subsequently dried in a Hetoac VR-1 freeze dryer (Heto Lab. Equipment AS, Denmark). The yield obtained was found to be 20% (w/w) of the dried plant.

**Preparation of the fractions**

About 60 g of freeze dried extract was dissolved in distilled water, and then placed in a sonicator at 45°C for 20 min to facilitate dissolution. Thereafter the solution was transferred into a 1000 ml separating funnel and extracted with n-hexane (200 ml portions) for 48 hours. The n-hexane portions were collected and labelled as n-hexane soluble portion. This portion was then evaporated using the rotary evaporator to obtain a semi solid gel which was labelled n-hexane extract.

The same procedure was repeated for ethyl acetate, chloroform and n-butanol solvents by taking their relative polarity into consideration. As described earlier similar fractions were pooled together evaporated and the residue kept and labelled until when it was needed.

**Animals**

Swiss albino mice of either sex weighing between 20–30 g were obtained from the Animal Research and Services Centre, Universiti Sains Malaysia. The animals were acclimatized to laboratory conditions for seven days prior to the experiments. During acclimatization, 12 mice were housed per polycarbonate cage, with free access to normal diet (48% carbohydrate, 23% crude protein, 3% crude fat, 8% crude ash, 5% crude fibre and 13% moisture) and tap water ad libitum. The food pellets for the experimental were purchased from Gold Coin Holdings Sdn. Bhd. Malaysia. The animals were maintained on a natural light and dark cycle. All procedures were performed according to the guidelines of care and use of laboratory animals as approved by the Animals Health and Wellness Unit, Universiti Sains Malaysia.

**Acute toxicity studies in mice**

The method described by Lorke was used; three groups of three animals were treated with the methanolic stem bark extract of *P. kotschyi* at doses of 10, 100, and 1000 mg/kg body weight i.p and observed for 24 hours for signs of toxicity. In the second phase, four groups of one animal each were treated with the methanolic stem bark extract at doses of 140, 225, 370, and 600 mg/kg i.p. The LD50 value was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (0/1 and 1/1).

**Acetic acid induced writhing test**

The method of Koster et al. as modified by Yam et al. was adopted for the study of the analgesic effect of *P. kotschyi* in mice. Thirty albino mice of both sexes were randomly divided into five groups of six mice per group and treated as follows: group 1 mice received 10 ml/kg of normal saline. Group 2 mice received 10 mg/kg of indomethacin while groups 3, 4 and 5 mice were given 50, 100 and 200 mg/kg of *P. kotschyi* extract, respectively. One hour after administration of the drug and extract, each mouse was injected with (0.6% v/v) acetic acid (10 ml/kg, i.p). The number of writhing observed in each mouse was counted for 15 minutes and recorded. The percentage inhibition was calculated by comparing with control group by the standard formula: percentage inhibition = [(control mean − treated mean)/control mean] × 100.

**Tail flick test**

The method described by Yam et al. was used for this experiment. Twenty-five albino mice were randomly divided into five groups with five mice each, all fasted for 12 hours with clean drinking water provided ad libitum. The animals were pre-treated 60 minutes before tail flick test with 10 ml/kg n/saline solution for group A (negative control), 10 mg/kg (p.o) indomethacine for group...
B (positive control) and 50, 100, 200 mg/kg (p.o) of *P. kotschyi* extract for groups C, D and E, respectively. Each mouse was encased in a small aluminium chamber with the middle portion of the tail placed over the light beam of the tail flick apparatus. A maximum cut-off time of 10 seconds was observed, to minimize undue tissue damage as a result of over exposure of the tail to heat.

**Formalin induced test**

The method of Kamei et al. 23 was adapted with some modifications. A total of 30 male mice were used in the experiment. They were divided into five groups of six mice each. About 5% formalin solution was prepared by diluting with normal saline from the commercial 10% formalin solution. A 50-µl of the 5% formalin solution was injected into the plantar tissue of the mice right hind paw following a 1 hour interval of administration of normal saline, indomethacin (10 mg/kg) and *P. kotschyi* extract (50, 100 and 200 mg/kg) p.o to the negative control, positive control and test groups, respectively. The total number of flinches/licking of the hind paw was recorded by visual observation for 5 minutes period for a total of 2 hours following formalin injection.

**Hot plate test**

The method of Yam et al. 22 was adopted with some modifications, 42 mice (weighing between 24–34 g) were grouped into seven groups of six mice each. Group 1 received normal saline while group 2 received DF118 at a dose of 3 mg/kg body weight (p.o) as adapted from Kamei et al 23. Groups 3–7 received 200 mg/kg of the different solvent fractions (p.o). The latency of pain was recorded as the time taken to jump out of the glass chamber or licking of paw. The results were recorded after 15, 30, 45, 60 and 90 minutes post administration of extract or controls.

**Data analysis**

The result was presented as mean ± SEM and analysed using one-way analysis of variance (ANOVA). The difference between the means was tested with post hoc Duncan and t-test and values of P < 0.05 were considered statistically significant.

**RESULTS**

The methanol extract of *P. kotschyi* produced a dose-dependent inhibition of acetic acid induced writhing (Fig. 1), 200 mg/kg of the extract significantly (P < 0.05) reduced the number of writhes and produced an 88.1% inhibition. The effect was comparable to 10 mg/kg Indomethacin, a known analgesic/antipyretic agent.

*P. kotschyi* solvent fractions (Fig. 2) significantly reduced the number of writhes induced by acetic acid. The highest activity was observed with chloroform and N-butanol fractions at doses of 200 mg/kg. The percentage inhibition of writhing was 98.59 and 92.62, respectively.

The result in Table 1 showed a dose-dependent increase in the response time in the extract treated groups though the difference was not statistically significant at (P < 0.05).

From the results in Table 2, the chloroform and n-butanol fractions showed moderate antinociceptive activity relative to the control, though the effect was not statistically significant.

The results in Table 3 indicate that the methanol extract of *P. kotschyi* produced a dose dependent reduction in paw licking/flinching at the given test doses. The response was significant at P < 0.05.

The result in Table 4 indicates a significant reduction in the number of paw licking/flinching in all the

**Fig. 1** Graph of acetic acid induced writhing and percentage inhibition of writhes in the acetic acid induced mice antinociceptive model by treatment with the methanol extract of *P. kotschyi*.
Antinociceptive activity of the methanolic stem bark extract

Fig. 2: Graph of acetic acid induced writhing and percentage inhibition of writhes in the acetic acid induced mice antinociceptive model by treatment with *P. kotschyi* and its fractions.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Result of the tail flick experiment on <em>P. kotschyi</em> methanol extract.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Mean response time (s)</td>
</tr>
<tr>
<td>N/SAL</td>
<td>3.58 ± 0.60</td>
</tr>
<tr>
<td>IND 10 mg/kg</td>
<td>5.13 ± 0.99</td>
</tr>
<tr>
<td>PK 50 mg/kg</td>
<td>2.78 ± 0.51</td>
</tr>
<tr>
<td>PK 100 mg/kg</td>
<td>3.77 ± 0.50</td>
</tr>
<tr>
<td>PK 200 mg/kg</td>
<td>5.43 ± 0.80</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n = 6.

Table 2: Chloroform and N-butanol fractions of 200 mg/kg showed better antinociceptive effect than the positive control (indomethacin) in the tail flick antinociception studies, but the values were not statistically significant (P < 0.05).

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Showing the result of the early and late phase of the formalin induced antinociception on the methanol extract of <em>P. kotschyi</em> in mice.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Early stage</td>
</tr>
<tr>
<td>N/SAL</td>
<td>60.33 ± 6.71</td>
</tr>
<tr>
<td>IND</td>
<td>37.17 ± 13.23</td>
</tr>
<tr>
<td>PK 50</td>
<td>31 ± 6.55a</td>
</tr>
<tr>
<td>PK 100</td>
<td>17.67 ± 5.98b</td>
</tr>
<tr>
<td>PK 200</td>
<td>12 ± 30.86b</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n: 6, a: P < 0.05, b: P < 0.001, c: P < 0.0001.

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Showing the result of the early and late phase of the formalin induced antinociception on the extract and fractions of <em>P. kotschyi</em> in mice.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Early phase</td>
</tr>
<tr>
<td>N/S</td>
<td>64.5 ± 4.54</td>
</tr>
<tr>
<td>IND</td>
<td>60.67 ± 3.48</td>
</tr>
<tr>
<td>NHEX 100</td>
<td>29 ± 2.27b</td>
</tr>
<tr>
<td>NHEX 200</td>
<td>18 ± 5.14c</td>
</tr>
<tr>
<td>CHCL 3100</td>
<td>9.67 ± 3.61c</td>
</tr>
<tr>
<td>CHCL 3200</td>
<td>0c</td>
</tr>
<tr>
<td>NBUT 100</td>
<td>25.33 ± 10.98b</td>
</tr>
<tr>
<td>NBUT 200</td>
<td>6 ± 2.73c</td>
</tr>
<tr>
<td>R-AQ 100</td>
<td>16.5 ± 6.24c</td>
</tr>
<tr>
<td>R-AQ 200</td>
<td>9.17 ± 0.17c</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n = 6. a: P < 0.05, b: P < 0.001, c: P < 0.0001.
increase the response time in the tail flick experiment though the result was not statistically significant. In the hot plate experiment *P. kotschyi* methanol extract showed an insignificant increase in response time same as was the fraction.

Acetic acid-induced writhing reflex is a model of visceral pain which is highly useful for screening analgesic drugs and several chemicals such as phenylquinine and acetic acid could induce writhing reflex in laboratory animals.

The intraperitoneal administration of acetic acid produces writhing reflex is characterized by abdominal contractions, movements of the body as a whole, twisting of dorsoabdominal muscles and a reduction in motor activity and coordination. This effect is as a result of activation of the chemosensitive nociceptors in the animals, therefore the percentage reduction in abdominal contractions over a defined period of time is a reflection of the plant's antinociceptive effect.

One of the possible mechanisms underlying the analgesic mechanisms of *P. kotschyi* could be linked to the inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition; hence, a need to include other models that measure central analgesic activity.

**DISCUSSION**

The aqueous extract of *P. kotschyi* has been used traditionally for the treatment of pain conditions of different forms. In this study, we investigated its antinociceptive and anti-inflammatory properties. Antinociceptive effects of the plant extract and its fractions at the doses tested revealed that it significantly reduced the number of writhes, paw flicking/flinching in the formalin induced test in mice, this signifies that it possesses peripherally mediated activities. The extract dose dependently
that opiates selectively prolongs the reaction time of the
typical tail withdrawal reflex in mice\textsuperscript{36} while in the hot
plate model, the sensitivity of the mice’s paw to tem-
perature is the object of measurement\textsuperscript{37}.

In the present study, the extract demonstrated a
dose-dependent increase in reaction time in both crude
extract and fractions in the tail flick experiment, though
the result was not significant, the plant could be said to
have some central analgesic effects.

The tail flick and hot plate models have been used
to study centrally acting analgesics\textsuperscript{18}, the mechanism of
nociception in these models is based on the activities
of sensory nerves and the release of endogenous sub-
stances such as prostaglandins, histamines and leukot-
rienes are minimised\textsuperscript{39}. From the results of the present
study, though \textit{P. kotschyi} showed promising antinocicep-
tive actions in the tail flick and hot plate experiments, it
was less pronounced than the acetic acid-induced and
formalin induced models and this may suggest that the
analgesic activity of \textit{P. kotschyi} may not be fully medi-
atated through central mechanism. It was also observed
that \textit{P. kotschyi} crude methanol extracts and its fractions
showed both analgesic and anti-inflammatory effect sim-
ilar to NSAIDS; though the exact mechanism of action
was not well investigated it is safe to say that the plant
has both analgesic and anti-inflammatory effects which
buttresses its traditional use.

CONCLUSION

The present study and similar studies on the antino-
icceptive effect of \textit{P. kotschyi} indicates clearly that the plant
has both analgesic and anti-inflammatory effects sim-
ilar to NSAIDS especially indomethaine (used as con-
tral in this research). This study supports the traditional
claim of analgesic and anti-inflammatory activities of
the plant; further research is suggested to evaluate the
molecular mechanism of the analgesic activity.

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