EVALUATION OF ANTINOCICEPTIVE ACTIVITY OF CROTON Megalo carpus (HUTCH) Eurphobaceae

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ABSTRACT

The use of plant parts for a wide range of therapeutic purposes is commonly practiced in Africa where several plants are used as folklore remedies for fever, inflammation and pain. An example of plants whose parts are used for analgesia is Croton Megalo carpus however; scientific studies have not been done to evaluate the efficacy of the claimed antinociceptive activity. The aim of this study was to evaluate the antinociceptive activity of the stem, root bark and leaves extract using animal model. The antinociceptive activity was investigated using the chemical pain test models; writhing and the formalin tests as well as one thermal pain test model (tail Flick test). In all test, the extract exhibited highly significant (p < 0.001) antinociceptive effects. These results showed that the extracts of C. megalocarpus exhibited peripheral, inflammatory and central antinociceptive activity. Hence it probably contains phytochemicals that may be of value in development of a novel remedy for analgesia. However, further studies need to be done to elucidate nature and mechanism(s) of action of these metabolites.

Key words: Croton megalocarpus, Antinociceptive, Tail flick, Formalin, Pain, Writhing, Analgesic.

INTRODUCTION

Traditional medicine practiced in Kenya is widespread where about 400 different plant species are used in the practice (Kokwaro JO, 2003). There is however high reliance on traditional medicine in rural areas which is attributed to both economic and cultural factors (Aketch CA, 1992). Croton megalocarpus is a tree that belongs to the euphorbiaceous family and is widely distributed in East Africa. An extract from the plant is taken as a vermifuge, as a folklore remedy for cough, pneumonia, (Gacathi FN 1989; Bussmann RW 2006; Maroyi A, 2010), stomach-ache, fever, malaria, and abdominal complaint problems (Johns T et al., 1994; Bussmann RW 2006; Njoroge and Bussmann, 2006; Maroyi A, 2010) while the leaves and young twigs are applied to wounds (Njoroge and Bussmann RW, 2007; Maroyi A, 2010). It has also been used for diarrhea management in central Kenya (Njoroge and Kibunja, 2007). Both the aqueous and organic extracts of the roots and leaves were found to inhibit cyclooxygenase activity (Matu EN &Staden V, 2003). Qualitative phytochemical analysis of the extracts showed the presence of alkaloids, glycosides, terpenoids, flavonoids, flavones reducing sugars and saponins in the extracts (Waiganjo et al., 2013). This plant parts has multiplicity of folklore uses including painful states and injuries, however there is little or no scientific studies reported on the efficacy, mode of action or toxicity. The aim of this study was to investigate the effect of this plants extract on nociception using animal models.
MATERIALS AND METHODS

Collection of plant material
The plant parts were collected from Gatundu area of Kiambu County, about 45 km North of Nairobi (Kenya) in the month of July 2014. Identification and authentication was done by the University of Nairobi, School of Biological Sciences Herbarium and a voucher specimen WGG2014/01 reserved for reference.

Preparation of the plants extract
The stem, root bark and leaves were air dried under shade for two weeks away from direct sunlight. They were then mixed in equal proportions (w/w) then ground into powder in the chemistry department, University of Nairobi. About 150 grams of the powder was soaked in 300ml of a 1:1 mixture of dichloromethane and methanol for 48 hours then decanted to obtain the supernatant. The residual was subjected to the same procedure and the supernatant obtained. The supernatant was pooled together and concentrated using a rotor evaporator and then left in universal bottles for two weeks to dry. About 17.6 grams of the extract was obtained.

Experimental animals
Swiss albino mice (20-25g) of both sexes evenly distributed were used for all the studies. They were housed in cages at room temperature and 12-hour light-darkness cycle. They were fed on mice pellets (Unga Feeds) and water ad-libitum. The Principle of Laboratory Animal Care (NIH publication No. 85-23) guidelines and procedures were followed in this study (NIH publication revised 1985).

Drugs and Chemicals
The following standard drugs and reagents were used; methanol, dichloromethane, acetic acid, dimethyl sulfoxide (DMSO), formalin, morphine and acetyl salicylic acid (aspirin).

Antinociceptive assay
Writhing test
The acetic acid writhing test (Singh PP et al., 1983) test mice (n = 8) were injected intra-peritoneal with 50, 100 and 200 mg doses of the extract, the positive control group received 100 mg of aspirin and the negative control (vehicle ) 10% DMSO in normal saline. Sixty minutes after respective test solution administration, the mice were then injected intraperitoneally with 0.1 ml solution of 0.5% acetic acid then placed individually into perspex boxes 30 cm x 30 cm x 30 cm with mirror placed behind for ease of visualization. The writhes (abdominal constriction) resulting from injection of acetic acid were taken as an indication of pain and were counted twenty minutes following the injection. Reduction in number of writhes as compared to the vehicle treated group was an indication of analgesia.

Tail Flick Test
The tail flick test as described in Amour and Smith was used to assess the effect of the plant extract on thermal induced nociception. The test mice in groups (n = 8) were injected intraperitoneally with 50, 100 and 200 mg doses of the extract while the controls received 5 mg of morphine and the vehicle (10% DMSO) in normal saline. One hour later they individually underwent the tail flick procedure using a radiant heat analgesia meter (IITC Inc. Model 33). In the procedure, a beam of radiant heat was focused 5 mm from the tip of the animal’s tail and a timer started. The end point was when the animal flicked its tail away the time taken from application of the radiant heat to the flicking of the tail was taken as the latency of nociception. A cut of time of 20 seconds exposure was set to avoid tissue damage (Le Bars et al., 2001)

Formalin test
The formalin test as described by Hunskaar et al., 1985 was used to evaluate the antinociceptive effect of the plant extract. In the test, groups of mice (n = 8) were given i.p. injection of 50, 100 and 200 mg doses of the extract while the controls received 100 mg aspirin, 5 mg Morphine and vehicle (10% DMSO in normal saline). Sixty minutes later, each mice was injected with 0.1 ml of 5% formalin in the sub plantar region of the hind paw then individually placed in transparent cage observation chamber with mirrors to facilitate visualization. The time spent flinching, biting and licking of the injected paw was quantified as latency of nociception and was recorded for 30 minutes as follows. Early phase was measured between 0 – 5 minutes and the late phase between 15 – 30 minutes.

Statistical Analysis
The data for each set was pooled and expressed as the mean ± standard errors of the mean (S.E.M). One way analysis of variance (ANOVA) followed by Tukey’s Honest Significance post hoc test was used to compare test and control group values. Statistical significant was set at p < 0.05.
RESULTS

Writhing test
Table 1. The effect of *C. megalocarpus* extract on acetic acid induced writhing pain in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Writhes</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>21.87 ± 0.47</td>
<td></td>
</tr>
<tr>
<td>50 mg</td>
<td>6.5 ± 0.98</td>
<td>70**</td>
</tr>
<tr>
<td>100 mg</td>
<td>4.87 ± 1.34</td>
<td>77**</td>
</tr>
<tr>
<td>200 mg</td>
<td>2.625 ± 0.37</td>
<td>88**</td>
</tr>
<tr>
<td>Aspirin 100 mg</td>
<td>2.12 ± 0.22</td>
<td>90**</td>
</tr>
</tbody>
</table>

** p < 0.001

Tail flick test
Table 2. The antinociceptive effect of *C. megalocarpus* extract in the tail flick test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean latency of nociception</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>5.53 ± 0.12</td>
</tr>
<tr>
<td>50 mg</td>
<td>8.40 ± 0.39**</td>
</tr>
<tr>
<td>100 mg</td>
<td>8.91 ± 0.44**</td>
</tr>
<tr>
<td>200 mg</td>
<td>9.09 ± 0.50**</td>
</tr>
<tr>
<td>Morphine 5 mg</td>
<td>10.31 ± 0.65**</td>
</tr>
</tbody>
</table>

** p < 0.001

Formalin test
Table 3. The effect of various doses of *C. megalocarpus* extract on formalin induced nociception.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Early Phase</th>
<th>Late Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>152.5 ± 12.42</td>
<td>209.75 ± 16.74</td>
</tr>
<tr>
<td>50 mg</td>
<td>121.38 ± 5.87</td>
<td>149.91 ± 7.05**</td>
</tr>
<tr>
<td>100 mg</td>
<td>85.81 ± 9.62*</td>
<td>103.60 ± 10.96**</td>
</tr>
<tr>
<td>200 mg</td>
<td>49.83 ± 5.3**</td>
<td>16.03 ± 2.86**</td>
</tr>
<tr>
<td>Aspirin 100 mg</td>
<td>38.32 ± 5.93**</td>
<td>29.15 ± 3.31**</td>
</tr>
<tr>
<td>Morphine 5 mg</td>
<td>15.09 ± 4.5**</td>
<td>7.39 ± 1.59**</td>
</tr>
</tbody>
</table>

** P < 0.001

Fig 1. Antinociceptive effect of *C. megalocarpus* on acetic acid induced writhing pain in mice

Fig 2. Antinociceptive effect of *C. megalocarpus* in tail flick test

** p < 0.001

Fig 3. Antinociceptive effects of *C. megalocarpus* extract on formalin induced pain

** p < 0.001
DISCUSSION

All the three doses of the extract and aspirin caused highly significant (p < 0.001) antinociceptive effects compared with the vehicle in the writhing test (Fig. 1; Table 1). It is a model test for study of the peripheral antinociceptive effect of extracts and compounds. It involves stimulation of the cyclo-oxygenase (COX) and lipooxygenase (Ikeda et al., 2001) and indirectly leads to the release of endogenous nociceptive mediators (PGE₂, PGF₂α, serotonin, histamine, cytokines and eicosanoids) as well as other LOX products in peritoneal fluids that can induce various nociceptive neurons within the peritoneal cavity (Ikeda Y et al., 2001; Vasudevan M et al., 2006). The ability of the extract to attenuate the acetic acid induced abdominal constriction test suggests that its antinociceptive mechanism may involve, in part, its ability to inhibit cyclo-oxygenase enzyme in the peripheral tissues leading to decrease in prostaglandin synthesis and blockage of the pain transduction in primary afferent nociceptor (Mohd et al., 2012). The extracts exhibited highly significant antinociceptive effect in the test hence it is highly probable they exert peripheral action which includes the release of these mediators.

In the tail flick test, all the three doses, 50, 100 and 200 mg of the extract exhibited highly significant antinociceptive effects (p < 0.001) compared to the vehicle which was comparable to aspirin. However morphine showed higher level of activity than the herbal doses (Fig. 2; Table 2). The tail flick test is a spinally mediated nociceptive test commonly used to study pain mechanism (Bar L et al., 2001). In this study the extract was found to prolong the latency withdrawal time after radiant heat was directed to the tail of the test mice. Since the tail flick is a spinally mediated reflex, it is likely that C. megalocarpus extract acted via the central nervous system by blocking pain pathway at spinal level.

The 100 and 200 mg exhibited a highly significant (p < 0.001) effects compared to the vehicle while aspirin exhibited significant (p < 0.05) antinociceptive effects in the early phase of the formalin test. In late phase of the test, all the doses of the extract exhibited a highly significant (p < 0.001) antinociceptive effects compared to the vehicle effects that were comparable to that of aspirin and morphine (Fig. 3; Table 3). The formalin test, which represents a model of prolonged pain, is very useful in studies of pain mechanism and in the evaluation of analgesic drugs (Shibata M et al., 1989). The early phase, classified as neurogenic pain, is an acute response observed immediately after the administration of formalin as a result of direct action of formalin on nociceptors (Duboisson D & Dennis SG, 1977; Alreja M et al., 1984; Hunskaar et al., 1985 a; Hunskaar S and Hole L, 1987, Shibata M et al., 1989). The late phase, represents inflammatory pain, is a tonic response resulting from the inflammatory processes generated by the release of inflammatory mediators such as histamine, serotonin, prostaglandins, bradykinin (Vermaet. al., 2005) and activation of the dorsal horns pain pathways in the spinal cord (Hunskaar S & Hole OB 1987; Tang L et al., 2007). Centrally acting drugs such as opioids inhibit both phases while peripherally acting agents like non-steroidal anti-inflammatory drugs inhibit only the late phase (Mohd M et al., 2012). Aspirin has been found to inhibit both phases of pain in mice (Hunskaar S and Hole OB, 1987) which could be because of prostaglandin inhibition in CNS or increasing serotonin though it may be due to down regulation of 5-HT2 receptors that can centrally reduce pain. In this study, antinociceptive activity was observed in both phases compared to the vehicle, results that may suggest that the extract may have exerted its effect through both central and peripheral nociceptive mechanisms.

CONCLUSION

The leaf, stem and root bark extract of C. megalocarpus in the 1:1:1 ratio exhibited significant antinociceptive activity in animal the models of nociception. It inhibited the peripheral, chronic and central nociceptive activity. Therefore, the plant probably contains secondary metabolite(s) with antinociceptive effects which may be of great value in development of novel drugs for analgesia. However, further studies need to be done to elucidate nature and mechanism(s) of action of these metabolites.

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CONFLICT OF INTEREST

No interest.

REFERENCES


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