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Afr. J. Biomed. Res. Vol. 26 (December 2023); Supplement 1; 45 – 50

Research Article

Cnidoscolus aconitifolius Extract Represses Nociception Via Opiodergic and Cholinergic Pathways in Laboratory Mice

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ABSTRACT

Cnidoscolus aconitifolius plant has been shown to have antinociceptive effect, but the underlying mechanisms are unknown. This study examined the antinociceptive mechanisms of action *C. aconitifolius* by probing the opiodergic, cholinergic, and adrenergic systems. Behavioural assessments of pain were done using the paw licking test caused by formalin, abdominal writhing induced by acetic acid and tail-flick tests. Methanol extract of *C. aconitifolius* (MECA) significantly reduces nociception in the paw licking ibduced by formalin and abdominal writhing induced by acetic acid but not tail flick test. Pretreatment with opoidergic and cholinergic blockers reversed these effects but not adrenergic blocker. MECA can be said to potentiate its antinociceptive effect by acting peripherally in the manner of opioidergic and cholinergic agents.

Keywords: *Cnidoscolus aconitifolius , nociception, opiodergic system, cholinergic system, adrenergic system.*

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Received: December 2022; Accepted: June 2023

DOI: 10.4314/ajbr.v26iS1.7

INTRODUCTION

When noxious stimuli are encountered, the body's natural defense mechanism is activated, resulting in a sensation or emotional response. But the long-term impacts of chronic pain can have a significant impact on both clinical and economic outcomes (Mackey and Kao, 2019). Patients' reports of pain and anxiety serve as a kind of early warning system. Anguish and an emotional state of dangers linked to concern are generated by a sensory and perceptual experience. In order to avoid accidents and to survive, it warns of possible bodily harm (wieboda *et al* ., 2013). The periaqueductal gray region (PAG) get input from the amygdale, hypothalamus and rostral anterior cingulate cortex (rACC) , which in turn feeds the medulla via an opioid-sensitive descending circuit for pain modulation. For analgesia and antinociception, opiod receptops can be found in the Nervous system (Labuz *et al* ., 2016). An other mechanism implicated in antinociception is the cholinergic and adrenergic system, which can be found both in the spine and in the brain (Jiang *et al* ., 2018; Hayashida and Obata, 2019). The analgesia caused by 2-adrenoceptor stimulation in animal models of neuropathic pain is considered to involve spinal stimulation of acetylcholine release, rather than adrenergic suppression of acetylcholine release under normal conditions (Hayashida and Eisenach, 2010).

Pain can be treated using a wide range of medication classes. Nevertheless, the therapeutic use of analgesics is restricted because to their availability, adverse drug reactions, price and many drugs are not as efficacious as for all patients (Crofford, 2013). Tolerance and physical dependence can develop as a result of repeated use of a variety of medications, including opiates (Coluzzi *et al* ., 2017). Natural plant sources have been examined as prospective possibilities for the development of a medication with fewer side effects and higher tolerability for the treatment and management of pain (Anilkumar, 2010; Alexa-Stratulat *et al.*, 2017; Tamba and Alexa-Stratulat, 2017; Owemidu *et al* ., 2018). More than a quarter of all prescribed medications are derived from

medicinal plants, according to the International Agency for Research on Cancer (Sen and Chakraborty, 2017).

Western Nigeria is home to the *Cnidoscolus. aconitifolius*, better known as Chaya, tree spinach, or efo iyanaipaja (Yoruba). For the most part, the therapeutic properties of *C. aconitifolius* were overlooked. For more information, see Ross-Ibarra *et al* . (2002a). *Cnidoscolus aconitifolius* has previously been shown to have antinociceptive effects (Onasanwo, Oyagbemi and Saba, 2011). However, we don't yet know the exact mechanism behind this phenomena. In order to learn more about the antinociceptive mechanisms in *C. aconitifolius* lab mice, this research was carried out.

MATERIALS AND METHODS

Preparation of plant extracts: In Ibadan, Oyo State, Nigeria, fresh leaves of the *Cnidoscolus aconitifolius* plant were procured at a Bodija market. The leaves were powdered, dried by air drying, and weighed. A glass container containing 2 kg of the leaves was filled with enough methanol to cover the leaves for 3 days. Then, the methanol was drained and fresh methanol was added. Three more days were spent with this treatment. The filtrate was then concentrated at a lowered temperature of 104 °F in a rotating evaporator. A semi-solid paste was acquired and stored in the fridge for future use. Before use, the extract was freshly produced.

Animals: Swiss male mice weighing 20–29 g were the animals utilized in this study. The mice were housed in an animal facility with a 12-hour cycle of light and darkness. They have unlimited access to food and drink. All experimental protocols complied with the fundamentals of recommended laboratory animal use (NIH, 1996).

Analgesic activity

Test for paw licking caused by formalin: Following the oral administration of saline,Methanol ectract of *Cnidoscolus aconitifolius* (MECA) (50, 100 or 200 mg/kg) or Diclofenac (100 mg/kg), 60 minutes later , 20 µL of 2.5 % formalin solution was injected onto the plantar surface of the left hind paw. The experiment was conducted in a transparent plastic chamber (30 × 30 × 30) cm with a mirror positioned at the base (bottom) of the chamber to provide a clear view of the mice. An observer who was blind to the treatments recorded the percentages of paw licking from the moment formalin is injected for five minutes and later 15 minutes after formalin is injected for the next fifteen minutes. The formalin-injected paw was licked, which indicated pain. Following the injection of formalin, the immediate, acute nociceptive reaction within the first five minutes denoted the first phase which represented neurogenic pain responses, whereas between 15–30 minutes identified the chronic phase which represented inflammatory pain responses. Using the following formula, the % inhibition of nociception during the two stages was determined.

$$\%\ Inhibition = \frac{Control\ mean - Test\ mean}{Control\ mean} \times 100$$

Test of Tail-flick: According to a generally accepted approach (D'Amour and Smith, 1941), the efficiency of analgesics is determined by examining how the body responds to heat. This experiment made use of a tail flick apparatus (Ugo Basile, Italy). The animal was placed on the platform, the start button was clicked (time began), and the equipment focused a bright light beam on the animal's tail after treatment with saline,
C. aconitifolius extract (50, 100, or 200 mg/kg), or Diclofenac (100 mg/kg). When the animal flicks its tail, the timer stops, and the recorded time (delay) calculates the animal's pain threshold. For 120 minutes, the mice undergo this treatment again at intervals of 30 minutes. For 120 minutes, this process is done every 30 minutes.

Writhing test induced by Acetic acid: The animals were pre-treated with normal saline, *C. aconitifolius* extract (50,100, 200 mg/kg b.w.) or diclofenac 100 mg/kg. After 30 minutes, 0.1 ml/10g b.w. of 3 % acetic acid solution was given intraperitoneally (Taber *et al* ., 1969). For 30 minutes, the number of writhes was tallied and counted using the formula:

$$\%\ Inhibition = \frac{Mean\ no.of\ writhes\ (control) - Mean\ no.of\ writhes\ (Test)}{Mean\ no.of\ writhes\ (control)} \times 100$$

Mechanism of action: We tested for the involvement of the opioidergic, adrenergic and cholinergic systems in the mechanism of action of *Cnidoscolus aconitifolius* using the writing test induced by acetic acid. The most effective dose (200 mg/kg) from the previous experiment was used to investigate the possible mechanisms by which *Cnidoscolus aconitifolius* inhibits writhing caused by acetic acid. Various blockers were initially given to the mice intraperitoneally - Naloxone (non-selective opioid antagonist, 1 mg/kg), Atropine (2 mg/kg), a muscarinic cholinergic receptor antagonist or Prazosin (alpha 1-adrenergic receptor antagonist, 1 mg/kg). After 30 minutes, the mice received *Cnidoscolus aconitifolius* (200 mg/kg, orally). Thirty minutes after administration given the extract, 0.1 ml/10g b.w. of 3 % acetic acid solution as given and the number of writhes in was recorded by an observer blind to the treatments for 30 minutes.

Statistical Analysis: Mean±SEM is used to express the findings. Two-way analyses of variance was used to analyze the tail flick test, and then Tukey's post hoc analysis. One-way variance analysis and Tukey's post hoc analysis were used to analyze all other data. All statistical analyses were performed on Windows using GraphPad Prism version 5. The significance level was established at $P < 0.05$.

RESULTS

The formalin test's paw licking time was decreased by MECA: Figure 1A's One –way ANOVA results revealed a difference in the time of paw licking in the early stages of paw licking test induced by formalin that was statistically significant. Comparing diclofenac (100 mg/kg) to the control and MECA (50 mg/kg), diclofenac was able to shorten the duration of paw licking (p 0.05). MECA dosages of 100 mg/kg and 200 mg/kg resulted in a small but insignificant reduction when compared to control. Nevertheless, MECA dramatically decreased paw licking duration in the late period (Figure 1B). When compared to the control, all MECA doses significantly

shortened the time spent licking paws (p 0.01). These findings point to MECA's anti-inflammatory properties.

MECA increased response time in Tail-flick latency assay: Figure 2 from the two-way ANOVA analysis illustrates the significant treatment effect on the latency of response in the tail flick test. Basal response latency was similar among all the treatment doses. After 30 minutes of administration, there was a slight increase in latency in the MECA 100 and 200 mg/kg group compared to control. 60 minutes after administration, all treatment groups, including Diclofenac, showed slight increases in latency compared to control. MECA 50 mg/kg also tend to sustain these increases up to 120 minutes after administration compared to control. However, these changes were not significant.

MECA reduced abdominal writhing in the acetic acid-induced writhing test: MECA had a significant reduction effect on abdominal writhing. The number of writhes was significantly reduced at 200mg/kg when compared to control ($p < 0.01$) as shown in Figure 3. Among the three doses of MECA 200 mg/kg seem to have a greater effect in reducing the number of writhes ($p< 0.05$ MECA 50, 100 mg/kg vs 200 mg/kg). In addition, Diclofenac also significantly lowered the number of writhes when compared to control ($p < 0.05$).

MECA potentiate analgesic properties through opioidergic, cholinergic, but not adrenergic systems in the writhing test: To unravel the underlying mechanisms of the analgesic and antiinflammatory effects of MECA, we studied the involvement of the opioidergic, cholinergic, and adrenergic systems by blocking relevant receptors. As shown in Figure 4, the analgesic and antiinflammatory effects of MECA was reversed significantly. When pretreated with naloxone, the number of writhes increased significantly compared to animals that received MECA 200 mg/kg only ($p < 0.001$).

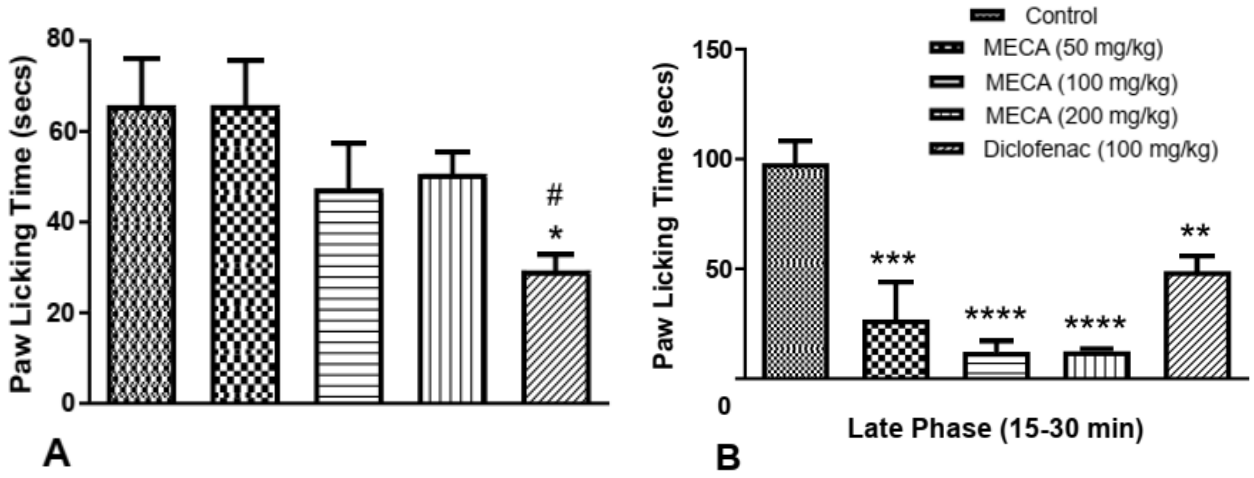

Figure 1:
MECA's impact on the length of time paws were licked during the formalin test's (A) early and (B) late phases. Mean and SEM are used to express data, n = 6 . *p <0.05, **p <0.01, ***p <0.001,
****p <0.0001 vs control; #p <0.05 against MECA 50 mg/kg.

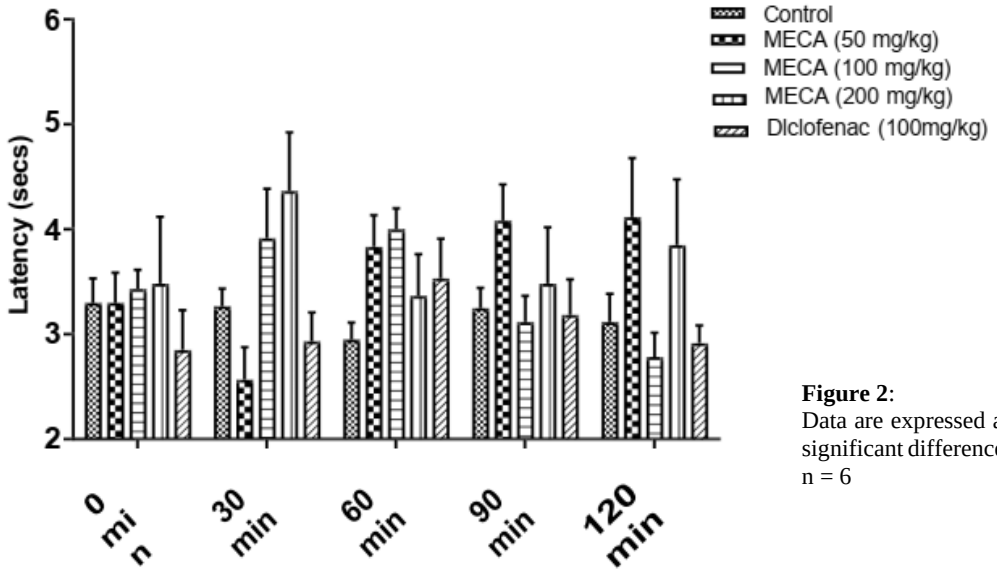

Figure 2:
Data are expressed as Mean ± SEM. There is no significant difference observed among the groups.
n = 6

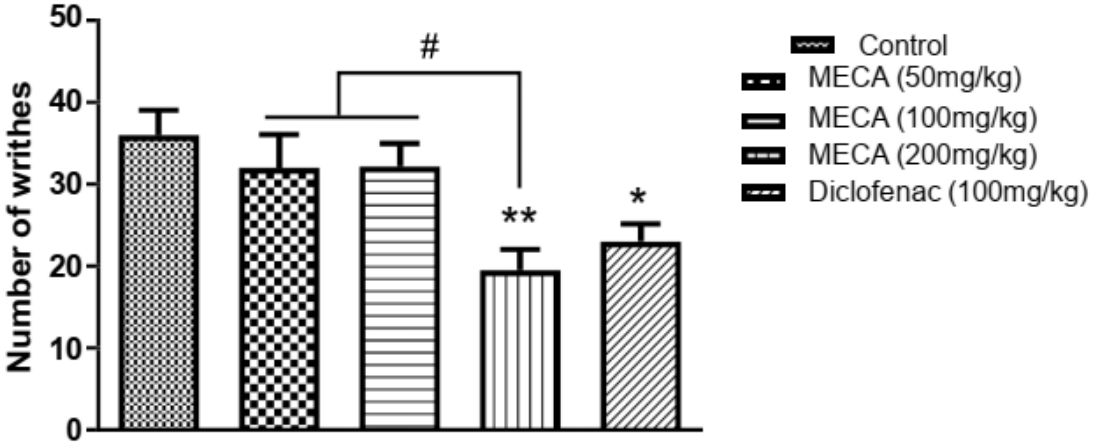

Figure 3:
MECA's impact on the number of writhes caused by acetic acid. Mean and SEM are used to express data, n = 6. *p <0.05, **p <0.01 vs control; #p <0.05 vs MECA 200 mg/kg.

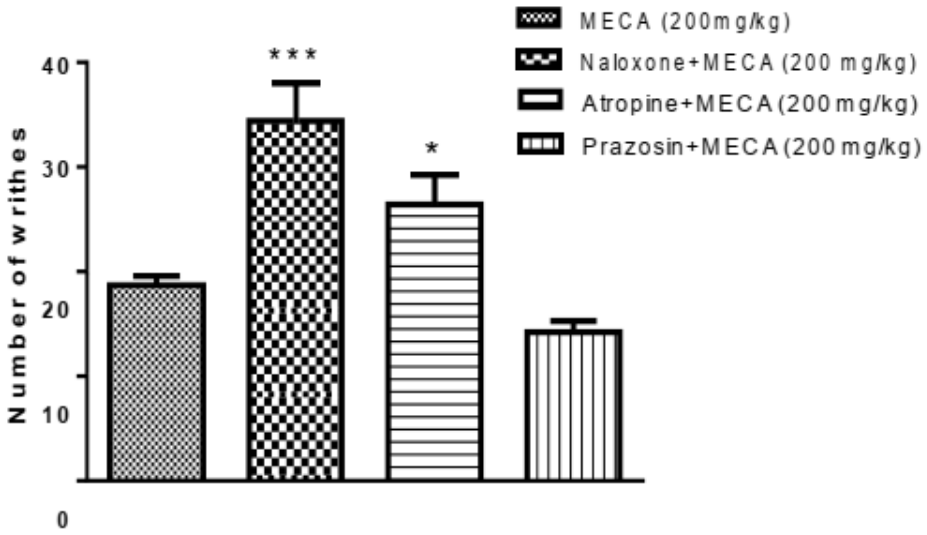

Figure 4:
Effect of MECA on abdominal writhing test induced by acetic acid after pretreatment with opioidergic, cholinergic, but not adrenergic receptor blockers. Mean and SEM are used to express data, n = 5. *p <0.05, ***p <0.01 vs MECA 200 mg/kg

Similarly, pretreatment with atropine also reversed the analgesic effect of MECA by increasing the number of writhes ($p < 0.05$). However, pretreatment with prazosin was not able to reverse these effects. Put together, these data suggest that MECA potentiates its analgesic effects via the opioidergic and cholinergic systems, but not adrenergic system.

DISCUSSION

In this paper, the probable mechanism of action of the methanol extract of *Cnidoscolus aconitifolius* and the involvement of the opioidergic, cholinergic, and adrenergic systems were investigated.

The biphasic formalin paw licking test's initial phase immediately affects nociceptors and stimulates prostaglandin production. (Hong and Abbott, 1995; Yin *et al* ., 2003). Opioid analgesics have been shown to have antinociceptive effects in both periods, but the second phase is more prominent (Le Bars, Gozariu and Cadden, 2001). For example, indomethacin, a nonsteroidal anti-inflammatory medicine (NSAID), is claimed to be beneficial only in the early phase, especially if formalin is administered at a high dosage (Yashpal and Coderre, 1998). Both MECA and Diclofenac failed to reduce the nociceptive activity caused by formalin in the study's initial phase. It was found that all doses of *Cnidoscolus aconitifolius* (MECA) and Diclofenac reduced nociception induced by formalin in the second phase

(inflammatory). There are anti-inflammatory activities in *Cnidoscolus aconitifolius* (MECA) methanol extract, as demonstrated by the results of this study.

Swelling linked with pain and inflammation is reduced by the use of nonsteroidal antiinflammatory drugs (NSAIDs) such as aspirin and indomethacin, which suppress prostaglandin production by directly targeting cyclooxygenase (COX) in the arachidonic acid metabolism (Amos *et al* ., 2001; Nwafor and Okwuasaba, 2003). While Diclofenac was found to have a dose-dependent anti- inflammatory impact, this study found that the *Cnidoscolus aconitifolius* methanol extract (MECA) had the same effect.

Tests for centrally acting analgesics like morphine use the tail-flick reflex, which is based on the spinal reflex and selective for centrally acting analgesics (Srinivasan *et al* ., 2003). Other brain structures may also be involved (Le Bars, Gozariu and Cadden, 2001). No significant differences were identified in the tail-flick test results as compared to a control group. Analgesic effects of MECA are not mediated through the *Cnidoscolus aconitifolius* central nervous system (CNS).

Irritants like acetic acid and phenylquinone are administered to mice in order to induce peripheral discomfort like writhes. A reduction in the frequency of writhing is evidence of the test compound's analgesic properties (Gawade, 2012). When administered intraperitoneally, acetic acid is thought to release inflammatory mediators from the nociceptive neurons, such as prostaglandins, bradykinin, and pro-inflammatory cytokines (Collier *et al* ., 1968; Salvemini *et al* ., 1995). (Ikeda *et al* ., 2001; Figueredo *et al* ., 2011).

Cnidoscolus aconitifolius ' methanol extract was found to have analgesic properties at 200 mg/kg. *Cnidoscolus aconitifolius* methanol extract has analgesic effects via reducing or suppressing the production of endogenous inflammatory mediators in nociceptive neurons, according to this findings. Which pathway is responsible for Cnidoscolus aconifolius methanol extract's anti-nociceptive activities? Several blockers were used in the mechanistic investigation, which demonstrated this.

Cnidoscolus aconitifolius ' methanol extract analgesic effects were efficiently inhibited by naloxone (non-selective opioid antagonist) and atropine (cholinergic receptor antagonist). This suggests that the *Cnidoscolus aconitifolius* ' methanol extract has analgesic properties that may be linked to the cholinergic and opioid systems.

As a medicinal herb, *Cnidoscolus aconitifolius* may have an analgesic impact on the peripheral nervous system. However, the opioidergic and cholinergic systems may be involved in the process by which it exerts its analgesic effect, with little participation from the adrenergic systems. In this work, *Cnidoscolus aconitifolius* ' analgesic qualities were investigated. A novel class of analgesics and anti-inflammatory drugs could be developed using *Cnidoscolus aconitifolius* ' isolated components.

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