



Analgesic and free radical scavenging activities of hydromethanolic extract of *Crateva adansonii* stem bark

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Received: March 31, 2015

Accepted: April 30, 2015

Published: May 08, 2015

ABSTRACT

Objective: *Crateva adansonii* is a moderately sized deciduous tree found throughout the tropics especially along the river banks. This study was aimed at the evaluation of the analgesic and antioxidant activities of the methanolic extract of *C. adansonii* stem-bark. **Methods:** The analgesic activity of *Crateva* extract was investigated using both chemical and thermal models of nociception in rodents while the antioxidant activity was evaluated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) photometric model. **Results:** The extract produced a minute concentration-dependent increase in free radical scavenging activities. The extract (100, 200, and 400 mg/kg) caused a significant ($P < 0.05$) dose-dependent reduction in the number of writhing in treated rats when compared to the negative control. The extract at 100, 200, 400 mg/kg, and pentazocine (3 mg/kg) increased the pain reaction time in the treated rats by 58.05%, 66.67%, 94.76%, and 79.40%, respectively, when compared to the negative control. **Conclusion:** The *C. adansonii* stem bark possesses analgesic activity against peripheral and central mediated pain sensation and also antioxidant properties. This study justifies the ethnomedical use of *C. adansonii* in pain treatment.

KEY WORDS: Acetic acid, analgesic, aspirin, *Crateva adansonii*, pentazocine, tail flick

INTRODUCTION

Medicinal plants have been used as a form of medication for the relief of pain throughout history [1]. The treatments of pain disorders are an area in which the practitioners of traditional medicine enjoy patronage and success [2]. Herbs are believed to be an important source of new chemical substances with potential therapeutic efficacy. Salicylic acid and morphine were originally derived from the plant sources, thus study of plant species traditionally used as pain killers should be encouraged as a useful research strategy in the search of new analgesic and anti-inflammatory drugs [3].

Crateva adansonii DC belonging to the family *Capparidaceae* is commonly called “Varun” or “garlic pear” in English [4,5]. *C. adansonii* is a moderately sized deciduous tree found throughout the tropics especially along the river banks. The leaves are trifoliate and ovate to oblong in shape. The flowers

are white or creamy and occur at the terminal corymbs. The barks are gray, smooth, and sometimes horizontally wrinkled [6]. Different parts of the plant are extensively used in folkloric medicine for the cure of many disease conditions. The powdered bark is used in the treatment of urinary, renal tubules, gastro-intestinal, and uterine affection [5]. In ethnomedicine, the plant is used in inflammatory conditions, asthma, snakebites, and as astringent [7]. In Senegal, the roots are used in the treatment of syphilis, jaundice, and yellow fever [8]. The scientific evaluation of the antimicrobial, anti-gout, and antitrypanosomal activities has been reported [4,9,10]. Abdullahi *et al.* [9] reported the presence of phenolics, alkaloids, flavonoids, and saponins in the leaves of *C. adansonii*.

Despite the widespread use of *C. adansonii* in ethnomedicine by many cultural groups in the relief of rheumatic and other pain conditions, there is a paucity of scientific information

on the analgesic and antioxidant properties of the plant. This study was designed to evaluate the analgesic and antioxidant activities of the methanolic extract of *C. adansonii* stem-bark.

MATERIALS AND METHODS

Collection and Identification of Plant Material

The stem barks of *C. adansonii* were collected in May, 2014 from Orba in Udenu local government, Enugu State, Nigeria. They were identified by Mr. A. O. Ozioko, a Taxonomist at Bioresource Development and Conservation Programme, Enugu state, Nigeria. A voucher specimen catalogued MOUAU/VPP/2014/012 was deposited in the departmental herbarium for reference purposes.

Preparation of the Plant Material

The stem bark of the plant were dried at room temperature on a laboratory bench and pulverized into coarse powder. The powdered plant material was extracted using cold maceration method in 80% methanol for 48 h with intermittent shaking at 3 h interval. The extract was filtered using Whatmann No. 1 filter papers. The filtrate was concentrated in a hot air oven at 40°C and the extract was stored in a refrigerator at 4°C as *Crateva* extract until required for the experiment. The percentage yield was calculated using the formula below:

$$(\text{Weight of extract/weight of starting plant material}) \times 100$$

Experimental Animals

Totally, 30 albino rats of both sexes weighing 100-130 g and 30 mice of both sexes weighing 28-34 g, sourced from the laboratory animal unit of the Department of Veterinary Physiology, Pharmacology and Biochemistry, Michael Okpara University of Agriculture Umudike, Abia State were used for the study. The animals were housed in aluminum cages at room temperature and under natural light/darkness cycles. The rats were supplied with clean drinking water and fed *ad libitum* with standard commercial pelleted grower feed (Vital feed® Nigeria). The rats were acclimatized for 2 weeks prior to the study. They were maintained in accordance with the recommendations of the Guide for the care and use of laboratory animals [11] and the experimental protocol was approved by the Institution's Ethical Committee.

Determination of Free Radical Scavenging Activities of *Crateva* Extract using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) Photometric Assay

The free radical scavenging activity of *Crateva* extract was analyzed by the DPPH photometric assay [12] using a spectrophotometer. The test extract (2 ml) at different concentrations (25, 50, 100, 200, and 400 µg/ml) were mixed with 0.5 mM DPPH (in 1 ml of methanol) in a cuvette. The absorbance at 517 nm was taken after 30 min of incubation in the dark at room temperature. The concentrations were

prepared in triplicates and the percentage antioxidant activity calculated as follows.

$$\% \text{ antioxidant activity (AA)} = 100 - \left(\frac{[\text{absorbance of sample} - \text{absorbance of blank}] \times 100}{\text{absorbance of control}} \right)$$

A volume of 1 mL of methanol plus 2.0 ml of the extract was used as the blank while 1.0 ml of the 0.5 mM DPPH solution plus 2.0 ml of methanol were used as the negative control. Ascorbic acid (vitamin C) was used as reference standard [13].

Effect of *Crateva* Extract on Acetic Acid-induced Abdominal Writhing in Mice

The method of Vale *et al.* [14] was used. Five groups of mice consisting of 6 mice each were fasted for 12 h but free access to tap water was allowed. Group A received distilled water (10 ml/kg) and served as negative control. Group B received aspirin (100 mg/kg) orally and served as positive control, while Groups C-E received 100, 200, and 400 mg/kg of *Crateva* extract by oral administration, respectively. 45 min later, the mice received 10 ml/kg of 0.7% acetic acid intraperitoneally. The number of writhing or abdominal stretches produced in each mouse was counted for 30 min.

Effects of *Crateva* Extract on Tail Flick Response in Rats

The experiment was carried out by measuring tail withdrawal time from hot water as described by Adzu *et al.* [15]. Thirty rats were randomly divided into 5 Groups (A-E) of 6 rats each and fasted for 12 h. The rats were treated as follows: Group A served as negative control and received distilled water (10 ml/kg) orally, Group B served as positive control and received pentazocine (3 mg/kg) intraperitoneally, while Group C-E received *Crateva* extract (50, 300 and 600 mg/kg, respectively) orally. 1 h post drug treatment about 3 cm of the tail of each rat was dipped into a water bath containing warm water maintained at a temperature of 50 ± 1°C. The time taken for the mouse to flick the tail known as the pain reaction time (PRT) was recorded for all the mice.

Statistical Analysis

Data obtained were presented as mean ± standard error of mean and analyzed using one-way Analysis of Variance of SPSS software. The variant mean was separated by least significant difference of the different groups. Significance was accepted at the level of $P < 0.05$.

RESULT

DPPH Radical Scavenging Effect

The extract produced a minute concentration-dependent increase in free radical scavenging activities. The effects of the extract were significantly ($P < 0.05$) lower when compared to the effects of the ascorbic acids. The IC₅₀ of the extract is >400 µg/ml [Figure 1].

Effect of *Crateva* Extract on Acetic Acid-induced Abdominal Writhing in Mice

The effects of *Crateva* extract on acetic acid induced writhing are presented in Table 1. The extract (100, 200, and 400 mg/kg) caused a significant ($P < 0.05$) dose-dependent reduction in the number of writhing in treated rats when compared to the negative control. The effects of the *Crateva* extract were comparable to that of aspirin (100 mg/kg). The extract (100, 200, and 400 mg/kg) and aspirin (100 mg/kg) produced 56.30%, 57.97%, 61.30%, and 50.93% reduction in the number of writhing respectively, when compared to the negative control.

Effects of *Crateva* Extract on Tail Flick Response in Rats

The pretreatment of the rats with *Crateva* extract (100, 200, and 400 mg/kg) caused a significant ($P < 0.05$) increase in the PRT in a dose-dependent manner when compared to the negative control group [Table 2]. The extract (100, 200, and 400 mg/kg)

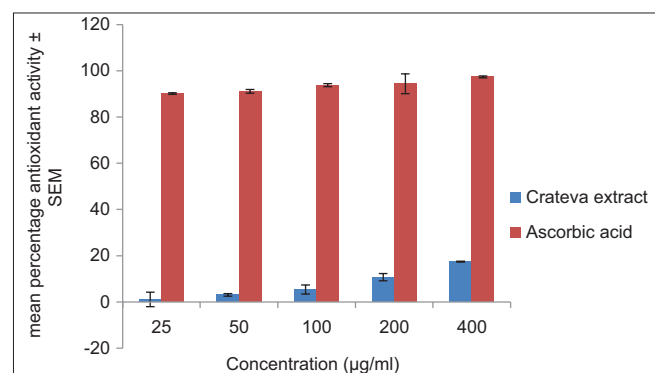


Figure 1: Effect of *Crateva* extract on 2, 2-diphenyl-1-picrylhydrazyl radical scavenging assay

Table 1: Effects of *Crateva* extract on acetic acid induced writhing reflex in mice

Treatment	Mean number of writhing ± SEM	% inhibition
Distilled water 10 ml/kg	135 ± 9.00	-
Aspirin 100 mg/kg	66.25 ± 0.98***	50.93
<i>Crateva</i> extract 100 mg/kg	59.00 ± 1.11***	56.30
<i>Crateva</i> extract 200 mg/kg	56.75 ± 1.56***	57.97
<i>Crateva</i> extract 400 mg/kg	52.25 ± 1.08***	61.30

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ when compared to distilled water treated group. SEM: Standard error of mean

Table 2: Effects of *Crateva* extract on tail flick response in rats

Treatment	Mean PRT ± SEM (sec)	% increase on PRT
Distilled water 10 ml/kg	2.67 ± 0.30	-
pentazocine 3 mg/kg	4.79 ± 0.53*	79.40
<i>Crateva</i> extract 100 mg/kg	4.22 ± 0.43*	58.05
<i>Crateva</i> extract 200 mg/kg	4.45 ± 0.49*	66.67
<i>Crateva</i> extract 400 mg/kg	5.20 ± 0.75*	94.76

* $P < 0.05$ when compared to distilled water treated group, PRT: Pain reaction time; Sec: Second; SEM: Standard error of mean

and pentazocine (3 mg/kg) increase the PRT in the treated rats by 58.05%, 66.67%, 94.76%, and 79.40% respectively, when compared to the negative control.

DISCUSSION

The analgesic activity of *Crateva* extract was investigated using both chemical and thermal models of nociception in rodents while the antioxidant activity was evaluated using DPPH photometric model. The choice of the doses used in this study was based on the report of previous studies [7]. Acetic acid-induced writhing test was used for detecting both the peripheral and central analgesia, whereas the tail flick test are most sensitive to central acting analgesic drugs. The extract demonstrated a weak antioxidant activity in the DPPH photometric assay, with 50% inhibitory concentration (IC_{50}) > 400 µg/ml.

Intraperitoneal injection of acetic acid induces the release of prostaglandins and sympathomimetic system mediators in the peritoneal fluid, which sensitize the peritoneal nociceptors [3]. The pretreatment of the mice with *Crateva* extract produced a dose-dependent analgesia comparable to the analgesia produced by the standard analgesic drug (Aspirin). This indicates that the *Crateva* extract and aspirin may have a similar mechanism of action. Aspirin is a non-steroidal anti-inflammatory drug that reduces the synthesis of prostaglandins and thromboxanes by irreversible inactivation of cyclooxygenase enzyme [16-18].

The *Crateva* extract caused a significant ($P < 0.05$) increase in PRT in the treated rats in a dose-dependent manner. The quality of the analgesia produced by the *Crateva* extract in the thermal model is comparable to the analgesia produced by pentazocine [Table 2]. This indicates that the *Crateva* extract has a central acting analgesic activity and may have a similar mechanism of action as pentazocine. Pentazocine is a synthetic opioid agonist-antagonist, which produces analgesia through interaction with μ and κ -receptors [19].

The analgesic and antioxidant activities of *Crateva* extract may be mediated by the phytochemical constituents [20]. Abdullahi *et al.* [9] reported the presence of saponins, flavonoids, alkaloids, and phenolics in *C. adansonii* leaves. The above listed phytochemical components of *C. adansonii* have been demonstrated to possess analgesic and antioxidant properties [20,21]. The antioxidant effect may help to counteract the adverse effect of oxidative stress that may arise due to pain sensation and drug biotransformation [22].

CONCLUSION

The *C. adansonii* stem bark possesses analgesic activity against peripheral and central mediated pain sensation and also antioxidant properties. This study justifies the ethnomedical use of *C. adansonii* in pain treatment. Further work is desired for the characterization of active analgesic principle.

ACKNOWLEDGMENT

The author appreciates the effort of Mr. A. O. Ozioko in the collection and identification of the plant sample.

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Source of Support: Nil, Conflict of Interest: None declared.