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Analgesic Effect of Methanolic Leaves Extract of *Crateva adansonii* in albino Rat

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Abstract Pain is the most common reason for physician consultation in most developed countries but treatment is becoming complex because of the side effects of analgesic pharmaceutical drugs. This has lead to an increase in the used of medicinal plants for pain and pain related conditions. Previous studies have demonstrated that *Crateva adansonii* stem-bark produced analgesic and anti-inflammatory effect but no data are available concerning the antinociceptive effect on the leaves. This study was aimed at investigating the antinociceptive effect of the methanolic leaves extract of *C. adansonii* using tail-flick and acetic acid-induced writhing methods in albino rat. The results showed that the pain reaction time (tail withdrawal) following administration of *C. adansonii* leaves was significantly increased ($p<0.05$) in a dose-dependent manner compared to the control. Also the extract at all doses caused a significant ($p<0.05$) dose-dependent reduction in the number of writhing when compared to the control. However, rats receiving 400mg/kg leaves extract showed no significant ($p>0.05$) difference in the pain reaction time and number of writhing respectively compared to the standard drug aspirin treated rats. In conclusion, *C. adansonii* leaves exhibited antinociceptive activity against central and peripheral mediated pain sensation. This further justifies the folkloric claim of this plant in pain treatment.

Keywords Pain, Acetic acid, antinociceptive, Aspirin, *Crateva adansonii*, Tail flick

Introduction

Medicinal plants are plants which contain substances that can be used for the therapeutic purposes in one or more of its organ or substances which are precursors for the synthesis of useful drugs [1]. Humans have used them throughout history to either cure or lessen symptoms from an illness. Traditional treatment using medicinal plants often vary from place to place for the treatment of a particular disease. According to the World Health Organization [2], 80% of the world population use medicinal plants in the treatment of diseases and in African countries, this rate is much higher. It has been estimated that up to 90% of the population in developing countries rely on the use of medicinal plants to help meet their primary health care needs [3]. Globally, so many factors have been attributed to the continual increase in the use of herbal medicine. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments [4, 5, 6].

Pain is an unpleasant sensory and emotional experience (personal experience that is influenced to varying degrees by biological, psychological, and social factors) associated with, or resembling that associated with, actual or

potential tissue damage [7]. Pain management is a global challenge, due to the high prevalence of chronic or acute pain worldwide, large medical burden, and disabling effects [8]. Many of the currently available pain therapies are either inadequate or cause uncomfortable to deleterious side effects [9, 10]. In recent times, large number of people patronize the use of herbal medicines due to the general believe that it is green, cheap and a safe treatment choice with fewer side effects than synthetic drugs. Although some herbal medicines have promising potential and are widely used, many of them remain untested and their use also not monitored [4].

Crateva adansonii is a medicinal plant used for the treatment of pain. It belonging to the family *Capparidaceae* is commonly called "Varun" or "garlic pear" in English [11]. Various parts of *Crateva adansonii* are used in traditional medicine [12]. Ground roots are taken against fever and applied externally to treat headache and swellings [13]. Root decoctions are taken to treat gastrointestinal complaints and rheumatism. Powdered root bark is administered as a remedy against carbuncles and anthrax [14]. Powdered or pounded stem bark is applied to cysts and swellings, whereas bark decoctions are taken to treat rheumatism and sterility, and as a tonic [15]. Scientific research on *C. adansonii* reported the stem bark extract to possess analgesic activity against peripheral and central mediated pain sensation, and also antioxidant properties [16] while the leaves where found to possess anti-inflammatory phytoconstituents [17]. In a continuous effort to explore more ethnoparmcological potential of *C. adansonii*, the present study is aimed at evaluating the analgesic activity of its leaves extract in animal models.

Materials and Methods

Plant material

The fresh leaves of *Crateva adansonii* were collected in April, 2017 at Jiga Birnin, Aliero Local Government, Kebbi State, Nigeria. The voucher specimen was prepared and authentication was done at the Herbarium Unit, Biological Sciences Department, Faculty of Science, Kebbi State University of Science and Technology, Aliero.

Preparation of Extracts

The collected *Creteva adansonii* leaves were air dried under shade and pulverized to small pieces using pestle and mortar. The powdered plant material was extracted using cold maceration method in 50% methanol for 72 hours with intermittent shaking at time interval. The extract was filtered using Whatmann No. 1 filter papers. The filtrate was allowed to dry with the use of heat produced by water bath at 45°C and the extract was stored in a refrigerator at 4°C as *Crateva* extract until required for the experiment

Phytochemical Analysis

Qualitative screening for the presence of phytochemicals in *C. adansonii* leaves were carried out using standard procedures [18, 19, 20].

Experimental Animals

Fifty (50) albino rats of both sexes weighing 150-200 g were used for the study. They were purchased from Nigerian institute of Trapanosomiasis Research (NITR), Kaduna State. The animals were housed in aluminum cages at room temperature. The rats were supplied daily with clean drinking water and fed with standard commercial pelleted grower feed (Vital feed® Nigeria). The rats were allowed to acclimatize for 3 weeks prior to the study.

Analgesic activity of *C. adansonii*

Tail flick method

The analgesic activity of *C.adansonii* leaves extract was examined using tail flick method. The method described by Adzu [21] was adopted. The albino rats selected for this study were fasted overnight but water allowed before administration of the plant extracts. They were divided into 5 groups (1-5) of 4 per cage and 100mg/kg, 200mg/kg, 400mg/kg of the methanolic extract of *C. adansonii* were administered to groups 2, 3 and 4 respectively, while 10ml/kg of distilled water and 100mg/kg of aspirin was administered to group 1 and 5 respectively. 30 minutes after drug administration the marked tail of 2cm was immersed into a water bath containing warm water maintained at a

temperature of 55 ± 1.0°C and the time taken for the rat to remove its tail out of the water bath was recorded. The latency was evaluated at 0, 30, 60, 90 and 120 minutes with 0 minute being the initial reading. Tail flick latency difference or mean increase in latency after drug administration was used to indicate the analgesia produced by test and standard drugs.

Acetic Acid-induced Abdominal Writhing

The method described by Zakaria [22] was adopted. The albino rats were divided into 5 groups of 4 per cage. Methanolic extract of *C. adansonii* (100mg/kg, 200mg/kg and 400mg/kg) were administered to groups 2, 3 and 4 respectively, while 10ml/kg of distilled water and 100mg/kg of aspirin was administered to group 1 and 5 respectively. Thirty (30) minutes later, rats in all groups were given 1% acetic acid (10mg/kg i.p). The total number of writhing following the acetic acid administered was recorded for 30 minutes, starting 5 minutes after injection. Antinociceptive activity was expressed as the percentage reaction or inhibition of the number of abdominal writhing (characterized by contraction of the abdominal musculature and extension of the hind limbs).

Statistical Analysis

Statistical analyses were performed by ANOVA followed by the Bonferroni test by using statistical software package, Graph Pad Prism; version 5.03. Values were expressed as mean ± SEM and the $P<0.05$ were considered as statistically significant.

Results

Percentage Yield

The *creteva adansonii* yielded 19.9g of hydro-methanolic extract which was deep brown and sticky with pleasant smell.

Phytochemical Analysis

The result of phytochemical analysis of hydro-methanolic extract of *Creteva adansonii* leaves is presented in table 1.

Table 1: Phytochemical screening of *Creteva adansonii* leaves

Phytochemicals	Results
Alkaloids	+
Tannins	+
Steroid	+
Terpenoids	+
Saponins	+
Quinnones	ND
Flavonoid	+
Phenols	+
Cardiac glycoside	+

+=Indicate the presences of phytochemicals ND=Indicate non detection of phytochemicals

Effects of *C. adansonii* Leaves Extract on Tail Flick Response in Rats

The tail withdrawal time following administration of *C. adansonii* leaves extract was significantly ($P<0.05$) increased in a dose- dependent manner (Table 2). Likewise, the standard drug (Aspirin 100mg/kg) exhibited analgesic activity which was significantly different ($P<0.01$) from the groups treated with 100-200mg/kg *C. adansonii* leaves extract. Maximum tail withdrawal time was observed in the extract treated group receiving 400mg/kg which was significantly different ($P<0.05$) from the standard drug treated group.

Table 2: Effects of *C. adansonii* Extract on Tail Flick Response in Rats

Treatment group	Dose (mg/kg)	Mean Pain Reaction Time (sec) ± SEM				
		0min	30min	60min	90min	120min
Control (dis.H_20)	10ml/kg	0.93±0.05	1.05±0.03	1.15±0.03	1.05±0.03	1.08±0.03
C. dansonii leaves extract	100mg/kg	2.80±0.27*	3.83±0.28*	5.18±0.36*	3.53±0.49*	2.68±0.24*
C. dansonii leaves extract	200mg/kg	2.53±0.41*	3.65±0.31*	5.30±0.34*	3.78±0.28*	2.75±0.13*
C. dansonii leaves extract	400mg/kg	8.00±0.41***	10.75±0.48***	12.75±0.63***	12.75±0.95***	9.50±0.29 ***
Aspirin	100mg/kg	4.75±0.25***	6.50±0.29***	8.00±0.41***	8.80±0.65***	7.00±0.41***

The results are presented as mean PRT±SEM. N=4 *P<0.05, **P<0.01, ***P<0.001, ns: P>0.05

Effect of *Creteva adansonii* Leaves Extract on Acetic Acid-induced Abdominal Writhing in Albino rat

The effects of *C. adonsonii* leaves extract on acetic acid induced writhing are presented in Table 3. 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 49.59%, 41.64%, 62.64%, and 59.34% reduction in the number of writhing respectively, when compared to the negative control.

Table 3: Effect of *Creteva adansonii* Leaves Extract on Acetic Acid-induced Abdominal Writhing in Albino rat

Groups	Dose (mg/kg)	No of abdominal writhing	% Inhibition
Control (dis.H_2O)	10ml/kg	30.75±3.731	0.00
C. dansonii leaves extract	100ml/kg	15.5±1.561**	49.59
C. dansonii leaves extract	200ml/kg	18.0±0.82**	41.64
C. dansonii leaves extract	400ml/kg	11.5±1.711***	62.64
Aspirin	100ml/kg	12.5±1.191**	59.34

The results are presented as mean number of abdominal writhing ±SEM. N=4 *P<0.05, **P<0.01, ***P<0.001

Discussion

Medicinal plants are very valuable and rich source to obtain the bioactive molecules. Phytochemicals such as flavonoids and tannins has in several literatures been implicated for their analgesic effects [23, 24, 25, 26]. Alkaloid has been used as CNS stimulant, topical anesthetic in ophthalmology, powerful painkillers, and antipyretic action among other use [27]. In the present study, phytochemical screening of the hydro-methanolic leaves extract of *C. adansonii* showed the presence of terpenoids, saponins, cardiac glycosides, alkaloids, flavonoids, saponins, tannins, steroids and phenols. The presence of these phytochemicals may be responsible for its analgesic effects.

Rodents are commonly used to study the pathophysiological mechanisms of pain as studies in humans may be difficult to perform and ethically limited [28]. Animal models of neuropathic pain have been essential in the exploration of molecular mechanisms of pain also for the analysis of novel analgesics in the treatment of chronic pain [29]. The analgesic activity of *C. adansonii* extract was investigated using both tail immersion (heat) and acetic acid-induced (chemical) methods in rodents. Acetic acid-induced writhing test is used for detecting both the peripheral and central analgesia, whereas the tail flick test are most sensitive to central acting analgesic drugs [30].

Tail flick test (using predetermined temperature or applying radiant heat to a small portion of the tail) is one of the most common tests based on a phasic stimulus of high intensity [31]. The reflexive response (flick or twitch) observed after tail immersion in a temperature controlled water bath is an indication of pain sensitivity in the rats and the time taken is the pain reaction time (pain threshold). The dose-dependent increase of pain reaction time in the present study implies that treatment with *C. adansonii* may be capable of decreasing pain sensitization.

Acetic acid-induced writhing reflex is a model of visceral pain which is highly useful for screening analgesic drugs [32]. Acetic acid induced pain is generated indirectly via endogenous mediators like prostaglandin, which stimulates peripheral nociceptive neurons. These neuronal fibers are sensitive to both narcotics and non-steroidal anti-inflammatory drugs [33]. In the present study, analgesic effect produced in *C. adansonii* treated rats may be attributed to inhibition of arachidonic acid release from tissue or through suppression of prostaglandin pathway.

Analgesic medications may be classified as non-opioid analgesics, which includes the nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin, paracetamol etc. and opioid analgesics such as morphine, tramadol etc. Opiod analgesics (narcotics) act centrally through CNS but do not produce an anti-inflammatory response while non-opioid analgesics (non-narcotics) act peripherally producing an anti-inflammatory effect. Aspirin and other non-steroid anti-inflammatory drugs (NSAIDs) are known to inhibit the activity of cyclooxygenase (COX) which leads to the formation of prostaglandins (PGs) that cause inflammation, swelling, pain and fever [34]. The pretreatment of the albino rat with *C. adansonii* extract produced an analgesia that was comparable to the analgesia produced by the standard analgesic drug (Aspirin). It could be suggested that *C. adansonii* leaves extract exhibited both peripheral and central analgesic effect as tail flick test is sensitive to centrally acting analgesic drugs.

Conclusion

The present findings indicate that *C. adansonii* possesses analgesic activity against peripheral and central mediated pain sensation in dose-dependent manner and could be attributed to the presence of its analgesia-related phytoconstituents. This effect was comparable to the standard drug aspirin at the highest dose of the extract only and may have a similar mechanism of action. This study validates the folkloric medicinal claim of the indigenes of Kebbi State.

References

[1]. Sofowora, A., Ogunbodede, E., & Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *African journal of traditional, complementary, and alternative medicines:* 10(5):210–229. https://doi.org/10.4314/ajtcam.v10i5.2

[2]. WHO. (2001). Legal Status of Traditional Medicine and Complementary/Alternative medicine: A world wide review. WHO Publishing 1.

[3]. WHO. (2002). Traditional medicine – growing needs and potentials. WHO Policy Perspectives Med.

[4]. Ekor M. (2014).The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacology.* 2013; 4: 177. doi: 10.3389/fphar.2013.00177

[5]. NHP (2016): Introduction and Importance of Medicinal Plants and Herbs. National health portal, India.https://www.nhp.gov.in/introduction-and-importance-of-medicinal-plants-and-herbs_mtl

[6]. Pearson H, Fleming T, Chhoun P, Tuot S, Brody C & Yi S. (2018) Prevalence of and factors associated with utilization of herbal medicines among outpatients in primary health centers in Cambodia. *BMC Complementary Alternative Medicine*. 18(1):114. doi:10.1186/s12906-018-2181-1

[7]. IASP (2020): IASP Announces Revised Definition of Pain. International association for the study of pain (IASP) Publication and News July 2020 from https://www.iasp-pain.org/PublicationsNews/ NewsDetail.aspx?ItemNumber=10475

[8]. Zeng F, Jian Kong & Ming Yi (2019). Traditional Medicine for Pain Management. HINDAWI Pain Research and Management / Published Special Issues / Special Issue

[9]. Loeser J D, Butler S H, Chapman C R & Turk K C, eds.(2001) Bonica's Management of Pain (Lippincott, Philadelphia).

[10]. Martel, M. O., Finan, P. H., Dolman, A. J., Subramanian, S., Edwards, R. R., Wasan, A. D., & Jamison, R. N. (2015). Self-reports of medication side effects and pain-related activity interference in patients with chronic pain: a longitudinal cohort study. *Pain*. 156(6), 1092–1100. https://doi.org/10.1097/j.pain. 0000000000000154

[11]. Igoli, N. P., Gray, A.I., Clements, C.J., Igoli, J.O., Uche, N. & Singla, R.K.. (2012). Scientific investigation of antitrypanosomal activity of Crateva Adansonii DC leaves extracts, *Indonesia Global Journal of Pharmaceutical Science.* 2(3):226-229.

[12]. Lemmens, R.H.M.J. & Bosch, C.H., (2013). Crateva adansonii DC. In: Schmelzer, G.H. & Gurib-Fakim, A. (Editors). Prota 11(2): Medicinal plants/Plantes médicinales 2. PROTA, Wageningen, Netherlands. Accessed 31 March 2017.

[13]. Facciola. S. (1998). Comucopia II, Kampong Publications, California. ISBN 0-9628087-2-5.
[14]. Nwosu M. (2000). Plant resources used by women as herbal medicines and cosmetics in Southeastern Nigeria. *Arztezeitschrift for naturopathy*, 41: 11.
[15]. Okoli RN, Aigbe O, Ohaju-Obodo JO, Mensah JK. (2007). Medicinal herbs used for managing some common ailments among Esan people of Edo State, Nigeria, *Pakistan Journal Nutrition* 6(5): 490-496.
[16]. Udeh N.E. and Onoja S.O (2015) Analgesic and free radical scavenging activities of hydromethanolic extract of *Crateva adansonii* stem bark. *Journal of Intercultural Ethnopharmacology.* 4(3): 224–227. doi: 10.5455/jice.20150430010855
[17]. Thirumalaisamy R, Ammashi S, and Muthusamy G (2018)**.** Screening of anti-inflammatory phytocompounds from *Crateva adansonii* leaf extracts and its validation by *in silico* modeling. *Journal of Genetic Engineering and Biotechnology*. 16(2): 711–719. doi: 10.1016/j.jgeb.2018.03.004
[18]. Sofowora, A., (1993). Medicinal Plants and Traditional Medicines in Africa. *Chichester John/Wiley & Sons, New York.* P *256.*
[19]. Harbourne, J.B. (1998). Phytochochemical methods: A guide to modern techniques of plant analysis. 3rd Edition. *Chapman and Hill, London*. P279.
[20]. Trease, G.E. & Evans, W.C. (1989). Pharmacognosy: a physician's guide to herbal medicine, 13th edition. *Bailliere Tindall, London*, Pp. 176-180
[21]. Adzu B, Amos S, Wambebe C & Gamaniel K. (2001) Antinociceptive activity of Zizyphus spina-christi root bark extract. Fitoterapia. 72:344–50
[22]. Zakaria Z.A., Abdul Ghani Z.D.F., Raden M., Nor R.N.S., Gopalan H.K., Sulaiman M.R., Mat-Jais A.M., Somchit M.N., Kader A.A., & Ripin J (2008). Antinociceptive, anti-inflammatory, and antipyretic properties of an aqueous extract of Dicranopteris *linearis* leaves in experimental animal models. *Journal of Natural Medicine*. 62:179–187.
[23]. Sannigrahi S., Mazumder U.K., Pal D., Mishra M., Maity S. (2011). Flavonoids of *Enhydra Fluctuans* exhibits analgesic and anti-inflammatory activity in different animal models. *Pakistan Journal of Pharmaceutical Sciences*, 24(3): 369–375.
[24]. Rajnarayana K., Sripal Reddy M. & Chaluvadi M.R. (2001) Bioflavanoids Classification, Pharmacological, Biochemical effects and Therapeutic potential. Indian Journal of Pharmacology 33: 2-16.
[25]. Navarro P., Giner R.M., Recio M.C., Máñez S., Cerdá-Nicolás M. & Ríos J -L. (2001). *In vivo* anti-inflammatory activity of saponins from *Bupleurum rotundifolium. Life Science.* 68(10):1199–206.
[26]. Ramesh M., Rao Y.N., Rao A.V.N.A, Prabhakar M.C., Rao C.S. et al. (1998) Antinociceptive and anti-inflammatory activity of a flavonoid isolated from Caralluma attenuate. *Journal of Ethnopharmacology* 62: 63-66.
[27]. Xu, F.Z., Xu, S.Z., Wang, L.J., Chen, C.T., Zhou, X.Q., Lu, Y.Z. & Zhang, H.H. (2011). Antinociceptive efficacy of verticinone in murine models of inflammatory pain and paclitaxel induced neuropathic pain. *Biological. Pharmaceutical Bulletin*. 34: 1377–1382.
[28]. Deuis J.R., Dvorakova L.S. & Vetter I. (2017). Methods Used to Evaluate Pain Behaviors in Rodents. *Frontiers Molecular Neurosciences.* https://doi.org/10.3389/fnmol.2017.00284
[29]. Mogil J.S., Davis K.D. & Derbyshire S.W. (2010). The necessity of animal models in pain research. *Pain*. 151:12–17.
[30]. D'Amour, F.E. & Smith, D.L. (1941). "A method for determining loss of pain sensation". *Journal of Pharmacology and Experimental Therapeutics.* 72 (1): 74–78.
[31]. Dzoyem J.P., McGaw L.J, Kuete V., Bakowsky U. (2017). Medicinal Spices and Vegetables from Africa: Therapeutic Potential Against Metabolic, Inflammatory, Infectious and Systemic Diseases. Chapter 9 - Anti-inflammatory and Anti-nociceptive Activities of African Medicinal Spices and Vegetables. Pages 239-270

[32]. Raquibul S.M., Hossain M.M., Aktar R., Jamila M., Mazumder M.E.H., Alam M.A., *et al*. (2010) Analgesic Activity of the Different Fractions of the Aerial Parts of *Commenila Benghalensis* Linn. *International Journal of Pharmacology*. 6(1):63–67

[33]. Choi J., Jung H.J., Lee K.T. & Park H.J. (2005) Antinociceptive and anti-inflammatory effects of the saponin and sapogenins obtained from the stem of *Akebia quinata*. *Journal of Medicinal Food*. 8(1):78-85. doi:10.1089/jmf.2005.8.78

[34]. Vane J.R & Botting J.R. (2003). The mechanism of action of aspirin. *Thrombosis Research.* 110(5–6): 255-258.