
Original Research Article

In vivo antinociceptive activity of the aqueous leaf extract of *Voacanga africana* Stapf (Apocynaceae) in mice

Ighodaro Igbe and Tarimobowei Edike

Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Edo State.PMB 1154. Nigeria

*For correspondence: Email: igbe.ighodaro@uniben.edu Tel: +2348166058559

Abstract

Purpose: To investigate the analgesic activity and phytochemical constituents of aqueous extract of *Voacanga africana* leaves and validate its use in folklore medicine.

Methods: Crude leaves were extracted by percolation with distilled water. The antinociceptive properties of the extract were determined using the acetic acid-induced writhing and the hot plate test methods in mice. Phytochemical screening was determined using standard methods.

Results: The phytochemical constituents of the aqueous extract of *Voacanga africana* leaves present were alkaloids, anthranoids, anthraquinones, cardiac glycosides and saponins. Oral administration of the

aqueous leaves extract (100, 200 and 400mg/kg) produced a significant ($P < 0.05$) analgesic activity in a dose dependent manner in acetic acid induced writhing test. The extract (200 and 400mg/kg) significantly ($P > 0.05$) prolonged the reaction latency time of pain in the hot plate test **Conclusion:** The study showed that the aqueous extract of *Voacanga africana* appears to possess analgesic activity that appears to be peripherally and centrally mediated, thus justifying its use in traditional medicine.

Keywords: Phytochemicals; writhing; latency time; dose dependent.

Indexing: Index Copernicus, African Index Medicus

Introduction

Medicinal plants are important sources of new chemical substances that potentially have strong therapeutic effects. In developing countries, most people are almost completely dependent on traditional medical practices for their primary health care needs and various plants are known to be the main source for drug therapy in traditional medicine [1].

Voacanga Africana Stapf (Apocynaceae) is a tree plant occurring in all of West Africa and as far as the Congo and even Tanzania. In Nigeria, it is called óvien-ibu (edo), petepete or akete (igbo), ako dodo or sherenkpen (Yoruba). Various parts of *Voacanga africana* have been widely used in Nigeria and some other African countries. In West Africa, the bark of *Voacanga africana* is often used as a stimulant and aid for hunting [2]. It is also reported to be a potent aphrodisiac. There is abundant white latex in the bark and other parts. The latex is applied to wounds in Senegal [2] and into a carious tooth in Nigeria

[3]. The milky latex of the plant is applied to wounds in Nigeria and Senegal. In animal studies, the root bark alkaloids of *Voacanga* species cause CNS-depressant, hypotensive, spasmolytic and cardiotoxic actions [4]. The seeds are suspected to be neurotoxic on some level, which may be due to the action of the major seed alkaloid tabersonine [4]. The latex or decoctions or infusions of the stem bark, leaves or roots are put on wounds, boils and sores and used to treat toothaches, gonorrhoea, eczema, fungal infections, scabies and ulcers in Cameroun [5, 6]. The seeds of *Voacanga* species are used in Europe due to their high tabersonine contents, as a precursor for vincamine which is used to treat neural deficiencies in the elderly [7]. The antioxidant and anti-inflammatory properties of a flavonoid fraction from the leaves of *Voacanga africana* has also been reported [8]. Thus the aim of this study was to investigate the antinociceptive activity of *Voacanga africana* leaves to justify its use in treatment of toothaches and inflammations.

Experimental

Plant collection and extraction

Leaves of *Voacanga africana* were collected on February, 2014 from Okhoro village, Benin City, Oredo Local Government Area of Edo State and were identified and authenticated by Prof. McDonald Idu of the Department of Plant Biology and Biotechnology, Faculty of Life Science. The leaves were air dried for about two weeks and pulverized by a mechanical grinder.

The powdered plant material (500g) was extracted with 3L of distilled water via hot maceration. The aqueous extract obtained was concentrated to dryness using a rotary evaporator (yield = 65.94g; 13.19%). The dried extract was stored in clean glass containers in the refrigerator at 4°C until used.

Phytochemical screening

Preliminary phytochemical screening was done on the leaves of *V. africana* using standard methods [9].

Animals

All experiments were performed using Swiss Albino mice (20-31g) of either sex procured from the Department of Pharmacology and Toxicology Animal House, University of Benin. The animals kept in plastic cages were maintained under standard controlled environment and were allowed free access to feed (top feeds® Grower mash, super-Deluxe Animal Feeds by Premier Feed Mills co. Ltd, Nigeria) and clean drinking water *ad libitum*. The handling procedures were conducted in accordance with the Faculty of Pharmacy, University of Benin Ethical committee on experimental animals. The animals were also allowed two weeks under these conditions to acclimatize before the commencement of the experiments.

Experimental design

Acetic Acid-induced writhing in mice

This experiment was based on the modification of the method described by [10]. Swiss Albino mice were randomly divided into five groups comprising of five animals each. The different groups of animals were given the leaf extract (100, 200 and 400 mg/kg) or acetylsalicylic acid (ASA) (100 mg/kg) or distilled water (5 ml/kg) an hour prior to injection of 0.6%v/v acetic acid (10 ml/kg) intraperitoneally. The number of writhes by each mouse was counted immediately after the acetic acid administration at intervals of 5 minutes for a period of 30 minutes [11].

Hot plate test

This hot plate test was used to measure the latencies of pain response according to method described by [12]. Swiss albino mice were divided into five groups of five mice each. The animals were individually placed on a hot plate maintained at a constant temperature of 55±1°C, the time interval from placement and shaking/licking of the paw or jumping was recorded as an index of response latency. The initial reaction time of each animal was determined and the cut off time was set at 30 seconds. The different groups of animals were given extract (100, 200, 400 mg/kg) or distilled water (5 ml/kg) orally. Morphine (4 mg/kg) given intraperitoneally was used as the standard. The animals were placed on the hot plate at 30, 60, 90 and 120 minutes after treatment and the time taken for either paw licking or jumping was recorded.

Statistical analysis

Results were expressed as the mean ± standard error of the mean (S.E.M). Comparison between the treatment groups was carried out using ANOVA followed by Tukey's post hoc test. Analysis was done using graph pad prism version 5.0. Results were considered significant when p<0.05

Results

Preliminary phytochemical screening of aqueous extract of *V. africana* revealed the presence of some active principles: alkaloids, flavonoids, anthranoids, anthraquinones, cardiac glycosides, saponins, starches and sugars.

Figure 1 shows the effect of the aqueous extract on acetic acid-induced mouse writhing. The extract produced a significant (p<0.05) dose dependent decrease in writhing at 100 mg/kg (54.4 ± 1.56), 200 mg/kg (48.2 ± 3.32) and 400 mg/kg (42.8 ± 2.85) when compared to the control (78.0 ± 3.83) after 30 mins. These results were however, not comparable to the effect of aspirin (34.4 ± 4.79; p<0.01).

In Table 1 showing the hotplate test, the aqueous extract of *V. Africana* significantly (p>0.05) increased reaction time at the 90 min only at doses of 200 mg/kg (8.84±1.02secs) and 400 mg/kg (8.00±1.71secs) compared to the control (4.74±1.07). Morphine significantly increased reaction time at 30 (11.50±1.77secs), 60 (13.06±2.17secs) and 90 mins (10.26±1.04secs) compared to the control.

Discussion

The acetic acid-induced writhing in mice is widely

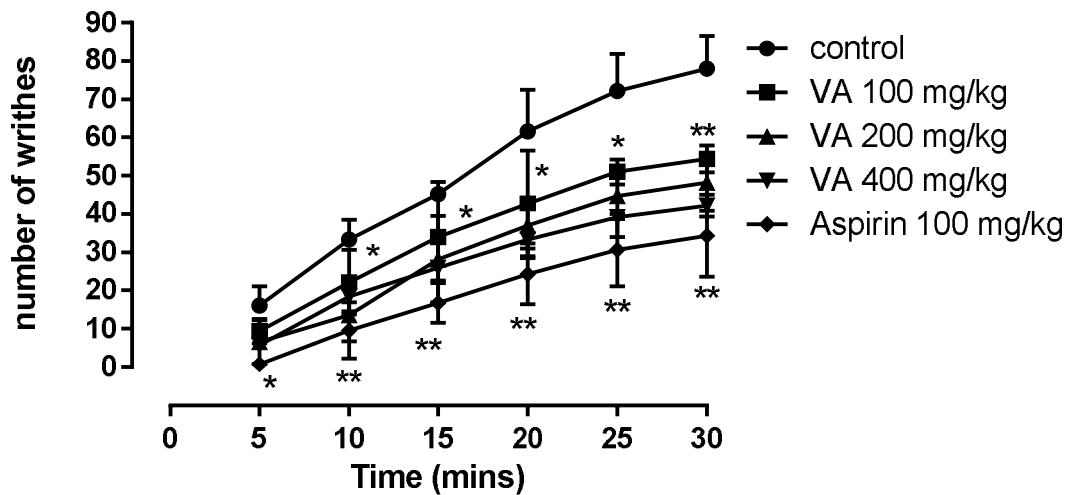


Figure 1: Effect of the aqueous leaf extract of *V. africana* (VA) on acetic-acid induced mouse writhing. *p<0.05, **p<0.01 compared to control (n = 5 for each group)

Table 1: Effect of the aqueous leaf extract of *V. africana* (VA) on hot plate test in mice

	Dose (mg/kg)	Reaction time (secs)					
		0 min	30 min	60 min	90 min	120 min	
Control	-	4.58±0.71	5.42±0.95	5.88±0.91	4.74±1.07	6.38±1.16	
<i>Voacanga africana</i>	100	5.04±0.44	5.16±1.98	8.90±1.40	5.78±0.34	8.50±1.13	
	200	5.68±0.83	7.60±1.23	7.98±0.85	8.84±1.02*	8.84±0.67	
	400	5.96±0.30	7.08±1.44	7.12±1.02	8.00±1.71*	7.90±1.37	
Morphine	4	6.90±0.92	11.50±1.77*	13.06±2.17**	10.26±1.04**	8.04±1.94	

*P<0.05, **P<0.01 compared to control (n = 5 for each group)

used for the evaluation of peripheral anti-nociceptive effect of compounds at dose levels that may appear inactive in other methods such as the tail-flick test [13, 14]. Pain sensation in acetic acid induced writhing method is elicited by triggering localized inflammatory response resulting in the release of free arachidonic acid from tissue phospholipid via cyclooxygenase (COX), and prostaglandin biosynthesis [15]. Hence, the acetic acid induced writhing has been associated with increased level of PGE2 and PGF2α in peritoneal fluids as well as lipoxigenase products [16]. The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability [17]. The acetic acid induced writhing method was found effective in evaluating peripherally active analgesics. The agent reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition [18, 19]

Oral administration of the aqueous extract of *V. africana* was seen to significantly reduce writhes induced by acetic acid at all doses and it was sustained throughout the 30 minutes period and the effect was dose-dependent. This observation suggests that the extract may contain analgesic principles acting through the prostaglandins pathway and thus maybe peripherally mediated.

The extract at doses of 200 and 400 mg/kg prolonged the latency time in mice in the hot plate test after administration only at the 90th min. The hot plate method is one of the most common tests of nociception based on a phasic stimulus on high intensity [20]. Pain induced by the main stimulus of the hot plate is specific for the centrally mediated nociception [21]. Preliminary qualitative phytochemical screening reveals the presence of alkaloids, anthranoids, anthraquinones, cardiac glycosides, saponins, starches and sugars in *V. africana*. Therefore, it is assumed that these compounds may be responsible for the observed analgesic activity. Flavonoids were reported to have a role in analgesic activity primarily by targeting prostaglandins [22, 23]. There are also reports on the role of alkaloids in their ability to inhibit pain perception [24, 25].

Conclusion

In conclusion, we can confirm that the aqueous leaf extract of *V. africana* possesses peripheral and probably some central analgesic properties. However, further studies are required to understand the precise mechanism.

Declarations

Acknowledgement

The authors wish to thank Prof McDonald Idu for helping to identify the plant.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Igbe conceived and designed the study. Igbe and Edike sourced, collected and extracted the plant material. Edike did the phytochemical analysis. Igbe and Edike performed all the pharmacological experiments and wrote the manuscript.

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