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Analgesic Effects of *Vigna unguiculata* subsepecies *dekindtiana* in Mice

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Authors' contributions

This work was carried out in collaboration among all authors. Author ATA designed the study, performed the statistical analysis, managed the literature searches, wrote the protocol and wrote the first draft of the manuscript. Authors MAA and GO assisted in the designing of the work and managed the analyses of the data. Author AOS managed the literature searches and the reviewed of the manuscript. All authors read, financed and approved the final manuscript.

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ABSTRACT

In the present study, we investigated the antinociceptive effects of the plant *Vigna unguiculata* spp *dekindtiana* using chemical and thermal tests in mice. The peripheral and the central analgesic activities of the methanol extract and its fractions were investigated in-vivo in albino mice using acetic acid induced-writhing test and hot plate models respectively. The result of the central analgesic effect showed that the methanol extract (VUME) at 400 mg/kg produced a significant ($p<0.05$) delay in reaction time in mice on hot plate compared to the control. Various fractions of the extract showed more potency compared to the crude extract. In acetic writhing model, the extract and the fractions demonstrated dose dependent reduction in writhing reaction induced by acetic acid in mice. The reduction was significant when compared to control which was suggestive of the

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analgesic effect of the plant. It was also seen that the extract and fractions showed an improved analgesic effect compared to diclofenac used as positive control in this model. Yohimbine (alpha adrenergic receptor antagonist) and cyproheptadine (serotonergic receptor antagonist) reversed the antinociceptic effect of the fractions in the hot plate model demonstrating the possibility of adrenergic and serotonergic involvement in eliciting the analgesic effect. Naloxone on the other hand, caused a reversal only in the butanol fraction meaning that this fraction may contain active principles that may mediate their analgesic effect through the opioid mechanism. In the writhing test, yohimbine abolished the analgesic effect of both hexane and butanol fractions. This may therefore, suggest that the analgesic effect of these fractions may be mediated through adrenergic pathway. In conclusion, the plant *V. unguiculata* subspecies *dekindtiana* possesses active principles with potential analgesic activity, establishing the folkloric use of the plant in managing pain.

Keywords: Vigna unguiculata spp dekindtiana; hot plate model; morphine; acetic writhing model; diclofenac.

1. INTRODUCTION

Plants have been in use since the time immemorial in preventive and curative health care systems in both developed and the developing world [1,2]. Many of these plants have been translated into modern medicinal agents that are sometimes used in the clinical set up in many nations of the world, for example, Cetrosema is used for wound healing [3,4]. The scientific evaluation and standardization of these herbal remedies, particularly those of plant origin, have made it increasingly feasible to transform traditional medicine into a modern industrial enterprise and this is capable of making significant contribution to both healthcare delivery systems as well as increasing economic growth of developing countries [1]. Medicinal plants as part of alternative medicine also provide a cheap, effective, accessible and affordable source of drug for the majority of the world population [5,6]. The active constituents from these plants can also provide lead agents and starting materials for the synthesis of the new drugs with improved pharmacological properties and with less adverse effects [3]. Many of the drugs that are used clinically to manage pain are either steroid-like corticosteroids or non steroidal, like aspirin and paracetamol. These orthodox drugs are characterized with serious or mild untoward side effects [7]. For example, the dependent and abuse tendencies and respiratory depressant effects of opioids and gastrointestinal side effects such as ulceration and bleeding of Non-steroidal anti-inflammatory drugs have been a serious concern to drug manufacturers and the health professionals [8]. These drugs are also characterized by toxic effects like renal failure, allergic reactions, hearing loss or increase in the risk of haemorrhage by drugs that affect platelet function. Therefore, the search for novel drugs with potent therapeutic efficacy and fewer side effects will be a worthwhile task to be embarked upon. The neurobehavioural as well as sedative effect of this plant has been investigated and reported [9]. The antidepressant effect and phytochemical estimation of the aqueous fraction of the plant had been investigated and reported by Akinpelu et al. [10]. *Vigna unguiculata* subspecies *dekindtiana* has been used for a long time as herbal medicine in Western part of Nigeria to manage pain and migraine headache and there has been no scientific studies *in vivo* from literature survey have previously been conducted to justify this folkloric use of this plant. Hence, the need to investigate the *in-vivo* analgesic effect of the methanol extract of the plant in this study in order to justify, or otherwise, its local use in the management of pain and headache.

2. METHODOLOGY

2.1 Preparation of the Plant Materials

The preparation of the plant extracts and fractions had been reported previously [9]. The plant materials were air-dried in the shade at room temperature, pulverized, extracted with absolute methanol and concentrated in vacuo. The extract was partitioned into n-hexane, ethylacetate, butanol and aqueous fractions. The extract and fractions were placed in a desicator (containing activated silica gel) until needed for experimental work. The methanol extract and fractions were solubilised with 2% tween 20 in normal saline while the aqueous fraction was dissolved in normal saline. The extract *Vigna unguiculata* (VU) used is VUME (methanol), and fractions

VUHF (n-Hexane), VUEF (ethyl acetate), VUBF (butanol) and VUAF (Aqueous) respectively.

2.2 Animals

Swiss albino mice of both sexes obtained from Empire Farm (Osogbo) were used for this experiment. The animals were obtained at about 6 weeks old and were kept in standard cages for mice in a well-lit animal house at the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife. The animals were fed with standard mouse chow, allowed free access to water and maintained under natural day/night condition for about 3 weeks before use.

2.3 Chemicals

The chemicals used in this experiment were as follows: acetic acid, naloxone (BDH Chemicals Ltd., England), diclofenac (Jawa Int. Ltd., India), normal saline (Unique Pharmaceuticals, Lagos, Nigeria); cyproheptadine, yohimbine, morphine (BDH Chemicals Ltd., England).

2.4 Acute Toxicity Test

The method described by Lorke [11] was used to determine the LD_{50}, which is the index of acute toxicity. Swiss albino mice (18-25 g) of either sex were used. This method involved an initial dose finding procedure, in which the animals were randomized into three groups of three animals per group. Doses of 10,100 and 1000 mg/kg of methanol extract (solubilised by 2% Tween 20 in normal saline) and fractions were administered orally (p.o.). The treated animals were monitored for general behavior continuously for 1 hour and then at interval every 1 hour for mortality for 24 hours. No mortality was observed after 24 hours hence the choice of 1600, 2900 and 5000 dose levels in the second phase of the test for oral administration. The same procedure was repeated for each of the fractions.

2.5 Effects of the Crude Extract and Fractions on Pain Reaction Time in Mice Hot Plate Model

Heat is more selective in the way it stimulates cutaneous receptors and can cause excitation of thermosensitive and nociceptive receptors. Hot plate model has been recognized as a tool to screen analgesic agents [12]. The analgesic potential of the plant extract and the fractions of *V. unguiculata* spp *dekindtiana* were evaluated in this model. The doses of 100, 200 and 400 mg/kg (5 mice per dose) of the methanol extract and fractions were administered orally to mice and each animal was placed on the hot plate maintained at a constant temperature of 55 ± 1°C and the reaction time was taken as the time it took the animal to lick its paws or jump from the surface of the plate [13]. In another experiment, naloxone (5 mg/kg, i.p.), yohimbine (1 mg/kg, i.p.) and cyproheptadine (0.5 mg/kg, i.p.) were administered intraperitoneally separately to mice 15 minutes before the oral administration of the extracts and fractions to test the possible mechanism of the effects observed.

2.6 Acetic Acid Induced Writhing Reflex Model

The analgesic property of the methanol extract and fractions was evaluated using acetic acid induced writhing reflex in mice [14,15]. In this method, acetic acid was administered intraperitoneally into mice and both abdominal constriction and the stretching of the hind limbs were taken as pain response to acetic acid. Male and female mice were randomly selected into five groups. The control, (group 1) received vehicle (2% Tween 20 in normal saline, 10 ml/kg p.o.), body weight, group 2 to 4 received the extract at doses of 100, 200 and 400 mg/kg and the last group received a standard anti-inflammatory drug, diclofenac (10 mg/kg body weight, i.p.) (5 mice per dose) [16]. After 60 minutes of drug administration, 1% acetic acid solution was administered to each mouse i.p. at the dose of 0.l ml/kg body weight. The number of writhing was counted for 15 minutes, starting from 6 minutes after acetic acid administration [17]. A significant reduction in the number of writhing in the groups treated with extracts compared to control was considered to be a positive analgesic response [18]. The same protocol was repeated for each of the fractions. The reaction time for diclofenac was taken after 30 minutes of drug administration.

3. RESULTS

3.1 Effect of Extract VUME and Fractions (VUHF, VUEF, VUBF and VUAF) on Reaction Time in Mice

The antinociceptive effect of the extract and fractions was evaluated in the hot plate model in mice which was maintained at a temperature of 55±1^{o}C and the time it takes the mouse to lick

the paw or to jump up is taken as the reaction time. The extract (VUME) and fractions (VUHF, VUEF, VUBF and VUAF) prolong the pain threshold as reflected in the increase in the reaction time compared to control as shown in Tables 1 to 5. In Table 1, VUME produced a significant prolongation of the reaction time at 60 and 90 minutes respectively. At 400 mg/kg, the extract caused significant prolongation in the reaction time at 60, 90 and 120 minutes. The VUHF, VUEF, VUBF in Tables 2, 3 and 4 respectively, produced significant prolongation in the reaction time in all doses compared to the control. There was an improvement in reaction time in these fractions compared to the extract. The VUAF demonstrated the least analgesic action compared to other fractions (Table 5). Naloxone (an opioid receptor antagonist) did not reverse the analgesic effect of the extract and fractions except butanol fraction (Table 8). On the other hand, yohimbine (alpha 2 adrenergic receptor antagonist) (Table 7) and cyproheptadine (serotonergic receptor antagonist) (Table 6) reversed the antinociception effect of the fractions.

Table 1. Effects of methanol extract in reaction time in mice on hot plate model

Treatment	Reaction time (minutes)				
	0	30	60	90	120
VEH 1	4.4 ± 0.4	5.4 ± 0.2	4.1 ± 0.3	3.6 ± 0.3	3.5 ± 0.4
VUME 100	3.5 ± 0.1	5.9 ± 0.5#	5.1 ± 0.1#	6.7 ± 0.5#	5.8 ± 0.4#
VUME 200	3.8 ± 0.3	7.7 ± 0.4*#	8.1 ± 0.4*	8.1 ± 0.2*#	6.8 ± 0.4#
VUME 400	3.2 ± 0.1	6.5 ± 0.4 #	7.4 ± 0.6*	7.1 ± 0.3*#	10.1± 0.5*
Morphine	3.3 ± 0.2	16.3 ± 0.9*	9.2 ± 0.8*	14.1 ± 1.1*	12.3 ± 0.5*

*The result is expressed as mean ± S.E.M (n=5). One way ANOVA revealed significant difference in reaction time in mice between the control group and various groups as indicated by asterisk at different time intervals. VUME: V. unguiculata methanol extract (100, 200 and 400 mg/kg, p.o.); VEH 1: vehicle (2% Tween 20 in normal saline, 10 ml/kg, p.o.); Morphine: (5 mg/kg, i.p.); *significant p<0.05 compared to VEH; #significant compared to morphine*

Table 2. Effects of n-hexane fraction on reaction time in mice on hot plate model

Treatment	Reaction time (minutes)				
	0	30	60	90	120
VEH 1	4.4 ± 0.4	5.4 ± 0.4	4.1 ± 0.3	3.6 ± 0.3	3.5 ± 0.4
VUHF 100	4.4 ± 0.1	9.3 ± 1.2*#	12.2 ± 0.7*	13.1 ± 1.4*	15.5 ± 0.4*
VUHF 200	4.0 ± 0.2	9.4 ± 1.0*#	11.5 ± 0.5*	13.3 ± 1.0*	11.1 ± 0.4*
VUHF 400	4.7 ± 0.3	7.2 ± 1.2*#	12.4 ± 1.7*	11.0 ± 1.3*	11.1± 0.5*
Morphine	3.3 ± 0.2	16.3 ± 0.9*	9.2 ± 0.8*	14.1 ± 1.0*	12.3 ± 0.5*

*The result is expressed as mean ± S.E.M (n=5). One way ANOVA revealed significance difference in reaction time in mice between the control group and various groups as indicated by asterisk at different time intervals. VUHF: V. unguiculata n-hexane fraction (100, 200 and 400 mg/kg, p.o.) VEH 1: vehicle (2% Tween 20 in normal saline, 10 ml/kg, p.o.); morphine: (5 mg/kg, i.p.); *significant p<0.05 compared to VEH 1; #significant compared to morphine*

Table 3. Effects of ethyl acetate fraction in reaction time in mice on hot plate model

Treatment	Reaction time (minutes)				
	0	30	60	90	120
VEH 1	4.4 ± 0.4	5.4 ± 0.4	4.1 ± 0.3	3.6 ± 0.3	3.5 ± 0.4
VUEF 100	4.4 ± 0.1	9.3 ± 0.9*#	11.8 ± 1.3*	10.1 ± 1.5*	12.3 ± 1.2*
VUEF 200	4.5 ± 0.6	7.6 ± 1.2*#	9.6 ± 0.9*	9.6 ± 0.9*	13.3 ± 1.8*
VUEF 400	3.7 ± 0.4	8.2 ± 0.8*#	11.0 ± 0.8*	9.1 ± 1.2*	14.1± 1.5*
Morphine	3.3 ± 0.2	16.3 ± 0.9*	9.2 ± 0.8*	13.9 ± 1.1*	12.3 ± 0.5*

*The result is expressed as mean ± S.E.M (n=5). One way ANOVA revealed significant difference in reaction time at different time intervals in mice between the control group and various groups as indicated by asterisk. VUEF: V. unguiculata ethyl acetate fraction (100, 200 and 400 mg/kg, p.o.); VEH 1: vehicle (2% Tween 20 in normal saline, 10 ml/kg, p.o.) Morphine: (5 mg/kg, i.p.) *significant p<0.05 compared to VEH 1; #significant compared to morphine*

Table 4. Effects of butanol fraction in reaction time in mice on hot plate model

Treatment	Reaction time (minutes)				
	0	30	60	90	120
VEH 1	4.4 ± 0.4	5.4 ± 0.4	4.1 ± 0.3	3.6 ± 0.3	3.5 ± 0.4
VUBF 100	4.4 ± 0.2	7.9 ± 1.1*#	9.5 ± 0.8*	10.4 ± 1.2*	11.0 ± 1.4*
VUBF 200	5.3 ± 0.7	10.8 ± 0.6*	15.5 ± 1.4*	14.7 ± 1.0*	15.9 ± 2.0*
VUBF 400	3.9 ± 0.2	8.5 ± 0.9*#	11.8 ± 1.2*	14.2 ± 0.4*	14.3± 1.5*
Morphine	3.3 ± 0.2	16.3 ± 0.9*	9.2 ± 0.8*	11.9 ± 2.3*	12.3 ± 0.5*

*The result is expressed as mean ± S.E.M (n = 5). One way ANOVA revealed significant difference in reaction time in mice between the control group and various groups as indicated by asterisk at different time intervals. VUBF: V. unguiculata butanol fraction (100, 200 and 400 mg/kg, p.o.); Morphine: (5 mg/kg, i.p.); VEH 1: vehicle (2% Tween 20 in normal saline, 10 ml/kg, p.o.); *significant p<0.05 compared to VEH 1; #significant compared to morphine*

Table 5. Effects of aqueous fraction in reaction time in mice on hot plate model

Treatment	Reaction time (minutes)				
	0	30	60	90	120
VEH 2	4.7 ± 0.2	4.6 ± 0.2	5.4 ± 0.3	4.9 ± 0.3	5.3 ± 0.4
VUAF 100	4.5 ± 0.7	8.0 ± 1.1*#	7.2 ± 0.6	7.4 ± 0.5 #	7.4 ± 0.8 #
VUAF 200	4.6 ± 0.5	7.7 ± 1.1#	7.7 ± 0.7	8.4 ± 1.1*	7.6 ± 1.0 #
VUAF 400	4.9 ± 0.2	8.4 ± 0.7*#	11.7 ± 1.8*	8.2 ± 0.9*	8.8± 0.6*
Morphine	3.3 ± 2	16.3 ± 0.9*	9.2 ± 0.8*	13.9 ± 1.1*	12.3 ± 0.5*

*The result is expressed as mean ± S.E.M (n=5). One way ANOVA revealed significant difference in reaction time between the control group and various groups as indicated by asterisk at different time intervals.; VUAF: V. unguiculata aqueous fraction (100, 200 and 400 mg/kg, p.o.) VEH 2: vehicle (normal saline, 10 ml/kg, p.o.); Morphine: (5 mg/kg, i.p.); *significant p<0.05 compared to VEH 2; #significant compared to morphine*

Table 6. Effect of cyproheptadine on reaction time in mice induced by *V. unguiculata* fractions on hot plate model

Treatment	Reaction time (minutes)				
	0	30	60	90	120
VEH	4.4 ± 0.4	5.4 ± 0.4	4.1 ± 0.3	3.6 ± 0.3	3.5 ± 0.4
VEH + CYP	5.2 ± 0.4	6.7 ± 0.6	6.8 ± 0.6	8.08 ± 1.0	6.3 ± 0.8
VUHF 200	5.0 ± 0.4	9.4 ± 1.0	11.5 ± 0.5	13.3 ± 1.0	11.1 ± 1.4
VUHF 200 + CYP	5.1 ± 0.4	6.6 ± 0.5	6.2 ± 0.7*	8.8 ± 0.9*	6.8 ± 0.8*
VUEF 200	4.5 ± 0.6	7.6 ± 1.1	9.7 ± 0.9	9.6 ± 0.9	14.1 ± 1.5
VUEF 200 + CYP	5.0 ± 0.6	6.0 ± 0.7	7.1 ± 0.7	8.0 ± 0.6*	6.1 ± 0.7*
VUBF 200	5.3 ± 0.7	11.0 ± 0.6	15.9 ± 1.5	14.7 ± 1.0	14.3 ± 1.4
VUBF 200 + CYP	4.9 ± 0.5	6.7 ± 0.3*	6.8 ± 0.3*	5.7 ± 0.5*	6.1 ± 0.8*

*The result is expressed as mean ± S.E.M (n=5). One way ANOVA revealed the effect of cyproheptadine on reaction time in mice of n-hexane fraction. Cyproheptadine reversed the analgesic effect of n-hexane fraction, ethyl acetate, and butanol fraction. VUHF: V. unguiculata n-hexane fraction (200 mg/kg, p.o.); VUEF: V. unguiculata ethyl acetate fraction (200 mg/kg, p.o.); VUBF: V. unguiculata butanol fraction (200 mg/kg, p.o.); CYP: cyproheptadine (0.5 mg/kg, i.p.); *significant p<0.05: antagonist versus treatment alone*

3.2 Effect of Extract (VUME) and Fractions (VUHF, VUEF, VUBF and VUAF) on Acetic Acid Induced Writhing Reflex in Mice

The analgesic potential of the extract and its fractions were evaluated using acetic acid induced writhing reflex. The extract and fractions significantly ($F(4, 20) = 96.5$, $p < 0.05$) reduced the number of writhes in mice. The reduction in writhe reaction in mice induced by VUME is dose dependent (Fig. 1). The reduction capacity of the number of writhes at 400 mg/kg was comparable to diclofenac (10 mg/kg, i.p.). In VUHF test, the least number of writhes was found at 200 mg/kg demonstrating the most effective dose. All doses

were significantly different from the control (F (4, 20) = 61.2, p<0.05). All doses in VUEF, VUBF and VUAF induced reduction in frequency of writhes that was significant when compared with control (F (4, 20) = 48.4, p<0.05, F(4, 20) = 74.4, p<0.05, F(4, 20) = 96.2, p<0.05). Yohimbine reversed the inhibition of abdominal writhes induced by VUHF and VUBF but did not affect the inhibition of abdominal writhes induced by VUEF.

Table 7. Effect of yohimbine on reaction time induced by *V. unguiculata* fractions in mice on hot plate model

Treatment	Reaction time (minutes)				
	0	30	60	90	120
VEH + YOH	4.3 ± 0.4	5.5 ± 0.7	4.8 ± 0.3	5.4 ± 0.8	7.2 ± 1.1
VUHF 200	4.0 ± 0.2	9.4 ± 0.1	11.5 ± 0.5	13.3 ± 1.0	11.1 ± 1.4
VUHF 200 + YOH	4.1 ± 0.6	5.6 ± 0.3*	7.2 ± 0.9*	6.1 ± 0.5*	7.3 ± 1.0*
VUEF 200	4.5 ± 0.6	7.5 ± 1.2	9.6 ± 0.9	9.6 ± 0.9	13 ± 1.8
VUEF 200 + YOH	3.8 ± 0.4	6.5 ± 0.8	5.6 ± 0.4*	4.9 ± 0.6*	6.1 ± 0.8*
VUBF 200	5.3 ± 0.7	11.1 ± 0.6	15.9 ± 1.5	14.7 ± 1.0	15.9 ± 2.0
VUBF 200 + YOH	4.7 ± 0.2	4.9 ± 0.5*	5.7 ± 0.5*	5.4 ± 0.7*	5.2 ± 0.7*

*Each bar is expressed as mean ± S.E.M (n=5). One way ANOVA revealed that yohimbine reversed the reaction time induced by n- hexane, ethyl acetate, and butanol fraction in mice; P<0.05; VUHF: V. unguiculata n-hexane fraction; VUEF: V. unguiculata ethyl acetate fraction; VUBF: V. unguiculata butanol fraction; YOH: yohimbine (1 mg/kg, i.p.); *significant p<0.05 difference: antagonist versus treatment alone*

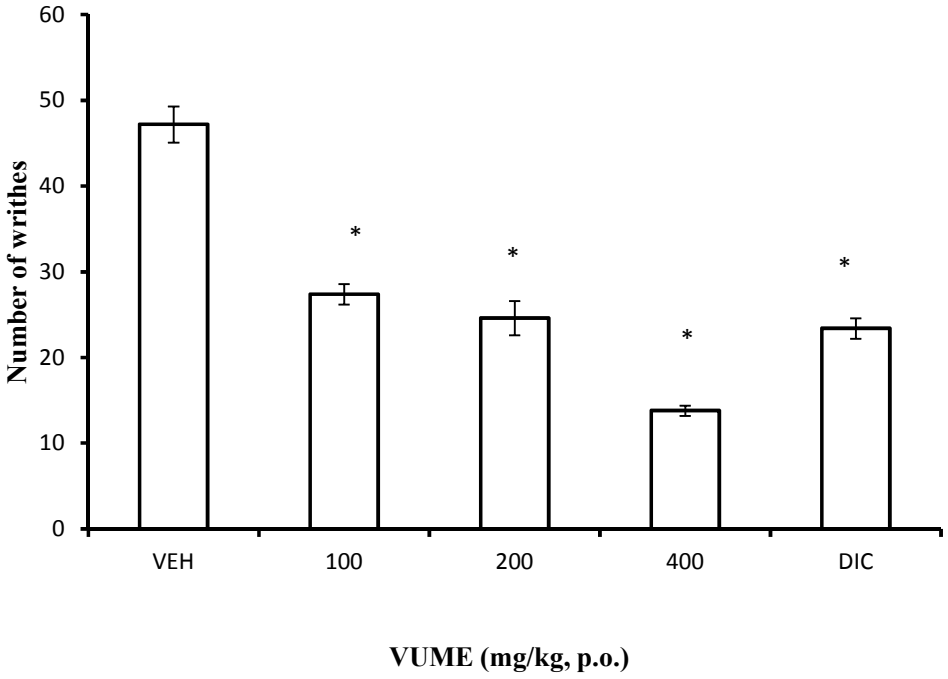

Fig. 1. Effect of methanolic extract on acetic acid induced writhing in mice

*Each bar is expressed as mean ± S.E.M (n=5). One way ANOVA revealed significant difference in the number of writhes among the groups compared with the control exhibiting decrease in the number of writhes by the extract. VUME: V. unguiculata methanol extract; Dic: diclofenac (10 mg/kg, i.p); VEH: vehicle (2% Tween 20 in normal saline, 10 ml/kg, p.o.); *significant p<0.05 compared to VEH*

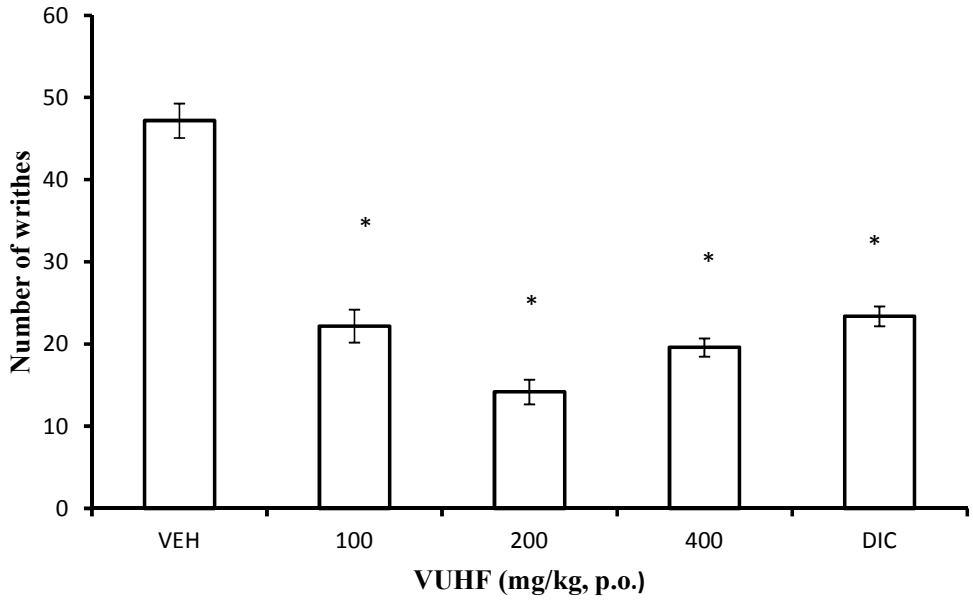

Fig. 2. Effect of n-hexane fraction on acetic acid induced writhing

*Each bar is expressed as mean ± S.E.M (n=5). One way ANOVA revealed significant difference in the number of writhes among the groups compared with the control exhibiting decrease in the number of writhes by n-hexane fraction. VUHF: V. unguiculata n-hexane fraction; Dic: diclofenac (10 mg/kg, i.p.); VEH: vehicle (2% Tween 20 in normal saline, 10 ml/kg, p.o.); *significant p<0.05 compared to VEH*

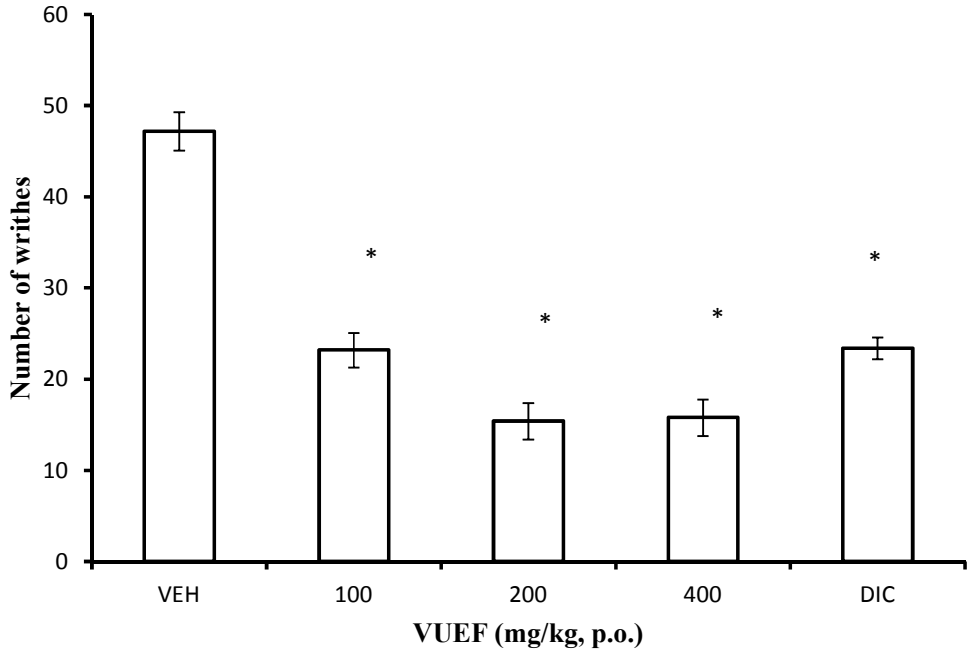

Fig. 3. Effect of ethyl acetate fraction on acetic acid induced writhing

*Each bar is expressed as mean ± S.E.M (n=5). One way ANOVA revealed significant decrease in the number of writhes by ethyl acetate fraction compared with the control. VUEF: V. unguiculata ethyl acetate fraction; DIC: diclofenac (10 mg/kg, i.p.); VEH: vehicle (2% Tween 20 in normal saline, 10 ml/kg, p.o.). *significant p<0.05 compared to VEH*

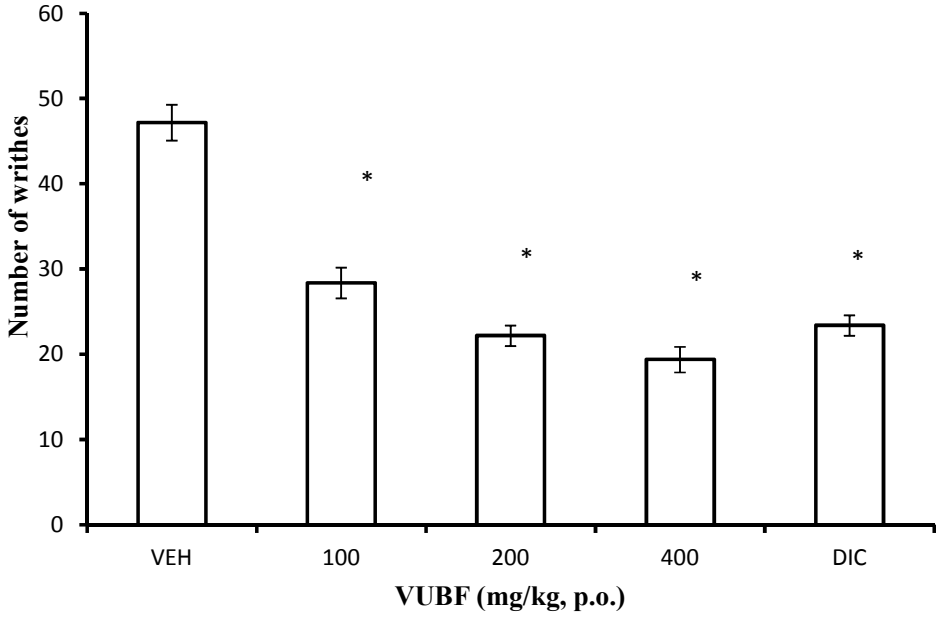

Fig. 4. Effect of butanol fraction on acetic acid induced writhing

*Each bar is expressed as mean ± S.E.M (n=5). One way ANOVA revealed significant difference in the inhibition of abdominal writhes induced by VUBF compared with the control. VUBF: V. unguiculata butanol fraction; DIC: diclofenac (10 mg/kg, i.p.); VEH: vehicle (2% tween 20 in normal saline, 10 ml/kg, p.o.).*significant p<0.05 compared to VEH*

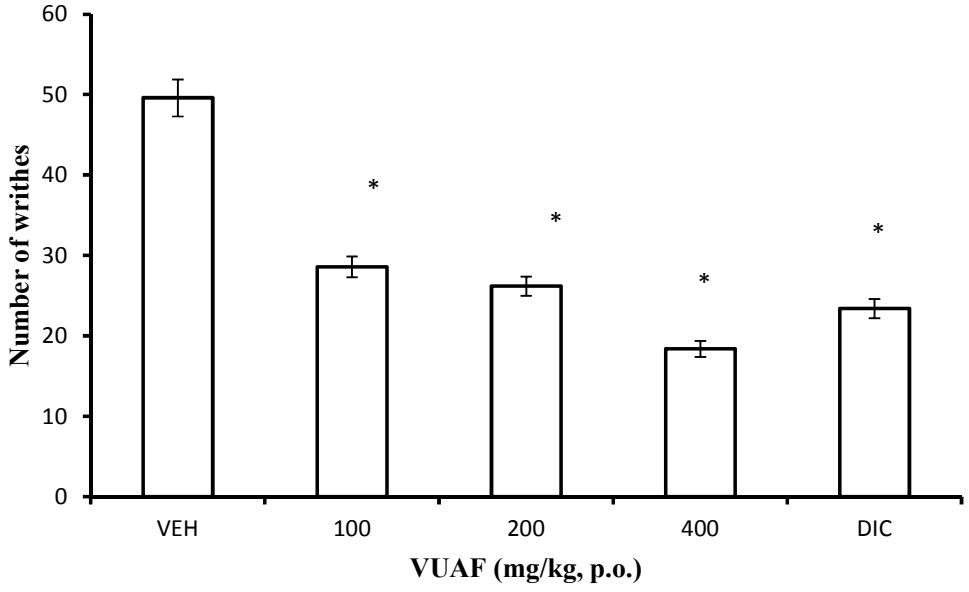

Fig. 5. Effect of aqueous fraction on acetic acid induced writhing

*Each bar is expressed as mean ± S.E.M (n=5). One way ANOVA revealed significant difference in the inhibition of abdominal writhes induced by VUAF compared with the control. VUAF: V. unguiculata aqueous fraction; DIC: diclofenac (10 mg/kg, i.p.); VEH: vehicle (normal saline, 10 ml/kg, p.o.); *significant p<0.05 compared to VEH*

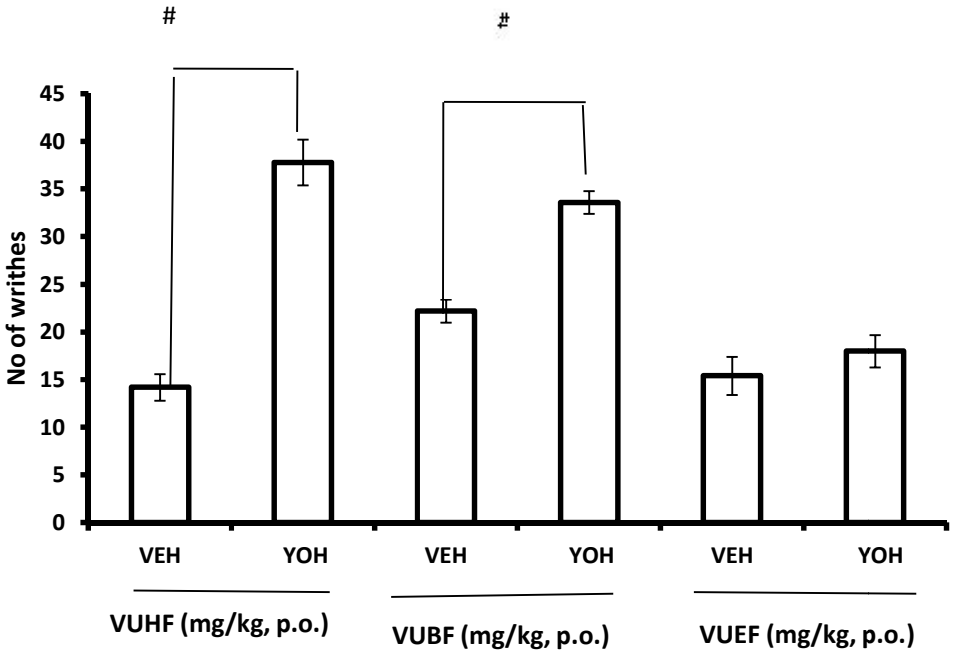

Fig. 6. Effect of Yohimbine on the Number of Writhes Induced by VUBF and VUEF in Acetic Acid Induced Writhing

Each bar is expressed as mean ± S.E.M (n=5). One way ANOVA revealed that yohimbine reversed the inhibition of abdominal writhes induced by n-hexane and butanol fractions but has no effect on VUEF. (F(5, 20) =33); VUHF: V. unguiculata n-hexane fraction (200 mg/kg, p.o.); VUEF: V. unguiculata ethyl acetate fraction (200 mg/kg, p.o.); VUBF: V. unguiculata butanol fraction (200 mg/kg, p.o.); YOH: yohimbine (1 mg/kg, i.p.); #significant p<0.05: antagonist versus treatment alone

Table 8. Effect of naloxone on reaction time induced by n-hexane fraction in mice on hot plate model

Treatment	Reaction time (minutes)				
	0	30	60	90	120
Morphine + NAL	4.8 ± 0.5	6.0 ± 0.3	5.9 ± 0.6	6.9 ± 0.9	8.2 ± 0.8
Morphine	3.2 ± 0.2	16.3 ± 0.9	9.2 ± 0.8	13.9 ± 1.1	12.3 ± 0.5
VUHF 200	4.0 ± 0.2	9.4 ± 1.0	11.5 ± 0.5	13.3 ± 1.0	11.1 ± 1.4
VUHF 200 + NAL	5.5 ± 0.6	8.6 ± 0.4	9.4 ± 1.6	11.9 ± 1.3	7.3 ± 1.6
VUEF 200	4.5 ± 0.6	7.5 ± 1.2	9.46 ± 0.9	9.6 ± 0.9	13.3 ± 1.8
VUEF 200 + NAL	5.4 ± 0.8	7.9 ± 2.2	13.6 ± 1.2	11.5 ± 0.9	11.5 ± 1.1
VUBF 200	5.3 ± 0.7	11.0 ± 0.6	15.9 ± 1.5	14.7 ± 1.0	15.9 ± 2.0
VUBF 200 + NAL	5.4 ± 0.6	8.4 ± 1.2	9.8 ± 1.0*	9.6 ± 1.5	12.0 ± 1.1

The result is expressed as mean ± S.E.M (n=5). One way ANOVA revealed that naloxone did not reversed the reaction time induced by n- hexane, ethyl acetate, and butanol fraction in mice; p>0.05; VUHF: V. unguiculata n-hexane fraction (200 mg/kg, p.o.); VUEF: V. unguiculata ethyl acetate fraction (200 mg/kg, p.o.); VUBF: V. unguiculata butanol fraction (200 mg/kg, p.o.); NAL: naloxone (5 mg/kg, i.p.)

4. DISCUSSION

Acetic acid induced writhing and thermal model were selected to investigate peripheral and central antinociceptive effects of the extract and fractions. Acetic acid induced abdominal constriction is used to evaluate superficial and visceral pain [19]. The intraperitoneal administration of acetic acid, unleashes the release of several mediators including,

bradykinin, substance P and prostangladins as well as cytokines such as IL-1β, TNF-α and IL-8 and these mediators activate chemosensitive nociceptors which contribute to the development of pain [20,21]. The number of abdominal constriction is a reflection of the intensity of the pain, hence, the higher the number of abdominal constriction, the higher the pain and *vice versa*. Any chemical agent that blocks or reduces the abdominal constriction is considered to possesses analgesic effect [22,23]. The result of acetic acid-induced writhing shows that VUME possesses strong analgesic effect and the inhibition of nociception is dose dependent (Fig. 1). The percentage inhibition induced by VUME at 100, 200, 400 mg/kg and diclofenac 10 mg/kg relative to the control are 41.9, 47.9, 70.8 and 50.4 respectively. The result showed that the highest dose of VUME induced a better inhibition of pain than the standard drug. The n-hexane fraction also inhibited the nociceptive response by a greater percentage compared to control. In this test, 200 mg/kg of the fraction induced the highest inhibition of nociception. The percentage inhibition induced byVUHF at 100, 200 400 and diclofenac 10 mg/kg compare to the control are 53.0, 69.9, 58.5 and 50.4 respectively (Fig. 2). In the same vein, all the test doses of VUEF produced inhibition of nociceptive response better than diclofenac (the standard drug) group (Fig. 3). The percentage inhibitions are 50.8, 67.4, 66.5 and 50.4 for VUEF at doses of 100, 200, 400 and diclofenac 10 mg/kg respectively. The butanol fraction also produced dose dependent inhibition of nociceptive response that was significantly different from control. The highest dose (400 mg/kg) produced the highest inhibition of the nociception in this fraction. VUAF also induced dose-dependent inhibition of nociceptive response with the highest inhibition at 400 mg/kg. Hexane fraction produced the highest inhibition while aqueous fraction induced the least inhibition in this model (VUHF>VUEF>VUBF>VUAF). The anti-nociceptive effect of extract and fractions are in agreement with the works of others [24,25,26, 27,28]. The significant increase in the percentage inhibition of abdominal constriction induced by the extract and its fractions might be due to the presence of active principles acting via the release of any of the mediators e.g. prostaglandin. The highest antinociceptive action was obtained in the crude extract (70.8) at 400 mg/kg which might be due to synergistic effect of various active principles that may be more abundant in the extract compared to the fractions alone. It is known that acetic acid induces pain by liberating endogenous substances that excite pain nerve endings [29]. There are claims that local peritoneal receptors are partly involved in the abdominal constriction response. Hence, the analgesic effect may be due to the blockade of these endogenous substances at the nerve ending. In another test involving the use of yohimbine, an alpha adrenergic antagonist, there was a reversal of the inhibitory effect produced by VUHF and VUBF while there was no change in inhibitory effect produced by VUEF. The alpha-2A and -2C subtypes are found mainly in the central nervous system while 2B receptors are found in the vascular smooth muscle where they mediate vasopressor effects. These receptors have been shown to inhibit adenylyl cyclase which in turn reduces the level of cyclic adenosine mono-phosphate and causing hyperpolarization of noradrenergic neurons in the medial dorsal pons, specifically in the locus coeruleus [30,31]. The stimulation of alpha-2 receptors in the dorsal horn of the spinal column has been reported to inhibit nociceptive neurons and reduce the release of substance P [31]. Therefore, the blockade of alpha 2A receptor will abolish the analgesic effect of this receptor. This effect may be responsible for the attenuation of analgesic effect of the fractions induced by yohimbine in this test. This therefore, suggests that the analgesic potential of both VUHF and VUBF may be mediated by adrenergic pathways [32].

The hot plate test measures the complex response to a non-inflammatory, acute nociceptive input and is one of the models normally used for studying central nociceptive activity [33]. It is an established fact that any agent that causes a prolongation of the hot plate latency must be acting centrally [34]. The ability of the extract to significantly prolong the reaction latency to thermally-induced pain in mice by the hot plate suggests that the extract may have a central analgesic activity. The results of this study indicate that the extract and its fractions possess central analgesic activity. VUME caused prolongation of the pain reaction time at different time intervals and different dose levels compared with control. The increase in the pain reaction time is a measure of antinociceptive activity of the extract. In n-hexane fraction, all test doses significantly prolonged the pain reaction time compared with the control. The analgesic property of this fraction at some time intervals was comparable to morphine group. The ethyl acetate fraction also produced strong analgesic property as it significantly prolonged the reaction

time at all doses from 30 minutes to 120 minutes. The highest antinociceptive activity was recorded at 120 minutes for all doses in this fraction. At this time 200 and 400 mg/kg of the fraction produced a superior analgesic property over morphine (5 mg/kg, i.p.). The butanol fraction also demonstrated a strong analgesic property by causing prolongation of the reaction time. The 200 mg/kg of this fraction (VUBF) produced the highest reaction time which was the most effective dose when compared to other fractions. The pain reaction time induced by VUAF showed weakest analgesic property compared with other fractions and morphine group; however, the 200 mg/kg at 60 minutes demonstrated analgesic effect that was significantly different from the control. On comparative basis, the relative potency of analgesic effect induced by the fractions is in the order of VUBF>VUHF>VUEF>VUAF. The study therefore showed that the analgesic effect of the extract and fractions in both models may be acting through central and peripheral mechanisms. Morphine activity is mediated by μ opioid receptors [35,36]. The mechanism of action underlying the central analgesic effect was evaluated using naloxone, yohimbine and cyproheptadine. Naloxone only reversed the analgesic effect of the butanol fraction suggesting that the analgesic activity of this fraction may be mediated via opioid receptor pathway. In the other tests, yohimbine and cyproheptadine reversed the increase in pain reaction time attenuating the analgesic effect of these fractions. The cellular and neural mechanisms underlying the drug action in the pain transmission may involve the interplay of a number of neurotransmitters and ion channels. It has been reported that agonists acting at α_2-adrenoceptor possess analgesic property [37,38]. Therefore any antagonist (for example yohimbine) that will block this α_2-adrenoceptor will definitely abolish or attenuate the analgesic property that its stimulation produces. Also the role of 5-HT in the management of pain has been established [39]. Goadsby, [40] reported the role of 5-HT receptors in nociceptive response as it relates to the pharmacology of head-ache. Therefore, the reversal of analgesic effect by cyproheptadine may be due to possible antagonism effect at 5-HT receptors. The study therefore, suggests the likely involvement of α_2 adrenergic and serotonergic neurotransmission pathways in the analgesic effect of the extract and fractions of this plant. The butanol fraction may also elicit its analgesic effect via the opioid receptor pathways.

5. CONCLUSION

The study justifies the folkloric use of extracts of this plant in the management of pain and migraine head-ache.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All the animal experiments were conducted according to the ethical norms approved by the Institutional Ethical Committee. The protocol for care of animals as approved by the Ethical Committee of the University (approval number PHP11/12/H/2765) was followed.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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