Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci.
https://doi.org/10.1007/s40011-024-01653-w

RESEARCH ARTICLE

Evaluation of Anxiolytic Potential of Extract and Fractions of *Vigna unguiculata* spp *dekindtiana* in Mice

Adekunle T. Adegbuyi[1] · G. Olayiwola[3] · Samuel S. Agboola[4] · Abayomi O. Sijuade[2] · Joseph O. Fadare[2]

Received: 14 July 2022 / Revised: 14 April 2024 / Accepted: 24 May 2024

Abstract Anxiety disorder is mental disease that is characterised by feeling of worry or fear that interferes with daily activity. The folkloric use of *Vigna unguiculata* spp *dekindtiana* has been reported in the management of neurological diseases such as epilepsy and anxiety. Although orthodox drugs are available for this disorder, medicinal plants have been proven to have curative potentials for this disorder with minimal side effect, hence this study. The anxiolytic property of the methanol extract and fractions of *Vigna unguiculata* spp *dekindtiana* was evaluated using elevated plus maze (EPM) and hole-board models in mice. The extract and fractions were administered orally and once daily to mice of both sexes (20.6 ± 1.5 g) at 50, 100, and 200 mg/kg dose levels. The probable mechanism of anxiolytic-like activity was assessed using flumazenil, yohimbine and cyproheptadine antagonists as receptor probes. One-way ANOVA was applied for the analysis of data and significance level was determined at $p < 0.05$. The results showed that the extract, the ethylacetate fraction and the aqueous fraction (VUA) significantly increased the frequency of entry and the time spent on the open arm of the EPM at 50 and 100 mg/kg significantly, suggesting the anxiolytic-like activity. The open arm avoidance index further validated the anti-anxiety property of the extract and fractions. These effects were reversed by flumazenil, a GABAergic receptor antagonist (3 mg/kg), yohimbine (1 mg/kg), an antagonist of alpha$_2$-adrenergic receptor, and cyproheptadine (0.5 mg/kg), a serotonergic receptor antagonist. There was also an increase in the frequency of head dips at 50 (except VUA) and 100 mg/kg, suggesting an anti-anxiety-like effect of the extract and fractions in the hole-board model compared to the control. The study concluded that the extract, aqueous and ethylacetate fractions of *V. unguiculata* spp *dekindtiana* exhibited an anti-anxiety effect in mice which was probably mediated through GABAergic, α_2-adrenergic and serotonergic pathways.

Keywords Anxiolytics · Elevated plus maze · Hole-board · Neurotransmitters *Vigna unguiculata* spp *dekindtiana*

Significance statement: Orthodox drugs for the management of anxiety disorders have some critical side effects that necessitate the search for the alternative ones with minimal side effects and, at the same time, cost-effective. In this regard, given the fact that medicinal plants have a plethora of claimed usefulness with minimal side effects coupled with beneficial natural products with synergistic roles, we investigated the anxiolytic roles of *Vigna unguiculata spp dekindtiana* in mice models. Before now, because of its folkloric curative claims, this plant has been used in traditional medicine for this purpose without any scientific documentation. It is, therefore, the objective of this study to validate this indigenous claim and possibly see if this plant can be studied further with the prospect of purifying the active principle for a possible formulation into an anxiolytic remedy.

✉ Adekunle T. Adegbuyi
adekunlegbuyi@gmail.com

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Federal University, Oye Ekiti, Nigeria

[2] Department of Pharmacology and Therapeutics, College of Medicine, Ekiti-State University, Ado Ekiti, Nigeria

[3] Department Clinical Pharmacy and Administration, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria

[4] Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria

Introduction

Anxiety is a prominent mental disorder that affects a large number of populations globally. The incidence is higher among women compared to men. Studies have shown that

Published online: 14 June 2024

the global pandemic such as covid-19 has contributed to the rise of the disorder among the population [1–3]. This condition induced autonomic and neuroendocrine activation which is shown in behavioural activities such as change in feeding habit, fight or flight for defensive purposes. [2, 4, 5]. The brain area responsible for this adaptive function such as expression of fear, defensive behaviour and aggression are the hippocampus and amygdala [2]. The roles of various neurotransmitters in the pathophysiology of anxiety disorders are significant. For example, the decrease in the inhibitory signalling by γ-aminobutyric acid (GABA) or increased excitatory neurotransmission by glutamate has been shown to cause an increase in activity of emotional processing in brain regions of patients with anxiety disorders [6, 7]. This pathological state affects adaptive function, quality of life and productivity at work [1]. Anxiety disorder being a chronic disease requires management for life and most clinically available drugs come with some untoward effects that make adherence to medication regimen difficult. Hence, this study was aimed at searching for new molecules with more tolerable profile [8, 9].

Medicinal plants has been used locally in the time past in many Nations to manage these behavioural disorders such as anxiety, depression, and amnesia [10]. The folkloric uses of the plant *V. unguiculata* spp *dekindtiana* have been investigated and documented in literature, for example, the antidepressant effect, the sedative and other behavioural potentials of the extract from this plant have been investigated and reported [11, 12]. The analgesic potential of the plant has been investigated and documented to support the local use of the plant in the management of pain and migraine [13]. The reports from literature indicated that methanol extract and fractions of the plant may contain some active principles that may interfere with the transmission of various neurotransmitters modulating these behaviours.

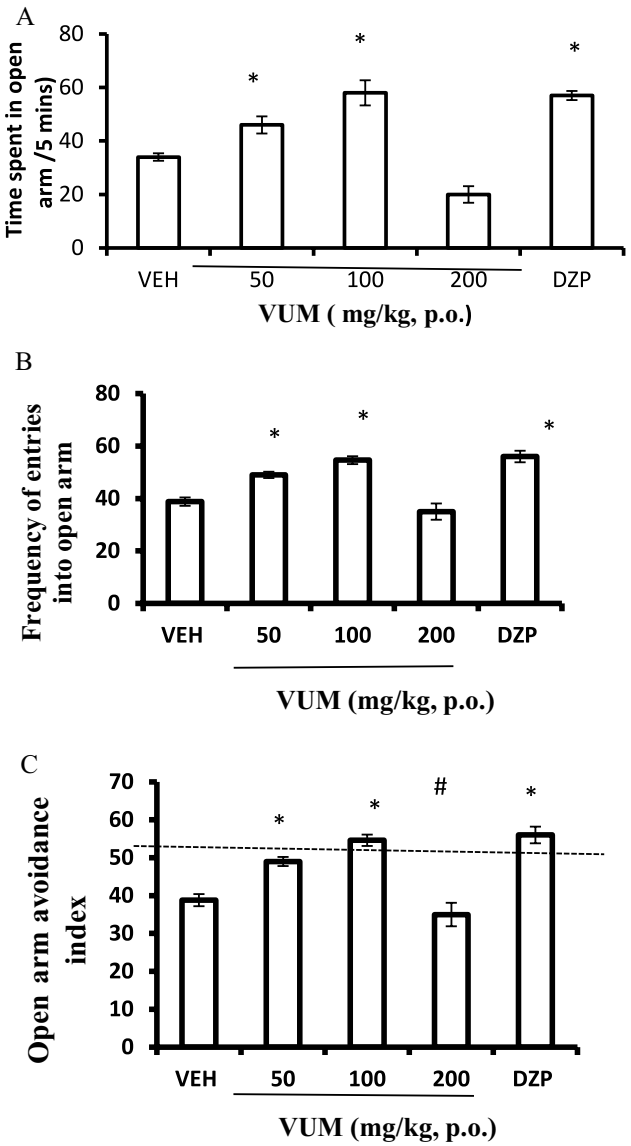

Fig. 1 Anxiolytic effect of VUM and DPM showing notable difference compared to VEH. Each bar represents mean ± standard error of mean. *significant $p < 0.05$ compared to VEH. **A** Time spent on open arms; **B** Number of entries into open arms; **C** Open arm avoidance index (IOAA) in mice

Material and Methods

Chemicals and Reagents

The chemical agents used in this study included: diazepam (Roche, Basel, Switzerland); cyproheptadine, yohimbine, (Sigma Chemicals Co, St. Louis, Missouri, U.S.A.); flumazenil (Hikma Pharmaceuticals, Portugal), normal saline (Unique pharmaceuticals, Lagos, Nigeria).

Extract Preparation

The process of extraction has previously been reported (2). Briefly, the leaves were air-dried and later pulverised into powder which was extracted with methanol and later fractionated into n-Hexane, butanol, ethylacetate and aqueous fractions. The residue of the extract and fractions were obtained by evaporation to dryness in rotary evaporator. The dry extract and the fractions were stored in the refrigerator until use.

Animals

A total of 80 Swiss mice (20.6 ± 1.5 g) were obtained and were maintained under natural day and night, fed with standard pellets from UAC Foods, Nigeria Limited and were allowed free access to water. The ethical guidelines

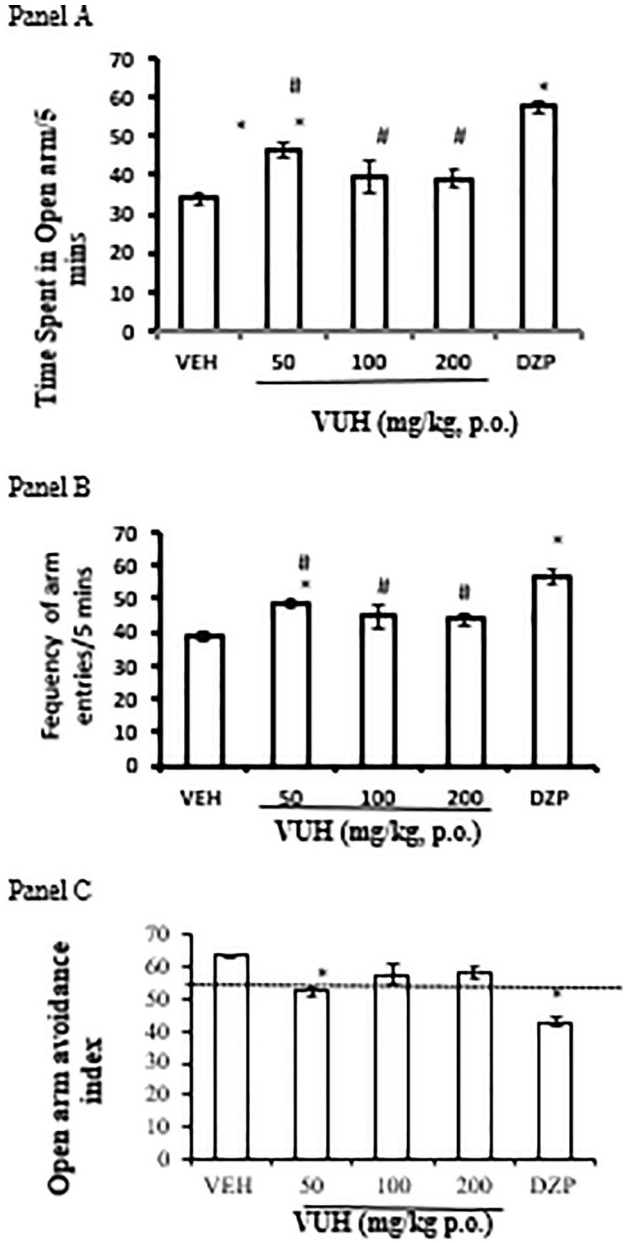

Fig. 2 VUH showed anxiolytic effect at 50 mg/kg. Each bar represents mean ± S.E.M.VUH: *significant $p < 0.05$ compared to VEH; #significant compared to DPM. **A** Time spent in open arms; **B** frequency of visit into the open arms; **C** Effect of VUH on IOAA in mice

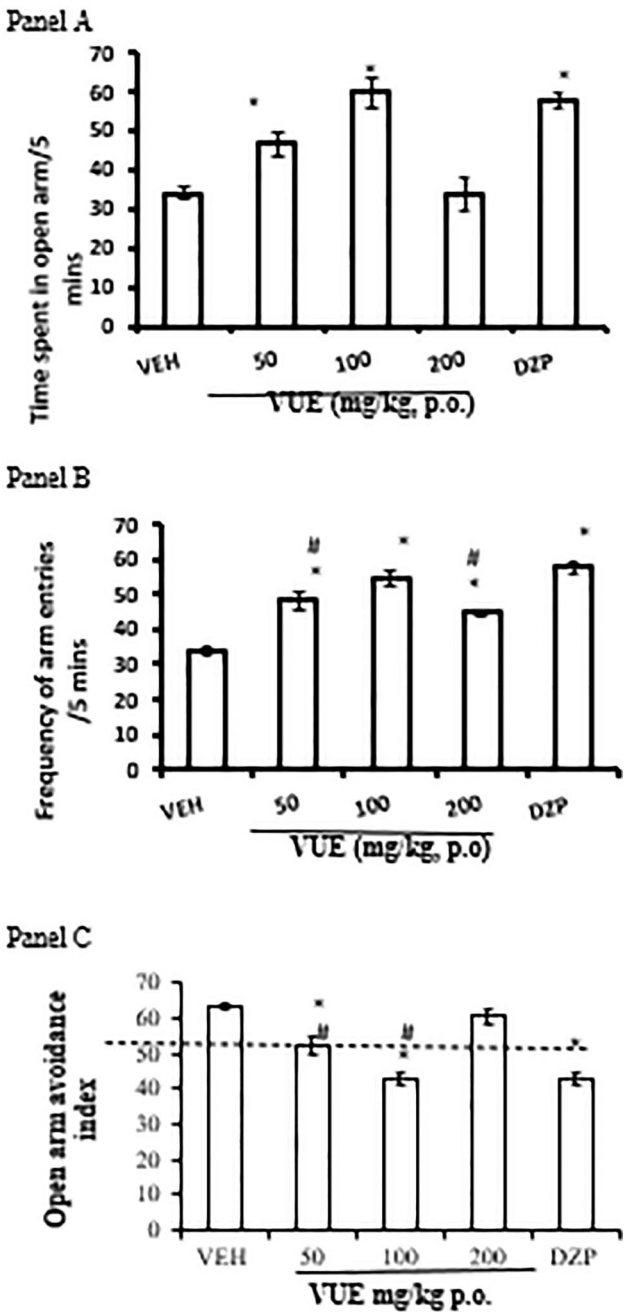

Fig. 3 Anxiolytic effect of VUE. Each bar represents mean ± standard error of mean.VUE at 50 and 100 mg/kg exhibited. *significant $p < 0.05$ compared to VEH; #significant compared to DPM. **A** Period of stay in the open arms; **B** Frequency of visit into the open arms; **C** Effect of VUE on OAAI in mice

for the use of animals in research given by the National Committee for Research Ethics in Science and Technology (NENT), 2018, were followed. The approval number obtained from Obafemi Awolowo University, Ile-Ife, Nigeria, for this work was PHP11/12/H/2765. The animals were randomised into five groups ($n = 5$) for extract, *n*-Hexane, butanol, ethylacetate and aqueous fractions. Group 1 received 2% Tween 20 in 0.9% saline solution orally, and groups two to four were treated orally with extracts and fractions while group 5 received diazepam (DPM), (1 mg/kg) intraperitoneally.

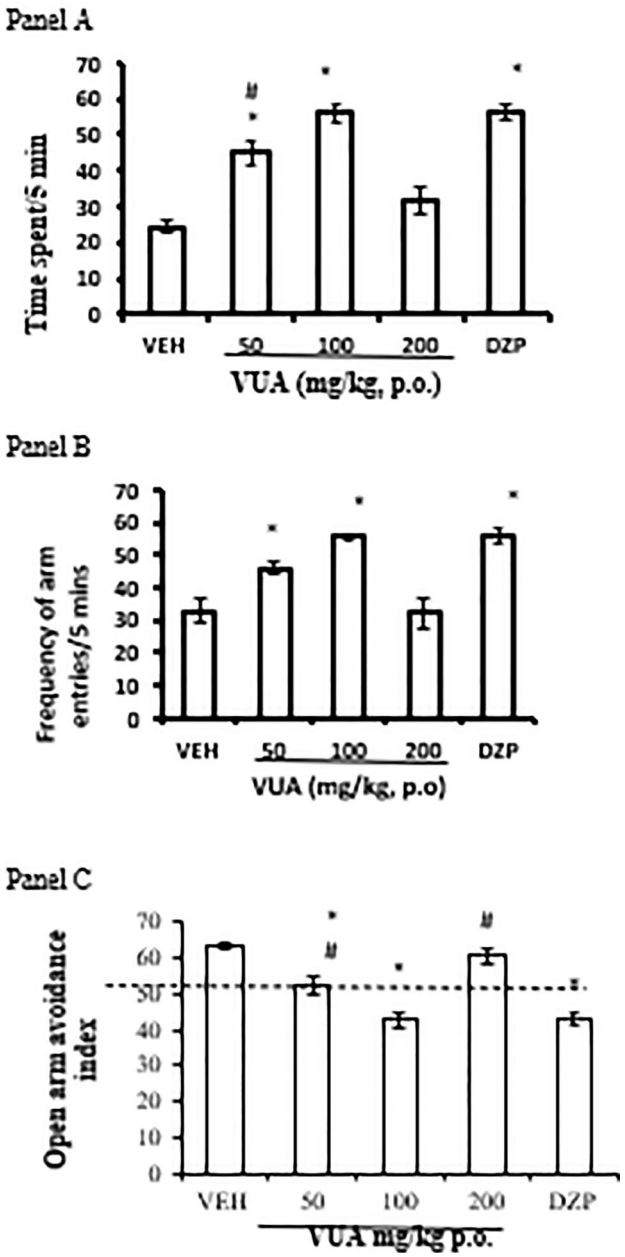

Fig. 4 Anxiolytic effect of VUA. Each bar represents mean ± standard error of mean. VUA at 50 and 100 mg/kg exhibited anxiolytic effect. *significant $p < 0.05$ compared to VEH. #significant compared to DPM $p < 0.05$. **A** Period of stay in open arms; **B** Frequency of visit in open arms; **C** Effect of VUA on OAAI in mice

Effects of the Extract and Fractions of V. unguiculata on Mice Using Elevated Plus Maze

The maze was made up of two arms (uncovered) with dimensions of 35 × 6 cm and two arms (covered) with dimensions of 35 × 6 × 15 cm in a plus sign arrangement and was raised to 40 cm above the floor. This model was used for the assessment of the anxiolytic potential of the methanol extract and fractions. Each mouse was positioned in the middle of the maze to face the uncovered arm. The mouse was permitted to move around inside the maze for 5 min and the number of visits into both arms as well as the time spent in each arm were documented [14, 15, 16]. The animals were administered with the vehicle (10 ml/kg, p.o.) or methanol extract of *V. unguiculata* (50, 100 and 200 mg/kg, p.o.) 60 min before their placement at the centre of the maze. The standard anxiolytic agent was administered intraperitoneally (i.p.) thirty minutes before placing the mouse on the maze. The preference of visits to the uncovered arm compared to enclosed part of the maze was taken as an indication of anxiolytic activity of the extract (VUM) [17]. The same process was repeated for each of the fractions at 50, 100 and 200 mg/kg (N-hexane fraction (VUH), ethylacetate fraction (VUE) and butanol (VUB)). 10 ml/kg of the vehicle (2% Tween 20 in 0.9% saline solution) served as negative control while 10 ml/kg of 0.9% saline solution served as control for aqueous fraction (VUA). Diazepam (1 mg/kg i.p.) served as standard reference drug. The mechanisms of the effects exhibited by extract and fractions were delineated by intraperitoneal injection of flumazenil (3 mg/kg), cyproheptadine (0.5 mg/kg) and yohimbine (1 mg/kg).

The open arm avoidance index (OAAI) was calculated using the formula: OAAI = 100–(% period stayed on the open arm + frequency of visit to the open arm)/2 [18, 19].

Effects of the Extract and Fractions of V. unguiculata on Mice Using Hole-Board Model

The model was a flat chamber made of wood with dimensions of 40 by 40 by 25 cm) (l × b × h) containing sixteen equidistant cavities of 1 cm in diameter and 2 cm in depth. One mouse was positioned at one end of the board and permitted to move unhindered from one end of the board to the other. The head dip into holes was an indication of exploratory behaviour of the mice [20]. The extract was administered orally to the mouse for 1 h before observing the frequency of head dip for 5 min. The doses employed in this experiment were 50, 100 and 200 mg/kg. The same protocol was employed for each of the fractions. The DPM (1 mg/kg, i.p.) was employed as standard reference anxiolytic agent while 2% Tween 20 in 0.9% saline solution served as control for extract and fractions except the aqueous fraction which had 0.9% saline solution as vehicle. Yohimbine (1 mg/kg, i.p.), cyproheptadine (0.5 mg/kg,i.p.) and flumazenil (3 mg/kg, i.p.) were antagonists administered, respectively, to investigate the possible mechanism of action.

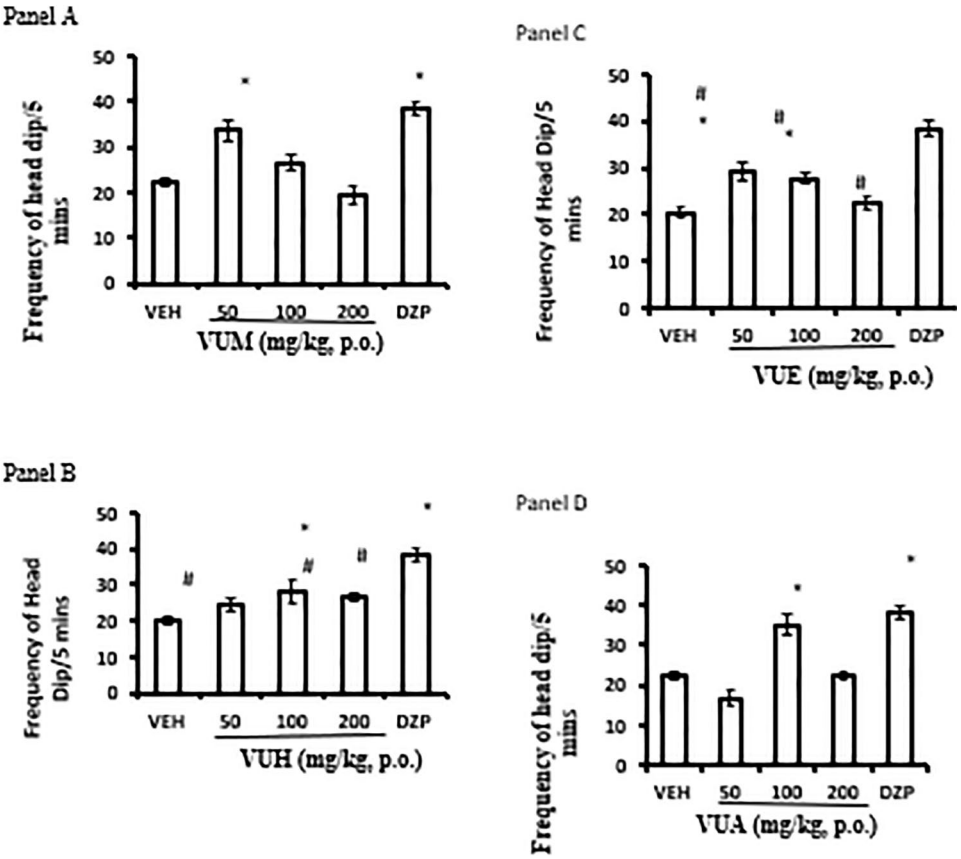

Fig. 5 Effects of VUM, VUH, VUE and VUA showing significant difference in mouse head dips in hole-board model compared to VEH. Each bar represents mean ± standard error of mean. *significant $p < 0.05$ compared to VEH, #significant compared to DPM $p < 0.0$

Statistical Analysis

The data were expressed as mean ± S.E.M and analysed using one-way analysis of variance. Student Newman–Keuls post hoc test determined the level of significance ($p < 0.05$).

Results

The result showed that there was an increase in both the frequency of entry and the time spent at the uncovered arm of the maze. The VUM at 50 and 100 mg/kg (Fig. 1) induced an increase in the frequency of the number of entries/visits into and period of stay at open arm of the maze compared to the control. The anxiolytic effect of the extract (VUM) at 50 and 100 mg/kg was significant compared to control [VEH: vehicle 10 ml/kg (2% Tween 20 in 0.9% saline solution)], $F(4.20) = 21.3$, $p < 0.05$; $F(4.20) = 27.9$, $p < 0.05$. This anti-anxiety effect induced by the extract at 100 mg/kg was similar of diazepam (DPM 1 mg/kg, a standard anxiolytic agent) (Fig. 1). The butanol fraction at 50 mg/kg of VUH fraction revealed an increase in the frequency of entries and time spent in the open arm compared to control (Fig. 2) ($F(4,20) = 8.0$, $p < 0.05$; $F(4,20) = 14.3$, $p < 0.05$). The VUH at 100 mg/kg in hole-board model revealed an increase in exploration behaviour of the animal (Fig. 5C) indication of anxiolytic-like effect ($F(4,20) = 12.1$, $p < 0.05$). The ethylacetate fraction (VUE), 50 and 100 mg per kg (Fig. 3) raised the mean number of visits into open arm in mice and were found to be significant compared to the control $F(4, 20) = 13.6$. In hole-board model (Fig. 5C), VUE 50, 100 and DPM 1 mg/kg raised the mean number of head dips significantly ($F(4, 20) = 21.1$, $p < 0.05$). In Fig. 4 panel A and C, doses of 50 and 100 mg/kg of VUA enhanced the frequency of visits and period of stay in the open arms but100 mg/kg of this fraction increased the frequency of the number of head dips in this model (Fig. 5 panel D). The possible mechanism of action of anxiolytic effect of these fractions was evaluated using flumazenil, cyproheptadine and yohimbine as shown in Figs. 6, 7 and 8. Flumazenil reversed the anxiolytic effect of VUM while the effect induced by VUE 100 mg/kg was reversed by cyproheptadine in the hole-board model. Cyproheptadine and Yohimbine reversed the increase in the mean number of entries and the time spent in the open arms in both VUM 100 and VUE at 100 and 200 mg/kg in the EPM model. The reversal of the anxiolytic effect of the VUE showed an anxiety index of 51.8 ± 1.7, 43.6 ± 2.8 and 72.2 ± 1.8, respectively. The anxiety index due to VEH (control) was 63.4 ± 0.7 while that of DPM (the standard anxiolytic drug) was 43.0 ± 1.5. The anxiety index for

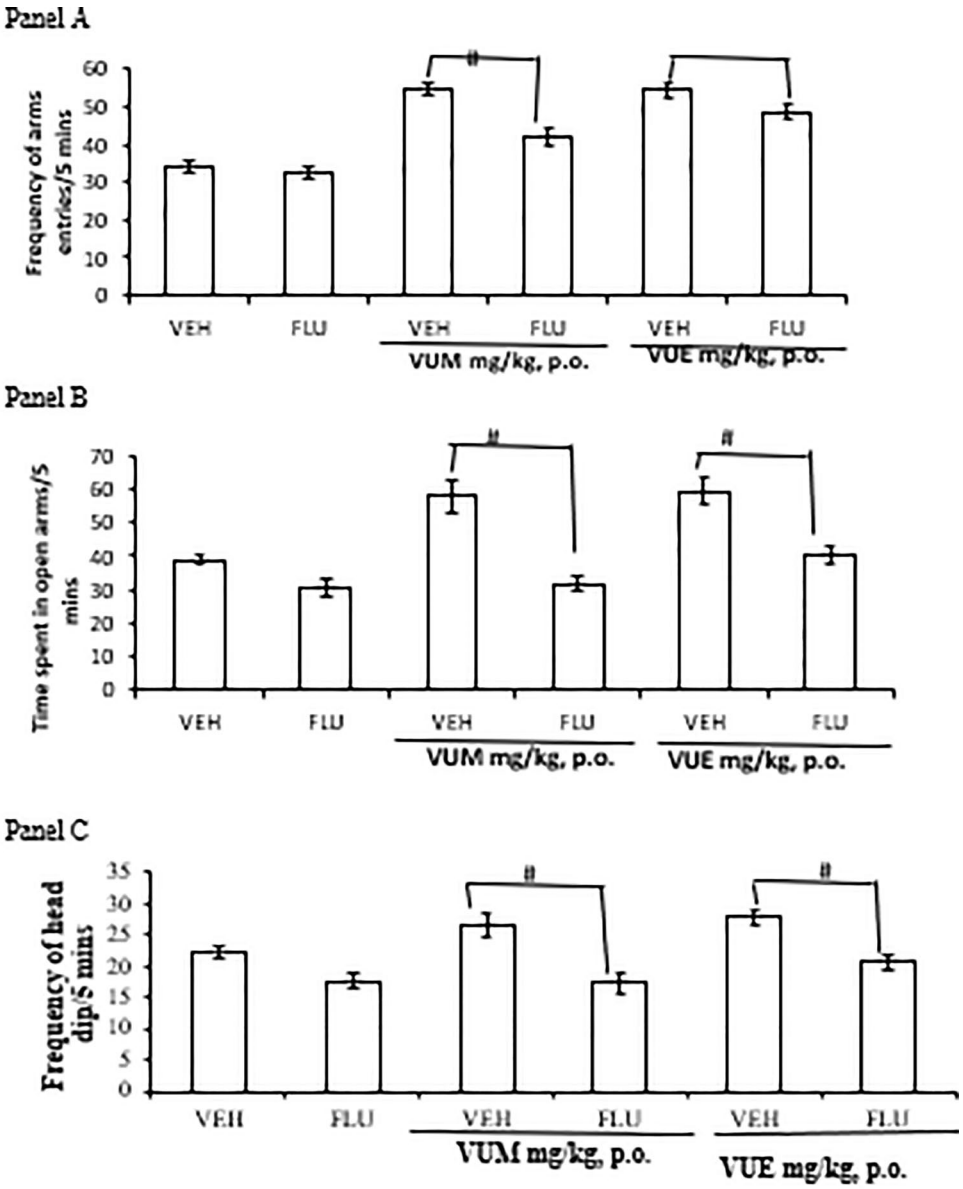

Fig. 6 Mechanism of action of anxiolytic effect of VUM and VUE using flumazenil. #significant $p < 0.05$: antagonist versus treatment alone

VUH at 50, 100 and 200 mg/kg were 52.4 ± 1.4, 57.8 ± 3.0 and 58.4 ± 1.9, respectively. The aqueous fraction showed 54.4 ± 2.1, 43.6 ± 1.5, 67.4 ± 4.0, respectively, at similar dosage levels. The higher the anxiety index, the less the anxiolytic value of the agent. It has been postulated that if any group is at least10 points less than the control's (vehicle), then the sample is said to be anxiolytic. Conversely, if the sample is at least 10 point greater than the control, then such agent is said to show anxiogenic effect. The result, therefore, showed that the VUM at 50 and100, VUH at 50; VUE at 50 and 100; VUA at 50 and 100 mg/kg and DPM possessed anxiolytic effect.

Discussion

Herbal medicines have being in use to manage various neurological disorders such as stress and anxiety and many of the active principles responsible for this activity have been isolated. Examples of such plants include *Passiflora incarnata, Cimicifuga racemosa.* The evaluation of the extract from this plant was premise on the use by the natives to manage epilepsy. The results showed that the extract produced a significant rise in both the percentage of time that the mouse spent in and the frequency of entry into the uncovered portion of the EPM as well as inducing an increase in the frequency of head dips on the hole-board [23]. These indicated that the extract and fractions of this plant possessed

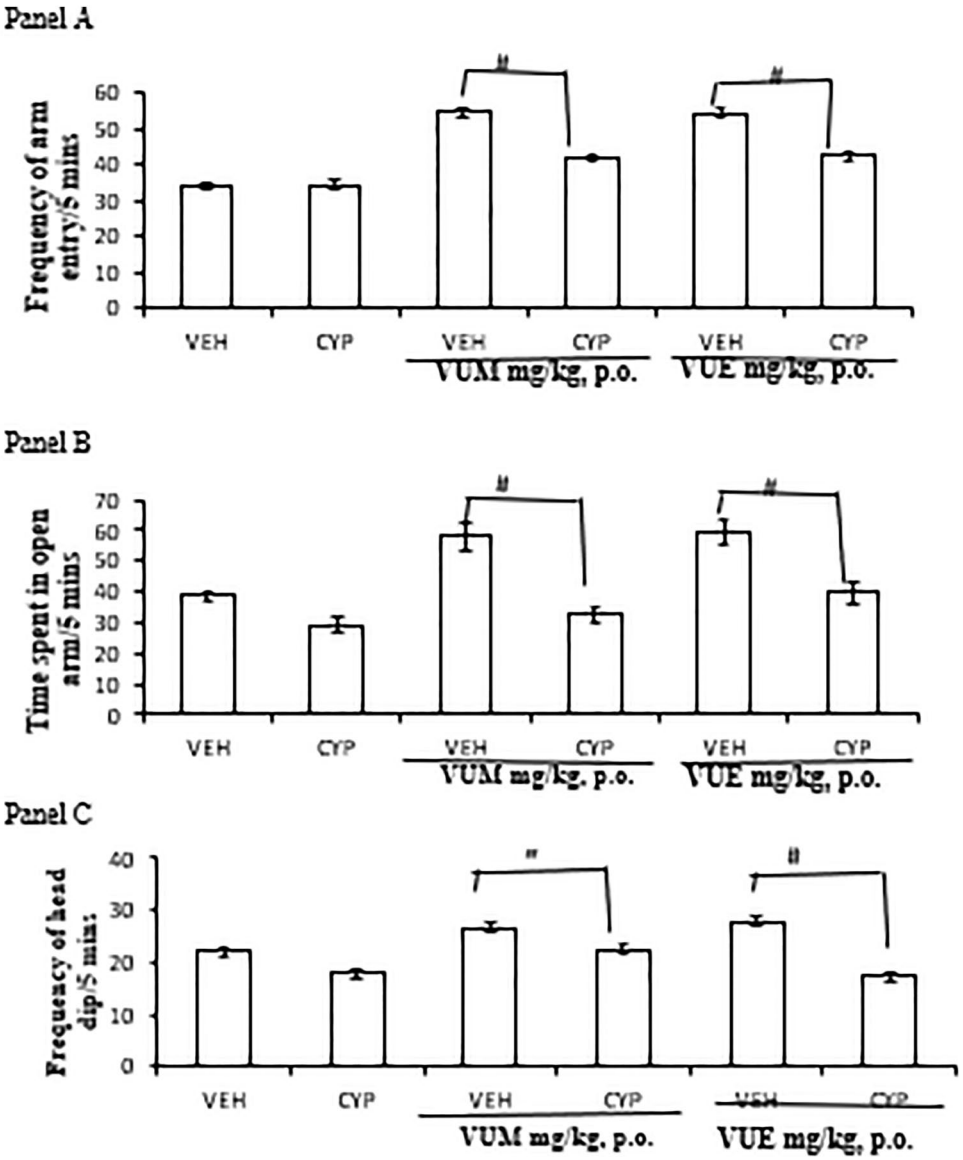

Fig. 7 Mechanism of action of anxiolytic effect of VUM and VUE using cyproheptadine. #significant $p < 0.05$: antagonist versus treatment alone

anxiolytic property similar to diazepam (a standard anxiolytic agent) used in this experiment. Usually, rodents have natural tendency to explore an enclosed portion of the maze than the opened portion [18, 19]. Therefore, any chemical agent that induces an increase in the number of visits as well as prolongs the period that rodents spend in the exposed portion of the maze is said to exhibit anxiolytic property whereas the agent that induces a reverse effect is said to be anxiogenic agent [20]. The ethylacetate fraction produced more convicing anxiolytic effect in both models compared to other fraactions. The anxiolytic action of this fraction is comparable to DPM (Fig. 5).

The Index of open arm avoidance (IOAA) was determined to further validate anxiolytic property of the extract. The interpretation of IOAA is such that, if any group is at least10 points less than the control's (vehicle), then the sample can be said to show anti-anxiety effect. On the other hand, if the sample is at least 10 points greater than the control's then, such sample is said to have shown an anxiogenic activity [21, 22]. In this study, the values of IOAA for the control, VUM, VUE, VUA and diazepam were 64%, 46%, 44%, 45% and 46%, respectively. Like diazepam, the OAAI for each of the extract and fractions was less than the control's by more than 10%, and as such they convincingly demonstrated an anxiolytic property (panel C in Figs. 1, 2, 3 and 4).

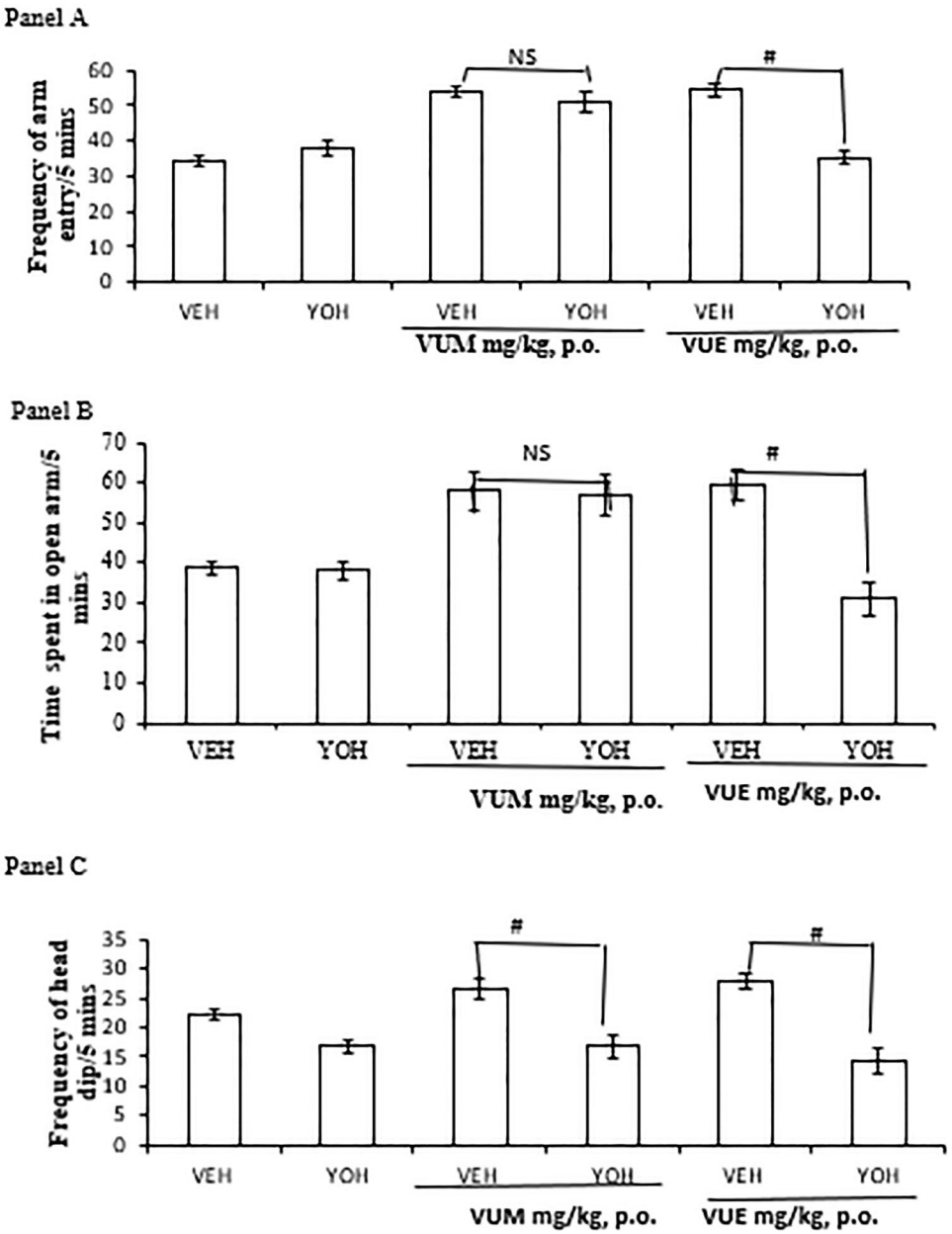

Fig. 8 Mechanism of action of anxiolytic effect of VUM and VUE using yohimbine. #significant $p < 0.05$: antagonist versus treatment alone

Neurotransmitters such as acetylcholine, noradrenaline, 5-HT (serotonin), dopamine, histamine and GABA that are found in hypothalamic–pituitary–adrenal axis and serotonergic systems have been shown to modulate anxiety disorders [1, 2, 5, 21]. This informed the use of some antagonists like yohimbine (alpha adrenergic antagonist), cyproheptadine (serotonin antagonist) and flumazenil (GABA antagonist) to delineate the possible mechanism of anxiolytic effect of the extract and fractions in this study (Figs. 6, 7 and 8). The reversal anxiolytic effect of VUM and VUE by flumazenil in both models was an indication that GABAergic neurotransmission may be involved in the anti-anxiety effect of extracts of this plant (Fig. 6 panel C). The reversed effect of cyproheptadine on VUE fraction may also suggest some constituents of this fraction may produce anti-anxiety effect via serotonergic pathway. In the same vein, Yohimbine reversal effect in both models may also suggest adrenergic involvement in the anti-anxiety effect since increase in cerebral noradrenergic activity has been shown to be closely related to anxiety-related behaviour [23, 24]. Thus, the result of this study therefore suggests the connection between GABAergic, serotonergic and alpha$_2$ adrenergic neurotransmission and the anxiolytic potential of VUE while GABAergic and serotonergic is suggested to be involved in anxiolytic effect of VUM. Some studies have shown that the action of anxiolytic drugs improved the response to GABA by opening of GABA$_A$-activated chloride channels [25–27]. Therefore, Gabaergic receptor stimulation may be one of

the possible mechanisms of action by which *V. unguiculata* spp *dekindtiana* crude extract and its fractions exerted their anxiolytic effect. From the results, the extract and pharmacologically active fractions of *V. unguiculata* spp *dekindtiana* can be utilised to manage anxiety-related disorders. The anti-agitation properties of this plant is similar to those of the essential oil of *Melissa officinalis* [28]. Further studies will be required to isolate the pharmacologically active molecules in the fractions that are responsible for this anxiolytic effect.

Conclusion

This work has shown that the methanol extract, ethylacetate and aqueous fractions of *V. unguiculata spp dekindtiana* possess anxiolytic activity in mice, thereby justifying the ethnomedicinal use in the treatment of anxiety disorders. The obtained results suggested the involvement of GABAergic, catecholaminergic and serotonergic pathways in the anxiolytic property of these phytoconstituents in mice.

Acknowledgements While the authors declare no conflict of interest, the authors acknowledged the contributions of the following technologists Mr Olaniyi Fadare, Mr Kehinde Omodara and Mr A.O. Owolabi of the Department of Pharmacognosy, Drug Production Unit and Department of Pharmacology, ObafemiAwolowo University, respectively.

Declarations

Conflict of interest There is no conflict of interest among the authors.

References

1. Charrey DS (2003) Neuroanatonical circuits modulating fear and anxiety. ActaPsychiatr Scand 417:38–50
2. Shin LM, Liberzon I (2010) The neurocircuitry of fear, stress and anxiety disorders. Neuropsychopharmacol Rev 35:169–191
3. Siegel RS, Dickstein DP (2012) Anxiety in adolescents: update on its diagnosis and treatment for primary care providers. Adolesc Health Med Ther 3:1–16
4. Steimer T (2002) The biology of fear and anxiety - related behaviors. Dialogues Clin Neurosci 4:123–137
5. Ferry B, Quirarte GL (2012) Role of norepinephrine in modulating inhibitory avoidance memory storage: critical involvement of the basolateral amygdala. Intect 204–229
6. Nutt DJ (2005) Overview of diagnosis and drug treatments of anxiety disorders. CNS Spectr 10(1):49–56
7. Sathya B, Ariharasivakumar G, Christopher VD, Subramani M, Magesh M (2011) Pychopharmacological evaluation of ethanolic extract of leaves of *Bauhinia tomentosa* L. in mice. Int J Pharm Technol 3(4):3693–3709
8. Baldessarini RJ (1996) Drugs and the treatment of psychiatric disorders. In: Hardman JG, Limbrid LE (eds) Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 9th edn. McGraw-Hill, New York, pp 399–427
9. Baldwin DS, Ajel K (2007) Development and evaluation of psychotropic drugs. Psychiatry 6:279–283
10. Petrovska BB (2012) Historical review of medicinal plants' usage. Pharmacognosy Rev 6(11):1–5
11. Akinpelu AL, Adegbuyi AT, Agboola SS, Olaonipekun JK, Olawun JI, Adegoke MA, Oyemitan AI, Olayiwola G (2017) Antidepressant Activity and Mechanism of Aqueous Extract of *Vigna unguiculata* ssp. *Dekindtiana* (L.)Walp Dried Aerial Part in Mice. Int J Neurosci Behav Sci 5(1):7–18
12. Adegbuyi TA, Akanmu MA, Olayiwola G (2019) Neurobehavioural effects of *Vigna unguiculata* spp *Dekindtiana* (L.) Walp in mice. J Drugs Pharm Sci 3(4):32–48
13. Adegbuyi TA, Akanmu MA, Olayiwola G, Sijuade OA (2020) Analgesic effects of vigna unguiculata subspecies dekindtiana in mice. J Adv Med Pharm Sci 22(7):10–22
14. Pellow S, Chopin P, File SE, Briley M (1985) Validation of open-closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 14:149–167
15. Trullas R, Skolnick P (1993) Differences in fear motivated behaviours among inbred mouse strains. Psychopharmacology 111(3):323–331
16. Akanmu MA, Olowookere TA, Atunwa SA, Ibrahim OI, Lamidi OF, Adams PA, Ajimuda BO, Adeyemo LE (2011) Neuropharmacological effects of Nigerian honey in mice. Afr J Tradit Complement Altern Med 8(3):230–249
17. Calzavara MB, Patti CL, Lopez GB, Abilio VC, Silva RH, Frussa-Filho R (2005) Role of learning of open arm avoidance in the phenomenon of one-trial tolerance to the anxiolytic effect of chlordiazepoxide in mice. Life Sci 76(19):2235–2246
18. Ishola IO, Chatterjee M, Tota S, Tadigopulla N, Adeyemi OO, Palit G, Shukla R (2012) Antidepressant and anxiolytic effects of amentoflavone isolated from *Cnestisferruginea* in mice. Pharmacol Biochem Behav 103:322–331
19. Cryan FJ, Sweeney FF (2011) The age of anxiety: role of animal models of anxiolytic action in drug discovery. Br J Pharmacol 164:1129–1161
20. Ennaceur A, Chazot PL (2016) Preclinical animal anxiety research-flaws and prejudices. Pharma Res Per 4(2):e00223. https://doi.org/10.1002/prp2.223
21. Thoeringer C, Erhardt A, Sillaber I, Mueller M, Ohl F, Holsboer F, Keck M (2009) Long-term anxiolytic and antidepressant-like behavioural effects of tiagabine, a selective GABA transporter-1 (GAT-1) inhibitor, coincide with a decrease in HPA system activity in C57BL/6 mice. J Psychopharmacol 24:733–743
22. Bystritsky A, Khalsa SS, Cameron ME, Jason S (2013) Current diagnosis and treatment of anxiety disorders. Pharmacy Ther 38(1):30–57
23. Goehler LE, Park SM, Opitz N, Lyte M, Gaykema RPA (2008) *Campylobacter jejuni* infection increases anxiety-like behavior in hole-board: possible anatomical substrates for viscerosensory modulation of exploratory behavior. Brain Behav Immunol 22(3):354–366
24. Crespi F (2009) Anxiolytic antagonize yohimbine-induced central noradrenergic activity: a concomitant *in vivo* voltammetry-electrophysiology model of anxiety. J Neurosci Methods 180(1):97–105
25. Foyet HS, Tsala DE, Bouba AA, Hritcu L (2012) Anxiolytic and Antidepressant-like effects of the aqueous extract of *Alafia multiflora* stem barks in rodents. Adv Pharmacol Sci. https://doi.org/10.1155/2012/912041
26. Nuss P (2015) Anxiety disorders and GABA neurotransmission: a disturbance of modulation. Neuropsychiatr Dis Treat 11:165–175
27. Rudolph U, Crestani F, Mohler H (2001) $GABA_A$ receptor subtypes: dissecting their pharmacological functions. Trends Pharmacol Sci 22(4):188–194

28. Abuhamdah S, Huang L, Elliot M, Howes M, Ballard C, Holmes C, Burns A, Perry E, Francis P (2008) Pharmacological profile of an essential oil derived from Melissa officinalis with anti-agitation properties: focus on ligand-gated channels. J Pharm Pharmacol 60:377–384