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Extract of the Leaves of *Hydrolea glabra* Schum. &Thonn. (Hydrophyllaceae), Exerts Anxiolytic Effect on Swiss Albino Mice

Chikaodinaka A. Anyanwu-Ndulewe[1], Aderonke A. Adepoju-Bello[1], Samuel Fageyinbo[2], HerbertCoker[1]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos*
[2]*Department of Pharmacology, Toxicology and Therapeutics, Faculty of Basic Medical Sciences, University of Lagos.*

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ABSTRACT

The plant,*Hydrolea glabra*, is used in neurodegenerative disorders associated with dementia, where personality changes present as symptoms such as anxiety, depression and sleep changes. This notwithstanding, no pharmacological studies have been carried out on the anxiolytic and sedative properties of the plant.

This research evaluated the anxiolytic effects of the extract of the plant, *Hydrolea glabra*, in order to validate the folkloric claims of its usefulness in the management of dementia. Methanol extract of the leaves of *Hydrolea glabra* was analysed for the phytochemical composition and the anxiolytic effect was evaluated, using the Elevated plus maze, Hole board and Phenobarbitone-induced sleeping time models in mice.

Phytochemical screening showed the presence of alkaloids, terpenoids, saponins, phenolics, condensed tannins, cardiac glycosides and reducing sugars. There was significant percentage increase (48%) in the time spent in the open arms, by the rats given the extracts, which was comparable to 42% of the standard Diazepam (1 mg/kg b.w) in the elevated plus maze model. The extract at 200mg/kg, also had a sedative effect by prolonging the phenobarbitone-induced sleeping time by 45% when compared to the standard, Diazepam.

The anxiolytic effect observed may substantiate the medicinal relevance of the plant, by providing the pharmacological basis for the use of this plant in folkloric medicine practice.

Keywords: Anxiolytic activity, *Hydrolea glabra*, exploratory behaviour, sedative activity, dementia.

Introduction

Anxiety is a common symptom among patients with cognitive impairment; occurring in a majority of patients with dementia of the Alzheimer's disease (AD) type and in 10% to 45% of patients with mild cognitive impairment (MCI).[1-4] Anxiety disorder is characterized by cognitive, somatic, emotional and behavioural alterations symptoms, as also seen in patients with dementia.[5]

In early Alzheimer's disease, anxiety is reported to predict a decline in learning as measured on the Auditory Verbal Learning Test.[6] High levels of anxiety in patients with mild cognitive impairment have an adverse effect on executive functioning. Vascular risk factors and poorer premorbid cognitive function are significantly associated with anxiety and decline in learning and memory is associated with high baseline anxiety score.[7] This may be for the reason that increased anxiety levels are hypothesized to increase amyloid beta, thereby increasing the rate of cognitive decline prior to the development of AD dementia.[8]

*Corresponding author. E mail: cndulewe@gmail.com

Tel: +2348037152220

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Neurodegenerative changes associated with dementia, can involve limbic areas such as the hippocampus and amygdala, which are implicated in the regulation of emotions, including anxiety. However, the presence of anxiety can be difficult to establish in people with dementia, especially when expressive or receptive language is impaired.[7]Starkstein*et al* reported a number of symptoms that can individually predict the presence of generalized anxiety disorder in dementia: excessive anxiety or worry that is difficult to control, restlessness, irritability, muscle tension, fears and respiratory distress. [9] Despite the accessibility to copious classes of drugs and advances in the treatment of anxiety and depression, full diminution of disease symptoms as well as clinical needs of a considerable number of patients are vaguely met.[10] Clinical use of these classes of drugs, such as Barbiturates, Benzodiazepines, monoamine oxidase inhibitors, selective serotonin reuptake inhibitors, and tricyclic antidepressants is limited by their characteristic side effects and poor tolerability profile.[11-14]The British Association for Psychopharmacology guidelines shows evidence in support of the use of diazepam and alprazolam in acute anxiety, but warned about the adverse effects.[15] The efficacy, duration of effects and side effects of these available drugs have constituted serious concern, hence the need for newer drugs. The diversity in neural targets however, makes phytomedicine a promising candidate for the treatment of these diseases.[10] Currently, the therapeutic value of medicinal plants comes to bear in the percentage of medical prescriptions (25%) derived from vegetal species.[16] The World Health Organization (WHO) in the guidelines on the conservation of medicinal plants, has stated that across the world, traditional medicine (TM) serves either as the mainstay of healthcare delivery or complement to it. Consequently, three-quarters of the world population depend on TMs for their primary healthcare needs.[17] Traditional medicine or nonconventional medicine, otherwise referred to as

complementary and alternative medicine (CAM) is an important and often underestimated part of health services.[18] It has a long history of use in health maintenance, disease prevention and treatment, involving the total sum of knowledge, skill, and cultural practices based on the theories, beliefs and indigenous experiences whether explicable or not towards diagnosis and treatment of physical and mental illness.[19] The integration of CAM and conventional medicine indicates inherent value of CAM in primary healthcare needs.[20,21]

*Hydrolea glabra (*fam. Hydrophyllaceae) Schum &Thonn, is an annual broadleaf herb, with thick, spongy hairless stem and leaves arranged throughout the stem. The flowers are blue and clustered in the axils of the leaves, along the stem. The shrub is usually found in swampy areas and rice farm land, especially in the West African coastal region. Its folkloric uses include the treatment of headaches and 'development of intelligence' among the Yoruba of South west Nigeria.[22,23] Despite the purported medicinal uses of the plant, there has been no report of the anxiolytic and sedative effects of the leave extract. This study therefore aims to determine these activities as a validation for its use in neurodegenerative disorders.

Materials and Methods

Plant material and preparation of extracts

Fresh whole plant of *H. glabra* Schumach&Thonn (Hydrophyllaceae) was purchased from a local herbs merchant from Mushin market in Lagos state. The plant was washed with tap water to remove all remnants of soil and dirt particles and then dried at room temperature in shade and away from direct sunlight. The plant was authenticated by MrOdewor, in the Herbarium of the Department of Botany, Faculty of Science of the University of Lagos, Nigeria, where a voucher specimen was deposited and assigned number LUH 4203. The dried plant materials were coarsely powered and stored in airtight bottles, until needed. A 125g weight of powdered leaves of the plant was extracted exhaustively, by cold maceration in 840ml of methanol for 72hours. The resulting extract was filtered through a Whatman filter paper, concentrated on a rotary evaporator at less than 40°C and was freeze-dried and used for further studies. The extract was suspended in distilled water, using 0.1% Tween 40, as suspending agent and administered to the mice at the appropriate dose levels by oral route of administration. Each suspension was freshly prepared before use.

Experimental Animals

Seventy-Five Swiss albino mice of either sex weighing between 18 – 24g were obtained from the Animal house of the Department of Pharmacology, Faculty of Basic Medical Sciences of the University of Lagos. The animals were housed under controlled room temperature (25±2°C) for a minimum of 7 days, to acclimatize. All animals were housed under standard environmental conditions: 25 ± 2°C and 12:12hr light/dark cycle and fasted overnight prior to the experiment. All the animals were given free access to feed and water *ad libitum* under strict hygienic conditions. All experimental procedures were approved by the Research, Grants and Experimental Ethics Committee of the College of Medicine, University of Lagos, Nigeria. All experiments were carried out during the light period.

Phytochemical analysis

The different extracts were subjected to phytochemical analysis for the qualitative determination of secondary metabolites such as tannins, phenolics, flavonoids, alkaloids, anthraquinones, glycosides, saponin and steroidal nucleus using the standard methods.[24,25]

Drugs and Chemicals

Diazepam tablets and injection (Roche Laboratories Ltd.), Phenobarbitone sodium injection (NATR, Sterop, Belgium) methanol and all other chemicals of highest available purity were obtained from Merck.

Animal grouping and treatment

The animals were divided into three groups of twenty-five mice each. All groups were further divided into five subgroups, each subgroup consisting of five mice. The five subgroups were used for the control, standard and three different doses of the test extract (50, 100 and 200mg/kg body weight) for each of the animal models of the experiment viz Elevated plus maze test, Hole-board test and Phenobarbitone induced sleeping time assay.

Elevated Plus-Maze

The Elevated Plus-Maze prototype was employed in the evaluation of the relative anxiety level in mice. The EPM is a wooden platform in the shape of a plus '+' sign, raised at a height of 50 cm, consisting of an open central area, which flows into two open arms and two closed arms, oppositely situated. The mice were randomly assigned to either control or treatment groups and were orally administered the corresponding doses of methanol extract of *H. glabra*, diazepam (1mg/kg b.w) and vehicle respectively, thirty minutes before the test. The mice were placed in the middle of the EPM, facing towards one of the enclosed arms and were observed by two persons, for five minutes, from both sides of the maze. Activities recorded included: (a) number of entries into the open arms, (b) time spent in the open arms of the maze, (c) number of entries into the closed arms and (d) time spent in the closed arms.[26]

Hole-Board Test

The hole-board test was employed in line with the described method by Ozturk *et al*.[27] For this test, a flat platform of 16 evenly spaced holes was used. Briefly five groups of five mice each were used. One group served as control and mice in the second, third and fourth groups received oral doses of *H. glabra* extracts (50, 100 and 200mg/kg b.w) in distilled water 30 min before the experiment. Mice in the fifth groups were orally administered the standard drug, diazepam, at a dose of 1mg/kg b.w. The mice were allowed to move freely on the platform and the number of nose dipping into the holes was counted for each animal for a five (5) minute period. To avoid disturbing environmental factors, the experimental procedure was carried out in a silent room and which allowed the mice to be isolated from visual environmental effects.

Phenobarbitone-Induced Sleeping Time Determination

Phenobarbitone-induced sleeping time was evaluated with a modification to the method described by Ghorbani*et al*.[28] Briefly, the animals were given (p.o) the vehicle and extracts and diazepam (10mg/kg b.wi.p). Thirty minutes later, phenobarbitone (30 mg/kg b.wi.p.) was injected to induce sleep. The mice were considered asleep if they lost mobility and righting reflex, when they were placed on their back. The animals however, were judged to be awake if they could return to an upright position. The time interval between injection of phenobarbitone and onset of sleep, determined by the loss of righting reflex, as well as the duration of sleep, which was determined by the time taken to regain righting reflex, were recorded.

Statistical Analysis

The results are presented as Mean ± SEM. The statistical analysis was performed using one-way ANOVA followed by the Dunnet's post hoc test for the Elevated Plus Maze and Hole Board test and one-way ANOVA followed by Tukey's post hoc tests for the Phenobarbitone sleeping time test, was adopted. For all tests, P values less than 0.05 were considered significant. All statistical analysis was performed using Graphpad 6.

Results and Discussion

Medicinal plants constitute a rich font of biomolecule with therapeutic values for the treatment of anxiety and depression.[10] The development of anxiolytic and antidepressant drugs of plant origin, takes advantage of multidisciplinary approach, including, but not limited to ethnopharmacological survey (careful investigation of folkloric application of medicinal plant), phytochemical and pharmacological studies. This study examined the effect of a *H. glabra* extract in three behavioural models of anxiety, namely, the Elevated plus maze, Hole board and Phenobarbitone induced sleeping time assays.

Phytochemical Screening

The preliminary phytochemical analysis of the leaf extracts of *H. glabra* was indicative of the presence of carbohydrates, condensed tannins, phenolic compounds, terpenoids, cardiac glycosides, saponins and alkaloids.

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Elevated plus maze (EPM)

Given the mice's primordial instinct for self-preservation in unpleasant situations by retreating into enclosed spaces, the Elevated Plus Maze paradigm presupposes that there would be less advances into the open arms from mice in the control group. [29] This observation therefore hints that the treated animals are less apprehensive or anxious in the untried environment. In the EPM model, the percentage of time in the open arms, frequency of open arm entries, and unprotected head dips are all validated measures of anxiety-like behavior.[30]Increases in these measures are indicative of reduced anxiety, whereas decreases compared to vehicle suggest increased anxiety.[31] In contrast, the frequency of closed arm entries is seen as an index of general activity.[32] From our study, there was no statistically significant difference observed in the duration of entries in the closed F (4, 18) = 0.59 p=0.68 and open arms F (4, 18) = 0.58 p=0.68 of the elevated plus maze. However, the reduction in the frequency of entry into the closed arms, was marginally significant, F (4, 18) =2.94 p=0.049, while there was no statistical significance on the effect of the treatments on the frequencies of entry into the open arms F (4, 18) = 2.132 p=0.119. The reduction in the mean number of entries into the closed arms was statistically significant for the standard anxiolytic drug Diazepam, compared to the control (p=0.023). Over all, there was a considerable reduction in the frequency of entries into both arms of the maze, for all groups given the extract, when compared to the control, but no statistical significance was observed of the groups (Figure 1). Although all treatment groups did not significantly alter the times spent in both arms, they did however, marginally increase duration in the open arms and decrease time spent in the closed arms when compared with the control, within the five minute testing time (Figure 2). Summarily, the mice treated with methanol extract of *H. glabra* entered the open arms with greater frequency and spent more time in the open, compared to control mice.

The percentage or ratio of open to total arm entries as well as time spent in the open arm seem to reflect anxiety better than the absolute number of open arm entries, which seems more reflective of general motor activity.[32] Furthermore, these percentages are said to be more sensitive to drug effect than the number of entries.[25] This is also displayed in the result (Figure 3), by the observed increase in the percentage time spent versus number of entries in the open arms (9% vs 3%) compared to the control and at all doses, including the standard anxiolytic drug Diazepam.

Effect of extracts on exploratory behavioural potential

Hole Board test

Head-dipping represents a behavioral pattern largely used to estimate anxiety level in rodents tested in hole-board (HB) apparatus, based on the hypothesis that the behavior of animals, exposed to a novel situation, originates from antagonism between an exploratory and an escape propensity.[33] Thus, according to this hypothesis, a high level of anxiety results in decreased head-dipping behavior, on the contrary, a low level one provokes an increase. Different authors however have contradictory results and opinions on this, ranging from increase,[34,35] decrease[25] or no variation[36] in frequency of head dipping following the administration of anxiolytic drugs.[37]The inverse relationship between head-dipping and anxiety has been shown to be true only in a certain range of anxiety level; with more aversive situations and consequent high anxiety level of the animals, the holes may represent a possible way to escape from the aversive environment instead of an explorable object. In this case the relation between anxiety state and head-dipping activity is directly and not inversely proportional.[38]

The one-way repeated measure analysis of the hole board procedure, did not result in a statistically significant reduction in the number of head dips in the holes within the five minute test duration F (2.07, 8.26) = 0.78 p=0.49, when compared to the vehicle. There was however, a decrease in the mean number of head-dips for all treated groups, compared to the vehicle (Figure 4), with the 50mg/kg treatment dose of the extract giving the highest mean value (*M*=13.40, SEM=1.81).

The result of this study supports the opinion above, as the frequency of head-dipping was seen to reduce in the standard anxiolytic drug diazepam, and higher doses of the extract (Figure 4), in contrast to the vehicle (9 vs 14 head-dips). This hypothesizes that the dosed mice are not fearful of the new environment and so are making no desperate effort to find an escape route.

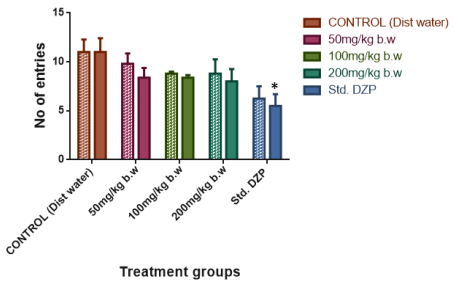

Fig.1. **Open and Closed Arms Enteries :** Number of entries (±SD, n =5 per group) into the open (patterned) and closed (plain) arms on the elevated plus maze following oral administration of *Hydrolea glabra* (50, 100, and 200mg/kg).*p<0.05, indicates significant differences from vehicle.

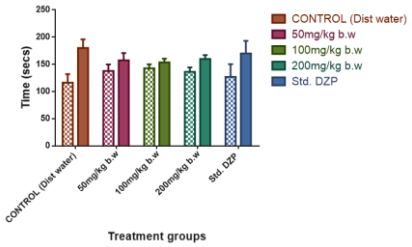

Fig. 2. **Open and closed arms duration :** Time (±SD, n = 5 per group) spent in the open (patterned) and closed (plain) arms on the elevated plus maze following oral administration of Hydrolea glabra (50, 100 and 200mg/kg).*p<0.05, indicates significant differences from vehicle.

Effect on phenobarbitone sodium-induced sleeping time

The treatment significantly altered the onset of sleep F (1.47, 7.36) =13.69 p=0.0046. The standard drug, Diazepam, showed a significant reduction in the onset of sleep, compared to all other groups, p<0.05. The duration of sleep was also significantly potentiated F (2.2, 11.0) = 10.76 p=0.0022 with a statistical significance achieved only at a dose of 200mg/kg for the extract. The test extracts, at all doses, considerably reduced the onset and increased the duration of sleep, in a dose dependent manner, with respect to the control (Figures 5 and 6).

The plant extracts at all doses, reduced the latency of sleep compared to the control (Figure 5), but much less than Diazepam. However, the potentiation of sleep was observed to a greater degree by the extracts than diazepam, when compared to the vehicle (Figure 6). This shows a CNS depressant effect exhibited by the plant, which may be sedative in nature. Benzodiazepine agonists, such as Diazepam, enhance the affinity of GABA for its receptor and hence, prolong pentobarbital-induced sleep duration.[39] The sleep-promoting neurons located in the anterior hypothalamus release gamma-aminobutyric acid (GABA) to suppress activity of wake-inducing areas of the brain.[40] Similarly, some medicinal plants interact with GABAergic system to induce their hypnotic effect.[41,27] Many neuroactive secondary metabolites (flavonoids and steroids) have been found to be ligands for the GABA receptors in the CNS; which led to the assumption that they may act as benzodiazepine-like molecules – which also act through GABA receptor.[42] Thus the putative metabolites in *H. glabra*, could likely be responsible for these CNS – depressant and anxiolytic-like activities of the plant.

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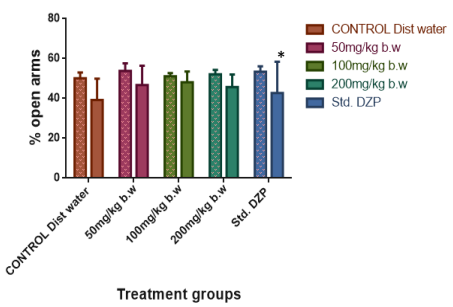

Fig. 3 Percentage entries and time (±SEM, n =5 per group) spent in the open arms on the elevated plus maze following oral administration of *Hydrolea glabra* (50, 100 and 200mg/kg).*p<0.05, indicates significant differences from vehicle.

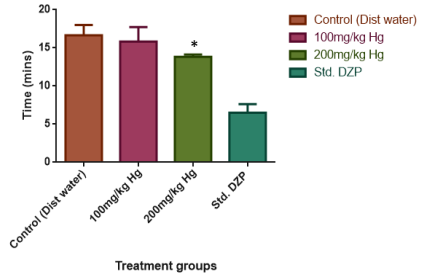

Fig. 5. **Onset of sleep :** Time taken (in minutes) (±SEM, n = 5 per group) for the mice to lose righting reflex and sleep, following the administration of Phenobarbitone sodium (i.p.), 30minutes after the oral administration of *Hydrolea glabra* (100 and 200mg/kg). *p<0.05, indicates significant differences from vehicle.

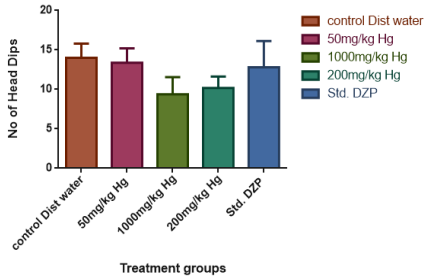

Fig. 4. The number (±SEM, n = 5) of head dips into the holes in the hole board, following oral administration of *Hydrolea glabra* (50, 100 and 200mg/kg).*p<0.05, indicates significant differences from vehicle.

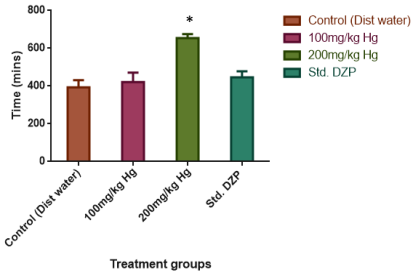

Fig. 6. **Duration of sleep :** Time taken (in minutes) (±SEM, n = 5 per group) to regain righting reflexes following the administration of Phenobarbitone sodium (i.p.), 30 minutes after the oral administration of *Hydrolea glabra* (100 and 200mg/kg).*p<0.05, indicates significant differences from vehicle

Conclusion

Medicinal plants provide ample opportunities for the development of anti-anxiety drugs, as shown by *H.glabra*, lowering anxiety-like behaviour in mice appreciably in all three behavioural tests. These results in part, show the pharmacological basis for the folkloric claims of the uses of the plant.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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