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SEDATIVE AND CENTRAL NERVOUS SYSTEM DEPRESSANT EFFECT OF *NYMPHAEA LOTUS* LINN *(*NYMPHAEACEAE*) RHIZOME EXTRACT*

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ABSTRACT

Nymphaea lotus also known as water lily, is a medicinal plant used for management of dyspepsia, enteritis, diarrhoea, hemorrhoids, fevers, insomnia, anxiety and other related disorders. The effect of methanol rhizome extract of water lily on sleep onset and duration as well as its CNS depressant activity was investigated. The sedative effect of the rhizome was assessed using diazepam- and ketamine-induced sleep time in mice and was validated using the beam-walk assay. CNS depressant effect was evaluated using Hole-Board Test (HBT), Open-Field Maze (OFM), Elevated-Plus Maze (EPM) and Light/Dark Box Maze (LDBM). In the diazepam-induced andketamine-inducedsleeping test, the extract significantly ($p< 0.05$) increased the mean duration of sleep but did not significantly alter sleep onset. In the beam walk assay, the extract significantly ($p \leq 0.05$) increased the number of foot slips and the time taken to cross the beam, decreased the number of head dips in the HBT, decreased the number of squares crossed and decreased the total number of central squares in OFM, increased time spent in the closed arm in EPM and increased the time spent in the dark compartment in LDBM. The results of this research suggest that Water lily possess sedative and CNS depressant effect.

KEYWORDS: ***Nymphaea lotus*; Sedation; CNS depressant; Sleep.**

INTRODUCTION

Sleep disorders significantly contribute to health burden in the world [1,2]. Various sleep related problems have been implicated in poor quality of life, motor vehicle accidents, mental health problems and cardiovascular disease [3]. Increased mortality, absenteeism and errors in work place, decrease in production and deterioration of personal and professional relationships have all been associated with sleep deprivation [4].

The direct and indirect costs of sleep problems are enormous. Several studies have shown the enormous challenges of sleep problems [5-7]. Sleep walking was associated with violence and the experience was psychologically disturbing [5] and sleep deficit was identified as a significant cause of road traffic accident [8].

SeveralCentral Nervous System (CNS) depressants have sedative/hypnotic effects. These drugs can slow brain activity, making them useful for treating anxiety, panic, acute stress reactions, and sleep disorders [9]. However, the use of these conventional sedative-hypnotics such as benzodiazepines, barbiturates, Z-drugs is associated with several unwanted effects including dependency/withdrawal, cognitive impairment, falls, fractures, parasomnias, and driving impairment and motor vehicle accidents [10]. Hence the search for better sources of sedatives.

Medicinal plants have always been the first treatment option for most indigenous people and

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Nymphaea lotus is one of such plants. *N. lotus* is a floating leafed macrophyte and water lily, native to Africa and specific areas in Europe [11]. The leaves, petioles, roots and seeds are all used in medicine in Nigeria [12]. The ethanolic extracts of the plant have been shown to have antimicrobial [13] and antibacterial [14] activities. The rhizome extract was reported to possess antidiarrhoeal activity [15]. It is used in the traditional medicine system as aphrodisiac, astringent, sedation, pain relieve and inflammatory diseases [16]. *Nymphaea lotus* produces a calming effect on the nervous system and is used for treatment of insomnia, anxiety and other related disorders [17]. There has been no documentation of the safety and efficacy of the leaf of *Nymphaea lotus* Linn in the treatment of insomnia anxiety, and this study was undertaken to fill this gap.

MATERIALS AND METHODS

Chemicals

Methanol, Distilled water, Chloroform (Sigma Chemical Co. USA), Concentrated Hydrochloric acid (BDH Ltd Poole, English), Drangendorff Reagent (BDH Ltd Poole, English), Ferric chloride (BDH Ltd Poole, English), Picric acid reagent (BDH Ltd Poole, England), Sulphuric acid, (BDH Ltd Poole, English), Chips of magnesium metal (BDH Ltd Poole, England), Ketamine Injection, Diazepam injection (Roche Ltd, Switzerland), Glacial acetic acid (Searle Essex, England).

Plant collection and identification

The whole plant, was collected in a pond at old Kano road, Dogarawa, Sabon Gari L.G.A in Zaria. The plant was identified and authenticated in the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University. A voucher number of 894 was allocated to the plant following comparison with an existing specimen.

Preparation of extract

The rhizome was separated, washed, shade-dried and reduced to coarse powder using mortar and pestle. One kilogram (1 kg) of the powdered rhizome was extracted with aqueous methanol (70%^{v}/v) using cold maceration for 72 hours with occasional shaking. After filtration, the extract was evaporated to dryness on a water bath at 40 ^{o}C. The percentage yield was 2.29 % $^{w}/_{w}$. The extract (AMNL) was stored in a desiccator until needed for use. A fresh solution of the extract was prepared for each study.

Animals

Male Swiss Albino mice (18-25 g) were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The animals were maintained under normal laboratory conditions and were placed on standard laboratory diet with water *ad libitum*. Ethical approval for the use of laboratory animals was obtained from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC), with the approval number of ABUCAUC/2019/30.

Phytochemical screening

The extract was subjected to preliminary phytochemical screening for the presence of secondary metabolites. using standard procedures [18].

Acute toxicity study

Lorke's method was used to evaluate the acute toxic effect of the extract [19].

Diazepam-induced sleeping time test in mice

The sleep inducing or potentiating effect of the AMNL was studied using diazepam induced sleeping time test [20]. 24 mice were divided into 4 groups of six (6) mice each. Mice in group I received normal saline (10 ml/kg). Group II to IV mice received graded doses (50, 100 and 200 mg/kg) of the extract. All mice were administered diazepam 20 mg/kg after 30minutes pretreatment period. Mice were subsequently placed in cages individually and observed for onset and duration of sleep. The interval between the administration of diazepam until the loss of righting reflex was recorded as the onset of sleep while the interval between the loss and recovery of the righting reflex was regarded as the duration of sleep [21].

Ketamine-induced sleeping time test

The method described by Mimura *et al.* [22] was adopted. Thirty (30) mice were divided into 5 groups of six (6) mice each. All drug treatments were carried out intraperitoneally. Mice in group I received normal saline 10ml/kg, mice in groups II, III and IV received 50, 100and 200 mg/kg of the extract respectively. Mice in group V received 2 mg/kg of diazepam. After 30minutes pretreatment period, all mice were injected with ketamine (100 mg/kg, *i.p*). The animals were subsequently placed individually in cages and the onset and duration of sleep in each mouse was noted and recorded. The interval between the administration of ketamine until the loss of righting reflex was recorded as onset of sleep while the interval from loss

to regaining of the righting reflex was considered the duration of sleep.

Beam walking assay for motor coordination

The mouse beam walking assay is a motor coordination test used to predict clinical sedation. The method described by Stanley *et al.* [23] was adopted for this study. Mice were trained to walk from a start platform along a ruler (80 cm long and 3 cm wide) elevated 30 cm above the bench by metal support to a goal box. Three trials were performed for each mouse and it was designed such that the mice were aware that a goal box was to be reached. Mice that successfully walked from one end of the ruler to the goal post were used for the study. Mice were grouped into 5 groups of six (6) mice each. Drug treatment was similar to that in the diazepam-induced sleep test. Thirty (30) minutes post-treatment, each mouse was placed at one end of the beam and allowed to walk to the goal box. Mice that fell were returned to the position they fell from, with a maximum time of 60s allowed on the beam. The number of foot slips (one or both hind limbs slipping from the beam) was recorded with the aid of a tally counter. The number of foot slips and time taken to reach the goal box was recorded.

Hole board exploratory activity in mice

Method adopted for this study was as described previously [24, 25]. The apparatus used was a white painted wooden board (60 cm x 30 cm) with 16 evenly spaced holes (1 cm diameter x 2 cm depth). Six groups of five (5) mice each were used for the study. Mice in group I received normal saline (10 ml/kg, *i.p*), mice in groups II, III and IV received 50,100 and 200 mg/kg of the extract respectively. Mice in groups V and VI received 0.5 mg/kg and 0.05 mg/kg of diazepam respectively. All drug treatments were via intraperitoneal route. The number of head dips in the hole at the level of the eyes was counted using a tally counter during a 5 minutes observation period for each mouse.

Open field test

Method described by Hall was adopted [26]. Awooden box (7× 72 × 36 cm) with one of the side walls made of transparent Perspex glass and illuminated with a 60W bulb suspended 100 cm from above was used. Mice were randomly divided into five (5) groups of six mice each. Grouping and treatment were similar to those in beam walk assay but with 0.5 mg/kg dose of diazepam in group V. The mice were placed individually at the corner of the arena and the number of central squares and total number of squares crossed was observed for 5 min period. Each mouse's activity was recorded using a video camera hung above the arena. After each individual test session, the floor was thoroughly cleaned with 70% ethanol.

Light and dark box maze

The method for light and dark exploration described by Crawley *et al.* [27], and modified by Adnaik *et al.* [28] was used. A box of size 46 x 27 x 30 cm consisting of two compartments (1/3rd black compartment and 2/3rd white compartment illuminated with 40W light source and connected through a small open doorway) was used. Drug treatment was similar to that in the open field test. After 30 minutes pre-treatment period, each mouse was placed individually in the center of the light compartment and observed for 5 minutes for the crossings between the two compartments and time spent in the light and dark compartments. The compartments were cleaned after each experimental episode with 70% alcohol to minimize odor cues. All drug treatments were via intraperitoneal route.

Elevated plus maze (EPM) test

The method was adapted from Adnaik *et al*. [28]. The EPM apparatus consisted of four arms - two open arms (35 cm × 5 cm) and two enclosed arms with high walls (30 cm × 5 cm × 15 cm). The arms were positioned at 90° relative to the adjacent arms and linked with a central area (5 cm × 5 cm) to form a plus sign. The apparatus was elevated to a height of 60 m above the floor. The maze floor and the walls of the enclosed arms were made of wood and painted black. The room was illuminated with a 60-W lamp at the central platform. Five (5) groups of six (6) mice each were used for the study and drug treatment was similar to that in the hole board exploratory test. Thirty (30) minutes after drug administration, each mouse was placed at the center of the elevated plus maze with its head facing the open arm. Every precaution was taken to ensure that no external stimuli, other than the height of the plus maze invoke maze anxiety. The behavior of each mouse was recorded using a video camera. The frequency and duration of entries into the open and closed arms were observed for 5 min. An entry was counted when all four paws of the mouse entered an open or closed arm. The arena was thoroughly cleaned after each experimental episode with ethyl alcohol to remove olfactory clue.

Statistical analysis

Values are expressed as percentages and Mean ± S.E.M. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Bonferroni post-hoc test for multiple comparisons in

IBM-SPSS version 23. P values ≤ 0.05 were considered statistically significant.

RESULTS

Phytochemical screening

The preliminary phytochemical screening of the extract revealed the presence of alkaloids, cardiac glycosides, saponin, tannins, flavonoids, terpenoids and carbohydrates. (Table 1).

Acute toxicity profile

The intraperitoneal LD_{50} of the extract was 774.59 mg/kg while the oral LD_{50} was greater than 5000 mg/kg.

Diazepam-induced sleep time

The extract at 100 and 200 mg/kg significantly ($p \leq 0.05$) increased the mean duration of sleep. The extract did not significantly alter the onset of sleep at doses of 50 and 100mg/kg when compared to normal saline. However, sleep onset was significantly reduced at 200mg/kg ($p < 0.05$) as shown in Fig.1.

Ketamine-induced sleep time

In ketamine induced-sleep test, the extract did not affect the onset of sleep (Figure 2). Sleep duration in extract treated groups was however, significantly increased at doses of 100 and 200mg/kg.

Beam-walk assay

The extract at all doses significantly ($p<0.05$) increased the number of foot slips compared to normal saline treated mice. The time taken to cross the beam was also significantly longer in extract-treated mice than in the normal saline group. The increase in foot slips and duration of beam walking observed in the extract treated mice were comparable to those observed in the diazepam-treated mice (Figures 3a and 3b)

Hole-board exploratory test

The extract significantly decreased ($p \leq 0.05$) the number of head dips in a dose-dependent fashion when compared to normal saline. Diazepam on the other hand, significantly increased the number of head dips when compared to normal saline ($p< 0.05$). (Figure 4).

Open-field maze

The extract at 100 and 200 mg/kg significantly ($p < 0.05$) decreased the number of squares crossed and the total number of central squares crossed while diazepam (2 mg/kg) significantly increased the number of squares and total number of centre-square crossing compared to normal saline ($p < 0.05$) (Table 2).

Light/dark exploratory maze

The extract (100 and 200 mg/kg) significantly ($p \leq 0.05$) and dose dependently increased the time spent in the dark compartment, decreased the time spent in the light compartment and decreased overall locomotor activity of the mice (Table 3).

Elevated plus maze

The extract significantly decreased the time spent in the open arm and significantly increased the amount of time spent in the closed arms when compared to normal saline. The extract did not affect the number of open -arm and close-arm entries (Table 4).

DISCUSSION

The diazepam-induced sleep test is one of the major tools for screening potential sedative and hypnotic compounds. Agents that decrease onset of sleep have the potentials to be used in sleep initiation while agents that prolong sleep duration have a high propensity for use in the maintenance of sleep. The plant extract therefore, may be used for both initiation and maintenance of sleep. The result obtained in the ketamine-induced sleep test further affirmed to that obtained in the diazepam-induced sleep test. Although the mechanism by which this extract caused sedation was not investigated, dissociative anesthetic drugs such as ketamine prevent the binding of glutamate, the major excitatory neurotransmitter in the CNS to the N-methyl-D-aspartate receptor, resulting in suppressed activities of thalamocortical and limbic system as well as the activities of the reticular activating systems nuclei [29] leading to sedation. The beam walk assay is developed to assess fine motor coordination and balance as well as predict clinical sedation [30]. The extract significantly increased the number of foot slips and the time it took the mice to complete the task. This indicates that the extract causes motor coordination deficit which further reaffirms the possession of sedative properties.

The hole-board test has been used in several experiments [31] to evaluate changes in the emotional state of animals in terms of changes in exploratory activity, i.e., total locomotor activity, numbers and duration of rearing and head-dipping, and latency to the first head-dipping. The extract significantly and dose-dependently decreased the number of head dips in the hole-board exploratory test, a reflection of CNS depression [31]. This finding was further corroborated by the activity of the extract

Table 1: Result of Phytochemical Screening

Phytochemical constituents	Inference
Alkaloids	Present
Cardiac glycosides	Present
Saponins	Present
Tannins	Present
Steroids	Absent
Terpenoids	Present
Carbohydrates	Present
Anthraquinones	Absent

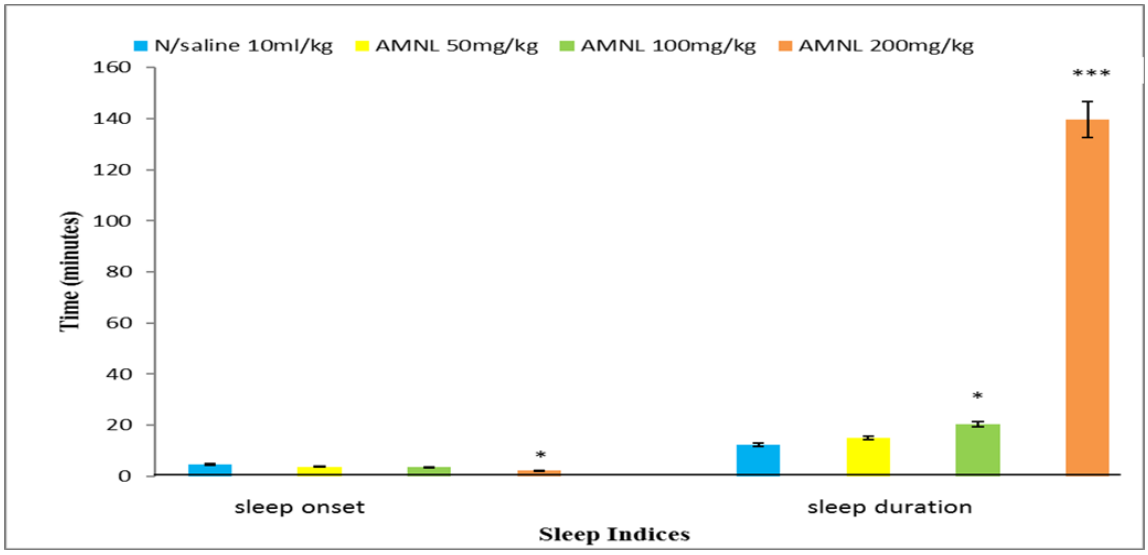

Figure 1: Effect of the extract on Diazepam-induced sleep in mice.
AMNL = Aqueous methanol rhizome extract of *Nymphaea lotus*; DZP = diazepam; N/Saline = normal saline; * $p \leq 0.05$; n=6

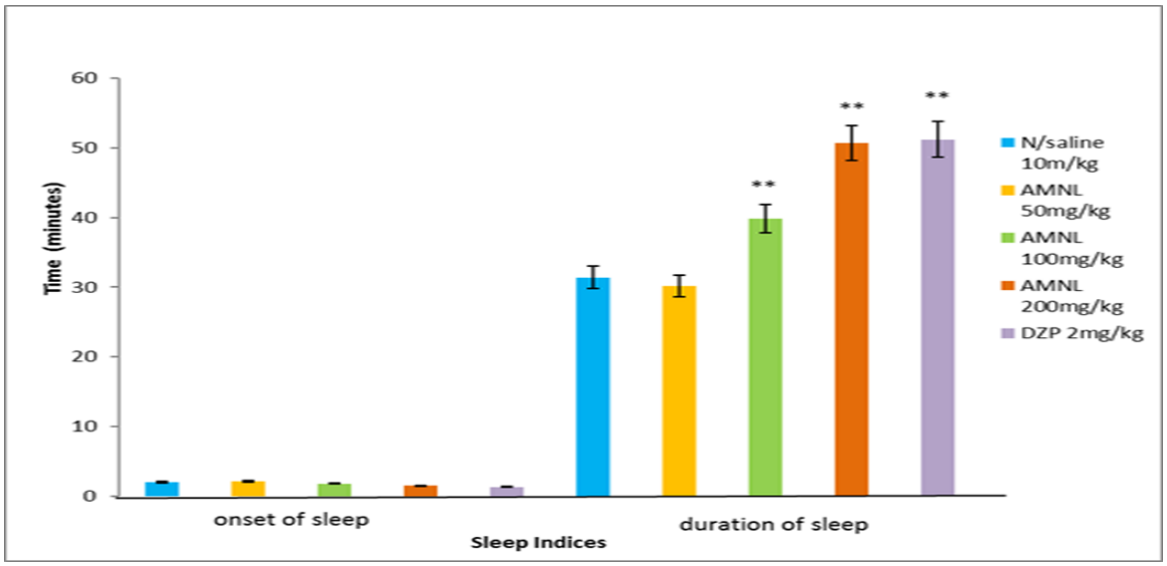

Figure 2: Effect of the extract on Ketamine-induced sleepin mice.
AMNL= Aqueous methanol rhizome extract of *Nymphaea lotus*; DZP = diazepam; N/Saline = normal saline. *$p \leq 0.05$; n=6.

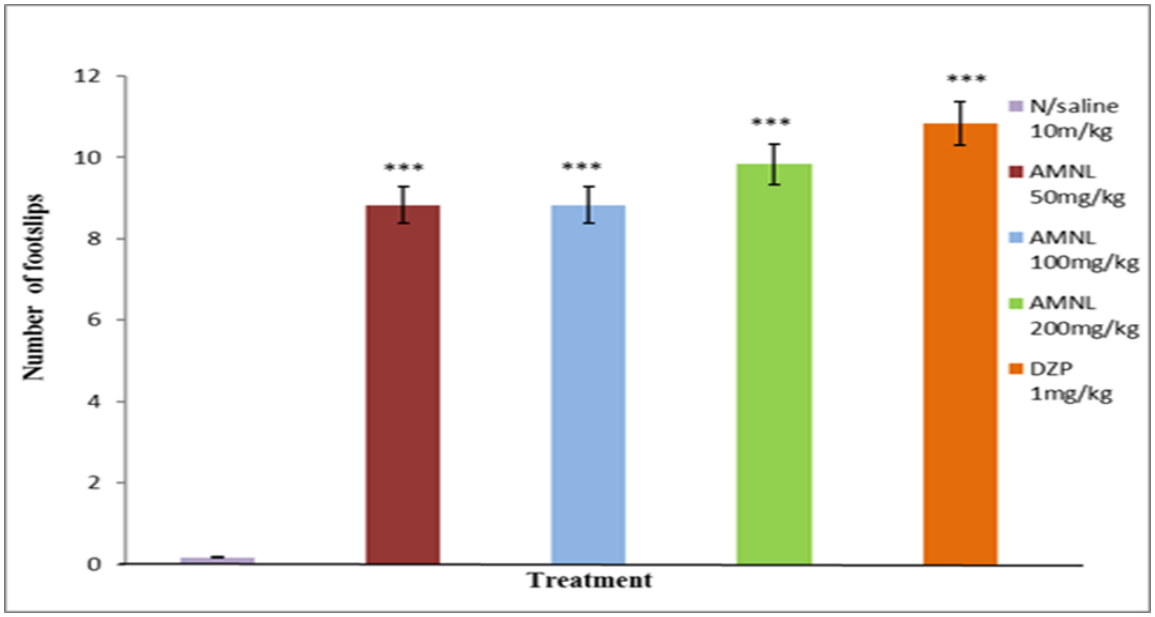

Figure 3a: Effect of the extract on motor coordination in mice using foot slips as an index of motor-coordination. *** $p<0.05$; n=6. Key: AMNL = Aqueous methanol rhizome extract of Nymphaea lotus; DZP = Diazepam; N/Saline = Normal saline.

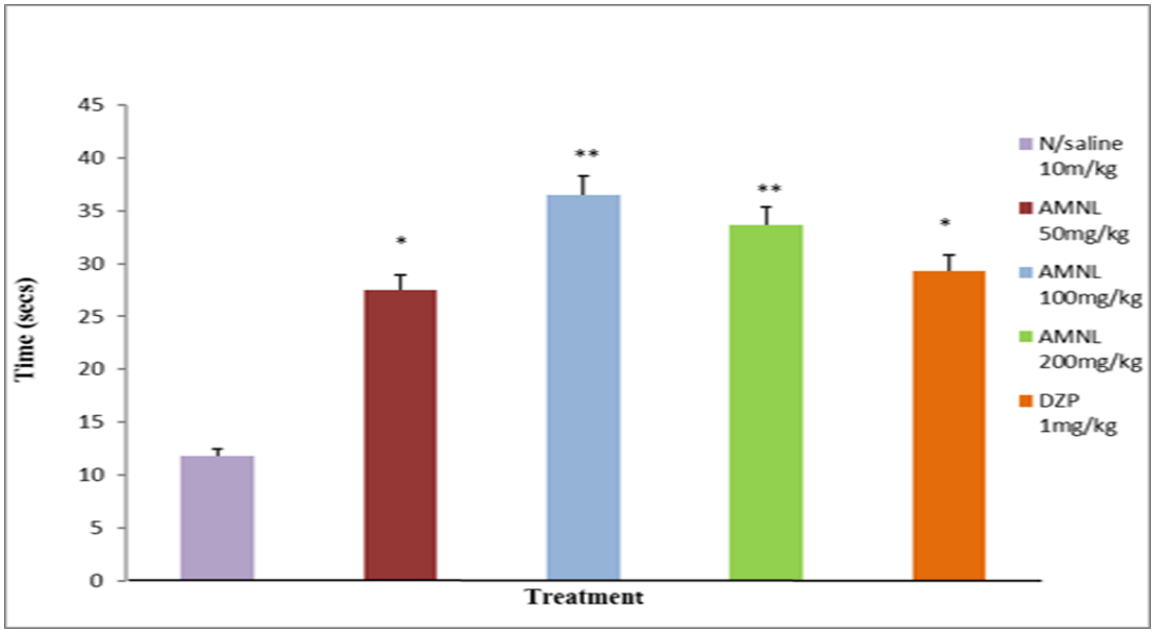

Figure 3b: Effect of the extract onmotor coordinationin mice using time taken to reach goalpost as an index of motor-coordination. *$p \leq 0.05$; n=6. AMNL: Aqueous methanol rhizome extract of Nymphaea lotus; DZP = Diazepam; N/Saline: Normal Saline.

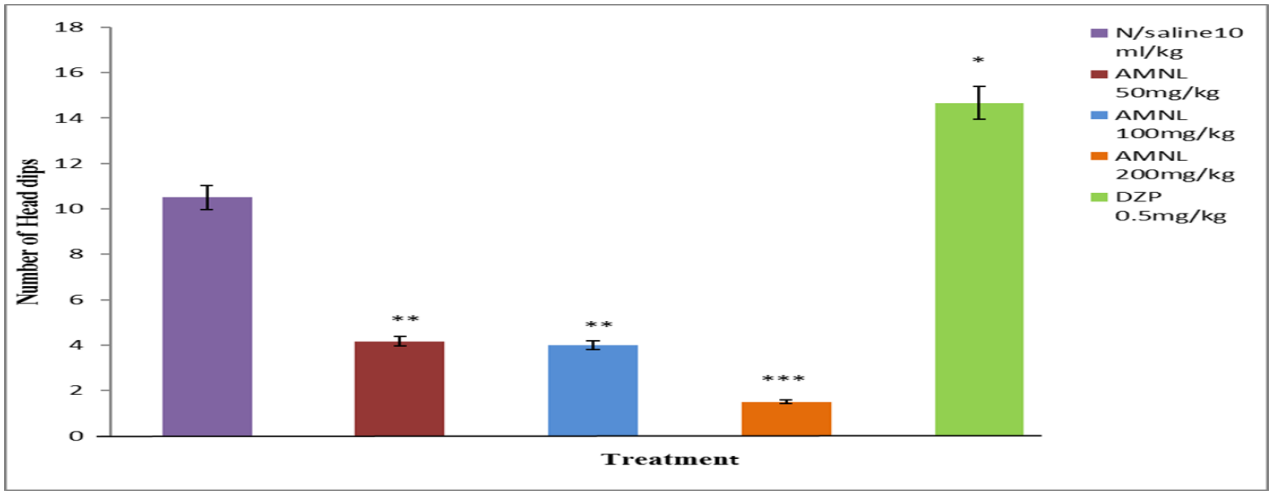

Figure 4. Effect of the extract on miceexploratoryactivityin hole board apparatus.
AMNL: Aqueous methanol rhizome extract of Nymphaea lotus; DZP = diazepam; N/Saline = normal saline. * $p \leq 0.05$ n=6

Table 2: Effect of the extract on micebehaviour in Open Field Test

Treatments	Dose (mg/kg)	Number of Centre Square Crossed	Total Number of Squares Crossed
Normal Saline	10ml/kg	1.0 ± 0.32	89.20 ± 5.60
AMNL	50	1.33 ± 0.62	54.33 ± 5.28
AMNL	100	1.17 ± 0.65	65.17 ± 2.60*
AMNL	200	0.33 ± 0.21*	44.67 ± 2.47**
DZP	0.5	2.33 ± 0.56*	102.33 ± 5.74***

AMNL: Aqueous methanol rhizome extract of Nymphaea lotus; DZP= diazepam; N/Saline: normal saline * $P \leq 0.05$, n=6

Table 3: Effect of the extracton mice behaviour in light and dark box

Treatment	Dose (mg/kg)	TLC (seconds)	TDC (seconds)	NOC
N/Saline	10ml/kg	77.67 ± 4.87	222.33 ± 4.87	4.00 ± 0.52
AMNL	50	77.83 ± 5.07	222.17 ± 5.06	4.50 ± 0.43*
AMNL	100	58.80 ± 5.51*	241.20 ± 5.51*	4.20 ± 0.37*
AMNL	200	40.00 ± 2.71*	260.00 ± 2.71*	3.83 ± 0.31*
DZP	0.5	156.33 ± 7.60*	143.67 ± 7.60*	4.83 ± 0.48**

TLC: Time spent in Light Compartment TDC: Time spent in dark compartment; NOC = Number of crossings; AMNL: Aqueous methanol rhizome extract of *Nymphaea lotus*; DZP = diazepam; N/Saline: normal saline. * $P \leq 0.05$; n=6

Table 4: Effect of the extract on Elevated Plus Maze

Treatment (mg/kg)	OAE	CAE	TTOA (s)	TTCA (s)
N/Saline	34.25±3.01	65.75±3.01	29.27±2.98	29.27±2.98
AMNL 50	38.24±4.76	61.75±4.76	26.11±4.04	73.87±4.04
AMNL 100	36.25±4.09	63.75±2.49	16.55±1.93	83.45±1.93
AMNL 200	31.01±2.49	68.99±2.49	13.67±1.98*	86.33±1.98*
DZP 0.5	58.01±2.53*	41.99±2.52*	50.44±2.25*	49.56±2.25*

Data wa OAE; number of open arm entries; CAE: number of closed arm entries; TTOA:Total time spent in the open arm; TTCA: Total time spent in the closed arm AMNL: Aqueous methanol rhizome extract of *Nymphaea lotus*; DZP: diazepam; N/Saline: normal saline. * $P \leq 0.05$, n=6.

in the OFT, EPM and LDBM. In the OFT, the extract at higher doses significantly decreased the total number of squares crossed while the number of central squares crossed was only mildly affected. Thigmotaxis (the tendency of a rodent to remain close to the wall) in the Open Field Maze is used to evaluate anxiolytic, anxiogenic/CNS depressant activities of pharmacological and even non-pharmacological treatments [32]. The degree of thigmotaxis has been validated as a measure of anxiogenic behavior in mice [33]. Thigmotaxis increases as anxiety levels rise. The extract therefore exhibited CNS depressant activity evident in the decrease in overall movement of the mice, though couldn't be said to have exhibited anxiolytic effect, yet lacked anxiogenic activity since it affected more of locomotory than behavioural activity of the mice.

In the light/dark box exploratory test, the extract significantly and dose-dependently increased the time spent in the dark compartment. The light and dark exploration test which was devised by Crawley is based on innate aversion of rodents to brightly illuminated areas and on the exploratory behaviour of rodents [34]. An increase in time spent in the open compartment is an indication of an anxiolytic effect and vice versa [35, 36]. The elevated plus maze test is one of the most widely used tests for measuring anxiety-like behavior. The test is based on the natural aversion of mice for open and elevated areas, as well as on their natural spontaneous exploratory behavior in novel environments [37]. The elevated plus maze test has a strong predictive validity for screening anxiolytic drugs [38-40]. Anxiolytic drugs specifically increase, and anxiogenic drugs specifically decrease, the number of entries into the open arms and the time spent there [39]. The extract did not significantly alter the number of open-arm and close-arm entries but at the highest dose, it was able to increase the duration of stay in the close-arm, an indication of mild anxiogenic activity.

The sedative effect of the extract may be linked to the presence of alkaloid [41], saponin [42], terpenoids [43] and cardiac glycosides [44].

CONCLUSION

Rhizome of N. *lotus* has sedative and CNS depressant properties with mild anxiogenic effect and may be explored in the management of disease conditions where CNS depression is of advantage.

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