Research Article

iMedPub Journals
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Annals of Clinical and Laboratory Research
ISSN 2386-5180

2018
Vol.6 No.1:223

DOI: 10.21767/2386-5180.1000223

Effect of Ethanol Extract Leaves *of Nymphaea Lotus* (Water Lily) on Fear and Anxiety Behaviour in Mice

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Received: January 23, 2018; **Accepted:** February 24, 2018; **Published:** March 05, 2018

Citation: Aduema W, Munachi I, Akunneh-Wariso C (2018) Effect of Ethanol Extract Leaves of *Nymphaea Lotus* (Water Lily) on Fear and Anxiety Behaviour in Mice. Ann Clin Lab Res Vol.6: No.1: 223.

Abstract

The aim of this study was to investigate the effect of the leave extract of *Nymphaea lotus* on neuro-behaviour using mice as experimental model on fear/anxiety. Twenty seven mice weighing between 20-29 g were randomly selected into A, B and C groups for the study. Prior to the assessment of these neurobehavioral parameters, the phytochemical screening of the plant and the LD50 were analyzed. The elevated plus maze was used to access anxiety/fear related behaviours. Our findings showed that *N. lotus* extract reduces fear/anxiety related behavior, this was so because, the time spent in the open arm was increased significantly ($p<0.01$ respectively) in the mice that were treated with the leave extract, the head dips showed similar results. However, the number of fecal boles, frequency of SAP and close arm duration were decreased in the mice treated groups compared to control ($p<0.01$ and $p<0.05$ respectively. In conclusion, the leaves of *Nymphaea lotus* may be responsible for the reduced level of anxiety and fear we found in the treated mice.

Keywords: Ethanol extract; *Nymphaea lotus*; Water lily; Fear; Anxiety behaviour; Mice

Introduction

Nymphaea lotus is a perennial plant that grows up to 45 cm in height; whose leaves float in water [1]. They are easily recognized by their aquatic habit, floating leaves, flamboyant flowers, several petals, many stamens and carpels and endospermous seeds with small embryo [2] This plant is mostly found in the Northern part of Nigeria. The plant contains many pharmacological and chemical important compounds which have been useful in the field of medicine [3]. Despite the immense technological advancement in modern medicine, many people still rely on traditional medicine and healing practices for their daily health practices [4,5]. This is owned to the fact that modern medicine leave resultant side effects coupled with the high cost in acquiring synthetic drugs. Moreover, there is an increasing incidence of neurobehavioral disorders among people of all ages. The side effects of many neuro-cognition enhancing drugs have left many individuals and patients with irreversible neurological damage [6]. This debilitating situation has led to the investigation of the medicinal properties of *N. lotus*. The validation of the therapeutic efficacy of *N. lotus* will provide novel means for the management/treatment of neurobehavioural disorders such as anxiety disorders [7-9].

Materials and Methods

Animal care

Twenty six (26) Swiss white mice were used in the study. The animals weighing between 20-29 g were housed in the Department of Physiology, Gregory University, Uturu for one week and later transported to the Department of Physiology, Abia state university, were they were kept for 14 day in a clean and well ventilated compartment for the animal to acclimatize before the experiment was carried out. The animals were feed properly and allowed drinking water adlibitum

Experimental design

The Twenty-six (26) Swiss mice were divided into three groups. A, B and C of 10 mice. Animals in group A received normal rodent chow and served as the control, group B and group C which serve as the low and high dose treated animals received 200 mg and 400 mg/kg of the leave extracts for the number of days that the experiment lasted.

Ethical approval

All authors hereby declare that principle of Laboratory animal care was followed. All animals have been examined and approved by the appropriate ethics committee.

Experimental procedure

- Each mouse was picked from its tail and then placed in the centre square between the open and close arm of the apparatus.

- Between each trial the apparatus is cleaned using a cotton wool soak in 70% ethyl alcohol in order to remove olfactory cues.
- Each mouse spent five minutes after which each mouse was removed from the apparatus and returned into their home cages.

Behaviors that were scored included: open arm entries (frequency and duration), closed arm entries (frequency and duration), head dipping, frequency of Stretch attend posture, grooming frequency, rearing; urination and defecation (number of fecal bole produced).

Results

Duration in the open arm

The open arm duration signifies the time spent by the mice in the open arm of the elevated plus maze **(Figure 1)** for the control, low dose and high dose diet of *N. lotus* which are 143.55 ± 8.28; 179.44 ± 4.71 seconds and 181.55 ± 10.72 seconds respectively. The graph in **Figure 2** shows that the treated groups of mice spent a higher time in the open arm when compared to control (p<0.01).

Figure 1 Elevated plus maze.

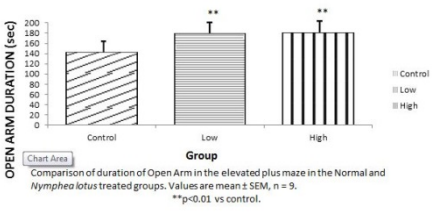

Figure 2 Comparison of duration of Open Arm in the elevated plus maze in the Normal and *Nymphea lotus* treated groups.

Closed arm duration

Figure 3 shows the close arm duration for the various groups, starting from the control, and then the treated groups (low and high dose). Their values were 147.88 ± 4.17; 127.33 ± 4.10 and 127.33 ± 2.19 seconds respectively. The close arm duration for the treated mice were significantly lower (P<0.001) compared to control. However, there was no significant difference among the treated groups.

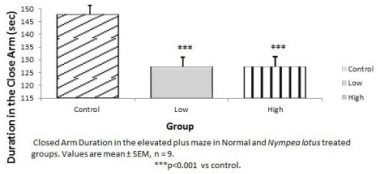

Figure 3 Closed arm duration in the elevated plus maze in normal and *Nympea lotus* treated groups.

Frequency of head dips

The frequency of head dips for the experimental animals were 9.88 ± 1.91; 20.44 ± 0.92/5 mins and 20.88 ± 2.40/5 min for mice fed control, low dose diet and high dose diet of *N. lotus*. The head dips for the mice administered the plant extract were higher when compared to the control (P<0.001) **(Figure 4).**

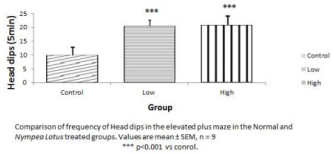

Figure 4 Comparison of frequency of Head dips in the elevated plus maze in the Normal and *Nympea Lotus* treated groups.

Frequency of stretch attend posture

The stretch attend posture values were found to be 4.00 ± 0.50; 1.88 ± 0.26; and 1.89 ± 0.20/5 mins respectively. The stretch attend posture was statistically lower in the treated mice (low and high dose) of *N. lotus* (P<0.01, P<0.001) compared to control **(Figure 5)**.

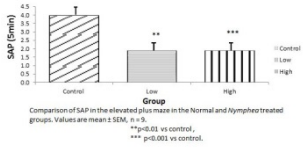

Figure 5 Comparison of SAP in the elevated plus maze in the Normal and *Nymphea lotus* treated groups.

Frequency of grooming

Grooming frequency values for the mice fed control, low dose and high dose treated were 2.55 ± 0.72; 2.44 ± 0.72 and 2.44 ± 0.41/5 mins respectively. Grooming frequency did not differ among the groups **(Figure 6).**

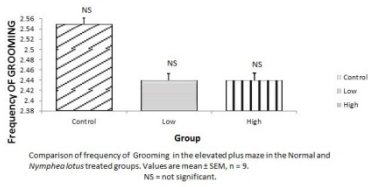

Figure 6 Comparison of frequency of grooming in the elevated plus maze in the normal and Nymphea lotus treated groups.

Defaecation frequency

Figure 7 compares the frequency of defecation among the groups. The values are: 2.88 ± 0.42; 1.77 ± 0.22 and 1.00 ± 0.23/5 mins respectively. The number of boles in the group of mice fed low dose and high dose of *N. lotus* was statistically lower (P<0.05 and P<0.01) compared to control.

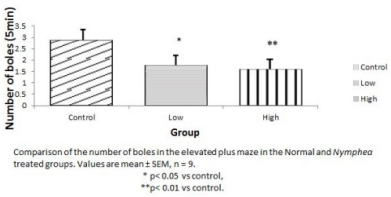

Figure 7 Comparison of the number of boles in the elevated plus maze in the normal and *Nymphea lotus* treated groups.

Discussion

The findings in the elevated plus maze shows that the treated mice turned out to be less fearful and anxious than the control because the treated mice spent more time in the open arm indicating absence of fear and anxiety [10]. The animals treated with doses of the extract also significantly spent less time in the arm that was closed, also indicating absence of anxiety/fear, the animals perhaps did not feel frightened from falling off the platform, thereby showing what the leaves of *N. lotus* can do, on the treated mice [11-14]. Findings from the elevated plus maze also showed that the animals treated with *N. lotus* extract had significantly more head dips [15]. Defecation frequency was significant less in the treated mice when compared to control, also indicating less anxiety and fear related behavior [16]. The amygdala control the level of Fear and anxiety mostly and the hypothalamus [17]. Stimulation of the amygdala for instance is associated with fear and feeling of terror in the animals [18]. Therefore, it is likely that the leaves of *Nymphaea lotus* contain a lot of chemical compounds, such as cardiac glycosides which has been reported to decrease contraction of the heart [19]. The leaves of *Nymphaea lotus* could be responsible for the anxiolytic properties found in the experimental animals my causing a decrease in the excitability of the cells of the amygdala, thereby increasing the level to which it response to stimuli, thus reducing the level of fear/anxiety in the experimental animals [20,21].

Conclusion

N. lotus reduces the level of fear/anxiety in animals. The plant also has low toxicity and reduced side effects, therefore pharmaceutical industries can use this plant in producing safe and efficient drugs for people with anxiety disorders. Based on the findings from this study, one can rightly say that *N. lotus* tends to have a significant effect on the nervous system by reducing the level of anxiety in animals. Therefore, cardiac glycoside which has been reported to decrease heart contraction and one of the constituents in *N. lotus* may be responsible for the decrease in anxiety/fear related behaviors in the experimental mice.

Acknowledgement

We acknowledged Mr. Uchenna Azunna and Mr. Nomso for their priceless support.

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