World Journal of Medical Sciences 14 (4): 135-140, 2017
ISSN 1817-3055

DOI: 10.5829/idosi.wjms.2017.135.140

Effects of Ethanol Extract of *Nymphaea lotus* Leaves (Water Lily) on Locomotor Behaviour in Swiss Mice

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Abstract: The effects of Nymphaea lotus (water lily) on locomotion in mice' was carried out with the aim of investigating whether the ethanol extract of *Nymphaea lotus* would have effect on neurobehavioral in mice, particularly locomotor behaviors. A total of twenty seven mice shared into three groups were used to carry out this study. Before the neurobehavioural parameters were assessed, the LD50 and phytochemical analysis of the plant was determined. The open field maze was used to assess locomotor relatedbehaviours. The results of the experiment showed that N. lotus extract had significant effect on locomotor behaviour by enhancing locomotion. The frequency of rearing in the open field was not significantly different in the low and high dose fed group compared to control. However, the frequency of line crosses frequency were increased in the test group ($p<0.001$) compared to control. This indicates an increase in locomotor behaviour in the test group. There was also a significant ($p<0.001$ and $P<0.01$) increase in the centre square entry and freezing duration in the open field maze for the low and high dose fed group, respectively when compared to the control. Therefore, the plant N. lotus enhances locomotion and can be used in the making of drugs and herbal mixtures for the treatment of locomotor disorders.

Key words: Nymphaea lotus · Locomotor · Open field maze · Mice

INTRODUCTION

Plant extracts, like other natural products, provide a wide range of opportunities for discovery of new drugs because of the availability of chemical diversity [1]. More than 80% of the world's population relies on traditional medicine for provision of their primary health care needs, according to the World Health Organization (WHO) 2011 Plants or herbs which are used for traditional medicine contain a wide range of substances that can be used to treat chronic and infectious diseases, including neurological disorders[2]. Many of today's synthetic drugs originated from the plant kingdom and only about two centuries ago, the major pharmacopoeias were dominated by herbal drugs. Herbal medicine went into rapid decline when basic and clinical pharmacology established themselves as leading branches of medicine. Nevertheless, herbal medicine is still of interest in many diseases, in particular, neurological disorders [3].

Nymphaea lotus (water lily) belongs to Nymphaeaceae family. It is a perennial plant that grows up to 45 cm in height; it is a herbaceous aquatic plant, whose leaves float or submerge in water [4]. This plant prefers clear, warm, still and slightly acidic water and is localized to Central and Southern Europe, Asia, the Middle East, North Africa, tropical mountains in Africa and West Africa especially in Nigeria. Many bioactive and pharmacologically important compounds have been obtained from the plant and used in medicine and pharmacy [5]. With the above in mind, the leaves of *Nymphaea lotus* were tested for antibacterial activity against some bacteria isolated from wounds, since it is being used in traditional medicine. The plant contains a number of bioactive phytochemical constituents and produces a calming and sedative effect on the nervous system, suggesting that the plant can therefore be used in treatment of disorders such as insomnia, anxiety and other related disorders [6].

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Locomotion, which isbehaviour under neural control, is the motor act that allows animals or humans to move through the environment. Locomotion is expressed in many forms and includes such acts as swimming, flying, walking, running and hopping. The motor act is essential for survival and allows individuals to find food, escape danger, mate and migrate to suitable environments [7]. Locomotor movements are different from other motor acts that the nervous system produces, like the knee reflex that is stereotypically repeated in response to sensory stimulation, or skilled movements, like playing tennis or the piano that are learned and involve a complex coordination of muscles often in non-repeated manners. It is a recurrent motor activity that involves sequential activity in limb and body muscles in a precise rhythm and pattern [7]. Although there is some adaptation and maturation of locomotion, it is an innate behavior that in vertebrates, including humans, is laid down in the nervous system before birth but often not executed before the appendages and postural activity is matured. The first precise description of locomotion came with the use of photographic techniques developed in the 1880s by E´tienne-Jules Marey and Eadweard James Muybridge that allowed viewers to capture snapshots of the different moments of locomotor movements in animals and humans [7]. These aggregated sequences of pictures showed the exact timing of the movements in detail that had not been seen before. A breakthrough in understanding the neural substrate for generating mammalian locomotion came from experiments carried out by the English neurophysiologist Thomas Graham Brown in the beginning of the twentieth century. He showed that flexor-extensor hind limb movements could be evoked in cats that had their spinal cords transected at the thoracic level and had sensory inputs removed. Graham Brown concluded that spinal cord neural networks can – when appropriately activated – organize rhythmic movements into alternating flexor and extensor activity in the absence of sensory influence. These findings clearly showed that the spinal cord itself contains a neural network that can generate rhythmic movements without sensory information. These ideas challenged the prevailing idea at that time, namely, that locomotion was a result of repeated reflex responses. Despite the clear evidence, Graham Brown's experiments were forgotten for almost 50 years until they were brought to light again by Swedish neurophysiologists in Gothenburg whose experiments demonstrated that rhythmic activity can be evoked in spinalized cats that are given L-DOPA, a precursor of noradrenalin. This neurochemical activation was sufficient to activate the dormant neural networks and produce rhythmic activity without the need for sensory inputs [7-11]. There is now overwhelming evidence from studies of many different vertebrates, including man, that the precise phasing and timing of locomotion is, for the most part, generated by circuits in the spinal cord. These networks are called central pattern generators or CPGs. Neurons in the CPG receive an input from the brain, from which they are able to produce the rhythm and pattern of activity that is conveyed to motor neurons and then to the muscles. Almost simultaneous with the rediscovery of the CPG in the cat in the 1960s, a Russian group found that electrical stimulation of a circumscribed area in the mesencephalon initiated locomotion in decerebrated cats. This region was named the mesencephalic locomotor region and has since been found in all vertebrates. Neurons in the mesencephalic locomotor region do not project directly to the spinal cord but mediate their effects through cells in the reticular formation in the lower brainstem. Neurons in the mesencephalic locomotor region itself are under the control of other brain structures that select the behavior. Although the CPG may produce a rhythm and pattern without sensory inputs, much research has shown that when actual locomotion is performed, sensory inputs from receptors in muscle and skin are active so that the locomotion can be adapted to the environment. These signals both influence the CPG activity in the spinal cord and are sent to supraspinal areas including cerebellum [7]. Therefore this study tends to find out the effect of ethanol extract of *Nymphaea lotus* on locomotor behaviour in mice.

MATERIALS AND METHODS

Acute Toxicity Test (LD_{50}): The determination of the toxicity levels were done through oral route and intra-peritoneal route. The mice were divided into 3 groups in two phases (low dose & high dose, 1 & 2 respectively) for each route of administration; Both routes has phase 1which consist of 3 groups of 3 mice each and Phase 2 consist of 3 groups of 1mouse each. Each group of mice administered orally were given a different dosage of the extract (10mg/kg, 100mg/kg, 1000mg/kg) for phase 1 (low dose) and 1600mg/kg, 2900mg/kg and 5000mg/kg for phase 2 (high dose). For the mice groups administered through intra-peritoneal route, 10mg/kg, 100mg/kg, 1000 mg/kg was given as low dose and 1000mg/kg, 1600mg/kg and 2900mg/kgfor high dose. The number of deaths inoral route for phase 1 was n=0 for all groups and for phase 2 alson=0for all groups. For intra-peritoneal route, the number of deaths (n) recorded in phase 1 was n=1 and n=1 for high dose was recorded within 24-72 hours.

Table 3.1: Oral route phase one

Group	Dose	Mortality
Group 1	10mg/kg	0/3
Group 2	100mg/kg	0/3
Group 3	1000mg/kg	0/3

Table 3.2: Oral route phase two

Group	Dose	Mortality
Group 1	1600mg/kg	0/1
Group 2	2900mg/kg	0/1
Group 3	5000mg/kg	0/1

Table 3.3: intraperitoneal route phase one

Group	Dose	Mortality
Group 1	10mg/kg	0/3
Group 2	100mg/kg	0/3
Group 3	1000mg/kg	1/3

Table 3.4: intraperitoneal route phase two

Group	Dose	Mortality
Group 1	1000mg/kg	0/1
Group 2	1600mg/kg	0/1
Group 3	2900mg/kg	1/1

Phytochemical analysis of the ethanol extracts of *Nymphaea lotus* leaves

Plant Constituents	Tests used	Occurrence
Alkaloids	Drangendorff's test	++
	Mayer's Reagent test	+
	Picric Acid test	-
Flavonoids	General test	++
Phenolics	Frothing test	++
Saponins	General test	+
Tannins	General test	+
Anthraquinones	General test	+++
Cardiac glycosides	Liberman' s test	+++
	Salkowski test	+++
Terpenes	Chloroform test	+++

Keys: + = Present in small concentrations
++ = Present in moderately high concentrations
+ + + = Present in high concentrations

Experimental Animals/ Groupings: Twenty-seven (27) Swiss mice weighing between 14 and 17g were randomly assigned into three groups A, B and C of 9 mice each. Group A was the control; groups B and C are low and high dosed respectively. Animals in group A were administered normal saline, group B animals received *ethanol extract of lotus* at a dose of 200 mg/kg and group C, received ethanol extract of *N.lotus* at 400 mg/kg. Extracts were administered via an oral cannula daily for a period of 14 days.

Experimental Design: The open field test was used and this test provided simultaneous measures of locomotion and exploration. The maze is constructed from white plywood with a 72 x 72cm floor and 36cm walls. One of the walls is made of clear plexiglas so that the mice could be visible in the apparatus. Blue lines are drawn on the floor with a marker and are visible through the plexiglas floor. The lines divide the floor into sixteen 18 x 18 cm squares. A central square (18 x 18cm) is drawn in the middle of the open field[8].The central square has sufficient space surrounding it to give meaning to the central location as being distinct from the outer locations [9].The open field maze was cleaned with 70% ethyl alcohol and permitted to dry between trials. Mice were placed in the anteroom or back into the colony room while cleaning the apparatus in bright light conditions.

Procedure:

- The Mice were carried to the test room in their home cages and tested one at a time for five minutes each.
- Mice were scooped up in a small plastic container from their home cage and placed at the centre square of the open field and allowed to explore the apparatus for five minutes while the behavior were scored.
- The behaviour scored included: line crossing, centre square entries; frequency and duration, rearing, stretch attend postures, grooming, freezing, urination, defecation.

Statistical Analysis: All results were shown as mean ± SEM. Differences between means of the two groups were compared using Student's *t* test or the Mann-Whitney *u* test, depending on whether the data were normally distributed. SPSS for Windows 11.5 software was used for statistical analysis. In all cases, significance level was set at $P<0.05$.

RESULTS

Open Field Maze

Line Crossing: Fig. 1 compares the frequency of line crosses in the three groups of mice. The number of lines crossed by the mice were, 48.33± 7.27 (control), 85.50 ± 5.72 (Low dose) and 84.50± 2.61(High dose). The frequency of line crossing in the open field maze was significantly higher ($P<0.001$) for the low and high dose treated groups compared to control. However, the frequency of line crosses for the low dose treated group was not significantly different compared to that of the high dose treated group.

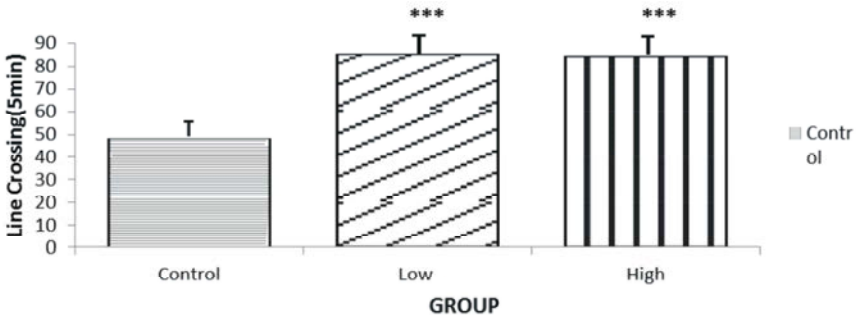

Fig. 1: Comparison of frequency of Line Crossing in the open field maze in control and Nymphea lotus-treated groups. Values are mean ± SEM, n = 9. *** p< 0.001 vs control

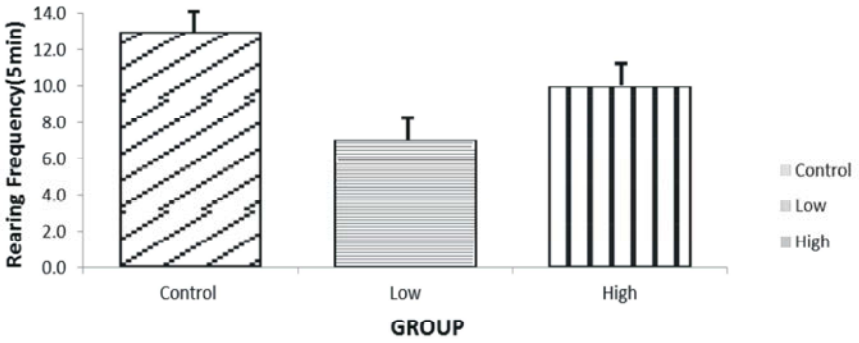

Fig. 2: Comparison of Rearing Frequency in the open field maze in control and Nymphea lotus-treated grous. Valus are mean ± SEM, n = 9

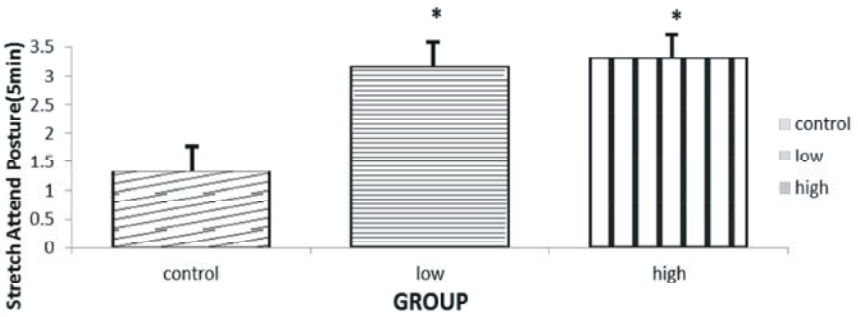

Fig. 3: Comparison ofStretch Attented Posture in the open field maze in control and Nymphea lotus-treated gtoups. Values are mean ± SEM, n = 9.. * p< 0.05 vs control

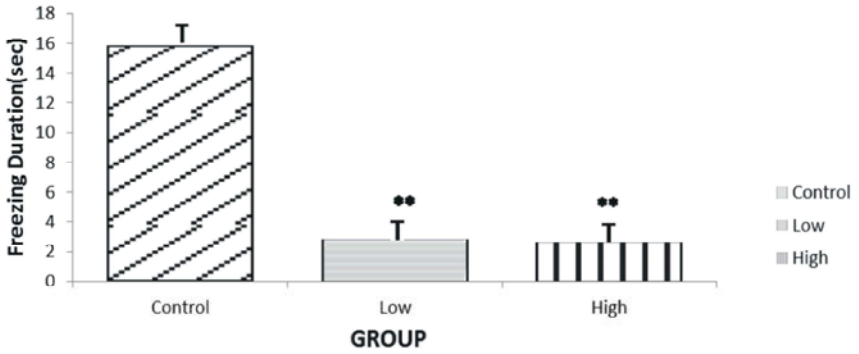

Fig. 4: Comparison of Freezing Duration in the open field maze in control and Nymphea lotus-treated groups. Values are mean ± SEM, n = 9. **P < 0.01 vs control

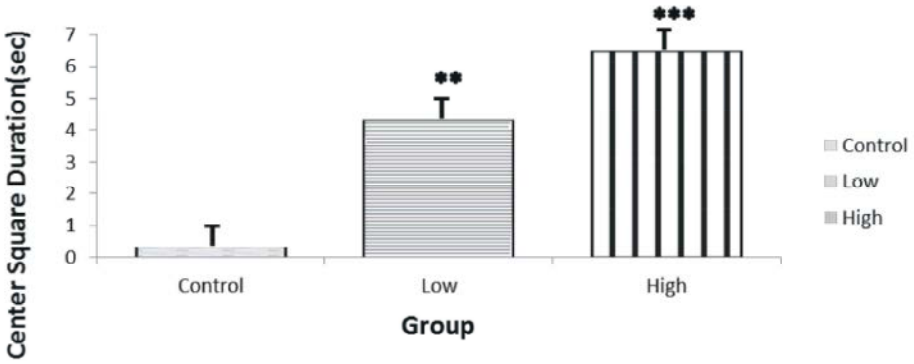

Fig. 5: Comparison of Center Square Duration in the open field maze in control and Nymphea lotus-treated groups. Values are mean ± SEM, n = 9. ** p< 0.01 vs control, ***p<0.001 vs control

Rearing Frequency: The frequency of rearing in the open field for control mice were, 13.00 ± 9.81 (control), 7.00± 2.30 (Low dose) and 10.00 ±2.59/5mins (High dose) respectively. The graph in Fig. 2 shows that the frequency of rearing among the groups, when compared was significantly not different.

Stretchattend Posture (SAP): Fig. 3. Compares the frequencies of the stretch attend posture (SAP) which is a measure of anxiety and locomotion in the three experimental groups. The values are: 1.33± 0.71(control), 3.17±0.17/5mins (Low dose) and 3.33± 0.33(High dose). The frequency of stretch attend posture (SAP) in the open field maze for the high dose group and low dose group were significantly higher (P<0.05) compared to control.

Freezing Duration: The freezing duration shown in Fig.4 were, 15.83± 5.53(control), 2.83±0.87/seconds (Low dose) and 2.67± 0.76/seconds (High dose). The freezing duration in the group of mice treated with *Nymphea lotus* for the low and high dose were significantly lower (P<0.01) compared to control.

Centre Square Entry: Fig. 5 compares the centre square entry between the three experimental groups of mice respectively. The centre square entry shown in Fig. 5 were, 0.33± 0.21(control), 4.33±0.49 (Low dose) /5mins and 6.50±0.42/5mins (High dose). The frequency of centre square entry in the open field maze showed that the low and high dose treated groups were significantly higher (P<0.01 & P<0.001, respectively) compared to control. However, there was no significant difference between the low and high dose treated groups.

DISCUSSION

The presence of various bioactive phytochemical constituents found in the Nymphaea lotus plant makes the plant a good material for making drugs for the treatment of various disorders [6]. Also, the result of the acute toxicity test shows that the Nymphaea lotus plant extract is safe even at high doses.

Based on the findings in the open field maze test for locomotion and exploration [10], whereby the mice treated with N. lotus extract appeared to be more mobile and faster than the mice that were not treated with the plant extract, this goes to show that the N. lotus plant extract significantly enhanced the locomotion of the N. lotus treated mice because in the test groups, line crossing was increased and freezing duration reduced. Centre square entry for the mice treated with the N. lotus extract in the open field maze was also increased. Since Locomotion is controlled by the central pattern generators in the spinal cord [11]. It is conceivable therefore, that the ethanol extract of the Nymphea lotus leave may have an stimulatory influence or effect on the motor areas of the nervous system such as the motor cortex, cerebellum or spinal cord which in turn caused the increased locomotor activities.

CONCLUSIONS

Based on the findings from this study, we may rightly suggest that N. lotus tends to have a significant effect on the nervous system by increasing locomotor behavior. Therefore, it may be useful in the treatment of locomotor disorders if our results are extrapolated to humans. However, we recommend that further studies be done

using this plant, such as finding out other ways by which Nymphaea lotus can be useful to the Nigerian society and the world at large.

ACKNOWLEDGEMENT

We acknowledged Mr. and Mrs. B.A.Aduema and Associate Prof.A.A.Nwankwo for their priceless support.

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