Pharmacological Evaluation of Nymphaea Lotus Seed Extract on Behavior and Motor Coordination in Animal Models

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ABSTRACT

This study aimed to investigate the potential of Nymphaea lotus seeds in enhancing behaviour and motor coordination. The plant material was collected, identified, and authenticated, and an extract was prepared through a standardized extraction process. Preliminary phytochemical studies revealed the presence of various active compounds. The extract was then subjected to in-vivo and in-vitro pharmacological screening using a Rota-Rod apparatus, Actophotometer, and various biochemical estimations. The locomotor activity of experimental animals was also assessed. Results showed that the extract significantly improved motor coordination and reduced oxidative stress and acetylcholinesterase activity in the brain. These findings suggest that Nymphaea lotus seeds may have potential as a natural supplement for enhancing behaviour and motor coordination. Further studies are needed to explore the underlying mechanisms of action and the long-term effects of the extract.

Keywords: Rota-Rod, Actophotometer, Locomotor activity, Biochemical estimations, Three-point bioassay

1. INTRODUCTION

The pharmacology of the brain in behavior and motor coordination is a critical area of research that has significant implications for the treatment of neurological and psychiatric disorders. The central nervous system is responsible for regulating a broad range of processes, including movement, cognition, and emotion [1]. The pharmacology of the brain is concerned with understanding how drugs and chemicals interact with the brain to affect these processes. This area of research has made significant strides in recent years, leading to the development of new treatments for disorders that affect Behavior and motor coordination, such as Parkinson's disease and depression [2]. Additionally, research in this area has the potential to enhance cognitive and physical abilities in healthy individuals, such as athletes or individuals seeking to improve their performance.

Nymphaea lotus seeds, also known as Indian lotus seeds, are an edible seed that has been used for centuries in traditional medicine and cuisine in various parts of Asia. These seeds are obtained from the water lily plant, which is native to many countries in Asia and Africa [3]. Nymphaea lotus seeds are a rich source of nutrients, including protein, fiber, and minerals, and are believed to have several health benefits [4]. They are often used in traditional medicine to treat conditions such as insomnia, anxiety, and diarrhea. In recent years, there has been growing interest in the potential health benefits of Nymphaea lotus seeds, leading to increased research into their nutritional value and potential medicinal properties [5].

Nymphaea lotus seeds contain several active constituents that may have potential effects on Behavior and motor coordination. One of the primary constituents is alkaloids, which are known to have central nervous system effects. These alkaloids include nuciferine, which has been found to have sedative effects and may reduce anxiety levels. Another alkaloid, aporphine, has been shown to have dopaminergic activity and may improve motor coordination. Nymphaea lotus seeds also contain flavonoids, which have been shown to have antioxidant and anti-inflammatory effects [6]. These compounds may help to protect neurons and improve overall brain function, potentially leading to improved motor coordination and cognitive function.

2. COLLECTION, IDENTIFICATION AND AUTHENTICATION OF PLANT MATERIAL

The seeds of *Nymphaea lotus* were collected from the local area of Ballia District, (U.P.), India.

The plant material, seeds of *Nymphaea lotus*, were identified and authenticated by, Dr. Priyanka Agnihotri, Scientist, Plant Diversity, Systematics & Herbarium Division, N.B.R.I., Lucknow. The plant specimen was authenticated in herbarium with Ref. No. LWG 55. The plant material was dried in shade. The seeds of *Nymphaea lotus* were crushed and then powdered with the help of manual homogenizer and mixer.

3. EXTRACTION

Extraction is a separation process, consisting of separating a substance from a matrix [7]. The powdered plant material (seeds of *Nymphaea lotus*) was extracted by maceration process, using water as the solvent. The quantity of the solvent used was 500 ml. The crude

extract obtained was collected and concentrated in a oven at 70ºC. The percentage yield of the crude extract of seeds was calculated by using the formula given below-

Percentage Yield = Weight of Extract (gm)/ Weight of Dry Powder (gm) ×100

3.1 PRELIMINARY PHYTOCHEMICAL STUDIES

The extract obtained from the seeds was analysed using various chemical tests in order to identify the different active phytoconstituents present.

4. MODELS FOR PHARMACOLOGICAL SCREENING

1. **IN-VIVO**
   (a) Rota-Rod Apparatus
   (b) Actophotometer

2. **IN- VITRO**

   Biochemical estimation of brain homogenate for:

   (a) Acetylcholinesterase.
   (b) Thiobarbituric Acid Reactive Substances.
   (c) Superoxide Dismutase.
   (d) Catalase.

3. **DETERMINATION OF POTENCY OF PLANT EXTRACT BY THREE-POINTBIOASSAY METHOD.**
   (a) Locomotor Activity.

4.1 Experimental protocol for pharmacological screening

Pharmacological screening of the aqueous extract of *Nymphaea lotus* seeds for evaluationof memory enhancing activity included the following-

- Experimental Animals.
- Experimental Design.
- Behavioral Assessment.
- Biochemical Estimation.
- Determination of Potency of Plant Extract by Three Point Bioassay Method.

The crude extract of *Nymphaea lotus* seeds was investigated for its potential towards memory enhancing. The activity of the extract of the seeds was evaluated in scopolamine induce memory impaired mice.

4.2 Experimental animals

Swiss Albino mice of either sex, weighing between 20-25 g, were utilized for the study. These mice were obtained from the Animal House at the Faculty of Pharmacy in BBDNIIT, Lucknow, U. P., India. The mice were housed in polypropylene cages with steel netting, in a temperature-controlled room with standard living conditions of 25 ± 5^0C and relative humidity of 55±5%, with a regular 12-hour light/dark cycle, and given unrestricted access to food and water. The treatment of all animals was conducted in accordance with humane guidelines set forth by the Institutional Animal Ethics Committee (IEAC). The Institutional Animal Ethics Committee approved the project proposal with Reg. No. 809/PO/Re/S/03/CPCSEA.

4.2.1 Experimental Design

Grouping and treatment protocol

A total of thirty animals were used in the study, and they were divided into five groups, each consisting of six animals [8].

Group 1: Positive control group (distilled water *p.o.*).
Group 2: Negative control group (distilled water *p.o.* + scopolamine *i.p.*).
Group 3: Standard group (piracetam at a dose of 200 mg/kg, *p.o.* +scopolamine *i.p.*).
Group 4: *Nymphaea lotus* seeds aqueous extract at dose 400mg/kg body weight *p.o.*
+scopolamine.
Group 5: *Nymphaea lotus* seeds aqueous extract at dose 600mg/kg body weight *p.o.* +scopolamine *i.p.*
In each group, all the mice were treated with once in a day respective dose as per their body weight. The doses were given at 1ml/100g body weight per oral (distilled water and extract of *Nymphaea lotus* seeds) for 6 days. The scopolamine 1 mg/kg *i.p.* was given on day 6 to each group.

4.3 Behavioral Assessment

The animals were trained for various task performance by conducting daily training trial for 15–30-minute time interval, it took 5 days to get the animals completely trained, during whichthey did not receive any drug. The trained animals were chosen for the study [9].

4.3.1 Rota-Rod Test

The evaluation of drug activity on motor coordination involved the use of a test to assess skeletal muscle relaxation. This was achieved by observing the ability of an animal to remain on a revolving rod after being administered a test compound. The endpoint of the study was determined as the dose at which 50% of the mice were unable to maintain their position on

the revolving rod. This test was utilized to assess the impact of drugs on motor coordination [10].

4.3.2 Locomotor Activity Test

The locomotor behaviour of the animals was monitored using an actophotometer. The animals were placed individually in the actophotometer and the basal activity score was recorded for a period of 5 minutes. Each animal was then administered the respective drug, and the activity score was recorded after 30 minutes and 1 hour. A decrease in activity score was considered as an indication of central nervous system (CNS) depression. This test was used to assess the impact of drugs on the CNS [11].

4.4 Biochemical Estimations

4.4.1 Preparation of homogenate: Brain tissue homogenate was prepared using 10% w/v ice cold saline solution, and then centrifuged at 4000 r.p.m. for 15 min at 4ºC. The supernatant was collected separately and further subjected to biochemical tests.

4.4.1.1 Estimation of Acetylcholinesterase Activity

To determine the enzyme activity, 0.5 ml of the supernatant liquid obtained from the brain homogenate was pipetted into a 25 ml volumetric flask, and then diluted with freshly prepared dithiobis nitricbenzoic acid (DTNB) solution. From the volumetric flask, two 4 ml portions were taken and transferred to two separate test tubes. To one of the test tubes, 2 drops of acetylcholine solution were added. Subsequently, 1 ml of substrate solution was pipetted into both test tubes, and then they were incubated for 10 minutes at 30°C. The change in absorbance per minute of the sample was measured using spectrophotometric colorimetry at 412 nm [12]. The enzyme activity was then calculated using the following formula:

R= C X Volume of Assay (3ml)/ E X mg of protein

Where**,** R= rate of enzyme activity in ‘n’ mole of acetylcholine iodide hydrolyzed/ minute/mgprotein
C= change in absorbance/ minute.
E= extinction coefficient (13, 600/ml/cm).

4.4.1.2 Measurement of Catalase Activity

To determine catalase activity, 50 µl of the supernatant was added to a cuvette containing 2.95 ml of a 19 mM/liter solution of H_2O_2 prepared in potassium phosphate buffer. The disappearance of H_2O_2 molecules was monitored at 1-minute intervals for 3 minutes at 240 nm [13]. Catalase activity was then calculated using the following equation:

Nanomoles of H_2O_2/ min/mg of protein =

$$\frac{\Delta A/min \times Volume\ of\ assay}{Volume\ of\ sample \times mg\ of\ protein}$$

Where; $\Delta A = A_0 - A_1$
A_0 is absorbance of sample at 0 min. and A_1 is absorbance of sample at 1 min.

4.4.1.3 Determination of Lipid Peroxidation

To measure the amount of Malondialdehyde (MDA) present in the sample, 1 ml of the suspension medium was taken from the supernatant of the 10% tissue homogenate. Then, 0.5 ml of 30% trichloroacetic acid (TCA) was added, followed by 0.5 ml of 0.8% thiobarbituric acid (TBA). The tubes were covered with aluminium foil and kept in a shaking water bath for 30 minutes at 80°C. After 30 minutes, the tubes were taken out and kept in ice-cold water for 15 minutes. They were then centrifuged at 3000 rpm for 15 minutes, and the absorbance of the supernatants was read at 540 nm at room temperature against an appropriate blank. The blank consisted of 1.0 ml distilled water, 0.5 ml of 0.8% TBA solution, and 0.5 ml of 30% TCA solution [14]. The amount of MDA present in the sample was calculated using the following equation:

$$\textbf{MDA level (nmol.)} = \frac{\textbf{volume of test solution – optical density}}{\textbf{0.56}}$$

4.4.1.4 Estimation of Superoxide Dismutase

The procedure for assaying superoxide dismutase (SOD) activity involved adding 100 µl of cytosolic supernatant to tris HCl buffer with a pH of 8.5. The final volume was adjusted to 3 ml with the same buffer, and then 25 µl of pyrogallol was added. Changes in absorbance at 420 nm were recorded at 1-minute intervals for 3 minutes. The presence of SOD inhibited the increase in absorbance at 420 nm after the addition of pyrogallol [15]. The amount of SOD required to cause 50% inhibition of pyrogallol auto-oxidation per 3 ml of assay mixture was defined as one unit of SOD, and calculated using the following formula:

$$\textbf{Unit of SOD per ml of sample} = \frac{(\mathbf{A} - \mathbf{B})}{(\mathbf{A} \times \mathbf{50})} \times \mathbf{100}$$

Where;

A = Change in absorbance per minute in control.
B = Change in absorbance per minute in test sample.
Data were expressed as SOD units per mg of protein.

4.5 Determination of Potency of Plant Extract by Three Point Bioassay Method

The three-point bioassay method combines the principles of matching and interpolation by using two doses of standard and one dose of the test. A logarithmic dose-response (LDR) curve is plotted using the response obtained from two different concentrations of standard solutions and the given test solution. From the linear part of the LDR curve, two standard doses (S1 and S2) are selected, and the corresponding responses are noted as S1 and S2. A test dose (T) is selected, which gives a corresponding response (T) that lies between S1 and S2 [16]. To reduce errors and avoid bias, the experiment is repeated using different orders of sequence, employing the Latin square method of randomization, as follows-

S_2 t S_1
S_1 S_2 t
S_2 S_1t

Calculation-

Potency of drug solution- $s1 \times \text{antilog}\left(\frac{T1-S1}{t1} \times \log s_2/s_1\right) \times \frac{c}{S2-S1}$

Where, s_1= lower dose of standard. S_2= higher dose of standard.
t_1=dose of test (lie between S1& S2). S_1=height of s_1 (mean height).
S_2=height of s_2 (mean height). T_1= mean of height of test.
C= concentration of standard solution.

5. RESULTS AND DISCUSSION

5.1 Results of Phytochemical Investigation of *Nymphaea Lotus* Seeds

Table 5.1.1 Percentage Yield of the Aqueous Extract of *Nymphaea lotus* Seeds (NLS).

S. No.	Extract	Weight (gm)	Percentage yield(w/w)
1.	Aqueous	2	4

5.1.2 Preliminary Phytochemical Screening

Table 5.1 Phytochemical Screening of *Nymphaea lotus* Seeds.

S. No.	**Class of compounds**	**Aqueous extracts**
1.	**Alkaloids**	**+**
2.	**Flavonoids**	**+**
3.	**Saponins**	**+**
4.	**Glycosides**	**+**
5.	**Tannins**	**+**
6.	**Amino acids**	**+**
7.	**Carbohydrate**	**+**
8.	**Fixed oil**	**+**
9.	**Protein**	**+**
10.	**Steroids**	**+**

(+) indicates presence of particular constituents

5.2 RESULTS OF BEHAVIOURAL ASSESSMENT

5.2.1 Rota-Rod Apparatus

Effect of aqueous extract of *Nymphaea lotus* seeds (NLS) on Motor Co-ordination

The Rota rod test is utilized to assess grip strength and motor co-ordination in experimental animals. The administration of scopolamine to mice resulted in impaired performance on the rota rod test, but there was no significant difference in performance in the group treated with the plant extract at a dose of 400 mg/kg body weight. However, at a dose of 600 mg/kg body weight, the extract significantly ($P<0.001$) improved performance on the rota rod test. Piracetam, a standard drug used for comparison at a dose of 200mg/kg body weight, significantly ($P<0.001$) improved rota rod performance as well.

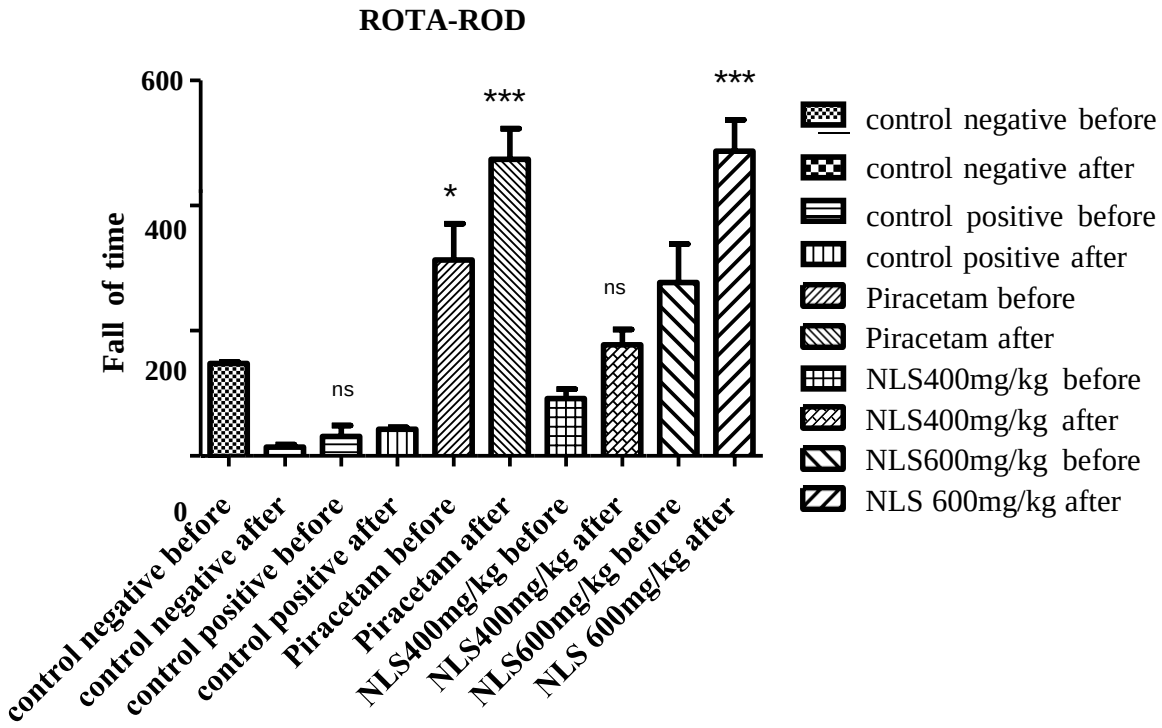

Fig 5.2.1 Effect of Aqueous extract of *Nymphaea lotus* seeds on Motor Co-ordination

The statistical analysis of the results was conducted using One Way Anova followed by Dunnett's t test. The values are presented as mean ± SEM with each group consisting of 6 animals. The significance level was indicated by *** for P<0.001, ** for P<0.05, and * for <0.01, while ns was used to indicate non-significant results. NLS 400 mg/kg represents the aqueous extract of Nymphaea lotus seeds at 400 mg/kg B.W. and NLS 600 mg/kg represents the aqueous extract of Nymphaea lotus seeds at 600 mg/kg B.W.

5.2.2 Actophotometer

Effect of Aqueous Extract of *Nymphaea Lotus* Seeds (NLS) on Locomotor Activity

The level of locomotor activity is a measure of alertness, and a spontaneous decrease in basal activity is associated with a reduction in anxiety. Animals treated with scopolamine showed a significant decrease in locomotor scores. The aqueous extract of Nymphaea lotus seeds at 600 mg/kg B.W. showed a significant ($P<0.05$) reversal of depressive behaviour induced by scopolamine, while NLS 400 mg/kg B.W. showed a significant ($P<0.01$) effect.

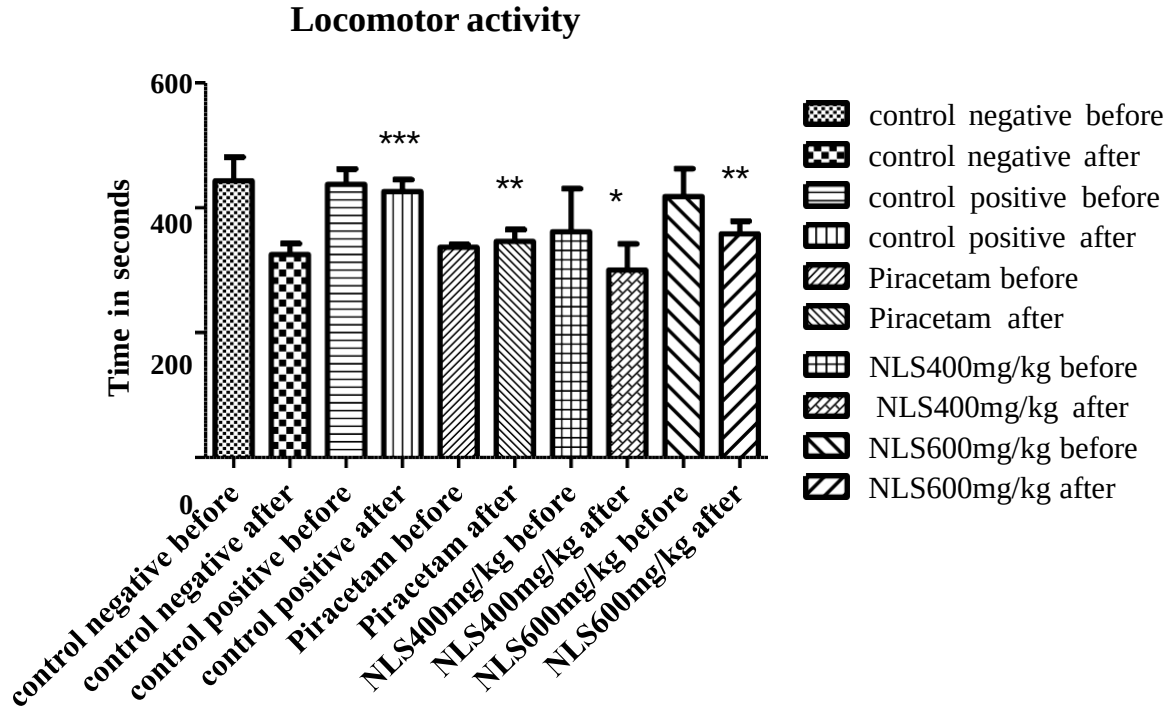

Fig. 5.2.2 Effect of Aqueous extract of *Nymphaea lotus* seeds on locomotor activity

The data was analysed using One Way Anova followed by Dunnett's t-test. The results are presented as mean ± SEM with 6 animals in each group. *** indicates $P<0.001$, ** indicates $P<0.05$, * indicates $P<0.01$, and ns indicates non-significant. NLS 400 mg/kg refers to the administration of aqueous extract of Nymphaea lotus seeds at a dose of 400 mg/kg B.W., and NLS 600 mg/kg refers to the administration of aqueous extract of Nymphaea lotus seeds at a dose of 600 mg/kg B.W.

5.3 RESULTS OF BIOCHEMICAL ESTIMATION

5.3.1 Effect on Acetylcholinesterase level in brain homogenate

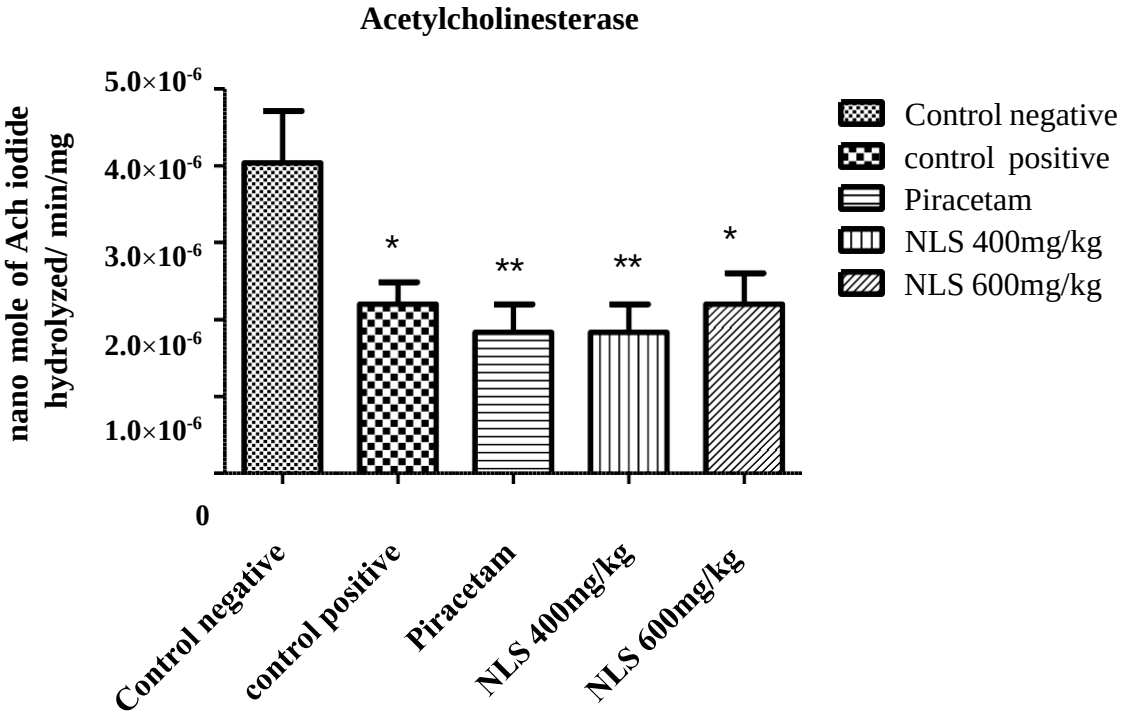

Fig 5.3.1 Effect of aqueous extract of *Nymphaea lotus* seeds on Acetylcholinesterase Activity

The statistical analysis was performed using One Way Anova followed by Dunnett's t test. The results are presented as mean ± SEM, and each group contained 6 animals. The significance levels were denoted by *** for P<0.001, ** for P<0.05, * for P<0.01, and ns for non-significant. NLS 400 mg/kg referred to the aqueous extract of Nymphaea lotus seeds administered at a dose of 400 mg/kg B.W., and NLS 600 mg/kg referred to the aqueous extract of Nymphaea lotus seeds administered at a dose of 600 mg/kg B.W.

The level of acetylcholinesterase in the brain was significantly increased in the scopolamine-treated group of animals compared to the control positive group. However, treatment with NLS at 400 mg/kg B.W. (P<0.05) and 600 mg/kg B.W. (P<0.01) significantly reduced the level of acetylcholinesterase in the brain compared to the scopolamine-treated group. Piracetam (200 mg/kg B.W.), used as a standard drug, also showed a significant (P<0.05) reduction in brain acetylcholinesterase. NLS at 400 mg/kg B.W. showed a more significant result compared to NLS at 600 mg/kg B.W.

5.3.2 Effect on Lipid Peroxidation

Lipid peroxidation is a process that generates degradation products and can cause damage to cell membranes. Malondialdehyde (MDA) is one of the major products of lipid peroxidation and can be detected using a thiobarbituric acid colour reaction. In this study, treatment with NLS extract at 600mg/kg B.W. showed a significant ($p<0.05$) decrease in MDA level compared to the scopolamine treated group. Piracetam showed even more significant ($p<0.001$) protection from oxidative stress by decreasing MDA level compared to the scopolamine treated group.

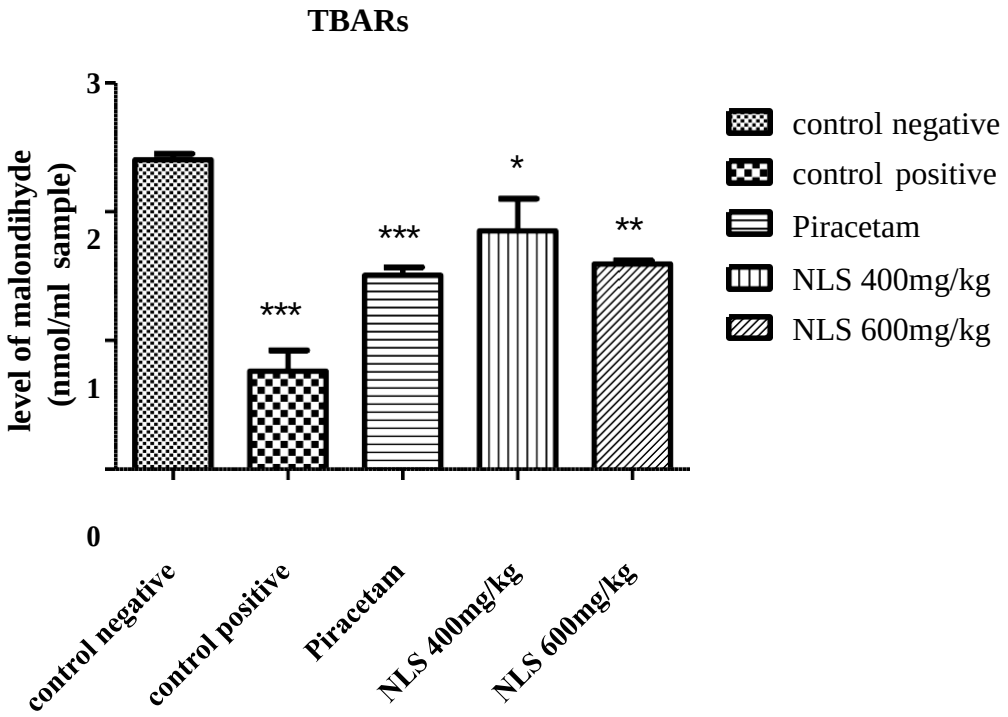

Fig.5.3.2 Effect of aqueous extract of *Nymphaea lotus* seeds on Lipid Peroxidation.

The results are statistically analyzed using One Way Anova followed by Dunnett's t test. Values are expressed as mean ± SEM with each group containing 6 animals. *** indicates P<0.001, ** indicates P<0.05 and * indicates <0.01 and ns indicates non-significant. NLS 400 mg/kg: aqueous extract of *Nymphaea lotus* seeds at 400 mg/kg B.W. and NLS 600 mg/kg indicates aqueous extract of *Nymphaea lotus* seeds at 600 mg/kg B.W.

5.3.3 Effect on Superoxide Dismutase level

Superoxide anions are precursors to active free radicals that can react with biological macromolecules and induce tissue damage. The scopolamine-treated group of animals showed increased oxidative stress, as evidenced by decreased levels of superoxide dismutase (SOD) compared to the control positive group. NLS treatment at 600 mg/kg B.W. resulted in a significant ($p<0.05$) increase in the percentage inhibition of superoxide compared to the scopolamine-treated group.

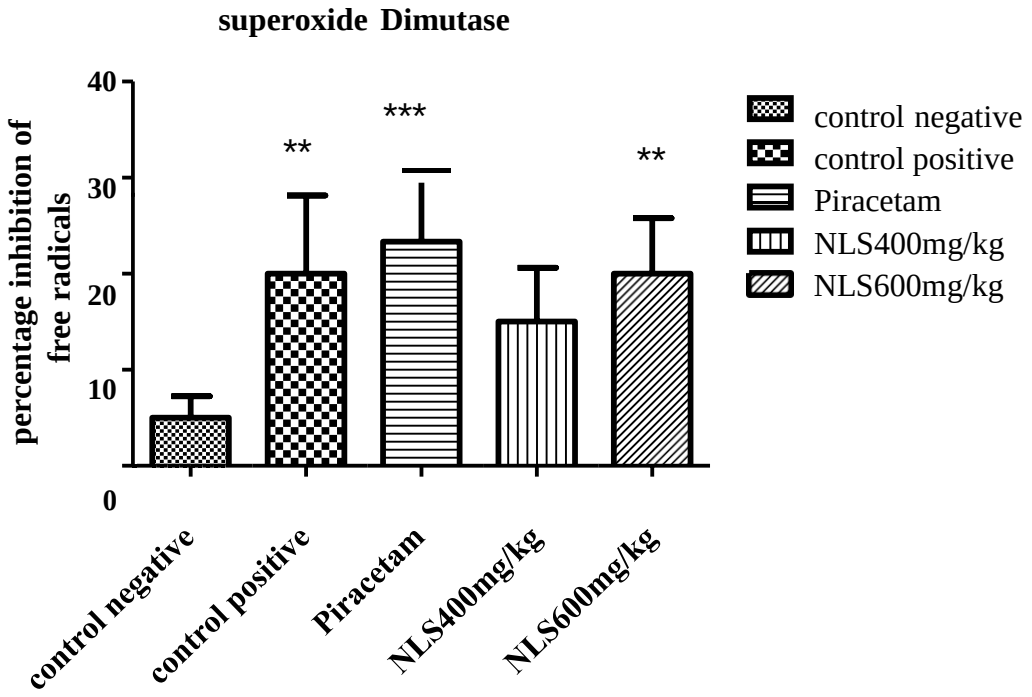

Fig.5.3.3 Effect of aqueous extract of *Nymphaea lotus* seeds on Superoxide dismutase

The statistical analysis was performed using One Way Anova followed by Dunnett's t test. The mean values with standard error of mean (SEM) are presented for each group consisting of 6 animals. The level of significance is indicated as follows: *** for $P<0.001$, ** for $P<0.05$, * for $P<0.01$, and ns for non-significant. The abbreviation NLS stands for the aqueous extract of Nymphaea lotus seeds, and NLS 400 mg/kg and NLS 600 mg/kg indicate the doses of 400 mg/kg and 600 mg/kg body weight, respectively.

5.3.4 Effect on Catalase level

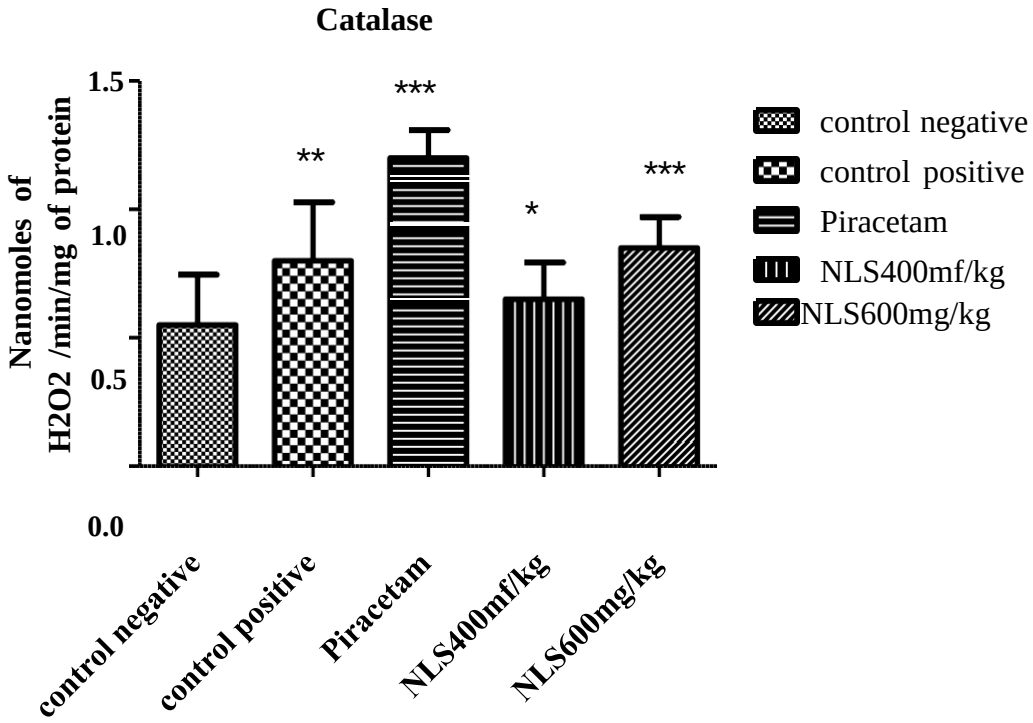

Fig.5.3.4 Effect of aqueous extract of *Nymphaea lotus* seeds on Catalase.

The results are statistically analyzed using One Way Anova followed by Dunnett's t test. Values are expressed as mean ± SEM with each group containing 6 animals. *** indicates P<0.001, ** indicates P<0.05 and * indicates <0.01 and ns indicates non-significant. NLS 400 mg/kg: aqueous extract of *Nymphaea lotus* seeds at 400 mg/kg B.W. and NLS 600 mg/kg indicates aqueous extract of *Nymphaea lotus* seeds at 600 mg/kg B.W.

The level of catalase was found to be reduced in the scopolamine-treated group compared to the control positive group. The extract of Nymphaea lotus seeds significantly increased the catalase level in the treated group. NLS 600 mg/kg B.W. showed a more significant (P<0.05) result as compared to NLS 400 mg/kg B.W. (P<0.01).

5.4 Determination of Potency of Plant Extract by Three Point Bioassay Method

Nymphaea lotus seeds extract's potency was checked by performing 3-point bioassay using Piracetam as standard on chicken ileum. The potency of the plant extract was found to be 0.995.

Fig.5.4 Three Point Bioassay

6. CONCLUSION

The results of this study demonstrate the potential of Nymphaea lotus seed extract in improving behaviour and motor coordination. The in vivo and in vitro models used in this study provide evidence of the extract's ability to improve motor coordination, reduce lipid peroxidation, and increase antioxidant enzyme activity. Moreover, the extract showed an inhibitory effect on acetylcholinesterase activity, indicating its potential in treating neurodegenerative disorders. These findings highlight the potential of Nymphaea lotus seeds as a natural source of bioactive compounds that could be used in the development of new drugs for the treatment of various neurological disorders. Further studies are warranted to explore the molecular mechanisms underlying the observed effects and to assess the safety and efficacy of the extract in clinical trials. Overall, this study provides evidence that Nymphaea lotus seeds could be a promising candidate for the development of new therapeutic agents for the treatment of motor and cognitive impairments.

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