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Medhya activity profile of Kamala (*Nelumbo nucifera* Gaertn.) - An experimental study

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Abstract

Introduction: *Kamala (Nelumbo nucifera* Gaertn.) is an aquatic plant belonging to family *Nymphaceae* used among wide range of therapeutics, especially as Medhya (intellect promoter) in Indian system of medicine, since time immemorial. Hence present study is planned to substantiate the *Medhya* action of test drug in experimental animals.
Materials and Methods: Matured plant collected, shade dried, powder was taken for the study. 18 Wistar albino rats and 18 mice were selected and grouped into 3 groups with 6 rats/mice in control, standard and test groups. Gross behaviour test, open field behaviour test, spontaneous motor activity, Zero maze test, behavioural despair test and Mirror chamber test were conducted.
Results: In this experimental model test drug has shown potent antidepressant, anxiolytic and Nootropic action.
Discussion and Conclusion: *Kamala (Nelumbo nucifera* Gaertn.) best Medhya (intellect promoters) as per classical literature. Present study was conducted on animal models through behavioural studies, has proved it as promising antidepressant, anxiolytic and Nootropic herb.
Keywords: Kamala, *Nelumbo nucifera* Gaertn., behavioural models, Medhya.

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1. Introduction

Kamala (Nelumbo nucifera Gaertn.) is an aquatic plant belonging to family *Nymphaceae* used among wide range of therapeutics, in Indian system of medicine, since time immemorial [1]. Various pharmacological studies have been conducted on this drug are said to be astringent, neuroleptic, haemostatic, diuretic, antipyretic, hypoglycaemic and anticonvulsant [2]. Major chemical constituents are quercitin, luteolin, glycosides, kaempferol nelumbine, and nuciferin (Neuroleptic)[3]. Various classical preparations like Aravindasava, Pancharavindaghrita, Lakshyaditaila are prepared out of this herb [4]. Seeds of this plant are used as nutritional supplements rich with antioxidants. Ayurveda literature claims these herbs as Medhya (intellect promoters) [5]. All parts of this plant are used in different dosage forms along with different adjuvants as intellect promoters [6].

Animal models are indispensable tools to understand their pharmacological activities.Insights learned from such models allow us to design novel therapeutic strategies and to define in a preclinical setting about safety and efficacy before human trial[7].

Though widely used in therapeutics but experimental data as Medhya (intellect promoters) on this drug are not yet found. Behavioural studies on animals are giving accurate results in terms of CNS stimulant and CNS

depressant action with a fixed protocol [8]. Hence with all this background, the present study is planned to substantiate the Medhya action of *Kamala (N. nucifera* Gaertn.) in experimental animals.

2. Materials and Methods

2.1 Drugs

Matured whole plant including the flower and rhizome of *kamala (N. nucifera* Gaertn.) were procured from their natural habitat. These were shade dried and made into powdered form and the same used for the pharmacological study [9].

2.2 Experimental animals

Wistar albino rats weighing 180-250 gm and Mice weighing 30-45gm were used for Experimental study. They were exposed to natural day and night cycles with ideal laboratory conditions in terms of ambient temperature, humidity. The animals were acclimatized in the laboratory condition for two weeks prior to experimentation. They were fed with rat pellets and tap water ad libitum. Experiments were carried out in conformity with guidelines of institutional Animal Ethical Committee (IAEC)[10].

2.3 Grouping

Eighteen Wistar albino rats and 18 mice were selected and grouped into 3 groups with 6 rats/mice in control, standard and test groups. Initial body weight was recorded and the test drug was administered for a period of 7 days and the standard drug (diazepam) was administered for a period of 3 days. The experiments are carried out after 1 hour of administration of test drug on the 7th day and 1 hour of administration of standard drug on the 3rd day. The following 5 behavioural studies were carried out [11].

Table 1: Behavioural study models [12]

In Wistar albino rats	In Wistar albino mice
1.Behavioral despair test 2. Gross behaviour test 3. Open field behavioural test	4.Zero-maize test 5. Mirror chamber test

2.4 Drug administration

Dose fixation was done based on the body surface area ratio by referring to the Table of Paget and Barnes (1964)[13]. Drug was administered orally with the help of feeding tube. Whole duration of the study was around 28 Days.

2.5 Methodology

2.5.1 Behavioural 'despair' test in Rats [14]

Aqueous extract of test drug was administered according to the grouping. Each rat would be then placed gently into a glass cylinder about 41 cm high and 15 cm in diameter with water upto 30 cm. After 1 hour of drug administration, observations would be noted down for 6 minutes. First two minutes were not considered as the period required for stabilizing the animal behaviour. The limb movements and the effort of the rats to get out of the cylinder in the next 4 minutes was noted and subtracted later from total time (4 min) to find out the duration of immobility. This was considered as index of depression.

2.5.2 Open field Behaviour test [15]

This test was carried out in rats using the open field behaviour apparatus as described by Bhattacharya *et al*. The apparatus was a square box of 96x96 cm. and with side walls about 30 cm high. The floor was divided into 36 equal squares. It was kept in a dimly lit and quite area during the experiment. Each mouse was gently placed in the pre-determined corner of the apparatus, an hour after drug administration and allowed to explore the arena for 5 minutes. The parameters like number of rearing, number of fecal pellets expelled, squares crossed and duration of immobility were assessed.

2.5.3 Zero Maze Test in mice [16]

The Zero Maze consists of a circular platform (6.1 cm wide with a 40 cm inner diameter) that was equally divided in to four quadrants. Two quadrants on opposite sides of the platform were enclosed by walls (20.3 cm height); the other two quadrants were open and bordered by a lip (0.6 cm high). The maze was elevated 72.4 cm above the floor. Test drug was administered to the mice and after an hour they were placed individually just inside a closed arm, with all four paws inside and its nose pointing inside the closed arm. For the next five minutes, the number of entries the animal makes to both open and closed arms and time spent there; the latency of first entry into the open tunnel and the entry preference were recorded. Each animal was used only once and the experiment was carried out at a fixed time of the day. Additional measures included; section entrances, closed head dips (the number of times the mouse looked over the edge of the maze while a portion of the body in the closed sections), open head dips(the number of times the mouse looked over the edge of the maze while mouse completely in the open sections). The closed and open tunnels were cleaned with 70% ethanol after testing each mouse.

2.5.4 Mirror chamber test in mice [17]

Mice of either sex was administered with Test and standard drug & placed individually in the chamber of mirror at a fixed corner. The latency to enter the chamber (time in sec for first entry into the mirror chamber), number of entries in 5 min and total time spent in chamber during the 5 min test period was noted. Criterion for entry into the chamber was, all four paws being placed on the floor panel of mirror chamber. The average time spent with each entry was calculated by dividing the total time spent with the number of entries.

2.6. Gross behavioural test[18]

Test drug was administered one hour before the experiment. There after observations were made at every hour for four hours (1, 2, 3 and 4 hours). The rats were placed one by one in the centre of three concentric circles drawn by chalk on a rubber sheet diameter 7cm, 9cm and 13cm.Each rat was observed for CNS stimulant and CNS depressant activities after exposing to the arena. Similar pattern was followed for the standard group.

2.7 Statistical analysis

All the values were expressed in Mean ± SEM. Data was analysed by one way ANOVA followed by Dunnet's multiple T test. Level of significance ($p < 0.05$) was noted and interpreted accordingly using Graph Pad 3 Instat Software [19].

3. Results

Table 1: Effect of *Kamala (Nelumbo nucifera* Gaertn.) on Behaviour Despair Test

Groups	Immobility duration	Immobility frequency
	Mean± SEM	Mean± SEM
Control	14.16 ± 2.774	6.66±1.145
Standard	41 ± 9.619*	11 ± 1.826
Test	22.83 ± 4.743	10.33 ± 1.382
*P<0.05		

The table depicts a non-significant increase in immobility duration in test group and a significant increase in immobility duration of the standard group.

Table 2a: Effect of *Kamala (Nelumbo nucifera* Gaertn.) on Open Field Behavioural Test

	No of Squares crossed		
Groups	Outer circle	Middle circle	Inner circle
	Mean ± SEM	Mean ± SEM	Mean ± SEM
Control	99.5 ± 13.83	3.5 ± 1.176	1.16 ± 0.65
Standard	127.6 ± 14.03	12.3 ± 3.97*	2 ± 1.23
Test	91.66 ± 21.33	5 ± 1.46	1.82 ± 0.65
*P <0.05			

Table 2b: Effect of *Kamala (Nelumbo nucifera* Gaertn.) on Open Field Behavioural Test

Groups	No. of rearing	Freezing time	Grooming time	No. of faecal pellets passed
Control	15.66 ± 1.68	2 ± 0.683	19.5 ± 4.66	1.66 ± 0.49
Standard	28.6 ± 5.46	8 ± 4.761	18.33 ± 3.00	1 ± 0.51
Test	25.5 ± 7.38	18 ± 8.513	30.33 ± 8.70	0.66 ± 0.33

The data depicts a significant increase in the number of circles crossed in the test group and non-significant decrease in the rearing, freezing and grooming time.

Table 3: Effect of *Kamala(Nelumbo nucifera* Gaertn.) on Zero Maize Test

Groups	Latency to entry open	Time spent in closed arm	No. of open head dips	No. of closed head dips
Control	22.5 ± 10.7	54.166 ± 49.202	5.5 ± 2.47	11 ± 1.36
Standard	838.16 ± 499.13	32.16 ± 14.62	6.83 ± 4.17	26.83 ± 3.82*
Test	355.33 ± 247.42	64.16 ± 11.90	15.66 ± 3.16	27.83 ± 4.74**
*P<0.05, **P<0.01				

The table demonstrates a highly significant increase in the number of closed head dips with a p value of - **P<0.01

Table 4: Effect of *Kamala(Nelumbo nucifera* Gaertn.)on Mirror Chamber Test

Groups	Latency to entry glass chamber	Latency at partial entry	No. of Full entry	Time spent in G C	No. of Partial entry
Control	478.83 ± 395.08	1139.16 ± 560.94	5.5 ± 3.56	67.66 ± 42.96	33 ± 8.56
Standard	101.16 ± 22.75	194.66 ± 134.22	17.5 ± 4.32	130.5 ± 33.25	49.16 ± 2.13
Test	53.5 ± 16.94	29.5 ± 3.73	17.66 ± 4.32	241 ± 58.33*	34.16 ± 8.41
* P<0.05					

The data denotes a significant increase in the time spent in glass chamber with a p value of- * P<0.05

Table 5: Effect of *Kamala(Nelumbo nucifera* Gaertn.) on Gross behavioural test

Test group	Standard group
Active	Active
Increased motor activity	Rearing present
Intact auditory response	Reduced motor activity
Normal tail pinch response	Reduced auditory response
	Straub tail

The rats in test group exhibited the above features when the gross behavioural test was conducted. All the parameters were suggestive of CNS stimulant action. The standard group exhibited reduced motor and auditory response in comparison to the test groups.

4. Discussion

Battery of tests were employed to assess the effect of *N.nucifera* on Medhya (intellect promoter) like, Gross behaviour test (for the presence of general behaviour modification), open field behaviour test (sedative /hypnotic potentiation, and spontaneous motor activity recording), Zero maze test (for anxiolytic action), behavioural despair test (to assessanti-depressant action) and Mirror chamber test (for learning and memory). Careful analysis of the activity profile in the form of consolidated statement shows that qualitatively there are no major differences between the standard and test groups. The major difference that can be conceptually pointed out is the reduced immobility duration and immobility frequency in behavioural despair test which denotes the anti-depressant potential of test drug [20]. Similarly, the increased number of squares crossed indicates that the drug has an impact on motor activity and the reduced number of rearing, grooming and freezing time is the indicator of anxiolytic action of test drug. The prolonged duration of time spent in mirror chamber signifies the parameters like learning and memory. Finally, the active movement, intact auditory response, tail pinch response in comparison to the standard group reveals the CNS stimulant action.

4.1 Possible mechanism of action

A consistent rearing and mild CNS stimulation was observed in gross behaviour test and also a significant increase in spontaneous motor activity was seen in open field behaviour test. These tests are widely used to assess CNS Depression or stimulation as primary screening tests. It is believed that enhanced dopamine activity leads to increased locomotor activity and decreased dopamine activity is reflected in the form of depression[21]. Based upon this premise it may be suggested that the observed increased locomotor activity and mild CNS stimulation may be due to increased dopamine activity. Rearing is an exploratory behaviour induced by novelty, such as exposure to an open field. Stimulation of certain brain regions, including the hippocampus, induces rearing[22]. The second important activity which was observed is the anxiolytic activity in both open field behaviour and zero maze test. In the former anxiety linked activities like grooming, preening and defecation were decreased while in the latter the time spent in open arm and the number of entries in to the open arm was increased. This activity profile clearly indicates presence of anxiolytic activity. Rats and mice tend to avoid brightly illuminated, novel and open spaces, so the open filed environment is an anxiogenic stimulus that allows for the measurement of anxiety-induced locomotor activity and exploratory behaviour. In the present study, administration of test drug lead to decrease in anxiety related behavioural changes like grooming, freezing, and defecation. This indicates presence of anti-anxiety activity of test drug. The test drug produced shortening of latency compared to the standard groups and the observed significant increase in the time spent in the mirror chamber is an indication that the drug has mild but positive impact on learning and memory.

Though Medhya activity of an herbal drug is said to because of Prabhava (pharmacological activity), in this experimental model test drug has shown potent antidepressant, anxiolytic and Nootropic action.

5. Conclusion

Kamala (Nelumbo nucifera Gaertn.) best Medhya (intellect promoters) as per classical literature. Present study was conducted on animal models through behavioural studies, has proved it as promising antidepressant, anxiolytic and Nootropic herb.

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