

## Comparison of hypnotic effect in fresh petal aqueous extract of lotus, (*Nelumbo nucifera* Gaertn) and diazepam powder on ornamental guppy fish, (*Poecilia reticulata*)

Senthil Bharath<sup>1</sup>, Tamilarasi<sup>1</sup>, Bala Mohan<sup>2\*</sup>, Duraiswamy Prabha<sup>2</sup>,

<sup>1</sup> PG & Research Department, Department of Zoology, Government Arts College, Coimbatore – 641 018, Tamil Nadu, India

<sup>2</sup> Department of Environmental Sciences, Bharathiar University, Coimbatore – 641 046, Tamil Nadu, India

### Abstract:

*Nelumbo nucifera* is naturally contained lots of natural drugs were mainly used so far in Ayurvedic and Chinese medicines to cure various diseases like strangury, vomiting, and skin diseases. The *Nelumbo nucifera* is also suited as an alternative to the chemical drug diazepam. Therefore, the present study was aimed to investigate the comparative efficiency of hypnotic effects from the fresh petal aqueous extract of *Nelumbo nucifera* and the chemical drug of diazepam fine powder. During the study period, fresh petal aqueous extract of the lotus was taken in three different concentrations such as 100mg/L, 200mg/L, and 500mg/L and diazepam 5 mg/L was tested against the Guppy fish, to analyze the sedative effect on *Poecilia reticulata*. After an hour of exposure, guppy fish were taken to assess for locomotory behaviour. Results from the present study revealed that lotus fresh petal extract of 500mg/L was reported to have a higher number of non-escaped fish (20 out of 40) when compared other two concentrations and next to diazepam states that lotus fresh petal extracts aqueous extract of 500 mg/L also have similar sedative/ hypnotic nature as that of diazepam. Moreover, diazepam powder 5 mg/L was also (19 out of 40) exposed to fish indicating a strong sedative effect. The present study concluded the fact of diazepam and lotus fresh petal extract possessed a valuable hypnotic/ sedative effect. By using valuable medicinal plant materials, may reduce the side effects and environmental friendliness to the ecosystem.

**Keywords:** Comparison; Lotus fresh petal extract; Diazepam powder; Guppy fish; Hypnotic effect

\*Corresponding author, Email address: [mohannethu300@gmail.com](mailto:mohannethu300@gmail.com)

## 1. Introduction

Lotus is a perennial aquatic plant. It belongs to the family of Nelumbonaceae, comprising only one genus *Nelumbo*, and it contains two species such as; *Nelumbo nucifera* and *Nelumbo lutea*. The *Nelumbo nucifera* has been widely used by indigenous communities as a diuretic, cardiac tonic (N' Guessan et al. 2021), vasodilator, anti-tumor, anti-bacterial, anti-helminthic, and in the treatment of strangury, vomiting, skin diseases, and nervous exhaustion (Mukherjee et al. 2009). The tremendous potential of *Nelumbo nucifera* has been used so far in traditional systems of medicine including Ayurveda and Chinese medicinal systems (Shahrajabian et al. 2022, Zheng et al. 2022). Moreover, the standardization of *N. nucifera* products must be pure and today's requisite to commercialize this herb's nutraceutical and pharmaceutical essence (Poumeni et al. 2017, Sridhar and Bhat, 2007). The *N. nucifera* develops promising lead compounds for the prevention and treatment of a plethora of diseases (Olas et al. 2005). The *N. nucifera* contains polyphenols compounds, and they can exhibit antioxidant activity which leads to many health benefits for human beings (Abderrahim et al. 2019, Yi et al. 2010), and apart from the biological activity of human beings, these chemicals function as a plant defense mechanism against to their predators. Therefore, many herbal products and spices serve as useful medicinal sources since antiquity human beings. The *Nelumbo nucifera* flowers are solitary, large, 10-25 cm in diameter, pink or pinkish. The flowers also contain glycoside kaempferol (kaempferol- 3- galactic-rhino glycoside). The stalks of *N. nucifera* have been used as one of the ingredients, have a unique fermenting medium, used for micro-biological screening (Liu et al. 2004), the flowers have a cooling effect, used as an astringent in diarrhea, also in cholera, and fever (Mukherjee et al. 1995, Paudel and Panth, 2015).

Diazepam is a benzodiazepine, most commonly used for "tapering". The benzodiazepine is dependent due to the drug's comparatively long half-life, allowing for more efficient dose reduction (Rada and Hoebel, 2005). Benzodiazepines have relatively low toxicity in overdose, and it is thought that benzodiazepines work by enhancing the activity of certain neurotransmitters in the brain (Cesarani et al. 2004, Mehta and Shank, 1995). Diazepam is used to treat anxiety disorders or alcohol withdrawal symptoms (Lader et al. 2009). Furthermore, diazepam is also mixed with other medications to treat muscle spasms and

stiffness, or seizures. The continuous intake of benzodiazepines can slow or stop your breathing especially if you have recently used an opioid medication, alcohol, or other drugs that may cause severe side effects in your body (Webster et al. 2008; Guo et al. 2018; Kindwall et al. 1999). The misuse of diazepam can cause addiction, overdose, or death, especially for children or other persons using this medicine without a doctor's prescription. In this present study, guppy fish was selected, because they are easily available in conditions, and world's most widely distributed tropical fish. Guppy fish is a member of the family of Poeciliidae, and they are highly adaptable and thrive in many different environmental and ecological conditions. The male guppies are smaller than females, have ornamental caudal and dorsal fins, and wild guppies generally feed on a variety of food sources, including benthic algae and aquatic insect larvae. The Guppies were used as model organisms in the fields of ecology, evolution, and behavioral studies. Therefore, the present study also chose guppy fish as experimental organisms, because they are easily available in conditions, and world's most widely distributed tropical fish. Understanding knowledge about medicinal plants is very important because recovering the conventional methods from ancient times is crucial. Therefore, the present aimed to investigate, the comparison of hypnotic effects in lotus fresh petal aqueous extract, (*Nelumbo nucifera*) and commercially available diazepam on guppy fish, (*Poecilia reticulata*).

## 2. Materials and methods

### 2.1 Fish collection and maintenance (Guppy Fish)

A total of 50 guppy fishes were collected from the golden aquarium fish shop in Gandhipuram. The collected guppy fishes were maintained in a rectangular glass tank (30 Liters) and they were checked regularly for infection and other unhealthy conditions. Fishes were fed with fish feed in pellet form from 7.00 to 8.00 am every day. The tank was cleaned and water was replenished daily during both acclimation and experimentation to eliminate metabolites and excretory products. The fish are maintained in this way and survived well without any disease problems. For all the groups the extract was given at 10-11 am for 1 hour during the experiment.

## 2.2 Preparation of lotus fresh petal extract and diazepam powder

Lotus petals were collected from the flower market and washed cleanly and made into an extract. The 10 grams of lotus petals were collected and ground well enough to produce extract which was added to 100ml of water. The pinkish colour extract was filtered and treated with the experimental fishes. The diazepam tablets (0.5 mg) were purchased from Super Medicals (P) Ltd on the prescription of the medical practitioner. The tablets were ground into a powder state to the required concentration for the treatment of the fish (Figure 1).



**Figure 1.** Preparation of lotus fresh petal extract and diazepam fine powder

## 2.3 Experimental Setup and data analysis

The *Poecilia Reticulata* fishes were divided into five separate groups and each group consists of 4 fishes (Figure 2).

Group I : Control fishes.

Group II : Lotus fresh petal extract (100mg/L) + Guppy Fishes.

Group III : Lotus fresh petal extract (200mg/L) + Guppy Fishes.

Group IV : Lotus fresh petal extract (500mg/L) + Guppy Fishes.

Group V : Diazepam (5mg/L) + Guppy Fishes.

A total of 5 buckets were taken for each group and filled with 1 liter of water, at the same time 20 guppy fishes were taken and separated into 5 groups (Groups I, II, III, IV, V). Each group consists of 4 fish for 1 hr. After the estimated time the fish in each group are experimented with individually for measuring the hypnotic effect of lotus petal extract in comparison with diazepam. The fish was taken in a 100ml beaker containing water individually and immersed in the glass tank consisting of normal water. The escape time of the fish was noted in seconds by using a stopwatch. A total time limit of 180 seconds is provided for every fish in a group. The fishes that did not come out of the immersed beaker within the time limit are noted as non-escaped fishes which show some sedative effects present in the fish. The escape time of each fish was noted and tabulated. The experiment was triplicate, due to the accuracy. The data of the experiment was entered in Microsoft Excel and analyzed as mean  $\pm$  SD.



**Figure 2.** Experimental design and Guppy fishes were introduced into experimental setups

### 3. Results and discussion

The effect of fresh petal aqueous extract of *Nelumbo nucifera* and the chemical drug of diazepam fine powder will affect the locomotory behavior of *Poecilia reticulata* during the study period (Table, 1). The comparison of the hypnotic effect in *Nelumbo nucifera* fresh petal aqueous extract, and the chemical drug of diazepam fine powder on *Poecilia reticulata* was analyzed for 10 days (Table 2), and time intervals on locomotory behavior of *Poecilia*

*reticulata* on the exposure of *Nelumbo nucifera* fresh petal aqueous extract and the chemical drug of diazepam fine powder and their average values are depicted on Table 3.

**Table 1.** The effect of lotus fresh petal aqueous extract and diazepam powder on *Poecilia reticulata* to analyze the locomotory behavior for a period of 10 days.

Days	Groups				
	Group I	Group II	Group III	Group IV	Group V
Day 1	20.23±1.05	21.22±0.35	48.81±2.32	29.12±1.02	65.77±4.50
Day 2	26.00±0.43	35.39±2.45	57.97±3.46	33.00±0.21	-
Day 3	48.58±6.41	23.50±1.02	39.61±2.30	32.05±1.25	26.00±2.65
Day 4	31.82±1.35	21.01±2.31	46.68±0.46	30.00±0.50	27.00±3.02
Day 5	29.40±1.32	30.34±2.42	23.50±0.21	39.56±1.12	29.50±0.54
Day 6	28.50±0.36	44.86±3.45	27.03±2.41	68.35±5.03	34.5±0.24
Day 7	49.00±4.21	37.69±3.52	47.50±2.84	30.50±2.42	29.00±1.32
Day 8	37.94±3.65	25.59±2.64	34.50±0.13	-	52.50±3.75
Day 9	44.00±2.41	42.77±0.32	51.41±3.46	30.81±2.89	27.96±1.47
Day 10	34.04±1.98	46.08±3.79	45.66±2.98	34.50±2.01	30.50±2.42

\* Group I : Control fishes; Group II : Lotus fresh petal extract (100mg/L) + Guppy Fishes; Group III : Lotus fresh petal extract (200mg/L) + Guppy Fishes; Group IV: Lotus fresh petal extract (500mg/L) + Guppy Fishes; Group V: Diazepam (5mg/L) + Guppy Fishes

**Table 2.** The Hypnotic Effect of *Nelumbo nuifera* and diazepam powder on *Poecilia reticulata*, was analyzed during the study period.

Days	Groups				
	Group I	Group II	Group III	Group IV	Group V
Day 1	1	2	-	2	1
Day 2	2	1	1	3	3
Day 3	1	2	1	2	2
Day 4	1	2	1	2	2
Day 5	1	1	2	3	2
Day 6	2	1	1	3	2
Day 7	2	-	-	2	2
Day 8	-	-	2	3	2
Day 9	2	-	-	1	1
Day 10	-	1	-	2	2

\* Group I: Control fishes; Group II: Lotus fresh petal extract (100mg/L) + Guppy Fishes; Group III: Lotus fresh petal extract (200mg/L) + Guppy Fishes; Group IV : Lotus fresh petal extract (500mg/L) + Guppy Fishes; Group V: Diazepam (5mg/L) + Guppy Fishes.

**Table 3.** Exposure of *Nelumbo nucifera* fresh petal aqueous extract and diazepam powder on *Poecilia reticulata* to analyze the time intervals and locomotory behavior during the study period.

Groups	No. of fishes escaped within the range of 180 seconds						Total no. of trials
	0-10 seconds	11-30 seconds	31-60 seconds	61-120 seconds	121-180 seconds	Not escaped	
Group I	10	7	8	4	5	06	40
Group II	1	5	6	10	6	12	40
Group III	1	4	6	7	7	15	40
Group IV	0	5	5	7	3	20	40
Group V	1	2	6	6	5	19	40



\* Group I : Control fishes; Group II : Lotus fresh petal extract (100mg/L) + Guppy Fishes; Group III : Lotus fresh petal extract (200mg/L) + Guppy Fishes; Group IV : Lotus fresh petal extract (500mg/L) + Guppy Fishes; Group V: Diazepam (5mg/L) + Guppy Fishes.

The present experiment was conducted for 10 days because continuous assessment is needed for scientific problems and they must contain high accuracy of data, therefore we selected 10 days and the 10 days is enough for the assessment of locomotory behavioural activity on guppy fish. In this present experiment, *N. nucifera* fresh petal extract in three different concentrations was chosen based on the previous report by Thamilarasi, 2018, Lazzarini et al. 1996, and Mukherjee et al. 2019, and they were tested against the guppy fish, assess the potential hypnotic effect and the results suggest that the fresh petal extract possesses a valuable hypnotic effect. Thamilarasi, 2018 revealed the anti-stress effect of lotus fresh petal effect on Wistar albino male rats. Sugimoto et al. reported that the methanolic extract of the embryos of *Nelumbo nucifera* seeds significantly inhibited locomotory activity in mice (Thamilarasi, 2018, Kim et al. 2006, Lazzarini et al. 1996). Rai et al. and Sugimoto *et al.* reported that embryos of seeds significantly decreased the rectal temperature in mice and it prolonged their sleeping time also. Therefore, the present study was undertaken to reveal the hypnotic effect of lotus fresh petal aqueous extract on the wells known as Guppy fish (Liu *et al.* 2004).

Guppy fish served as a subject for numerous behavioural studies related to predator avoidance mechanisms and evolution-related studies (Stevens et al. 2017, Chambel et al. 2015). The guppies are well known for their swift swimming and their very activeness. In the present investigation, guppies were exposed to fresh petal aqueous extract. After an assessment of the hypnotic effect, they were carried out to analyze locomotory behaviour. During the study period, the time taken for the escape behaviour from the beaker was noted. The present findings revealed that the fresh petal extract of lotus-exposed guppies took longer time than the control. Among the three concentrations of fresh petal extract of lotus, only 500 mg/L treated fish was observed higher time taken than the other groups when compared to 100 mg/L and 200 mg/L. Similarly, diazepam-treated guppy fish also took longer time than the control. The 500mg/L of fresh petal extract and diazepam treated on guppy fish exerted a similar kind of effect on the locomotory behaviour of guppy fish. This indicates that



diazepam and lotus fresh petal extract contains a strong sedative effect (Mukherjee et al. 2019, Rakotonirina et al. 2001). The total number of guppies that were not escaped from the beaker within the stipulated time that is 180 seconds was calculated, and it was noted that the maximum number was observed in the fresh petal extract of 500 mg/L such as 20 out of 40 exposed that indicates the strong sedative effect of fresh petal extract. The diazepam of 5mg/L is also reported to have a higher number of non-escaped fish (9 out of 40), and 200mg/L, and 100 mg/L are also very closely near to it. The locomotory behaviour of the guppies was highly affected in this experiment due to the exposure of lotus fresh petal aqueous extract, and it is clear that seeds of the lotus embryo and flower may also possess some compounds and due to its effect, the escaping behaviour of the guppies was significantly affected. Plants containing compounds such as flavonoids, terpenes, and saponins have been found to have a hypnotic effect (Wang et al. 2021). Behavioural studies have shown that several Methoxyphenols and alkylphenols have hypnotic and anticonvulsant properties (Jahani et al. 2019). In the present study, lotus fresh petal extract on guppy fish showed less activeness, decreased mobility, and no escapism from the beaker, it was very clear that the lotus fresh petal extract also exerts the same kind of effects on the central nervous system. It has been reported that Saponins could have effects like sedative and hypnotic (Shen et al. 2021). Some others have found that Eugenol has anti-convulsant, analgesic, and local anesthetic effects (Jo et al. 2021). Many findings have confirmed the therapeutic nature of the lotus plant. The lotus contained some secondary metabolites such as, coumarins, flavonoids, sterols, saponin, alkaloids, anthraquinones, tannins, terpenes, and cyanogenic glycosides are responsible for hypnotic effects. The lotus fresh petal aqueous extract contained of nuciferine, roemerine, anonaine, pronuciferine, and N-nornuciferine. Two benzyloquinoline alkaloids, (+)-1(R)-coclaurine, and (-)-1(S)-norcoclaurine, were also found in leaf extract of *N. nucifera*, and these compounds are responsible for the sedative effect on guppy fish. In addition, six non-phenolic bases were identified: roemerine, nuciferine, anonaine, pronuciferine, N-nornuciferine, and liriodenine, and two phenolic bases, artemepavine and N-methyl-coclaurine, were also found in *N. nucifera* leaf extract. The leaves also contain a glycoside, nelumboside, and flavonoids such as quercetin, and leucoanthocyanidin which were identified as leucocyanidin, and leucodelphinidin. The presence of some other flavonoids in the leaves such as quercetin 3-O- $\alpha$ -arabinopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -

galactopyranoside, quercetin-3-O- $\beta$ -D-glucuronide (32), rutin (33), (+)-catechin, hyperoside, isoquer-citri, and astragalin has also been reported by Kashiwada, et al. (2005) and Ohkoshi, et al. (2007). In this experiment, the comparison hypnotic effect of a fresh petal extract of lotus and the commercially available chemical drug diazepam with a simple experimental procedure. The present study reveals that both diazepam and lotus petal extract 500 mg/L possess a strong hypnotic sedative effect. Since the diazepam drug exerts side effects, lotus petal naturally suits as an alternative to the chemical drug of diazepam. The beneficial role of plants in health care, sustainability, economic, environmental friendliness, and ecological restoration have compelled mankind to shift to the use of medicinal plants to solve various global issues like chronic disorders, and neurological diseases such as Alzheimer's (Temviriyankul et al. 2020), and also in the management of several environmental issues including pollution, disease outbreaks, epidemics, and global warming. Results of the present study support the hypothesis that lotus fresh petal aqueous extract possesses a strong hypnotic effect on guppy fish.

#### 4. Conclusion

In the present study, lotus fresh petal aqueous extract was taken in three different concentrations such as 100mg/L, 200mg/L, and 500mg/L and diazepam 5 mg/L were tested against Guppy fish, for the potential hypnotic effect, and the results from the study revealed that the fresh petal extract of lotus was possessed a strong hypnotic effect, and the guppies were exposed to fresh petal extract. During the study period, locomotory behaviour was also analyzed, and the time taken for the escaping behaviour from the beaker was noted. The present study concluded that the fresh petal extract exposed to guppies took longer time than the control, and diazepam was also near close to it. The locomotory behaviour of the guppies was highly affected in this experiment due to the exposure to lotus fresh petal aqueous extract, and it is clear that *N. nucifera* fresh petal extract has a strong sedative effect, and the escaping behaviour of the guppies was significantly affected. The present experiment reveals the fact of *Nelumbo nucifera* is naturally suited as an alternative to the chemical drug diazepam.

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