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## Memory enhancing effect of *Nelumbo nucifera* seedpod extract and its active component quercetin in noise stress-exposed rats

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### ABSTRACT

Stress is an unavoidable phenomenon which disturbs the physiological homeostasis. Noise is the highest encountered stressor that affects the well-being of mankind. Hippocampus, a region of the brain crucial for memory is highly influenced by stress and thereby deteriorates memory. Male albino Wistar rats of 11-12 weeks were used in this study. *Nelumbo nucifera* (NNE) 400 mg/kg body weight (BW) and its active principle Quercetin (QRCT) (40 mg/kg bw) were administered orally to the animals 10 days before noise stress exposure of (100dB 4h/d). Ability to retain information by Learning and spatial memory was assessed using eight-arm radial maze apparatus. It was observed that after noise stress exposure errors in working, reference memory and time period to move into all baited arms were increased; however, the drug pre-treated animals showed a decrease in all the above parameters. The observations in the present work have proved that NNE and QRCT groups were effective in ameliorating the noise stress-induced changes, but the active component quercetin group had a higher protective impact on the changes.



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### INTRODUCTION

Stress is the reaction of the organism to a stimulus which disturbs the physiological equilibrium. It was shown that noise increases stress reaction which influences various body systems. All living organisms are being affected by acute or chronic stressful events in their entire span of life. The stressor has an impact on stressful experiences affecting the brain, cognition and behaviour. Noise

is a stressor which is the most widespread environmental source which induces irritability and influences cognitive processing (Smith *et al.*, 1983) mental and physical health (Clark, 1984). Background noise impairs one's ability to concentrate and can severely disturb our daily activities. The brain recognizes the sound levels reacts within seconds by releasing hormones and helps the body to adjust and overcome the stressful situation (Samson *et al.*, 2006).

Attention is the ability of the brain in processing information, and working memory is retaining learnt information in an accessible state are the important cognitive capabilities of the brain. The process of relationship between these is not well understood (Miyake, A., 1999) though there had been many studies done to assess it. Noise influences the cognitive abilities of the brain amongst which problem-solving, attention, work, and memory are more strongly affected.

Numerous studies in animals have shown that noise exposure causes an impact on spatial memory. HPA axis dysregulation occurs due to chronic stress which causes a decrease in spatial memory as opposed by direct impact on hippocampal morphology. Hippocampus has a complementary role to the mPFC and in that retrieval of recent memories is strongly activated by HIP which is also supported by other studies (Frankland *et al.*, 2004; Takashima *et al.*, 2006b, Maviel *et al.*, 2004; Takehara *et al.*, 2003).

Nelumbo nucifera Gaertn or sacred lotus is of family Nelumbonacea, used in Chinese, Thai, Indian and system of medicine. Many parts of NN are edible like rhizome, leaves, seed and flower which is being consumed by countries like Japan, South Korean and Asian. Various diseases like infection, diarrhoea, body heat imbalance, hypertension, cancer and so on are treated with different parts of NN (Sridhar and Bhat 2007). In vitro data shows that the NNE and its active component QRCT exhibited strong antioxidant activity. Based on this, the study was to investigate memory enhancing property of NN and its active component QRCT in noise-exposed rats by radial maze apparatus.

## MATERIALS AND METHODS

### Experimental Design

Experimental procedures were approved by the Institutional Animal Ethical Committee (IAEC) (TMC/IAEC/01/003), Tagore Medical College and Hospital, Chennai, India and Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. Experimental animals were Male albino rats (Wistar strain) of 11 -12 weeks of age was maintained under standard laboratory conditions with food and water ad libitum. Animals were divided into twelve groups with each group containing six rats.

The different groups, drug injected, doses, and stress procedure are given in Table 1.

### Preparation of the Nelumbo nucifera seedpod Extract

The NN seedpod extract was prepared by the methods (Lu and Foo 2001, Finose and Gopalakrishnan 2014 (11, 12). The extract was prepared by taking 15g of dried fine powder of NN seedpod and extracting it with 150ml ethanol (75%), acetone, and aqueous extract for 1min using Turax mixer (13, 000rpm) and soaked overnight at room temperature.

In a Buchner funnel Whatman No. 1 filter paper was kept, filtered and the filtered solution was evaporated under vacuum by using a rota-

evaporator at 40°C to a constant weight finally dissolved in respective solvents. The concentrated extracts were stored in airtight containers below 10°C.

### Noise Stress Procedure

White noise generator (0-26 kHz) produces a sound which is amplified by an amplifier (40W) connected to two loudspeakers (15W) located 30cm above the animal cage. The intensity of sound was assessed by a Sound level meter and maintained at 100 dBA intensity (Archana and Namasivayam 2007).

The Ventilation system in the room produced a background noise of  $44 \pm 2$  dBA. Animals were kept in noise chambers (100 dBA 4h/d). The level of noise detected in Discotheque, and some industrial workplaces were 100dBA which was chosen as noise level (Cohen and Weinstein 1981).

### Spatial memory testing

**Eight-arm radial maze (EARM):** Preparation of animals for the Eight-arm radial maze (Figure 1).



**Figure 1: Eight-arm radial maze (EARM)**

The ability to learn and spatial working memory was assessed as described by (Spritzer *et al.*, 2011) (Endo *et al.*, 1996; Nishimura *et al.*, 1999) by using an apparatus designed for evaluating memory such as radial maze apparatus. The apparatus has an octagonal central platform, 33.5 cm wide, around which are arranged 60 cm long by 12 cm wide arms made of gray vinyl chloride plates installed in a soundproof room. Prior to training rat diet was maintained to achieve 85% ad libitum weights. Food reinforcement was given by using Chocolate flavoured crisp rice cereal. Animals were acclimatised for the environment for nearly 3 days in the maze for a period of 10 minutes each day. To initiate the training procedure food pellets were scattered throughout the maze on the first day so that the animals can explore the apparatus and followings which food was placed in small cups. The rats were allowed to adapt themselves every

day with food for nearly 15 days between 10:00 a.m. and 12:00 p.m. h (n =6).

After adaptation, the rats were trained by placing them in the central platform to find out a piece of cereal kept in four (baited) of the eight arms. The room had many visual cues kept in appropriate places. The training procedure was completed after the rats finished moving into all the four baited arms or after a duration of 10 min.

### Parameters assessed

- Errors in reference memory (ERM), i.e. each entry into arms without food
- Errors in working memory (EWM), i.e. each, re-entries into already visited baited arms.
- The duration in seconds for moving in baited arms.

The training session for rats was 25–28 until a criterion of 0–2 errors was reached then followed by exposure to noise stress. Retrieval of spatial memory task was assessed during noise stress procedure on the 1st, 5th, 10th, 15th, 20th, 25th and 30th day of the noise stress exposure (after an hour) and control animals.

## RESULTS

### Interpretation

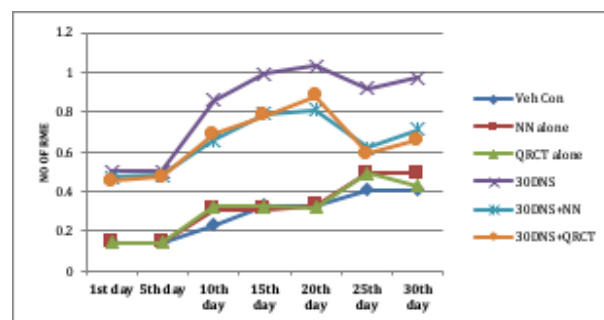
The errors in reference memory (ERM) increased on the 1st day (P<0.05) 5th day (P<0.05), 10th day (P<0.001) 15th day (P<0.05) 20th day (P<0.001) 25th day (P<0.001) 30th day (P<0.05) of stress exposure rats compared to vehicle control group. Treatment with QRCT alone and NNE alone to non-stressed animals showed no significant changes in reference memory errors when compared to control animals. The noise-exposed animals, which were treated with QRCT showed fewer difference errors in working memory (ERM) (P<0.05) on the 1st, 5th, and 10th day whereas on the 15th day (P<0.001) significant difference were observed in QRCT treated rats compared to that of stress control rats. The drug control groups showed significant difference similar to that of the vehicle control group as compared with stress-exposed rats.

The EWM increased on the 1st day, 5th day, 10th day, 15th day, 20th day, 25th day but not significant whereas on the 30th-day significant difference was observed (P<0.001) of noise stress exposure rats compared to control group. Treatment with QRCT alone and NNE alone to non-stressed animals showed no significant changes in EWM when compared to control animals. Animals exposed to noise which were treated with QRCT showed no significant difference in the EWM on the 1st and 10th, 15th, 20th, 25th and on the 30th day (P<0.05)

significant difference were observed compared to that of stress control rats. The noise-exposed animals, which were treated with NNE showed no significant changes in the EWM on the 1st, 5th, 10th, 15th, 20th day whereas on the 25th day (P<0.02), 30th day (P<0.05) significant difference were observed compared to that of stress control rats. The drug control groups showed a significant difference (P<0.001) similar to that of the normal control group as compared with stress-exposed rats.

The time period taken to visit all baited arms increased on the 1st day (P<0.05) 5th day (P<0.05), 10th day (P<0.01) 15th day (P<0.001) 20th day (P<0.001) 25th day (P<0.001) 30th day (P<0.001) of noise stress exposure rats compared to control group. Treatment with QRCT alone and NNE alone to non-stressed animals showed non-significant changes in the time taken to visit the final arms in comparison to control animals. The noise-exposed animals, which were treated with QRCT showed the difference (P<0.05) in the time taken to reach the baited arms on the 1st and 10th, 15th, 20th, 25th and 30th day (P<0.001) significant difference were observed compared to that of stress control rats. The noise-exposed animals, which were treated with NNE showed the non-significant difference in the time taken to reach the all the arms on the 1st, 5th, and 10th day whereas on 15th, 20th, 25th, 30th day (P<0.001) significant difference were observed compared to that of stress control rats. The drug control groups showed significant difference similar to that of the saline control group as compared with stress-exposed rats.

## DISCUSSION



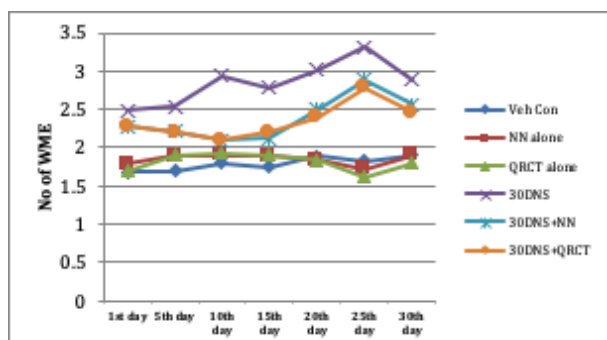
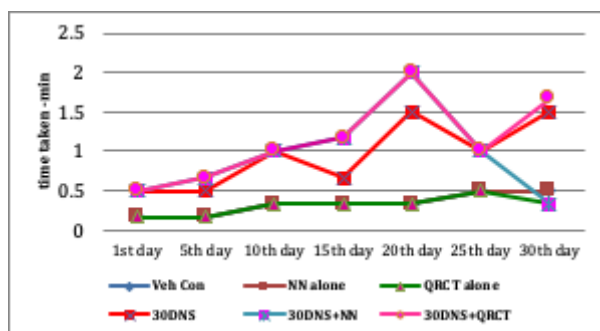
**Figure 2: Effect of NNE and QRCT on RME**

Stress can be beneficial to the organism and hence biologically important to normalise the system to some extent. The importance of both the beneficial and deleterious effects of acute and chronic stress on cognitive function such as learning and memory have been studied by many authors, which has formed a basis to unveil the role of stress on the hippocampus (McEwen 2007, De Kloet *et al.*, 2005, Lupien *et al.*, 2009, Howald 2008). It was observed

**Table 1: Experimentation group**

S.No	Experimental groups	Drug injected	Dosage of drug Volume/dose	No of days	Noise stress (4h/d)
1	Vehicle Control	Saline	1ml/day	40	No
2	NN alone	NN	400mg kg <sup>-1</sup> (ml day <sup>-1</sup> )	40	No
3	QRCT alone	QRCT	40mg kg <sup>-1</sup> (ml day <sup>-1</sup> )	40	No
4	1 DNS	Saline	1ml/day	1	Yes
5	NN alone + 1DNS	NN	400mg kg <sup>-1</sup> (ml day <sup>-1</sup> )	1	Yes
6	QRCT alone+1DNS	QRCT	40mg kg <sup>-1</sup> (ml day <sup>-1</sup> )	1	Yes
7	15 DNS	Saline	1ml/day	15	Yes
8	NN alone + 15DNS	NN	400mg kg <sup>-1</sup> (ml day <sup>-1</sup> )	15	Yes
9	QRCT alone+15DNS	QRCT	40mg kg <sup>-1</sup> (ml day <sup>-1</sup> )	15	Yes
10	30 DNS	Saline	1ml/day	30	Yes
11	NN alone + 30DNS	NN	400mg kg <sup>-1</sup> (ml day <sup>-1</sup> )	30	Yes
12	QRCT alone+30DNS	QRCT	40mg kg <sup>-1</sup> (ml day <sup>-1</sup> )	30	Yes

that, rats exposed to noise showed a significant impairment in memory tasks, from day one of exposure and subsequently following 15 and 30 days. It was evident from the increase in error in reference memory (Fig-2), working memory (Fig-3) and time duration to visit all the baited arms (Fig-4). Similar reports were also made by the previous studies (Manikandan *et al.*, 2006, Samson J *et al.* 2007, Chengzhi *et al.*, 2011).

**Figure 3: Effect of NNE and QRCT on WME****Figure 4: Effect of NNE and QRCT on time taken to visit baited arms**

In this study, Plasma corticosterone level was significantly increased in all the noise-stress groups (1 day, 15 days and 30 days) animals. Pretreatment with NNE and QRCT in rats could able to overcome the stress-induced loss in memory, and this was evident from their increase of performance in a memory task. This is also an indicator of the neuroprotective role of the NNE

and QRCT in preventing the increase in the level of corticosterone due to its anxiolytic activity. Manikandan *et al.* 2005, Thawatchai Prabsattroo *et al.*, 2016 have attributed the loss of memory in noise stressed rats to the increase corticosterone levels in their study.

Recently it was shown in a study that HIP of rats is affected by oxidative stress by injecting corticosterone (Sato *et al.* 2010), atrophy of HIP occurs when more amounts of corticosterone are released by strong stress (Hoschl C and Hajek 2001). The above studies show that the increase in corticosterone might induce oxidative stress which leads to tissue damage. The important role of QRCT metabolites is that they not only scavenge free radicals directly but also regulate the signalling cascade mechanism. In a recent study, it was demonstrated that oral administered action of QRCT affected HPA axis response via an increase in MAP kinase signalling in hypothalamus and decrease in corticosterone (Kawabata 2010).

Glucocorticoids modulate the expression of NMDA receptors in the hippocampus which is cause for the plasticity, atrophy and neuronal death HIP (McEwen 1999). Due to these contributing factors which can cause atrophy of neurons in mPFC and HIP may lead to memory impairment in a noisy environment.

## CONCLUSION

*N. nucifera* extract act as a cognitive enhancer agent against stress-related brain damage and memory deficit and hence has a neuroprotective role. The importance of the drug is associated with improved oxidative stress status. Thus they can also provide benefits as neuroprotection and memory enhancer against stress-induced memory deficit.

Mostly the naturally available drugs are cost effective and easy to avail. Therefore they may

serve as an alternative potential neuroprotective and memory enhancing agent.

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