Enhancement of memory in rats with *Centella asiatica*

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**Abstract**

Numerous medicinal plants are mentioned in ancient Indian literature as cognition enhancers. Among these plants, Centella asiatica (CeA) has been used to improve memory. This study demonstrated the effect of CeA on learning ability and memory in male Wistar rats. The rats were trained at various periods by intragastric administration of CeA to the test group at a dosage of 2mg / 0.5 ml of distilled water/day (12mg/ kg body weight) for 10 days and compared with the control group by an Operant conditioning technique, n = 6 in both groups. The two important parameters studied were latency period and the total number of bar pressings which correspond to memory retention and task learning respectively. The results were analyzed using the paired Student’s t-test. The CeA treated group showed a significant decrease in the latency period as compared to controls which suggested an improvement in retaining the learnt task as good retention of memory (P < 0.05). The number of bar pressings did not change significantly in the test group, due to lack of CeA action on the learning process. The results conclude that the CeA had facilitated the retention of a learnt task for a longer period as good retention of memory but did not accelerated the learning process as expected.

**Key words:** Centella asiatica (CeA), Operant conditioning technique, Learning, Memory, Latency period

**Introduction**

There are several thousand drug yielding plants all over the world, which are useful for Ayurvedic treatment. Most of the medicinal plants are known and utilized by Tabibs (herbal physicians) and Ayurvedic Vaids (physicians). Among these plants Centella asiatica (CeA) has been an important constituent of the Ayurvedic Materia Medica. This is commonly known as Vallarai in Tamil, Mandukaparni in Sanskrit and the Indian pennywort in English [1].

Leaves of CeA are used in medical aids, which improve general mental ability of mentally retarded children [2], memory enhancement [3] and as a tonic to improve memory [4]. Most of the studies were also carried out on neonatal rats to view the active brain growth with CeA [5].

Many Experiments have been conducted to prove the effect of CeA on memory retention through Y-maze and T maze methods which are widely used for discrimination learning and spatial alternation tasks [6]. The present study used an Operant conditioning technique [7], which provides a precise and reliable method for controlling behaviour. The effect of the CeA on memory was analyzed by training the animals at various periods with and without the administration of CeA.

**Materials and methods**

Experimental animals were male Wistar rats weighing between 150 and 180gms. The rats were maintained under standard laboratory conditions with 25°C, 40% relative humidity and 12 h dark/light cycle and allowed to have adequate food and water. The rats were divided into two groups, control (n = 6) and test (n = 6).

**Dosage of drug**

The commercially available CeA leaf powder was used, which was administrated intragastrically (i.g) to the test group, at a dosage of 2mg / 0.5 ml of distilled water/day (12mg/ kg body weight) for 10 days. The control group received equal volume of saline vehicle.

**Operant conditioning method**

The development of operant conditioning procedure is closely associated with the experimental analysis of behaviour by Skinner and his associates [8, 9].
Mode of assessment
Operant conditioning test was performed under appetitive motivation in 24 hours food-deprived rats with food as a reward while training the animals. The test was carried out on alternate days. The test group received the drug from the first day onwards. After ten days of training, the drug was discontinued for the test group and the training was also discontinued for both groups. This assessment was repeated on the 21st, 31st, 41st, and 51st day.

Apparatus
Perspex box was 30 X 25 X 20cm with a 2cm diameter circular opening on the roof. A 20cm long, slanting tube with 1.5cm inside diameter was connected from the outside to this circular opening, for introducing food pellets into the box. In this apparatus, there was a balance like arrangement over the roof, with weights hanging on the outside and perspex flat bar placed inside.

Procedure
A bar pressing is a simple model of operant behaviour. In this method a hungry rat was placed in a box in which turn spontaneously pressed it and became more frequent when followed by reinforcement. Often the untrained rat was shaped to perform the desired bar pressing response by the procedure of successive approximation, because food was delivered whenever the hungry rat pressed it to get the reward.

Parameters studied
Latency period
The time taken by the animal for the first bar pressing, from the time it was introduced into the Operant conditioning box. This corresponds to memory retention, (Figure 1 and Figure 2).

Total number of bar pressings
Total number of bar pressing for 15min by each rat. This corresponds to the learning process, (Figure 3 and Figure 4).

Statistical analysis
All values are given as means ± SEM. The results were analyzed using a paired Student’s t-test. Probability value of P < 0.05 was considered statistically significant.

Results
Comparison of Latency period between the control and the test group on the initial day as well as on the 10th day (Figure 1): The values for latency period of the control group on the initial day and 10th day were 172 ± 45 sec and 145 ± 39 sec. The test group showed a significant decrease of latency period value while comparing the initial day with the 10th day, i.e., 200 ± 48 sec to 92 ± 21 sec (* P < 0.05 with paired Student’s t - test, mean ± SEM, n = 6).

Comparison of latency period between the control and the test group on the 21st, 31st, 41st, and 51st day (Figure 2): The latency period was found to be significantly lower in the test group as compared to controls on the 21st, 31st, 41st, as well as on the 51st day, the values were given respectively 178 ± 43 sec and 52 ± 5 sec; 345 ± 86 sec and 132 ± 12 sec; 676 ± 94 sec and 60 ± 2 sec; 820 ± 74 and 23 ± 4 sec (*P < 0.05 with paired Student’s t - test, mean ± SEM, n = 6).

Comparison between the control and the test group on the initial day as well as on the 10th day for control and test (Figure 3): Total number of bar pressing on the initial day of control and the test were 2 ± 0.6 / 15min and 3 ± 0.8 / 15min. The values on the 10th day for control and test were 5 ± 1 / 15min and 4 ± 1 / 15min (mean ± SEM, n = 6).

Comparison between the control and the test group on the 21st, 31st, 41st, and 51st day (Figure 4): Total number of bar pressing was compared between the control and the test on 21st, 31st, 41st and 51st day. The values were given respectively 6 ± 2 / 15min and 7 ± 1 / 15min; 5 ± 0.6 / 15min and 5 ± 0.6 / 15min; 4 ± 0.6 / 15min and 7 ± 2 / 15min; 1 ± 0.2 / 15min and 7 ± 2 / 15min. Total number of bar pressing is significantly high on 51st day (*P < 0.05 with paired Student’s t - Test, mean ± SEM, n = 6).

Figure 1. Latency period of the Centella asiatica treated test group was compared to the control on the initial and 10th day. The Centella asiatica treated test group showed a significant decrease of latency period on the 10th day than control (*P< 0.05 with paired Student’s t- test; n=6).
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Figure 2. The latency period of the Centella asiatica treated rats was compared to controls on the 21<sup>st</sup>, 31<sup>st</sup>, 41<sup>st</sup>, and 51<sup>st</sup> day. The latency period of the Centella asiatica treated test group was significantly reduced when compared to controls on the 21<sup>st</sup>, 31<sup>st</sup>, 41<sup>st</sup>, as well as on 51<sup>st</sup> day as a good retention of memory (*P< 0.05 with paired Student’s t-test; n=6).

Figure 3. Total number of bar pressings of the Centella asiatica treated rats was compared with the controls on the initial and 10<sup>th</sup> day. Total numbers of bar pressing were not significantly different from each other on the initial day as well as on the 10<sup>th</sup> day (n=6).

Figure 4. Total number of bar pressings of the Centella asiatica treated group was compared with the controls on the 21<sup>st</sup>, 31<sup>st</sup>, 41<sup>st</sup>, and 51<sup>st</sup>. A significant increase was found in the test group on 51<sup>st</sup> day (*P< 0.05 with paired Student’s t-test; n=6).

Discussion

The important higher functions of the nervous system are learning and memory. These two are associated with intellectual processes in the cerebral cortex.

Learning refers to the more or less permanent modifications of behaviour which occur as a result of practice, experience or observation. Memory is the ability to record life experiences, to change one’s behaviour according to environmental conditions. Memory is the mental ability that enables one to retain and to recall through unconscious associative processes, previously experienced sensations, impressions, ideas, concepts, and all information that has been consciously learnt [10].

Memory is composed of three processes, i.e. registration, consolidation and retrieval [11]. It is caused by changes in the capability of synaptic transmission from one neuron to another as a result of previous neural activity. Because of consecutive changes new pathways would develop, for conduction of signals through the neural circuit of the brain. These new pathways are called memory traces.
ter being established they can be activated by active thinking to reproduce the memories [12].

Storage of memory may be localized in the temporal lobe, the hippocampus, and certain midline diencephalic structures such as the dorsomedial nucleus of the thalamus [5]. Rao et al., [13] showed that the CeA fresh leaf extract has neuronal dendritic growth-stimulating properties. There was a significant increase in the dendritic length and branching points in hippocampal CA3 neuronal dendritic arborization. Treatment with fresh leaf juice from CeA would bring about structural changes in the hippocampal CA3 pyramidal neurons in young growing rats.

In this study the comparison of latency period between the control and the test group on the initial day as well as on the 10th day that the results demonstrated the good retention of memory with the CeA treated test group by significantly decrease in the latency period on the 10th day (Figure 1). Similarly on the 21st, 31st, 41st, and 51st days also proved the significant improvement of retaining the learnt task as 10th day (Figure 2). This suggests an enhancement of retaining the learnt task or an increased consolidation of memory by the phenomenon of rehearsal.

On the other hand, the comparison of total number of bar pressing on the initial day as well as on the 10th day which did not show any significant change between the control and test group. This shows that the CeA has not accelerated the learning process on the 10th day (Figure 3). While comparing on the 21st, 31st, 41st, and 51st day, there is no significant change between the groups till 41st day. Somehow the significance was appreciated only on the 51st day; further study is required to explain this phenomenon (Figure 4). This result illustrates that CeA has not accelerated the learning process.

Conclusion

The above results illustrated that the CeA has not accelerated the learning process, but rather significantly facilitated the retention of learnt task as a good retention of memory for longer period which provides the further support for the earlier reports on cognitive enhancing ability of CeA.

Acknowledgements

I am grateful to late Dr. A. Namasiyayam, former Professor and Head, Department of Physiology, Dr. ALM PG IBMS, Taramani, Chennai – 113, for his valuable guidance and suggestion in this research work during the course of study.

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