Research Article

Effect of *Cyperus rotundus* Root Extract in Midazolam Induced Memory Loss in Mice

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ABSTRACT

The mysterious organ called Brain receives information through five senses and interprets it and store it in the form of memory. Various pats and lobes are for different functions. Different areas of the brain are responsible for different types of memory. In such case by taking Indian medicinal system into consideration the current work performed to examined the effect of *Cyperus rotundus* roots ethanolic extract on Midazolam induced acute memory loss. Evidence concerning states that Midazolam can be used postoperatively to forget the pain, in the dose of 2 mg/kg Midazolam can induce acute memory loss and *cyperus rotundus* is traditionally used as a memory enhancer. In performed work by using Elevated plus maze apparatus for mice the Transfer latency was measured at the time of acquisition, consolidation and retrieval in mice in arranged groups. It was found that the ethanolic extract of *cyperus rotundus* significantly decreases the Transfer latency in Elevated plus maze for mice which measures the increase in memory in at the time of retrieval. The goal of the study is use of *cyperus rotundus* alternatively to the allopathic medicines as a memory enhancer but it requires more work to understand the responsible chemical constituent and mechanism of action for further development of the drug.

Keywords: midazolam, memory loss, *Cyperus rotundus,* nootropic.

INTRODUCTION

Memory is very important part of Cognition for which the brain plays interesting games of neurotransmitter with billion of neurons. The limbic system constitutes the networks which works for memory. Different types of memory is associated with different parts of the brain.[1] Short term memory is associated with prefrontal cortex, Long term memory occurs in Hippocampus and temporary lobe, Skill memory processed in cerebellum. The disturbance in such area leads to amnesia and hence memory loss. There are many types of memory such as working, episodic, declarative etc. These types of memory can be studied by different animal models. Elevated plus maze was first used by Pellow in 1985.[2] Anti anxiety drugs can be studied by this apparatus, the general principles includes the more 'anxious' the subjects are, the less likely they will explore an uncomfortable, risky or threatening environment.[3] For memory the latency is the factor which can measured. The memory loss by Midazolam is one of the side effects of benzodiazepine anti – anxiety agents which is still controversial but it can use acutely postoperatively to forget the pain and which can cause amnesia or memory loss at the dose of 2 mg/kg due to long term potentiation of GABA. In humans, Benzodiazepine induced Anterograde amnesia have been emphasized by some authors.[4] *Cyperus rotundus* is used as nootropic from ancient times in india. It exerts Anti oxidant,[5] Anti diabetic,[6] Wound healing,[7] Anti diarrhoel,[8] Anti malarial etc activities. In present work we determined the effect of *Cyperus rotundus* extract on Aquisition, Consolidation and retrieval in midazolam induced memory loss in mice.

MATERIALS AND METHODS

Animals

Swiss albino mice was brought from serum institute, Hadapsar, Pune and housed in Sinhgad college of Pharmacy, Vadgaon, Pune. Animals were kept in the cages in the laboratory at approximately 24°C, under 12 h light/ dark cycle for a week before experimentation. Tap water and food pellets were available at libitum. For All procedures involving animals was approved by Institutional Animal Ethics Committee (SCOP/IAEC/2012-2013/32). All animals were native to the experimental apparatus and different animals were used for each test.

Elevated plus maze test

Spatial memory and anti anxiety drugs can be evaluated by Elevated plus maze apparatus. The maze was made up of wood and consisted of two open and two close arms such that two open arms were opposite to each other. The arms were connected by central platform. The principle of the test was the memory loss leads to the increase in the transfer latency of mice from open to close arm and memory enhancer drug should lead to the decrease in transfer latency.

To perform the procedure each mouse was placed at the end of the open arm facing towards the environment and the transfer latency i.e. the time taken by the animal to move in one of the closed arm (all four paws inside was measured. If the mouse did not enter in close arm in 90 sec it was eliminated from the study). Mice were allow to be in the maze for familiarization and returned to home cage. Experiment was conducted in the noon in natural light.[9]

Drugs

Midazolam was a gift from Wockhardt, Aurangabad. Midazolam was dissolved in the saline. Roots of *cyperus rotundus* extract was collected, authenticated from Agharkar institute, Pune. The extract of *Cyperus roundus* was prepared in ethanol by cold maceration process.

Drug administration

Animals were divided into the groups, Control group received the saline, in diseased control group received 2 mg/kg Midazolam, Treatment group 1 received *cyperus rotundus* ethanolic extract 100mg/kg and midazolam after 30 minutes, Treatment group 2 received extract 200mg/kg and midazolam. Midazolam was given i.p. and extract was administered orally.

Statistics

One way ANOVA followed by dunnett's multiple comparison tests was used to compare the difference between acquisition & consolidation and acquisition & retrieval on day and day 2. Also comparison was done between Acquisition, consolidation and retrieval in all groups on day 1 and day 2.

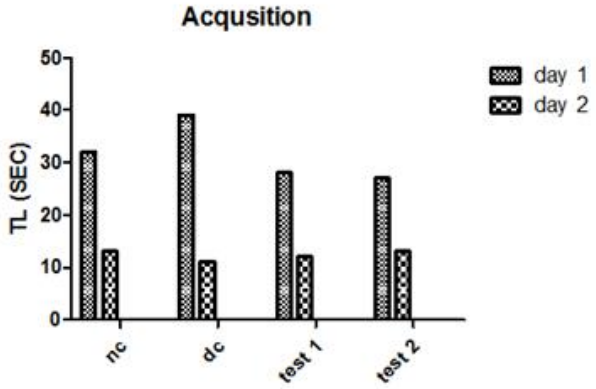

Figure 1: Effect on Acquisition in various groups

Effect of control, midazolam and cyperus rotundus in 100 mg/kg and 200 mg/kg on acquisition. P < 0.01 when acquisition on day 1 and day 2 were compared by two – way ANOVA.

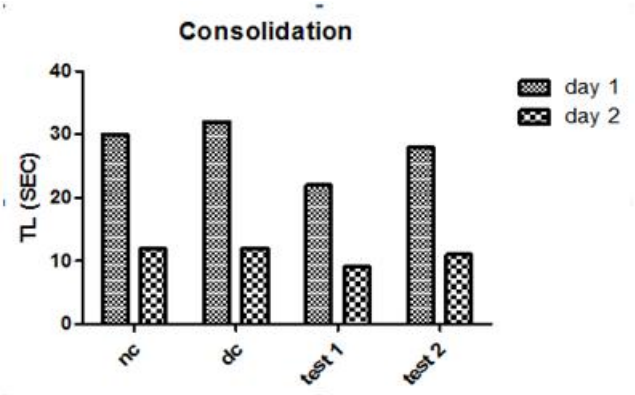

Figure 2: Effect on Consolidation in various groups

Effects of control, midazolam and cyperus rotundus in 100mg/kg and 200 mg/kg doses on consolidation. P< 0.01 when consolidation on day 1 and day 2 were compared.

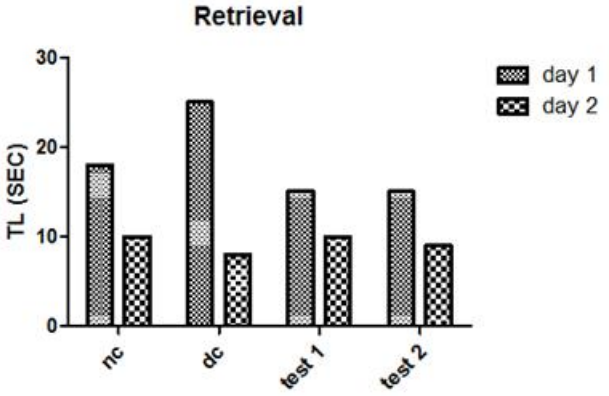

Figure 3: Effect on Retrieval in various groups

Effects of control, midazolam and cyperus rotundus in 100mg/kg and 200 mg/kg doses on Retrieval. P< 0.05 when consolidation on day 1 and day 2 were compared.

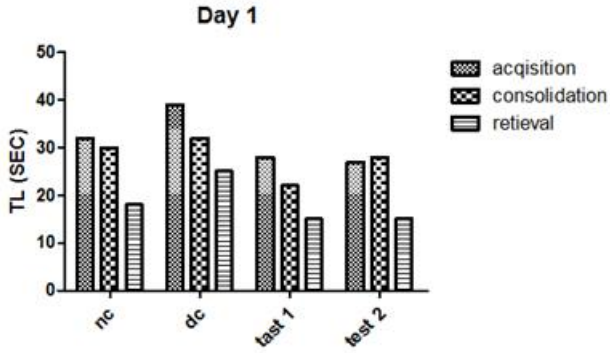

Figure 4: Effect on Acquisition, Consolidation and Retrieval in various groups on Day 1

Effect of control, midazolam, treatment groups on acquisition, consolidation and retrieval. By one – way ANOVA and Dunnett's test as a post test, P<0.05 when acquisition was compared to retrieval but the difference between the effect of acquisition and consolidation was not significant.

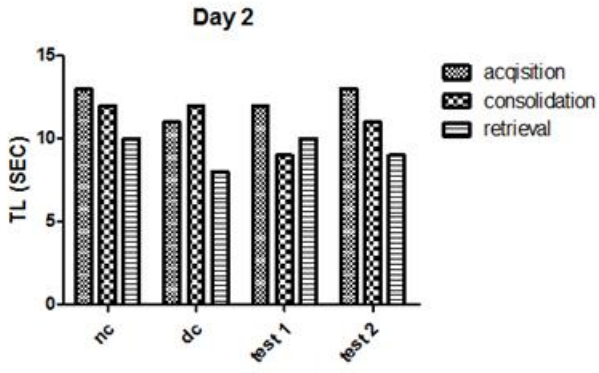

Figure 5: Effect on Acquisition, Consolidation and Retrieval in various groups on Day 2

Effect of control, midazolam, treatment groups on acquisition, consolidation and retrieval. By one – way ANOVA and Dunnett's test as a post test, P<0.05 when acquisition was compared to retrieval but the difference between the effect of acquisition and consolidation was not significant.

RESULTS

Graph pad prism 5 software was used to study and analyze the results which shows that there is significant difference at p< 0.01 in Acquisition, consolidation and p<0.05 retrieval in two days observation by using two-way Analysis of variance .

The increase in transfer latency in diseased control group that is Midazolam induced group shows the memory loss as compared to normal control group. The decrease in transfer latency in test 1(100mg/kg) and test 2 (200mg/kg) group than diseased control shows increase in memory.

There was significant difference p< 0.05 in acquisition and retrieval on day 1 and day 2 when analysed by ANOVA and Dunnett's test but there was not significant effect in acquisition and consolidation.

DISCUSSION

As per ancient medicinal systems, *cyperus rotundus* is considered to have a nootropic activity. It was proved that sub hypnotic doses of Midazolam affects the acquisition mechanism of conditioned taste aversion memory in rats, resulting the suppression of CTAM.[10] Atropine can be used against cold hypothermia induced by midazolam[11], Midazolam influences active avoidance , retrieval and acquisition rate in rats,[12] etc. From such study the antioxidant Cyperus rotundus[17] was investigated by us for its memory enhancing activity by using elevated plus maze and by induction of memory loss by Midazolam. The results analysed by Graph pad prism 5 shows the significant nootropic activity of the herbal drug after acute administration of Midazolam to induce memory loss. Transfer latency was the measure of memory during the experiment which decreases after in Midazolam induced group and increases significantly in *cyperus rotundus* treated group. It was concluded that *cyperus rotundus* shows significant nootropic activity on retrieval and non significant on consolidation. Total oligomeric Flavonoids from cyperus rotundus ameliorates neurological deficits, excitotoxicity, and behavioural alterations, induced by cerebral ischemic- reperfusion injury in rats[13], The fundamental assumption to use the EPM test for memory process evaluation is based on the behavioural principle that animals should learn and remember the location that provides them the sense of safety.[14,15] Aniracetam is one of the nootropic augments long term potentiation against midazolam[16]

CONCLUSION

The performed study proves the Indian traditional medicinal system which said nagarmotha as a memory enhancer and it can be used as alternative for short and long term memory or semantic memory[20] in the future, *Cyperus rotundus* needs more research on nootropic activity due to its neurobiological activities.[19]

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