Protective Effect of Ethanolic Extract of Curcuma Longa on Learning and Memory in Restrain Stressed Mice

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Abstract: This study aimed to evaluate the effect of ethanolic extract of Curcuma longa (CLE) on learning and memory in restrained stress induced cognitive dysfunction in male albino swiss mice. Eight groups of mice were treated with either Tween 80 or CLE (250 and 500gm/kg) orally for twenty eight days. Out of these six groups of mice were restrained concurrently in wire mesh for 5 hours daily during last 7 days of drug therapy. Following last stress session, the learning and memory was assessed by elevated plus maze (EPM) and passive avoidance paradigm (step through latency STL) test for all mice. CLE 500gm/kg significantly improved (p<0.001) learning and memory in chronic restrained group of mice on both the test models. The present findings indicate that ethanolic extract of CLE was effective in preventing the cognitive deficits in stress. This beneficial effect could provide a new dimension to the therapy of cognitive dysfunction.

Keywords: Curcuma longa, restrained stress, Cognition, Albino mice.

INTRODUCTION

In modern life style, stress is a common phenomenon and it has realized that stress is playing a causative role in precipitating several diseases. There are few studies which implicated that in laboratory animals, chronic stress is associated with morphological and functional alteration of brain structures (hippocampal neuron) involved in cognitive process Chronic stress induced cellular generation of reactive oxidative damage in neural tissues. Such a cumulative oxidative damage to brain (hippocampus) is a contributing factor for decrement in motor and cognitive performance in aging brain [1].

And so antioxidants boost cognitive ability by counteracting oxidative damage. Considering multiple hypotheses about mechanisms involved in the process of cognitive dysfunction like inflammation, oxidative stress, mitochondrial dysfunction and axonal transport deficits [2-5] it seems the need for searching an alternative therapy that may provide symptomatic relief [6].

In folk medical practice many plants are used as brain tonic but most of them are not scientifically validated. Earlier studies have shown that Curcuma longa (Turmeric) has ulcer protective, hepatoprotective, anticancerous, anti allergic and wound healing properties [7]. In the past decade, the antioxidant, antianxiety, antidepressant properties of curcumin (the polyphenolic nonflavone compound of curcuma longa) have been reported from various studies [8-11] few studies have also reported the neuroprotective effect of curcumin [12-16]. With this view the present study was undertaken to explore the effect of ethanolic extract of Curcuma longa (CLE) on learning and memory of restrained stressed mice.

MATERIALS AND METHODS

Animals: Male Albino Swiss mice of 6-8 wks old were selected for this study. Animals were kept in polypropylene cages and maintained under standard housing condition (room temperature 24- 27°C, relative humidity 60 – 70%) with 12 hours light and dark cycle. The food and the water were available ad libitum. Protocol of this study was approved by Institutional Animal Ethical Committee (IAEC). Experiments were conducted as per CPCSEA guidelines.

Plant material and preparation of extract

Curcuma longa (Turmeric) rhizomes were purchased from local market. The dry rhizomes were powdered and subjected to soxhlet extraction in ethanol for 72 hours. The extract was dried and stored for further study.
Experimental design

For each experimental model, four groups of mice were randomly divided to receive the drugs and vehicle orally for 28 days as per the following experimental design. Three groups of mice were subjected to five hours of daily restrain stress in a wire mesh for 7 consecutive days i.e. from day 21 to 28th day of drug treatment. Drug treatment was given one hour before the stress session.

<table>
<thead>
<tr>
<th>Group(n=6)</th>
<th>Drug and Doses (mg/kg)</th>
<th>Group</th>
<th>Drug and Doses(mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal + Tween 80-0.2 ml</td>
<td>V</td>
<td>Normal + Tween 80-0.2 ml</td>
</tr>
<tr>
<td>II</td>
<td>Stress + Tween 80-0.2 ml</td>
<td>VI</td>
<td>Stress + Tween 80-0.2 ml</td>
</tr>
<tr>
<td>III</td>
<td>Stress + C.LE 250mg/kg</td>
<td>VII</td>
<td>Stress + C.LE 250mg/kg</td>
</tr>
<tr>
<td>IV</td>
<td>Stress + C.LE 500mg/kg</td>
<td>VIII</td>
<td>Stress + C.LE 500mg/kg</td>
</tr>
</tbody>
</table>

Table-1

Following the last stress session, group I- IV and group V – VIII were subjected to elevated plus maze (EPM) and passive avoidance paradigm (PAP) respectively.

Elevated plus maze (EPM) test [19]

The plus maze consists of two open {16(length) x 5(width)} and two closed arms {16(length) x 5 (width) x 12 (height) cm} for mice facing each other with an open roof. A fine white line may be drawn in the middle of the floor of each enclosed arm. The entire maze is elevated at a height of 25 cm for mice from the surface. Mice were placed individually at the end of the one of the open arm facing away from the central platform and transfer latency (TL) i.e. the time it took to move from open arm to either of the closed arm (till all the four paws entered the closed arm) was recorded. Following first trial (acquisition) the mouse were allowed to explore the maze for 10 seconds. 24 hours of the first trial TL was again recorded which was served as the parameter of retrieval.

Passive avoidance paradigm (PAP): [20]

The apparatus consists of a small chamber connected to a large dark chamber. The small chamber is illuminated with a 7 W/12V bulb. The mice were allowed to explore the maze for one minute.

They were given an acquisition trial followed by retention trial at 24 hour and 48 hour latter. In 1st trial the mice were placed individually in the illuminated chamber and the latency to enter the dark compartment was recorded (step through latency – STL), animals those do not step through the door within a cut off time (90sec) were excluded. Immediately after the animal entered the dark compartment the door was shut and an unavoidable foot shock of 1mA for 1 second was delivered through grid floor. The mice were then quickly remove from the chamber and transferred to home cage. The cut off time on 2nd day trial (retrieval) was fixed at 30sec. The longer STL at 24 hour and 48 hour interval following shock was the parameter of positive retention.

STATISTICAL ANALYSIS

The data were analyzed by one way ANOVA followed by Tukey’s multiple t – tests. P<0.05 was considered as the minimum level of significance.

RESULTS

Effect of stress on acquisition and retrieval time

In stress, there was a significant increase (p<0.001) in acquisition time (52.5 ± 2.08sec) and retrieval time (50.67 ± 2.29sec) from normal control group (23.67± 1.28sec - acquisition time and 16.83±2.07sec - retrieval time) on EPM test (Table-II). On passive avoidance task, the Step through latency for acquisition was 90.25 ±5.36 sec which was significantly (P <0.01) more than that of control group (30.75 ± 5.39 sec) But retrieval time on 24 hours (128.25 ± 5.46 sec) and 48 hours (110.00 ± 5.40 sec) were significantly less (P< 0.05) than that of normal control group (24 hrs-179.75 ± 8.91 sec and 48 hrs-156.25 ± 4.73 sec) (Table – III).

Effect of CLE on acquisition and retrieval time

On EPM test, 500mg/kg CLE showed a significant improvement (P<0.01) in acquisition (38.0 ± 1.24 sec) and retrieval time (33.17±2.43 sec) from respective stress control group. II (Table-II). As observed in passive avoidance task, the STL for acquisition with CLE 500mg/kg were significantly less than stress control group (Table – III).

DISCUSSION

In the present study an attempt was made to explore the effect of aqueous extract of Curcuma longa on learning and memory in normal and stressed mice. Male mice of adult age group were included in this study because a different profile of AchE activity in brain areas and related behavioral changes among male and female rats were observed.[21] As chronic restrained stress simulates clinical stress immobilization in wire mesh model was adopted in the present work.
This experiment indicated that 7 days of restrain stress results in an impaired acquisition and retention of spatial long term memory and short term memory as assessed by performance in elevated plus maze (EPM) and passive avoidance paradigm (PAP) task respectively. These finding are in agreement with a previous study which was assessed on T-maze task in rats [22].

In the present work, both learning and memory performance was significantly improved with CLE 500 mg/kg treated mice when experimented on EPM test and PAP task. A similar observation was reported by Ying Xu et al. 2006 who explained that on chronic administration of curcumin the chronic stress induced down regulation of brain derived neurotrophic factor proteins and reduced phosphorylated CREB/CREB ratio were blocked [23]. Also, it modulated the effects on HPA axis and neurotrophic factor expression and reversed the effects of chronic stress on behavior. A recent report on the antidepressant effect of aqueous extract of Curcuma longa in mice was explained due to MAO A and MAO B inhibition. Multiple neuromodulatory factors and hormones are involved in stress response. So, the mechanism of protective role of CLE against stress induced cognitive deficits needs a parallel evaluation of various neurobiochemical analysis.

Hippocampus is the main centre for mediation cognitive function such as learning and memory process. Sunanda et al have reported that chronic restrain stress causes dendritic atrophy of CA3 pyramidal neural of the hippocampus [1]. Even stress induced rise in ROS is a neurodamaging factor so the agents having antioxidant, anti-inflammatory property are likely to prevent the oxidative stress induced neurodegeneration. Thus the anti-inflammatory and antioxidant property of curcuma longa could impart a possible mechanism of improvement of learning and memory (long-term and short-term) in stress induced memory impaired animals. In the study of Cheang-Su Eun et al. 2017 the protective effect of fermented Curcuma longa on memory dysfunction in oxidative stress induced neuronal death and scopolamine induced amnesia in mice was explained through its anti-inflammatory effect [24].

CONCLUSION

The present study indicates that Curcuma longa reverses the cognitive deficits in stressed mice. Hence it can offer a possibility of delaying the onset and reducing the severity of neurodegenerative disorders. Further studies on other experimental models and extensive clinical trials are awaited to confirm these results.

REFERENCES

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2. Esiri MM. The interplay between inflammation and neurodegeneration in CNS disease. Journal of Neuroimmunology. 2007 Mar;184(1-2):4-16.
6. Golde TE. The therapeutic importance of understanding mechanisms of neuronal cell death.

### Table-III: Effect of CLE on Step through Latency (STL) in Passive Avoidance Paradigm

<table>
<thead>
<tr>
<th>Group No</th>
<th>Drugs and Dose (mg/kg)</th>
<th>Time in seconds±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>Normal control – Tween 80 0.2 ml</td>
<td>30.75 ± 5.39</td>
</tr>
<tr>
<td>VI</td>
<td>Stress control</td>
<td>90.25 ±5.23***</td>
</tr>
<tr>
<td>VII</td>
<td>Stress +CLE. – 250</td>
<td>66.00 ± 8.67</td>
</tr>
<tr>
<td>VIII</td>
<td>Stress +CLE. – 500</td>
<td>26.00 ± 5.61</td>
</tr>
</tbody>
</table>

\[*P < 0.05, ** p< 0.01, *** - P <0.001 when compared with normal control group V. a p <0.001 when compared with stress control group VI.\]

## Table-II: Effect of CLE on acquisition and retrieval time in elevated plus maze test

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug and dose (mg/kg)</th>
<th>Time in seconds (mean± SEM)</th>
</tr>
</thead>
</table>
| I     | Normal control :Tween 80-0.2 ml | 23.67±1.28 24 hrs Acquisition time:
| II    | Stress control: Tween 80-0.2 ml | 52.5±2.08 24 hrs Retrieval time:
| III   | Stress+ C.LE. – 250 | 49.67±2.4 33 hrs Acquisition time:
| IV    | Stress +C.LE. – 500 | 38.0±1.24 33 hrs Retrieval time:

**P <0.01 when compared with normal control group I. b - p <0.001 when compared with stress control group II.**