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Original Article

Improvement in Cognitive Parameters Among Offsprings Born to Alcohol Fed Female Wistar Rats Following Long Term Treatment with *Centella Asiatica*

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Abstract

Prenatal ethanol exposure causes cognitive impairments in rats. This study was designed to evaluate the effect of *Centella Asiatica* (*CeA*) in offspring of alcoholic rats. Pregnant rats in alcoholic group were orally fed with 30% alcohol at a dose of 5 g/kg body weight during their gestation period. Pregnant rats in control group were given water. Offspring from alcoholic group were divided into treated group and untreated group. Offspring in treated group were orally given whole plant aqueous extract of *CeA* at a dose of 20 ml/kg body weight. Offspring in control and untreated group were fed with water. Cognitive studies (Morris Water Maze, Passive avoidance test, Elevated Plus Maze) were started from 75th day of postnatal life. Treatment with *CeA* increases the learning capacity ($P<0.05$), spatial memory ($P<0.05$), memory retention ($P<0.05$) and decreases the anxiety ($P<0.05$) like behavior in offspring of alcoholic rats. The present study showed that treatment with *CeA* can improve cognitive functions in offspring of alcoholic mothers.

Introduction

Prenatal exposure to alcohol causes mental retardation and congenital malformation in the newborns. Its teratogenic action affect the developing organ system in the fetus. The extent and severity of the child's condition depends on several factors such as quantity of the alcohol taken by the pregnant mother, frequency, and the period of pregnancy (1). The major symptoms include prenatal and post natal growth retardation, facial abnormalities, poor development of skeletal system, and central nervous system damage. Structural and functional changes in the brain regions including basal ganglia, corpuscallosum, cerebellum and hippocampus may be the cause for behavioral dysfunctions and cognitive impairments like attention deficits, hyperactivity, anxiety, motordysfunction, mental retardation, poor memory and learning deficits in those offspring (1).

In Ayurveda (Indian system of medicine) a number of medicinal plants are used individually or in combination for the treatment of various diseases. *Centella Asiatica (CeA)* is a herb which grows in wet places (in most of tropical Asian countries). It is

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used in ayurvedic medicine either as a whole plant or individual parts in fresh or extract form (2). The major constituents in *CeA* are triterpinoids, saponins including asiaticoside, centelloside, madecassoside, and asiatic acid. In addition it also contains other components including volatile oils, flavonoids, tannins, phytosterols, amino acids and sugars (3). It has been reported that *CeA* is used as a brain tonic and is very useful in improving cognitive functions (4-7). Previous reports showed that *CeA* leaf extract effect the hippocampal CA3 and amygdaldendritic neuronal arborization in neonatal rats (8, 9).

It is clear that alcohol consumption during gestation period causes behavioral dysfunctions in their offspring. Our previous study reported that prenatal exposure to alcohol increases the mortality rate and decreases the post natal weight gain and memory retention in their young adult offspring (10). The cognition enhancing property of *CeA* in offspring of alcoholic rat is not yet studied to best of our knowledge. Therefore the present study was designed to investigate the cognitive enhancing and anxiolytic property of *CeA* in rats which were exposed to alcohol during their gestation period.

Materials and Methods

Animal: Housing and administration of alcohol

Male and female Wistar rats were used for this study. The rats were maintained in normal room temperature and were fed with standard food pellet (Amruth laboratory, Maharashtra, India) and tap water *ad libitum*. Breeding and maintenance of the animals were done as per the guidelines of Government of India for use of Laboratory animals. Institutional Animal Ethical Committee (IAEC) approval had been taken before starting the work. Virgin female rats weighing 150-200 g were used for the study. Female rats were divided into two groups, control group (n=6) and alcoholic group (n=6). Pregnant rats in alcoholic group were administrated with 30% alcohol at a dose of 5 g/kg body weight during their gestation period (11). Rats in control group were given tap water. Pups were kept with their mothers during weaning period, immediately after that pups were separated and kept in separate cages.

Grouping of offspring: Offspring were selected randomly for behavioral studies on post natal day 75. Each group contained 8 rats (4 male+4 female).

Offspring from control mothers were considered as control group and they were fed with tap water. Offspring from alcoholic mothers were divided into two group, treated group and untreated group. Offspring in treated group were fed with whole plant aqueous extract of *CeA* at a dose of 20 ml/kg body weight (12) from post natal day 22. Offspring in untreated group were fed with tap water only.

Extraction procedure

Plants were collected from Mangalore vegetable market and were authenticated by Dr. Krishna Kumar G, Professor of Botany, Mangalore University, and Mangalore, India. The plants were dried under shade and powdered. Coarse powder of the plant was boiled with 1 gram of dry weight of CeA in 10 ml of distilled water for half an hour (13). This was cooled and filtered through a clean cloth. The filtrate was again boiled over a low flame and reduced to 100 ml and fed as such.

Behavioral studies

Morris Water Maze (MWM):

MWM was performed to assess the spatial learning and memory in animals. The water maze apparatus (Techno, Lucknow, India) consists of a circular water tank of 1.83 m in diameter, painted black and filled with water to a depth of about 40 cm. Tank was divided into 4 quadrants by imaginary lines. There was 4"×4" size escape platform submerged in one of the quadrant, which is known as target quadrant. The top surface of the platform was hidden approximately 1 cm below the surface of the water. The rats were trained in the water maze in 10 sessions on 6 consecutive days, with 2 sessions for 4 consecutive days except on the first day and last day where only one session was given. Each session consists of 4 trials. In each trial, time taken to reach the hidden platform was recorded. If the rat was unable to find the platform within 1 min, the training session was terminated and a maximum score of 1

min was assigned. Twenty-four hours after the last session, rats were subjected to memory retention test. This session was the probe trial and duration was 30 sec (14). Here time taken to reach the target quadrant and the time spent in the target quadrant was noted. Longer the time taken to reach the target quadrant and lesser time spent in the quadrant is an indicative of memory impairment.

Passive avoidance

To assess this test modified procedure of Buresova O and Bures J was used (15). Passive avoidance test determines the ability of a rat to remember a foot shock delivered 24 h prior to the memory retention test. The apparatus consists of two compartments: (I) bright, larger compartment and (II) dark smaller compartment. The smaller compartment was equipped with a grid floor which attached to a foot shock source. Experiment begins by placing a rat in the illuminated larger compartment for exploration. The door between the two compartments remained open at this time. The rat was allowed to explore both the compartments for 5 min which was followed by three test trials of 5 min each. At the end of 3rd test trail, as soon as the animal stepped into the dark compartment, the door between the two compartments was closed and a single foot shock was delivered through the grid floor (1.5 mA, 1 sec). The rat was held in the dark compartment for an additional 10 sec, to allow the animal to form an association between the properties of the chamber and the foot shock. It was then returned to its home cage. Memory retention test was done 24 h after foot shock. The rat was placed in the bright compartment and the time taken (the step-through latency) for it to enter the dark compartment for the first time was recorded up to 300s cut off. Longer the latency to enter the dark chamber better is the memory (16).

Elevated Plus Maze (EPM)

Open arm exploration in the EPM were assessed as an indication of anxiety-like behavior. EPM test was carried out as described in Burne et al (17). The apparatus consists of two open arms (50 x 10 cm) and two closed arms (50 x 10 x 40 cm), extended from a central platform (10 x 10 cm) and the maze was elevated to a height of 50 cm from the ground. At the start of testing, the animal is placed in the middle and is faced towards one of the closed arm. The animal is then allowed to freely explore the elevated plus-maze for 5 minutes. Percentage time spent on the open arms and percentage of entry into the open arm was recorded as an indication of anxiety like behavior.

Statistics

Statistical analysis was done using SPSS 16th version. Data was expressed as mean±SEM. Statistical analysis for multiple comparisons was performed by one-way analysis of variance (ANOVA) followed by post hoc test. $p<0.05$ was considered as statistically significant.

Results

Morris Water Maze (MWM) (Fig. 1)

Escape latency (Latency to reach the platform quadrant during the learning session)<

Testing the escape latency in animals from three groups, (viz. Control, untreated offsprings of alcoholic mothers and the treated offsprings of alcoholic mothers, (with *Centella Asiatica* extract) showed highly significant intergroup differences in the Morris Water Maze test ($p<0.001$). The shorter latency was more obvious in the trial from third day onwards up to 6th day.

Probe trial: (Fig. 2) When the time spent in the platform quadrant was tested, there was a statistically significant difference among the groups (One Way ANOVA followed by post hoc Tukey's test $p<0.001$). The rats in control group and CeA treated group spent more time in platform quadrant as compared to the untreated group. Offsprings in untreated group spent significantly less time in the platform quadrant as compared to offspring in control group ($p<0.001$). CeA treated group showed statistically significant memory retention since they spent more time the platform quadrant as compared to the untreated group ($p\leq0.01$).

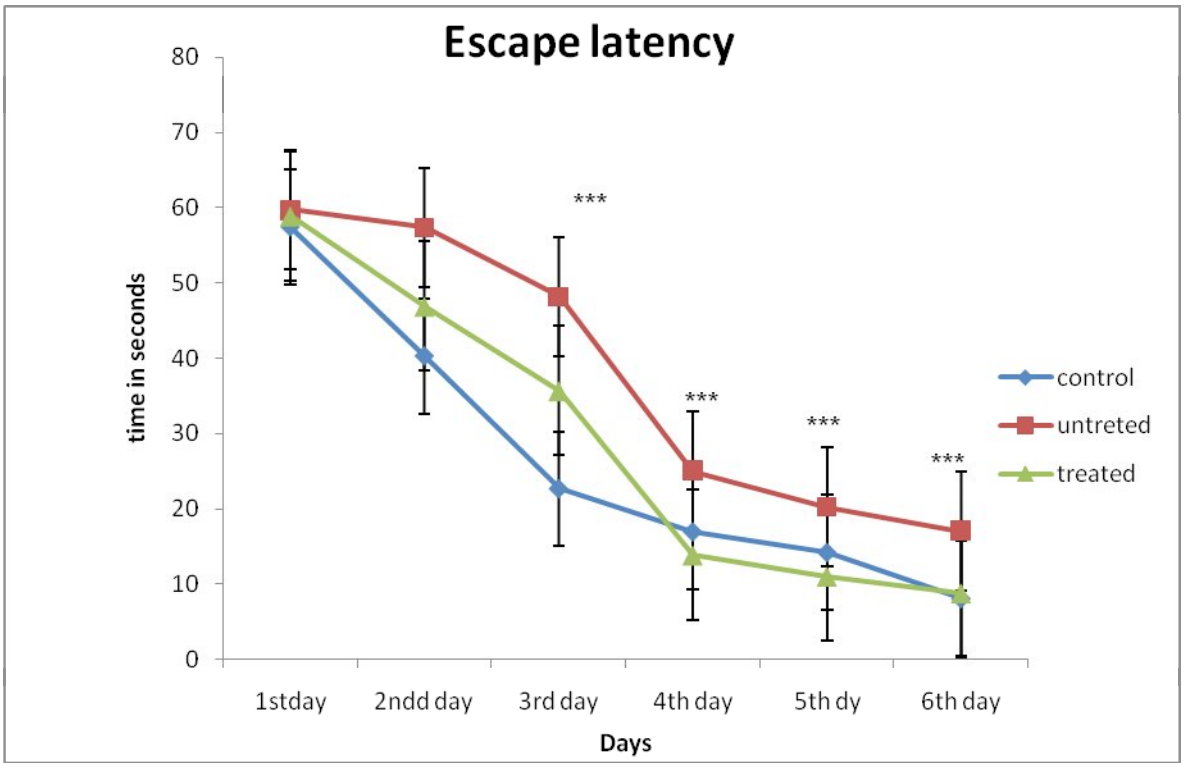

Fig. 1 : Effect of CeA treatment on escape latency in the MWM test. Statistical analysis was done by One Way ANOVA followed by post hoc Tukey's test (pd"0.05). ***p≤0.001.

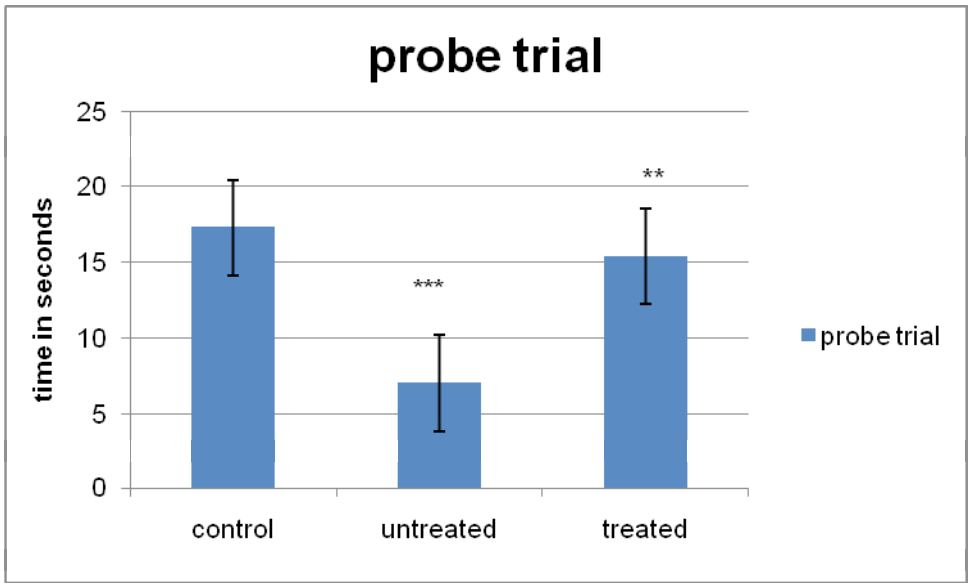

Fig. 2 : Effect of CeA treatment on memory retention in the probe trial of MWM test. Statistical analysis was done by One Way ANOVA followed by post hoc Tukey's test (p≤0.05). **p≤0.01, ***p≤0.001

Passive Avoidance test: Latency to enter into the Dark Chamber (LDC) (Fig 3)

Passive avoidance test conducted in the three groups of animals showed significantly increased latency to enter into the dark chamber by the CeA treated group when compared to the untreated animals from the alcoholic mothers (p<0.001).

The latency was lesser in untreated offsprings. There was no statistical difference in latency, when the normal animals and CeA treated groups were compared.

Elevated Plus Maze test: (Fig. 4, 5)

Elevated Plus Maze Test (EPM): In EPM test offspring of control group and *CeA treated* group spent more time in the open arm (Fig. 4), showed more number of entries (Fig. 5) into the open arm and as compared to the offspring of untreated group. (control Vs untreated $p<0.001$; treated Vs untreated $p<0.01$).

Discussion

Prenatal exposure to alcohol was known to adversely affect the normal functions of brain. In this study we found that treatment with whole plant aqueous extract

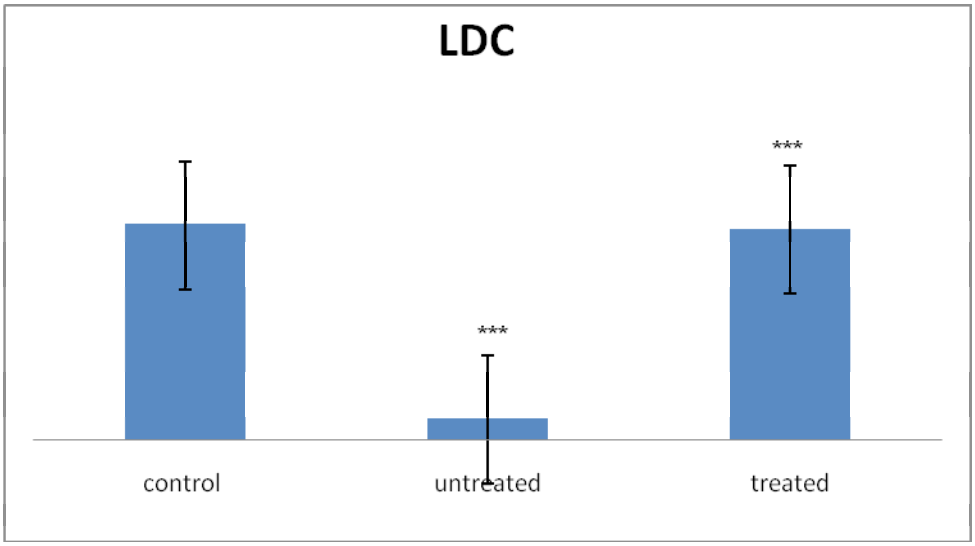

Fig. 3: Statistical analysis was done by One Way ANOVA followed by post hoc Tukey's test $p<0.001$, **$p\leq0.01$, ***$p\leq0.001$

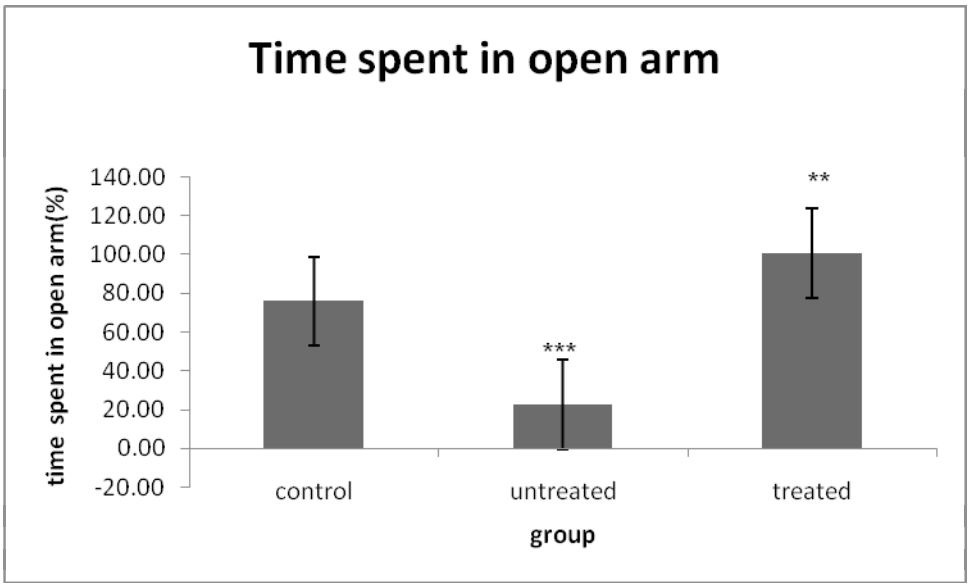

Fig. 4: Statistical analysis was done by One Way ANOVA followed by post hoc Tukey's test. $p<0.05$). **$p\leq0.01$, ***$p\leq0.001$

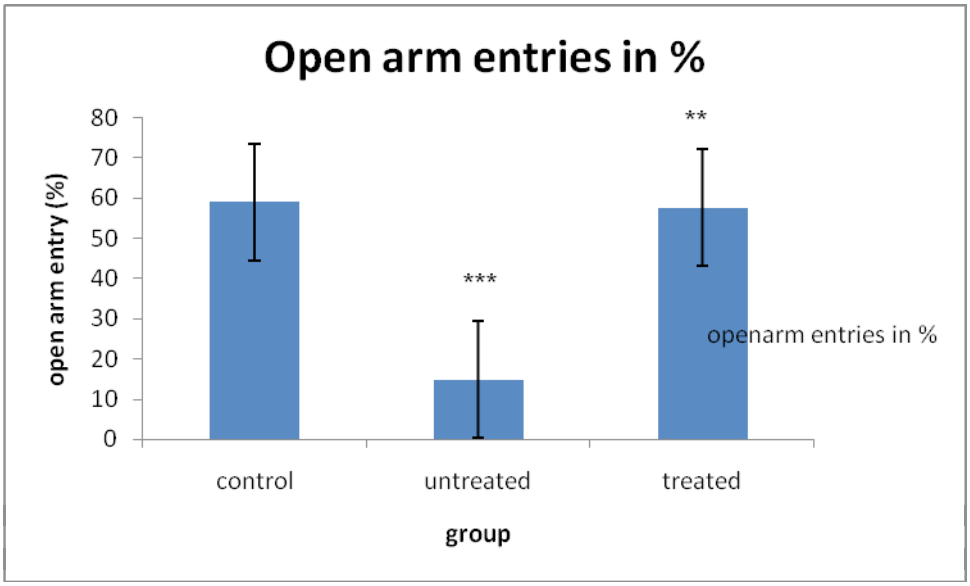

Fig. 5 : Statistical analysis was done by One Way ANOVA followed by post hoc Tukey's test p≤0.05, **p≤0.01, ***p≤0.

of *CeA* (20 ml/kg body weight for 2 month) decreases the effect of prenatal alcoholic exposure and improves the cognitive and behavioral functions in offspring of alcoholic mothers.

Ninety days old offspring of alcoholic mothers in *CeA* treated group showed an increased learning capacity and spatial memory as compared to the offspring in untreated group. Previous study reported that 3 months old normal mice treated with *CeA* for 15 days showed enhanced learning and memory in radial arm maze test (18). Other studies showed that treatment with *CeA* improves memory in normal rats (19, 20). In our study offspring of alcoholic mother in *CeA* group showed increased learning capacity and spatial memory in MWM test. Decreased memory retention was found in offspring who were exposed to alcohol during their intrauterine life (21, 22). In our study we found that effect of maternal alcohol consumption persist in the later life of their offspring. In this particular study we treated the offspring of alcoholic mother with *CeA* for 2 month. We observed that *CeA* treatment minimizes the prenatal alcoholic effect and offspring of the treated group showed improved memory as compared to the offspring of untreated group. Previous studies reported that treatment with *CeA* improves cognitive function in epileptic model rats (23). Our work also agrees with the cognitive enhancing property of *CeA*. EPM test is used to assess the anxiety like behavior in rodents. In this study we have seen that offspring in untreated group showed decreased exploration in the open arm indicating their increased anxiety like behavior. Previous studies reported that offspring of alcoholic mother showed increased anxiety in their later life 24, 25). Whereas offspring of CeA (aqueous extract) treated group showed significantly decreased anxiety, spent more time in the open arm as compared to the offspring of untreated group. These results are in conformity with other reports demonstrating the anxiolytic action of extracts of *CeA* in different models of rats (26, 27).

Conclusion

The present study shows that treatment with aqueous extract of *CeA* significantly improves the cognitive function and decrease the anxiety in offspring of alcoholic mothers. This could explain the neuro protective actions of *CeA*. The localization of action of *CeA* may require further study. From the present study, it can be conclude that administration of whole plant aqueous extracts of *CeA* for a longer period will improve cognitive dysfunction in offspring of alcoholic mother.

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