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NEUROPROTECTIVE EFFECT OF *CELASTRUS PANICULATUS* SEED OIL ON ALUMINIUM CHLORIDE-INDUCED NEUROTOXICITY IN ZEBRAFISH MODEL

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ABSTRACT

Celastrus paniculatus seed oil is distinguished for its ability to protect nerve cells. The plant's therapeutic benefits have been indicated in conditions such as depression, cognitive deficits, arthritis, and pain. This study aims to evaluate the neuroprotective effects of Celastrus paniculatus seed oil on neurotoxicity induced by Aluminium chloride in zebrafish by assessing various behavioral parameters, including the fish's height in the tank, locomotion, color preference, shoal cohesion, and light/dark responses. The zebrafish were divided into four groups, each containing six individuals, and neurotoxicity was generated using Aluminium chloride after they were exhibited to $AlCl_3$ for one hour; subsequently, the fish were placed in tanks treated with seed oil. Behavioral assessments were performed. Compared to the control group, the movement of the fish diminished throughout the $AlCl_3$. However, movement was restored upon treatment with Celastrus paniculatus seed oil. The $AlCl_3$ exposure avoided the zebrafish from exploring the dark area of the tank. When exposed to $AlCl_3$, the fish unchanged primarily in the upper section of the tank, but after administration of the oil, their

movement began to regain. Different colors triggered varied responses in the zebrafish. Under normal conditions, the red section of the tank was frequently visited, while the yellow area was largely ignored. When subjected to $AlCl_3$, the fish began to visit the yellow area, but after treatment with the seed oil, most of the fish reappeared to visiting the red section instead of the yellow. Zebrafish typically show a strong tendency towards the dark area. The exposure to Aluminium chloride limited zebrafish movement to the dark zone.

KEYWORDS: Neurotoxicity; Aluminium chloride; Zebrafish; Neuroprotective.

INTRODUCTION

The phrase "neurotoxicity" characterizes the detrimental effects on the brain or peripheral nervous system due to toxic substances. Toxicological factors can adversely effect the functions of the nervous system. The ability of a treatment to avert neuronal cell death by agitating and blocking the harmful processes that lead to cellular dysfunction. Neurotoxicity exhibits in various forms, such as neuronopathy, axonopathy, myelopathy, and transmission toxicity. Sources of neurotoxicity comprise organ transplantation, radiation treatment, cosmetics, heavy metals like lead, mercury, and cadmium, insecticides, chemotherapy drugs aimed at targeting rapidly dividing cells, viral infections, and the combustion of plastics. Celastrus paniculatus (CP), known in India as Malkangni or Jyotishmati, is rich in phytochemicals with therapeutic benefits. The plant shows numerous pharmacological properties, including anti-inflammatory, sedative, antifatigue, and analgesic effects, among others. Exposure to aluminium chloride has been clinically studied and experimentally validated to impair cognitive functions such as memory and learning. Danio rerio, a freshwater fish that belongs to the minnow family, Cyprinidae, has showed as a widely used model organism for investigating basic biological inquiries. Zebrafish models have proven effective in immitating the pathological features of Alzheimer’s disease (AD) and Tauopathy. The neuroprotective effects of Celastrus paniculatus seed oil against aluminium chloride-induced neurotoxicity were evaluated in a zebrafish model by measuring behavioral parameters, including the height of the fish in the tank, locomotion, color preference, shoal cohesion, and results from the light/dark test.

MATERIALS AND METHODS[8]

Fish models

Adult, healthy Zebrafish of both sexes were obtained from approved animal breeders, and water in the aquarium tank is aerated and maintained at a temperature of 26±20^{o} C and fed twice a day with fish feed.

Chemicals

Aluminium chloride: analytical grade, procured from NICE chemicals (P)Ltd, Edapally, Kochi, Kerala.

Tween 20: Central drug house (P) Ltd, New Delhi-110002.

Dimethyl sulfoxide (DMSO) - Merck life science (P) Ltd, Pirojshanagar, Mumbai.

Drugs

C.paniculatus seed oil (CPO)- Deve herbes UG-12 Westend mail Janakpuri, New Delhi, India.

Table 1: Study Design.

Sl. No	Groups	Treatment	No. of animals
1	Group I	Normal control	6
2	Group II	$AlCl_3$ treated	6
3	Group III	$AlCl_3$ + CPO	6
4	Group IV	Neostigmine	6

Four groups of six animals were divided. The water in the tank had a PH of 5.8, a temperature of 26 ± 20 °C, and was continuously aerated. Zebrafish were given aluminium chloride to cause neurotoxicity. Fish were continuously checked for neurotoxicity after 1.5 g of aluminium chloride was added to the glass tank. 0.5 ml of CPO was added. The following behavioral tests were conducted: light/dark test, color effect, locomotion effects, delay to travel high in tank, and shoal cohesiveness.

EVALUATING PARAMETERS

Height of the fish in the tank

Anxiety was measured using the position (bottom × middle × upper levels). During a one-minute examination, fish were noted based on the following scores: Movement is restricted to the bottom third of the tank; (2) the lower two-thirds of the tank are selected; (3) the three-

thirds are investigated at similar periods; (4) the upper two-thirds are desired; and (5) only in the upper third.

Locomotion

A general measure of behavioral exhilaration or inhibition was thought to be locomotion. The following scores were used to contrast this to "internal control" fish: (1) almost immobile; (2) slower than normal; (3) normal; (4) increased movement; and (5) intense locomotion.

Color Preference

Zebra fish manifested how they reacted to various colors. They were seen moving to a particular color. Scores were designated based on the fish's preference for the red and yellow regions.

Shoal cohesion

It is the word used to explain the tendency of zebra fish to swim in groups. In a number of fish species, this behavioral technique emerges to be effective against animals. shoal cohesion was calculated as an individual parameter for each fish by contrasting to “internal control” fish (i.e. a group of three untreated fish habituated in an independent tank) and were evaluated as following: (1) complete lack of group cohesion or fish interaction; (2) loose or partial shoaling behavior; (3) normal distance and shoaling behavior compared to ‘internal control’; and (4) increased shoal cohesion.

Light/dark test

It was noticed that zebra fish had appeared a marked preference for dark zones. Based on similar results shown by rodents toward brightly illuminated areas, this test in particular is used for the observation of anxiolytics effect in rodents. The apparatus was filled with 4 cm of water. This shallow tank blocks the bottom-dwelling, which is a well-confirmed anxiety behavior in a new environment. Thus, the main safe strategy is black preference, which is the characteristic used in this task.[8]

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RESULTS AND DISCUSSION

1. Height of the fish in the tank

The height up to which the fish moved was taken as an index of anxiety, as it was examined in the case of Zebra fish, the location near the wall versus the position in centre of an open field. Results were acquired by examining the fish for 5 and 10 minutes.

Table 2: The height of the fish in the tank in 5 and 10 minutes of various groups.

Groups	Height of the fish (5minutes)						Height of the fish (10 minutes)					
Control	3.5	3.2	3.1	3.5	3.1	3.2	4	3.8	3.7	3.9	3.8	3.7
$AlCl_3$	0.1	0.5	0.9	1	0.2	0.3	1.5	1.2	1.4	0.9	0.8	0.7
CPO	2.5	2.3	2.5	2.4	2.4	2.3	3	3.1	2.9	3	3	2.8
Neostigmine	3	2.8	3.1	3	3	3.1	3.5	3.4	3.2	3.1	3	3.2

The table depicts the height of the fish in the tank after 5 and 10 minutes in various groups. The heights of the fish in the control, Aluminium chloride, CPO treated, and Neostigmine groups were measured. Two Way ANOVA was used to perform statistical analysis on the values using the GraphPad prism 9 software.

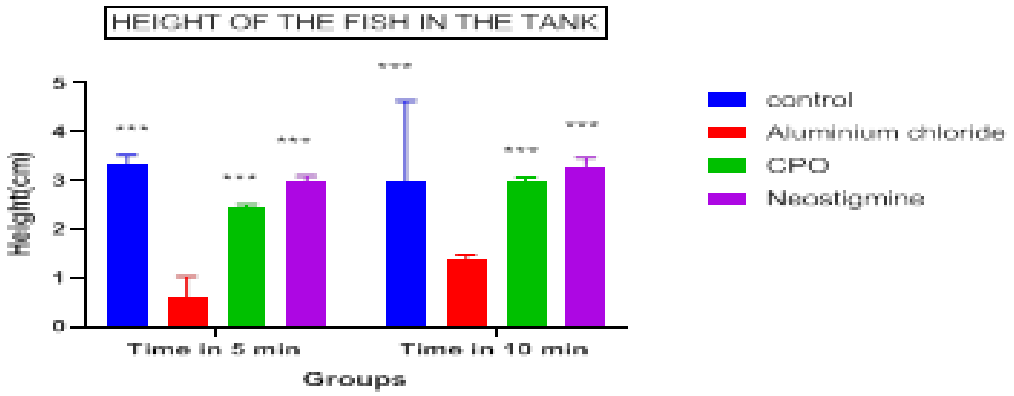

Fig. 1: The height of the fish in the tank in 5 minutes and 10 minutes. Values are statistically evaluated by two-way ANOVA. The values were stated as Mean ± SEM P-value P<0.001.

The graph showed that there was significant difference between the treatment group and the $AlCl_3$ treated group. After the exposure to $AlCl_3$ there is a significant difference in CPO treated group and the standard group. The treatment with CPO shows a statistically significant difference in height as compared with $AlCl_3$ treated group.

2. Locomotion

The general index of behavioral excitation/inhibition was considered locomotion. It was thought to be the criterion for normal behavior. In compared to the control group, mobility was reduced during the treatment with Aluminum chloride. The movement is regained when it is treated with CPO. When exposed to Aluminium chloride, movement is reduced; however, when treated with Neostigmine, movement is restored to normal levels.

3. Color Preference

The table depicts number of visits of zebrafish towards red and yellow region of different groups. TWO WAY A NOVA was used to perform statistical analysis on the values using the GraphPad prism 9 software.

Table 3: Number of visits of fish in various groups (Red and Yellow region).

GROUPS	RED						YELLOW					
Control	35	36	34	32	32	36	15	15	12	14	15	12
AlCl3	20	16	19	18	18	21	28	30	28	26	25	26
CPO	25	26	27	26	25	23	18	19	16	15	18	18
Neostigmine	30	28	31	29	30	26	16	18	16	20	19	18

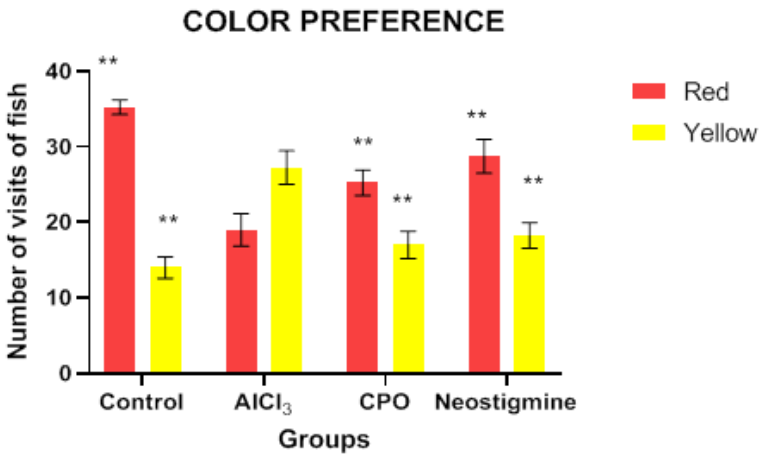

Fig. 2: Number of visits of fish towards red and yellow region. Values are statistically evaluated by Two Way Anova. Values are expressed as Mean ± SEM P value, P < 0.001 P = 0.0070, P< 0.001 as compared to Aluminium chloride.

4. Shoal cohesion

Fish prefer to swim in groups, a phenomenon known as "shoal cohesion." Zebra fish are typically observed swimming in groups. Their swimming habit significantly changed after receiving aluminium chloride treatment.

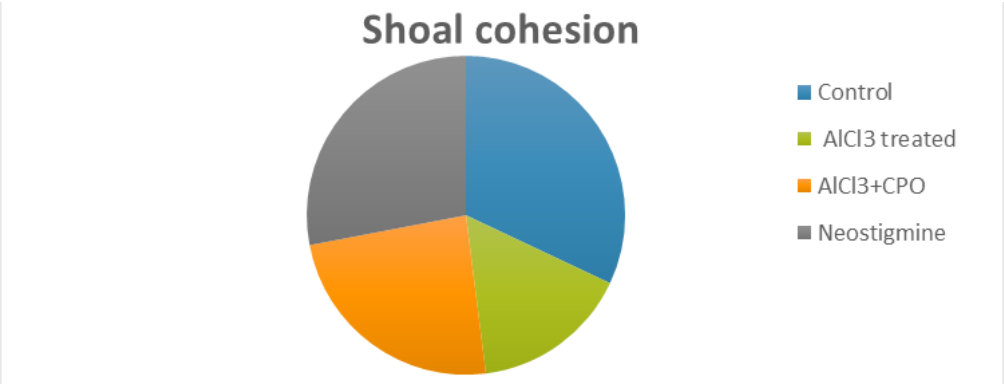

Fig. 3: Shoal Cohesion.

5. Light/dark test

Zebrafish show a prominent preference for dark zone. The treatment with Aluminium chloride cause movement of zebrafish blocked to dark zone. When treated with CPO the fish tends to move towards the dark region.

CONCLUSION

Zebrafish were prevented from entering the dark zone by the AlCl3 treatment. When fish are treated with CPO, their mobility gradually returns, but when they are treated with AlCl3, it is limited to the upper part of the tank. Zebrafish responded differently to different hues. Under normal conditions, the yellow area of the tank was virtually ignored while the red area was often frequented. The fish frequented the yellow zone when exposed to $AlCl_3$, but after treatment with CPO, the majority of the fish visited the red region rather than the yellow. Zebrafish have a strong preference for the dark zone. The treatment with Aluminium chloride causes zebrafish motility to be limited to the dark zone. It shows the neuroprotective activity of Celastrus paniculatus against aluminium chloride induced Neurotoxicity model.

Author contributions

Deepa, Athira and Shilpa Conceptualized, drafted and edited the manuscript. Sibi P I assisted in the literature search, supervised the work, provided guidance, reviewed and edited the paper. All authors have read and agreed to the published version of the manuscript.

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Declaration of conflict of interest

The authors declared that there is no conflict of interest.

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