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Studies on Neuroprotective effect of Cheilocostus Speciosus leaves on Aluminum chloride-induced Alzheimer's in Swiss Albino Mice

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ABSTRACT: Cheilocostus Speciosus, commonly known as Crepe ginger, is an herbal plant with medicinal value and traditionally was one of the most important plants used to treat various diseases. C. speciosus has ribosomes that are aphrodisiac, bitter, have an astringent property, and are also used as a tonic. In this investigation, our main goal is to identify whether C. speciosus ethanolic extract of leaves has neuroprotective activity or not. The result demonstrated that the leaf extract of C. speciosus has neuroprotective properties against Alzheimer's disease produced by $AlCl_3$. The research showed that C. speciosus shows toxicity at 1100mg/kg and good results at 300mg/kg, and 600mg/kg, according to which the dose was selected. The plant was extracted using the process of Maceration and the solvent used is Ethanol. Alzheimer's was induced using $AlCl_3$ at the dose of 2mg/kg. The animals were divided into 5 groups containing 3 animals in each group and the strain used was Swiss Albino mice. The Control group was administered with the vehicle, the diseased group was induced $AlCl_3$, the standard group was treated with Donepezil tartrate (10mg/kg), test group A was administered with Plant extract (300mg/kg), Test group B was administered with plant extract (600mg/kg).
In vivo tests were carried out such as behavioral study: Morris water maze test, Y-maze test, Forced swim test, and Rota rod test. The study was carried out for 14 days, at the 15th day 1 animal from each group was sacrificed and went for histopathological study. The result showed that plant extract (600mg/kg) showed more positive results than that of extract(300mg/kg) and can be easily compared with the standard.

KEYWORDS: Cheilocostus Speciosus, ethanolic extract, Alzheimer's, Maceration, Swiss Albino mice.

I. INTRODUCTION

The Alzheimer's Disease and Related Disorders Association (ADRDA) workgroup and the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) set criteria in 1984 for the clinical diagnosis of Alzheimer's disease (AD).These standards have been in place without change for more than 25 years, are widely accepted, and are incredibly helpful. But in the 27 years that have passed, significant progress has been made in our knowledge of AD, our capacity to identify the disease's pathophysiological mechanism and our conception of the disease's clinical spectrum **[1].**

Alzheimer's disease is a clinical-pathological condition in which neuropathological lesions characteristic of the illness cause clinical dementia. The number of plaques required for a diagnosis varies according to age and the availability of a clinical history of dementia, according to current neuropathological criteria. I shall make the case for a formal division of pathological evaluation from clinical data because I think it is conceptually incorrect to link neuropathological diagnosis to clinical data **[2].**

Aluminum (Al) is a common household element that is readily absorbed through the skin, consumed, inhaled, and injected intramuscularly. Known to be a strong neurotoxic metal, aluminum can penetrate the blood-brain barrier (BBB), build up in the brain, and cause neurodegenerative illnesses.Apoptosis in the hippocampus, the development of amyloid-β protein, axonal terminal loss, neurofibrillary tangle formation, and symptoms resembling Alzheimer's disease (AD) have all been observed in animals exposed to aluminum (Al) **[3].** Al exposure-induced free radicals and oxidative stress may be the cause of many of these illnesses. In addition, Al-treated animals have demonstrated deficiencies in cognition, movement, and memory; learning

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impairment; anxiety-like behavior; reduced neurotransmitter levels; changes in antioxidant stress indicators; and inflammation in the brain's cortex and hippocampus **[4].**

The drug donepezil was created to address the drawbacks of tacrine and physostigmine. Based on the cholinergic theory, it is used. Chemically distinct from other cholinesterase inhibitors, donepezil is a reversible acetylcholinesterase inhibitor based on piperidine. Its development was intended to treat Alzheimer's disease (AD) symptoms. With a far lower affinity for butyrylcholinesterase, which is primarily found in the periphery, donepezil is extremely selective for acetylcholinesterase. Clinical trials in phases I and II showed donepezil's advantageous pharmacokinetic, pharmacodynamic, and safety characteristics **[5].**

For individuals with renal and hepatic failure or the elderly, there is no need to adjust the donepezil dosage. In patients with mild to moderate AD, donepezil dramatically improved global function and cognition, according to pivotal phase-III trials conducted in the US, Europe, and Japan **[6].** Donepezil preserved overall and cognitive function in long-term trials for up to a year before the slow decline resumed. Most of the side effects of donepezil are minor, momentary, and cholinergic in origin, and the medication is often well tolerated. Laboratory indicators, particularly liver function, do not change in a way that is clinically meaningful when taking donepezil **[7].**

A significant ornamental and therapeutic plant, Cheilocostus Speciosus is used to treat a variety of illnesses. Numerous pharmacological properties of the plant have been discovered, including antioxidant, antihyperglycemic, larvicidal, antibacterial, antifungal, anticholinesterase,antipyretic,antihyperglycemic, anti-inflammatory, analgesic, antipyretic, antidiuretic, antistress, and estrogenic action. Cheilocostus Speciosus is a stimulating herb that clears toxins. Its rhizomes are bitter, astringent, acrid, cooling, aphrodisiac, purgative, anthelminthic, depurative, febrifuge, expectorant, and tonic. It also possesses anabolic and anti-fertility qualities. Rhizomes are also used to treat conditions like rheumatism, dropsy, jaundice, pneumonia, and urinary tract infections. Leaves are used to treat mental illnesses **[8].**

II. MATERIALS AND METHODS

Collection and Authentication of plant: The leaves of Cheilocostus Speciosus were collected from the local area of Haldwani,Uttrakhand,India.The plant was authenticated by Dr. Kailash C. Bhatt, Department of Botany, ICAR- National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110012, India.

Extraction methodology: After gathering the leaves, they were carefully cleaned with water, chopped into bits, and allowed to air dry at room temperature for three to four weeks. Using a mechanical grinder, the materials were ground into a coarse powder once they had dried. Ethanol was used in a Soxhlet apparatus to extract roughly 75g of the powdered sample. The crude extract was then obtained by evaporating the solvent at 50°C in a rotary evaporator with reduced pressure. This was done to preserve the extract for use in future in-vivo pharmacological research.

Experimental animals:A healthy adult female Swiss albino mouse weighing 25–30 grams were procured from Baddi University of Emerging Sciences & Technology's Animal House.

The mice lived in polypropylene cages furnished with bedding made of rice husk and were kept in a 12/12 light-dark cycle at a temperature of 24 ± 2°C and a relative humidity of 30–70%. They were given water at will and fed a typical laboratory diet. The CPCSEA criteria were adhered to and the experimental protocols were authorized by the Institutional Animal Ethics Committee (Approval No: BUEST/SPES/IAEC/2022/003).

Toxicity Study: The stem, leaves, and flowers were shade-dried and ground into a coarse powder using a grinder. Using maceration equipment, 50% ethanol was recovered from the 5000 g of powdered plant material. A viscous dark green extract was produced by evaporating the aqueous extract. Using Indonesian herbal pharmacopes as a guide, the ethanolic extract was examined as a standardized extract. There were no bacterial or fungal pollutants (E. Coli, Pseudomonas aeruginosa, Salmonella typhi, S. aureus, and Candida albicans), nor were there any contamination of arsenic (As), cadmium (Cd), mercury (Hg), or lead (Pb). To assess the subacute toxicity test of C. speciosus, one study trial has been conducted. Male mice were given C. speciosus at 275–1100 mg/kg/day for 90 days. Daily measurements of food and drink intake were made, and daily observations were made of toxic symptoms. After the investigation, the animals were slaughtered, and the weights of the important organs were measured and histologically analyzed. The administration of C. speciosus ethanolic

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extract at 275–1100 mg/kg/day for 90 days did not significantly alter any of the parameters, except the test animals' blood glucose and cholesterol levels decreased [9,10]. To ascertain the safety of C. speciosus, various extracts derived from each component of the plant were analyzed independently to calculate the extracts' LD50 and safety thresholds.

$AlCl_3$-induced neurotoxicity: Swiss albino mice of female sex (25-30g) were used. All the animals were divided into five groups, each group consisting of 3 animals and they received the treatments as follows:

Group I: Control (Vehicle (0.1% CMCp.o.)

Group II: Aluminum chloride ($AlCl_3$) (2mg/kg p.o) for 14 days

Group III: Standard (Donepezil tartrate + $AlCl_3$) (10mg/kg p.o+2mg/kg) for 14 days

Group IV: Ethanolic extract of Cheilocostus Speciosus (300mg/kg p.o) + $AlCl_3$ (2mg/kg p.o) for 14 days

Group V: Ethanolic extract of Cheilocostus Speciosus (600mg/kg p.o) + $AlCl_3$ (2mg/kg p.o) for 14 days

Blood and Organ Collection: At the end of the treatment, i.e., 14 days, animals were fasted overnight with water ad libitum before being sacrificed on the 15th day. On the 15th day, the animals were sacrificed by cervical dislocation. The whole brain from the sacrificed animal of all the groups was excised and washed with normal saline and then placed in (10% v/v) formalin solution.

Histopathological evaluation: One animal from each group was sacrificed at the end of the experiment, and a systematic histopathology investigation was done on it. The liver, which was the target organ, was removed and fixed in a 10% neutral buffered formalin solution. Tissue samples were washed in tap water overnight, dehydrated in ethyl alcohol of increasing intensity, cleaned in xylene, and embedded in paraffin for histological processing. Hematoxylin and eosin (HE) staining was carried out on 3–4 µ thick tissue sections that were cut from paraffin-embedded tissue blocks and placed on glass slides. Then, under a light microscope, the H and E-stained sections were checked for any pathological changes. The microphotography was performed using Nikon Eclipse 200 Microscope, in Japan.

III. RESULTS:

The microscopic findings in the brain tissue of all groups indicate the potential pharmacological effects of the plant extract prepared from C. speciosus leaves. The control group's brain tissue section underwent microscopy, and there were no abnormalities visible, and the neurons and neuron fibers appeared normal (Fig.18). However, when we compared microscopic findings between the diseased and control groups, we discovered that substantial histopathological abnormalities were brought on by brain damage by the administration of $AlCl_3$. Significant memory impairment increased oxidative stress markers and the presence of amyloid plaques (Fig.19). Necrosis of the neurons and neuron fibers in the cerebellum structure was observed. The portion of the brain from the standard treatment group only displayed slight histological changes when compared to the diseased group (Fig.20). Compared to neurochemical abnormalities, conservation. Neurons and neuron fibers showed mild deterioration. Neuron cells only show a mild form of oxidative stress markers compared to brain serum, which shows reduced oxidative stress markers. This preservation of the brain tissue of standard group samples can be attributed to the pretreatment with Donepezil tartrate, which is a well-known neuroprotective agent being used in various neurotoxicity studies. The brain's histological changes in the C. speciosus treatment group 1, i.e., 300 mg/kg, were mild to moderate, as seen in (Fig.21). Moderate improvement in cognitive function and reduction in oxidative stress compared to the $AlCl_3$-Induced Group. However, the brain's histological changes in the C. speciosus. treatment group 2, i.e., 600 mg/kg, was inconsistent and mild. Although rarely showing brain serum balance, the neuron and neural fibers in (Fig.22) are otherwise well preserved. More pronounced improvements in cognitive function and biochemical markers compared to both Test Group 1 and the $AlCl_3$-Induced Group, indicating a dose-dependent effect. Group 5 treated with a greater dose of C. speciosus leaf extract, which is comparable to the treatment dose of the standard drug, had the greatest neuroprotective effect. When increasing amounts of plant extract were given, a dose-dependent neuroprotectiveeffect was seen. Thephytochemicals included in the leavesextract,such as alkaloids, flavonoids, saponins, terpenoids, and tannins, among others,

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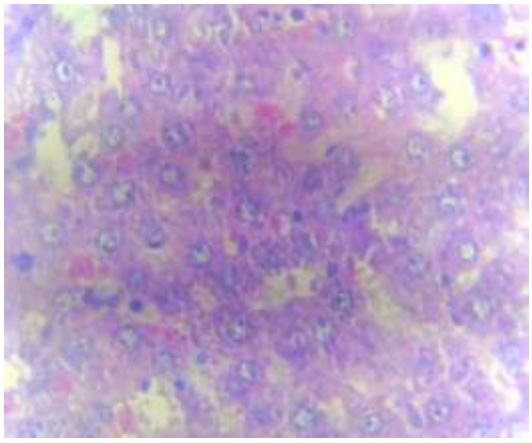
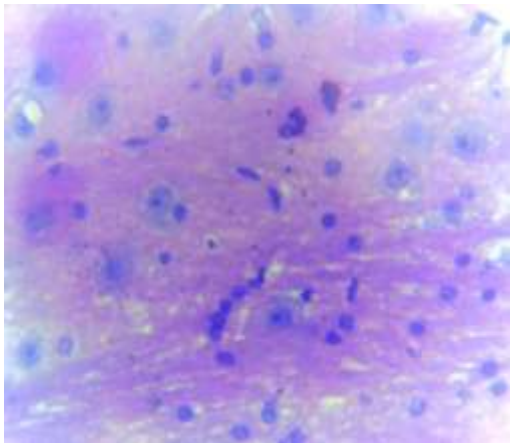
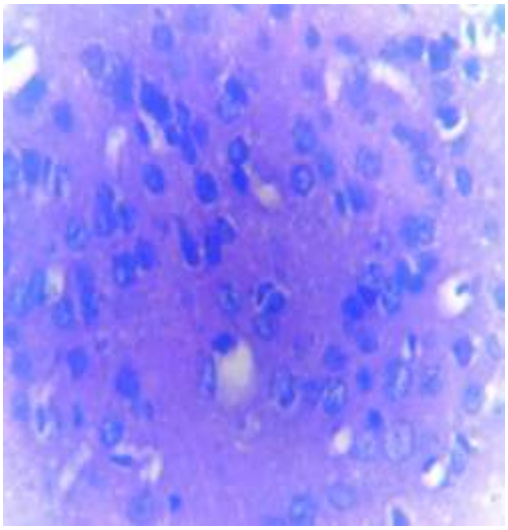
may be the cause of the neuroprotective activity of the C. speciosus plant.



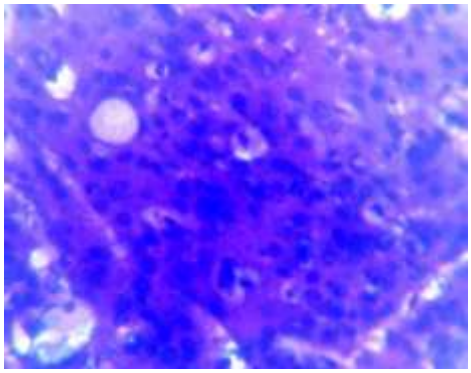
(A) CONTROL GROUP



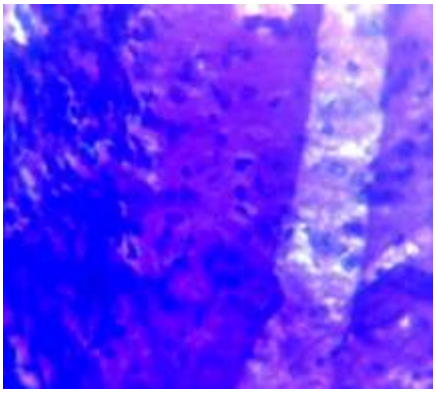
(B) DISEASED GROUP



(C) STANDARD GROUP



(D) TREATMENT GROUP (300mg/kg)



(E) TREATMENT GROUP (600mg/kg)

IV. CONCLUSION

Determining if an extract prepared from the leaves of the plant Cheilocostus Speciosus has neuroprotective qualities was the primary goal of this study. The same plant has been the subject of numerous studies that have revealed a range of pharmacological characteristics. The results demonstrated that the plant extract treatment increases the brain's capacity for repair and renewal. The reason behind the plant extract's potential stability of membranes could be due to the existence of phytochemicals and pharmaceutical outcomes. The antioxidant and free radical-scavenging abilities of phytoconstituents could be the possible mechanism. Further research is necessary to fully understand the advantageous effects of the Cheilocostus Speciosus plant on several organs.

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