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Memory Enhancing Activity of Ethanolic Leaf Extract of *Jacquemontia Caerulea* and *Allium Fistulosum* Against $AlCl_3$ Induced Dementia in Swiss Albino Mice

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ABSTRACT

OBJECTIVE: The main objective of the present study was to evaluate the nootropic activity of *Allium fistulosum* and *Jacquemontia caerulea* on the Swiss albino mice.

MATERIALS & METHODS: The leaves from both the plants i.e., *Allium fistulosum* and *Jacquemontia caerulea* were collected, shade dried for 15 days and were coarsely powdered using a blender. The plant materials were then taken up for extraction using ethanol and water in 70:30 ratio respectively. The extraction was carried out by maceration technique followed by distillation and subsequently stored in a refrigerator. AFJCELE (Allium fistulosum Jacquemontia caerulea ethanolic leaf extract) was thus used to assess the nootropic activity using donepezil (5 mg/kg, p.o.) as standard and ALCl3 (100 mg/kg, p.o) was used to induce memory loss in mice. Effect of drugs on behaviour, learning and memory of mice was evaluated by utilizing Elevated plus maze model and Y- maze model.

RESULTS: AFJCELE showed a significant a decrease in transfer latency in Elevated plus maze model which depicts an improvement in cognition while in Y- maze model, it was found to have increased the spontaneous alteration percentage as well as alteration of behaviour response was significant.

CONCLUSION: The study thus concluded that on administration of AFJCELE there was a significant increase in learning and memory in mice. Thus, the extract is found to have therapeutic effect against memory loss or amnesia.

KEYWORDS: Nootropic activity, Allium fistulosum Jacquemontia caerulea ethanolic leaf extract (AFJCELE), Amnesia, AlCl3, Donepezil.

1. INTRODUCTION

It has been reported according to WHO that about 450 million of population suffer from psychological and behavioural disorders [1]. One of the most common age-related psychological problem is dementia which is a typical feature of Alzheimer's disease (AD) [2-4]. In a brain affected by dementia, cell death happens more rapidly and worsens over time. The damaged brain cells have difficulty transmitting

messages to each other. This affects the clarity of thought, logic, behaviour, communication and mood. Blood clots and infections in the brain are also associated with dementia [5]. Alzheimer's disease is a neurodegenerative disorder which is caused due to accumulation of amyloid B protein and forms plaques and neurofibrillary tangles throughout the cerebral cortex of the brain. It is a neurological condition wherein the demise of synapses causes cognitive decline and intellectual decrease [6]. It was observed that due to decrease of Acetylcholine in the brain of patient's with AD is the main factor responsible for dementia [7]. Studies among people who were treated with contaminated dialysis have shown an increase in the amount of aluminium in the brain. This was believed to be as a result of inadequately monitored dialysis which then led to encephalopathy related dementia. Methods of dialysis have since been improved and doctors are better able to predict and prevent this form of dementia [8].

Agents or substances which are used for enhancing memory, thus used in treatment of Alzheimer's disease are called Nootropic drugs, which belong to psychotropic agents class [9]. These drugs are also known as smart drugs and are beneficial in improving mental functioning such as memory, increase in blood circulation and oxygen supply to brain [10]. Many synthetic drugs such as piracetam, rivastigmine, donepezil are used to improve cognition and also enhance memory in patient's with AD[11].

Plants offer a large variety of secondary metabolites that play an important role in metabolic pathways. Some species of plants are used for particular diseases in a specific region, the same plant is used in different ailments in some other region. India has generous medicinal plant flora of about 2500 species. Among these 2000 to 3000 at least 150 species are commercially used on a large scale. Plant-based **"nootropics"** are a diverse group of natural drugs that can improve cognitive abilities through various physiological mechanisms, especially in cases where these functions are weakened or impaired. The plant kingdom provides most of the currently available nootropics of natural origin. Various formulations possess antioxidant activity that protects brain tissue from neurotoxicity and improves the brain's oxygen supply. They can induce the synthesis of neuronal proteins, nucleic acids, and phospholipids for constructing and repairing neurohormonal membranes. These natural compounds can potentially be present in a great variety of herbs, shrubs, and even some trees and vines [12]. In this study leaves from two plant species i.e. Allium fistulosum and Jacquemontia caerulea were used for assessing memory enhancing activity. Both these plants are known to have potential therapeutic value. Allium fistulosum has shown anti-inflammatory, analgesic as well as antioxidant activity [13-15]. On the other hand, Jacquemontia caerulea has proven wound healing, anti-microbial, anti-inflammatory and analgesic activity [16,17].

2.MATERIALS & METHODS

2.1.COLLECTION, IDENTIFICATION AND EXTRACTION OF MEDICINAL PLANTS

The two species of plants I.e., Allium fistulosum and Jacquemontia caerulea were collected, shade dried for 15 days and were coarsely powdered using a blender. The plant materials were taken up for extraction using ethanol and water in 70:30 ratio respectively. The extraction was carried out by maceration technique followed by distillation and subsequently stored in a refrigerator.

2.2.PREPARATION OF EXTRACT:

Each 85 g of leaves of Allium fistulosum and Jacquemontia caerulea were collected and were dried in sun shade, coarsely powdered and extracted using maceration process. The extract was then subjected to

distillation and heated on water bath for semisolid consistency and then placed in a refrigerator for 15 days. The obtained extract was filtered and concentrated (yield 8.9%).

POWDERED LEAVES OF ALIUM CAERULEA

POWDERED LEAVES OF JACQUEMONTIA FISTULOSUM

MACERATION PROCESS

2.3.DISTILLATION EXTRACTION

The mechanism for this technique is similar to the Soxhlet extraction process. This method is commonly used to isolate volatile and non- volatile components present in the aromatic plants. In distillation extraction, the sample and water solvent are placed in a retort, heat is applied in order to vaporize the mixture. Extracted oil is then transported by steam in the vapor phase into the condenser where the condensate liquid mixture is formed. The liquid mixture then flows down into a separator where water and the essential oil are separated by difference in their densities.

DISTILLATION EXTRACTION

2.4. EXPERIMENTAL ANIMALS

Swiss albino mice weighing 15-30 gm were procured from the animal house of Shadan college of Pharmacy, Peerancheru, Hyderabad. Animals were housed under standard conditions of temperature and relative humidity (30-70%) with a 12:12 light: dark cycle. The animals were given standard diet and water. The Institutional animal' ethics committee (IAEC) had approved the experimental protocol from Shadan Institute of medical sciences and care was taken as per guidelines of CPCSEA, Department of Animal Welfare Government of India.

2.5.PRELIMINARY PHYTOCHEMICAL SCREENING

The above prepared extract, ALJCELE was screened for phytochemical constituents present in it such as flavonoids, alkaloids, glycosides, carbohydrates, saponins, etc.

To confirm the presence of alkaloids Wagner's test, Mayer's test, Hager's test and Dragendroff's test were used. The extract was treated with Mayer's reagent upon which yellow color precipitate was formed indicating presence of alkaloids. While on treatment of extract with Wagner's reagent, Hager's reagent and Dragendroff's reagent showed brown or reddish precipitate, red precipitate respectively confirmed the presence of alkaloids in the extract.

The presence of flavonoids was assessed by carrying out lead acetate and alkaline reagent test. In alkaline reagent test, few drops of sodium hydroxide solution was added to the extract leading to formation of intense yellow color, which becomes colorless on addition of dilute acid, thus confirming presence of flavonoids. In lead acetate test, when the extract was treated with few drops of lead acetate solution yellow color precipitate was formed thus indicating presence of flavonoids.

Glycoside presence was confirmed by Borntrager's test. Formation of rose pink color indicates presence of glycosides.

The presence of saponins was confirmed by froth and foam test wherein the extract when diluted with distilled water to 20 ml showed formation of 1cm layer of foam thus confirming presence of saponins.

Ninhydrin test, Million's test, Biurette's test and Xanthoprotein test were performed to assess the presence of proteins in extract. The change in color to purple, white then to brick red, , violet or pink and white to yellow respectively confirmed the presence of amino acids.

2.6.ACUTE TOXICITY STUDY

The acute toxicity test was done following the OECD-423 guidelines. Six albino female mice were taken, the weight of each was in between 20-30 gm. The extract was given to the mice at a dose of 2000 mg/kg. The mice were then examined for any behavioral changes, clinical signs and mortality after administration of test dose at time interval of 30min., 1h, 6h, 24h, 48h and 72h upto a period of 14 days. Irwin test was performed to predict the potential therapeutic activity and to select doses for subsequent efficacy tests.

2.6.1 IRWIN TEST

The mice are administered the test substance and then observed for the next several hours and on the following day. Clinical signs are grouped into several categories in an order to highlight the particular characteristics of the test substance [20].

Category		Symptom
Death		
Excitation		Convulsions Tremor Straub tail Increased activity Jumping Increased fear Increased reactivity to touch Increased abdominal muscle tone Aggression
Stereotypy		Head-twitches Stereotypies(head movement) Stereotypies (chewing) Stereotypies (sniffing) Scratching
Motor		Catalepsy Akinesia Abnormal gait (rolling) Abnormal gait (tip-toe) Motor incoordination
		Loss of traction Loss of grasping

Sedation	Decreased activity Decrease fear Decreased reactivity to touch Decreased abdominal muscle tone
Pain	Writhing Analgesia
Autonomic	Ptosis Exophthalmia Myosis/mydriasis Piloerection Defection/diarrhea Salivation Lacrimation
Other measure	Increased/decreased respiration Hypothermia/hyperthermia

2.7.EVALUATION METHODS FOR MEMORY ENHANCING ACTIVITY

2.7.1 Y MAZE TEST:

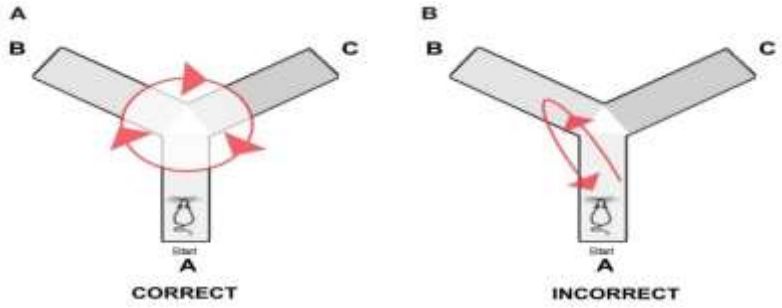


Fig no-5.5 SAMPLE OF Y-MAZE APPARATUS

PRINCIPLE

Y Maze Spontaneous Alternation is a behavioral test for checking the willingness of the mice to explore new environment. The mice typically prefer to investigate a new arm of the Y - maze rather than going back to one it had previously visited. Different parts of the brain including the hippocampus, the septum, the basal forebrain, and the prefrontal cortex are involved in this test [21].

ANIMAL GROUPING-

Six Swiss albino mice were randomized into 6 groups (G1 -G6), each with 6 animals.

GROUP -1: Animals in G1 were treated with vehicle and served as normal.

GROUP -2: Animals in G2 served as positive controls and only received Aluminum chloride AICI3 p.o.

GROUP-3: Animals in G3 were treated with Donepezil 5 mg/kg+ Aluminium chloride 100 mg/kg p.o.
GROUP-4: Animals in G4 were treated with 125 mg/kg extract + Aluminium chloride 100mg/kg p.o.
GROUP-5: Animals in G5 were treated with 250mg/kg extract + Aluminium Chloride 100 mg/kg p.o
GROUP-6: Animals in G6 were treated with 500mg/kg extract+ Aluminum chloride 100 mg/kg p.o. daily for 21 days to induce learning deficiency and dementia.

PROCEDURE

Y -MAZE

The Y-maze is used to assess short term memory in the mice. Y-shaped maze has three identical arms which are at a 120° angle from each other. After introducing to the center of the maze, the animal is given free access to all three arms. If the animal chooses a different arm than the one it had arrived from, then this choice is called as an alteration. This is considered as a correct response, whereas returning to the Arm it had previously visited is considered an error. The. percentage of alternation is calculated from total number of arm entries and the sequence in which the animal had entered [22].

2.7.2 ELEVATED PLUS MAZE-
PRINCIPLE

Elevated Plus maze is one of the most widely used models for evaluating anxiety related behaviors in rodents. It consists of two opposite open arms (50 cm x 12 cm) and two opposite closed arms which is surrounded by 50 cm high walls of similar dimensions. The middle section allows the animal to move from one arm to the other and also consists of a square with dimensions of 12 x 12 cm. The maze was elevated at a height of 50 cm above ground and the open arms are equipped with 0.5 x 0.5 cm lengths to make sure that the animals do not fall down the maze. The placement and lighting conditions were kept similar for every trial [23].

ANIMAL GROUPING-

Six Swiss albino mice were randomized into 6 groups (G1 -G6), each with 6 animals.

GROUP -1: Animals in G1 were treated with vehicle and served as normal.

GROUP -2: Animals in G2 served as positive controls and only received Aluminum chloride $AlCI_3$ P.O

GROUP-3: Animals in G3 were treated with Donepezil 5 mg/kg + Aluminium chloride 100 mg/kg P.O.

GROUP-4: Animals in G4 were treated with 125mg/kg extract + Aluminium chloride 100mg/kg p.o.

GROUP-5: Animals in GS were treated with 250mg/kg extract + Aluminium Chloride 100 mg/kg p.o

GROUP-6: Animals in G6 were treated with 500mg/kg extract+ Aluminium chloride 100 mg kg p.o daily for 21 days to induce spatial memory (short term memory).

ELEVATED PLUS MAZE

PROCEDURE

In the Elevated Plus maze model, the animal is placed in the center of the maze, typically towards a closed arm. When released, the animal explores the maze for a particular period of time, mostly for around 5 minutes. Two measures or indices of anxiety are then recorded.

1. The number of entries made by the animal in each arm which is often demonstrated as the proportion of entries in the open arms.
2. The time invested in each arm is demonstrated as a percentage of total time spent on all arms.
   Mice were placed at the center of maze facing towards the open arm. They were then noted for behavior changes like postures, grooming, head dips stretch, line crosses and freezing, rearing for 5 minutes which were then recorded. After 5 minutes, the mice were taken out from the maze by holding their tails and were kept back in cage. The frequency of urinations and defecations were recorded and then the maze is cleaned before introducing the next mice.

The scoring of behavior is as follows

Open arm entries: frequency with which animal enters the open arms such that all its four paws should be inside the open arms.

Closed arm entries: frequency with which animal enters the closed arms such that all its four paws are in the closed arms.

Open arm duration: length of time the animal spent in open arms.

Closed arm duration: length of time the animal remains in closed arms.
Head dipping: frequency with which animal lowers its head over the sides of the open arms towards the floor.
Stretch attends posture: frequency for which the animal expresses forward elongation of the head as well as shoulders followed by retraction from original position.
Rearing: time for which animal stands on its hind legs
Grooming: the duration of time for which animal licks and scratches itself while being stationary.
Urination: Total number of puddles or streaks of urine.
Defecation: Total number of fecal bolus produced [24].

3.RESULTS AND DISCUSSION

TABLE NO. 3.1- PRELIMINARY PHYTOCHEMICAL SCREENING RESULTS

PHYTOCHEMICAL TESTS	RESULTS (COMPOUND)
1)Carbohydrates	+
a) Molish's Test	+
b) Fehling's Test	+
c) Legal's Test	+
d) Bontrager's Test	-
2)Fixed Oils and Fats	+
a) Filter paper Test	+
b) Saponification Test	+
3)Proteins and Free Aminoacids	+
a) Millon's Test	+
b) Biuret Test	-
c) Ninhydrin Test	+
4)Tannins and Phenolic Compounds	+
a) Ferric chloride Test	+
b) Lead Acetate Test	-
5)Phytosterols	+

a) Salkowski Test	+
b) Liebermann Burchard Test	+
6)Gums and Mucilages	-
7)Flavonoids	+
a) Shinoda Test	+

Note: + indicates presence and
- indicates absence of phytoconstituents

TABLE NO. 3.2: ACUTE TOXICITY TESTING

Response	**Type**	**Result**
Behavioral	Alertness	+
	Stereotypy	-
	Irritability	+
	Touch	-
	Pain	-
	Spontaneous Activity	- +
	Grooming	-
	Restlessness	+
Neurological	Righting Reflex	+
	Limp Tone Grip Strength	+ +
	Twitching	-
	Abdominal Tone	- -
	Pinna Reflex	+
	Cornea Reflex	+
	Straub's Tail	+
	Tremors	-
	Convulsions	-
Autonomic	Writhing	+
	Defecation	-
	Urination	-
	Piloerection	-

	Heart Rate	+
	Respiration	+
	Pupil Size	-
	Skin Color	-

= normal
+ Increased/ present
-Decreased /Absent

TABLE NO. 3.3: EFFECT OF AFJCEE OF SPONTANEOUS ALTERATION BY Y-MAZE APPARATUS

Groups	Treatment	DAY 1	DAY 2	DAY 5	DAY 9
I	**Normal saline**	5.333±0.333	6.0±0.577	5.333±0.333	5.66±0.333
II	**AlCl3 100 mg/kg**	4.333±0.882	4.0±0.577	4.333±0.333	5.0±0.577
III	**Donepezil 5ml/kg + Alcl3 100mg/kg**	5.66±0.333	5.33±0.333	5.667±0.333	5.0±0.333
IV	**Extract 125 mg/kg + Alcl3 100 mg/kg**	5.66±0.66	6.0±0.333	6.0±0.333	6.0±0.577
V	**Extract 250 mg/kg +Alcl3 100mg/kg**	6.0±0.577	6.00±0.333	6.333±0.333	6.33±0.333
VI	**Extract 500 mg/kg+ Alcl3 100mg/kg**	6.0±0.577	6.33±0.333	6.667±0.333	6.66±0.333

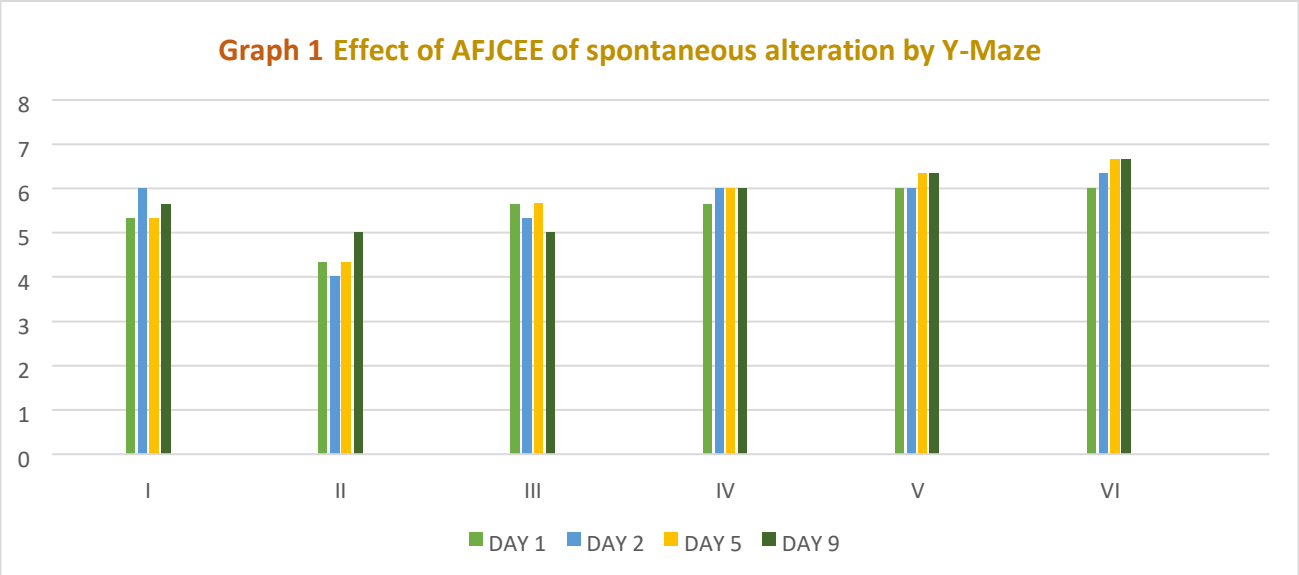
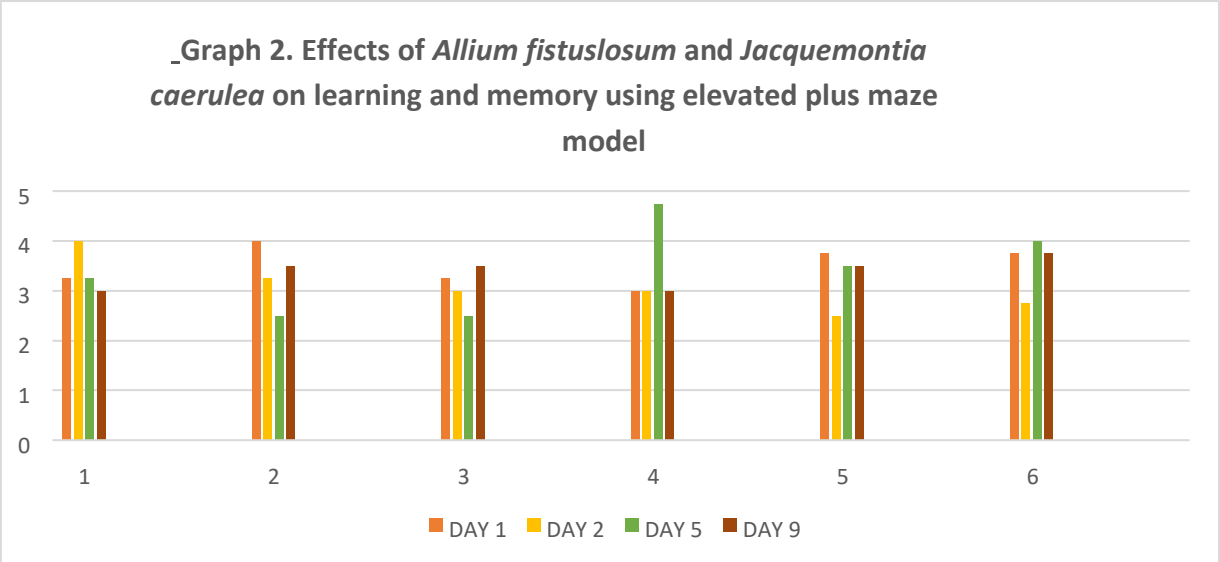


Table 3.4: Effects of *Allium fistuslosum* and *Jacquemontia caerulae* on learning and memory using elevated plus maze model

Group	Treatment(mg/kg)	DAY 1	DAY 2	DAY 5	DAY 9
I	**Control**	3.25±0.75	4.0±0.408	3.25±0.89	3.0±0.577
II	**AlCl3 100 mg/kg**	4.0±0.707	3.25±0.75	2.5±0.957	3.5±0.645
III	**Donepezil 5ml/kg + Alcl3 100mg/kg**	3.25±0.25	3.0±0.577	2.5±0.645	3.5±0.289
IV	**Extract 125 mg/kg + Alcl3 100 mg/kg**	3.0±0.408	3.0±0.577	4.75±0.75	3.0±0.577
V	**Extract 250 mg/kg +Alcl3 100mg/kg**	3.75±0.479	2.5±0.289	3.5±0.866	3.5±0.479
VI	**Extract 500 mg/kg+ Alcl3 100mg/kg**	3.75±0.479	2.75±0.479	4.0±0.408	3.75±0.408



4.DISCUSSION AND CONCLUSION:

In Indian system of medicine, we mostly use plant and their derivatives. Plants offer a wide variety of secondary metabolites which play an important role in metabolic pathways and also are extensively used in treating various diseases and serve as good source of nutrients.

Nootropics are also known as smart drugs, memory enhancers, cognitive enhancers. These are drugs or substances which are purported to improve mental functions such as cognition, attention, motivation and concentration.

Plant based nootropics are a diverse group of natural drugs which improve cognitive ability through various physiological processes.

AlCl3 induces dementia in experimental animals as well as in humans. Alzheimer's Disease is characteristic of neurological tangles formation in brain, cholinergic neuronal terminal loss in hippocampus and cortex, β-amyloid protein aggregation, development of oxidative stress and neuronal apoptosis in hippocampus which is a site for memory formation and synaptic plasticity.

It is well known that flavonoids are potent sources for promoting expression of brain derived neurotropic factor (BDNF), which is crucial to adult neurogenesis, synaptic growth and neuronal survival. Thus, we selected two plants, i.e., *Jacquemontia caerulea* and *Allium fistulosum* which have flavonoids and have significantly shown an increase in cognition as well as memory.

It is thus through evaluation of memory enhancing activity concluded that AFJCLEE showed significant increase in memory as well as cognition in $AlCl_3$ induced dementia in Swiss albino rats.

5. ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST: All authors approve the final manuscript and declare that there are no conflicts of interests.

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