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Pharmacological Evaluation of *Crossandra infundibuliformis* Leaves Extract in the Treatment of Sleep Deprived Dementia in Rats

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Abstract: *The current study evaluated the effects of the Methanolic Extract of Crossandra infundibuliformis leaves (MECI) on cognitive impairment induced by sleep deprivation in Sprague-Dawley rats. Rats were subjected to 21 days of Rapid Eye Movement (REM) sleep deprivation using Modified Multiple Platform Method (MMPM). Subsequently, they were treated with MECI (200 and 400 mg/kg), Donepezil (5 mg/kg), or vehicle for 21 days. Cognitive function was assessed using the Elevated Plus Maze (EPM) and Morris Water Maze (MWM) apparatus. Sleep-deprived control rats exhibited significant learning and memory deficits. Treatment with MECI at both doses significantly improved cognitive performance, reducing transfer latency in the EPM and escape latency in the MWM, while increasing retention time in the MWM. Phytochemical screening of MECI revealed the presence of alkaloids, tannins, phenolic compounds, flavonoids, saponins, and terpenoids. These findings suggest that Crossandra infundibuliformis leaves ameliorate cognitive impairment associated with sleep deprivation.*

Keywords: *Crossandra infundibuliformis*, Sleep deprived dementia, Alzheimer disease, Transfer latency, Escape latency, Retention Time

I. INTRODUCTION

The human brain is perhaps the most complex of all biological systems, with the mature brain composed of more than 100 billion information-processing cells called neurons. The brain is an organ composed of nervous tissue that commands task-evoked responses, movement, senses, emotions, language, communication, thinking, and memory. *(Maldonado KA et.al., 2023)*

Neurons are electrically excitable cells that transmit signals throughout the body. Neurons employ both electrical and chemical components in the transmission of information. *(Ludwig PE et.al., 2023)*

A common layman's conception of memory is the simple storage and retrieval of learned information that often evokes comparison to a filing system. Our everyday experience of memory, however, is in fact a complicated multifactorial process that consists of both conscious and unconscious components, and depends on the integration of a variety of information from distinct functional systems, each processing different types of information by different areas of the brain (substrates), while working in a concerted fashion. In other words, memory is not a singular process. It represents an integrated network of neurologic tasks and connections. *(Raslau F D, et.al., 2014)*

Dementia

Dementia refers to a syndrome that is characterized by progressive deterioration of cognitive function. The neuropsychiatric symptoms include apathy; agitation and depression. As the disorder progresses, the patient gradually becomes dependent on others to perform routine daily activities. The terms senile and pre-senile dementia have been used

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to differentiate between patients under or over 65 years. Disorders that may produce dementia syndrome are, Cortical dementia: Alzheimer's disease, Fronto-temporal dementia, Dementia with Lewy bodies; Subcortical dementia: Parkinson's disease, Huntington's disease, Wilson's disease, etc; Mixed dementia : Vascular dementia etc. *(Nitin Bansal et.al.,2014)*

Alzheimer's disease (AD) (named after the German psychiatric Alois Alzheimer) is the most common type of dementia and can be defined as a slowly progressive neurodegenerative disease characterized by neuritic plaques and neurofibrillary tangles , as a result of amyloid-beta peptide's (Aβ) accumulation in the most affected area of the brain, the medial temporal lobe and neocortical structures. *(Zeinab Breijyeh et.al.,2020)*

Epidemiology shows the number of dementia patients is projected to reach 152 million by mid-century worldwide, with the greatest increase expected in low-and middle-income countries. *(Zhang X. X. et.al.,2021)*

Sleep, Sleep deprivation and Dementia

Sleep is a critical function that is necessary for optimal cognitive functioning at all ages, but as age advances, there is an increased likelihood of sleep disturbances resulting in changes to the quality and quantity of sleep. As age increases, a number of changes occur to disrupt sleep that may result in fatigue, irritability, headaches, depression, excessive daytime sleepiness, and changes in motor and cognitive functioning among other effects. Sleep disturbances are also common to many neurodegenerative diseases and may include difficulty with insomnia, hypersomnia, parasomnias, excessive and abnormal nocturnal motor activity, disruption of circadian rhythmicities, and respiratory dysregulation. As the geriatric population continues to increase, so does the prevalence of dementia. *(Verna R Porter et.al.,2015)*

The pathophysiology of dementia is broadly thought to be related to the aggregation and accumulation of misfolded proteins. There are many hypothesis such as Amyloid hypothesis, Tau hypothesis, Oxidative stress, and neuro inflammation which contribute to development dementia (memory and learning impairement) *(Bruno P. Imbimbo et.al.,2005)*

Many researchers' studies have verified the relationship between sleep measurements, Aβ protein levels, and cognitive dysfunction in older patients, however, those studies has indicated that poor quality of sleep is related to a poor cognitive parameters. The abnormal accumulation of Aβ and tau proteins is a significant source of synaptic dysfunction, neurotransmission deterioration, and sleep problems, both of them playing a critical part in the development of Alzheimer's disease. Changes in sleep seem to precede the onset of cognitive symptoms in patients with AD, and sleep quality decline further in parallel with both cognitive disorders and the progression of the chronic disease. *(GaiedChortane et.al.,2023)*

Current Treatment

Given the limitations of current pharmacological treatments for dementia, which primarily offer symptomatic relief without altering disease progression, there is a growing interest in exploring alternative and complementary therapies, particularly those derived from medicinal plants (Herbal and medicinal plant remedies). Many plants are being investigated for their potential neuroprotective and cognitive-enhancing properties.*(Arpita Roy et.al.,2018).*

Rational for selecting *Crossandra infundibuliformis* species

Crossandra infundibuliformis (firecracker flower) is a plant traditionally used in various systems of medicine. Preliminary phytochemical investigations have revealed the presence of diverse bioactive compounds in its leaves, including tannins, saponins, alkaloids, flavonoids, steroids, glycosides, terpenoids, and carbohydrates, anthraquinones, phenolic compounds. *(Ch. Sajeena et.al.,2024)*

It is shows antimicrobial activity, wound healing activity, hepatoprotective activity, antibacterial activity, antituberculocis activity, antisolar activity, antioxidant activity, Anti-arthritic Activity, Antidiabetic Activity, anti-inflammatory activity, Antifungal and Anticandidal Activity, Anticancer and Insecticidal, Antiulcer Activity etc. The extract from the Firecracker plant is also used to treat minor headaches, aperitif, fever, pain, and also wound healing. *(Prajwal Chavhan et.al.,2023)*

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The phytochemicals present in *Crossadra infundibuliformis* may known for their antioxidant and anti-inflammatory properties, which could potentially be beneficial in mitigating neurodegenerative processes.

In this context, the current study aims to pharmacologically evaluate the efficacy of the MECI leaves in treating cognitive impairment induced by sleep deprivation in rats. It hypothesized that the bioactive compounds present in the leaf extract could attenuate the cognitive deficits resulting from REM sleep Deprivation, potentially offering a novel therapeutic avenue for conditions where sleep disruption contributes to cognitive decline. The study employed a rat model of sleep-deprived cognitive impairment and assessed learning and memory functions using the Elevated Plus Maze and Morris Water Maze apparatus.

II. PLANT PROFILE

Figure No. 1: *Crossandra infundibuliformis*

Kingdom	*Plantae*
Division	*Tracheophyta*
Order	*Lamiales*
Family	*Acanthaceae*
Genus	*Crossandra*
Species	*Infundibuliformis*

Table No. 1: Taxonomical Classification of *Crossandra infundibuliformis (Prajwal Chavhan et.al.,2023)*

III. MATERIALS AND METHOD

3.1 Materials

Chemicals and reagents: Petroleum ether, Methanol, Mayer's reagent, α-napthol, Conccentrated sulphuric acid, Glacial acetic acid, Ferric chloride, Ninhydrin solution, Lead acetate, Ammonia solution, and Donepezil used as standard drug to cure the experimental rats.

Apparatus and Instruments: Sleep Deprivation Appratus (Modified Multiple Platform Method), Morris water maze apparatus, Elevated plus maze apparatus

3.2 Method

Collection & Authentication of Plant Materials

Crossandra infundibuliformis was collected from Nursery, Yavatmal, Maharashtra, India. The plant material was identified and authenticated by Vasantrao Naik College of Agricultural Biotechnology, Yavatmal. (Ref No. VNCABT/Ytl/Hort/1859/2024. Date : 24/12/2024)

Extraction of *Crossandra infundibuliformis* Leaves

Leaves of *Crossandra infundibuliformis* plant were collected, dried in shade and coarsely powdered. The powdered leaves of *Crossandra infundibuliformis* were extracted in petroleum ether by using Soxhlet apparatus for removing out fat

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contents. Then powder was again dried and then extracted by methanol using Maceration. Percent Practical yield of MECI was found to be 8.4 % w/w.

Phytochemical Screening

MECI was subjected to phytochemical screening for the detection of various active constituents. This screening shows the presence of tannins, saponins, alkaloids, flavonoids, cardiac glycosides, terpenoids, and phenolic compounds.

Figure No. 2 : Phytochemicals screening of MECI for Alkaloids, Phenolic compound Cardiac glycosides, Terpenoids, Flavonoids

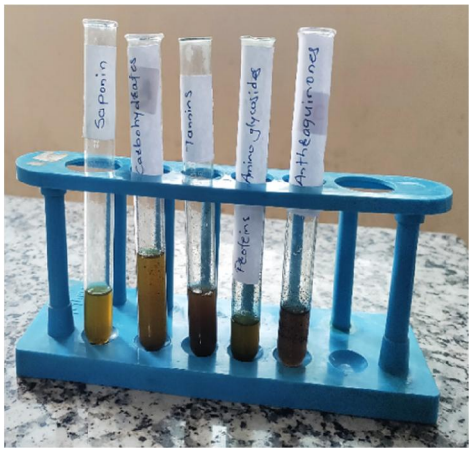

Figure No. 3 : Phytochemicals screening of MECI for Saponin, Carbohydrates, Tanins, Proteina and Amini acids, Anthraquinon

3.3. Experimental Design

Experimental Animals

8 weeks old healthy Female Sprague-Dawley rats (weighing 170-220 gm) were used for this study. Rats were housed in polypropylene cages with wire mesh top and husk bedding and maintain under control condition of light (12h – light, 12h - dark), temperature (25 ± 2 ˚C), and humidity (60 ± 5 %) and fed with a standard pellet diet and water. The experiments were performed for 24 hrs. The rats were housed and treated according to the rules and regulations of CPCSEA and IAEC. The protocol for all the animal study was approved by Institutional Animal Ethics Committee (IAEC) with research project number 650/Po/Re/2002/2025/CCSEA/10. Date : 09/01/2025)

Animal Groups

For this study, rats were divided into following groups (n = 6)

- **Group 1 (Vehicle Control) :-** Rats received only normal saline solution for 21 days.
- **Group 2 (Negative Control) :-** Memory & learning impairment in rats was produced by using Sleep Deprivation for 21 days.
- **Group 3 (MECI 200 mg/kg) :-** Memory impaired rats were treated with MECI (200 mg/kg) orally for 21 days.
- **Group 4 (MECI 400 mg/kg) :-** Memory impaired rats were treated with MECI (400 mg/kg) orally for 21 days.
- **Group 5 (Standard) :-**Memory impaired rats were treated with Donepezil (5 mg/kg) orally for 21 days.

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Memory and learning impairment were produced by using
Sleep Deprivation (SD) Method

Animals were subjected to SD using Modified Multiple Platform Method (MMPM). Briefly, the animals were placed on platforms (15 platforms; 20 cm high and 5 cm diameter, 7 cm apart edge-to-edge) surrounded by water [(24+- 1)o C] in an aquarium where water and food were provided to animals. The water level in the aquarium was kept about 4 cm below the edge of the platform. This method have been reported to interfere with total sleep, but it mainly eliminates REM sleep. Loss of muscle tone during REM sleep caused animals to fall into the water and waken. *(A.M. Aleisa et.al.,2011)*

Learning & Memory Impairment State was Checked in all Animals by Different Animal Models Before & After Administration of Sleep Deprivation

All animals in each group were assessed for the learning and memory impairment state by following animal models.
Elevated plus maze apparatus
Morris water maze apparatus
Reading of all animals in each group was noted down. These readings were referred as a 0 day before administration of Sleep deprivation. These reading were compared with the readings of animal model on day 3, day 7 and day 21 after administration of Sleep deprivation.

Study of Learning and Memory Impairment State on O Day , Day 3 , Day 7 &Day 21 by Following Model

Elevated Plus Maze Apparatus

The EPM apparatus consists of two intersecting arms that form the shape of a "+". Two opposing closed arms are constructed with high walls and are often termed alleys.A typical rat maze is raised approximately 70 cm above the floor, with each arm measuring 10 cm wide by 45 cm in length with the walls of the enclosed arms approximately 30 cm high.Observations are typically performed by video recording the test sessions, with the video camera mounted directly above the maze. The basic test is performed by placing the mouse or rat in the center of the maze, typically facing a closed arm. Upon release, the animal is free to explore the apparatus for a set period of time, usually five minutes. Two measures or indices of anxiety are then recorded transfer latency (TL) *(Guy B Mulder et.al.,2004)*

Morris Water Maze Apparatus

The MWM apparatus consisted of a circular water pool (120 cm diameter, 60 cm deep) divided into four equal quadrants. The water was made opaque by the addition of a non-toxic white colorant. A platform (10 cm diameter) was submerged 1 cm below the water surface in the center of one of the quadrants (the target quadrant).Each rat underwent four training trials per day for four consecutive days. In each trial, the rat was placed in the water at one of the four randomly chosen starting points (North, South, East, or West) facing the wall of the pool and allowed to swim to find the hidden platform. The time taken to find the platform was recorded as the escape latency (EL), with a maximum cutoff time of 60 seconds. If a rat failed to find the platform within 60 seconds, it was gently guided to it and allowed to stay there for 20 seconds. The escape latency was recorded for each trial. An hours after the last training trial, a probe trial was conducted. The platform was removed from the pool, and each rat was allowed to swim freely for 60 seconds. The retention time (RT), defined as the time spent in the target quadrant where the platform was previously located, was recorded as a measure of spatial memory. *(Sahba Jafarian et al.,2019)*

Statistical Analysis

All data were expressed as the mean ± standard deviation. For statistical Analysis of the data, group mean were compared by one-way (ANOVA) followed by Dunnett's test, $p<0.01$ was considered as significant value.

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IV. RESULTS

4.1 Estimation of Behavioral Study

A. Elevated Plus Maze Apparatus

Transfer Latency

Table No. 4.1 : Effect of MECI on transfer latency (TL) of rats in EPM apparatus

Sr. No.	Groups	Transfer latency in seconds on Day 0	Transfer latency in seconds on Day 3	Transfer latency in seconds on Day 7	Transfer latency in second on Day 21
1.	Normal Control	38.76 ± 1.86	38.97 ± 1.19	39.64 ± 2.25	39.55 ± 2.14
2.	Negative Control	46.29 ± 2.91^{ns}	46.33 ± 1.61^{ns}	66.33 ± 2.84$^{@}$	79.45 ± 2.90$^{@}$
3.	MECI(200 mg/kg)	41.39 ± 2.28^{ns}	41.40 ± 1.66^{ns}	61.32 ± 2.77**	56.09 ± 2.80**
4.	MECI(400 mg/kg)	42.67 ± 2.24^{ns}	42.68 ± 1.14^{ns}	53.27 ± 2.89**	44.05 ± 2.95**
5.	Donepezil (5mg/kg)	44.25 ± 2.50^{ns}	44.27 ± 1.18^{ns}	46.33 ± 2.58**	32.41 ± 2.65**

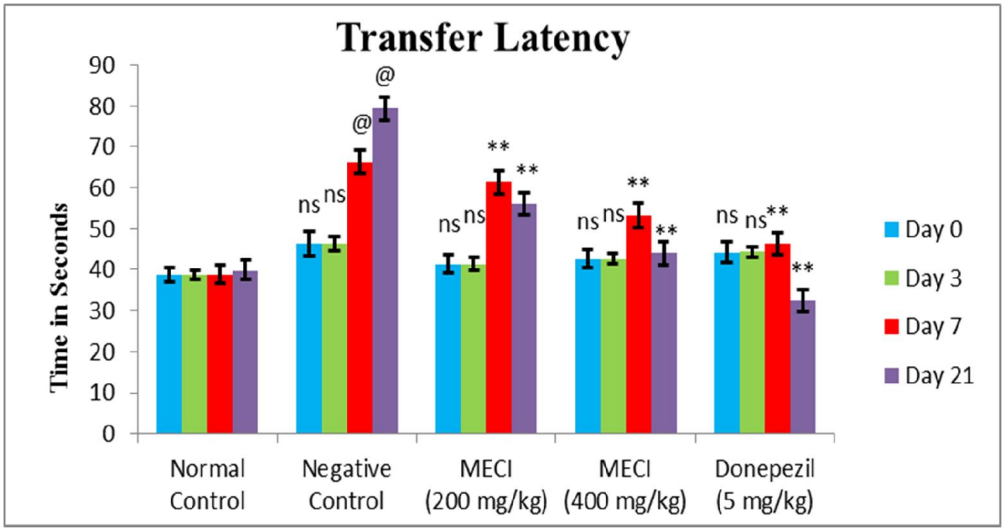

Figure No. 4.1 : Effect of MECI on transfer latency of rats in EPM apparatus

All values are expressed as Mean ± SD $^{@}$ p<0.01 Significant increase in transfer latency was observed when compared to normal control group. ** p<0.01 Significant decrease in transfer latency was when observed compared to negative control group.

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B. Morris Water Maze Apparatus

1. Escape Latency

Table No. 4.2 : Effect of MECI on escape latency (EL) of rats in MWM apparatus

Sr. No.	Groups	Escape latency in seconds on Day 0	Escape latency in seconds on Day 3	Escape latency in seconds on Day 7	Escape Latency in second on Day 21
1.	Normal Control	24.46 ± 0.93	23.74 ± 1.10	24.93 ± 1.49	23.93 ± 1.50
2.	Negative Control	34.81 ± 1.91^{ns}	34.90± 1.42^{ns}	58.45± 1.63$^{@}$	61.81± 1.53$^{@}$
3.	MECI(200 mg/kg)	36.62 ± 1.74^{ns}	36.75± 1.25^{ns}	50.32± 1.87**	37.75± 1.88**
4.	MECI(400 mg/kg)	34.07 ± 2.01^{ns}	34.25± 0.96^{ns}	44.85± 0.72**	31.00± 0.79**
5.	Donepezil (5mg/kg)	35.88 ± 1.60^{ns}	35.97± 1.92^{ns}	40.80± 2.55**	26.67± 2.59**

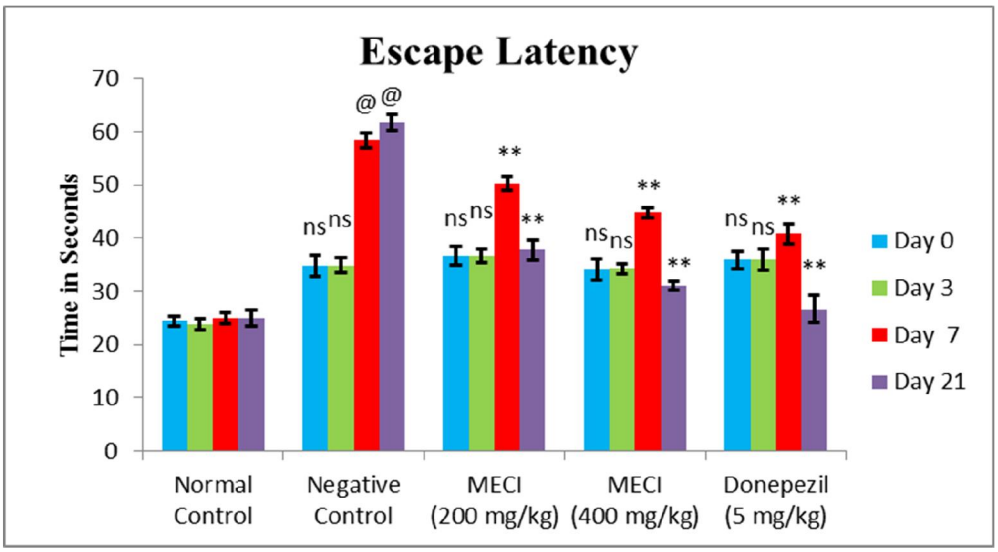

Figure No. 4.2 : Effect of MECI on escape latency of rats in MWM apparatus

All values are expressed as Mean ± SD $^{@}$ $p<0.01$ Significant increase in escape latency was observed when compared to normal control group. $^{**}p<0.01$ Significant decrease in escape latency was when observed compared to negative control group.

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2. Retention Time

Table No. 4.3 : Effect of MECI on retention time (RT) of rats in MWM apparatus

Sr. No.	Groups	Retention Time in seconds on Day 0	Retention Time in seconds on Day 3	Retention Time in seconds on Day 7	Retention Time in second on Day 21
1.	Normal Control	52.89 ± 1.37	56.12 ± 0.71	57.16 ± 1.26	58.15 ± 1.16
2.	Negative Control	52.66 ± 2.34^{ns}	52.67 ± 1.64^{ns}	49.40 ± 2.97$^{@}$	42.23± 2.94$^{@}$
3.	MECI(200 mg/kg)	54.62 ± 2.05^{ns}	54.63± 0.93^{ns}	69.33 ± 1.55**	78.22± 1.50**
4.	MECI(400 mg/kg)	57.58 ± 2.52^{ns}	57.59± 1.90^{ns}	75.33 ± 1.98**	82.33± 1.90**
5.	Donepezil (5mg/kg)	58.55 ± 0.72^{ns}	58.56± 1.95^{ns}	80.44 ± 1.69**	85.55± 1.70**

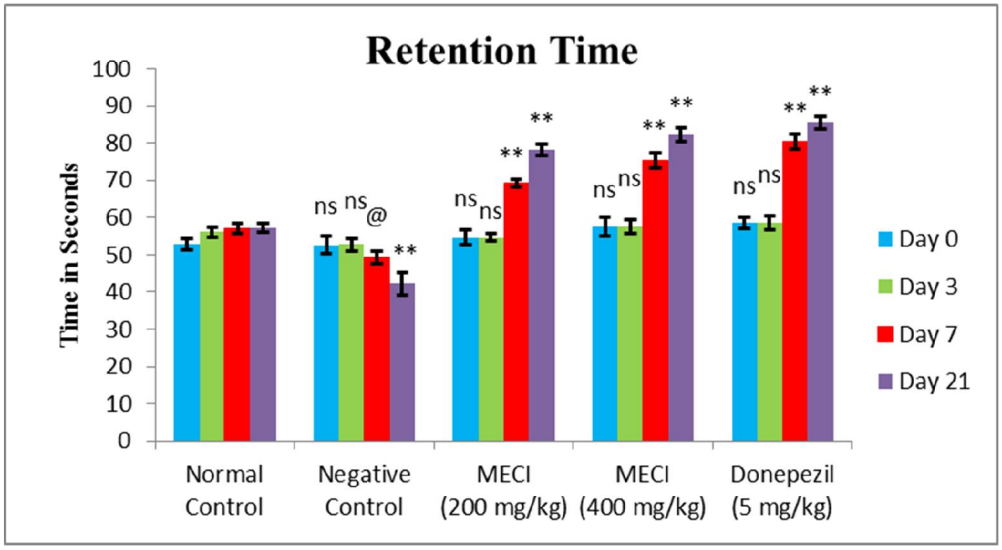

Figure No. 4.3 : Effect of MECI on retention time of rats in MWM apparatus

All values are expressed as Mean ± SD $^{@}$ $p<0.01$ Significant decrease in retention time was observed when compared to normal control group. $^{**}p<0.01$ Significant increase in retention time was observed when compared to negative control group.

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V. DISCUSSION

Current study investigated the potential of the MECI leaves (MECI) to alleviate cognitive impairment induced by sleep deprivation in rats. Sleep deprivation for 7 days resulted in significant learning and memory deficits, as evidenced by increased transfer latency in the Elevated Plus Maze (EPM) and increased escape latency with decreased retention time in the Morris Water Maze (MWM).

In elevated plus maze apparatus, there was significant ($p<0.01$) increase in TL in negative control group as compared to normal control group on Day 7 and Day 21. There was significant ($p<0.01$) decrease in MECI 200 mg/kg, MECI 400 mg/kg and Donepezil (5 mg/kg) treated group at Day 7 and Day 21 when compared to negative control group.

In morris water maze apparatus, there was significant ($p<0.01$) increase in EL in negative control group as compared to normal control group on Day 7 and Day 21 . There was significant ($p<0.01$) decrease in MECI 200 mg/kg, MECI 400 mg/kg and Donepezil (5 mg/kg) treated group at Day 7 and Day 21 when compared to negative control group.

In MWM in memory impaired rats, there was significant ($p<0.01$) decrease in RT in negative control group as compared to normal control group on Day 7 and Day 21. There was significant ($p<0.01$) increase in MECI 200 mg/kg, MECI 400 mg/kg and Donepezil (5 mg/kg) treated group at Day 7 and Day 21 when compared to negative control group.

Phytochemical screening of MECI revealed the presence of various compounds, including alkaloids, tannins, phenolic compounds, flavonoids, saponins, and terpenoids, which are known to possess neuro-protective properties. The observed beneficial effects of MECI are likely attributable to the synergistic action of these phytochemicals, potentially through mechanisms involving antioxidant and anti-inflammatory actions

Current study revealed that MECI leaves exhibits learning and memory enhancing activity due to the presence of flavonoids as a major constituent.

VI. CONCLUSION

The present finding indicates that the MECI leaves (200 mg/kg) exhibits significant mild to moderate learning and memory enhancing activity and MECI leaves (400 mg/kg) showed significant improvement in learning and memory enhancing activity in rats. It can be conclude that MECI leaves can be utilized for the treatment of Sleep deprived Dementia.

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