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Memory Enhancing Activity of *Dendrobium macraei* Lindl. in Swiss Albino Mice

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Memory is not a single process, but rather a series of interactive processes beginning when we are exposed to new information, which is registered by the brain, encoded, and in the right conditions, stored for later retrieval. Short and long term memory loss may result from deteriorating cerebral mechanisms due to different causes having impact on the quality of life. Memory enhancers can improve thinking, memory, and alertness in people with Alzheimer's disease or dementia that affect the mind or memory.
Objective: In the present study, an attempt has been made to highlight the importance of the plant *Dendrobium macraei* Lindl. (family- Orchidaceae) in the field of traditional medicines which is traditionally used as memory enhancer.
Methods: The present study comprises *in vivo* memory enhancing activity evaluation in mice using various memory models such as elevated plus maze model and Morris water maze model. Piracetam was used as standard drug. Effects of various extract viz. petroleum ether, chloroform, ethyl acetate, methanol and aqueous extract of *D. macraei* were evaluated for memory enhancing activity.

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Results and Conclusion: Amongst all extracts tested, only ethyl acetate and methanol extract of *D. macraei* showed significant short term and long term memory enhancing activity using elevated plus maze model and Morris water maze model in mice respectively. Ethyl acetate extract (200 mg/kg, p.o.) showed marked significant (P<0.05) effect as compared to methanol extract (200 mg/kg, p.o.) in both the models. Therefore, it would be worthwhile to explore the potential of this plant in the management of dementia.

Keywords: Dendrobium macraei; memory; memory enhancement; piracetam; elevated plus maze model; Morris water maze model.

1. INTRODUCTION

Memory is the ability of an individual to record sensory stimuli, events, information, *etc.*, retain them over short or long periods of time and recall the same at a later date when needed. Poor memory, lower retention and slow recall are common problems in today's stressful and competitive world. Age, stress, emotions are conditions that may lead to memory loss, amnesia, anxiety, high blood pressure, dementia, or to more ominous threats like schizophrenia and Alzheimer's diseases (AD) [1]. AD is a neurodegenerative disorder characterized by a progressive loss of memory and cognition [2]. Reducing oxidative stress by anti-oxidants, protecting brain inflammatory lesions using anti-inflammatory drugs and facilitation of brain cholinergic neurotransmission with anti-cholinesterases are some positive approaches to management of AD [3]. The nature provides a new opportunity to regain one's full mental capacity. A number of herbs traditionally employed in the Indian System of Medicine "Ayurveda," have yielded positive results.

The plant *Dendrobium macraei* is the important botanical source of Ayurvedic drug Swarna Jivanti (common name) belonging to family Orchidaceae. It is an epiphyte with creepy rhizome and pendulous stem [4]. The plant is sweet with a flavor, cooling, alterative, astringent to the bowels, brain tonic, aphrodisiac, expectorant, useful in asthma, bronchitis, 'tridosha', throat troubles, fevers, burning sensations, biliousness, diseases of the eye and the blood. The plant is stimulant and brain tonic [5]. It is reported to contain alkaloids, carbohydrates, flavonoids, steroids, tannins and phenolic compounds. Jibantine, resinous principles α and β jibantic acid and diosgenin derivatives like denfigenin and defuscin as steroids are reported as chief constituents in this plant [6]. Swarna Jivanti is one of the important *Rasayana* drugs in Ayurveda. Traditionally, it is being used as a memory modulator. Therefore, the present study was undertaken to investigate the effect of *D. macraei* on learning and memory in mice. Both short term memory and long term memory were evaluated using different models of memory.

2. MATERIALS AND METHODS

2.1 Plant Material

Plant material (*Dendrobium macraei*, whole plant) (Plate 1) was procured from Kankhal, near Haridwar, Uttarakhand, India. The plant was identified and authenticated at the Herbarium of Council of Scientific and Industrial Research - National Institute of Science Communication and Information Resources (CSIR- NISCAIR), Delhi vide reference no. NISCAIR/RHMD/Consult/ 2015/2565-144.

Plate 1. Whole plant of *D. macraei*

2.2 Animals

The experimental animals (Swiss albino mice of either sex, 20-25 g) was used in the present study after taking approval from the Institutional Animal Ethical Committee of Sardar Bhagwan Singh Post Graduate Institute of Biomedical Sciences and Research, Balawala, Dehradun-

248161, Uttarakhand, India for carrying out biological studies. The animals were housed under standard and easily adaptable conditions, room temperatures and a 12 h light: dark cycle. They were provided with food and water *ad libitum*. The animals were acclimatized under these conditions for 6 days prior to the experiment.

2.3 Drugs and Chemicals

Piracetam (ALO'S Pvt. Ltd., India) and Diazepam (Yarrow chem., India) were used as standard drugs. Distilled water was used as vehicle for preparing the various test doses of different extracts of *D. macraei*.

2.4 Acute Toxicity Study

The acute toxicity study was carried out as per Organization for Economic Cooperation and Development (OECD) 423 Guidelines, 2001. Swiss albino mice were used for the study. The acute toxicity study revealed the nontoxic nature of all the extracts even at highest starting dose of 2000 mg/kg body weight of animal for oral route of administration.

2.5 Preparation of Extracts

Dried parts of whole plant were pulverized using a mechanical grinder. Powdered material was subjected to successive soxhlet extraction by solvents in increasing order of polarity viz. petroleum ether, chloroform, ethyl acetate, methanol and distilled water. Before each extraction the powdered material was dried in hot air-oven below 50℃. Finally, marc was boiled with distilled water for 4 h to obtain the aqueous extract. All extracts were concentrated in a rotary vacuum evaporator (40ºC), freeze-dried and stored at 4ºC until further use in the experiment.

2.6 Standardization of Extracts

2.6.1 Determination of total phenolic content [7]

2.6.1.1 Preparation of standard

10 mg of gallic acid was dissolved in 100 ml of 50% methanol (100 µg/ ml) and then, further diluted to 1, 2, 4, 8, 16 µg/ml. 1 ml aliquot of each dilution was taken in a test tube and diluted with 10 ml distilled water. Then, 1.5 ml of Folin Ciocalteu reagent was added and allowed to incubate at room temperature for 5 minutes. 4 ml of 20% w/w sodium carbonate solution was added in each test tube and then, further adjusted with distilled water up to the mark of 25 ml, agitated and left to stand for 30 min at room temperature. Absorbance of standard was measured at 765 nm using spectrophotometer against blank (distilled water).

2.6.1.2 Preparation of test

One mg /ml of all plant extracts were prepared in methanol and then, diluted with 10 ml distilled water. Then, 1.5 ml of Folin Ciocalteu reagent was added and allowed to incubate at room temperature for 5 min. 4 ml of 20% w/w sodium carbonate solution was added in each test tube and then, further adjusted with distilled water up to the mark of 25 ml, agitated and left to stand for 30 min at room temperature. Absorbance of test samples was measured at 765 nm using spectrophotometer against blank (distilled water). The total phenolic content was expressed in milligrams of gallic acid equivalents per gram of extract.

2.6.2 Determination of total flavonoid content

2.6.2.1 Preparation of standard

10 mg rutin was dissolved in 100 ml of 80% methanol (100 µg/ ml), and further diluted to 7.5, 15, 30, 60, 120 µg/ ml. The diluted standard solutions (0.5 ml) were separately mixed with 1.5 ml of methanol (95%), 0.1 ml of $AlCl_3$ (10%), 0.1 ml of 1 M (Potassium acetate) and 2.8 ml of distilled water. Absorbance at 415 nm was recorded after 30 min of incubation against blank (distilled water). A standard calibration plot was generated at 415 nm using known concentrations of quercetin.

2.6.2.2 Preparation of test

The total flavonoid content of all extracts of *D. macraei* was estimated according to the aluminium chloride method as follows: Aliquots of extract 1 mg/ ml solutions was taken and the diluted standard solutions (0.5 ml) were separately mixed with 1.5 ml of methanol (95%), 0.1 ml of $AlCl_3$ (10%), 0.1 ml of 1 M (Potassium acetate) and 2.8 ml of distilled water. Absorbance at 415 nm was recorded after 30 min of incubation against blank (distilled water). The concentrations of flavonoid in the test samples was calculated and expressed as mg quercetin equivalent/g of sample [8].

The total flavonoid content was expressed in milligrams of rutin equivalents per gram of extract.

2.7 Experimental Protocol

Mice were divided into six groups consisting of 5 animals per group. Group I animals were treated with control vehicle (Distilled water) taken as control group, Group II animals were administered with Diazepam (D) (4 mg/kg i.p.) which is served as inducing group. Group III, IV, V were treated with ethyl acetate and methanol extract at a dose of 50, 100, 200 mg/kg p.o. respectively along with diazepam served as test groups and group VI was given Piracetam 200 mg/kg p.o. with diazepam as positive control or standard group. All the extracts and standard drug treated animals were subjected to Diazepam (D) (4 mg/kg i.p.) 60 minutes before administration of extract and Piracetam, except the first group which served as vehicle control. The protocol is summarized in Table 2.

2.8 Models for Memory Enhancing Activity

2.8.1 Elevated plus-maze (EPM) test

The EPM consists of two open arms (16 × 5 cm) and two enclosed (covered) arms (16 × 5 × 12 cm) and the arms were extended from a central platform and the maze was elevated to a height (25 cm) from the floor. On the first day, each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was taken as the time taken by the mouse to move into any one of the covered arms with all its four legs. TL was recorded on the first day. If the mouse did not enter into one of the covered arms within 90 sec, it was gently pushed into one of the two covered arms and the TL was assigned as 90 sec. The mouse was allowed to explore the maze for 10 sec and then was return to its home cage. Memory retention was examined 24 h after the first day trial on the second day [9,10].

2.8.2 Morriz water maze test

Morris water maze was employed to evaluate learning and memory. It consists of a circular water tank (diameter 150 cm and height 45 cm), filled with water maintained at 25℃. The water was made opaque with a white coloured dye (milk). The tank was divided into four equal quadrants with the help of two threads, fixed at right angle to each other on the rim of the pool. A platform (10 cm^2) of approximately 29 cm height was located in the centre of one of these four quadrants. The position of platform and clues was kept consistent throughout the training session.

- **Acquisition trial:** Each animal was subjected to four consecutive trials on each day with an interval of 5 min, during which mouse was allowed to escape on the hidden platform and was allowed to remain there for 20 sec. In case of the inability of the animal to locate the hidden platform within 90 sec., it was gently guided by hand to the platform and allowed to remain there for 20 sec. Escape latency time (ELT) to locate the hidden platform in water maze was noted as an index of acquisition and learning. In the preliminary study, trial was conducted to familiarize the mice with the task and was not counted. Mouse was subjected to acquisition trials for four consecutive days. Starting position on each day to conduct four acquisition trials was changed as follows:

  Day 1 Q1 Q2 Q3 Q4
  Day 2 Q2 Q3 Q4 Q1
  Day 3 Q3 Q4 Q1 Q2
  Day 4 Q4 Q1 Q2 Q3

- **Retrieval trial:** On the next day, platform was removed and each mouse was allowed to explore the pool for 90 s. Mean time spent of the animal in each of four quadrants was noted. The mean time spent by the mouse in target quadrant for searching the hidden platform was noted as an index of retrieval. The experimenter always stood at the same position. Care was taken that relative location of water maze with respect to other objects in the laboratory, serving as prominent visual clues was disturbed during the total duration of study [11].

2.9 Statistical Analysis

The values were represented as mean ± SEM. (n=6) statistically significant differences between groups were calculated by using ANOVA, followed by Post hoc Tukey's multiple range test where $P<0.05$ was considered statistically significant.

3. RESULTS

In the acute toxicity test observation indicated that there was no death at 2000 mg/kg dose after 72 hr. Therefore, the drug was found safe for further investigations.

Total phenolic content was determined in various prepared extracts by taking the standard plot of gallic acid as standard drug (Fig. 1). Total phenolic content in ethyl acetate extract found in higher amount as compared to methanol extract which is shown in Table 1. Total flavonoid content was determined in various prepared extracts by taking the standard plot of rutin as standard drug (Fig. 2). Total flavonoid content in ethyl acetate extract estimated higher than methanol extract (Table 1).

In elevated plus maze model, the effect of EA (ethyl acetate) and M (methanol) extract (doses-50, 100 or 200 mg/kg administered orally for 7 successive days) of *D. macraei*, piracetam (200 mg/kg, p.o.) which was used as a standard drug and diazepam (D) (4 mg/kg, i.p.) was used as inducing (memory impairment) drug were evaluated on transfer latency of mice using elevated plus-maze after trial period of mice of about 7 days. The whole study during the test shows reflected retention of information or memory in mice. Transfer latency on 1st day of drug treatment reflected learning behaviour of animals, whereas transfer latency of next day of activity period reflected retention of information or memory.

The effect of vehicle, diazepam control, EA & M (50 mg/ kg, 100 mg/kg, and 200 mg/kg) and piracetam were evaluated at 1st day & after 24 hr of activity duration of administration of drugs. Transfer latency on 1st day of drug treatment reflected learning behaviour of animals, whereas transfer latency of next day reflected retention of information or memory. Diazepam (4 mg/kg i.p) group showed a significant increase in transfer latency values on acquisition as well as on the retention days as compared with vehicle control mice, indicating impairment in learning and memory. EA & M at dose level of 50, 100, 200 mg/kg orally demonstrated significant decrease in transfer latency on transfer latency on 1st day and 24 hr after in elevated plus maze test as compared to diazepam control and successfully reversed memory deficit induced by diazepam ($p<0.05$). Piracetam used as standard drug at a dose of 200 mg/kg also improved learning and memory in mice and reversed the amnesia induced by diazepam (Table 3, Figs. 3, 4). The results obtained were statistically significant ($P<0.05$).

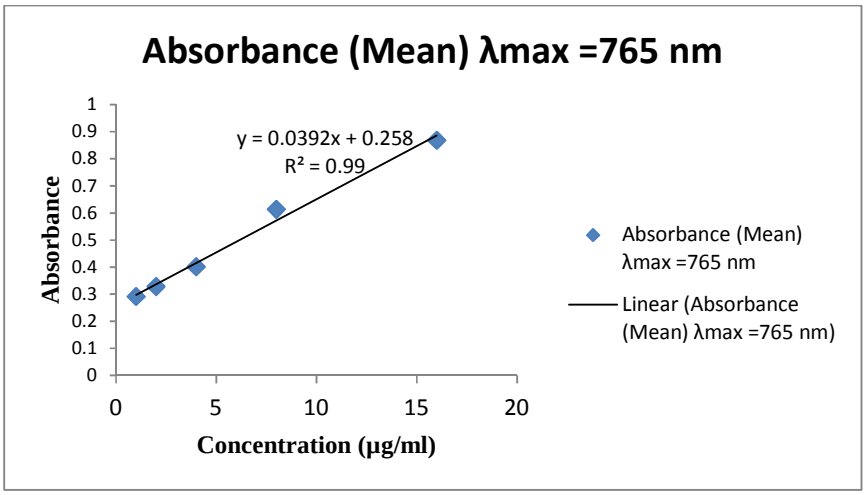

Fig. 1. Calibration curve of standard gallic acid for determination of total phenolic content in *D. macraei*

Table 1. Total phenolic and flavonoid content in different extracts of *D. macraei*

S. no.	Plant extracts	% total phenolic content (mg/g)	% total flavonoid content (mg/g)
1.	Ethyl acetate extract	6.4256%	3.575%
2.	Methanol extract	4.301%	2.087%

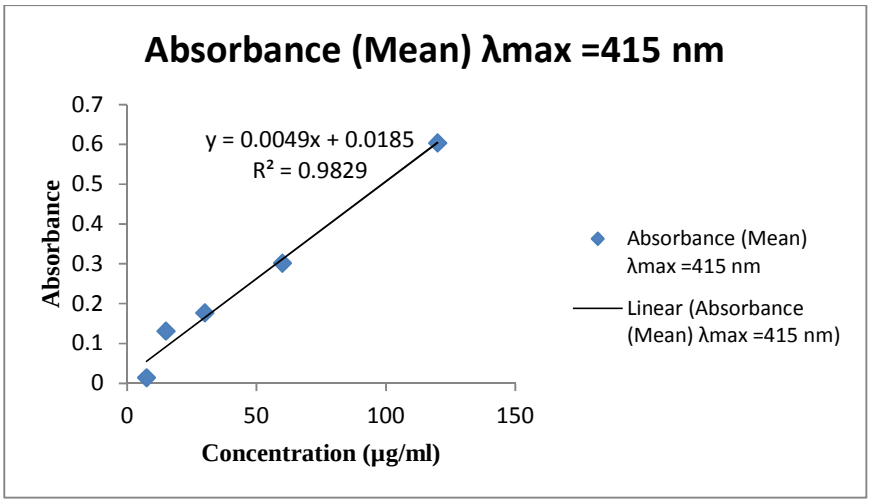

Fig. 2. Calibration curve of standard rutin for determination of total flavonoid content in *D. macraei*

Table 2. Study protocol design (n=6)

S. no.	Groups	Treatment (mg/kg)		Remarks
1.	I	Distilled water		Vehicle control
2.	II	Diazepam (D) (4 mg/kg, i.p)		Inducing group
3.	III	D + Ethyl acetate extract (50 mg/kg, p.o.)	D + Methanol extract (50 mg/kg, p.o)	Test
4.	IV	D + Ethyl acetate extract (100 mg/kg, p.o.)	D + Methanol extract (100 mg/kg, p.o)	Test
5.	V	D + Ethyl acetate extract (200 mg/kg, p.o.)	D + Methanol extract (200 mg/kg, p.o.)	Test
6.	VI	D + Piracetam (200 mg/kg, p.o.)		Standard

Table 3. Effect of different extracts of *D. macraei* on transfer latency in mice using an elevated plus-maze model

Sr. no.	Groups	Treatment (mg/kg)	Transfer latency time (seconds)	Average no. of entries in closed arms
1.	I	Distilled water	45.4 ± 6.735	2.0 ± 0.199#
2.	II	Diazepam (D) (4 mg/kg i.p.)	73.2 ± 8.135#	4.6 ± 0.244
3.	III	D + Ethyl acetate extract (50 mg/kg, p.o.)	42.4 ± 1.802	2.6 ± 0.316
		D + Methanol extract (50 mg/ kg p.o)	45.6± 0.748	2.9 ± 0.244
4.	IV	D + Ethyl acetate extract (100 mg/kg, p.o.)	30.1 ± 1.869*	1.8 ± 0.199
		D + Methanol extract (100 mg/kg, p.o.)	38 ± 1.427	2.2 ± 0.199
5.	V	D + Ethyl acetate extract (200 mg/kg, p.o.)	24.2 ± 1.580*	1.2 ± 0.199
		D + Methanol extract (200 mg/kg, p.o.)	29 ± 1.378*	1.6 ± 0.199
6.	VI	D + Piracetam (200 mg/kg, p.o.)	25 ± 4.06*	1.1 ± 0.244

*All values are expressed as mean ± SD, n=6, *denotes P< 0.05 compared with control group of mice (#). # denotes P< 0.05 compared with vehicle control group of mice*

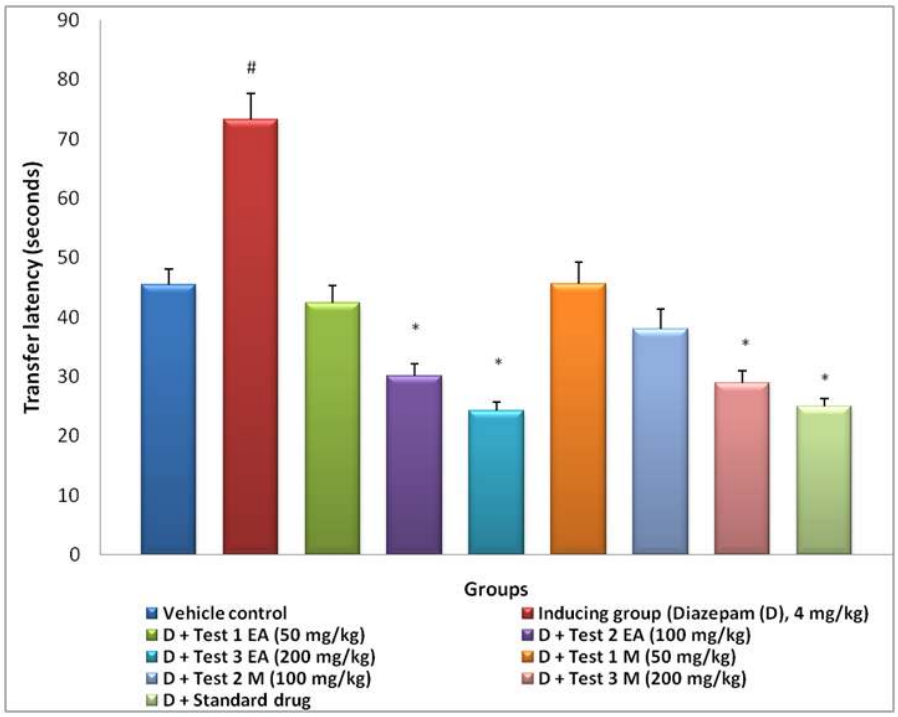

Fig. 3. Effect of EA (ethyl acetate) and M (methanol) extract (doses- 50, 100 or 200 mg/kg administered orally for 7 successive days) of *D. macraei* on transfer latency of mice using elevated plus-maze. Piracetam (200 mg/kg, p.o.) was used as a standard drug. Diazepam (D) (4 mg/kg, i.p.) was used as inducing (memory impairment) drug

*All values are expressed as mean ± S.D., n=5. *Denotes P < 0.05 compared with control group of mice (#)*

In Morris water maze model, the effect of EA (ethyl acetate) and M (methanol) extract (doses- 50, 100 or 200 mg/kg administered orally for 7 successive days) of *D. macraei*, Piracetam (200 mg/kg, p.o) which was used as a standard drug and Diazepam (D) (4 mg/kg, i.p.) was used as inducing (memory impairment) drug were evaluated on escape latency of mice using Morris water-maze in target quadrant (Q4) on 7th retrieval day after trial period of mice of about 7 days. The whole study during the test shows reflected retention of information for a long term period or increased memory in mice. Escape latency on 1st to 7 consecutive days of drug treatment or during training sessions reflected learning behaviour of animals, whereas escape latency of 7th retrieval day of activity period reflected retention of information or memory. The effect of vehicle, diazepam control, EA & M (50 mg/ kg, 100 mg/kg, and 200 mg/kg) and piracetam were evaluated at 7th retrieval day & after 7 days training sessions. Escape latency on 7th consecutive days during training sessions of drug treatment reflected learning behaviour of animals Diazepam (4 mg/kg i.p.) group showed a significant increase in escape latency values on acquisition as well as on the retention days as compared with vehicle control mice, indicating impairment, whereas escape latency of 7th retrieval day reflected retention of information or memory in learning and memory. EA & M at dose level of 50, 100, 200 mg/kg orally demonstrated significant decrease in escape latency during training session days and at 7th retrieval day after drug administration in Morris water maze test as compared to diazepam control and successfully reversed memory deficit induced by diazepam (P<0.05). Piracetam was used as standard drug (at a dose of 200 mg/kg p.o.) also improved learning and memory in mice and reversed the amnesia induced by diazepam (Table 4, Fig. 5). The results obtained was statistically significant (p<0.05).

4. DISCUSSION

Global scenario of persons afflicted by mental disorders is alarming. About 500 million people suffer from neurotic, stress related and somatoform problems, 200 million from mood

disorders, 83 million from mental retardation, 30 million from epilepsy, 22 million from dementia, and 16 million from schizophrenia. Amongst them dementia are serious medical illnesses that have affected 1/8th of total population worldwide irrespective of gender, age, religion, nationality and profession. Alzheimer's diseases (AD) is a progressive and fatal neurodegenerative disorder manifested by cognitive and memory deterioration, progressive impairment of routine activities of living, and a variety of neuropsychiatric symptoms and behavioural disturbances [12]. The clinical features of AD are an amnesic type of memory impairment, deterioration of language and visuospatial deficits. Motor and sensory abnormalities, gait disturbance and seizures are uncommon until the late phases of the disease [13]. Despite the severity and high prevalence of this disease, Allopathic system of medicine is yet to provide a satisfactory antidote. Therefore, we were motivated to explore the new approach in Indian traditional system to manage this deadly disease (AD). In the present study, we have focused upon exploring the potential of *Dendrobium macraei* which is traditionally used for the treatment of various ailments, especially in CNS disorders as nervine tonic, memory enhancer and stimulant. Despite a long history of traditional uses the plant has never been subjected to CNS activity studies. Thus, it was considered worthwhile to evaluate *D. macraei* for memory enhancing activity.

The plant extracts were standardized on the basis of total phenols and flavonoid compounds. Quantification of total phenolic content is based on colorimetric measurements. Total phenolic content was determined in various prepared extracts by taking the standard plot of gallic acid as standard drug which is further compared with extracts total phenolic content in which ethyl acetate extract shows high amount of total phenolic content (6.4256%) as compared to methanol extract (Table 1). Flavonoids were the most important plant pigments for flower coloration, producing pigmentation. They may also act as chemical messengers, physiological regulators, and cell cycle inhibitors, and to protect body against reactive oxygen species. Total flavonoid content was determined in various prepared extracts by taking the standard plot of rutin as standard drug which is further compared with extracts total flavonoid content in which ethyl acetate extract shows high amount of total flavonoid content (3.575%) as compared to other methanol extract (Table 1).

Table 4. Effect of different extracts of *D. macraei* on escape latency in mice using Morris water-maze test on 7thretrieval day after training sessions in target quadrant (Q4)

Sr. no.	Groups	Treatment (mg/kg)	Escape latency (seconds)	Average no. of entries taken by mice to find the hidden platform
1.	I	Distilled water	55.3 ± 2.549 ^{c}	2.2 ± 0.199 ^{c}
2.	II	Diazepam (D) (4 mg/ kg, i.p.)	79.8 ± 6.529 ^{a}	4.5 ± 0.316 ^{a}
3.	III	D + Ethyl acetate extract (50 mg / kg, p.o.)	44.2 ± 1.87	2.9 ± 0.316
		D + Methanol extract (50 mg / kg, p.o.)	50.5 ± 1.224	3.6 ± 0.244
4.	IV	D + Ethyl acetate extract (100 mg / kg p.o.)	34.5 ± 1.029 ^{b}	2.4 ± 0.223^{b}
		D + Methanol extract (100 mg / kg, p.o.)	43.3 ± 0.979	2.9 ± 0.316
5.	V	D + Ethyl acetate extract (200 mg / kg, p.o.)	27.8 ± 0.969 ^{b}	1.2 ± 0.199 ^{b}
		D + Methanol extract (200 mg / kg, p.o.)	36.4 ± 1.224 ^{b}	1.9 ± 0.244 ^{b}
6.	VI	D + Piracetam (200 mg / kg, p.o.)	27.1 ± 1.157 ^{b}	1.8 ± 0.373 ^{b}

All values are expressed as mean ± S.D, n=6.

a= P<0.05 vs. time spent in target quadrant in control mice (c); b= p< 0.05 vs. time spent in target quadrant in inducing group (a); c= p< 0.05 vs. time spent in other quadrants (Q1, Q2, Q3) in control mice

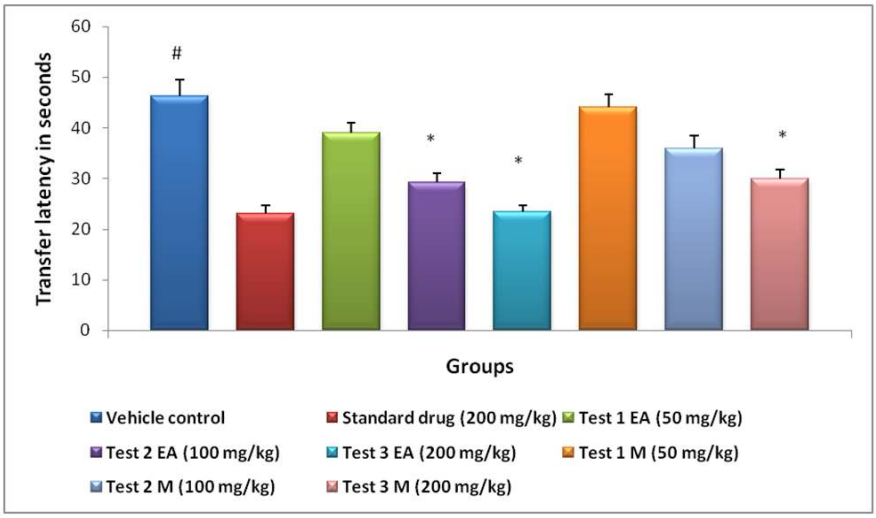

Fig. 4. Reversal of diazepam (4 mg/kg, i.p.) induced amnesia by EA (ethyl acetate) and M (methanol) extract (doses- 50, 100 or 200 mg/kg administered orally for 7 successive days) of *D. macraei* on transfer latency of mice using elevated plus-maze. Piracetam (200 mg/kg, p.o.) was used as a standard drug. Diazepam (D) (4 mg/kg, i.p.) was used as inducing (memory impairment) drug

*All values are expressed as mean ± S.D., n=5. *Denotes P< 0.05 compared with control group of mice (#)*

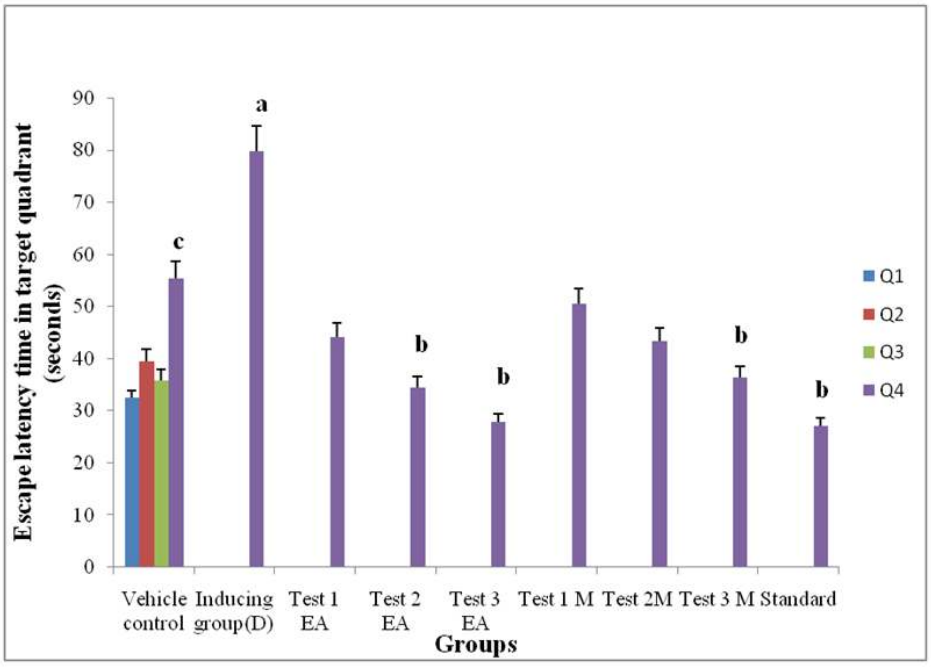

Fig. 5. Effect of EA (ethyl acetate) and M (methanol) extract (doses: 50, 100 or 200 mg/kg administered orally for 7 successive days) of *D. macraei* on Morris water maze model on 7th retrieval day in target quadrant (Q4). Piracetam (200 mg/kg, p.o.) was used as a standard drug. Diazepam (D) (4 mg/kg, i.p.) was used as inducing (memory impairment) drug

All values are expressed as mean ± S.D., n=5. a= P<0.05 vs. time spent in target quadrant in control mice (c); b= p< 0.05 vs. time spent in target quadrant in inducing group (a); c= P< 0.05 vs. time spent in other quadrants (Q1, Q2, Q3) in control mice

In vivo memory enhancing activity was evaluated in mice by using elevated plus maze model and Morris water-maze model. These models are widely employed for evaluating the effect of drugs on learning and memory [10,14,15]. In elevated plus maze, decrease in transfer latency on 2nd day (i.e., 24 h after the first trial) indicated improvement of memory and *vice versa*. In Morris water maze, a decrease in escape latency during training and increase in time spent in target quadrant during retrieval indicated improvement of learning and memory respectively; and *vice versa* [16].

Effects of the petroleum ether, chloroform, methanol and aqueous extract of *D. macraei* were evaluated for memory enhancing activity. Amongst all extracts tested, only ethyl acetate and methanol extract of *D. macraei* showed significant short term memory enhancing activity using elevated plus maze model in mice. Ethyl acetate extract (200 mg/kg dose) with transfer latency time of 24.2 ± 1.580 seconds showed marked significant effect as compared to methanol extract (200 mg/ kg dose) with transfer latency time of 29 ± 1.378 seconds which is clearly depicted in Tables 3 and 4.

In the present study piracetam (200 mg/ kg) was used as a standard memory enhancement drug. It is a derivative of the neurotransmitter gamma-aminobutyric acid. At a neuronal level, piracetam modulates neurotransmission in a range of transmitter systems (including cholinergic and glutamatergic), has neuroprotective and anticonvulsant properties, and improves memory. Furthermore, its efficacy is documented in cognitive disorders and dementia, vertigo, cortical myoclonus, dyslexia, and sickle cell anemia [17,18]. In the current study, ethyl acetate and methanol extract showed significant memory enhancing activity which was almost similar to piracetam.

In Morris water-maze model long term memory of mice was evaluated in mice in which ethyl acetate and methanol extracts were used to evaluate the memory enhancement in mice as both the extracts shows high content of total phenolic and flavonoid content among which ethyl acetate extract (200 mg/ kg dose) with escape latency time of 27.8 ± 0.969 seconds which shows marked significant effect in comparison to methanol extract (200 mg/ kg) dose with transfer latency time of 36.4 ± 1.224 seconds in which the escape latency of 7th retrieval day in quadrant 4 (Q4) which was clearly depicted in Table 4.

5. CONCLUSION

The present study establishes that the plant *Dendrobium macraei* contains high content of phenols and flavonoids which are present in methanol and ethyl acetate extract. Ethyl acetate extract showed more significant effect of memory enhancement as compare to methanol extract. Therefore, *D. macraei* appears to be a promising candidate for improving memory, and it would be worthwhile to explore the potential of this plant in the management of Alzheimer patients. Nevertheless, further studies are needed to explore the full potential of *D. macraei* in memory deficits. Future prospects of the current investigations include firstly bioactivity directed fractionation of bioactive ethyl acetate extract of the plant with a view to isolate and characterize bioactive fraction/constituent(s), and also explore mechanism of action involved in memory enhancing activity.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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