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Sapogenin Mixture and Methanolic Extract Obtained from Leaves of *Dragea volubilis* Improve Memory in Young and Aged Mice

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ABSTRACT

The methanolic extract prepared in a Soxhlet apparatus after de-fatting with petroleum ether was fractionated using a butanol and water mixture to obtain a crude sapogenin glycosidic mixture. Methanolic extract of *Dragea volubilis* and sapogenin mixture (active fraction-AF) ameliorated memory in young and aged animals in exteroceptive and scopolamine-induced amnesia in mice behavioural models. A significant transfer latency (TL) reduction in the elevated plus maze was observed. It also demonstrated improvement in locomotor activity. These observations suggest that the methanolic extract and a fraction obtained from *D. volubilis* had the potential as a nootropic and it should be explored further for evaluation as a cognition enhancer.

Key words: MDV, *Dragea volubilis,* Alzheimer's disease (AD), AF

INTRODUCTION

Many neurological disorders like Alzheimer's and Parkinson's disease affect memory by different mechanisms. Neurological disorders are still a major challenge due to the complex mechanisms of action. Cognitive functions are associated with the central cholinergic system. Impairment of memory is associated with Alzheimer's disease (AD) (Beg *et al.*, 2018). Drugs like donepezil are used for some indications in AD but these are not effective to stop the progression of the disease (Tahami Monfared *et al.*, 2022). Aducanumab proved its efficacy in clinical trials. It halts the progression of AD by reduction of amyloid β plaques. Aducanumab is approved by USFDA for its role. Neurological diseases still pose a major challenge, therefore, all useful approaches and strategies must be explored to prevent or treat neurological diseases (Tolar *et al.*, 2020). Natural antioxidants are fruitful in neutralizing free radicals, which are harmful to the brain. Medicinal herbs are useful in neurological disorders due to their multifactorial mechanisms. Bioactive secondary metabolites obtained from plants such as flavonoids and phenols are useful in their anti-aging effects. Plant extracts and compounds obtained from it have a relatively higher safety without adverse effects. *Dragea volubilis* is also known as wattakaka *volubilis*. Many compounds yielded on chemical exploration of *D. volubilis*, mainly polyhydroxy pregnane glycosides (Phuong *et al.*, 2020). *D. volubilis* (L.F.) Benth is a fairly large woody plant that is abundantly found in south India (Amalraj *et al.*, 2021). The plant is used in wound healing and as a neuroprotective, dyslipidemic and antidiabetic (Thuy *et al.*, 2021). Many activities are due to its active components like poly pregnane glycosides (Thuy *et al.*, 2021). However, no study was done to investigate the effect of *D. volubilis* methanolic extract and crude sapogenin glycosidic mixture (AF) in *in vivo* experimental models through different learning parameters in mice. Elderly mice mimic natural models for aging-related complications resembling AD. Scopolamine an anti-muscarinic is also used to mimic amnesia. Therefore, the present study was sighted to explore the effect of methanolic extract and active fraction obtained from *D. volubilis*.

MATERIALS AND METHODS

The leaves of *D. volubilis* (L.F.) Benth Ex Hook (Asclepiadaceae) collected from Nilgiri hills of Thoothukudi Tamil Nadu were authenticated from Punjabi University as specimen no 112. Swiss mice were used in this study. The

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two age groups were selected with an average age of three months and weight of around 25 g and aged eight months weighing around 28 g. Animals were procured from the animal house of the CRI, Kasauli (H. P., India) as per rules. All experiments were carried out as per the protocol vide no. RIP/IAEC/2019-20/03.

Leaves obtained from *D. volubilis* were shade-dried and defatted with petroleum ether and further extracted in a Soxhlet extractor with methanol. The methanolic extract was further fractionated using a butanol and water mixture to obtain a crude sapogenin glycosidic mixture (AF). In the present study, two doses of the methanolic extract were used i.e. 100/200 mg/kg and AF 5 mg/kg.

Behavioural models included transfer latency (TL) elevated plus-maze, and locomotor activity. The exteroceptive and interoceptive memory model of young and old mice is depicted in Tables 1 and 2.

An elevated plus-maze can assess learning and memory in mice, which serves the exteroceptive behavioural model. The same procedure was followed with animals with the administration of scopolamine in mice. MDV extracts 100 and 200 mg/kg were administered along with donepezil 5 mg/kg for 14 days in different groups of young and old mice. Acquisition of memory was tested on day 13 after administration of extracts followed by retention on day 14. Briefly, every mouse of all groups was positioned at the edge of the open arm facing away from the center on days 13 and 14 for the behavioural study. TL is an observation of the movement of an animal in secs from open to close arm with all its four legs. TL was observed to interpret memory improvement. A decrease in transfer latency stipulated memory improvement (Habibyar *et al.*, 2016).

Locomotor activity is time tested method for the assessment of behaviour. In this study, locomotion activity was assessed, by using an actophotometer on the 0, 13th and 14th days. Stimulant drugs increasd activity, whereas depressant drug reduced locomotor activity. Here several digital counts were recorded as a measure of locomotion. Each mouse was subjected to an activity cage after 30 min of oral administration of extracts for 10 min. Locomotor activity was recorded in extract-treated groups and was compared with a control group to signify a change in locomotor activity (Flaive *et al.*, 2020). All the results were expressed as the mean±S. E. M. The data were

Table 1. Exteroceptive memory model

Groups	Mice	Treatment	Dose and route	No. of animals	No. of days
I	Young mice	Normal saline (NS)	5 ml/kg/p.o	06	14
	Old mice			06	
II	Young mice	DNZ	5 mg/kg/p.o	06	14
	Old mice			06	
III	Young mice	MDV-LD (extract)	100 mg/kg/p.o	06	14
	Old mice			06	
IV	Young mice	MDV-HD (extract)	200 mg/kg/p.o	06	14
	Old mice			06	
V	Young mice	AF (crude glycosidic mixture)	5 mg/kg/ p.o	06	14
	Old mice			06	

Table 2. Interoceptive memory model

Groups	Mice	Treatment	Dose and route	No. of animals	No. of days
I	Young mice	Normal saline (NS)	5 ml/kg/i.p	06	14
	Old mice			06	
II	Young mice	Scopolamine+NS	2 mg/kg/i.p	06	14
	Old Mice			06	
III	Young Mice	DNZ+scopolamine	5 mg/kg/p.o,	06	14
	Old mice		2 mg/kg/i.p	06	
IV	Young mice	MDV-LD (extract)+scopolamine	100 mg/kg/p.o,	06	14
	Old mice		1 mg/kg/i.p	06	
V	Young mice	MDV-HD (extract)+scopolamine	200 mg/kg/ p.o,	06	14
	Old mice		2 mg/kg/i.p	06	14
VI	Young mice	AF(crude glycosidic mixture)+Sco	5 mg/kg/ p.o	06	14
	Old mice		2 mg/kg/i.p	06	14

analyzed using ANOVA (one-way analysis of variance) and Student's (unpaired) t-test (post hoc Tukey's test).

RESULTS AND DISCUSSION

Behavioural studies were done to assess the memory enhancement activity of MDV extract and AF with DNZ and the evaluation of reversal in scopolamine-induced amnesia. There was no significant difference in the acquisition trial on the 13th day in the TL of MDV as compared to normal saline. Similar trends were indicated in young and old mice. A significant decrease in (P<0.01) was observed in TL with MDV and AF treated groups in young and old mice in the exteroceptive and in animals administered with scopolamine on day 14th. It was also observed that MDV produced a reduction in transfer latency in a dose-dependent manner. Similar results were shown by DNZ in all models. The scopolamine group showed an increase in transfer latency (TL; Figs. 1 and 2).

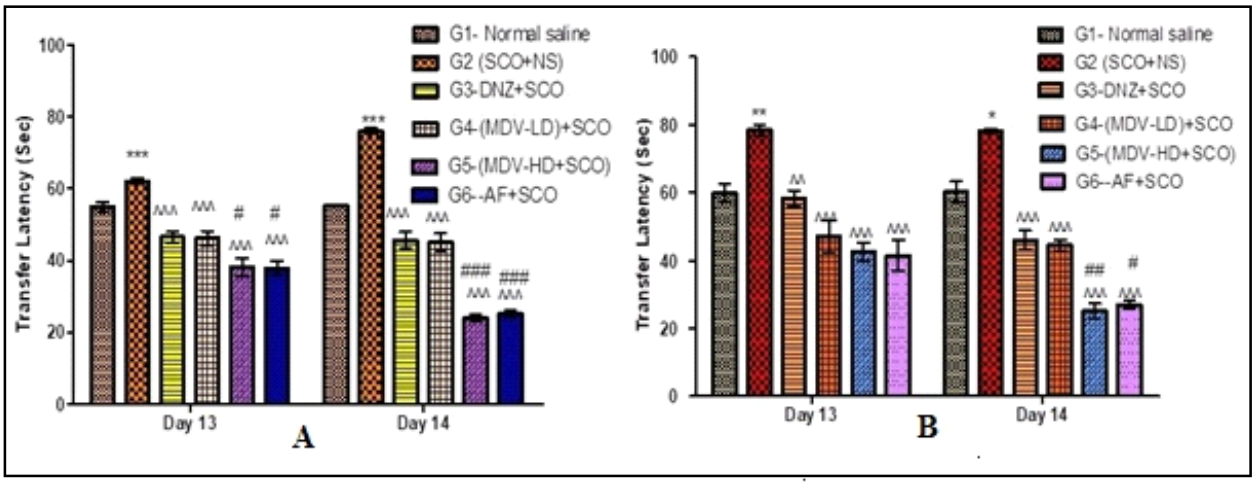

Fig. 1. Effect of MDV (100 and 200 mg/kg), DNZ 10 mg/kg, and AF 5 mg/kg on transfer latency in an interoceptive model, where, A and B denoting study in young and old mice, respectively, as compared to control group of young and old mice on 13th and 14th day. Data expressed as means±SEM; ***P<0.001,**P<0.01 and *P<0.05 as compared to the normal saline (control) group on the same day of the treatment; ### (P<0.001), ## (P<0.01) and # (P<0.05) as compared to the donepezil treated group within the same day of the treatment; ^^^ (P<0.001) and ^^ (P<0.01) as compared to the scopolamine treated group within the same day of the treatment.

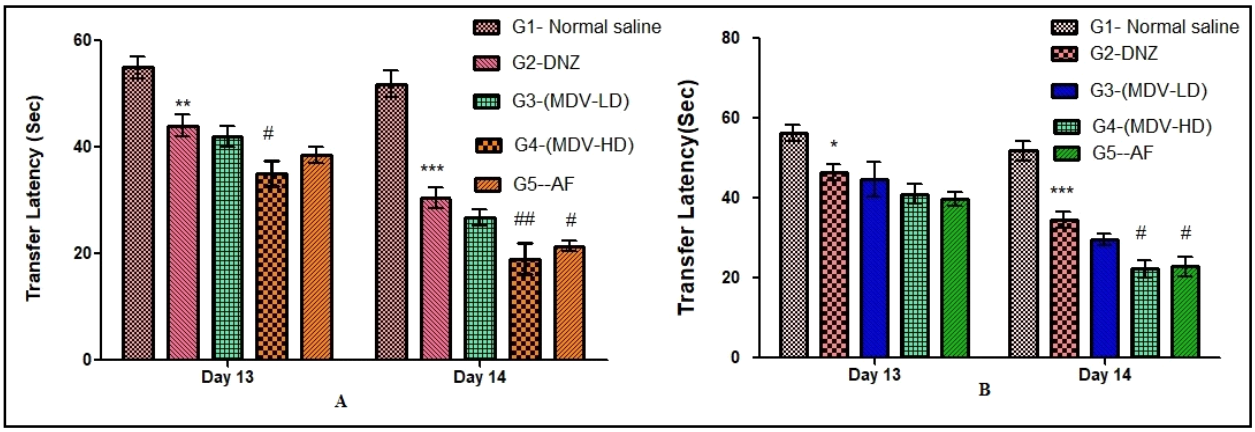

Fig. 2. Effect of MDV (100 and 200 mg/kg), DNZ 10 mg/kg, and AF 5 mg/kg on transfer latency (in seconds) in exteroceptive model, where, A and B denoting study in young and old mice, respectively, as compared to control group of young and old mice on 13th and 14th day. Data expressed as means±SEM. ***(P<0.001), **(P<0.01) and *(P<0.05) as compared to the control group within the same day of the treatment; # (P<0.05) as compared to the Donepezil treated group within the same day of the treatment; ## (P<0.01) as compared to the Donepezil treated group within the same day of the treatment.

Locomotor activity was done on 0, 13th and 14th days of the study. It was measured as mean values for all groups of several crossings±standard error mean. It was observed that MDV groups showed better performance as compared to all other groups (Figs. 3 and 4).

Alzheimer's disease is still an ailment in which diffuse abnormalities occur in the brain (Soria Lopez *et al.*, 2019). Multiple neuronal pathways are affected due to the disruption of cholinergic neurotransmission that greatly affects memory (Ozben and Ozben, 2019). Cognition disturbances are often seen in old age. Normal aging also affects memory so the aged animal model is useful in studying neurological disorders and behavioural studies (Lane *et al.*, 2018). The involvement of oxidative stress is well known to cause neurodegeneration and subsequently impaired memory and cognitive processes (Poprac *et al.*, 2017; Fracassi *et al.*, 2021). The generation of free radicals in the brain is associated with many neurological disorders (Wojtunik-Kulesza *et al.*, 2016). The brain has a very high consumption of oxygen so the generation of free radicals disrupts its functioning and affects cognition (Budzynska *et al.*, 2015). Many well-known complications are associated with neuronal destruction. Oxidation in the brain is the major event responsible for aging and destruction of neurons which in this case is

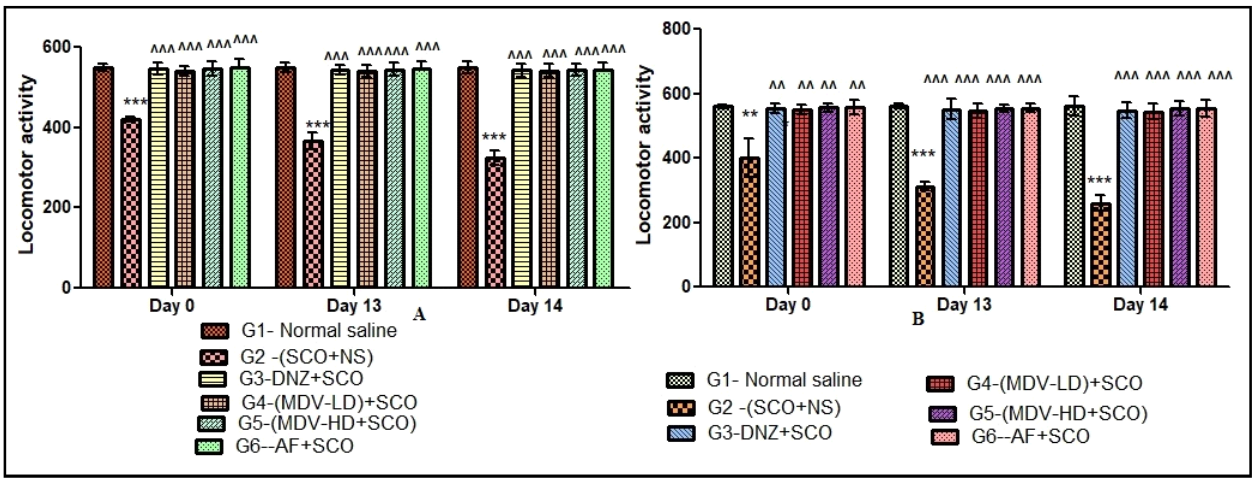

Fig. 3. Effect of MDV (100 and 200 mg/kg), DNZ 10 mg/kg, and AF 5 mg/kg on locomotor activity (in seconds) in an interoceptive model, where, A and B denoting study in young and old mice, respectively, as compared to control group of young and old mice on 13th and 14th day. Data expressed as means±SEM; *** (P<0.001) and ** (P<0.01) as compared to the normal saline (control) group on the same day of the treatment; ^^^ (P<0.001) and ^^ (P<0.01) as compared to the scopolamine treated group within the same day of the treatment.

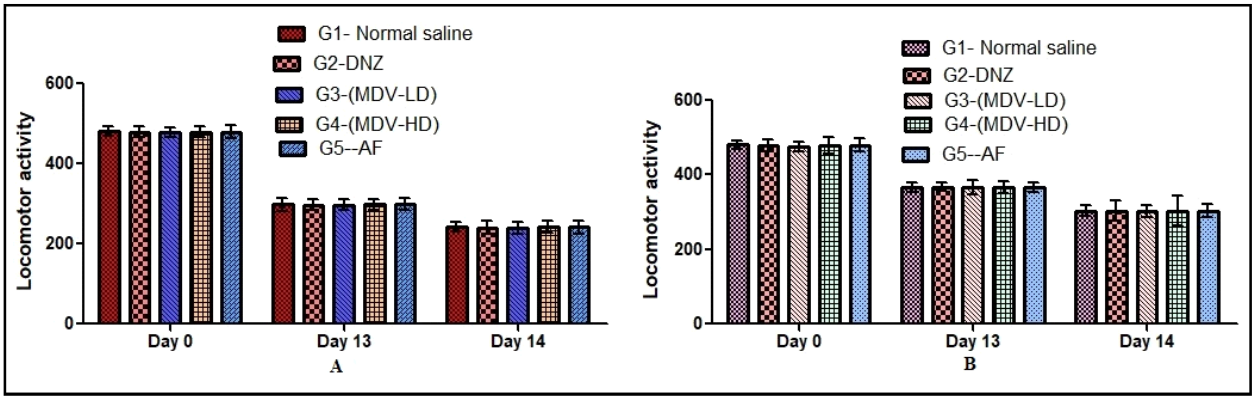

Fig. 4. Effect of MDV (100 and 200 mg/kg), DNZ 10 mg/kg, and AF 5 mg/kg on locomotor activity (in seconds) in an exteroceptive model, where, A and B denoting study in young and old mice, respectively, as compared to control group of young and old mice on 13th and 14th day. Data expressed as means±SEM. No significant difference was observed in various groups.

also seen in AD (Huang *et al.*, 2016). In the present study memory in behavioural parameters with MDV showed its potential as a nootropic. Cognition improvement potential is indicative of promising prospects in AD. The level of reinstatement or betterment in behavioural parameters with MDV and AF on account of accordant training and observation gives the basis for MDV extract as a prospective anti-AD. In the present study, the effects of MDV extract AF and DNZ on the behavioural, parameters were evaluated. This study showed that MDV extract significantly decreased TL in the elevated plus-maze model, both in young and old mice MDV proved to improve TL. The results stipulated that there was reversal of memory with MDV and AF. Locomotor activity is an important parameter in behavioural studies so in the present study it was assessed on days 0, 13th and 14th of study. Locomotion is a parameter of normalcy. The observation of the normal locomotor activity is indicative of validity of other parameters. A change in the locomotor parameter can lead to dissension in TL.

CONCLUSION

The observations of the present study revealed the potential of MDV extract and AF in enhancing memory and nootropic activity. MDV extract and AF have shown improvement in the behavioural model which suggests its usefulness as a potential memory enhancer. Hence, further elaboration of this product can be developed to be used as potential anti-Alzheimer's features.

ETHICAL APPROVAL

The study protocol was approved by the Institutional Animals Ethics Committee (IAEC) vide no. RIP/IAEC/2019-20/03.

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REFERENCES

Amalraj, S., Mariyammal, V., Murugan, R., Gurav, S. S., Krupa, J. and Ayyanar, M. (2021). Comparative evaluation on chemical composition, *in vitro* antioxidant, antidiabetic and antibacterial activities of various solvent extracts of *Dregea volubilis* leaves. *South Afr. J. Bot.* **138**: 115-123.

Beg, T., Jyoti, S., Naz, F., Rahul, Ali, F., Ali, S. K., Reyad, A. M. and Siddique, Y. H. (2018). Protective effect of kaempferol on the transgenic drosophila model of Alzheimer's disease. *CNS Neurol Disord Drug Targets* **17**: 421-429.

Budzynska, B., Boguszewska-Czubara, A., Kruk-Slomka, M., Skalicka-Wozniak, K., Michalak, A., Musik, I. and Biala, G. (2015). Effects of imperatorin on scopolamine-induced cognitive impairment and oxidative stress in mice. *Psychopharmacology* (Berl.) **232**: 931-942. *https://doi.org/10.1007/s00213-014-3728-6.*

Flaive, A., Fougère, M., van der Zouwen, C. I. and Ryczko, D. (2020). Serotonergic modulation of locomotor activity from basal vertebrates to mammals. *Front. Neural Circuits* **2020**. *https://doi.org/10.3389/fncir.2020.590299.*

Fracassi, A., Marcatti, M., Zolochevska, O., Tabor, N., Woltjer, R., Moreno, S. and Taglialatela, G. (2021). Oxidative damage and antioxidant response in frontal cortex of demented and non-demented individuals with alzheimer's neuropathology. *J. Neuroscience* **41**: 538-554.

Habibyar, A. F., Sharma, N. and Khurana, N. (2016). PASS assisted prediction and pharmacological evaluation of hesperidin against scopolamine induced amnesia in mice. *Eur. J. Pharmacol.* **789**: 385-394.

Huang, W. J., Zhang, X. and Chen, W. W. (2016). Role of oxidative stress in Alzheimer's disease (review). *Biomed. Rep.* **2016**. *https://doi.org/10.3892/br.2016.630.*

Lane, C. A., Hardy, J. and Schott, J. M. (2018). Alzheimer's disease. *Eur. J. Neurol.* **2018**. *https://doi.org/10.1111/ene.13439.*

Ozben, T. and Ozben, S. (2019). Neuro-inflammation and anti-inflammatory treatment options for Alzheimer's disease. *Clin. Biochem.* **2019**. *https://doi.org/10.1016/j.clinbiochem.2019.04.001.*

Phuong, P. T., Thuy, N. T. K., van Huan, N., Nhiem, N. X. and van Kiem, P. (2020). Pregnanes from the leaves of *Dregea volubilis*. *Vietnam J. Chem.* **58**: 386-391. *https://doi.org/10.1002/vjch.202000006.*

Poprac, P., Jomova, K., Simunkova, M., Kollar, V., Rhodes, C. J. and Valko, M. (2017). Targeting free radicals in oxidative stress-related human diseases. *Trends Pharmacol. Sci.* **2017**. *https://doi.org/10.1016/j.tips.2017.04.005.*

Soria Lopez, J. A., González, H. M. and Léger, G. C. (2019). Alzheimer's disease. In: *Handbook of Clinical Neurology*. Elsevier B. V., pp. 231-255. *https://doi.org/10.1016/B978-0-12-804766-8.00013-3.*

Tahami Monfared, A. A., Byrnes, M. J., White, L. A. and Zhang, Q. (2022). Alzheimer's disease: Epidemiology and clinical progression. *Neurol. Ther.* **2020**. *https://doi.org/10.1007/s40120-022-00338-8.*

Thuy, N. T. K., Phuong, P. T., Hien, N. T. T., Trang, D. T., Huan, N., van, Anh, P. T. L., Tai, B. H., Nhiem, N. X., Hung, N. T. and Kiem, P. van (2021). Pregnane glycosides from the leaves of *Dregea volubili*s and their α-glucosidase and α-amylase inhibitory activities. *Nat. Prod. Res.* **35**: 3931-3938.

Tolar, M., Abushakra, S., Hey, J. A., Porsteinsson, A. and Sabbagh, M. (2020). Aducanumab, gantenerumab, BAN2401 and ALZ-801–The first wave of amyloid-targeting drugs for Alzheimer's disease with potential for near term approval. *Alzheimers Res. Ther.* **2020**. *https://doi.org/10.1186/s13195-020-00663-w.*

Wojtunik-Kulesza, K. A., Oniszczuk, A., Oniszczuk, T. and Waksmundzka-Hajnos, M. (2016). The influence of common free radicals and antioxidants on development of Alzheimer's disease. *Biomedicine and Pharmacotherapy* **2016**. *https://doi.org/10.1016/j.biopha.2015.12.024.*