Int. J. Pharm. Investigation, 2022; 13(1):162-172.
www.jpionline.org

Original Article

Therapeutic Potential of *Dragea volubilis* Leaf Extract against Scopolamine-induced Memory Impairment in Young and Aged Mice

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ABSTRACT

Background: Treating neurological disorders is still a major challenge, especially diseases like alzheimer's. *Dragea volubilis* is a wonder herb with many pharmacological activities like neuroprotective, antioxidant, anti-tumor, and anti-inflammatory. *Dragea volubilis* is synonymously known as *Wattakaka volubilis*. Chemical exploration of *Dragea volubilis* has yielded polyoxypregnane glycosides. **Objectives:** This study focused to explore the cognition-enhancing and neuroprotective functions of chloroform extract of *Dragea volubilis* in young and old mice in interoceptive and exteroceptive models. **Materials and Methods:** The chloroform extract of *Dragea volubilis* (CDV) was prepared by the soxhlation method. The antioxidant, anticholinesterase, and behavioral parameters were assessed through avoidance, elevated plus, and morris water maze. *In-vivo* studies were conducted by dividing the animals into 4 groups exteroceptive and 5 interoceptive(scopolamine-induced amnesia) 6 animals in each group. The scopolamine was administered at 2mg/kg and the extract of *Dragea volubilis* was administered at two different doses (100, and 200 mg/kg) for 14 days. Animals were sacrificed after behavioral parameters and brains were isolated for antioxidants and acetylcholinesterase assays. **Results:** A marked increase in step-down latency (SDL- passive avoidance) and a significant reduction in transfer latency (TL) in the elevated plus maze were noticed with the extract. It also demonstrated significant improvement in memory in the morris water maze (MWM) test in both the training and retention trials. CDV inhibited acetylcholinesterase and reduced thiobarbituric acid reactive substance activity (TBARS) and increased glutathione (GSH) and catalase in the mice brain showing significant antioxidant properties. **Conclusion:** It can be concluded with results that CDV has significant potential as a nootropic and antioxidant with neuroprotective properties.

Keywords: Antioxidants, CDV, *Bacopa monnieri*, *Dragea volubilis*, Alzheimer's disease.

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Received: 28-05-2022 ;
Revised: 06-08-2022 ;
Accepted: 30-09-2022.

INTRODUCTION

The most important component of the brain is memory. Many brain disorders affect memory. Neurological disorders like Alzheimer's disease (AD) and Parkinson's disease affect memory by different mechanisms. Regulation of cognitive functions is associated with the central cholinergic system. Progressive impairment of cognition, memory, and behavioral functions is a hallmark feature of AD.[1] Many medicines like acetylcholinesterase inhibitors donepezil and galantamine are approved for some indications in AD. There is a remarkable development in the therapy of AD. Aducanumab showed promising results in halting the progression of AD. Alternative therapeutic approaches and multi-targeted strategies are much needed to prevent or treat neurological diseases.[2] Oxidative stress greatly affects the brain because the brain is the highest consumer of oxygen. Natural antioxidants can play a definite role in the neutralization of free radicals. Multiple pathological or neurotoxic pathways can lead to AD.[3] Medicinal herbs and derived agents have proved their usefulness in the treatment of neurological disorders. Antioxidants obtained from plants and bioactive secondary metabolites derived from them such as flavonoids and phenols are useful in their anti-aging effects. Plant extracts have relatively higher safety with no or fewer adverse effects.[4] *Dragea volubilis* synonymously known as *Wattakaka volubilis* on chemical exploration leads to the isolation of polyhydroxy pregnane glycosides, steroid pregnane content and progesterone-resembling compounds. The plant is a plentiful source of phytoconstituents like drevogenins,dregeosides, hyperoside, and kaempferol. *Dragea volubilis* (L.f.) is a fairly large woody plant that is extensively distributed in southern parts of

DOI: 10.5530/223097131525

India. A wide variety of pharmacological activities were reported with *Dragea volubilis* including neuroprotective, paralytic disorders, anti-leukemic, dyslipidemia, and antidiabetic.[5,6] However, no compendious study was piloted to explore the effect of *Dragea volubilis* extract against memory impairment in *in-vivo* experimental models through different learning parameters in mice. Scopolamine is an anti-muscarinic drug that is a useful tool for studying cognitive impairment in young and elderly mice. It is associated with a reduction of acetylcholine levels which produces amnesia. There are three useful parameters in the process of learning and memory which are considered for evaluation i.e., acquisition, consolidation, and recall of the learned task.[7,8] Therefore, this study was sighted to investigate the effect of CDV. Anti-amnesic effects of *Dragea volubilis* were studied in exteroceptive and scopolamine-induced amnesia (interoceptive) in young and old mice.

MATERIALS AND METHODS

Materials

Dragea volubilis (L.F) *(Asclepiadaceae)* leaves were collected from Nallur, Thoothukudi, Nilgiri Hills, Tamil Nadu by Dr. V ChellaDurai and authenticated by Dr. Avneet Pal Singh (Asst Prof) Punjabi University Patiala vide specimen no: 115 dated 02-12-2016. All chemicals used were of analytical grade and utilized as received from different sources. Scopolamine hydrobromide (SCO), thiobarbituric acid (TBA), glutathione, DTNB, acetylthiocholine were purchased from Sigma Aldrich (Bangalore, India).

Animals

In this study, swiss mice of either sex were used. Young mice with average weight of 25 g and were 3 months old and old mice were 28 g in weight and were more than 8 months old. Procurement of animals in good physical condition was done from the animal facility of the Clinical Research Institute, Kasauli (H.P, India) as per rules. Animals were accustomed for 5 days to the new climatic conditions before behavioral studies. The animals were allowed access to food and water. Standard conditions of 12 hr light and dark cycle were followed. Experiments were carried out in a uniform time frame i.e., during the daytime from 0900 to 1800 h. All norms of experimentation were followed as per the approved protocol of study by the IAEC vide no: - RIP/IAEC/2019-20/03. All experiments were carried out with appropriate care and handling instructions as per the specification of CPCSEA (Reg. No. 874/PO/ac/05/CPCSEA).

Table 1: Exteroceptive memory model in young mice and old mice (8 months old).

Groups	Mice	Treatment	Dose and route	No. of Animals	No. of Days
Gp-I	Young mice	Normal Saline	5ml/kg/i.p	06	14
	Old mice			06	
Gp-II	Young mice	BME	120mg/kg/p.o	06	14
	Old mice			06	
Gp-III	Young mice	CDV-LD (extract)	100 mg/kg/ p.o	06	14
	Old mice			06	
Gp-IV	Young mice	CDV-HD (extract)	200 mg/kg/ p.o	06	14
	Old mice			06	

Table 2: Interoceptive memory model in young mice and old mice (8 months old).

Groups	Mice	Treatment	Dose and route	No.of Animals	No.of Days
Gp-I	Young mice	Normal saline (NS)	5ml/kg/i.p	06	14
	Old mice			06	
Gp-II	Young mice	Scopolamine + NS	2mg/kg/i.p	06	14
	Old mice			06	
Gp-III	Young mice	BME + scopolamine	120mg/kg/p.o, 2mg/kg/i.p	06	14
	Old mice			06	
Gp-IV	Young mice	CDV-LD (extract) + scopolamine	100 mg/kg/ p.o, 2mg/kg/i.p	06	14
	Old mice			06	
Gp-V	Young mice	CDV-HD (extract) + scopolamine	200 mg/kg/ p.o, 2mg/kg/i.p	06	14

Preparation of extract

Shade-dried leaves obtained from *Dragea volubilis* were sequentially extracted in a Soxhlet extractor with petroleum ether and chloroform. Extracts were dried by a rotary evaporator and preserved for study. In the present study, two doses of chloroform extract were used i.e., 100mg/kg and 200mg/kg.[9,10]

Experimental Design and Treatments

Behavioural Test / Laboratory models

Behavioral models include observation of passive avoidance apparatus (Step down latency-SDL), elevated plus-maze (Transfer latency- TL), escape latency (EL)-morris water maze, and locomotor activity. Behavioral study in interoceptive models includes amnesia induced by scopolamine in young and old mice. Exteroceptive and interoceptive memory models for young mice (average age 3 months old) and old mice (average age 8 months old) are depicted in Tables 1 and 2.

Assessment of nootropic activity by evaluation of behavioral parameters

Passive avoidance (observation of pattern in step down latency)

The observation of the step-down parameter was done in an apparatus called passive avoidance. During the experimentation, the cage was brightened with a bulb (15W) and an electric shock was delivered to the floor. In the SDL observation, the acquisition trial was performed followed by the retention trial on days 13th and 14th consecutively. SDL was recorded for each mouse on day 13th in presence of shock and on day 14th in absence of shock. A maximum time of 300 sec was observed. A marked increase in SDL value showed betterment in memory. The experiment was repeated on the same animals after the wash-out period with the administration of scopolamine at 2mg/kg half an hour before experimentation. A notable increase in SDL signifies a memory improvement.[11,12]

Elevated plus Maze (EPM-Observation of transfer latency)

Exteroceptive evaluation of behavior in terms of memory in mice can be done by EPM.CDV extract 100mg/kg and 200mg/kg were administered along with BME 120mg/kg for 14 days in all groups. Acquisition of memory was tested on day 13th after administration of extracts followed by retention on day 14th. TL is an observation of the movement of an animal in secs from open to close arm with all its four legs. A decrease in TL stipulates memory improvement.[13]

Morris water maze (observation of escape latency)

EL can be done in the morris water maze which is designed as a pool round in shape. All experimentation was done in dim light. A submerged platform was placed inside the desired quadrant (TQ). The position of the platform was kept the same during the training session. Training trials were started from the 5th day to the 7th day. In these trials, animals were allowed to locate the platform. Animals were placed in different locations in these trials. Animals were allocated 120 sec to locate the platform. EL is the time taken by the animal to locate the hidden platform and is a parameter of spatial reference memory. Spatial working memory was done on the 8th day in the absence of a platform. On 8th day, the animal was placed in any of the compartments to explore it for 300 sec except the target quadrant i.e., where a platform was placed. The mean time spent in the target compartment was recorded as probe or retrieval. The animals were tested in acquisition and retention trials (T1 and T2) on the 11th, 12th, 13th and 14th day of experimentation. Time spent in the desired compartment was recorded.[14,15]

Locomotor function

Locomotor activity is time tested method for the assessment of behavior. In this study, locomotion activity was assessed by using an actophotometer on the 0, 13th and 14th day. Each mouse was subjected to an activity cage after 30 min of oral administration of extracts for 10 min.[16]

Biochemical estimation

Animals were sacrificed after finishing behavioral parameters. Isolation of the brain was done immediately. The isolated brains were preserved and separation of the hippocampus and cortex was done. Biochemical estimation i.e. thiobarbituric acid reactive substance activity (TBARS), glutathione (GSH), Catalase and acetylcholinesterase (AChE) was done with the supernatant.[17-19]

Measurement of thiobarbituric acid reactive substance activity

TBARS gives a measurement of lipid peroxidation of the brain. The TBARS value was expressed as nmol/ mg of protein.[20]

Measurement of glutathione activity

The GSH content in tissue was estimated using the method of reduced glutathione content evaluated in brain tissue. Results were indicated in µmol of reduced glutathione/mg of protein.[21]

Measurement of catalase activity

The catalase activity was estimated utilizing the millimolar extinction coefficient of hydrogen peroxide (H_2O_2).[20] Results expressed as µmol of H_2O_2 oxidized/ min/ mg of protein.

$$\text{CAT activity} = \frac{\delta \text{O.D.}}{\varepsilon \text{ X Volume of sample (mL) X mg of protein}}$$

where 'δ O.D.' = change in absorbance per minute'ε' = extinction coefficient of H_2O_2.

Measurement of acetylcholinesterase activity

AChE is an important parameter because acetylcholinesterase hydrolyzes acetylcholine. These were evaluated based on the development of yellow color due to thiocholine reactions to dithiobis nitrobenzoate ions. The level of thiocholine formation from acetylcholine iodide in the presence of brain cholinesterase can be estimated using a spectrophotometer at 420 nm with the formula below

$$R = \frac{\delta \text{ O.D. X vol. of assay}}{\varepsilon \text{ X mg of protein}}$$

where 'R' is the rate of enzyme activity in 'n' mole of ACh hydrolyzed/minute /mg of protein; 'δ O.D.' is the change in absorbance/minute; 'ε' is extinction coefficient (13600/M/cm).[22]

Statistical analysis

The results were presented as the mean ± S.E.M. One-way analysis of variance ANOVA and Student's (unpaired) *t*-test (post hoc Tukey's test) were used for analyzing data. Results were considered statistically significant at $p<0.01$, and $p<0.001$ values.

RESULTS

Interpretation of behavioral models

The behavioral parameters were done to evaluate the memory-enhancing effect of CDV extract with *Bacopa monnieri* extract (BME) andthe evaluation of reversal in SCO-induced amnesia.

Evaluation of step down latency

A significant reduction in SDL was observed in the group trated with SCO only whereas an increase in SDL was seen with CDV and BME.A significant ($p<0.01$) increase in SDL was observed in CDV treated group as compared to the control group during retention on the 14th day (Retention trial) in young and old mice both exteroceptive and interceptive as shown in Figure 1.

Evaluation of transfer latency

A decrease in ($p<0.01$) TL was observed in CDV and BME-treated groups in the exteroceptive and interceptive model during the retention test on the 14th day. CDV produced a reduction in TL

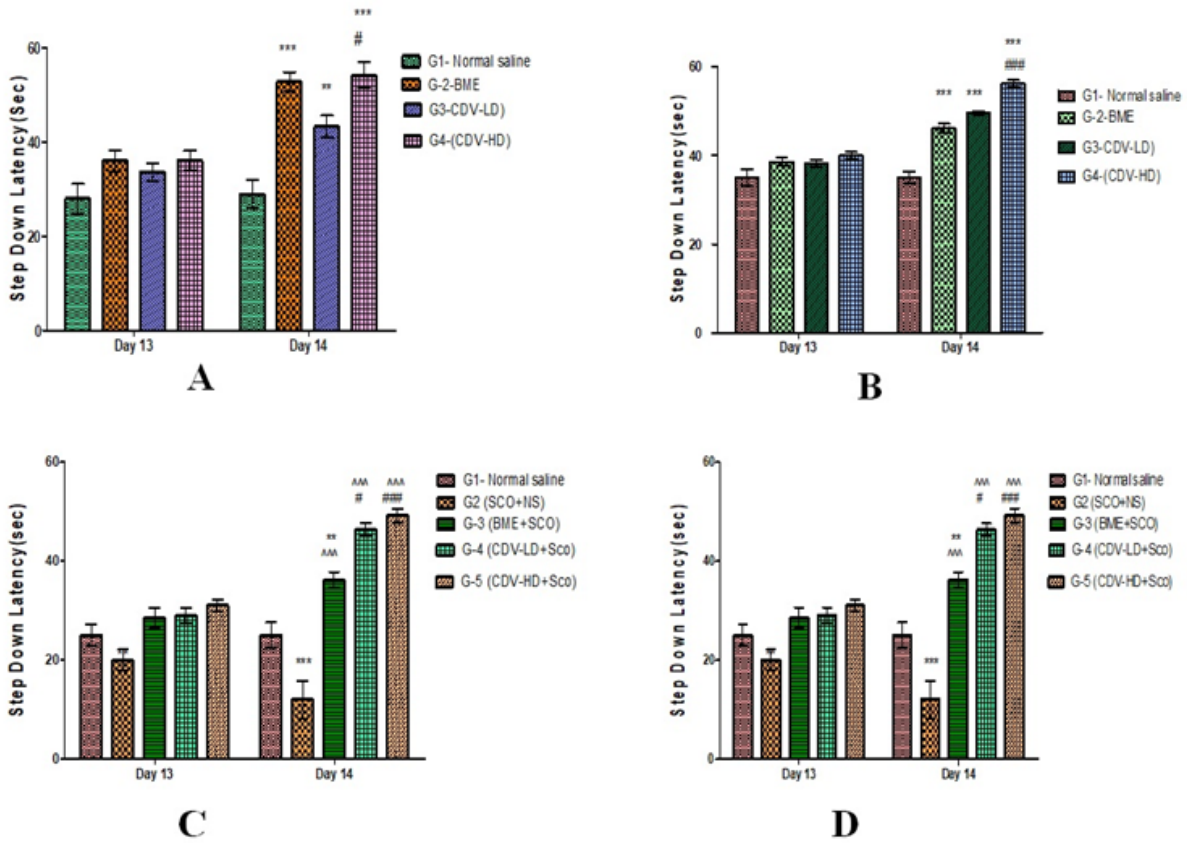

Figure 1: The effect of CDV (100 mg/kg and 200 mg/kg), BME 120 mg/kg on SDL in an exteroceptive model where A: Young mice, B: Old mice, and in interoceptive model where C: Young mice, D: Old mice as compared to control group of young and old mice on 13th and 14th day. Data expressed as means ± SEM; *** represents $p<0.001$and ** represents $p<0.01$ as compared to the normal saline (control) group on the same day of the treatment; ###represents ($p<0.001$) as compared to the BME treated group within the same day of the treatment; ^^^ represents ($p<0.001$) as compared to the SCO treated group within the same day of the treatment. ###represents (P<0.001) as compared to the *Bacopa monnieri* extract (BME) treated group within the same day of the treatment; # represents (P<0.05) as compared to the BME treated group within the same day of the treatment; ^^^ represents (P<0.001) as compared to the scopolamine treated group within the same day of the treatment;

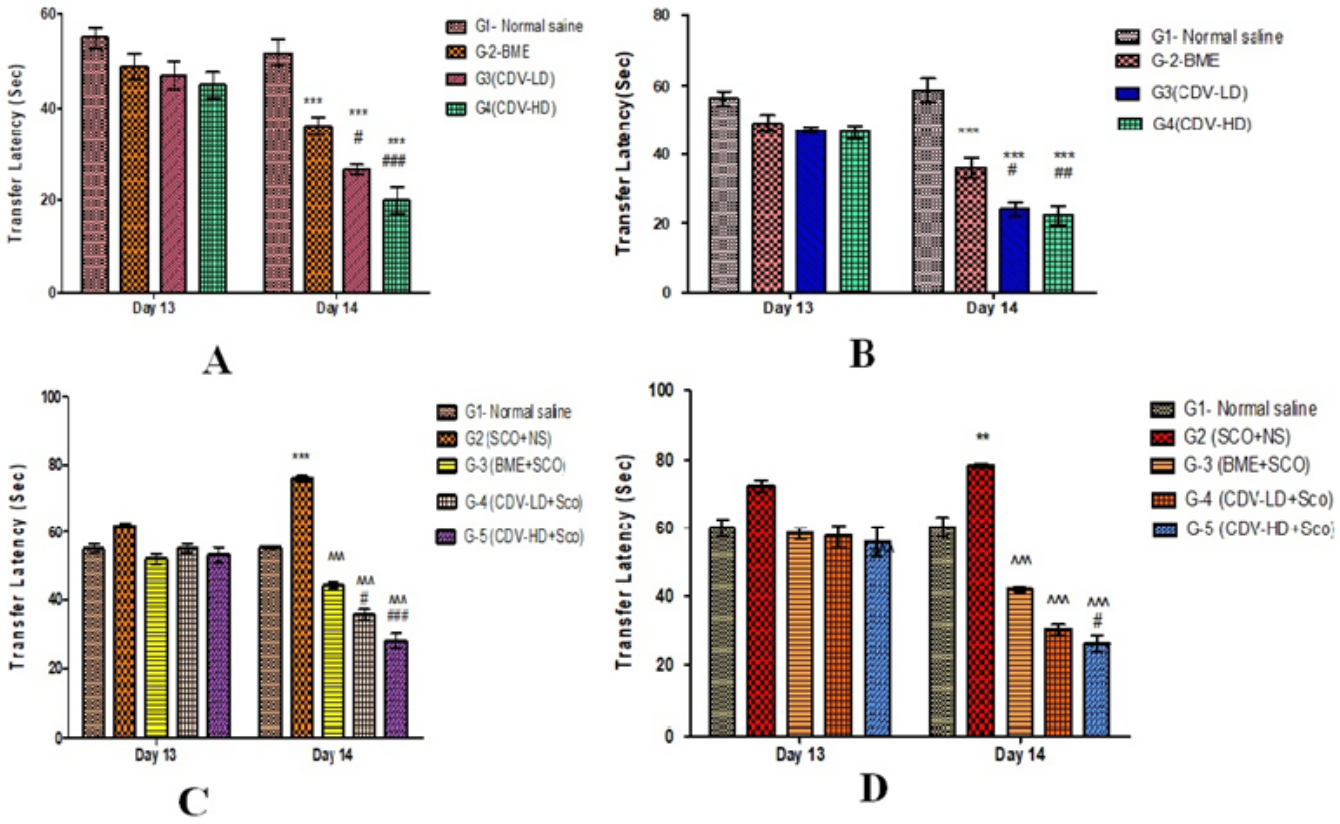

Figure 2: The effect of CDV (100 mg/kg and 200 mg/kg), BME 120 mg/kg on TL (Transfer latency in seconds) in exteroceptive model where A: Young mice, B: Old mice and in interceptive model where C: Young mice, D: Old mice as compared to control group of young and old mice on 13th and 14th day. Data expressed as means ± SEM; *** represents *P*<0.001 as compared to the normal saline (control) group within the same day of the treatment; ** represents *P*<0.01 as compared to the normal saline control group within the same day of the treatment; ### represents (*P*<0.001) as compared to the *Bacopa monnieri* extract (BME) treated group within the same day of the treatment; # represents (*P*<0.05) as compared to the BME treated group within the same day of the treatment; ^^^ represents (*P*<0.001) as compared to the scopolamine treated group within the same day of the treatment.

in a dose-dependent manner followed by BME in all models as represented in Figure 2.

Evaluation of EL- MWM in relation to spatial reference memory (SRM)

Significant (p<0.01) reduction in escape latency were observed on days 5th, 6th, and 7th for all the groups as compared to the control for acquisition trials. A significant (p<0.001) reduction in EL was seen in CDV treated group as compared to the SCO treated group as depicted in Figure 3.

In relation to Time spent in the target quadrant in sec (TSTQ) and Spatial working memory (SWM)

Day 8th probe trial indicating the effect of CDV, and BME on TSTQ in all models. Figure 4 depicts CDV-treated groups spending more time in the desired quadrant in comparison to control and SCO groups. There was a significant (p<0.01) reduction in EL with CDV in the probe trial as compared to normal saline in all models of young and old mice. During training probe trial 2, EL for all the groups was significantly (p<0.01) lesser than the EL of the normal saline (control) group. There was a significant (p<0.001) reduction in EL with the CDV group as compared to the SCO group in all populations of mice. The effect of CDV on EL was found to be better than BME on EL (Figure 5, 6).

Locomotor activity

Test of locomotion activity was done on 0, 13th and 14th days of the study. It was observed that CDV groups have shown better performance as compared to all other groups shown in Figure 7.

Biochemical estimation

Estimation of TBARS activity in both the cortex and the hippocampus

The CDV-treated group showed a significant (p<0.001) reduction in TBARS levels as compared to the SCO treated group indicated in Figure 8.

Estimation of AChE activity in both the cortex and the hippocampus in cortex and hippocampus

Significant (P<0.01) reduction in AChE activity was observed with CDV extract in both hippocampus and cortex. SCO treated groups showed an increase in AChE activity in comparison to control (Figure 9).

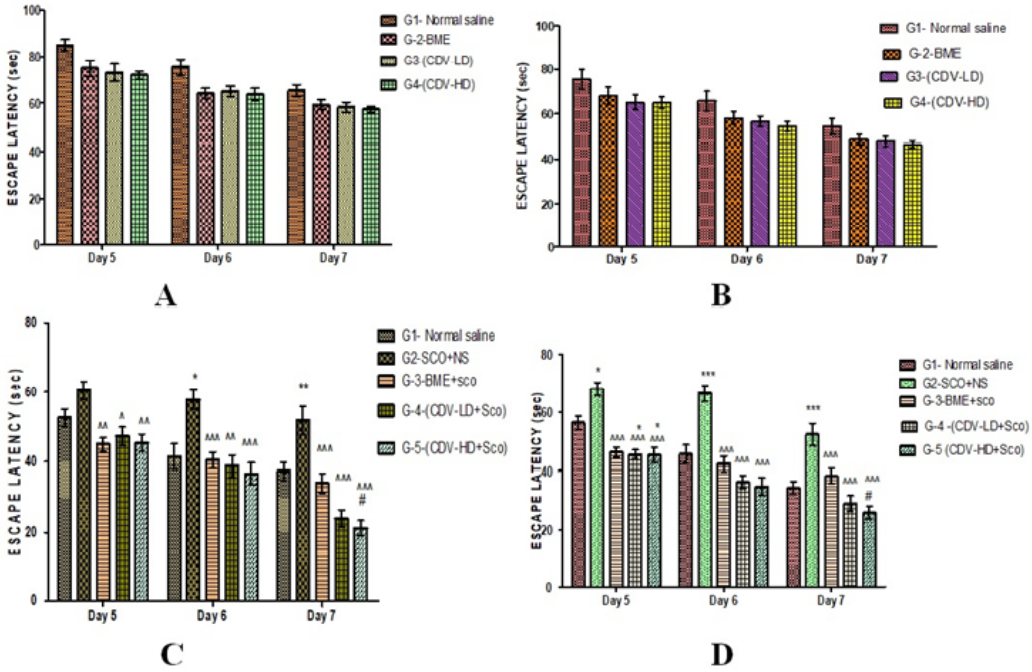

Figure 3: Acquisition Trial indicating the effect of CDV (100 mg/kg and 200 mg/kg), BME 120 mg/kg on EL (escape latency in sec) in exteroceptive model where A: Young mice, B: Old mice and in interceptive model where C: Young mice, D: Old mice as compared to control group of young and old mice on 13th and 14th day. Data expressed as means ± SEM; *** represents P<0.001 as compared to the normal saline (control) group within the same day of the treatment; ** represents *P*<0.01 as compared to the normal saline control group within the same day of the treatment; * represents *P*<0.05 as compared to the normal saline control group within the same day of the treatment; # represents (*P*<0.05) as compared to the *Bacopa monnieri* extract (BME) treated group within the same day of the treatment; ^^^ represents (*P*<0.001) as compared to the scopolamine treated group within the same day of the treatment; ^^ represents (*P*<0.01) as compared to the scopolamine treated group within the same day of the treatment; ^ represents (*P*<0.05) as compared to the scopolamine treated group within the same day of the treatment.

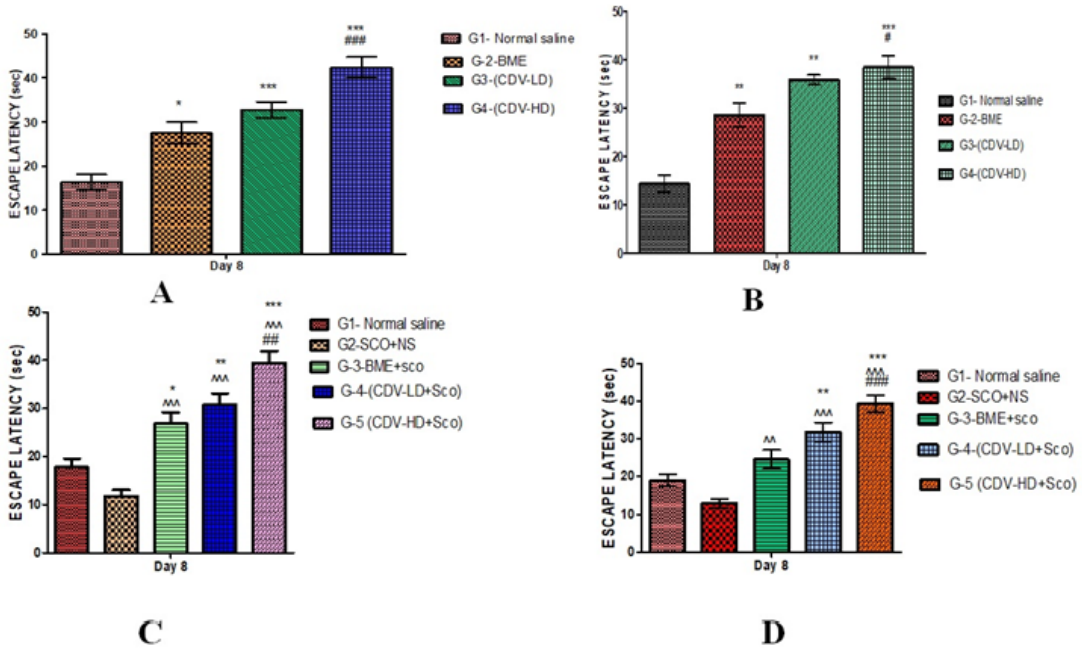

Figure 4: Probe Trial indicating the effect of CDV (100 mg/kg and 200 mg/kg), BME 120 mg/kg on TSTQ (Target spent in target quadrant in sec) in exteroceptive model where A: Young mice, B: Old mice and in interceptive model where C: Young mice, D: Old mice as compared to control group of young and old mice within the same day of the treatment. Data expressed as means ± SEM; *** represents *P*<0.001 as compared to the normal saline (control) group within the same day of the treatment; ** represents *P*<0.01 as compared to the normal saline control group within the same day of the treatment; * represents *P*<0.05 as compared to the normal saline control group within the same day of the treatment; ### represents (*P*<0.001) as compared to the *Bacopa monnieri* extract (BME) treated group within the same day of the treatment; ##represents (*P*<0.01) as compared to the BME treated group; #represents (*P*<0.05) as compared to the BME treated group within the same day of the treatment; ^^^ represents (*P*<0.001) as compared to the scopolamine treated group ; ^^ represents (*P*<0.01) as compared to the scopolamine treated within the same day of the treatment.

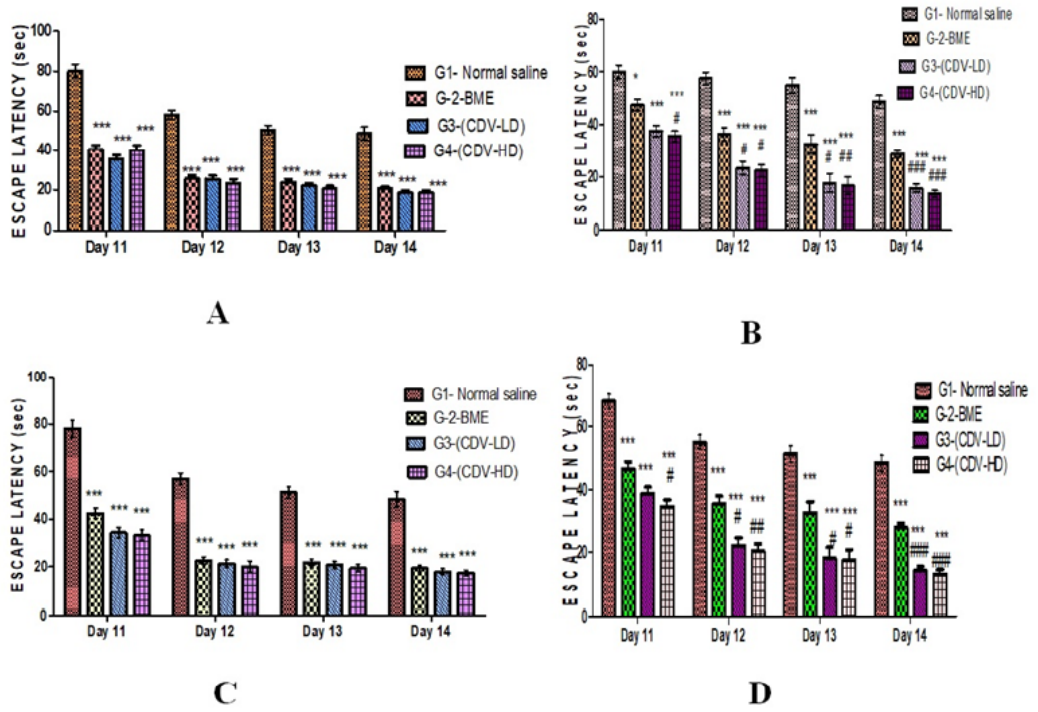

Figure 5: The effect of CDV (100 mg/kg and 200 mg/kg), BME 120 mg/kg on EL (escape latency in seconds) in an exteroceptive model where A and B indicate Trial I and II in Young mice respectively and C and D indicates Trial I and II in Old mice respectively as compared to control group of young and old mice within the same day of the treatment. Data expressed as means ± SEM; *** represents $P<0.001$ as compared to the normal saline (control) group within the same day of the treatment; * represents $P<0.05$ as compared to the normal saline control group within the same day of the treatment; $^{###}$represents ($P<0.001$) as compared to the Bacopa monnieri extract (BME) treated group within the same day of the treatment; $^{##}$represents ($P<0.01$) as compared to the BME treated group within the same day of the treatment; $^{#}$represents ($P<0.05$) as compared to the BME treated group within the same day of the treatment.

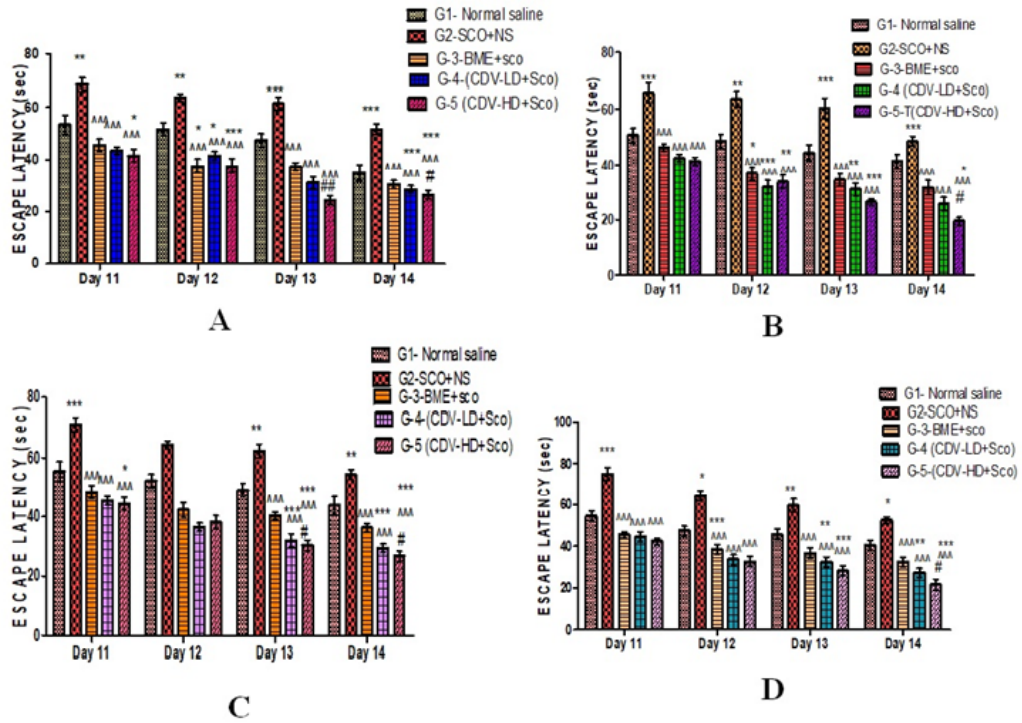

Figure 6: The effect of CDV (100 mg/kg and 200 mg/kg), BME 120 mg/kg on EL (escape latency in seconds) in interoceptive model where A and B indicates Trial I and II in Young mice respectively and C and D indicates Trial I and II in Old mice respectively as compared to control group of young and old mice within the same day of the treatment. Data expressed as means ± SEM; *** represents $P<0.001$ as compared to the normal saline (control) group within the same day of the treatment; ** represents $P<0.01$ as compared to the normal saline (control) group within the same day of the treatment; * represents $P<0.05$ as compared to the normal saline control group within the same day of the treatment; # represents ($P<0.05$) as compared to the *Bacopa monnieri* extract (BME) treated group within the same day of the treatment; ^^^ represents ($P<0.001$) as compared to the scopolamine treated within the same day of the treatment.

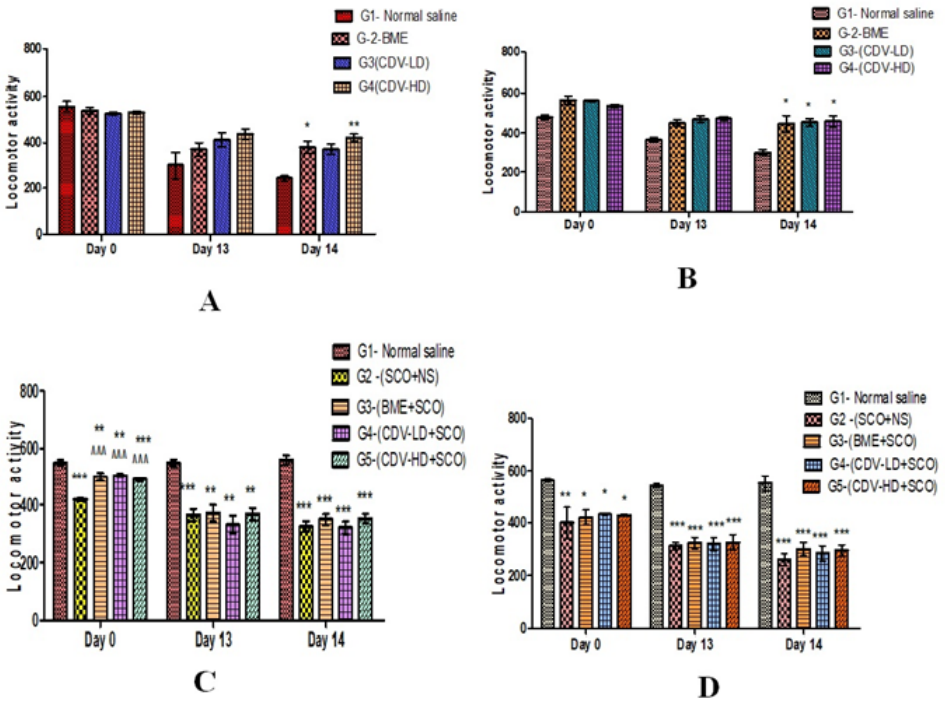

Figure 7: The effect of CDV (100 mg/kg and 200 mg/kg), BME 120 mg/kg on Locomotor activity (in seconds) in exteroceptive model where A: Young mice, B: Old mice and in interceptive model where C: Young mice, D: Old mice as compared to control group of young and old mice on 0, 13th and 14th day. Data expressed as means ± SEM; *** represents *P*<0.001 as compared to the normal saline (control) group within the same day of the treatment; **represents *P*<0.01 as compared to the normal saline (control) group within the same day of the treatment; * represents *P*<0.05 as compared to the normal saline control group within the same day of the treatment; ^^^ represents (*P*<0.001) as compared to the scopolamine treated within the same day of the treatment.

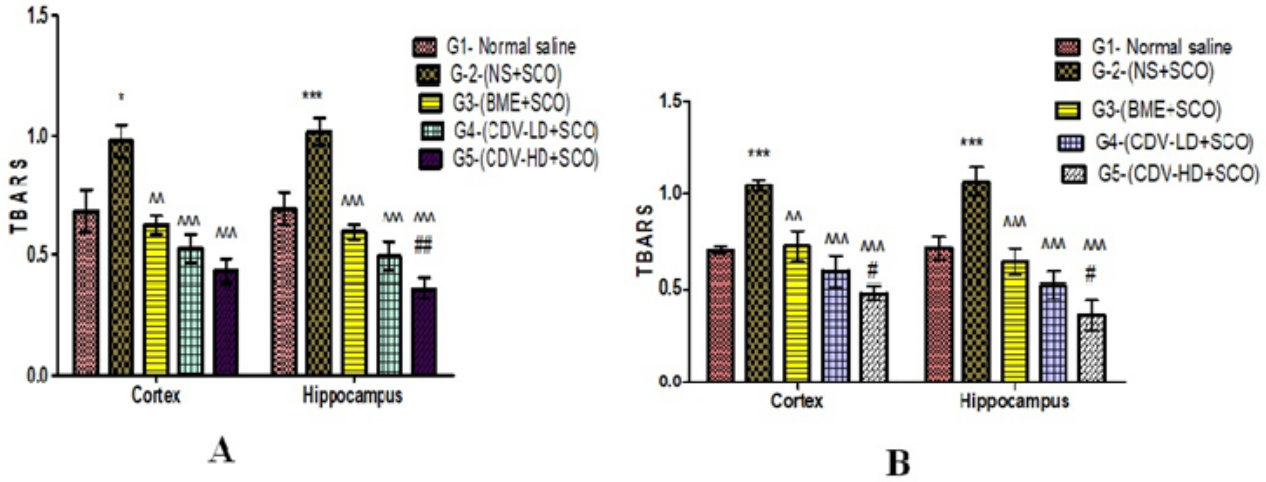

Figure 8: Effect of CDV (100 mg/kg and 200 mg/kg), BME 120 mg/kg on TBARS activity in A: young and B: old mice. Data expressed as mean ± S.E.M. of each group show TBARS activity/min/mg protein .*** represents (*p*<0.001) and * represents (*p*<0.05) as compared to the control group within the same day of the treatment; ^^^ represents (*p*<0.001)and ^^ represents (*p*<0.01) as compared to the scopolamine treated group within the same day of the treatment; ##represents (*p*<0.01) and # represents (*p*<0.05) as compared to the BME treated group within the same day of the treatment. ##represents (P<0.01) as compared to the *Bacopa monnieri* extract (BME) treated group within the same day of the treatment; #represents (P<0.05) as compared to the *Bacopa monnieri* extract (BME) treated group within the same day of the treatment.

Estimation of Catalase activity in both the cortex and the hippocampus

CDV extract shows a significant (*p*<0.01) increase in cortex and hippocampus catalase activity in comparison to the control group and a significant (*p*<0.001) increase as compared to groups treated with SCO (Figure 10).

Estimation of GSH activity in both the cortex and the hippocampus

CDV extract shows a significant (*P*<0.01) increased GSH levels in the cortex and hippocampus in comparison to the control group and a significant (*p*<0.001) increase as compared with SCO treated group as shown in Figure 11.

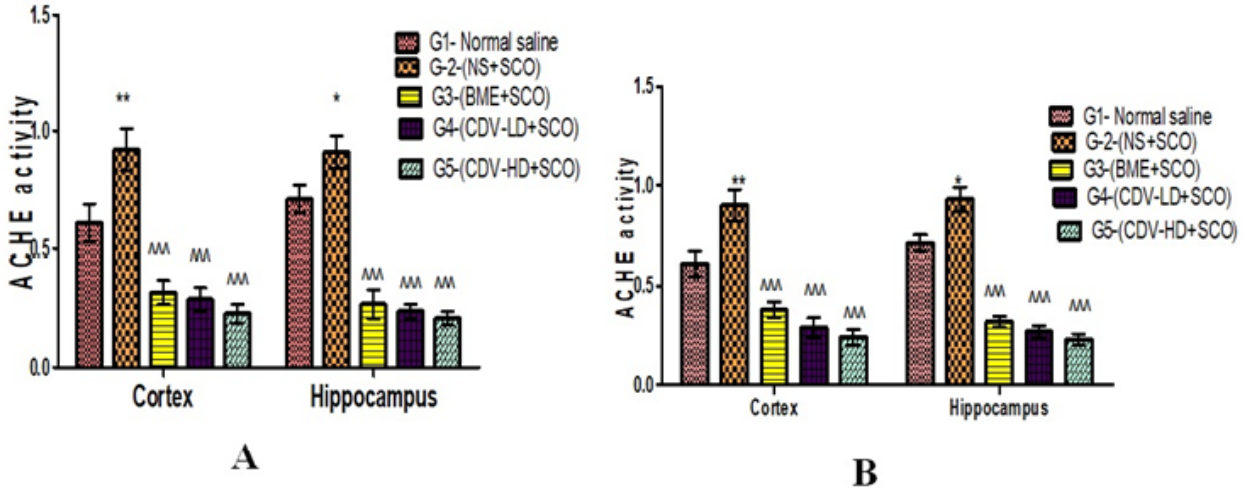

Figure 9: Effect of CDV (100 mg/kg and 200 mg/kg), BME 120 mg/kg on ACHE activity in A: young and B: old mice respectively. Data expressed as mean ± S.E.M. of each group show ACHE activity/min/mg protein. *represents (P<0.05) as compared to the control group within the same day of the treatment; ** represents (P<0.01) as compared to the control group within the same day of the treatment; ^^ represents (P<0.01) as compared to the scopolamine treated group within the same day of the treatment; ^^^ represents (P<0.001) as compared to the scopolamine treated group within the same day of the treatment.

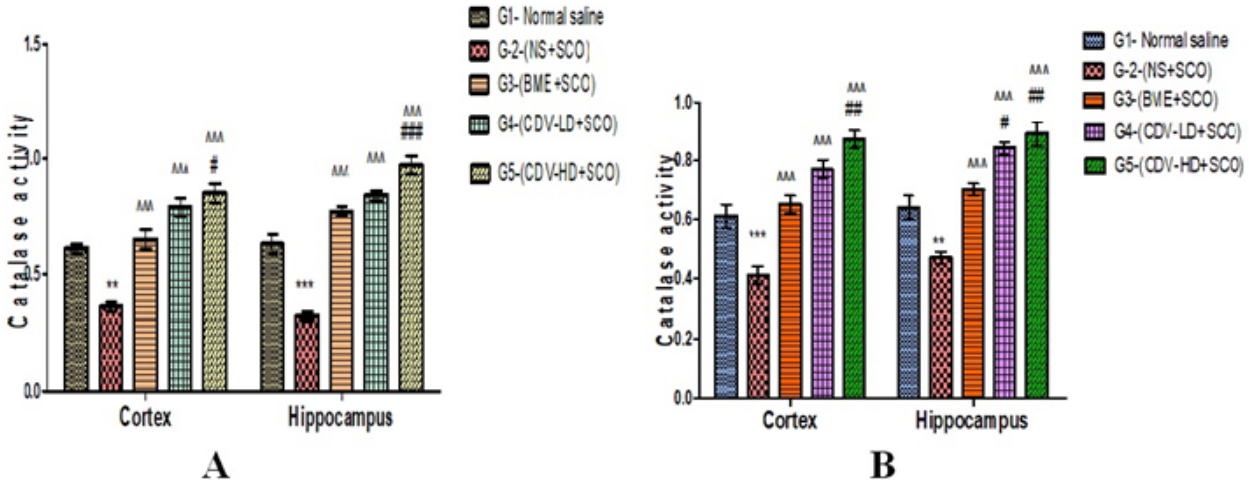

Figure 10: Effect of CDV (100 mg/kg and 200 mg/kg), BME 120 mg/kg Catalase activity in A: young and B: old mice respectively. Data expressed as mean ± S.E.M. of each group show Catalase activity/min/mg protein. ** represents (P<0.01) as compared to the control group within the same day of the treatment; *** represents (P<0.001) as compared to the control group within the same day of the treatment; ^^^ represents (P<0.001) as compared to the scopolamine treated group within the same day of the treatment. ###represents (P<0.001) as compared to the BME treated group within the same day of the treatment; ##represents (P<0.01) as compared to the BME treated group within the same day of the treatment; #represents (P<0.05) as compared to the BME treated group within the same day of the treatment.

DISCUSSION

AD is still a complex phenomenon in which diffuse abnormalities occur in the brain.[23] Cognition disturbances are often seen in old age.[24] Normal aging also affects memory so the aged animal model is useful in studying neurological disorders and behavioral studies.[25,26] Scopolamine acts through a non-selective centrally acting muscarinic receptor antagonist. It's administration is also linked with the generation of free radicals and is reported in many studies.[27-29] Neuronal systems get damaged with Oxidative stress affecting memory thereby the destruction of the neuronal pathways.[30,31] It was seen in the current study that scopolamine increases oxidative stress.[32,33] The improvement of cognition in behavioral models with CDV proved it as a nootropic and useful in AD owing to its antioxidant mechanism. Cognition enhancement is indicative of useful features in AD.[34,35] The level of reinstatement or betterment in behavioral parameters with CDV on account of accordant training and observation gives the basis for CDV extract as a prospective anti-AD drug. The findings indicated that CDV produced a significant reverse swing in memory and cognitive loss due to aging and amnesia caused by scopolamine. The results stipulated that reversal of memory was comparable to BME and better than normal saline and scopolamine treated group in both young and old mice.

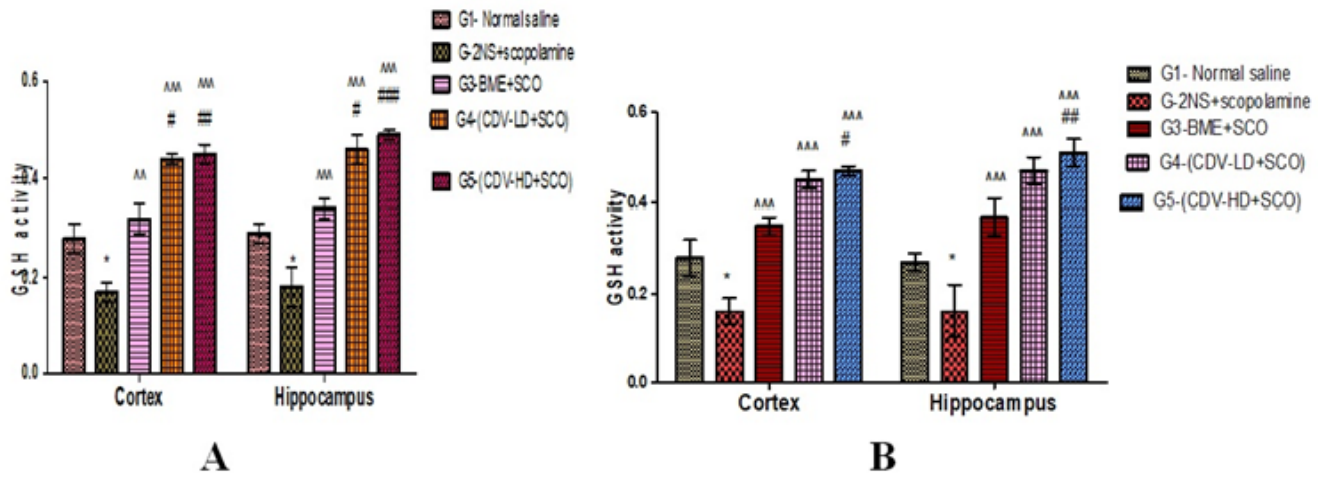

Figure 11: Effect of CDV (100 mg/kg and 200 mg/kg), BME 120 mg/kg on GSH activity in A: young and B: old mice respectively. Data expressed as mean ± S.E.M. of each group show GSH activity/min/mg protein. *represents (P<0.05) as compared to the control group within the same day of the treatment; ^^ represents (P<0.01) as compared to the scopolamine treated group within the same day of the treatment; ^^^ represents (P<0.001) as compared to the scopolamine treated group within the same day of the treatment; . ###represents (P<0.001) as compared to the BME treated group within the same day of the treatment; ##represents (P<0.01) as compared to the BME treated group within the same day of the treatment; #represents (P<0.05) as compared to the BME treated group within the same day of the treatment.

All groups were better than scopolaminein locomotion. So it is evidenced that CDV has potential as a nootropic agent. CDV showed its antioxidant potential and anticholinesterase activity. Owing to strong antioxidant properties as evidenced by results CDV a is a perfect candidate as an anti-AD drug.

CONCLUSION

The findings of the current study explored the cognition-enhancing potential of chloroform extract of *Dragea volubilis* due to its antioxidant, and neuroprotective activity. It reversed scopolamine-induced memory loss and has shown improvement in the behavioral model which suggests its usefulness as a potential neuroprotective agent. Hence further investigation of this product can be developed to be used as potential anti-Alzheimer's features.

Data Availability

The authors confirm that the data supporting the findings of this research are available within the article.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

Ethical Approval

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

ACKNOWLEDGEMENT

Authors are grateful to the Department of Research, Innovation and Consultancy (RIC), I. K. Gujral Punjab Technical University, Kapurthala, Punjab for providing access to reputed scientific journals via Open Athens account to carry out this research work.

ABBREVIATIONS

AD: Alzheimer's disease; **CDV:** Chloroform extract of *Dragea volubilis;* **SDL:** Step-Down Latency; **TBARS:** Thiobarbituric acid reactive substance activity; **GSH:** Glutathione; **EL:** Escape Latency; **MWM:** Morris water maze; **TL:** Transfer latency; **SCO:** Scopolamine hydrobromide; **TBA:** Thiobarbituric acid; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **BME:** *Bacopa monnieri* extract; **SRM:** Spatial reference memory.

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Cite this article: Sharma A, Gill N. Therapeutic Potential of Dragea volubilis Leaf Extract against Scopolamine-induced Memory Impairment in Young and Aged Mice. Int. J. Pharm. Investigation. 2023;13(1):162-72.