

# Exploring the Potential of *Desmodium gangeticum* (L.) DC. Extract against Spatial Memory Deficit in Rats

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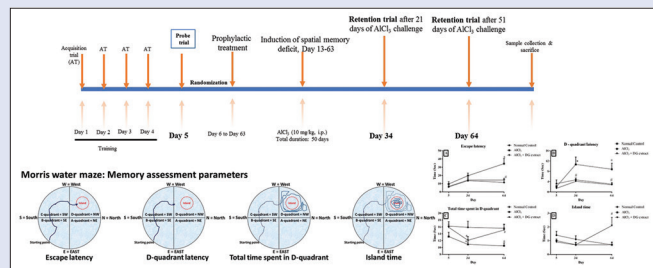
## ABSTRACT

**Background:** A few published reports demonstrated the neuroprotective effect of *Desmodium gangeticum* (L.) DC. in an acute model of dementia. **Objective:** The purpose of the present study was to evaluate the preclinical efficacy of *D. gangeticum* against chronic dementia when administered prophylactically. **Materials and Methods:** Chronic spatial memory deficit was induced in rats by aluminum chloride (AlCl<sub>3</sub>, 10 mg/kg, i.p.). Treatment with hydroalcoholic whole plant extract of *D. gangeticum* (DG extract) was initiated 2 week before AlCl<sub>3</sub> challenge and continued till the 51<sup>st</sup> day after the challenge, orally at the dose of 400 mg/kg/day. The spatial memory was assessed by Morris water maze test. Hippocampal and frontal cortex acetyl cholinesterase (AChE) and oxidative stress were assessed in diseased rat brains. **Results:** Chronic administration of AlCl<sub>3</sub> produced spatial memory deficit in rats. Memory impairment was manifested in rats as an increase in escape latency and D-quadrant latency whereas a decrease in total time spent in D-quadrant. These behavioral alterations were reversed significantly by the treatment with DG extract. In addition, DG extract significantly increased the island time, indicating memory improvement. DG extract corrected the declined AChE in frontal cortex and altered frontal cortex/hippocampus catalase activity. Phytochemical investigation of the DG extract revealed large content of saponins among the other phytochemicals such as tannins, alkaloids, and flavonoids. **Conclusion:** These results indicate the possible prophylactic potential of saponin-rich DG extract against chronic memory deficit in rats. **Key words:** Acetyl cholinesterase, aluminum chloride, dementia, *Desmodium gangeticum* (L.) DC, oxidative stress

## SUMMARY

- Hydroalcoholic whole plant extract of *Desmodium gangeticum* (L.) DC. (DG extract) protects against chronic memory deficit in rats

- Chronic oral treatment with DG extract reversed the altered brain acetyl cholinesterase activity and catalase.



**Abbreviations used:** AChE: Acetyl cholinesterase; AD: Alzheimer's disease; AlCl<sub>3</sub>: Aluminum chloride; Aβ: Amyloid-beta; *D. gangeticum*: *Desmodium gangeticum* (L.) DC; DG extract: Hydroalcoholic whole plant extract of *Desmodium gangeticum*; DTNB: 5,5'-Dithiobis-(2-nitrobenzoic acid); H<sub>2</sub>O<sub>2</sub>: Hydrogen peroxide; MWM: Morris water maze; NMDA: N-methyl-D-aspartic acid.

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## INTRODUCTION

Dementia, an age-related neurodegenerative disorder, which occurs due to hindered neuronal transmission expedited by neuronal damage or death, is manifested as memory loss, mental confusion, and inability to speak or sometimes comprehend simple day-to-day situations. Alzheimer's disease (AD) is one of the major causes of dementia, involving amyloid-beta (Aβ) plaques deposition, leading to neurodegeneration.<sup>[1]</sup>

Aluminum is a redox mineral, the physiological role of which is unknown. However, in case of chronic consumption, it accumulates maximally in the brain, particularly in the hippocampus region. It has been reported to increase Aβ protein deposition<sup>[2]</sup> and hyperpolarization of tau protein in the hippocampus, leading to dementia-like symptoms.<sup>[3]</sup> Thus, chronic aluminum chloride (AlCl<sub>3</sub>) administration has been utilized as a preclinical tool to produce AD-related dementia in experimental animals.<sup>[4-8]</sup>

Limitations of existing therapeutic modalities led into the search of naturally obtained extract (s) or phytoconstituents for the treatment of

dementia and neurodegenerative disorders.<sup>[7,9-11]</sup> The Indian traditional medical system or Ayurveda provides numerous treatment modalities for dementia. *Rasayana* is considered helpful in age-related problems and may treat dementia. Some herbal preparations such as *Dashmolarishta* and *Dashmolakawath*, mentioned in Ayurveda, have been found to treat nervous disorders.<sup>[12]</sup> *Desmodium gangeticum* (L.) DC. is mentioned in the Ayurveda as a treatment for various neurological disorders<sup>[13]</sup> and forms an important constituent of *Dashmolarishta* and *Dashmolakawath*.

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*Desmodium gangeticum* (L.) DC. (*Fabaceae*) is commonly known as Shalparni in Sanskrit and is abundantly found throughout India. *D. gangeticum* is distributed in the Himalayan territory up to an altitude of 5000 feet and also in China, Philippines, and tropical Africa. Conventionally, this plant exerts various pharmacological actions such as diuretic, astringent, anthelmintic, anti-inflammatory, anti-bacterial, anti-diabetic, anti-ulcer, locomotor, and wound-healing activities.<sup>[14,15]</sup> Different extracts from roots and aerial parts of *D. gangeticum* have been previously reported to reverse scopolamine-induced acute amnesia in mice.<sup>[13,15]</sup> In another research, the leaf and root aqueous extract of *D. gangeticum* was found to inhibit whole brain acetyl cholinesterase (AChE) activity and to enhance the acetylcholine content in the same experimental model,<sup>[16]</sup> which ultimately resulted in increased cholinergic transmission in the brain.<sup>[16]</sup> However, these effects have not been tested in a chronic study. Thus, there is a need to explore the chronic effect of *D. gangeticum* in dementia, especially when administered prophylactically, to prevent or delay the progression of dementia. Based on all these findings, the present study was designed to study the effect of *D. gangeticum* on AlCl<sub>3</sub>-induced dementia in rats.

## MATERIALS AND METHODS

### Experimental animals

The study protocol was approved by the Institutional Animal Ethics Committee, Kasturba Medical College, Manipal University (IAEC/KMC/66/2016), and performed as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals. The study was carried out in 3-month-old male Wistar rats, weighing 250–300 g in-bred in the Central Animal Research Facility, Manipal. They were kept under standard husbandry condition (room temperature 22°C ± 1°C and humidity 55%). The animals were maintained in polyacrylic cages (3 animals/cage) with 12-h light and dark cycles. The entire experiments were carried out from 9:00 to 14:00 h.

### Chemicals and reagents

AlCl<sub>3</sub> was purchased from Loba Chemie Pvt. Ltd. 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB) and acetylthiocholine iodide were purchased from Sigma Aldrich Chemicals Pvt. Ltd. All other laboratory chemicals used in the study were of analytical grade.

### Plant extract

For the current study, the hydro-alcoholic whole plant extract of *D. gangeticum* (L.) DC. (DG extract) was provided by Dr. Rajesh Kumar Ganjhu, Scientist cum Entrepreneur, Radhika Ayurveda Research and Development, Pune, Maharashtra, India. The extraction was carried out as per the standard operating procedures at NABL Accredited and GMP Certified Company-Ayushraj Enterprises Pvt. Ltd, Jaipur, Rajasthan, India. The *D. gangeticum* (L.) DC. plant specimen was deposited and authenticated at Central National Herbarium-Botanical Survey of India,

Howrah-711103 (Letter Ref. No. CNH/Tech IV/Repository/2015/17 dated December 21, 2015. The collection site of the plant was Khunti District of Jharkhand State - 835 210, India.

### Phytochemical estimation

Gravimetric analysis of DG extract for the qualitative and quantitative estimation of the phytochemicals was carried out in Ayushraj Enterprises Pvt. Ltd, Jaipur, Rajasthan, India.

### Dosing and treatment schedule

As reported earlier,<sup>[8,10]</sup> from day 1 to 4, acquisition trial was performed using Morris water maze (MWM) [Figure 1]. On Day 5, a probe trial was done. Animals were randomized based upon the escape latency during probe trial on Day 5 and were divided into three groups (normal control, AlCl<sub>3</sub> control, and AlCl<sub>3</sub> + DG extract [400 mg/kg p. o.]). Dose of DG extract was based upon published report.<sup>[15]</sup> All the animals were treated with vehicle/extract (p.o.), respectively starting from day 6 to 63. From day 13, treatment was continued along with AlCl<sub>3</sub> challenge (10 mg/kg, i.p.) till Day 63. Retention trials were performed on day 34 and 64 (21<sup>st</sup> and 51<sup>st</sup> day, respectively, after AlCl<sub>3</sub> administration) to check the memory impairment in animals.

### Spatial memory assessment

The MWM test was developed by "Richard Morris."<sup>[17,18]</sup> As described earlier,<sup>[6-8,10]</sup> the maze consists of a circular tank (150 cm in diameter and 40 cm in height) filled with water, approximately half of the entire volume. The maze was divided into four spaced points, East (E), West (W), North (N), and South (S) to make quadrants (NE, SE, SW, and NW). In the acquisition trial, a platform was kept hidden 0.5–1 inch below the water, in the NW (D-quadrant). Rats were trained for 4 days by giving four trials per day, to find out the hidden platform. During the trials, animal was allowed to reach the hidden platform starting from all four different quadrants. In each trial, animals were kept slowly in the in the water maze (facing wall of the tank) and allowed to swim for 1 min to find out the hidden platform. After reaching the platform, the animals were allowed to stay on it for 30 s and then removed from the platform and dried with the towel. If animals failed to find the platform within 60 s, they were guided to the platform.

In the case of the probe (on Day 5) and retention trials (on Day 34 and 64), the platform was absent and the ability of the animals to find the initial position of the platform was assessed (one trial per animal/day). The trial was conducted for 60 s by placing the animal in SE, i.e., B-quadrant. Rest of the procedure was similar to acquisition trial. The retention trial was done after inducing the disease with AlCl<sub>3</sub> as described in Figure 1. During probe and retention trials, the parameters such as escape latency (latency of first entry to the area where the platform was hidden during acquisition trial), D-quadrant latency (latency of first entry

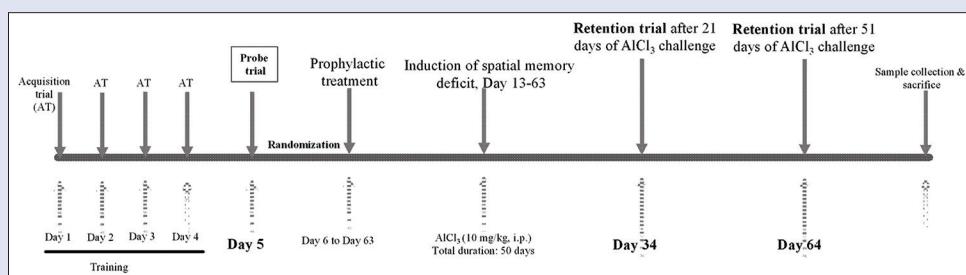


Figure 1: Scheme for the induction of memory deficit and treatment schedule

to target quadrant “D”), total time spent in D-quadrant (total time spent in the area of target quadrant “D”), island time (total time spent in area where the platform was hidden during acquisition trial), and swimming speed were recorded. The trial data were recorded through a video camera, which was fixed above the center of the maze and connected with the computerized tracking device (AnyMaze software, UgoBasile, Italy).

## Brain biochemical estimation

### Isolation of cortex and hippocampus from brain

After the completion of the experimental period, the rats were sacrificed by euthanasia. The brain of each rat was quickly removed, washed well with ice-cold saline to remove blood, kept on an ice-cooled plate, and then dissected into the hippocampus and frontal cortex by following the procedure by Glowinski and Iversen<sup>[19]</sup> and further stored at  $-20^{\circ}\text{C}$ .

### Homogenate preparation for biochemical estimations

Homogenates of tissue samples (10% w/v) were prepared in ice-cold 0.1 M phosphate buffer (pH 7.4) using a homogenizer fitted with Teflon plunger at a speed of 8000 rpm. The homogenates were centrifuged at 15,000 rpm at  $4^{\circ}\text{C}$  for 15 min to collect the supernatant for analysis.

### Estimation of total protein

Total protein was estimated in samples using Pierce<sup>®</sup> BCA Protein Assay Kit following the protocol by Thermo Scientific, USA. Bovine serum albumin was used as the standard.

### Estimation of acetylcholinesterase activity

The levels of AChE were determined by Ellman's method.<sup>[20]</sup> Briefly, the assay mixture contained 134  $\mu\text{l}$  of the supernatant, 866  $\mu\text{l}$  of phosphate buffer (0.1 M), 33  $\mu\text{l}$  of DTNB, and 7  $\mu\text{l}$  of acetylthiocholine iodide. The absorbance was measured at 412 nm over a period of 4 min. The concentration of AChE activity in the supernatant was expressed as  $\mu\text{moles}$  of acetylthiocholine iodide hydrolyzed/min/mg of protein.<sup>[8,10]</sup>

### Estimation of catalase activity

Catalase activity was measured by determining the decomposition of its substrate hydrogen peroxide ( $\text{H}_2\text{O}_2$ ).<sup>[21]</sup> Catalase decomposes

$\text{H}_2\text{O}_2$  and produces a continuous decrease in absorbance at 240 nm. The difference in absorbance ( $\Delta A$ ) per unit time is a measure of the catalase activity. The assay mixture contained 930  $\mu\text{l}$  of  $\text{H}_2\text{O}_2$  and phosphate buffer (50 nM, pH 7.0), 20  $\mu\text{l}$  of supernatant to determine the catalase activity. The concentration of the catalase in the supernatant was expressed as  $\mu\text{moles}$  of  $\text{H}_2\text{O}_2$ /decomposed/min/mg of protein.<sup>[10,22]</sup>

## RESULTS

### Phytochemical profile

Qualitative and quantitative gravimetric analysis of the DG extract revealed the presence of tannins (7.16 g/100 g), saponins (27.37 g/100 g), alkaloids (0.78 g/100 g), and flavonoids. Among these phytoconstituents, saponins were present maximally in DG extract.

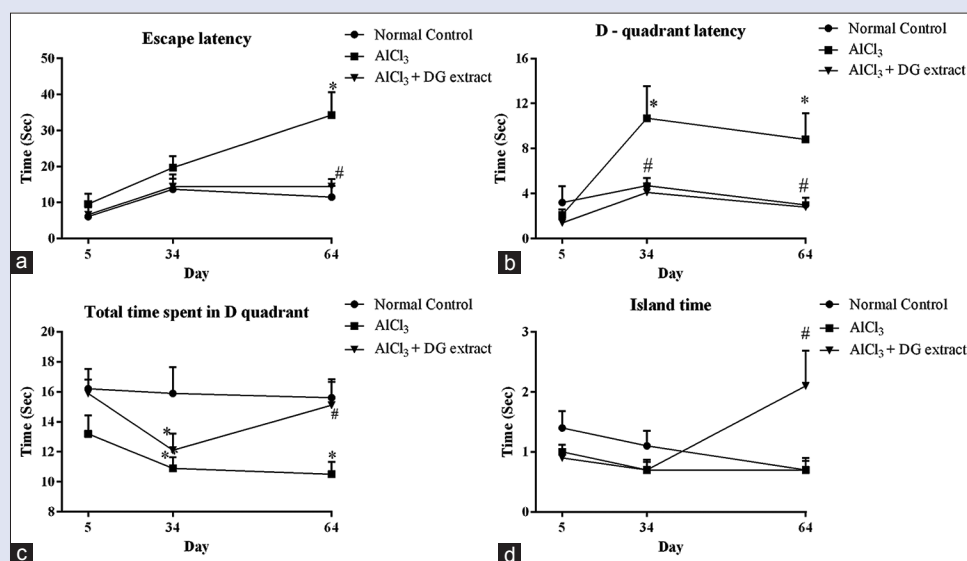
### Hydroalcoholic whole plant extract of *Desmodium gangeticum* (L.) DC. protected rats against aluminum chloride-induced memory deficit

$\text{AlCl}_3$  administration increased the escape latency and D-quadrant latency in rats on 21<sup>st</sup> and 51<sup>st</sup> day. Prophylactic treatment with DG extract reversed the elevated escape latency and D-quadrant latency on Day 51 and 21, respectively [Figure 2a and b].

Further, chronic administration of  $\text{AlCl}_3$  to rats significantly reduced the total time spent in D-quadrant on Day 21 and 51 as compared to normal control but did not affect the island time. Prophylactic treatment with DG extract significantly raised total time spent in D-quadrant and the island time on day 51 as compared with  $\text{AlCl}_3$  group [Figure 2c and d].

### Effect of hydroalcoholic whole plant extract of *Desmodium gangeticum* (L.) DC. on mean swimming speed of rats

There was no difference in the mean swimming speed of  $\text{AlCl}_3$ -treated rats compared to control animals or among the rats treated with DG extract compared to  $\text{AlCl}_3$ -treated animals [Figure 3].



**Figure 2:** Effect of hydroalcoholic whole plant extract of *Desmodium gangeticum* on (a) escape latency, (b) D-quadrant latency, (c) total time spent in D-quadrant, and (d) island time against aluminum chloride-induced dementia in rats. Data represented as mean  $\pm$  standard error of the mean,  $n = 6$ . \* and # represent  $P < 0.05$  as compared with normal control and aluminum chloride, respectively

### Effect of hydroalcoholic whole plant extract of *Desmodium gangeticum* (L.) DC. on brain acetyl cholinesterase activity

No significant change was observed in hippocampus AChE activity of  $\text{AlCl}_3$  group as compared to the control group. However,  $\text{AlCl}_3$  administration significantly reduced the AChE activity in frontal

cortex as compared to the control group. Treatment with DG extract significantly increased AChE activity in frontal cortex as compared to  $\text{AlCl}_3$  group [Figure 4a and b].

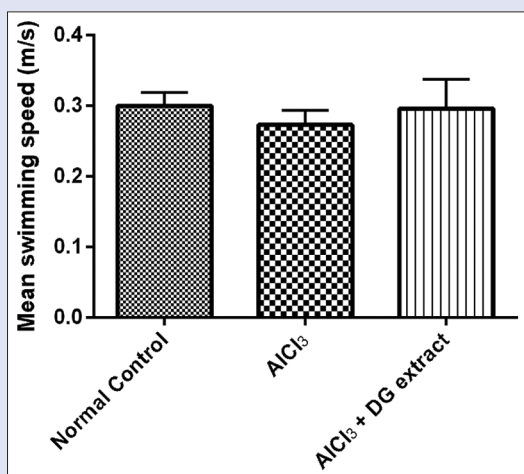
### Effect of hydroalcoholic whole plant extract of *Desmodium gangeticum* (L.) DC. on brain catalase activity

Chronic  $\text{AlCl}_3$  administration significantly increased the catalase activity in the hippocampus of  $\text{AlCl}_3$  control group as compared to normal controls. These elevated levels were significantly reversed in the rats treated with DG extract as compared to  $\text{AlCl}_3$  group. Further, no significant change was observed in frontal cortex catalase activity of animals after chronic  $\text{AlCl}_3$  administration [Figure 4c and d].

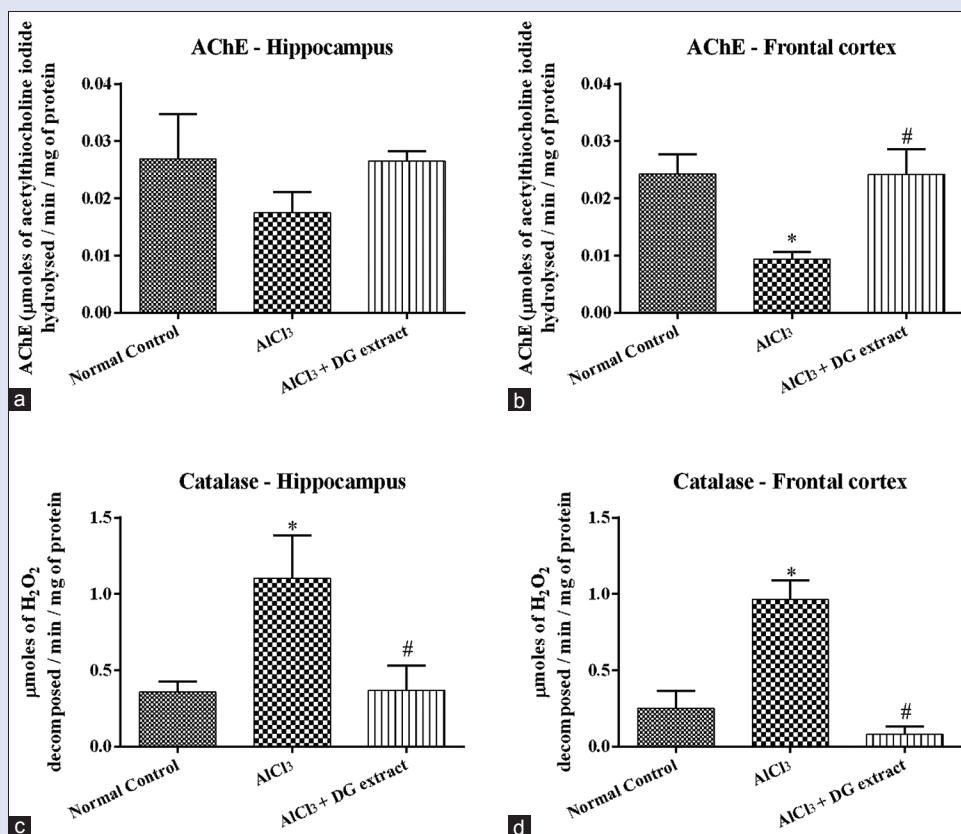
## DISCUSSION

The present study was aimed at evaluating the prophylactic potential of the DG extract against memory deficit in rats. For this purpose,  $\text{AlCl}_3$ -induced dementia was used as the pharmacological tool. The spatial memory of the experimental animals was evaluated for the degree of dementia using MWM test. The MWM trial is strongly correlated to the hippocampal synaptic plasticity and NMDA receptor function.<sup>[17]</sup> Thus, the loss in spatial memory is associated with the abnormal synaptic plasticity particularly in the hippocampus.

Post- $\text{AlCl}_3$  administration from day 21 onward, the animals experienced spatial memory deficit in MWM test, manifested as an increase in D-quadrant latency and a decrease in total time spent in D-quadrant. In addition, a significant increase in escape



**Figure 3:** Effect of hydroalcoholic whole plant extract of *Desmodium gangeticum* on mean swimming speed of rats. Data represented as mean  $\pm$  standard error of the mean,  $n = 6$



**Figure 4:** Effect of hydroalcoholic whole plant extract of *Desmodium gangeticum* on hippocampus (a and c)/frontal cortex (b and d) acetyl cholinesterase (a and b) and catalase (c and d) activity in aluminum chloride-induced dementia in rats. Data represented as mean  $\pm$  standard error of the mean,  $n = 6$ . \* and # represent  $P < 0.05$  as compared with normal control and aluminum chloride, respectively

latency was observed on Day 51. These findings were in line with the previous report where  $\text{AlCl}_3$  administration resulted in memory deficit in rats.<sup>[6,7]</sup> The doses of  $\text{AlCl}_3$  were selected based upon the previous report from our laboratory.<sup>[10]</sup> Prophylactic treatment with DG extract (400 mg/kg) showed significant improvement in reversing  $\text{AlCl}_3$ -induced changes in escape latency, D-quadrant latency and total time spent in D-quadrant. Further, there was no significant difference in the swimming speed of the animals across the groups. These data suggest that the animals' swimming activity remained normal and the change in memory function was not associated with swimming disability. Thus, aluminum or extract at the tested doses did not affect the motor coordination which might have caused impaired swimming and delay in reaching to target quadrant. These results indicate that DG extract improved the spatial memory of animal and showed neuroprotective action against  $\text{AlCl}_3$ -induced neuro-behavioral changes when administered prophylactically.

The neuroprotective action of DG extract was further studied by estimating the oxidative stress markers and AChE activity in hippocampus and frontal cortex. *D. gangeticum* has been reported to possess cholinesterase inhibition and anti-oxidant capacity.<sup>[23]</sup> However, there are insufficient data on the chronic effect of *D. gangeticum* on these markers. In contrast to an earlier report,<sup>[10]</sup> where whole brain AChE activity was increased, in the present study, frontal cortex and hippocampus AChE activity were found to be decrease in  $\text{AlCl}_3$ -challenged rats. These results are in line with the previously published report from our lab, where a decline in AChE activity was observed in  $\text{AlCl}_3$  model of dementia.<sup>[6,8]</sup> Pretreatment with DG extract corrected the  $\text{AlCl}_3$ -induced decline in AChE activity in the cortex. Intriguingly, there was no significant change in hippocampal AChE activity between the groups.

Oxidative stress has been associated with dementia and neurodegenerative disorder such as AD.<sup>[10,24]</sup> In hippocampus and frontal cortex of diseased rats, the increased catalase activity was observed, which is in contrast to an earlier report.<sup>[6]</sup> This could be due to a low dose of  $\text{AlCl}_3$  used in the current study as compared to earlier reported method<sup>[6]</sup> where at 175 mg/kg p.o. for 60 days,  $\text{AlCl}_3$  showed a decline in catalase activity. Catalase is an antioxidant enzyme, which corrects the oxidative stress. Elevation in catalase activity in the diseased group is a sign of mild oxidative stress, which was reversed significantly by the DG extract. This supports the protective mechanism of extract to correct oxidative stress in the brain. Various phytoconstituents such as pterocarpan (gangetin, gangetinin, desmocarpane, and desmodin), alkaloids, flavonoid glycosides (Rutin, quercetin-7-O- $\beta$ -D-glucopyranoside, and kaempferol-7-O- $\beta$ -D-glucopyranoside.), sterols and phenolic acids (caffeic acid and chlorogenic acid) have been reported in *D. gangeticum*.<sup>[22]</sup> Among the phytochemicals determined by gravimetric analysis of DG extract (tannin, flavonoid, saponin, and alkaloids), in the present study, saponins were found to be the major constituents. Published report on amelioration of dementia by timosaponins<sup>[25]</sup> indicates the possible role of major constituents saponins for the observed efficacy of DG extract. However, the efficacy of DG extract could also be associated due to the combined effects of all the present constituents. A detailed characterization of extract and bioactivity-guided fraction will help in identifying the active principle for anti-dementia activity of DG extract.

## CONCLUSION

DG extract showed a neuroprotective effect against  $\text{AlCl}_3$ -induced dementia in rats by correcting the  $\text{AlCl}_3$ -mediated spatial memory deficit with reversal of hippocampal and frontal cortex AChE and catalase activity. This suggests the role of DG extract in modulating cholinergic

and oxidative stress. Limitations of the current study involve the inability to evaluate the molecular markers related to dementia. Future research is warranted for detailed characterization of extract and envisages the molecular mechanism of action.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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