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Effect of Polyherbal Formulation on Learning and Memory in Animal Models of Alzheimer's Disease

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Abstract

Objective: - The current study was investigated on sumanas (SMS) a polyhebral formulation against colchicines induced animal model of Alzheimer's disease. **Methods**: -The memory enhancing activity of SMS was demonstrated by in vivo methods like Morris water maze and elevated plus maze using colchicines induced model cause cognitive impairment and memory demolition. The cognitive impairment activity of SMS was evaluated by serum levels GSH, LPO, CAT, AChE content of the brain. **Results and Discussion**: - the Effect of Sumanas in colchicine induced Alzheimer model has showed, a significant alteration in biochemical parameters i.e., serum enzymes namely, LPO, GSH, CAT, AChE levels and in was observed. Oral administration of Sumanas (25, 50, 100 mg/Kg) was found increases LPO, CAT and AChE levels by dose dependent manner. By this alteration in biochemical estimation confirmed that SMS has positive effective in treatment of AD. The observed neuroprotective property of herbal formulation SMS was due to presence of phytochemicals. **Conclusion**: - SMS was already used herbal medicine in the cognitive patients which shown improved the cognitive impairment through memory enhancing property. Due to lack of evidence the study was undertaken and concluded by estimation of biochemical markers. SMS has found significantly neuroprotective in colchicines induced Alzheimer's disease.

Keywords
Alzheimer's disease, Sumanas, Colchicine, Cognitive impairment, Memory enhancer.

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INTRODUCTION

Alzheimer's disease (AD) is a degenerative cerebral infection disease, most common type of dementia that usually starts in old age. These outcomes of symptoms were reformist cognitive decline, progressive memory loss, impended thinking, disorientation, and changes in character, mood swing and marked histological changes by the degeneration of brain neurons particularly in the cerebral cortex. The cerebral cortex made with neurofibrillary tangles and plaques which including beta-amyloid protein[1].

Alzheimer's disease is a progressive and disabling brain disorder. Which were gradually loss tangles and plagues or formation of abnormal protein around brain cells. Due to the deposition of protein cause destroy memory and thinking skills and, ultimately, it lost the capacity to carry out the simplest tasks. In most people symptoms is first appear in the late onset age of mid-60s. Most often AD happens between an individual's 30s and mid-60s. The main cause of dementia is wildly recognized in older adults[2].

AD is characterized by behavioral changes, decline in memory loss and dementia. The plaques formation, neurofibrillary tangles accumulation, and cholinergic brokenness are major illness of the disease. Examination of clinical and preclinical study highlights that neuronal death, synaptic loss, neurochemical impermanent and main

neurotransmitter i.e. Acetylcholine factors are fundamental variable cause cognitive and dementia symptoms[3]. Dr.Alois Alzheimer is first identified the disease in 1906. He was observed the neuronal changes in brain of women; she died due to mental illness. Her symptoms and signs resemble to cognitive decline and behavioral changes. Once her brain was subjected to examine, he found abnormal clumps (now called amyloid plaques) and tangle fibers (now called neurofibrillary). These are main feature to cause AD[4].

Currently, the treatment of Alzheimer's disease is non tolerable and allopathic drugs for treatment of Alzheimer's disease is not adequate and not much benefited. The polyherbal formulation, Sumanas (SMS) used by Ayurvedic practitioner to treat memory loss cases. The component of this includes, Bramhi, Shankhapushpi, Jatamamsi, Sarpagandha, Parasika Yavani and Vacha. The Bramhi (Bacopa monnieri)[5], Shankhapushpi (Clitoria ternatea)[6], Jatamamsi Nardostachys (jatamansi)[7], Sarpagandha (Rauwolfia serpentina)[8], Parasika Yavani (Hyoscyamus niger)[9], Vacha (Acorus calamus)[10] reported to possess memory enhancing activity in AD.

However, no scientific data is available for the treatment of Alzheimer's disease using Sumanas. Hence, the current research is aimed to scientifically validate the memory enhancing property of Sumanas (SMS) in experimental animal models of AD. The current research programmed is an attempt to evaluate the anti-Alzheimer activity of SMS against Colchicine induced Alzheimer's disease model.

MATERIALS AND METHODS:

Collection of SMS

SMS powder collected from pavaman Pharmaceuticals, vijayapur, Karnataka, India

Surgery and administration of Colchicine at intracerebroventricular:

Animal was anesthetized using an andesitic compound i.e thiopental sodium (45 mg/kg). The head was kept on board and made a small incision at midline sagittal of scalp. Two holes are made using driller in skull for implantation of injection cannula at position of 0.8mm posterior bregma, 1.8mm lateral sagittal suture and 3.6mm cortical surface. The scalp was tied by suture by applying gentamicin at surgical area to prevent sepsis. The animals were kept in animal house with soft bedding. Special care to be taken during period of behavioral study by provided water and food to rats. Rats were infused with ICV with either artificial cerebrospinal fluid (ACSF; in mmol/l: 147 NaCl, 2.9 KCl, 1.6 MgCl2, 1.7 CaCl2, and 2.2 dextrose) or 15 µg colchicine dissolved in ACSF. The solution was injected to each animal of group using hamilton microsyringe in a injection cannula and syringe was kept for 2min to diffusion and prevents damage[11].

Colchicine induced Alzheimer's disease model.

The animal was divided into five groups:

Groups	Treatment
Sham	Received 5µl of 0.9% normal saline and 0.2% ascorbic acid by stereotaxic injection into both the lateral cerebral ventricles and distilled water orally for 28 days.
control	Received 5µl of Colchicine (15µg/rat) in ACSF (Artificial Cerebrospinal Fluid) and 0.2% ascorbic acid by stereotaxic injection into both the lateral cerebral ventricles and distilled water orally for 28 days.
SMS 25mg/kg	Received 5µl of Colchicine (15µg/rat) in ACSF (Artificial Cerebrospinal Fluid) and 0.2% ascorbic acid by stereotaxic injection into both the lateral cerebral ventricles and SMS 25mg/kg orally for 28 days.
SMS 50mg/kg	Received 5µl of Colchicine (15µg/rat) in ACSF (Artificial Cerebrospinal Fluid) and 0.2% ascorbic acid by stereotaxic injection into both the lateral cerebral ventricles and SMS 50mg/kg orally for 28 days.
SMS 100mg/kg	Received 5µl of Colchicine (15µg/rat) in ACSF (Artificial Cerebrospinal Fluid) and 0.2% ascorbic acid by stereotaxic injection into both the lateral cerebral ventricles and SMS 100mg/kg orally for 28 days.

Behavioral Assessment of Cognitive Performance
Elevated Plus Maze Paradigm[12]
The elevated plus maze was designed in such a way that it consisted of two black open arms in opposite direction (50 × 10 cm), crossed with two closed walls of the same dimensions with 40 cm high walls. The arms related to a central square of dimensions 10 × 10 cm. The entire maze was elevated to a height of 50 cm from the floor. Evaluated the memory was test on day 13 after once colchicine administration. Individual Animal was placed in one open arm away from central square face should be towards central square. Record the time to move from open arm to close arm as the initial transfer latency (ITL).
Animal was allowed for 20 seconds once ITL was recorded and then returned to the home cage. If the animal is unable to reach closed arm within 90 seconds, it was guided on the back into one of the enclosed arms and the ITL set as 90 seconds. Placed individual rat at open arm to check the Retention of memory and noted the retention latency was on day 14 and day 21 of ITL and is called as the first retention transfer latency (1st RTL) and second retention transfer latency (2nd RTL), respectively.

Spatial Navigation Task[13]
The Morris water maze was used to evaluated acquisition and retention of a spatial navigation task. Water maze was designed, and it is circular pool (180 cm in diameter and 60 cm in height) with black color bottom. Pool was filled with opaque water by adding a nontoxic dye about height of 40cm and maintain temperature of28 ±2◦C kept at test room. Divide the pool into 4 equal quadrants such that compass (N, S, E, and W). The moveable round shaped platform (9cm diameter) placed inside pool of 2cm above the water level. During the training period platform will keep above the water range and platform position was changed. Leave the rat at one quadrant and place platform in another quadrant. The platforms do not give local cues of guide for climbing. In principle, rat swims and identifies the platform over time finally climbing on platform. Note down time to reach the platform in training and observational periods.

Assessment of Gross Behavioural Activity[14].
The gross behavior activity was done on 1st, 7th, 14th and 21st days for the colchine induced animals. Animal was kept in closed a square (30cm) area by passing infrared light sesnsitive photocell using an digital actophotometer. Observe animal for 5min inside square expressed the value in counts/5min.

Dissection and Homogenization.
On the last day, after the finishing of behavioral assessment animals subjected to scarification. Brain was dissected out and identify cerebellum was discarded. Brain was rinsed with saline water and put into ice cold water. A homogenate (10 % w/v) is prepared in 0.1M phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 rpm for 15 minutes and aliquots of supernatant was separated and used for biochemical estimation.

Biochemical Tests: -
1. Lipid peroxidation[15]
2. Estimation of Reduced glutathione[16]
3. Catalase activity[17]
4. Acetylcholinesterase (AChE) Activity[18]

RESULT
Colchicine induced Alzheimer's disease model: -
Effect of SMS on memory performance in elevated plus maze.
In the elevated plus maze, on the day 13 it was observed that, a relatively stable mean ITL for all rats and no any significant variation in performance. Within 90 s all the rats entered closed arm in EPM. The control group rats showed pitiable performance during the experiment and did not show any change in the mean retention transfer latencies on days 14 and 21 as compared to pre-training latency on day 13, indicates that colchicine- induced marked cognitive impairment (Table 1). Following training, sham-operated, SMS treated rats entered into closed arm quickly and the mean retention transfer latencies (1st RTL and 2nd RTL) to enter into closed arm on days 14 and 21 were shorter as compared to ITL of each group. SMS (25, 50, 100 mg/kg) significantly improved the colchicine-induced cognitive impairment compared to control group by reducing mean retention latencies on days 14 and 21 in dose dependent manner.

Table 1 Effect of SMS on memory performance in Elevated plus maze.

Groups (mg/kg)	Mean transfer latency (in seconds)		
	ITL	1st RTL	2nd RTL
Sham	61.37±2.80	23.08±1.93	18.22±3.28
Control	63.15±2.93	83.28±1.77[a]	74.54±1.49[a]
SMS (25mg)	67.33±2.71	49.33±0.98*	43.07±0.56*
SMS (50mg)	64.39±2.28	39.62±0.78**	31.44±0.91**
SMS (100mg)	65.90±2.41	32.18±0.69***	25.82±0.83***

The initial transfer latencies (ITLs) on day 13 and retention transfer latencies on days 14(1st RTL) and 21(2nd RTL) following colchicine injection were observed. All the values are expressed as mean±SEM, n=6, ᵃp<0.001, as compared to sham group and *p<0.05, **p<0.01, ***p<0.001, (One way Analysis of Variance [ANOVA] followed by Dunnett's test for multiple comparisons) as compared to control group.
Note: SMS: Sumanas; ITL: Initial transfer latencies; 1st RTL: Retention transfer latencies on day 14 and 2nd RTL: Retention transfer latencies on day 21.

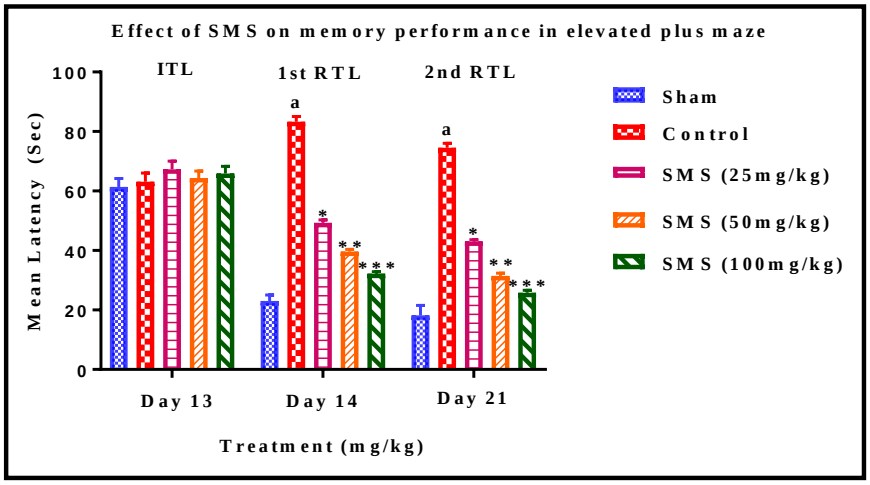

Figure 1 Effect of SMS on memory performance in elevated plus maze.

Fig.1. Histogram showing effect of SMS on memory performance in elevated plus maze. All the values are expressed as mean±SEM, n=6, ᵃp<0.001, as compared to sham group and *p<0.05, **p<0.01, **p<0.001, (One way Analysis of Variance [ANOVA] followed by Dunnett's test for multiple comparisons) as compared to control group. Note: ITL: Initial transfer latencies; 1st RTL: Retention transfer latencies on day 14 and 2nd RTL: Retention transfer latencies on day 21.

Effect of SMS on spatial navigation task

Control animals showed an early increase in escape latency, which decrease with persistent training during the acquisition of a spatial navigation task on day 13. There was a significant difference in the mean IAL of control group to Sham group on day 13 indicating colchicine-induced impaired acquisition of spatial navigation task. The low dose of SMS (25mg/kg) treatment had less effect on the IAL as compared to control group (P<0.05). In contrast, SMS (50mg/kg and 100mg/kg) treatment significantly decreased the IAL to reach the platform in the pre-trained rats as compared to colchicine-injected rats on day 13. Following training, the mean retention latencies (1st and 2nd RL) to escape onto the hidden platform was significantly decreased in sham- group on days 14 and 21, respectively as compared to IAL on day 13. SMS (25, 50 and 100 mg/kg) treatment showed a significant decrease in the 1st and 2nd RL, on days 14 and 21, compared to control group (Table 2) indicating significant improvement in the retention performance of the spatial navigation task. This effect was found to be dose dependent.

Effect of SMS on special navigation task

Table 2 Effect of SMS on special navigation task

Groups (mg/kg)	Mean transfer latency (in seconds)		
	ITL	1st RTL	2nd RTL
Sham	48.82 ± 2.83	13.18 ± 1.29	20.83± 2.8
Control	71.2 1± 2.13	78.21 ± 1.78a	65.42 ± 1.3a
SMS (25mg)	72.31 ± 2.72	43.29 ± 1.81*	35.05 ± 0.6*
SMS (50mg)	68.92 ± 2.48	38.92 ± 1.51**	31.44 ± 1.5**
SMS (100mg)	73.33 ± 2.13	28.01± 0.82***	25.82 ± 1.8***

The initial transfer latencies (ITLs) on day 13 and retention transfer latencies on days 14(1st RTL) and 21(2nd RTL) following colchicine injection were observed. All the values are expressed as mean±SEM, n=6, ᵃp<0.001, as compared to sham group and *p<0.05, **p<0.01, ***p<0.001, (One way Analysis of Variance [ANOVA] followed by Dunnett's test for multiple comparisons) as compared to control group.
Note: SMS: Sumanas; ITL: Initial transfer latencies; 1st RTL: Retention transfer latencies on day 14 and 2nd RTL: Retention transfer latencies on day 21.

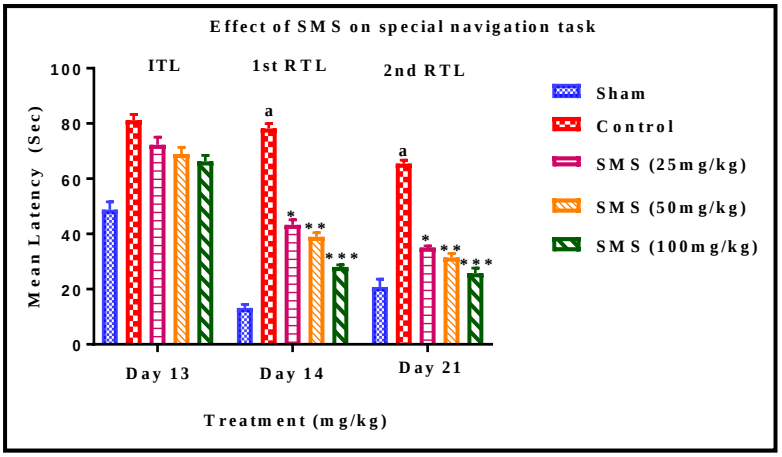

Figure 2 Effect of SMS on special navigation task

Fig.2. Histogram showing effect of SMS on special navigation task. All the values are expressed as mean±SEM, n=6, ᵃp<0.001, as compared to sham group and *p<0.05, **p<0.01, **p<0.001, (One way Analysis of Variance [ANOVA] followed by Dunnett's test for multiple comparisons) as compared to control group. Note: ITL: Initial transfer latencies; 1st RTL: Retention transfer latencies on day 14 and 2nd RTL: Retention transfer latencies on day 21.

Effect of SMS on gross behavioral activity

In the current sequence of experiments, each rat showed relatively stable mean score of gross behavioral activity (locomotor activity) without any significant variation on day 13. The mean locomotor scores in sham and control group rats remain unchanged from the mean scores of gross behavioral activities observed on day 13. SMS (25mg/kg) was less significant compared to SMS (50 and 100mg/kg) rats in gross behavioral activity on day 14 and 21 (Table 3).

Effect of SMS on gross behavioral activity

Table 3 Effect of SMS on gross behavioral activity

Groups	Counts /5 min (in seconds)		
mg/kg	ITL	1st RTL	2nd RTL
Sham	311.0±41.2	275.3±24.9	268.2±13.2
Control	298.5±30.9	259.0±21.2	217.1±12.3
SMS (25mg)	283.4±21.9	253.2±23.1	248.3±15.6
SMS (50mg)	302.2±29.3	261.4±21.3	256.2±13.9
SMS (100mg)	309.9±32.9	272.7±21.4	265.0±11.3

The initial transfer latencies (ITLs) on day 13 and retention transfer latencies on days 14(1st RTL) and 21(2nd RTL) following colchicine injection were observed. All the values are expressed as mean±SEM, n=6.

Note: SMS: Sumanas; ITL: Initial transfer latencies; 1st RTL: Retention transfer latencies on day 14 and 2nd RTL: Retention transfer latencies on day 21.

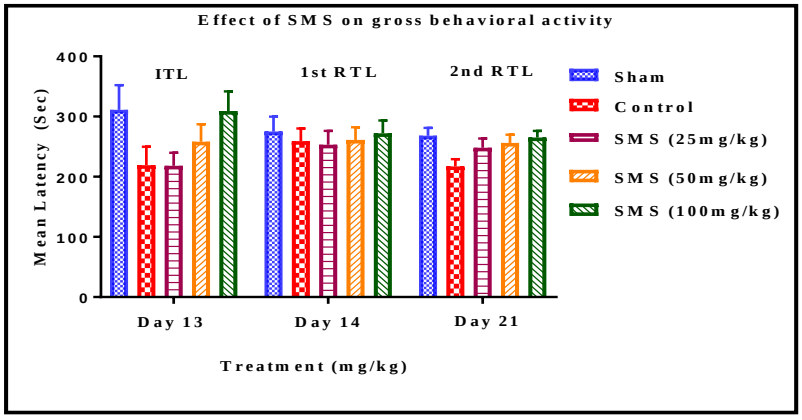

Figure 3Effect of SMS on gross behavioral activity

Fig.3. Histogram showing effect of SMS on gross behavioral activity. All the values are expressed as mean±SEM, n=6. Note: ITL: Initial transfer latencies; 1st RTL: Retention transfer latencies on day 14 and 2nd RTL: Retention transfer latencies on day 21.

Effect of SMS on brain lipid peroxidation level

Intracerebroventricular administration of colchicine had increased LPO level in brain as compared to sham group. SMS (25mg/kg) treated animals showed less significant (P<0.05) effect on LPO level. However, SMS (50 and 100mg/kg) administration significantly reduced LPO levels (P<0.001) (Table 5). This effect was found to be dose dependent decrease in LPO levels.

Effect of SMS on brain GSH and catalase levels

Central administration of colchicine caused significant depletion of GSH, and catalase levels as compared to Sham operated groups. However, SMS (25, 50 and 100 mg/kg) treatment significantly increase in catalase level and restored the GSH content as compared to control in a dependent manner (Table 5).

Effect of SMS on acetyl cholinesterase levels

Intracerebroventricular administration of colchicine had severe effect on brain acetyl cholinesterase levels as compared to sham group. Central colchicine injection showed significant increase in the brain acetyl cholinesterase activity as compared to sham group ($p > 0.001$). SMS at low dose (25mg/kg) showed slight change in the brain acetylcholinesterase activity therefore less significant ($p > 0.05$). However, high dose of SMS (50 and 100mg/kg) significantly decreased acetyl cholinesterase activity ($p < 0.001$) compared to control group (Table 5).

Table 5 Effect of SMS in colchicine induced oxidative stress parameters in rat brain.

Groups (mg/kg)	LPO (nmoles/mg of protein)	GSH (nmoles/mg of protein)	CAT (U/mg of protein)	AChE (µM/mg of protein)
Sham	83.2±2.54	15.8±1.37	0.521±0.056	0.11±0.21
Control	172.8±1.60[a]	9.6±0.60[a]	0.143±0.009[a]	0.46±0.18[a]
SMS (25mg)	140.4±2.43*	11.5±2.67*	0.170±0.018*	0.22±0.31*
SMS (50mg)	112.7±7.61**	14.8±1.98**	0.423±0.065***	0.19±0.13**
SMS (100mg)	94.9±3.79***	16.4±2.87***	0.503±0.21***	0.13±0.14***

All the values are expressed as mean±SEM, n=6, [a]p<0.001, as compared to sham group and *p<0.05, **p<0.01, ***p<0.001, (One way Analysis of Variance [ANOVA] followed by Dunnett's test for multiple comparisons) as compared to control group. Note: SMS: Sumanas tablet; ITL: Initial transfer latencies; 1st RTL: Retention transfer latencies on day 14 and 2nd RTL: Retention transfer latencies on day 21; LPO: Lipid Peroxidation; GSH: Reduced Glutathione; CAT: Catalase.

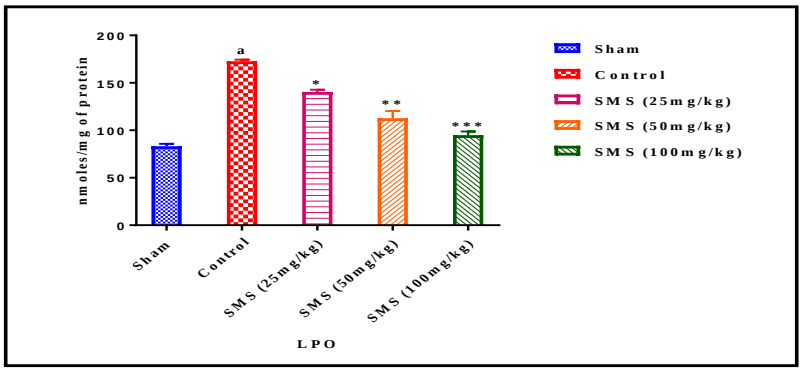

Figure 4 LPO

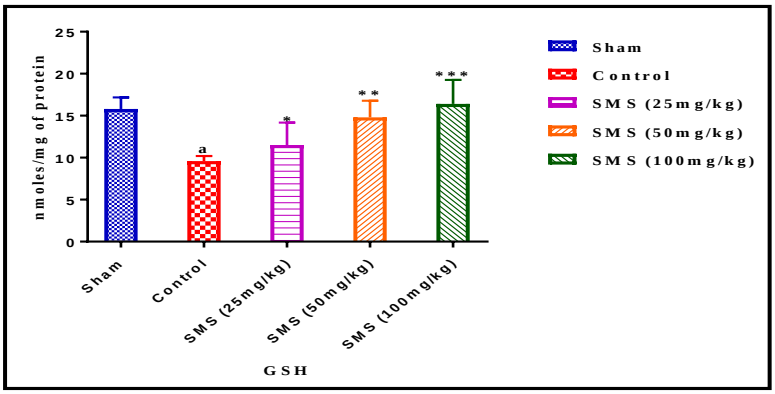

Figure 5 GSH

Fig. 4 & Fig. 5. Histograms showing effect of SMS on LPO and GSH levels against colchicine induced AD. All the values are expressed as mean±SEM, n=6, [a]p<0.001, as compared to sham group and *p<0.05, **p<0.01, **p<0.001, (One way Analysis of Variance [ANOVA] followed by Dunnett's test for multiple comparisons) as compared to control group.

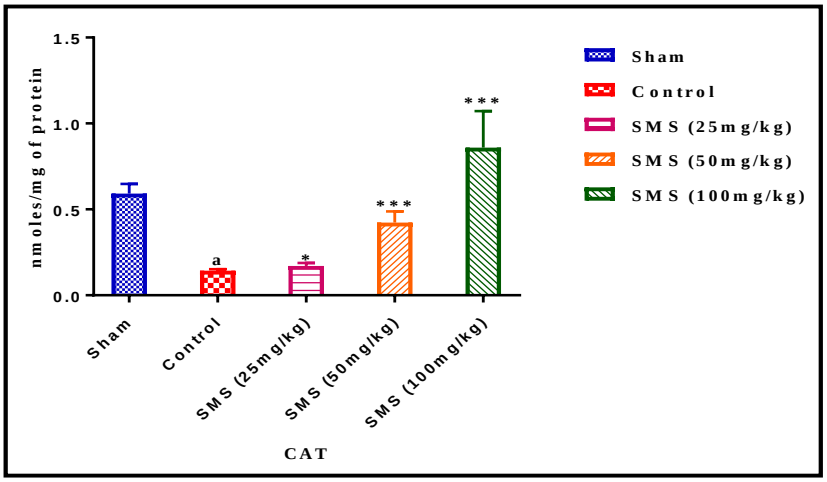

Figure 6 CAT

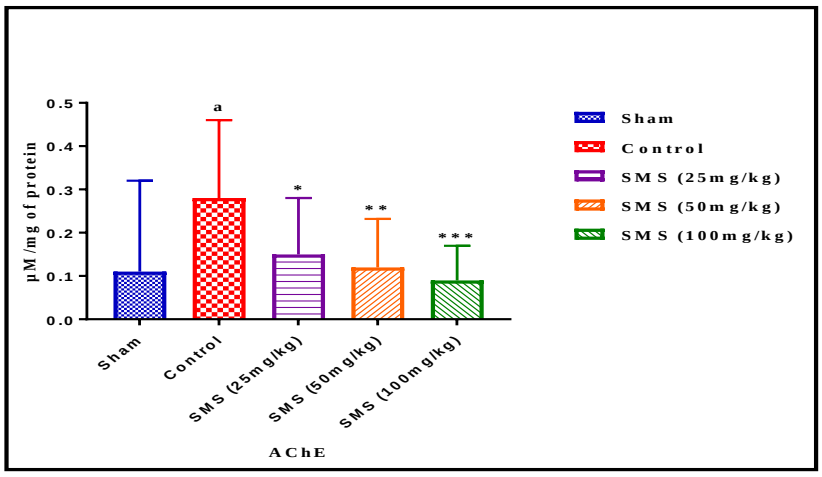

Figure 7 AchE

Fig. 6 & 7. Histograms showing effect of SMS on CAT and AChE levels against colchicine induced AD. All the values are expressed as mean±SEM, n=6, [a]p<0.001, as compared to sham group and *p<0.05, **p<0.01, **p<0.001, (One way Analysis of Variance [ANOVA] followed by Dunnett's test for multiple comparison) as compared to control group.

CONCLUSION

Colchicine is an alkaloid phytochemical compound from lily family plants. Colchicines has potent drug that caused dementia by inactivating cholinergic neurons at cholinergic site and inhibited reversibally[19]. Colchicine is also used as a neurotoxin which leads to destration of hippocampus it causes degeneration of tau protein by binding to tubulin, which directly increases AChE activity that causes i.e., learning impairment and memory loss. The colchicines already reported that was cause cell death due administration by Intracerebroventricular also cognitive impairment[20]. Colchicines also cause oxidative stress and increases free radical generation[21]. The ICV administration of colchicines (15µg) which can cause inactivation and decreased memory in both MWM and EPM paradigms[22]. The reported colchicines also significantly cause impairment of memory by estimated the decreased GSH, CAT enzyme levels because increase in the free radical generation[23] but the levels of these enzymes was restored by the administration of SMS in graded doses. . The LPO level was increased because of increased in MDA levels which are responsible for the oxidative damage in rats[24] but this enzyme level was successfully decreased by oral administration of SMS in graded dose. The major therapeutic target to treat AD is by decreasing acetyl cholinesterase activity.

Therefore, AChE inhibitors are the common choices of drugs in the management of AD which act by decreasing its break down rate hence increase the production and availability of Ach. Hence boost the cholinergic neurotransmission in forebrain regions and reduce the loss of brain cells. In current

investigation, Intracerebroventricular administration of colchicine caused significant increase in acetyl cholinesterase activity hence learning and memory defects occurred. This was overcome by the treatment of SMS in graded dose. As per the cholinergic hypothesis[25,26], the cognitive enhancement is mainly due to the development of procholinergic compounds. The research reports specify that, the use of anti-cholinesterase drugs enhance the acetylcholine concentration at synapse by inhibiting its metabolism hence these are the main class of therapeutics for the management of AD. Acute and chronic treatment with Anti-ChE drugs has demonstrated improved cognitive function in animal models[27]. In our study also, the drug has shown improved performance in behavioral tasks such as in elevated plus maze[28], Morris water maze[29]. This improvement in the memory by the Sumanas may be due to its effect on the biochemical parameters which was altered by colchicine.

The SMS has shown effective in prevention of cognitive impermanent and oxidative stress[30]. SMS has good antioxidant property acts as immunomodulatory by increase superoxide dismutase (SOD), glutathione peroxidase, and glutathione in experimental animal's model. SMS has decrease LPO level in hydrogen peroxide induced oxidative stress[31].

Hence it was confirmed that, the Anti- Alzheimer property of SMS was due to its memory enhancing property because it contains chief chemical constituents like alkaloids131,132,133, volatile oil134,135, saponin136, flavonoids137etc, these chemical constituents showed synergic effect, hence successfully act as memory enhancer in the management of animal models of AD.

CONCLUSION

In conclusion, the results of current study scientifically validate the traditional use of SMS as a suitable polyherbal formulation for the treatment of AD in animal models by improving cognitive impairment through memory enhancing property which was established by the estimation of various biochemical markers in chemical (colchicine and scopolamine) induced animal models employed. And further histological studies also confirmed the same. It also concluded that use of heavy metals (Al, Cu) can cause AD which is managed by SMS.

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