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ANTIAMNESIC ACTIVITY OF GUGGUL EXTRACT ON SCOPOLAMINE INDUCED AMNESIA IN MICE

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ABSTRACT

Objective of this study was to evaluate Guggul extract for the treatment of Alzheimer's disease using scopolamine induced amnesia in mice on Morris water maze. Guggul extract (50mg/kg) was administered orally for fifteen successive days followed by Scopolamine (0.4 mg/kg i.p.) from 15th to 18th day in mice. Morris water maze was employed to evaluate learning and memory using parameter like Escape Latency Time (ELT), Time Spent in Target Quadrant (TSTQ) and determination of brain Acetylcholinesterase level. Scopolamine was used to induce amnesia in mice and the activity was compared with standard drug Piracetam. Guggul extract significantly improved learning and memory in mice and reversed the scopolamine induced amnesia. Guggul extract when co administered with Piracetam (200mg/kg) has shown synergistic activity. Guggul extract is a known hypolipidemic agent and has shown excellent activity in scopolamine induced amnesia when given orally for 15 successive days in mice. It has shown synergistic effect with Piracetam and further detailed studies are required to exploit Guggul extract as new therapeutic agents for antialzheimer's disease.

Key words: Amnesia, Guggul extract, Morris water maze, Scopolamine.

INTRODUCTION

Alzheimer's disease is a neurodegenerative disorder of the central nervous system associated with progressive cognitive and memory loss. Alzheimer's disease produces an impairment of cognitive abilities that is gradual in onset but relentless in progression. Impairment of short-term memory usually is the first clinical feature, whereas retrieval of distant memories is preserved relatively well into the course of the disease. Alzheimer's disease is a devastating neurological disorder that affects more than million people worldwide.[1,2,3] Nootropic agents such as piracetam[4], pramiracetam, aniracetam[5] and choline esterase inhibitors like Donepezil are being primarily used to improve memory, mood and behavior. However, the resulting adverse effects associated with these agents have limited their use. [6,7] Therefore, it is worthwhile to explore the utility of traditional medicines for the treatment of various cognitive disorders. Gugulipid, an ethyl acetate extract of the resin of plant *Commiphora whighitii* is an established hypolipidemic agent in clinical practice. The major constituent of gugulipid is guggulsterone.[8] This study was undertaken to evaluate the potential benefits of Guggul extract alone and in combination with Piracetam using mice as animal model for the treatment of Alzheimer's disease.

MATERIALS AND METHODS

Animals and experimental design: Albino Mice (25-30gm) were procured from Animal House, JSS Medical College, Mysore, Karnataka, India. They were acclimatized for laboratory condition for 7 days and randomly divided into five groups each having six animals. The animals were housed under standard laboratory conditions and maintained under a 12-h light- dark cycle and had free access to drinking water and diet for one week. Institutional Animal

Ethical Committee approval was taken prior to the experiment (No. 035/2009).

Treatment:

Group I: Vehicle (5% Gum Acacia -1ml/kg body wt.) was administered orally to mice for fifteen successive days.

Group II: 5% Gum Acacia (1ml/kg body wt.) was administered orally for fifteen successive days followed by Scopolamine (0.4mg/kg i.p) after 45 min of administration daily from 15th day to 18th day.

Group III: Piracetam (200mg/kg i.p) was administered for fifteen successive days to mice. Scopolamine was injected i.p after 45 min of administration daily from 15th day to 18th day.

Group IV: Guggul extract (50mg/kg) was administered orally for fifteen successive days and Scopolamine was injected (i.p) after 45 min of administration daily from 15th day to 18th day.

Group V: Piracetam (200mg/kg) was administered i.p after 45 min administered Guggul extract (50mg/kg) for fifteen successive days to mice. Scopolamine was injected i.p after 45 min of administration daily from 15th day to 18th day.

Scopolamine induced amnesia: Each animal was subjected to four consecutive trials each day with a gap of 5 min for four consecutive days, during which they were allowed to escape on to the hidden platform and to remain there for 20 seconds. If the mouse failed to find the platform within 120 sec, it was guided gently on to the platform and allowed to remain there for 20 sec. Escape latency time (ELT) is defined as the time taken by the animal to locate the hidden platform was choosen as parameter for learning and memory. ELT was noted as an index of learning. On 18th day the platform was removed. Mouse was placed in water maze and allowed to explore the maze for 120 sec. Each mouse was subjected to four such trials and each trial was started from a different quadrant. Mean time spent in all the three quadrants i.e. Q1, Q2 and Q3 was recorded and the time spent in target quadrants (TSTQ) in search of the missing platform provided as an index of retrieval. Care was taken not to disturb the relative location of water maze with respect to other objects in the laboratory. [9]

Estimation of Brain AChE Activity: On the 18th day animals were euthanized by cervical dislocation carefully to avoid any injuries to the brain tissue. The whole brain AChE activity was measured using the Ellman method. [10] The end point was the formation of the yellow color because of the reaction of thiocholine with dithiobisnitrobenzoate ions. The rate of formation of thiocholine from acetylcholine iodide in the presence of tissue cholinesterase was measured using a spectrophotometer. The sample was first treated with 5, 5-dithionitrobenzoic acid (DTNB), and the optical density (OD) of the yellow colour compound formed during the reaction at 420 nm every minute for a period of 3 min was measured. Protein estimation was done using Auto analyzer (Micro lab 300). AChE activity was calculated using the following formula:

$$R = \frac{\delta \text{ O. D.} \times \text{Volume of Assay (3 ml)}}{E \times \text{mg of protein}}$$

Where R is the rate of enzyme activity in 'n' mole of acetylcholine iodide hydrolyzed per minute per mg of protein. δ OD is the change in absorbance per minute and E is the extinction coefficient, which is 13 600 M^{-1} cm^{-1}

Drugs and Chemicals: Scopolamine, 5, 5-dithionitrobenzoic acid (DTNB), Acetylcholine iodide were purchased from Sigma Aldrich. Piracetam was obtained as a gift sample from Torrent Pharmaceutical Ltd. Gujarat, India. Guggul extract was obtained as a gift sample from Chaitanya Agro Herbals. Mysore, India. Potassium dihydrogen phosphate and disodium hydrogen phosphate were purchased from RANKEM. Gum acacia was purchased from Laboratory of Apex chemicals, Bombay.

Data Analysis: All the results were expressed as Mean ±SEM. All the data were analyzed using ANOVA followed by Turkey multiple comparison test (Graph Pad Prism 5). P <0.001 was considered significant.

RESULTS

The antiamnesic effects of Guggul extract are presented in Table 1, 2 and 3. It was observed that when Scopolamine administered, it has significantly (P < 0.001) increased ELT value (32.5±1.83) as compared to the normal group (Table 1). When the Piracetam was administered for fifteen days at the dose of 200mg/kg, it has significantly (P < 0.001) decreased ELT value (17.5±0.61) as compared to the Scopolamine treated group. It was observed that administration of Guggul extract at the dose of 50 mg/kg resulted in a significant decreased ELT value (18.8±0.70) as compared to the Scopolamine treated group. It has shown effect similar to that of Piracetam. Administration of Guggul extract in combination with Piracetam resulted in a significant (P < 0.001) decreased ELT value (15.4±0.68) as compared to the Piracetam group and Scopolamine group. It showed that, when the Guggul extract co-

administered with Piracetam, produces synergistic effect.

It was observed that administration of Scopolamine resulted in a significant ($P < 0.001$) decreased TSTQ value (30.4±0.89) as compared to the normal group (Fig 2). Chronic administration of Piracetam for fifteen days at the dose of 200mg/kg, has resulted in significant increased TSTQ value (59.4±0.86) as compared to the Scopolamine group. Guggul extract resulted in a significant ($P < 0.001$) increase in TSTQ (58.5±1.47) as compared to the Scopolamine treated group (Table 2). This shows that, the Guggul extract has potent antiamnesic activity similar to that of Piracetam. Co-administration of Guggul extract in combination with Piracetam resulted in a significant ($P < 0.001$) increase TSTQ (61.5±0.71) as compared to the Scopolamine group. This shows the synergistic effect and the activity was more than the Piracetam treated group (59.4±0.86). Guggul extract has synergism with Piracetam.

In this study we have determined the level of AChE in the whole brain homogenate of all group animals, which was used to assess the nootropic activity (Table 3). It was observed that administration of Scopolamine resulted in a significantly ($P < 0.001$) increased AChE value s(205±4.19) as compared to the normal group (Fig 4). When the Piracetam was administered at the dose of 200mg/kg, it has significantly decreased AChE value (130±0.77) as compared to the Scopolamine treated group. The activity of AChE after administration of Guggul extract at the dose of 50 mg/kg has resulted in a significantly decreased AChE value (123±1.12) as compared to the Scopolamine treated group. Guggul extract in combination with Piracetam resulted in a significant decreased AChE value (127±1.15) as compared to Scopolamine group. The activity was found to be more than the Piracetam treated animals; which shows the synergistic effect with Piracetam.

Guggul extract has potent nootropic activity as that of Piracetam on scopolamine induced amnesia in mice and also shows synergistic effect.

DISCUSSION

Dementia is a clinical syndrome characterized by the development of multiple cognitive defects that are severe enough to interfere with daily social and professional functioning. [11] Alzheimer's disease related dementias are neurodegenerative conditions characterized by progressive brain dysfunction occurring in a step-wise biologic sequence: neuronal injury, synaptic failure and neuronal death. Neurofibrillary tangles, amyloid plaques and degeneration of cholinergic neurons are the pathological hallmarks of AD. [12] To improve cholinergic transmission, different strategies are adopted, including increase of Ach synthesis, the augmentation of pre synaptic Ach release, another stimulation of cholinergic post synaptic muscarinic and nicotinic receptors and the inhibition of Ach synaptic degradation by employing cholinesterase inhibitors. [13] Despite the availability of various treatment strategies, the severity and prevalence of this disease is not yet under control. Therefore, alternative and complementary medicines including herbal supplements are being utilized in the management of this disease. [14,15]

The nootropic effect of Guggul extract was assessed by using *in vivo* model of Scopolamine induced amnesia in mice by Morris water maze. Scopolamine produces amnesia by blocking the muscarinic acetylcholine receptors in the brain. [16] Scopolamine induced amnesia is a well accepted models of amnesia and it has been used by researcher for screening the drug candidates for the treatment of dementia or Alzheimer's disease. [17] In this study we have evaluated well known phytomedicine Guggul extract for possible application for the treatment of Alzheimer's disease.

It was observed that Scopolamine treatment resulted in a significant ($P < 0.001$) decreased ELT (8.1%) (Fig 1) as compared to the normal group (40.5 %). When animals were treated with Piracetam at the dose of 200mg/kg, it has produced increased (31.3%) ELT as compared to the Scopolamine treated group (8.1%). Guggul extract at the dose of 50 mg/kg resulted in increased (23.2%) activity as compared to the scopolamine treated group in ELT. The % of activity was more in the animals treated with combination of Guggul extract with Piracetam. The activity in ELT was found to be 45.9 %, which is more when compare to the Piracetam (31.3%) or Guggul (23.2%) alone treated groups. This study clearly shows that, Guggul extract has synergistic affect (ELT) with Piracetam in Scopolamine induced amnesia in mice. The Guggul extract has shown improvement in learning and memory in mice.

The mice administered Scopolamine pre-treated with vehicle has shown amnesic activity by decreasing the Time Spent in Target Quadrant (Table 2). TSTQ was less in amnesic animals when compared with treated groups. It was observed that administration of scopolamine resulted in a significant ($P < 0.001$) decreased TSTQ value (30.4±0.89) as compared to the normal group (68.4±0.48) The animals pretreated with Piracetam at the dose of 200mg/kg, has significantly increased TSTQ value (59.4±0.86) as compared to the scopolamine group. It was observed that administration of Guggul extract resulted in significantly ($P < 0.001$) increased TSTQ value (58.5±1.47) as compared to the scopolamine treated

group. Administration of Guggul extract in combination with Piracetam has produced more TSTQ (61.5±0.71) as compared to the Piracetam and scopolamine treated group. The study showed that, when the Guggul extract co-administered with Piracetam, it produces synergistic effect.

The brain acetylcholine level is responsible for memory [18] and level of acetylcholine is depends on the activity of metabolizing enzyme acetylcholine esterase (AChE). The brain acetylcholine level determines the memory functions and Acetylcholine Esterase enzyme are responsible for deactivating brain Ach. [19] More the AChE activity less will be the Ach level, in term less will be the memory. In this context, the AChE activity was measured in all group animals to know the level of acetylcholine.

There will be depletion of level of Ach in animals treated with scopolamine, the same thing was revealed when scopolamine administered at the dose of 0.4 mg/kg (Table 3). It was observed that administration of scopolamine resulted in a significant increased AChE value (205±4.19) as compared to the normal group. When the animals were pretreated with Piracetam before the challenge of scopolamine, has significantly (P < 0.001) decreased AChE value (130±0.77) as compared to the scopolamine group. The Guggul extract at the dose of 50 mg/kg has resulted in a significant decreased AChE value (123±1.12) as compared to the Scopolamine treated group. Administration of Guggul extract in combination with Piracetam resulted in a significant decreased AChE value (127±1.15) as compared to scopolamine group. It showed when the Guggul extract co-administered with Piracetam it produces synergistic effect.

The major components of Guggul extract include Guggulipid and Guggul sterone. These ingredients exhibit several pharmacological properties. Gugulipid as an anti dementia property and it is a cognitive enhancer. [8]

CONCLUSION

When Guggul extract was given orally for fifteen successive days before the challenge of Scopolamine on 15th to 18th day, showed good antiamnesic activity. Guggul extract is a hypolipidemic agent and can be exploited as antialzheimer's agent and also shows significant synergistic effect when co-administered with Piracetam.

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Figure 1. Effect of Guggul extract and in combination with Piracetam on Scopolamine induced amnesia in mice.

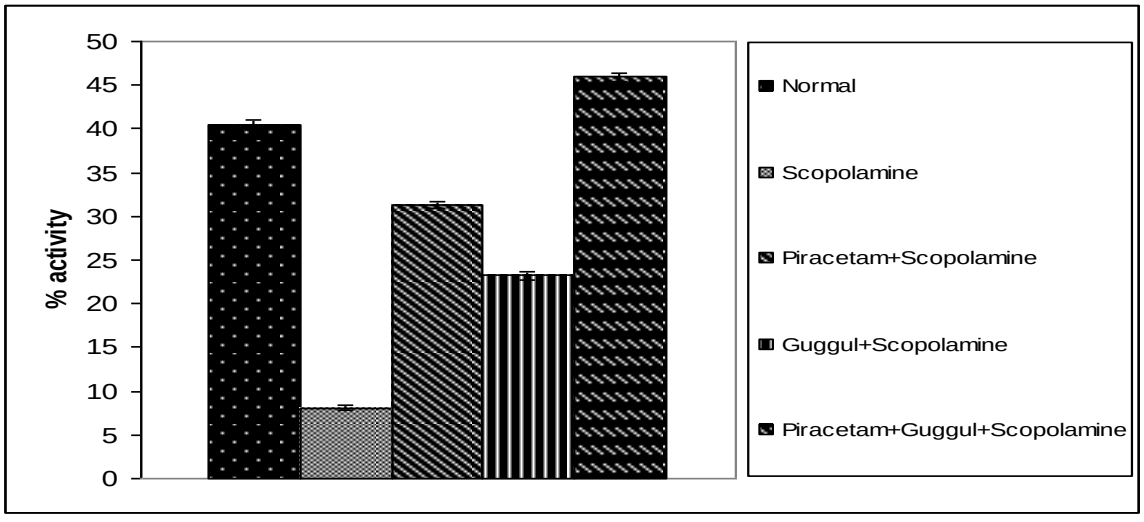

Figure 2. Effect of Guggul extract and in combination with Piracetam on Scopolamine induced amnesia in mice.
(Time Spent in Target Quadrant in second)

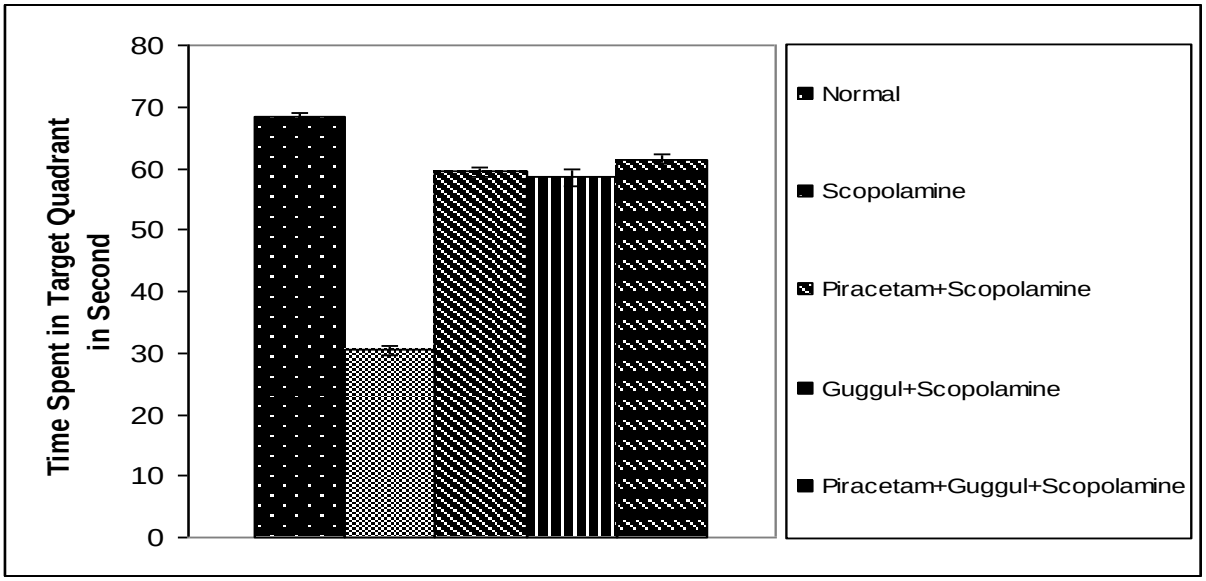

Figure 3. Effect of Guggul extract and in combination with Piracetam on Scopolamine induced amnesia in mice.
(AChE activity in µ mol)

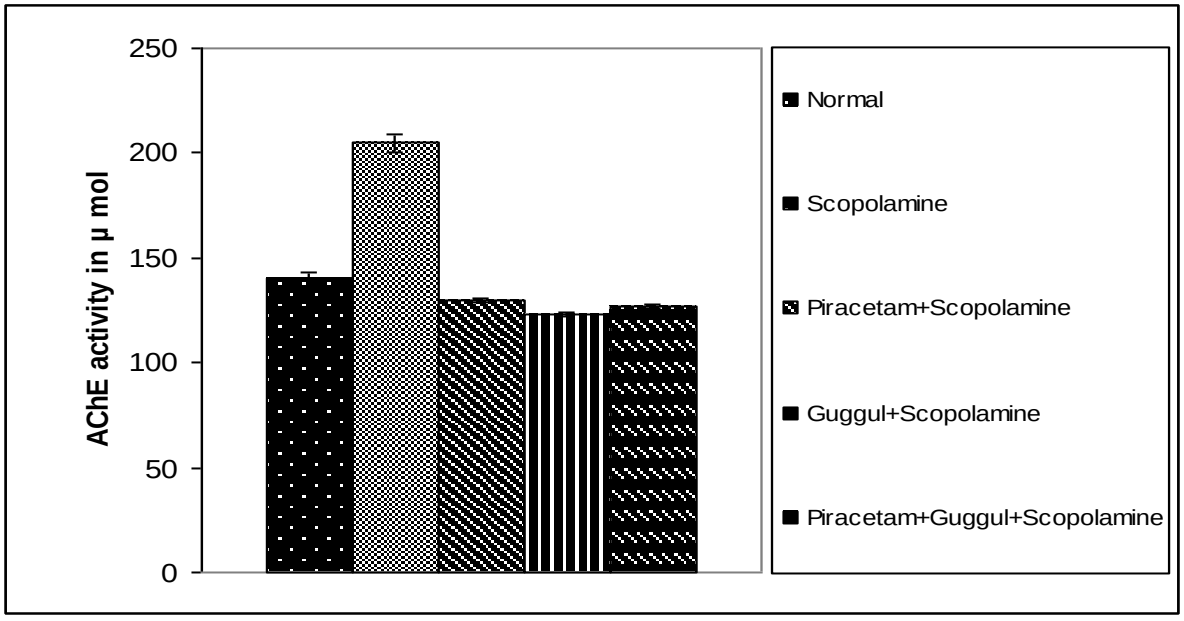

Table 1. Effect of Guggul extract and in combination with Piracetam on Scopolamine induced amnesia in mice using Morris water maze. (Escape Latency Time in Second)

SI No.	Group	Before Scopolamine Treatment (In Second)	After Scopolamine Treatment (In Second)
1	Normal	39.5±0.94	23.5±1.00
2	Scopolamine	35.4±1.35	32.5±1.83[a]
3	Piracetam+Scopolamine	25.5±0.85	17.5±0.61[b]
4	Guggul+Scopolamine	24.5±0.56	18.8±0.70[b]
5	Piracetam+Guggul+Scopolamine	28.5±0.84	15.4±0.68[b]

Values are expressed as Mean±SEM, n=6

Significant [b]P < 0.001 compared with Scopolamine treated group.

Significant [a]P < 0.001 compared with normal group animals.

Table 2. Effect of Guggul extract and in combination with Piracetam on Scopolamine induced amnesia in mice using Morris water maze. (Time Spent in Target Quadrant in second)

SI No.	Group	TSTQ (In Second)
1	Normal	68.4±0.48
2	Scopolamine	30.4±0.89[a]
3	Piracetam+Scopolamine	59.4±0.86[b]
4	Guggul+Scopolamine	58.5±1.47[b]
5	Piracetam+Guggul+Scopolamine	61.5±0.71[b]

Values are expressed as Mean±SEM, n=6

Significant [b]P < 0.001 compared with Scopolamine treated group.

Significant [a]P < 0.001 compared with normal group animals.

Table 3. Effect of Guggul extract and in combination with Piracetam on Scopolamine induced amnesia in mice using Morris water maze. (AChE activity in µ mol).

SI No.	Group	AChE activity (µ mol)
1	Normal	140±3.39
2	Scopolamine	205±4.19[a]
3	Piracetam+Scopolamine	130±0.77[b]
4	Guggul+Scopolamine	123±1.12[b]
5	Piracetam+Guggul+Scopolamine	127±1.15[b]

Values are expressed as Mean±SEM, n=6

. Significant [b]P < 0.001 compared with Scopolamine treated group.

Significant [a]P < 0.001 compared with normal group animals

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