ORIGINAL ARTICLE

Psychopharmacological investigations on the benefits of *Ageratum conyzoides* in the modulation of neurodegenerative disorder of Alzheimer's type

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Alzheimer's disease is a neurodegenerative disease of the central nervous system that leads to dementia, behavioural and cognitive impairments. The study was aimed to investigate the effect of *Ageratum conyzoides* (AC) on memory and whole brain acetylcholinesterase activity in mice by employing the exteroceptive behavioural models like elevated plus maze and Morris water maze and interoceptive behavioural models like scopolamine and natural ageing inducing amnesia. AC (250, 500 and 750 mg/kg, orally p.o.) produced a dose-dependent improvement in learning capability and retention memory of both young and aged mice. Furthermore, it also reversed the scopolamine (0.4 mg/kg, i.p.) and natural ageing-induced amnesia in young and old mice respectively. One more important thing about AC is that it increases the acetylcholine indirectly by reducing the whole brain acetylcholinestrease activity. Hence AC may have a beneficial effect in the management of neurodegenerative disorder of Alzheimer's type.

Key words: Acetylcholinestrease, aged mice, *ageratum conyzoides*, scopolamine

INTRODUCTION

Alzheimer's disease, the most common form of dementia, is characterized by memory impairment, cognitive decline and impairment of functions such as memory and language. By the year 2050, an estimated 30% of the population in Western Europe will be above the age of 65 years and up to 10% will have Alzheimer's disease (AD). The annual cost of an AD patient care is estimated to be US $ 35,000. This means that the overall cost of the care for these patients exceeds that for cancer and heart disease together.[1] Epidemiological studies have revealed that between two-thirds of aged population across the world are affected by cognitive decline.[2,3]

Age, stress and emotions are conditions that may lead to loss of memory, amnesia, anxiety, dementia, or to more ominous threats like AD.[4] Natural ageing is one of the factors which deteriorate memory in humans. However, oxygen free radicals, the harmful byproducts of oxidative metabolism, lead to organic damage to the brain as the brain is highly vulnerable to oxidative stress due to relatively high rate of oxygen free radical generation which may be responsible to the development of dementia or AD.[5,6]

So reducing oxidative stress by anti-oxidants, protecting brain inflammatory lesions using anti-inflammatory drugs and facilitation of brain cholinergic neurotransmission with anti-cholinesterase are some positive approaches to management of AD.[7] A number of herbs traditionally employed in the Indian System of Medicine 'Ayurveda' have yielded positive results.

In many developing nations of the world, a large number of people still rely heavily on traditional healers and medicinal plants to meet their daily primary healthcare needs.[8] *Ageratum conyzoides* Linn. (AC) is an annual herbaceous plant that belongs to the family of Asteraceae, with a long history of traditional medicinal use in folk remedies including treatment of ulcers, febrifuge, colic, anti-inflammatory, analgesic, etc. and in some African countries the plant is used for treatment of infectious and mental diseases too.[9,10] Simultaneously *Ageratum conyzoides* is proven scientifically for its anti-inflammatory, antioxidant, gastroprotective and hypoglycaemic and antihyperglycaemic activities.[11-13] The plants with anti-inflammatory and antioxidants show a protective effect against cognitive disorders. Hence the current study was undertaken to investigate the effect of AC in memory deficits-associated dementia

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of AD and whole brain acetylcholinestrease (AchE) activity by employing both exteroceptive and interoceptive behavioural models in mice.

MATERIALS AND METHODS

Animals

All the experiments were carried out using male, Swiss albino mice procured from Bioneeds laboratory animals and preclinical services, Bangalore, Karnataka, India. Young (3-4 month old) mice weighing between 20 and 22 g and aged (12- to 15-month-old) mice weighing between 30 and 35 g were used in the present study. The animals had free access to standard food pellets and water, and they were housed in a natural (12 hours each) light-dark cycle. The animals were acclimatized for 5 days to the laboratory conditions before conducting behavioural experiments. Experiments were carried out between 09:00 hours and 18:00 hours. The experimental protocol was approved by the Institutional Animal Ethics Committee (SETCP/IAEC/2008-2009/242) and the care of laboratory animals was taken as per the guidelines of CPCSEA.

Drugs and Chemicals

Scopolamine hydro bromide obtained from Sigma Aldrich, Ltd., Louis, MO, USA. PiracetamNootrpil®, UCB India Pvt. Ltd., Vapi, Gujarat, DTNB (5,5-dithiobis-2-nitro benzoic acid) and acetylcholine iodide (Hi-Media, India). All other chemical substances used were of analytical grade.

Plant Material

The potential Indian medicinal plant *Ageratum conyzoides* was collected from Khanapur forests and authenticated by qualified taxonomist, Dr. S. S. Hebbar, Department of Botany, Karnataka University Dharwad (KUD) Dahrwad. A voucher specimen No. SETCPD/Ph.cog/herb/2008/10 is available in our department for further reference.

Preparation of Extracts

Ageratum conyzoide was collected during July-August and properly washed with running tap water, rinsed with distilled water and kept for shade drying. The whole plant was powdered and exhaustively extracted with 95% ethanol using a Soxahlet apparatus. It was then concentrated in vacuo to a thick syrup consistency and kept in a freezer. The percentage yield was found to be 16%. Fresh dilutions of extract were made with 2% 'tween-80 in 0.9% NaCl) and utilized for the experiment. Then extract of *Ageratum conyzoides* was subjected to qualitative chemical tests to detect the presence of various phytoconstituents.[14]

Acute Toxicity Study

Healthy Swiss albino mice were starved overnight were divided into six groups (n=6) and were orally fed with the ethanol extract of *Ageratum conyzoides* in increasing dose levels of 100, 500, 1000, 3000, 6000 and 10,000 mg/kg body weight.[15] The mice were observed continuously for 2 hours for behavioural, neurological and autonomic profiles and after a period of 24 and 72 hours for any lethality or death.[16]

Experimental Protocols

Elevated plus maze protocol

The elevated plus maze (EPM) model was used in plane young mice [Table 1], scopolamine-induced amnesia of young mice [Table 2] and age-induced amnesia of aged mice [Table 3]. The elevated plus maze served as the exteroceptive behavioural model (wherein the stimulus existed outside the body) to evaluate learning and memory in mice. The apparatus consisted of two open arms (16 cm × 5 cm) and two covered arms (16 cm × 5 cm × 12 cm). The arms extended from a central platform (5 cm × 5 cm), and maze was elevated to a height of 25 cm from the floor. Normal control groups received only 0.9% NaCl of 10 ml/kg,p.o. Vehicle control groups received a 2% Tween-80 at a dose of 10 ml/kg,p.o. Standard (Piracetam) control groups received daily

Table 1: Effect of *Ageratum conyzoides* on transfer latency time of elevated plus maze in normal young mice

Group	Treatment	Dose (mg/kg)	TLT (16th day)	TLT (17th day)
I	Normal control	10 ml/kg, p.o.	25.57±0.5	22.19±0.8
II	Vehicle control	10 ml/kg, p.o.	23.79±1.2	20.94±0.9
III	Piracetam (std)	400 mg/kg, i.p.	16.64±1.1***	15.62±1.1***
IV	ACEt	250 mg/kg, p.o.	20.41±0.9*	18.88±0.9*
V	ACEt	500 mg/kg, p.o.	18.98±0.1**	17.61±1.4**
VI	ACEt	750 mg/kg, p.o.	17.64±1.5***	16.56±1.2***

Each group comprised six animals. Each value represents mean±S.E.M. of sex mice. P>0.05 is considered as Non significant. ***P<0.001, **P<0.01, *P<0.05 compared to normal control. One-way ANOVA followed by Tukey's post-test; TLT – Transfer latency time

Table 2: Reversal of scopolamine-induced amnesia by *Ageratum conyzoides* of EPM model in young mice

Group	Treatment	Dose (mg/kg)	TLT (16th day)	TLT (17th day)
I	Normal control	10 ml/kg, p.o.	25.57±0.5	22.19±0.8
II	Vehicle control	10 ml/kg, p.o.	23.79±1.2	20.94±0.9
III	Scopolamine control	0.4 mg/kg, i.p.	40.40±1.3###	39.29±0.8###
IV	Piracetam+scop.	400 mg/kg, i.p.	19.92±1.4***	18.87±0.8***
V	ACEt+scop.	250 mg/kg, p.o.	23.05±0.7***	21.02±1.0***
VI	ACEt+scop.	500 mg/kg, p.o.	22.18±0.7***	20.37±0.4***
VII	ACEt+scop.	750 ml/kg, p.o.	21.40±1.0***	18.69±0.7***

Each group comprised six animals. Each value represents mean±S.E.M. ***P<0.001 compared to normal control. ###P<0.001 compared to the normal control group. One-way ANOVA followed by Tukey's post-test; EPM – Elevated plus maze; TLT – Transfer latency time;

administration of Piracetam (400 mg/kg,i.p.) for consecutive 15 days. The *Ageratum conyzoides* Ethanolic extract (ACEt) groups received daily ACEt at a dose of 250, 500 and 750 mg/kg,p.o. for 15 consecutive days. Scopolamine was administered to respective groups on the 16th day at a dose of 0.4 mg/ kg,i.p. after 90 minutes of last dose of ACEt; then mice were exposed to training session after 45 minutes of scopolamine induction to measure the transfer latency time (TLT) as an indication of learning,for which each mouse was placed at the end of open arm, facing away from the central platform. TLT was taken as the time taken by the mouse to move into one of the covered arm with all its four legs. If the animal did not enter into one of the covered arm within 90 seconds, it was gently pushed into one of the two covered arms and the TLT was assigned as 90 seconds. The mouse was allowed to explore the maze for 10 seconds and then returned to its home cage. Memory retention was examined 24 hours, i.e. on the 17th day.[17]

Acetylcholinestrease activity in the aged mice protocol

Normal young control groups received only 0.9% NaCl at a dose of 10 ml/kg,p.o. The aged control group also received only 0.9% NaCl at a dose of 10 ml/kg,p.o. The aged vehicle control group received a 2% Tween-80 at a dose of 10 ml/kg,p.o.The aged+standard (Piracetam) control group received daily administration of Piracetam (400 mg/kg,i.p.). The aged+*Ageratum conyzoides* ethanolic extract (ACEt) group received daily administration of ACEt at a dose of 250, 500 and 750 mg/kg,p.o. for consecutive 15 days. All the above-said treatment was given for 15 consecutive days. On the 16th day after 90 minutes of the treatment with all the drugs, the animals were killed by cervical dislocation carefully to avoid any injuries to the tissue. The whole brain was removed and Acetylcholinestrease (AChE) activity was measured using the Ellman method.[18] This was measured on the basis of the formation of yellow colour due to 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 412 nm every minute for a period of 3 minutes was measured. Protein estimation was done using Folin's method. AChE activity was calculated using the following formula:

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

$$E \times \text{mg of protein}$$

where R is the rate of enzyme activity in 'n' mole of acetylcholine iodide hydrolyzed/min/mg protein, δO.D. is the change in absorbance/min and$E$is theextinction coefficient (=13600/M/cm).

Table 3: Reversal of age-induced amnesia by *Ageratum conyzoides* of EPM model in aged mice

Group	Treatment	Dose (mg/kg)	TLT (16th day)	TLT (17th day)
I	Young control	10 ml/kg, p.o.	25.57±0.5	22.19±1.9
I	Aged control	10 ml/kg, p.o.	35.53±1.1###	33.27±0.9###
III	Aged vehicle control	10 ml/kg, p.o.	34.92±0.7	32.83±0.8
IV	Piracetam+aged	400 mg/kg, i.p.	21.82±0.7***	17.52±1.1***
V	ACEt+aged	250 mg/kg, p.o.	23.35±1.4***	17.34±0.9***
VI	ACEt+aged	500 mg/kg, p.o.	23.22±1.7***	16.97±0.9***
VII	ACEt+aged	750 ml/kg, p.o.	22.24±0.5***	16.40±0.8***

Each group comprised six animals. Each value represents mean±S.E.M. ***P<0.001 compared to aged control. ###P<0.001 compared to the young control group. One-way ANOVA followed by Tukey's post-test ; EPM – Elevated plus maze; TLT – Transfer latency time

Morris water maze experimental protocol

The Morris water maze (MWM) model was used in plane young mice [Table 4] and scopolamine-induced amnesia of young mice [Table 5]. Normal control groups received only 0.9% NaCl of 10 ml/kg,p.o. Vehicle control groups received a 2% 'tween-80 at a dose of 10 ml/kg,p.o. Standard (Piracetam) control groups received daily administration of Piracetam (400 mg/kg,i.p.) for consecutive 20 days. The *Ageratum conyzoide* sethanolic extract (ACEt) groups received daily administration of ACEt at doses of 250, 500 and 750 mg/kg,p.o. for 20 consecutive days. In MWM amnesia was induced to young mice by scopolamine injection at a dose of 0.4 mg/kg b.w., i.p., after 90-minute administration of last dose of test extract and standard drug administration

Table 4: Effect of *Ageratum conyzoides* on escape latency time and TSTQ of MWM in normal young mice

Group	Treatment	Dose (mg/kg)	ELT (21st day)	ELT (24th day)	TSTQ (25th day)
I	Normal control	10 ml/kg, p.o.	89.06±2.6	40.23±1.1	61.01±0.9
II	Vehicle control	10 ml/kg, p.o.	86.83±2.1	38.89±0.7	60.77±0.7
III	Piracetam (std)	400 mg/kg, i.p.	74.10±1.9**	33.09±2.4**	72.94±2.1**
IV	ACEt	250 mg/kg, p.o.	80.35±1.5	37.30±2.4	57.89±3.5
V	ACEt	500 mg/kg, p.o.	75.94±3.7*	35.51±1.0	60.53±2.2
VI	ACEt	750 mg/kg, p.o.	76.34±3.6*	32.52±0.9**	70.40±2.0*

Each group comprised six animals. Each value represents mean±S.E.M. **P<0.05 compare to normal control; ***P<0.001 compared to normal control. One-way ANOVA followed by Tukey's post-test ; TSTQ – Time spent in target quadrant; MWM – Morris water maze; ELT – Escape latency time

Table 5: Reversal of scopolamine-induced amnesia by *Ageratum conyzoides* of ELT and TSTQ in young mice

Group	Treatment	Dose (mg/kg)	ELT (21st day)	ELT (24th day)	TSTQ (25th day)
I	Normal control	10 ml/kg, p.o.	89.06±2.6	40.23±1.1	61.01±0.9
II	Vehicle control	10 ml/kg, p.o.	86.83±2.1	38.89±0.7	60.77±0.7
III	Scopolamine	0.4 mg/kg, i.p.	111.30±4.1^{++}	97.48±4.5^{+++}	32.44±2.4^{+++}
IV	Piracetam+scop.	400 mg/kg, i.p.	91.87±4.2**	61.09±1.6***	56.03±1.2$^{###}$
V	ACEt+scop.	250 mg/kg, p.o.	94.89±3.4*	60.09±1.3***	52.42±1.6$^{###}$
VI	ACEt+scop.	500 mg/kg, p.o.	93.05±4.5*	60.56±1.8***	54.86±2.3$^{###}$
VII	ACEt+scop.	750 mg/kg, p.o.	88.11±1.9***	60.27±1.7***	57.21±2.5$^{###}$

Each group comprised six animals. Each value represents mean±S.E.M. $^{*}P<0.05$ compare to normal control. $^{***}P<0.001$compared to scopolamine control. $^{###}P<0.001$ compared to the scopolamine control group. $^{+++}P<0.001$ compared to the normal control group. One-way ANOVA followed by Tukey's post-test ; ELT – Escape latency time; TSTQ – Time spent in target quadrant

on 21st and 24th days. Then after 45 minutes of scopolamine injection animals were exposed to training session. During the training session (25th to 24th day) the ACEt and piracetam administration were continued. The retention memory was measured after 24 hours later (on the 25th day), by measuring the time spent in target quadrant (TSTQ) as an index of retrieval memory without giving any kind of drug to any one of groups of animals.

The MWM test was employed to assess learning and memory of the animals. MWM is a swimming model where the animals learn to escape on to a hidden platform. It consisted of a circular water tank (150 cm diameter, 45 cm height), filled with water (30 cm depth) maintained at 25°C. Water was made opaque with a white-coloured non-toxic dye. The tank was divided into four quadrants with the help of two threads, fixed at right angle to each other on the rim of the pool. A platform (10 cm^2) of 29 cm height was located in the centre of one of these four quadrants which one referred as the target quadrant. The position of the platform was kept unaltered throughout the training sessions. In the present study the target quadrant was Q4. Each animal was subjected to four consecutive trials on each day with a gap of 5 minutes for four consecutive days, during which they are allowed to escape onto the hidden platform and to remain there for 20 seconds. In case the animal was unable to locate the hidden platform within 120 seconds, it was gently guided to the platform and allowed to remain on the platform for 20 seconds. Escape latency time to locate the hidden platform in water maze was taken as an index of acquisition or learning. The starting position on each day to conduct four acquisition trials was changed as described below and Q4 was maintained as the target quadrant in all the acquisition trials. The starting point for dropping the mice into water maze on day 1 for four consecutive acquisition trials was in the sequence Q1, Q2, Q3 Q4 and so on. The sequence change in starting point was as follows.

Day 1: Q1, Q2, Q3, Q4
Day 2: Q2, Q3, Q4, Q1
Day 3: Q3, Q4, Q1, Q2
Day 4: Q4, Q1, Q2, Q3

Mean escape latency time (ELT) was calculated for each day of the trial. On the fifth day the platform was removed, and each mouse was allowed to explore the pool for 120 seconds. The animal was subjected to four such trials with 5-minute interval time and each trial had a different starting point covering all the four quadrants. The mean time spent by animal in all four quadrants was recorded. The time spent in the target quadrants Q4 compared to time spent in other quadrants in search of missing platform was taken as an index of retrieval. Care was taken that the relative location of water maze with respect to other objects in laboratory serving as visual clues was not disturbed during the total duration of the study. All the trials were completed between 09:00 and 17:00 hours.

Statistical Analysis

The data were analysed statistically using analysis of variance (ANOVA) followed by Tukey's post-test. Values are expressed as mean±standard errors of mean (S.E.M). $P<0.05$ is considered as significant. Statistical comparisons were performed by Tukey's post-test using Graph Pad Prism version 4.0, USA.

RESULTS

Acute Toxicity Studies

All the doses of (100, 500, 1000, 3000, 6000 and 10,000 mg/kg) of AC employed for acute oral toxicity studies were found to be non-toxic. AC does not show a toxic effect even at high dose (10,000 mg/kg) per orally.

Photochemical screening

The freshly prepared ethanolic extract of *Ageratum conyzoides* was qualitatively tested for the presence of chemical constituents. These phytochemicals were identified by characteristic colour changes using standard procedures. The screening results show the presence of triterpenoids, alkaloids, flavonoids, tannins and phenolic compounds.

EPM exteroceptive behavioural model

Treatment with Piracetam (400 mg/kg, i.p.) for 15 days decreased TLT on 16th and 17th days compared to the normal

control group indicating improvement in both learning and retrieval memory respectively. There is no significant difference in TLT on 16th and 17th days in between vehicle control and normal control groups indicating that there is no influence of vehicle *per se* in learning and retrieval memory. Pretreatment with ACEt (250, 500 and 750 mg/kg orally) decreased the TLT on 16th and 17th days in normal young ($P<0.05$, $P<0.01$, $P<0.001$ respectively) when compared to control groups. Higher dose of ACEt (750 mg/kg, p.o., $P<0.001$) significantly enhanced learning and retrieval memory in normal young mice and its results are comparable to standard drug. The whole results are summarized in Table 1.

Scopolamine (0.4 mg/kg,i.p.) increases TLT significantly ($P<0.001$) in the scopolamine control group on the 16th day and 24 hours later, i.e. on the 17th day, which may be considered as interruption in learning and retention of memory compared to normal control. Pretreatment with Piracetam (400 mg/kg, i.p.) for 15 days decreased TLT on 16th and 17th days compared to the scopolamine group, indicating a reversal effect of amnesia concerned with both learning and memory. Pretreatment with ACEt (250, 500 and 750 mg/kg,p.o) also significantly decreased ($P<0.001$) the TLT on 16th and 17th days in young when compared to scopolamine control groups. Seventeenth-day TLT of Piracetam and ACEt shows more prominent decreasing in TLT than the 16th-day TLT. The detailed results are summarized in Table 2.

In age-induced amnesiac model, especially the aged control group shows a significant ($P< 0.001$) increase in TLT when compared with young control mice indicating that the natural ageing process may interfere with learning and memory. Pretreatment with Piracetam (400 mg/kg, i.p.) for 15 days daily significantly decreases the TLT on 16th and 17th days compared to theaged control group, indicating a reversal effect of amnesia concerned with both learning and memory. Pretreatment with ACEt (250, 500 and 750 mg/ kg,p.o) also significantly decreased ($P<0.001$) the TLT on 16th and 17th days in aged mice when compared to the aged control group. But the aged vehicle control group did not show any significant ($P>0.05$) decrease in TLT when compared to the aged control group, indicating that there is no involvement of vehicle itself in memory and retention. However, with standard drug and ACEt extracts of different doses it showed a decrease in TLT on 16th and 17th days indicating that they reverse the amnesic effect induced by natural ageing. All the results are summarized in Table 3.

Acetylcholinestrease activity

In the present study, the aged group of animals shows significant ($P<0.001$) elevation in whole brain activity compared to the young group of mice. Piracetam (400 mg/kg, i.p.) and ACEt (250, 500 and 750 mg/kg,p.o) significantly ($P<0.001$) lowered this activity indicating the counteracting action of age-induced acetylcholinestease activity. This AchE-lowering capacity of standard and ACEt indirectly involved in the improvement of Ach is an important neurotransmitter involved in the cholinergic system of learning and memory. The results are represented in the Table 6.

MWM exteroceptive behavioural model

There was a significant fall in ELT of the standard drug- and ACEt-treated group compared to normal young control mice especially with ACEt 750 mg/kg,p.o. which shows learning ability (acquisition) of mice. The vehicle control group did not show any extra improvement in the learning significantly ($P>0.05$) indicatingthat there is no influence of vehicle in improvement of learning and retrieval memory. Further there is a significant rise in TSTQ (as a memory of retrieval) of standard drug and ACEt of 750 mg/kg on the 25th day compared to TSTQ of the normal control group of mice. Even though there is a decrease in ELT and an increase in TSTQ of ACEt of 250, 500 mg/kg, it is non-significant compared to the normal control group of mice. The detailed results are summarized in Table 4.

Scopolamine treatment in mice significantly increased ELT ($P<0.001$) and decreased TSTQ ($P<0.001$) during the learning/ acquisition trial. Pretreatment in the standard and ACEt groups at all the doses reverses the scopolamine-induced amnesia by decreasing the ELT ($P<0.001$) as an index of acquisition and increasing the TSTQ ($P<0.001$) as an index of retrieval memory compared to that in the scopolamine group. The detailed results of this model are represented in the Table 5.

DISCUSSION

AD is a progressive and fatal neurodegenerative disorder manifested by cognitive, memory detoriation, progressive impairment of routine activities of living, a variety of neuropsychiatric symptoms and behavioural disturbances.[19] Impairment of short-term memory usually is the first clinical feature, as the condition progresses, additional cognitive abilities are impaired, among them the ability to calculate, exercise Visio-spatial skills, and use common objects and

Table 6: Effect of *Ageratum conyzoides* on age-induced changes in whole brain AchE activity

Group	Treatment	Dose (mg/kg)	AchE (µ moles)
I	Young control	10 ml/kg, p.o.	130.80±1.3
II	Aged control	10 ml/kg, p.o.	173.0±2.6###
III	Aged vehicle control	10 ml/kg, p.o.	168.4±3.5
IV	Piracetam+aged	400 mg/kg, i.p.	138.9±3.9***
V	ACEt+aged	250 mg/kg, p.o.	136.9±5.2***
VI	ACEt+aged	500 mg/kg, p.o.	133.0±4.6***
VII	ACEt+aged	750 ml/kg, p.o.	129.7±6.2***

Each group comprised six animals. Each value represents mean±S.E.M. ***$P<0.001$ compared to aged control. ###$P<0.001$ compared to the young control group. One-way ANOVA followed by Tukey's post-test; AchE – Acetylcholinestrease

tools (ideomotor apraxia).[20] Acetylcholine is one of the most important neurotransmitter involved in the regulation of cognitive function. Defectiveness of acetylcholine in the cholinergic forebrain ultimately leads to dementia.[21] Memory impairments in patients with the senile dementia are due to a selective and irreversible deficiency in the cholinergic functions in the brain.[19] Hence there is extensive evidence indicating that decreased brain cholinesterase activity leads to improvement in memory.[4,19,22-25]

The epidemiological studies have almost confirmed that the non-steroidal anti-inflammatory drugs reduce the incidence of AD.[26,27] One more important factor which may lead to the development of the AD in elderly patients is oxygenfree-radicals.[28,29] Hence the use of acetylcholinesterase inhibitors, anti-oxidants and anti-inflammatory agents serves as the rationale in the treatment or management of the AD.

By observing the above results it seems that the AC treatment improves learning/acquisition and retention of memory by attenuating the TLT, ELT and TSTQ and AchE in behavioural models. Data from literature show the AC has got antioxidant and antiflammatory effects,[30] and phytochemical investigation shows the presence of triterpenoids, alkaloids, flavonoids, tannins and phenolic compounds.

By considering the fact of dementia, associated with increased brain oxidative stress during brain ageing, this memory-enhancing and retention power of the memory effect might be due to any one or combination of these phytochemicals.

Since ACEt exhibited cognitive improvement, it might be clinically useful in the control of age-related memory disorders like AD. A successive study is under process to establish the precise nature of active constituents as well as their mechanism of action.

CONCLUSION

Finally the authors suggest that ACEt effectively improves the learning ability and retentioning of memory along with increase in the acetylcholine indirectly by decreasing the AchE activity of whole brain. Hence it might be worthwhile to explore ACEt as a potential herbal medicine in the management of AD.

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