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Effect of *Convolvulus pluricaulis* Choisy. and *Asparagus racemosus* Willd on learning and memory in young and old mice: A comparative evaluation

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A dose dependent enhancement of memory was observed with *A. racemosus* and *C. pluricaulis* treatment as compared to control group when tested on second day. *A. racemosus* and *C. pluricaulis* at the dose of 200 mg/kg, po showed significantly higher percent retentions, than piracetam. Multiple treatment with *A. racemosus* and *C. pluricaulis* for three days also demonstrated significant dose dependent increase in percent retentions as compared to control group. The effect was more prominent with *C. pluricaulis* as compared with piracetam and *A. racemosus*. A significantly lower percent retention in aged mice was observed as compared to young mice. Aged mice (18-20 months) showed higher transfer latency (TL) values on first and second day (after 24 h) as compared to young mice, indicating impairment in learning and memory. Pretreatment with *A. racemosus* and *C. pluricaulis* for 7 days enhanced memory in aged mice, as significant increase in percent retention was observed. Significantly higher retention was observed with *C. pluricaulis* (200 mg/kg; po) as compared with piracetam (10 mg/kg/; po). Post-trial administration of *C.pluricaulis* and *A. racemosus* extract demonstrated significant decrease in latency time during retention trials. Hippocampal regions associated with the learning and memory functions showed dose dependent increase in AChE activity in CA 1 with *A. reacemosus* and CA3 area with *C. pluracaulis* treatment. The underlying mechanism of these actions of *A. racemosus* and *C. pluricaulis* may be attributed to their antioxidant, neuroprotective and cholinergic properties.

Keywords: *Asparagus racemosus, Convolvulus pluricaulis,* Elevated plus maze, Learning, Memory

A wide variety of clinical syndromes like head trauma, convulsive disorders and neurodegenerative diseases manifest cognitive or memory dysfunctions[1]. Cognitive and behavioural impairments are also shown by most common dementia in elderly. Although primary cause of these remains unclear, recent studies have suggested that free radicals produced during oxidative stress or inflammatory processes are pathologically important[2-4]. Currently the mainstays of pharmacological treatment for the cognitive deficits are donepezil, galantamine, rivastigmine and memantine-4, which are being primarily used to improve memory, mood and behavior[5]. However, the resulting adverse effects associated with these agents have limited their use[5,6]. Therefore, it is worthwhile to explore the utility of traditional medicines for the treatment of various cognitive disorders.

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Medhya rasayana are Ayurvedic preparations known to improve memory, intelligence[7,8]. Some of these rasayanas *Withania somnifera*[9], *Asparagus racemosus*[10], *Convolvulus pluricaulis*[11], *Tinospora cordifolia*[12] and *Centella asiatica*[13] are commonly prescribed by the Ayurvedic practitioners for treatment of cognitive disorders or improving learning and memory. However, there has not been sufficient investigation to establish the biological effects of these plants in improving cognitive deficits or memory disorders. Therefore it is necessary to evaluate the beneficial effects of these plants on learning and memory by means of proper animal learning model with various sample treatments. In present study, *A. racemosus Willd.* and *C. pluricaulis Choisy* were selected for evaluation of their effects on learning and memory in mice. Both plants possess antioxidant, free radical scavenging activity and neuroprotective activities[14,15]. Free radical reactions are implicated in memory deficit, neurodegenerative diseases and also in aging[2-4,16].

Present study has been undertaken to investigate the effects of the extracts of *A. racemosus* and *C. pluricaulis* on learning and memory in young and old mice with a specific aim to evaluate their possible efficacy in treatment of memory disorders.

Materials and Methods

Animals—Male Laka strain young (2 to 3 months old) and aged (18-20 months old) mice bred in Central Animal House facility of Punjab University, Chandigarh were used. The animals were housed under standard laboratory conditions maintained under a natural light and dark cycle, and had free access to food and water. Animals were acclimatized to laboratory conditions before the experiment. Each animal was used only once. All the experiments were carried out between 0900 and 1500 hrs. The experimental protocols were approved by the Institutional Animal Ethics Committee.

Preparation of plant extracts—A. racemosus roots were collected from Herbal garden of Maharana Partap Agriculture University, Udaipur. The roots were dried under shade, pulverized by a mechanical grinder and stored in a closed container for further use. The air dried and powdered material (500 g) was extracted with 90% v/v ethanol in a Soxhlet extractor for 35 h. The solvent was evaporated at low temperature under reduced pressure in a rotavapor, to obtain dry mass (29.22 g) completely free from the solvent.

C. pluricaulis was collected from Maharana Bhupal College ground of M L S University, Udaipur. Air-dried, coarsely powdered whole plant material (1 kg) of *C. pluricaulis* was imbibed in ethanol (95%) for 24 hr. This moistened drug was extracted with ethanol (95%) at 70°C using a Soxhlet apparatus for 48 h. The ethanolic extract was concentrated using vacuum and stored in a refrigerator. The yield of total ethanolic extract was 22.6%. Both extracts were subjected to phytochemical analysis as per protocols of Chhabra *et al*[17]. *A. racemosus* extract gave positive tests for polyphenols, flavanoids, tannins, saponins and glycosides and *C. pluricaulis* extract showed the presence of alkaloids (large quantity), a moderate quantity of tannins, and a small quantity of sugars. The semisolid extracts of both the plants were kept in refrigerator and dissolved in distilled water just before the administration.

Both the plants were authenticated by Professor K.G. Ramawat (Laboratory of Ethnobotanical Research, Department of Botany, Faculty of Science, M L S University, Udaipur, Rajasthan, India) through comparison with a voucher specimen present in the herbarium.

Drugs and chemicals—Piracetam (Sigma, USA) was diluted in normal saline and injected, ip, with dose volume of 1 ml/100 g. All the drugs were administered in the morning session (between 08.00-09.00 hrs) on each day.

Elevated plus maze—The elevated plus maze served as the exteroceptive behavioral model (wherein the stimulus existed outside the body) to evaluate learning and memory in mice[18]. 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 the maze was elevated to a height of 25 cm from the floor. On the first day, each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was taken as the time taken by the mouse to move into any one of the closed arms with all its four paws in. TL was recorded on the first day. If the mouse did not enter into one of the covered arms within 90 sec, it was gently pushed into one of the two covered arms and the TL was assigned as 90 sec. The mouse was allowed to explore the maze for 10 sec and then was returned to its home cage. Memory retention was examined 24 h after the first day trial on the second day.

Tissue preparation for microscopy—Control and treated mice treated were sacrificed by rapid decapitation. Brain was dissected out as rapidly as possible, rinsed briefly in cold distilled water and then placed in chilled 10% calcium formal for 16 h at 4°C. Next day, brain tissue was transferred to cold sucrose solution (10, 20 and 30% w/v) for 24 h in each. Tissues were rinsed briefly in cold distilled water and cryostat sectioning was done at 20µm thickness[12].

Preparation of incubation media and histochemical detection of acetylcholinestrase (AChE)—Method of Karnovasky and Roots [19] was used to study AChE. Acetylthiocholine iodide (5 mg) was dissolved in 6.5 ml of 0.1 *M* acetate buffer of *p*H 6.0, followed by 0.5 ml of 0.1 *M* sodium citrate (0.198/10 ml), 1.0 ml of 30 m*M* copper sulphate (75 mg/10 ml), 1.0 ml of distilled water and 1.0 ml of 5 m*M* potassium ferricyanide with stirring. The final incubation media was clear yellow green in color. Sections were placed in freshly prepared incubation media for 40-45 min until brown colour of reaction was developed. The

stained sections were rinsed briefly in distilled water and were mounted on albumin coated slides. Slides were air dried, and were passed in alcohol series for dehydration, cleared in xylene and mount in DPX for microscopy.

Experimental protocols

Mice were divided into two main groups pre-trial (A) and post trial (B) groups. In pre-trial group animals were again subdivided into acute study group (a), sub chronic study group (b) and chronic study group (c). The acute study group mice (a) were further divided in to six groups I to VI. Sub chronic study group mice were divided in to six groups VII to XII and chronic study group mice were divided in to 6 groups XIII to XVIII. In case of post trial group mice were divided in to six groups XIX to XXIV. Each group consisted of a minimum of five to seven animals. Separate animals were used for each experiment.

(A) Effect of pre-trial administration of plant extracts on learning and memory

(a) Acute study in young mice—Groups I: control group (n=8) received distilled water orally. Groups II: given Piracetam (10 mg /kg, ip) Group III, IV received *A. racemosus* (100 and 200 mg/ kg, po) and V and VI received *C. pluricaulis* (100 and 200 mg/ kg, po) respectively. In all groups TL was noted 60 min after drug administration and again after 24 h.

(b) Sub-chronic study in young mice—Groups VII: control group. Given DW orally for 3 days. Group VIII: Given Piracetam (10 mg/ kg, ip) for 3 days to young mice. Group IX, X received *A. racemosus* (100 and 200 mg/ kg, po) and XI and XII *C. pluricaulis* (100 and 200 mg/ kg, po) respectively for 3 days. The last dose was given 60 min before subjecting the animals to elevated plus maze test. TL was noted on the third day and again after 24 h in all groups.

(c) Chronic study in aged mice—Group XIII: control group for aged mice, given distilled water orally for 7 days. Group XIV: given Piracetam (10 mg /kg, ip) to aged mice for 7 days. Groups XV, XVI were given *A. racemosus* (100 and 200 mg/ kg, po) and XVII and XVIII *C. pluricaulis* (100 and 200 mg/ kg, po) for 7 days to aged mice. TL was noted in all groups after 60 min of administration on the 7th day and again after 24 h.

(B) Effect of post-trial administration of plant extracts on memory

Group XIX: control group for young mice, received distilled water orally immediately after noting first day TL. Group XX: given Piracetam (10 mg/kg. ip) immediately after noting first day TL. Group XXI, XXII were given *A. racemosus* (100 and 200 mg/ kg, po) and XXIII and XXIV *C. pluricaulis* (100 and 200 mg/kg, po) respectively, after noting first day TL. TL was again noted in all the groups after 24 h on second day.

Statistical analysis—All the values were expressed as mean ± SE. The data were analyzed by using ANOVA followed by Dunnett's test. Significance of the data were accepted at $P<0.05$.

Results

Effect of pre-trial administration of plant extracts on memory

A dose dependent enhancement of memory was observed with *A. racemosus* and *C. pluricaulis* ($P< 0.05$) as compared to the vehicle treated control group when tested on second day. *A. racemosus* (200mg/kg, po) and *C. pluricaulis* (200 mg/kg, po) showed significantly higher percent retentions (73.2 and 81.42% respectively) than piracetam, 48.7% ($P < 0.05$) (Fig.1).

Multiple treatment with *A. racemosus* and *C. pluricaulis* for three days demonstrated significant dose dependent increase in percent retentions as compared to control group $P< 0.05$), similar to that observed in Piracetam treated animals. The effect was more prominent with *C. pluricaulis* (84.2%) as compared with Piracetam (75.9%) (Fig. 2).

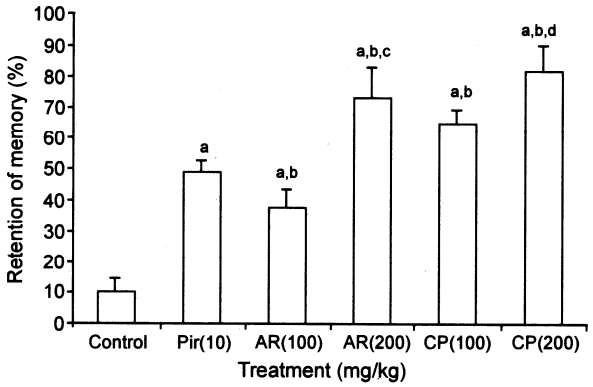

Fig. 1—Effect of single pretreatment of *C. pluricaulis* (CP; 100 and 200 mg/kg, po) or *A. racemosus*, (AR; 100 and 200 mg/kg,po) for one day, on percent retention of memory using elevated plus-maze paradigm Values are expressed as mean ± SE, ANOVA followed Dunett's test. *P* values: <0.5 as compared to [a]control (young mice), [b]piracetam, [c]*A. racemosus* (100 mg/kg, po), [d] *C. pluricaulis*(100 mg/kg, po).

A significant lower percent retention (3.7%) in aged mice was observed as compared to young mice (18.3%). Aged mice (18-20 months) showed higher transfer latency (TL) values on first day and on second day (after 24 h) as compared to young mice, indicating impairment in learning and memory. Pretreatment with *A. racemosus* and *C. pluricaulis* for seven days enhanced memory as significant increase in percent retention as compared to control aged mice ($P < 0.05$) was observed. Significantly higher retention (55.3%; $P < 0.05$) was observed with *C. pluricaulis* (200mg/kg; po) as compared with piracetam (10mg/kg, ip) which was 42.84% (Fig. 3).

Effect of post-trial administration of plant extracts on memory

All the groups were given respective treatments after training them on elevated plus maze. During acquisition trials, latency times were similar across all experimental groups. However, post trail administration with *C. pluricaulis* and *A. racemosus* demonstrated significant decrease in latency times during retention trials. The percent retention of memory after 24 h in control group was significantly less than ($P < 0.05$) that of the *C. pluricaulis* and *A. racemosus* treated groups during these trials (Fig. 4).

Effect of plant extracts on AChE —CA1 region of hippocampus of control mice showed weak AChE staining (Fig. 5A). In *A. racemosus* treated group, moderate increase in AChE staining was observed after 200 mg/kg dose (Fig. 5C). At 100 mg/kg dose (Fig. 5B) the AChE activity was similar to control group. Similar changes were observed in CA3 area of *C. pluricaulis* treated group. In CA3 region (Fig. 5 E and F) a significant dose dependent increase in AChE activity was observed as compared to control (Fig. 5D).

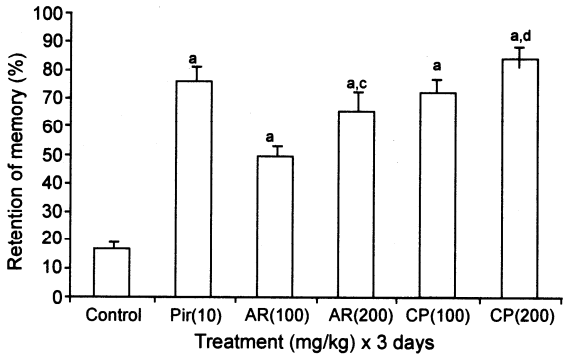

Fig. 2—Effect of pretreatment of *C. pluricaulis* (CP; 100 and 200 mg/kg, po) or *Asparagus racemosus*, (AR; 100 and 200 mg/kg, po) for three days on percent retention of memory using elevated plus- maze paradigm. Values are expressed as mean ± SE, ANOVA followed Dunett's test. *P* values: <0.5 as compared to [a]control (young mice), [b]piracetam, [c]*A. racemosus* (100 mg/kg, po), [d]*C. pluricaulis*(100 mg/kg, po).

Discussion

The present work was undertaken to study the effects of *A. racemosus* and *C. pluricaulis* on learning and memory in young and aged mice. Normal ageing is known to deteriorate memory in human beings. In the present study, aged-animals showed impaired memory as percent retention significantly decreased as compared to control young mice. Both acute

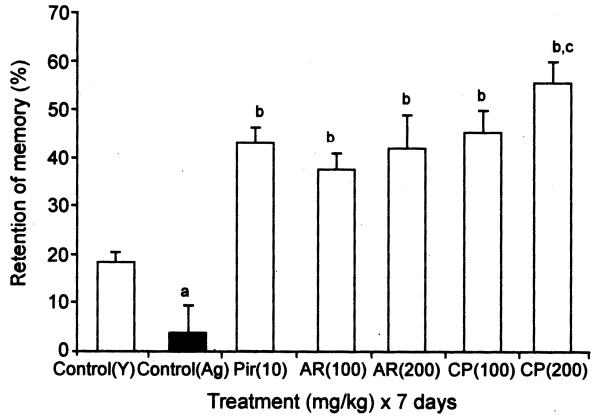

Fig. 3—Effect of pretreatment of *C. pluricaulis* (CP; 100 and 200 mg/kg,p.o) or *A. racemosus*, (AR; 100 and 200 mg/kg, po) for seven days on percent retention of memory using elevated plus-maze paradigm. Values are expressed as mean ± SE, ANOVA followed Dunett's test, *P* values: <0.5 as compared [a]control (young mice) [b]control (aged mice), [c]piracetam (10 mg/kg; ip).

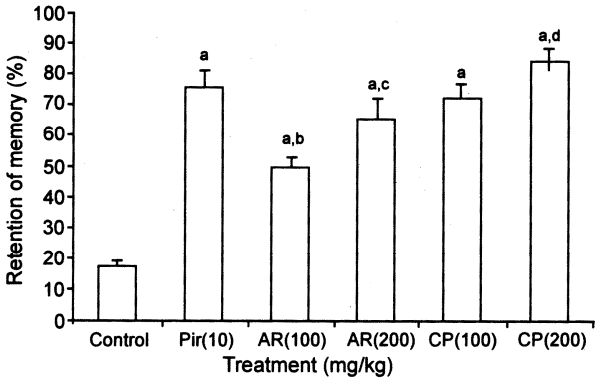

Fig. 4—Effect of post trail administration of *C. pluricaulis* (CP; 100 and 200 mg/kg, po) or *A. racemosus*, (AR; 100 and 200 mg/kg, po) on percent retention of memory using elevated plus- maze paradigm. Values are expressed as mean ± SE, ANOVA followed Dunett's test. *P* values: <0.5 as compared [a]control (young mice), [b]piracetam, [c]*A. racemosus* (100 mg/kg, po), [d]*C. pluricaulis* (100 mg/kg,p.o.).

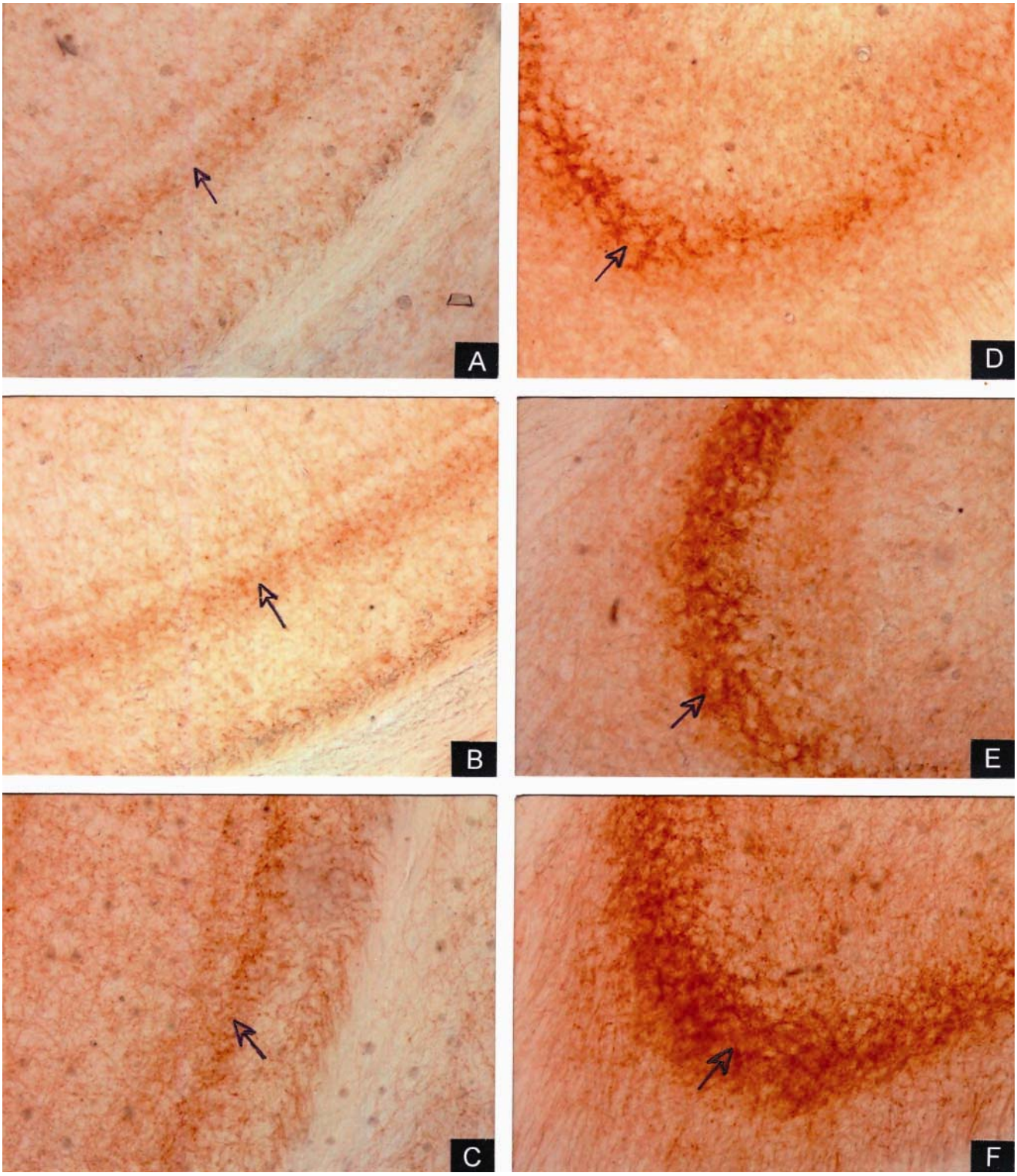

Fig. 5—Effect of *A. racemosus* and *C. pluricaulis* extract on AChE activity of hippocampal sub region of aged mice a: CA1 sub region of control mice showing weak activity; b: CA1 sub region of mice receiving 100 mg/kg, po *A. racemosus* extract; c: CA1 sub region of mice receiving 200 mg/kg, po *A. racemosus* extract; d: CA3 sub region of mice showing weak activity; e: CA3 sub region of mice receiving 100 mg/kg, po *C. pluricaulis* extract; f: CA3 sub region of mice receiving 200 mg/kg, po *C. pluricaulis* extract. a–f: 40X

and chronic treatment with *C. pluricaulis* and *A. racemosus* produced a significant reduction in the transfer latency when tested after an interval of 24 h in elevated plus maze, indicating that both extracts improved the ability to retrieve information and therefore, strengthens explicit memory of young and aged mice. Although *C. pluricaulis* showed more significant improvement than the *A. racemosus* in aged mice.

Several reports suggested that aging and age associated disorders leads to impairment in memory tasks relative to their younger counterparts and progressive deterioration of memory[20,21] associated with gradual loss of cells in specific areas of the brain[19]. Earlier studies have shown that antioxidant-rich diets improve cerebellar physiology and motor learning in aged-rats[22,23], thereby result in enhanced memory, reduced brain damage and improved neuronal functions. Their profound free radical scavenging action could insulate neuronal tissues from degeneration probably by preserving these areas from perturbations of oxidative stress[23]. Antioxidative and neuroprotective effect of *A. racemosus* root extract has been demonstrated against kainic acid-induced neurotoxicity[23] and chronic stress-induced neurodegeneration in rats[14,15]. Antioxidative activity of *C. pluricaulis* has been also reported. Pretreatment with *C. pluricaulis* extract, is known to significantly decrease norepinephrine levels in limbic region of rat brain which may be suggestive of its memory enhancing effect[11,25]. In another study, dietary feeding of *C. pluricaulis* increased protein synthesis in the hippocampus, which is indicative of enhanced learning and memory in experimental animals[11]. Based on these considerations, it is suggested that memory enhancement and neuroprotection conferred by *C. pluricaulis* and *A. racemosus* in present study is due to their established antioxidative and neuroprotective action.

In present study, significant increase in AChE activity in CA1 area of *A. recemosus* and in CA3 area of *C. pluricalus* fed animals was evident, suggesting altered cholinergic functions in these areas. Parent and Baxter[26] reported that upregulation of AChE activity is important reversing the memory deficits. Taranalli and Cheeramukuzhy[27] in *Clitorea terneata* fed animals and Shinomol and Muralidhara[28] in *Centella asiatica* fed animals reported increased AChE activity in CA3 area. It has been suggested that increased AChE levels may reflect increased acetylcholine release which facilitate synaptic transmission in these brain areas and thus enhancement of cognitive functions could be attributed to the plants extracts to elevate AChE activity in related areas. CA1 and CA3 areas of hippocampus are earlier shown to be related with learning and memory. Gilbert *et al.*[29] showed that CA1 area is related to temporal processing. Lee and Kesner[30] discussed that CA3 mediate short term memory while CA1 mediate intermediate-term memory.

Thus, it may be concluded that *C. pluricaulis* and *A. racemosus* has shown a promising memory-enhancing effect in both young and aged mice. Although, the exact mechanism of memory strengthening effect of both the plant extracts was not explored in the present study, it appears to be related to a combination of a variety of its properties such as antioxidant, anti-inflammatory, neuroprotective and cholinergic properties of these plants.

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