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# Reversal of Scopolamine-Induced Deficits in Navigational Memory Performance by the Seed Oil of *Celastrus paniculatus*

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GATTU, M., K. L. BOSS, A. V. TERRY, JR., AND J. J. BUCCAFUSCO. *Reversal of scopolamine-induced deficits in navigational memory performance by the seed oil of* Celastrus paniculatus. PHARMACOL BIOCHEM BEHAV **57**(4) 793–799, 1997.—*Celastrus paniculatus* (CP), a medicinal plant from India has been reputed to be useful as a pharmaceutical aid for learning and memory. We investigated the effects of the seed oil of CP on the 6 day performance of young adult rats in a navigational memory task--the Morris water maze. Chronic oral (gavage) daily treatment with CP, (50, 200, or 400 mg/kg) for 14 days completely reversed the scopolamine (0.5 mg/kg)-induced task performance deficit. On the other hand, acute treatment (single injection prior to scopolamine treatment) with CP (200 mg/kg) did not significantly reverse the scopolamine-induced impairment in maze performance. Alone, CP produced a slight, but significant improvement in maze performance on the first day of testing. Acute treatment or chronic 14 day treatment with CP resulted in no significant alteration in normal locomotor activity in an open field. Moreover, CP did not alter the scopolamine-induced increases in locomotor activity. Chronic treatment with CP did not alter brain acetylcholinesterase levels and no signs of cholinergic overstimulation were ever noted during or after treatment. Thus, the seed oil of CP, when administered chronically, selectively reversed the impairment in spatial memory produced by acute central muscarinic receptor blockade, supporting the possibility that one or more constituents of the oil may offer cognitive enhancing properties. The neural mechanism underlying the reversal of scopolamine's mnemonic effects by CP is not yet known, but it is not related to an anticholinesterase-like action. © 1997 Elsevier Science Inc.

Learning memory  Medicinal plant  Scopolamine  Muscarinic receptors  Locomotor activity

## INTRODUCTION

TRADITIONAL or folk medicines have been widely employed for centuries, and they remain one important source for the discovery of new bio-active compounds. Ayurveda, an ancient traditional system of medicine that has been practiced in India since 200 B.C. employs a large number of medicinal plants used in the prevention and treatment of a wide number of diseases. One of these includes the plant *Celastrus paniculatus* Willd. (CP), a plant known for centuries as "the elixir of life". According to Ayurveda, depending upon the dose regimen, CP may be employed as a stimulant-nerve tonic, rejuvenant, sedative, tranquilizer and diuretic. It is also used in the treatment of leprosy, leucoderma, rheumatism, gout, paralysis and asthma. Most of the claims for this plant have not been substantiated in rigorous scientific settings. This includes the purported property of CP germane to this study—its ability to stimulate the intellect and sharpen the memory. There have been pharmacological studies to suggest that the oil obtained from the seeds of CP possess sedative and anticonvulsant properties (15,17). Sheath and coworkers (33) isolated an active fraction of the oil that they termed Mal-IIIA, and suggested that this component exhibits the sedative and tranquil-

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izing effects observed in rats and mice. Sporadic reports in the scientific literature also exist to suggest that the neet oil of CP or its extracts exhibit the following pharmacological actions: anti-viral (7), anti-bacterial (28), analgesic (2), anti-inflammatory (2,11), anti-malarial (5), anti-fatigue (19), anti-spermatogenic (36), insecticidal (4), hypolipemic (22), and, potentiation of barbiturate sleeping time (3). The indigenous peoples that employ CP also know this preparation as a general cognitive enhancing agent. Several reports are now available to support this later notion in the laboratory setting. Karanth and coworkers (20), and Nalini and coworkers (26), reported that rats treated with CP improved their performance in raised platform shock-avoidance paradigm and two-way passive avoidance paradigm, respectively. Nalini and coworkers (25) reported that chronic treatment with CP oil produced improvement in I.Q. scores and decreased the content of catecholamine metabolites, vanilylmandelic acid and homovanillic acid in the urine of mentally retarded children. Similar neurochemical results were also observed in rats, wherein chronic treatment with CP decreased the brain content of norepinephrine, dopamine, and serotonin, and decreased their respective metabolites in the brain and urine (26).

Pharmacologic blockade of central muscarinic receptors produces a short-term amnestic response in a wide variety of animal models performing a variety of tasks challenging learning and recall (13,14,16,23). Scopolamine is particularly effective when administered just before task training (13). One of the most widely employed paradigms used to assess working memory in rodents is the Morris water maze spatial navigational task (24). Administration of low doses of scopolamine have been shown to effectively interfere with task performance. One other advantage of the use of scopolamine is that the drug evokes characteristic changes in locomotor activity in an open field (1,29,32,35). The effects of scopolamine on maze performance and locomotor activity can be reversed by the administration of classical (centrally-acting) muscarinic receptor agonists, particularly inhibitors of acetylcholinesterase (32). Thus, the seed oil of CP was examined for its ability to reverse the effects of either or both the scopolamine-induced impairment of water maze performance and increased open field locomotor activity in adult rats. We also determined whether *in vivo* administration of CP could inhibit brain acetylcholinesterase activity measured *ex vivo*.

## METHODS

### *Animals*

Male, Wistar rats (275–375 g) were obtained from Harlan Sprague–Dawley Inc., (Indianapolis, IN), housed individually in controlled-temperature environment with a 12 h light/dark cycle. Food and water available ad lib. Behavioral experiments were conducted during the light cycle.

### *Drugs*

The seed oil of *Celastrus paniculatus* Willd. was obtained in encapsulated form from BAN Pharmacy, Rajkot, India. Each capsule contained 300 mg of oil. Oil was removed from capsule and mixed with peanut oil to make the appropriate concentrations. Scopolamine hydrobromide was obtained from Sigma chemical Co., St. Louis, MO.

### *Water Maze Task*

Maze testing was performed in a circular plastic pool (diameter: 180 cm, height: 76 cm) (Bonar Plastics, Noonan, GA) with the inner surface painted black. The pool was filled to a depth of 35 cm of water (maintained at 25 ± 1°C) that covered an invisible black) 10 cm square platform. The platform was submerged approximately 1 cm below the surface of the water and placed in the center of the northeast quadrant. The pool was located in a room with a number of extra-maze visual cues, including brightly colored geometric images (squares, triangles circles etc.) that were hung on the wall. Diffuse lighting and black curtains were used to hide the experimenter and the awaiting rats. Swimming activity of each rat was monitored *via* a ccTV camera mounted overhead, that relayed the latency to find the platform as well as the distance traveled to a video tracking system (Poly-Track, San Diego Instruments, San Diego, CA).

For the hidden platform test, each rat was given 4 trials per day for 6 consecutive days. Each rat's latency for the given day was calculated by averaging the four trials. A trial was initiated by placing the rat in the water facing the pool wall in one of the four quadrants (designated NE, NW, SE, SW). The daily order of entry into individual quadrants was randomized such that all 4 quadrants were used once every day. For each trial, the rat was allowed to swim a maximum of 90 s, in order to find the hidden platform. When successful the rat was allowed a 30 s rest period on the platform. If unsuccessful, within the allotted time period, the rat was given a score of 90 s and then physically placed on the platform and also allowed the 30 s rest period. In either case the rat was immediately given the next trial (Inter Trial Interval [ITI] = 30 s) after the rest period. On the seventh day, a highly visible (neon colored) cover was attached to the platform which was raised above the surface of the water (approximately 1.0 cm). Lighting was changed such that extra-maze cues were no longer visible. Each rat was given one trial in order to acclimate to the new set of conditions and to visually locate the platform. This was accomplished by lowering the rat into the water in the NE quadrant and allowing the animal to locate the platform. No time limit was placed on this first trial. The rat was then immediately given a second trial in the same manner and the latency to find the platform measured as a test of visual acuity.

### *Locomotor Activity*

Automated open field locomotor activity was measured using an Omnitech Digiscan (model CCDIGIO) optical animal activity monitoring system that employs horizontal and vertical banks of photo beam sensors to monitor several categories of animal movement with time. The animal was placed in a clear test cage (40 × 40 × 30) and the trial initiated immediately. Locomotor activity was monitored continuously for 30 min and the data were accumulated and processed in a spread sheet format. The following parameters were recorded: horizontal activity, movement time, number of stereotypy movements, and vertical activity. A complete description and definition of each parameter has previously been published (30).

### *Acetylcholinesterase Assay*

Brain tissue derived from CP treated rats was homogenized (10 ml/g tissue) in 100 mM phosphate buffer (pH 7.0). Acetylcholinelerase activities were determined spectrophotometrically in whole tissue homogenate by the method of Ellman and coworkers (12). Brain homogenate (100 μl) was added to a reaction mixture (50 mM Tris-HC1, pH 8.0) containing 75 mM acetylthiocholine iodide as substrate, and 6.9 mM dithiosnitrobenzoic acid in a total volume of 3 ml. The change in absorbance at 412 nm was recorded for 2 min.

EXPERIMENT 1

Rats were randomly assigned to two groups: one group was treated with CP at a dose of 200 mg/kg/day (by oral gavage); and the other group was treated with peanut oil as a vehicle for 14 days. The water maze experiment was initiated on the 15th day of treatment. On day 15, the peanut oil group was subdivided into control (C) and scopolamine (S) groups, whereas the CP treated group was subdivided into CP alone (CP) and CP + Scopolamine treated (CPS) groups. Twenty min prior to each day's maze testing session vehicle, CP, vehicle + scopolamine (0.5 mg/kg, IP), or CP (200 mg/kg) + scopolamine were administered to (C), (CP), (S) or (CPS) groups, respectively. At the completion of the experiment, the rats were decapitated and the brain tissue was used to evaluate the acetylcholinesterase activity. In a second series, using the same experimental protocol, separate groups of rats were administered 50 or 400 mg/kg of CP prior to, and during the scopolamine challenge. Each rat's body weight and normal ongoing behavior was repeatedly observed throughout the experiment to detect any potential toxic effect of CP.

Using the same protocol as described above, a new group of rats was tested in the openfield locomotor activity task for 30 min to determine the effect of chronic treatment of CP on motor activity.

*Experiment 2*

The effects of a single acute administration of CP (200 mg/kg) on water maze performance was assessed in this experiment. As described above, a new group of rats was divided in to 4 groups, C, S, CP and CPS, and drugs or vehicle were administered 20 min prior to testing. One week after completion of the water maze experiment, rats were tested in the openfield locomotor activity experiment.

*Statistics*

Comparisons between the means of several populations were performed by using a two-way ANOVA with repeated measures in a paired design, and the differences considered significant at the $p < 0.05$ level. If data sets failed tests of normality or of equal variances, the Kruskal-Wallis one way analysis of variance on ranks was used to determine the statistical difference between groups. For data sets derived from behavioral experiments that were shown to be significantly different by ANOVA they were also shown to be significantly different according to the Kruskal–Wallis test. The Student–Newman–Keul test was employed as a post hoc test, except for the data derived from acetylcholinesterase levels for which a Student's *t* test was used.

RESULTS

Once daily, oral treatment with CP (50–400 mg/kg) over 14 days produced no significant effect on body weight compared to the vehicle group. Animals appeared to behave normally in their home cage and test environments. Also, there were no obvious signs of "cholinergic" toxicity (tremor, convulsions, salivation, fasiculations, lacrimation etc.) observed after administration of CP at any dose or regimen.

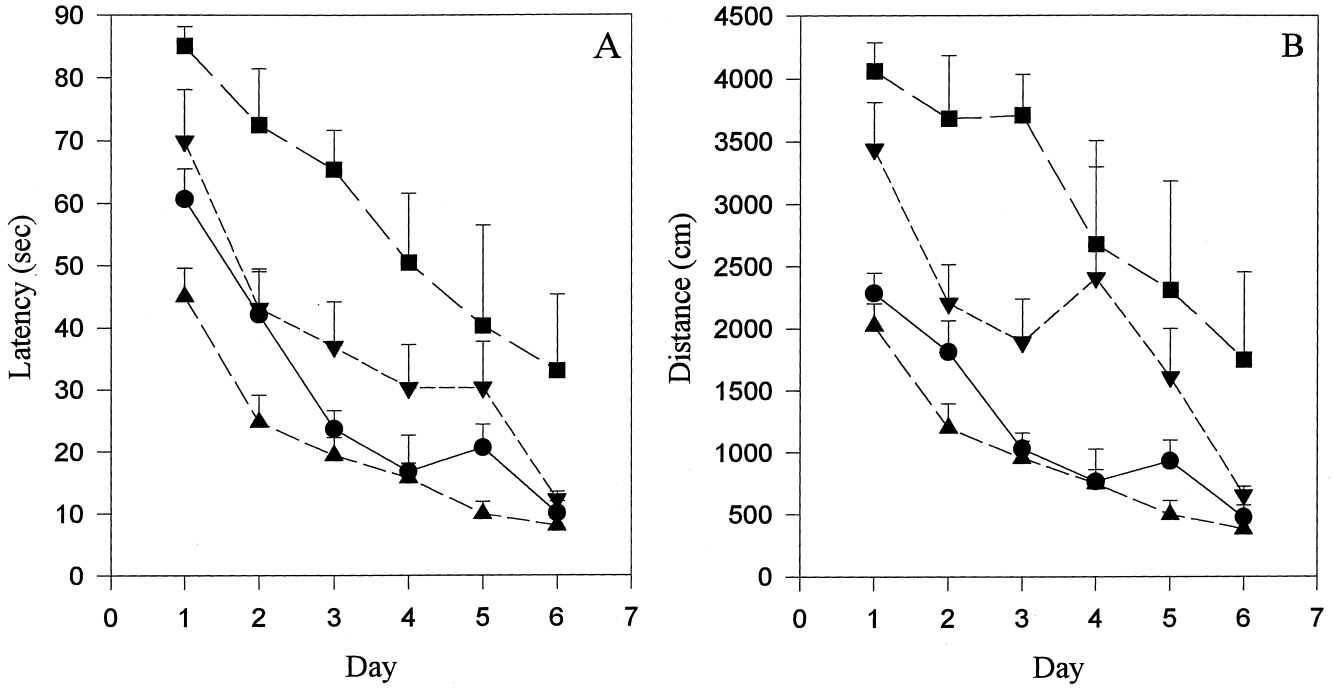


FIG. 1. The effect of daily oral administration of 200 mg/kg of CP for 14 days prior to, and during 6 days of water maze testing on the scopolamine-induced (0.5 mg/kg, IP) performance deficits in rats. A. Mean swimming latencies. B. Mean swimming distance. Each value represents the mean ± SEM of 6 rats. (■) - Chronic treatment: 14 days of CP vehicle (peanut oil); Pre-test treatment: scopolamine alone. (▼) - Chronic treatment: CP; Pre-test treatment: scopolamine and CP; (●) - Chronic treatment: vehicle; Pre-test treatment: saline, IP (▲) - Chronic treatment: CP; Pre-test treatment: CP. Each pre-test drug regimen was administered 20 min before maze testing. The scopolamine alone group was significantly different from each of the other groups ($p < 0.05$).

TABLE 1

EFFECT OF CP ON SCOPOLAMINE TREATED AND UNTREATED RATS IN OPEN-FIELD LOCOMOTOR ACTIVITY PERFORMANCE

| Activity | Con | Sco | CP | CP+S |
|---|---|---|---|---|
| A. Chronic Study | | | | |
| Horizontal activity | 9187 ± 994 | 14830 ± 1456* | 12152 ± 907 | 13735 ± 1390* |
| Movement time (s) | 515 ± 57 | 795 ± 81* | 623 ± 45 | 712 ± 72 |
| Number of stereotypy | 220 ± 15 | 266 ± 19* | 272 ± 7* | 275 ± 9* |
| Vertical activity | 2647 ± 261 | 3873 ± 359 | 3273 ± 284 | 3607 ± 350 |
| B. Acute Study | | | | |
| Horizontal activity | 11343 ± 1005 | 22284 ± 2607* | 10254 ± 629 | 24288 ± 2598* |
| Movement time (s) | 886 ± 86 | 1429 ± 70* | 811 ± 49 | 1473 ± 43* |
| Number of stereotypy | 220 ± 13 | 308 ± 9* | 181 ± 9 | 299 ± 10* |
| Vertical activity | 2243 ± 298 | 2268 ± 3651 | 2319 ± 453 | 3198 ± 606 |

Values are mean ± SEM of 6 rats.
*Significantly different than control (C) rats ($p < 0.05$).

No significant differences are present between either control and CP group or scopolamine (Sco) and CP + Scopolamine treated groups. Values are mean ± SEM of 6 rats.
*Significantly different than control (Con) and CP treated rats ($p < 0.05$).

*Experiment I*

Over the 6 days of water maze testing, rats treated chronically with vehicle and administered scopolamine prior to maze testing (S) exhibited significantly longer swim latencies in comparison with either control rats not administered scopolamine (C), or compared with the two groups receiving the chronic CP regimen (CP or CPS) ($F(3, 20) = 12.39$; $p < 0.0001$). Impairment was evident even on the first day of testing in that the (S) group required 85 s on average to locate the hidden platform. In contrast, the (C), (CP) and (CPS) groups required only 60, 45 and 69 s respectively, to locate the platform. Since 4 trials were run per day, it was possible to examine task performance on the very first trial before learning could take place. Under these conditions the 4 experimental groups did not exhibit significantly different swim latencies. For the (C), (S), (CP) and (CPS) groups the mean swim latencies on the very first trial were, respectively, 78 ± 13, 90 ± 0, 77 ± 9 and 81 ± 9 s. Chronic daily administration with 200 mg/kg CP for 14 days produced a statistically significant decrease in the latency to find the platform on the first day but no difference was observed on subsequent days. As shown in Fig. 1A, chronic treatment with CP significantly reversed the impairment in maze performance produced by scopolamine [(CPS) vs. (S)]. Post hoc analysis indicated that significance differences were observed between (CPS) and (S) over first four days of water maze experiment. Swim latencies exhibited by all groups of rats were gradually, but significantly decreased from day 1 to day 6 ($F(5, 15) = p < 0.0001$). This decrease in swim latencies over the 6 test days indicated that all of the rats eventually learned the task irrespective of drug treatment.

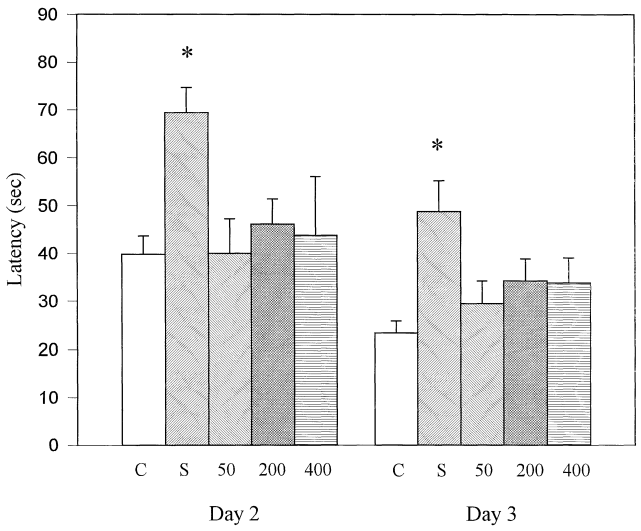


FIG 2. The effect of daily oral administration of CP for 14 days prior to, and during 6 days of water maze testing on the scopolamine-induced (0.5 mg/kg, IP) performance deficits in rats. Only days 2 and 3 of maze testing are presented. Each value represents the mean ± SEM of 6–12 rats. (C) - Chronic treatment: CP vehicle (peanut oil); Pre-test treatment: saline, IP (S) - Chronic treatment: 14 days of CP vehicle (peanut oil); Pre-test treatment: scopolamine alone. (50) - Chronic treatment: 50 mg/kg CP; Pre-test treatment: scopolamine and 50 mg/kg of CP. (200) - Chronic treatment: 200 mg/kg CP; Pre-test treatment: scopolamine and 200 mg/kg CP. (400) - Chronic treatment: 400 mg/kg CP; Pre-test treatment: scopolamine and 400 mg/kg CP. The scopolamine alone group was significantly different from each of the other groups ($p < 0.05$).

Consistent with the swim latency results, the distances traveled by the (S) group rats were significantly longer than either control rats not administered scopolamine (C), or the other two groups rats that received the chronic CP regimen (CP or CPS) ($F(3, 20) = 14.98$; $p < 0.0001$). With some minor exceptions, the between-group differences obtained for the swim distance data were similar to those obtained for the swim latency data (Fig. 1B). There were no significant between-group differences observed for the animals performing the visual acuity task administered on the seventh day of testing. Group means for this task were 14,10, 9, and 7 s for (C), (CP), (S) and (CPS), respectively. The cholinesterase activity of frontal cortex homogenates were not significantly different between (C) and CP groups (0.0069 ± 0.001 and 0.0061 ± 0.0003 μmol/min, respectively).

For the locomotor activity experiment, all 4 experimental groups received their respective regimens 20 min prior to test-

ing. Horizontal activity (horizontal beam breaks), movement time, and the number of stereotyped movements (repetitive breaking of the same beam) were significantly enhanced by scopolamine. In contrast, vertical activity (vertical beam breaks) was not significantly affected by the muscarinic receptor antagonist (Table la). Chronic pre-treatment with CP slightly increased all locomotor activity parameters compared to control but no significant differences were observed. Unlike, its anti-scopolamine effect on water maze performance, the chronic CP regimen did not significantly affect the increase in horizontal motor activity and stereotypy produced by scopolamine (Table la).

Using the same experimental paradigm described above, a second series of experiments was performed in which two additional doses (chronic) 50 mg/kg and 400 mg/kg of CP were evaluated for their ability to reverse scopolamine-induced deficits in water maze performance. The data for the second and third days of maze testing are presented in Figure 2 along with those for the 200 mg/kg dose for comparison. As with the 200 mg/kg dose of CP, both the 50 and the 400 mg/kg doses completely reversed the scopolamine deficit in maze performance ($F$ (5, 48) = 8.89; $p < 0.0001$). All 3 doses appeared to be maximally effective in this regard, and as such, there was no apparent dose-response relationship over this range of doses. The data suggest that CP may exhibit a wide therapeutic window (Fig. 2).

*Experiment 2*

In an acute experiment where rats received either drugs or vehicle 20 min prior to maze testing, scopolamine treated rats (S) exhibited significantly longer swim latencies in comparison with either vehicle (C) or (CP) treated rats ($F$ (3, 20) = 6.37; $p < 0.01$). Single dose (acute) treatment with CP (200 mg/kg) 20 min prior to testing was not as effective as chronic administration in reversing the scopolamine-induced deficits in water maze performance. In fact, the learning curve (swim latencies) for the combined CP and scopolamine treatment (CPS) was slightly, but not significantly improved compared with the scopolamine (S) group (Fig. 3A). Also, for acute administration, CP alone (CP) had no effect on swim latencies in comparison with the (C) group. As with the results from the chronic study, no significant differences were obtained among mean swim latencies for the 4 experimental groups on the very first trial of the first day of testing. Essentially identical results were obtained when swim distances were compared among the groups Fig. 3B). Finally, acute administration of CP alone (CP) had no effect on locomotor activity in comparison with the (C) group. As with the chronic administration regimen, acute CP was completely ineffective in reversing scopolamine-induced increases in locomotor activity (Table 1b).

## DISCUSSION

At least four previous studies exist that have examined the effects of CP on behavioral tasks that involve learning and memory (20,21,25,26). In one such study (20), rats were treated with 400 mg/kg ofCP (by oral gavage) once daily for 3 days. The animals were then given 10 trials in a raised platform shock-avoidance task. Each trial was spaced 5 min apart. The CP treated rats exhibited a significantly increased learning curve compared with vehicle treated animals in the avoid-

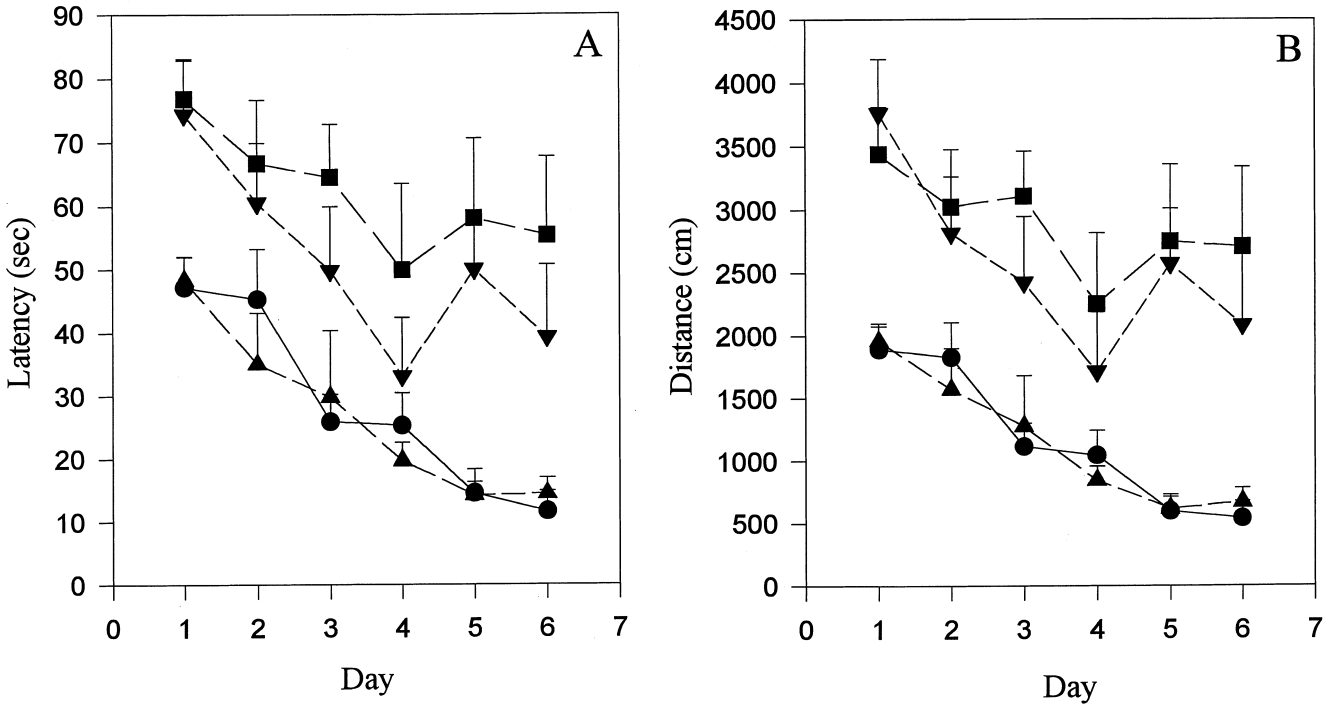


FIG 3. The effect acute administration of 200 mg/kg of CP prior to, and during 6 days of water maze testing on the scopolamine-induced (0.5 mg/kg IP) performance deficits in rats. A. Mean swimming latencies, B. Mean swimming distance. Each value represents the mean ± SEM of 6 rats. (■) - scopolamine alone. (▼)- scopolamine and CP; (●)- saline, IP (▲) - CP alone. Each pre-test drug regimen was administered 20 min before maze testing. The scopolamine alone and the scopolamine + CP groups were significantly different fiom the saline and CP alone groups ($p < 0.05$).

ance paradigm. In another study, rats treated daily with 850 mg/kg of CP oil for 15 days exhibited a significant improvement in their retention times in a two-way passive avoidance task. CP also produced a significant decrease in the content of norepinephrine, dopamine and serotonin, and certain of their respective metabolites in both brain and urine (26).

The results of the present study support the reports of the ancient Ayurvedic literature, and confirm, the results of the previous studies mentioned above in that they are consistent with the ability of chronic CP administration to enhance the performance of subjects engaged in memory-related tasks. Chronic treatment with 200 mg/kg of CP alone resulted in a small degree of enhancement in water maze acquisition. A marked degree of task enhancement was not expected since the rats were young and presumably cognitively unimpaired. Moreover, we used relatively low doses of CP (50–400 mg/kg/day) compared to those used in the studies cited above. However, even the 50 mg/kg/day dose regimen was effective in improving maze performance when performance was impaired by central muscarinic receptor blockade with scopolamine. The selectivity of CP in affecting the cognitive aspects of this task was suggested by the ability of the drug to reverse the scopolamine-induced impairment of maze performance, but not the scopolamine-induced increase in locomotor activity. Neither scopolamine nor CP regimens affected the level of performance of the first trial of the maze task; one that presumably does not involve learning. It is not likely, therefore, that the drug treatments altered task performance by affecting non-mnemonic aspects of water maze learning.

Chronic treatment with CP reversed the scopolamine-induced deficits in maze performance, whereas the acute treatment with CP was much less effective in this regard. This finding is consistent with those of the more recent pharmacological studies employing CP. For example, chronic administration of the drug from 3 to as long as 45 days has been demonstrated to be required for maximal drug responses (8, 20,26). However, we cannot at this time rule out the possibility that the lack of an anti-scopolamine action to acute CP administration is related to the fact that in the acute paradigm the rats were not handled or administered solutions orally over 15 days as they were in the chronic study. It is yet to be proven, but perhaps more likely, that CP induces some slowly adaptive cellular change within the CNS.

In the present study, all three doses 50, 200 and 400 mg/kg/day of CP reversed the scopolamine deficits but no dose-response relationship was observed. Possibly the range of doses used in this experiment might have been too narrow to exhibit a dose-response. Clearly, the 50 mg/kg regimen produced a maximal scopolamine reversal. Although a separate experimental series using either the 50 or 400 mg/kg regimens of CP alone was not performed, Nalini and coworkers (26) reported that 850 mg/kg/day of CP for 15 days, did significantly improve performance by rats in passive avoidance paradigm. Although these two experiments were conducted using two different paradigms, the data suggest that higher doses of CP may directly enhance memory-related task performance in rats. Incidentally, chronic CP administration was associated with no observable side effects in the animals, even with the 400 mg/kg dose regimen.

The mechanism of action by which CP enhances learning and memory performance in behavioral tasks, and by which it reverses scopolamine-induced learning deficits is as yet unknown. CP did not appear to inhibit brain cholinesterase activity, nor were there any symptoms of cholinergic receptor stimulation exhibited by the animals at any time during treatment. Moreover, if CP were acting as a cholinergic agonist, we would have expected to obtain a reversal of scopolamine's amnestic actions after the acute administration of the material. In the present experiment, CP antagonized scopolamine's actions in the water maze but not in the locomotor activity task. Similarly, 5-$HT_3$ antagonists have been reported to reverse the scopolamine produced deficits in learning and memory models but not the motor activity (9). In addition, both CP and 5-$HT_3$ antagonists share several pharmacological actions such as lowering blood pressure (27,31,33), anxiolytic (33,18) and analgesic (2,34) effects, decreased dopamine levels (26,10), decreased aggression (33,18) and improved cognitive performance (20,21,25,26 6). Moreover, 5-HT related drugs often exhibit a significant delay in the onset of clinical benefit, also reminiscent of the effects of CP. On the basis of the results from previous studies and those of the present work, it is possible that one or more of the constituents of CP oil may posses serotonergic receptor antagonistic properties. Further experiments will be necessary to confirm this possibility.

The results of this study are consistent with the possibility that there is a basis for the contention derived mainly from anecdotal reports that CP may enhance learning and memory in humans. Furthermore, this plant seed oil may be more effective in individuals who are cognitively impaired as a result of chemical or organic brain damage as compared with normal subjects. In the least, these data may provide the impetus for further study of the material, and isolation of its active components.

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