Investigation of the possible role of Shankapushpi in the attenuation of ECT induced amnestic deficits

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ABSTRACT

Introduction: Shankapushpi (Evolvulus alsinoides and others) has received mention in traditional Indian writings as a potential enhancer of cognitive functioning. This study used an animal model to examine whether Shankapushpi improves learning and memory and attenuates anterograde and retrograde amnesia associated with electroconvulsive shocks (ECT).

Materials and Methods: Adult, male, Sprague Dawley rats (n=64) were treated with an aqueous extract of Shankapushpi or vehicle all through the 13-day study. From Days 1 to 8, the animals received Shankapushpi or vehicle alone. On Days 8–10, the animals were trained in a T-maze. On Day 11, they received two true or sham 30 mC ECS 5 h apart. On Day 12, recall of pre-ECT training was examined, and on Day 13, new learning was assessed.

Results: Shankapushpi-treated rats did not show better learning during the pre-ECT phase (Days 8–10). Seizure duration was not influenced by Shankapushpi treatment (Day 11). Shankapushpi did not attenuate ECS-induced retrograde amnesia (Day 12). ECS did not impair new learning, and the effect of Shankapushpi herein, therefore, could not be ascertained (Day 13).

Conclusions: In this animal model of learning and memory, and of ECS-induced retrograde amnesia, Shankapushpi was found to have no favorable or unfavorable effects on either cognitive or seizure duration parameters. These findings diminish expectations of cognitive and anticonvulsant benefits of simple Shankapushpi decoctions in clinical settings, but do not preclude benefits with extracts obtained and concentrated by using other methods.

Key words: Animal model, anterograde amnesia, electroconvulsive therapy, learning, memory, retrograde amnesia, Shankapushpi, T–maze

INTRODUCTION

India has a rich heritage in herbal medicine. According to Ayurveda, an ancient, traditional and alternative form of medicine prevalent in the Indian subcontinent, herbal extracts from Ashwagandha, Brahmi, Jatamansi, Jyotishmati, Mandukparni, Shankhapushpi and others singly or in combination were employed as rejuvenators or as antidotes for diverse disorders and diseases.[10] A current priority in neuropsychiatric research is the identification of medicinal substances that improve cognition in conditions such as age-related cognitive decline and the different dementias. Shankapushpi is a candidate herb that has been used for conditions related to the central nervous system; for example, along with turmeric in milk, Shankapushpi has long been used as a rejuvenator, mental stimulant and tranquilizer.[2]

In Ayurvedic practice, Shankhapushpi has been suggested
for neuropsychiatric states such as epilepsy, psychosis, insomnia, fatigue, low energy levels, memory loss, anxiety, stress, neuroticism and nervous debility. Therapeutic effects of Shankhapushpi have been claimed against a wide range of disorders, including anorexia, abdominal disorders, hypertension, stomatitis, piles, sterility in women, hematemesis and skin diseases.\[3\]

There is plurality regarding the botanical identity of Shankhpushpi with named botanical sources, including *Convolvulus pluricaulis*, *Evolvulus alsinoides*, *Clitorea ternatea* and *Canscora decussata*, all of which are indigenous to the Indian subcontinent.\[14\] *C. ternatea* are slender twiners, the chemical constituents of which include kaempferol and its glucoside clitorin. Its roots are used as a cathartic and diuretic, and contain taraxerol. A lactone, aparajitin, is present in the leaves, while the seeds contain the acylmioieties ternatins, beta-sitosterol, gamma-sitosterol, hexacosanol and an anthoxanthin. The defatted seeds contain a phenol glycoside, an alkaloid, ethyl alpha-D-galactopyranoside and rho-hydroxycinnamic acid.\[12\] Thus, the whole plant has been reported to possess medicinal properties.\[8\]

*Canscora decussata* Schult are small erect herbs, the phyoconstituents of which include the phenolic compounds scopoletin and mangiferin, alkaloids, xanthones, sterols, flavonoids and triterpenoids.\[6,7\] An ethanolic extract from the aerial parts of this plant showed muscle relaxant activity in an animal model.\[8\] The powdered plant as well as the alcoholic extracts attenuated chronic (but not acute) convulsive activity in rodents,\[9\] but total xanthones as well as mangiferin (which is the major and most polar xanthone of *Canscora decussata*) have no acute anticonvulsant action.\[10\]

*Convolvulus pluricaulis* is a perennial wild herb that grows under xerophytic conditions in India.\[11\] Its chemical constituents include the alkaloid shankhpushpine, the tropane alkaloids convolamine and scopoletin, ceryl alcohol, the flavonoid kaempferol and the steroids phytosterol and beta-sitosterol. The plant is suggested to have nootropic, anti-stress, anxiolytic, antidepressant, anticonvulsant, tranquilizing and sedative properties.\[13\] Ethyl acetate and aqueous fractions of the ethanolic extract of this plant were both found to significantly improve learning and memory in active and passive avoidance models.\[13\] Shankhpushpi extracted from *Convolvulus pluricaulis* has also been shown to possess anticonvulsant activity in different animal models.\[13,14\]

The bioactive constituents of *Evolvulus alsinoides* include the alkaloids betaine, shankhpushpine and evolvine. An ethanolic extract yielded four compounds – scopoletin, umbelliferone, scopolin and 2-methyl-1,2,3,4-butanetetrol.\[6\] Importantly, Shankhpushpi prepared from *E. alsinoides* has been reported to possess all the beneficial therapeutic properties that have been reported with other botanical sources of the herb.\[14\]

Kotiyal et al. examined which botanical source provided a more active and potent form of Shankhpushpi: *Convolvulus pluricaulis* or *E. alsinoides*. Extracts were obtained by pulverizing the plant material to a coarse powder and subjecting this to soxhlet extraction using 95% ethanol as the solvent. The extract of *E. alsinoides* was claimed to provide greater nootropic effects in rats than the extract of *Convolvulus pluricaulis*.\[14\]

Despite assertions in the alternative medicine literature, little empirical evidence exists documenting the effects of Shankhpushpi on fundamental central nervous system (CNS) functions. Given that *E. alsinoides* may provide the best source of the herb, and that learning and memory are impaired in diverse neuropsychiatric disorders, we sought to examine in animal models whether an aqueous extract of *E. alsinoides* improves learning and memory or reverses disturbances thereof. We posed the following specific questions:

1. Does Shankapushpi improve learning?
2. Does Shankapushpi attenuate retrograde amnesia induced by electroconvulsive shocks (ECS)?
3. Does Shankapushpi attenuate anterograde amnesia after ECS?

As a secondary objective, we also sought to assess whether Shankapushpi has anticonvulsant activity as evidenced by abbreviation of ECS seizure duration. We chose to study an aqueous extract because, traditionally, the consumption of a decoction (as opposed to consumption of the raw herb) would have been the only means of medicinal use of the plant.

**MATERIALS AND METHODS**

Adult, male, Sprague Dawley rats weighing 180–220 g at intake were housed four per cage with free access to tap water and standard laboratory diet. The rats were maintained in a controlled, disturbance-free environment all through the experiment. The study procedures adhered to the institutional guidelines on ethical animal handling that were in force at the time the study was conducted.

The rats were divided into two groups. One group received Shankhapushpi in an aqueous formulation and the other group received vehicle (water) alone in an identical volume. Treatment with Shankapushpi or vehicle commenced and continued until the end of the study. Days 8–10 were devoted to training in the T-maze.

### Table 1: Study flow chart

| Day | Activity |
|-----|----------|
| 1   | Treatment with Shankapushpi or vehicle commenced and continued until the end of the study |
| 8–10| Training in the T-maze |
| 11  | Two true or sham electroconvulsive shocks, 5 h apart |
| 12  | Assessment of recall of pre-ECS training in the T-maze |
| 13  | Assessment of new learning in the T-maze |

ECS – Electroconvulsive shocks
The Shankapushpi formulation was prepared freshly, each day, as follows: 1 g of Shankapushpi obtained from the Gujarat State Forest Department was soaked overnight in 16 mL of water. In the morning, the mixture was boiled down to a volume of 4 mL to extract the soluble ingredients. Next, the preparation was filtered and the residue was discarded. The filtrate was made up to 5 mL and was administered to the rats in the dose of 1 mL/kg by slow intraoral syringing through a wide-bore, smooth-tipped needle inserted into the posterior part of the pharynx. Administration of Shankapushpi/vehicle extended from the beginning to the termination of the experiment.

From Days 1 to 4, the rats received unrestricted feeds in addition to the daily Shankapushpi/vehicle dosing. From Day 5 onwards, the rats were kept on a 1-h restricted feeding schedule per day. On the days of T-maze learning, the feeding was permitted only after the maze trials were completed.

On Days 8–10, the rats were exposed to the T-maze. Each day, half of the animals in each group were trained to run into the left arm of the maze while the other half were trained to run into the right arm. A food pellet served as a reward. Animals alternated between left and right arms so that scent and other markings did not bias movements (the apparatus was also cleaned after every rat exposure to reduce the effect of such markings on subsequent animal behavior). On each of these three days of maze exposure, the direction of arm learning was kept constant – i.e., animals that were trained to run into the left arm continued to be trained to run to the left, while animals that were trained to run into the right arm continued to be trained to run to the right.

Each day, each animal received continued training trials on the T-maze until it attained satisfactory learning, defined as nine correct arm entries across 10 consecutive trials. A maximum of 25 trials was permitted. Each animal had two learning scores: the number of trials taken for satisfactory learning (taken as 25 in animals that did not learn) and the number of wrong arm entries during the period of learning.

On Day 11, half of the animals in the Shankapushpi group and half of those in the vehicle group received a suprathreshold electroconvulsive shock (ECS) circa 10 a.m. and, again, 5 h later, circa 3 p.m. The ECS were administered through saline-soaked earclip electrodes at a dose of 30 mC of charge, using the Nivique ECT device (Nivique Meditech, Bangalore, India). The ECS stimulus settings were 0.8 mA pulse amplitude, 1.5 ms pulse width, 62.5 Hz (bidirectional) pulse frequency and 0.2 s pulse duration.

Seizure duration was timed (with a stopwatch) by an experienced observer, and was defined as extending from the commencement of passage of current TO the termination of movements, or the onset of asynchronous movements, whichever occurred earlier. All seizures were at least 15 s in motor duration. In the event that a convulsion failed to develop, the stimulus was repeated at a dose of 60 mC of charge (stimulus duration increased to 0.4 s).

The remaining animals in each of the Shankapushpi and vehicle groups received sham ECS. The procedure was identical to that for ECS, except that no current was passed.

On Day 12, the animals were re-exposed to the T-maze to assess the extent to which the ECS had disturbed their recall of the pre-ECS training. The direction of arm learning was the same as that maintained pre-ECS. Thus, Day 12 scores reflected ECS-induced retrograde amnesia and the degree to which Shankapushpi offered protection against this effect.

On Day 13, each animal was trained to run into the opposite arm of the T-maze. The criterion for satisfactory learning remained the same. Thus, Day 13 scores reflected the degree to which ECS interfered with new learning (ECS-induced anterograde amnesia) and the degree to which Shankapushpi offered protection against this effect.

**Statistical analysis**

Data were analyzed using repeated measures multivariate analysis of variance with Pillai’s trace as the statistical criterion. In these analyses, group (drug vs. vehicle) and ECS (true vs. sham) were the between-subjects factors and time (occasion of assessment) was the within-subjects factor. Baseline learning scores were used as a covariate in the assessment of post-ECS learning.

**RESULTS**

**Effect of Shankapushpi on learning**

[Table 2] displays the data from the pre-ECS phase for the number of trials taken by the rats to achieve the criterion of satisfactory learning in the Shankapushpi and vehicle groups. There was a significant main effect for time (Pillai’s statistic=0.24, F=9.43, df=2,61, P<0.001), indicating that the rats showed significant learning across the three days of training. The main effect for drug (F=1.70, df=1,62, P=0.20) and the drug x time interaction (Pillai’s statistic=0.09, F=2.95, df=2,61, P=0.06) were non-significant, indicating that Shankapushpi did not improve learning performances.

[Table 3] displays the data from the pre-ECS phase for the number of wrong arm entries during the learning trials in the Shankapushpi and vehicle groups. There was a significant main effect for time (Pillai’s statistic=0.25, F=10.28, df=2,61, P<0.001), indicating that the rats showed a significant decrease in the number of wrong arm entries across the three days of training. The main effect for drug (F=0.81, df=1,62, P>0.30) and the drug x time interaction
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(Pillai’s statistic=0.07, F=2.40, df=2,61, P=0.10) were non-significant, indicating that Shankapushpi did not reduce the error rates during training.

In summary, the results of the pre-ECS phase indicate that this extract of Shankapushpi does not enhance T-maze learning in rats, as defined by the number of trials required for satisfactory learning and the number of wrong arm entries during the period of training.

Effect of Shankapushpi on ECS seizure duration

The seizure duration data for rats treated with Shankapushpi or vehicle are presented in [Table 4]. The main effect for time was non-significant (F=2.59, df=1,32, P>0.10), indicating that there was no change in seizure duration across time. The main effect for drug (F=0.28, df=1,32, P>0.50) and the drug x time interaction (F=2.22, df=1,32, P>0.10) were both non-significant, indicating that Shankapushpi had no effect on seizure duration.

In summary, these data suggest that this extract of Shankapushpi has no effect on seizure duration and may hence have no anticonvulsant activity.

Effect of Shankapushpi on ECS-induced retrograde amnesia

[Table 5] presents the data for learning on the first day post-ECS, when the rats were trained to run into the same arm as that during their pre-ECS training.

| Group                      | Day 1      | Day 2      | Day 3      |
|----------------------------|------------|------------|------------|
| Shankapushpi               | 11.36 (2.74)| 10.21 (1.37)| 9.57 (1.17) |
| Vehicle                    | 12.19 (4.29)| 10.14 (1.25)| 10.67 (3.11)|

Statistical inferences: Main effect for time, P>0.001; Main effect for group and group x time interaction, P>0.05; ECS – Electroconvulsive shocks

For the number of trials to satisfactory learning, there was a significant main effect for ECS (F=10.79, df=1,59, P=0.002). This indicates that ECS-treated rats required significantly more trials for satisfying learning, implying ECS-induced retrograde amnesia. The main effect for drug (F=1.99, df=1,59, P>0.10) and the drug x ECS interaction (F=0.54, df=1,59, P>0.40) were non-significant, indicating that Shankapushpi did not attenuate ECS-induced retrograde amnesia.

For the number of wrong arm entries, there was a significant main effect for ECS (F=9.72, df=1,59, P=0.003). This indicates that ECS-treated rats made significantly more errors, implying the occurrence of retrograde amnesia. The main effect for drug (F=1.92, df=1,59, P>0.10) and the drug x time interaction (F=1.03, df=1,59, P>0.30) were both non-significant, indicating that Shankapushpi did not attenuate ECS-induced retrograde amnesia.

In summary, this extract of Shankapushpi does not attenuate ECS-induced retrograde amnesia as operationalized in this study using the T-maze.

Effect of Shankapushpi on ECS-induced anterograde amnesia

[Table 6] presents the data for learning on the second day post-ECS, when the rats were trained to run into the opposite arm as that during their pre-ECS training.

| Group                      | No. of trials (n) | No. of wrong arm entries (n) |
|----------------------------|-------------------|------------------------------|
| Shankapushpi/true ECS (n=16)| 13.06 (2.95)     | 2.69 (1.49)                 |
| Shankapushpi/sham ECS (n=12)| 14.17 (4.28)     | 3.17 (2.12)                 |
| Vehicle/true ECS (n=18)     | 15.00 (4.03)     | 3.28 (1.78)                 |
| Vehicle/sham ECS (n=18)     | 14.50 (4.30)     | 3.22 (2.21)                 |

Statistical inferences: For both number of trials for satisfactory learning and wrong arm entries, the main effect for drug and the drug x time interaction were non-significant (P>0.20); ECS – Electroconvulsive shocks

For the number of trials to satisfactory learning (F=0.04, df=1,60, P>0.05) and for the number of wrong arm entries (F=0.13, df=1,60, P>0.50), the main effect for ECS was not significant. For both trials (F=1.47, df=1,60, P>0.20)
and wrong arm entries (F=0.51, df=1.60, P>0.40), the main effect for drug was not significant. The drug x ECS interaction was likewise non-significant for both variables (F=0.65, df=1.60, P>0.40 and F=0.30, df=1.59, P>0.50, respectively). These findings indicate that, in this model of anterograde amnesia, ECS did not impair new learning nor did Shankapushpi influence new learning.

In summary, as studied in this experiment, Shankapushpi has no effect on new learning after ECS.

**DISCUSSION**

In ancient Indian writings, many herbal substances have been suggested to improve CNS functioning; examples include Brahmi (*Bacopa moniera*), Jal-Brahmi (*Centella asiatica*), Ashwagandha (*Withania somnifera*) and others.[4] Shankpushpi is one such substance;[4] however, Shankapushpi has been far less studied, which is why we chose to focus on this herb in our investigation. We specifically selected the *E. alsinoides* plant because a previous investigation suggested that this could be the most bioactive herb from among the different sources.[4] We specifically chose to study an aqueous extract because alcoholic extracts have already been studied and because, in traditional domestic use, it is likely that aqueous methods were employed to prepare decoctions of the herb for consumption.[4]

We found that in a T-maze model of learning and memory, an aqueous extract of Shankapushpi did not improve learning and memory in Sprague Dawley rats. Whereas, two ECS administered 5 h apart induced significant retrograde amnesia, whereas Shankapushpi did not attenuate or worsen the degree of amnesia. Shankapushpi did not influence ECS seizure duration either. Finally, Shankapushpi did not influence new learning after ECS; however, the role of Shankapushpi in ECS-induced anterograde amnesia could not be determined because ECS, as administered in this study, did not result in impaired learning.

These findings are disappointing. It is usually easier to demonstrate cognitive benefits in animal models than in clinical contexts.[15] Therefore, if Shankapushpi is ineffective in an animal model, chances are that it would be of little benefit in persons with cognitive dysfunction. There are limitations to this conclusion. For example, our findings were obtained from a specific model of learning and memory and impairment thereof. It may be useful to study the benefits of Shankapushpi in other cognitive models, such as those based on active or passive conditioned avoidance, and it may also be useful to study Shankapushpi in other models of cognitive impairment, such as those associated with aging. We also acknowledge the possibility that a higher dose of Shankapushpi extract could have yielded positive findings, and that other sources of Shankapushpi, or combinations of sources, may provide cognitive benefits. Was our model of ECS too toxic for an effective cognitive enhancer to demonstrate benefits? It is unlikely, because only two ECS were administered, which, in fact, did not even result in significant anterograde amnesia. Previous studies have administered up to six ECS or more.[15]

We intended to study the possible benefits of Shankapushpi in ECS-induced anterograde amnesia as well as in ECS-induced retrograde amnesia. However, our failure to induce anterograde amnesia, a known difficulty in animal research, resulted in our inability to draw conclusions from our findings. Therefore, we cannot comment on the possible role of Shankapushpi in facilitating new learning after an insult to the CNS (such as may result from the use of ECT).[16] The subject requires further study.

As an afternote, the absence of effect of Shankapushpi on ECS seizure duration suggests that the herb may not be useful in patients with epilepsy; however, the data do not suggest a risk in epileptic patients, either. A limitation to this conclusion is that seizures in epilepsy result from lowered seizure threshold and antiepileptic drugs raise this seizure threshold. We examined seizure duration, a variable which is influenced by different mechanisms from those which affect the seizure threshold.

In conclusion, we found that an aqueous extract of Shankapushpi does not enhance learning or reverse ECS-induced retrograde amnesia in a T-maze. These findings do not encourage the use of simple Shankapushpi decoctions for domestic use as a cognitive enhancer in clinical contexts, but do not preclude the study of extracts obtained and concentrated by using other methods.

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