COMPARATIVE EVALUATION ON COMMERCIAL SOURCES OF INDIGENOUS MEDICINE SHANKHPUSHPI FOR ANTI-STRESS POTENTIAL A PRELIMINARY STUDY

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Summary

The present study was undertaken to determine a comparative account of the effects of methanolic extract of the aerial parts of the plants available as commercial sources of Shankhpushpi in India and one of its marketed formulation (Brand name- Shankhpushpi), on the experimental induced stress in albino rats. The parameters selected were CAR, stress induced epinephrine level and potentiation of barbiturate induced hypnosis for the purpose of present investigation. All the examined plant extracts were effective against experimental stress, and the results are comparable with marketed formulation. Out of the plant tested, the methanolic extract of C*onvolvulus pluricaulis* (100 mg/kg.b.w.) in rats has shown significant anti-stress activity.

Keywords: *Convolvulus pluricaulis*, Anti-stress, Shankhpushpi, CAR, Epinepherine.

List of Abbreviations: MEEA: Methanolic Extract of *Evolvulus alsinoids.;* MECP: Methanolic Extract of *Convolvulus pleuricaulis;* MECT: Methanolic Extract of *Clitorea ternatea;* SS: Shankhpushpi Syrup.

Introduction

The CNS acting drugs are invaluable therapeutically; because they can produce specific physiological and psychological effects [1]. All critical analysis on commercial and other information available on traditionally known CNS active herbal remedies indicate that the most popular amongst such remedies are those which are clinically and preclinically the most well studied ones, and which are also recommended for therapeutic purposes by the health authorities of many Western and other countries outside the USA [2]. Shankhpushpi is a drug of ayurvedic 'Medhya Rasayana' category which was used to boost memory and intellect. In India, *Convulvulus pluricaulis* Choisy*., Evolvulus alsinoides* Linn., and *Clitoria ternatea* Linn. and *Canscora decussata* Schult. are generally used as shankhpushpi by practitioners of ayurveda [3-10].

Ayurvedic medicine regards *Evolvulus alsinoides* highly effective for impairment of the central nervous system. Laboratory studies revealed the herb as anticatatonic and central nervous system depressant with an LD_{50} of 450 mg/kg [11]. The cyto-protective effects of *E. alsinoides* on hippocampal cells in mice suggested that in addition to improving memory the drug also has cytoprotective anti-stress effects [12].

The use of *C. pluricaulis* as an anti-anxiety and antidepressent was also suggested [13-14]. A review on number of '*Medhya rasayana*' drugs which are supposed to counteract the effects of stress by tranquilizing action and reported its tranquilizing activity mentioning *C. pluricaulis* [15]. The beneficial effect of *C. microphyllus* in memory potentiation in rats was also studied [16]. Antihypertensive and potentiation of barbiturate induced hypnosis activity of *C. pluricaulis* was reported to be highest during spring season [17]. Alcoholic as well as methanol extract of roots and aerial parts of *Clitorea ternatea* were shown to possess anxiolytic, antidepressant tranquilizing, sedative, anticonvulsant and antistress activity [18-20]. With a view to evolve a comparative account for their activities, based on the scientific basis, some of the available commercial plants as Shankhpushpi, were selected and subjected to investigations on experimental induced stress related conditions.

Materials and methods

Plant Materials

The entire herbs of *Evolvulus alsinoids* Linn*, Convolvulus pleuricaulis* Choisy and *Clitorea ternatea* Linn were collected from the campus and identified by the Botany Department, M. S. University, Baroda, Gujarat, India. The specimens of the plants were also preserved in lab.

Marketed formulation

Shankhapushpi (Unjha Pharmacy, India): syrup. Contain 6 species:
Convolvulus pluricaulis- Centella asiatica - Nardostachys jatamansi - Nepeia hindostana -Nepeia elliptica- Onosma brateatum, was purchased from the local market of Vadodara, Gujarat, India.

Animals

Young growing albino rats (150-200gm) of either sex were procured from Zydus Healthcare, Ahemdabad, India. The animals were kept on a 12 h light/dark cycle, at a room temperature of 22 °C, with free access to food (Kisan Feed India Ltd., Bombay, India) and water. The animals were acclimatized for a minimum period of 7 days. Experiments were conducted between 0900 and 1400 h. The animals were used according to the guidelines of "Committee for the Purpose of Control and Supervision of Experiments on Animals" (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, New Delhi.

Drugs and chemicals

Sodium bicarbonate, Folins reagent, Epinepherine and Pentobarbitone sodium were purchased from Sigma Aldrich, St. Louis.

Preparation of extracts

Aerial parts of *E. alsinoides* and *C. pluricaulis* were shade-dried at room temperature. The shade-dried plant material was coarsely powdered and subjected to extraction with petroleum ether in a Soxhlet apparatus. The defatted marc of all the three drugs was subjected to methanolic extraction [21]. These methanolic extracts were utilized for the further investigation. Extraction was done as per the Ayurvedic Pharmacopoeia of India [22]. All chemicals used for the purpose were of analytical grade.

Chromatographic studies of extracts

Thin-layer chromatography
Out of the various solvent systems tried, Toluene: Chloroform: ethanol (28.7: 57: 14.5) gave the best resolution (number of spots *E. alsinoides* = 11, *C. pluricaulis* = 12, *C. ternatea* = 9, Shankhpushpi Syrup = 11). The detecting reagent was anisaldehyde in sulphuric acid followed by heating at 110°C for 5 min.

Administration of the extracts
Suspensions of the methanolic extract were prepared in distilled water using Tween 80 (0.2% v/v) as the suspending agent. The extracts were administered in a dose of 100 mg/kg to rats by oral route, 45 min before the test procedures. Control groups were given only the vehicle (0.2% v/v Tween 80 solution) in volume equivalent to that of the plant extracts. Marketed Shankhpushpi syrup (100mg/kg.) by oral route was used as the reference for comparison.

Activity parameters
Methanolic extracts of *C. pleuricaulis*, *E. alsinoids* and *C. ternatea* as well as marketed Shankhpushpi formulation of were screened using method reported by Wroblewski and La Due, 1956 & Asratyan and Simnov, 1982 [23-24].

Conditioned avoidance response (CAR) - induction of experimental stress
Cook's and Weidley pole climbing apparatus was used for the present study [25]. Thirty young growing albino rats of either sex were selected. All these animals were individually trained to jump to the pole for the avoidance of electric shock followed by the bell sound, in order to avoid the following electric shock. After optimum training, they were divided into five groups with six animals of each group.

Group 1- Control receiving vehicle.
Group 2- Methanolic extract of *E. alsinoids* (100 mg/kg/ml/ p.o.)
Group 3- Methanolic extract of *C. pleuricaulis* (100 mg/kg/ml/p.o.)
Group 4- Methanolic extract of *C. ternatea* (100 mg/kg/ml/p.o.)
Group 5- Shankhpushpi syrup (100 mg/kg/ml/p.o.)

All the extracts and formulation were given in the mentioned dose for a period of 30 days, 45 minute prior to training. On 30^{th} day, activity of animals of different groups was evaluated, in terms of the time consumed by the individual animals to reach their goals. Object of this experiment was to study the comparative avoidance response for the stress (Electric shock) on treated animals with various plant extract, as well as marketed formulation.

Determination of epinephrine content in blood serum by colorimetric method
Epinephrine - a catecholamine hormone secreted by the adrenal medulla and a central nervous system neurotransmitter released by some neurons. It is stored in chromaffin granules and is released in response to hypoglycemia, stress, and other factors. It is a potent stimulator of the sympathetic nervous system (adrenergic receptors), and a powerful vasopressor, increasing blood pressure, stimulating the heart muscle, accelerating the heart rate, and increasing cardiac output. It is used as a topical vasoconstrictor, cardiac stimulant, systemic antiallergic, bronchodilator, and topical antiglaucoma agent. It was also known as *adrenaline* (Great Britain). Epinephrine content in blood serum was determined by method of Ghosh *et al.,* 1951 in same animals receiving 30 days of treatment [26].

Collection of blood and separation of serum
Blood was withdrawn by puncturing retro orbital sinus, in clean dry test tube and allowed to clot at room temperature for 30 minutes. The serum was separated by centrifuging the clot at 2500 rpm for 10 minutes. The serum was then treated with mixture of acetonitrile: methanol (2:0.1) and shaken properly to precipitate the proteins. This was then subjected to centrifugation and the supernatant liquid was taken for the estimation of epinephrine.

Preparation of standard curve
Stock solution containing 1mg/ml of standard epinephrine was prepared in 0.2 N HCl. Aliquots containing 0.1-0.5 ml of epinephrine were transferred to 10 ml volumetric flask. Then 0.25 ml of folin's phenolic reagent (1 N), 0.75 ml of 5% sodium hydroxide, and 0.5 ml of 10% sodium bicarbonate was added to each and volume was made to 10ml with distilled water. The blue colour developed was measured within 90 seconds at 685 nm using Systronics 105 spectrophotometer. A blank solution was also prepared using same quantities of reagent except epinephrine.

Colorimetric estimation of Epinephrine in blood serum
0.1 ml of supernatant liquid obtained after precipitation of proteins from serum was taken in 2 sets of test tubes. One set of test tubes were cooled to 15^{o} C for 5 minutes, then 0.5ml of 10% solution of sodium bicarbonate added very slowly and contents were mixed by gently rotating the tube. Too second set of test tubes 0.5 ml of 10 % solution of sodium bicarbonate and 0.75 ml of 5 % sodium hydroxide solution were added and contents were mixed as per first. Both the sets of tubes were kept at 30 ^{o}C for 30 minutes. After 30 minutes 0.25 ml of Folins reagent was added in both the tube and finally volume was adjusted up to 10 ml with distilled water. The blue colour developed was measured within 90 seconds at 685 nm using Systronics 105 spectrophotometer for both the sets.

Potentiation of barbiturate induced hypnosis
All the five groups used for conditioned avoidance response were treated with 25 mg/kg Pentobarbitone sodium intraperitonially. The duration of loosing of righting reflexes to gaining of righting reflexes was recorded [18].

Results

Conditioned avoidance response (CAR)
Results of condition avoidance response in *Figure 1* shows there is significant improved in retention time for climbing the pole by MECP treated group in comparison to others. Response towards stress induced by electric shock was comparable with other treated group, itself suggest the potent anxiolytic action of the MECP towards stress.

Determination of epinephrine content in blood serum by colorimetric method
Results of present studies *Figure 2* suggested that there is significant lowering of epinephrine level in MECP treated group, which was the positive measure for the anxiolytic action. Increase in epinephrine content with stress by electric shock in different groups was lowered down in blood serum level in treated group according to degree of its action, which was comparable with vehicle treated group.

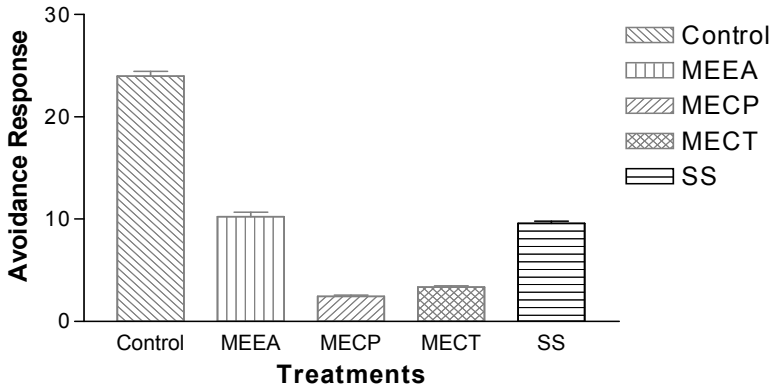

Figure 1: Effect of various aqueous extracts of shankhpushpi on CAR in RATS
All the values are in Mean ± SEM; ***p<0.001, **p<0.01, *p<0.5 compared to vehicle. All Data were analyzed by one way ANOVA; followed by bonferonni multiple comparison tests.

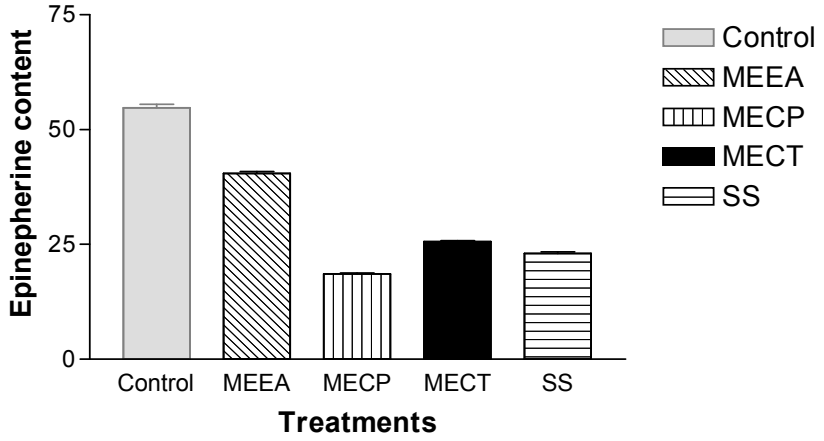

Figure 2: Effect of various aqueous extracts of shankhpushpi on epinephrine content in serum of rats
All the values are in Mean ± SEM; ***p<0.001, **p<0.01, *p<0.5 compared to vehicle. All Data were analyzed by one way ANOVA; followed by bonferonni multiple comparison tests.

Potentiation of barbiturate induced hypnosis
Result of present studies *Figure 3* shows that there is increased in sleeping time, group treated with MECP. Relaxation in stress in compare to vehicle treated improved sleeping time in extract treated group. MECP potentiates more prolonged sleeping time among extracts.

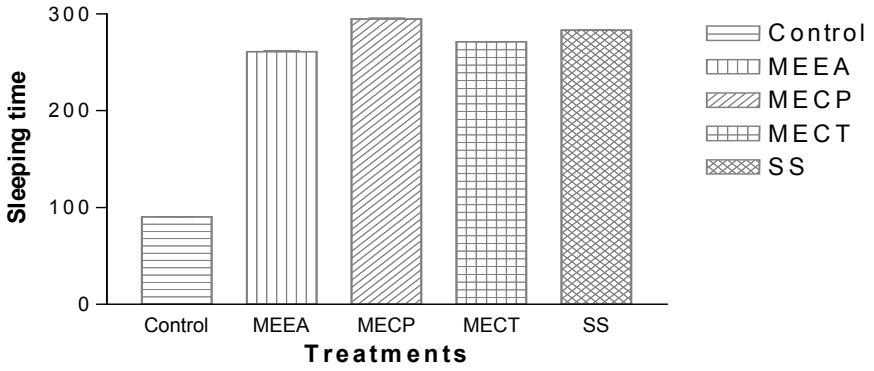

Figure 3: Effect of various aqueous extracts of shankhpushpi on Pentobarbitone induced hypnosis.
All the values are in Mean ± SEM; ***p<0.001, **p<0.01, *p<0.5 compared to vehicle. All Data were analyzed by one way ANOVA followed by bonferonni multiple comparison test

Discussion

The action of the nervous system and its subtle disruptive functioning caused by xenobiotics could be evaluated through the performance of animals in several behavioral tests [27-28]. In the Ayurvedic system of medicine, Shankhpushpi is considered as 'Medhya Rasayana' –meaning a drug which rejuvenate, maintain and potentiates intellect and memory [4, 10]. The drug shankhpushpi has been studied in the past, for its chemical composition. A significant hypotensive effect has also been reported in this drug [17, 29-31]. In the present study comparison parameters for the assessment of CNS activity among these suggest the comparative effectiveness of these commercial sources on stress condition, Epinephrine level (Level increased with stress), and sleeping time. In one of the earlier study by Prasad *et al*., 1974 suggested the role of all the endocrine and metabolic changes in the body, after stress; are mediated through the neurohumoral action. During study they found that, there is an increase in the acetylcholine level after stress, which was followed by gradual decline after subsequent period. They also found that the level of epinephrine level was higher in the blood for 2 hrs during the course [29]. Similarly in our study the level of epinephrine was found to be higher during the course of experiments in animals. But the beneficial effects of one month treatment with this drug provides significant relief in symptoms leading to improved mental function studied in terms of stress in conditioned avoidance response. Retention of stress was observed in treated groups compared to control group, as the time required to climb the pole on hearing the bell sound was found

to be decreased in case of treated groups. Reduction in epinephrine content was seen in case of animals receiving 30 days of treatment compared to the control group to substantiate the claims made. Prolongation of Pentobarbitone induced hypnosis was seen in case of animals receiving long term treatment. No significant prolongation in sleeping time could be seen in group receiving 3 days of treatment. Reduction in catecholamine level may be responsible for sedative effect of the drug. Presence of Scopoletin in the *Convolvulus pluricaulis* and preparation containing this may provide a lead for its utilization as a biomarker [32].

Conclusion

In the present study comparison parameters for the assessment of CNS activity among these suggest the comparative effectiveness of most commonly available commercial sources of shankhpushpi on stress condition, Epinephrine level (Level increased with stress), and sleeping time. As the result of present study shown potent effectiveness of *Convolvulus pluricaulis* to CNS other then two, itself suggest to use this rather then other in ayurvedic formulation, claims for CNS. As the controversy is concerned with the particular traditional name among these plants source with desired activity was resolved to some extent in the present preliminary studies. The methodology may also helpful in future for the preliminary screening of the drugs claiming, its effect on CNS. But there is still need to explore more precise parameters to develop exact mechanism of action for the activity.

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