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ROLE OF SANKHAPUSHPI SWARASA AS MEDHYA RASAYANA IN ALBINO RATS - A BEHAVIOURAL AND HISTOLOGICAL APPROACH

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ABSTRACT

Central nervous system consists of the brain and the spinal cord. It is the seat of learning, memory and intelligence. Memory is the process in which information is encoded, stored and retrieved. Neurons are the core components of the brain and spinal cord of the central nervous system. The higher functions of the brain such as learning, memory and intelligence are also experienced on the connection between neurons. In *Ayurveda* classics it is mentioned that *Sankhapushpi* (*Clitoria ternatea*) as a *Medhyadravya*. So this was an attempt made to observe whether there are any structural changes in CNS as well as behavioural changes in rats by intake of *Sankhapushpi swarasa* that may help the *Ayurveda* community to see the effect of *Sankhapushpi swarasa* as a *Medhyarasayana.* 16 albino rats of either sex was randomly selected and grouped into 2. Rats were weighed on the first day of experiment and the dose of *Sankhapushpi* was fixed and was administered for 2 months daily. During the procedure of 2 months, the rats of all the 2 groups were simultaneously subjected to various behavioural tests and were observed. On the 61st day all the animals were sacrificed by ether anaesthesia and the parts of CNS were collected and processed for histological study. Statistically significant result were seen in Open field and Behavioural despair tests with $p<0.05$. Histological examination revealed some changes in one rat in the test formulation administered group in the form of hemorrhage and infarct formation in parts of brain and some disturbance in the organization of gray matter in spinal cord. Since it was observed only in one rat not in other rats indicates that it is not linked to the test formulation administration.

Keywords: *Sankhapushpi, Medhya, Open field, Behavioural despair.*

INTRODUCTION

Ayurveda, the view of ancient scholars giving due emphasis for the maintenance of health as well as cure of diseases enabled them to enquire in depth about the mind and intellect. The analysis of mind and intellect from different angles and their explanations are abundantly available in *Ayurvedic*

literatures which provide evidence of the quantum of thoughts, crystal clear vision as well as experience of the ancient scholars.

A long healthy life has been the cherished wish of man since antiquity. Accordingly, if longevity is desired there must be a system of rejuvenation. *Rasayana Tantra* is one which deals with delaying ageing, increasing intellect and strength, prolongation of life and curing of disorders. *Rasayana* therapy has been described as a systemic and scientific medical discipline for people who desire longevity and great results were claimed by this therapy.

Ayurveda has its own principle which can prove a great solution for many problems concerned with mind and body. It specially defines a class of CNS modulating drugs called *MedhyaRasayana*. *MedhyaRasayana* described in *samhithas* are primarily meant for enhancement of intellectual function, which require reconsideration and application in present scenario as it can be powerful solution for many of the psychosomatic problems.

The changes in lifestyle and circumstances have changed the face of modern man. For the successful survival of man in this competitive world there is a need for promotion of mental health and proper management of various psychosomatic problems. *Medha* affects the *hitaayu* and *sukhaayu* which is essential for fulfilment of the motive of *Ayurveda*. The promotion of *Medha* has become a necessity of everyone. This is possible when the concept of *Medhya Rasayana* is understood properly.

In *Ayurveda* classics it is mentioned that *Sankhapushpi* (*Clitoria ternatea*) as a *Medhya dravya*[1]. Also *Acharya Charaka* commented that *Sankhapushpi* is the drug par excellence for the promotion of intellect[2]. For an experience to become part of memory, it must produce persistent structural and functional changes that represent the experience in brain[3].

So this was an attempt made to observe whether there were any structural changes in CNS as well as behavioural changes in rats by intake of *Sankhapushpi* that may help the *Ayurveda*community to see the effect of *Sankhapushpi* as a *Medhya Rasayana* which was the main intension of the study

METHODOLOGY

Materials and methods

Experimental studies were conducted on 200g-250g weighing Wistar albino rats that were bred in SDM Research Centre, SDM Ayurveda College Udupi. They were given standard food pellets and water as food. These rats were subjected to a routine cycle of 12hrs of light and darkness within a controlled environment of temperature and humidity.

INCLUSION CRITERIA:

- Healthy albino rats weighing 200g-250g
- Rats from either sex were selected.

EXCLUSION CRITERIA:

- Unhealthy and injured rats.
- Pregnant rats.
- Rats which were under other clinical trials.

Animal grouping

Group	Drug Used	Group	Number Of Rats
G-1	Normal Diet With Water	Control Group	8
G-2	Normal Diet With Water And *Sankhapushpi*	Trial Group	8

METHOD OF COLLECTION OF DATA: -

Animal species : RATS

Strain : Wistar albino

Source: Animal house attached to SDM Research Centre,
SDM Ayurveda College, Kuthpadi, Udupi

Numbering and identification: The animals were marked with saturated picric acid solution in water for proper identification. The marking within the cage is as follows.

Animal marking: Head, Neck, Body, Tail.

Place of work - pharmacology laboratory of S.D.M. Centre for Research in Ayurveda & Allied Sciences Kuthpadi, Udupi.

Ethical clearance - The experiment was carried out in conformity with the Institutional Animal Ethics Committee (IAEC) after obtaining its permission with reference number: **SDMCRA/IAEC/AL-SR-01**

Materials

Drug: - *Sankhapushpi* grown in and around Moodbidri was used for the trial.

EQUIPMENT:

1. Syringe (3ml)
2. Gavage needle no: 14
3. Weighing balance
4. Pair of gloves
5. Stop watch
6. Blotting paper
7. Glass beaker
8. Picric acid (to stain rats for identification)

DOSE:

Normal human dose: – 1 *karsha* (12g)

Animal dose:-

Standard dose conversion formula=Human dose x 0.018 x 5/kg body weight

ROUTE OF ADMINISTRATION: Oral route

PRE PROCEDURE:

a) Housing condition

The rats were housed in the animal house of SDM AyurvedaCollege. These were kept in a cage made of Poly Propylene with stainless steel top grill. 4 animals were housed in a cage. For one week prior to dosing allowed acclimatization of them to laboratory conditions. Temperature in the experimental animal room was maintained at 23 ± 10ºC, with humidity between 50-70%. Daily lighting sequence of 12 hours light and 12 hours dark was maintained.

b) Feeding and Water

The rats were fed with rats pellet (Sai durga feed, Bengaluru) and water throughout the study period.

c) Bedding

Dry husk of paddy were used and was changed daily.

d) Grouping of animals

16 Wistar albino rats of either sex were randomly grouped into 2 groups. In each group the animals were marked with yellow colour to different body parts to permit individual identification. Each group of 4 animals were kept in separate polypropylene cages denotes the number from 1 to 4 respectively

e) Dose

Normal human dose: – 1 *karsha* (12g)

Animal dose:-

Standard dose conversion formula=Human dose x 0.018 x 5/kg body weight

f) Drug administration

A pilot study was done before commencing the experiment with *Sankhapushpi Swarasa* where there was incidence of death of rats due to aspiration. The main objective of the study was to find the effect of Sankhapushpi. Hence it was decided to administer Sankhapushpi in *Swarasa* form as it was feasible for rats.

Group 2 was administered with *swarasa* of Sankhapushpi for 60 days including experiment day in the morning session between 9-10 am orally. The test drug was evaluated for its effect on gross behaviour, open field and behaviour despair on the 15^{th}, 30^{th}, 45^{th} and 60^{th} days. On 61^{st} day all animals were sacrificed under over dose of ether anaesthesia. The head was opened through midline incision to record the autopsy changes followed by dissecting out the brain and spinal cord. The brain and spinal cord was transferred into sample bottles filled with 10% formalin for the purpose of histological study. The details of the methods employed for assessing behavioural changes were as follows:

Gross Behaviour

Effect of *Sankhapushpi* on the Gross behaviour of rats was assessed by administering it to the rats in graded dose. Behavioural changes at different time intervals after drug administration was assessed by multidimensional observation procedure described by Clara Morpurgo 1971.

This was assessed in both groups of rat (trial and control). In control group only tap water was given. The procedure involved assigning scores on 0-3 point scale as per the average intensity of the phenomenon observed. The test drug was administered one hour before the experiment. There after observations were made four times (1, 3, 4 and 24 hours). The rats were placed one by one in the centre of three concentric circles drawn by chalk on a rubber sheet diameter 7cm, 9cm and 13cm. The following was the group of activities that was recorded after exposing each to test arena.

CNS DEPRESSION

- Hypo activity
- Passivity
- Relaxation
- Narcosis
- Ataxia

ANS EFFECTS

- Ptosis
- Exophthalmus

CNS STIMULATION

- Hyper activity
- Irritability
- Stereotypy
- Tremors
- Straub tail
- Analgesia

Behavioural 'despair' test in Rats

Drug was administered according to the grouping. Each rat was placed gently into a glass cylinder about 41 cm high, 15 cm in diameter, filled up to 30 cm with water. After 1 hour of drug administration, observations were noted down for 6 minutes. First two minutes was not considered for reading and was considered as the period required for stabilizing the animal behaviour. The limb movements and the effort of the rats to get out of the cylinder in the next 4 minutes was noted and subtracted later from total time (4 min) to find out the duration of immobility. This was considered as index of depression.

Open field Behaviour

This test was carried out using the open field behavior apparatus as described by Bhattacharya et. al., 1993. The apparatus is a square box of 96x96 cm and with side walls about 30 cm high. The floor is divided into 36 equal squares. It was kept in a dimly lit and quite area during the experiment. Each rat was gently placed in the pre- determined corner of the apparatus an hour after drug administration and allowed to explore the arena for 5 minutes. The following parameters were noted:

- The number of rearing.
- Number of fecal pellets expelled.
- Number of squares crossed.
- Duration of immobility (freezing time).
- Time of initiation.
- Any other type of behavior (grooming, preening, sniffing etc)

OBSERVATION AND RESULTS

EFFECT OF *SANKHAPUSHPI* ON GROSS BEHAVIOUR

Decreased motor activity was observed in 1 out of the 8 rats during the 45th day of the experiment after administration of the test drug.

Depression in respiration was observed in 4 out of the 8 rats during the 45th day of the experiment after administration of the test drug.

Blanching was observed in 1 out of the 8 rats during the 60th day of the experiment after administration of the test drug.

Cyanosis was observed in 2 out of the 8 rats during the 30th day of the experiment after administration of the test drug.

Increased sensitivity for the tail pinch response was observed in 1 out of the 8 rats after 3h, 3 out of the 8 on day 15 and day 60, 5 out of the 8 on day 30 and 4 out of the 8 on day 45, after administration of the test drug.

Pilo erection was observed on 1 out of the 8 rats on the 30th day after administration of the test drug.

Irritability was observed on 1 out of the 8 rats on 24h, day 15 and day 45. Also observed on 3 out of the 8 rats on day 30 and on 4 out of the 8 rats on day 60 after administration of the test drug.

Nasal secretion was observed on 1 out of the 8 rats on day 15, 3 out of the 8 rats on day 45 and 6 out of the 8 rats on day 60 after administration of the test drug

Rearing was observed on 1 out of the 8 rats on day 30 after administration of the test drug.

EFFECT OF *SANKHAPUSHPI* ON OPEN FIELD TEST OF RATS

: On day 15

Group	Outer circle	Middle circle	Inner circle	No: of rearing	No: of faecal pellet	No: of grooming	Duration of freezing
Control	57.4 ± 9.6	1 ± 0.77	0.2 ± 0.20	10.6 ± 0.60	1 ± 0.44	4 ± 1.39	63.4 ± 25.39
Trial	60.87 ± 10.63	5.16 ± 0.40**	2 ± 0.83	23.6 ± 3.54**	0.4 ± 0.24**	42 ± 3.60**	7.33 ± 3.32*

DATA: MEAN ± SEM * Significant (p<0.05) ** Very Significant (p<0.001)

: Onday 30

Group	Outer circle	Middle circle	Inner circle	No: of rearing	No: of faecal pellet	No: of grooming	Duration of freezing
Control	76.62 ± 15.30	3.62 ± 1.97	0.62 ± 0.42	11 ± 2.15	1.87 ± 0.77	37 ± 7.3	21.5 ± 7.10
Trial	37 ± 12.40	0.67 ± 0.49	0	10.67 ± 2.70	2.5 ± 0.56	24.3 ± 8.70	9.2 ± 2.6

DATA: MEAN ± SEM * Significant (p<0.05) ** Very Significant (p<0.001)

: Onday 45

Group	Outer circle	Middle circle	Inner circle	No: of rearing	No: of faecal pellet	No: of grooming	Duration of freezing
Control	98.62 ± 12.41	3.25 ± 1.33	0.62 ± 0.32	11.62 ± 2.23	1.87 ± 0.95	13.37 ± 2.93	10 ± 7.86
Trial	43.67 ± 10.63**	0	0	7.67 ± 3.24	1.83 ± 0.54	30.83 ± 4.75**	16.5 ± 4.79

DATA: MEAN ± SEM * Significant (p<0.05) ** Very Significant (p<0.001)

: On day 60

Group	Outer circle	Middle circle	Inner circle	No: of rearing	No: of faecal pellet	No: of grooming	Duration of freezing
Control	15.4 ± 5.07	1 ± 0.76	0.25 ± 0.25	1.6 ± 0.50	4.6 ± 1.40	2.4 ± 7.36	23 ± 3.93
Trial	49.2 ± 8.59**	0	0	10.8 ± 3.18*	3 ± 0.89	38.2 ± 3.98	2 ± 1.37**

DATA: MEAN ± SEM * Significant (p<0.05) ** Very Significant (p<0.001)

EFFECT OF *SANKHAPUSHPI* ON BEHAVIOURAL DESPAIR TEST OF RATS ON DAY 15, 30, 45 & 60

Day	Immobility time (sec)		Frequency	
	Control	Trial	Control	Trial
15d	37 ± 7.05	18.66 ± 2.66*	17.4 ± 1.47	15.4 ± 1.50
30d	27.12 ± 6.23	27.83 ± 5.02	16 ± 2.45	11 ± 1.40
45d	23.37 ± 9.75	14.17 ± 3.30	8.12 ± 1.83	7 ± 0.86
60d	39.12 ± 20.13	8.5 ± 6.34*	8.4 ± 1.50	2 ± 0.70**

DATA: MEAN ± SEM * Significant ($p<0.05$) ** Very Significant ($p<0.001$)

HISTOLOGICAL FINDINGS:

Normal cyto-architecture was observed in both trial and control groups except moderate infarct formation in cervical region of spinal cord in trial group as well as changes in gray matter in cervical region of spinal cord of control group was noted.

DISCUSSION ON EXPERIMENTAL STUDY

In the present study an attempt has been made to evaluate the *Medhya* effect of *Sankhapushpi in* Wistar albinorats by conducting Gross behaviour, Open field and Behavioural despair tests respectively by assessing the behavioural changes. All the tests were conducted on the 15th, 30th, 45th and 60th days of the experiment duration. Though many research efforts have been undertaken to test the extracts and isolates from this renowned plant study using the traditional form of its administration have not been reported hence this study on *Sankhapushpi*. Further instead of single dose the effect of repeated dosing was assessed since in clinical settings it is seldom given as a single dose. The parameters assessed mainly focused on effect on general behaviour, assessment of anti-anxiety and anti-depressant activities.

Extracts of *Clitoria ternatea* have been used as an ingredient in *'Medhya Rasayana'* as a rejuvenating recipe used for treatment of neurological disorders and considered as wholesome for intellect. *Clitoria ternatea* has been shown to improve learning, memory and also increase the acetylcholine content of the hippocampus in rats[4].

The major phytoconstituents found in *Clitoriaternatea* are the pentacyclictriterpenoids such as taraxerol and taraxerone. Phytochemical screening of the roots shows the presence of ternatins, alkaloids, flavonoids, saponins, tannins, carbohydrates, proteins, resins, starch, taraxerol and taraxerone. Leaves contain 3 monoglucoside, 3-rutinoside, 3-neohisperidoside, 3- o- rhamnosyl Glycoside, kaempferol- 3- o-rhamnosyl, aparajitin, beta-sitosterol, and essential oil[5]. There may be anxiolytic effect for the drug due to the presence of kaempferol- 3- o-rhamnosyl as a chemical constituent. *Clitoria ternatea* was found to possess nootropic, anxiolytic, antidepressant, anticonvulsant and antistress activity. *Clitoria ternatea* significantly increased acetylcholine content in the hippocampi of rats. Increase in acetylcholine content in their hippocampus may be the neurochemical basis for their improved learning and memory[6].

Gross behaviour

This assessment was done on the first day and twenty four hours after administration of trial drug upon the trial group rats. Parameters assessed or the observations were depicting CNS stimulating factors such as irritability and an increased response to pain. The test was followed on the 15th, 30th, 45th and 60th days of the experiment. It is a simple but versatile test which provides useful data on a drug's influence on the CNS. Though which the drug or formulations for CNS stimulation, depression, stereotypy, analgesic, and autonomic nervous system effects can be assessed. Analysis of the data shows that the only effect observed was irritability behaviour in some rats in test formulation administered group with increased nasal secretion. The parameters mentioned above were not affected. This clearly

shows that the drug per se has no impact on observable behaviour.

Open field test

Rats do like to remain or hide in a secure place where there won't be any interference. Usually they prefer dark areas. The open field test is done in a dimly lit room using the apparatus. The area is set so for the rats to feel as if it is in a secure place. This extensively used test provides information about the manner in which the drug or formulations influences the CNS and the behavioural pattern.

Total number of squares crossed – provides information about CNS stimulation or depression. Increase, generally indicates stimulation, decrease in the crossings indicates depression. However, when the action is complex like stereotypy the horizontal movements may be less and rearing and grooming may become higher. Faecal expulsion- more expulsion indicates anxiety and less indicates anxiolytic activity.

Duration of freezing (indicator of anxiety) - longer duration is an index of anxiety whereas shortened duration indicates anxiolytic activity. The data generated during the present study were analyzed in the background of the above presumptions.

Number of squares crossed - a complex time dependent activity pattern was recorded. During the phase I study conducted on 15th day, significant increase in the crossings in the middle squares was observed which indicates decreased anxiety, while the general crossings were not affected to significant extent. This indicates selective probable anti-anxiety activity, which is corroborated by anxiety specific parameters like freezing time. However, these effects were not observed during 30th day of testing. On 45th day of testing significant decrease was observed in relation to the heightened activity in control group. On 60th day increase was observed in relation to the control group in which the activity was much less in comparison to earlier testing phases. This indicates that the test formulation has complex time and dosing frequency dependent changes on the CNS and hence close clinical observation would be useful to fine tune the therapeutic utility.

Effect on rearing and grooming - Number of rearing and grooming significantly increased on 15th day of testing which was not seen on 30th day. Grooming behaviour tended to be much higher during 45th and 60th day of testing also. While rearing behaviour episodes remained constant however, the rearing was comparatively higher on 60th day in comparison to the control group in which decrease in total activity was observed. These lowered values in control group may be due to other extraneous influences.

Continuous rearing along with grooming may be indicative of stereotypy. Increased grooming with increased faecal pellet expulsion and freezing duration may be indicative of anxiety. Further some of the authors[7] are of the opinion that grooming in open field test is interplay between freezing, rearing and crossings. It is explained that once exposed to the novel situation in Open Field Test the rats tend to freeze which later is replaced by exploratory behaviour like rearing and grooming. The above activity profile indicates probably the test formulation may produce weak stereotypy. However, it may also be linked to significant decrease in the duration of freezing time indicative of anxiolytic activity. This seems more plausible because increased rearing was seen mainly during the first phase.

Anxiety related parameters - faecal pellet expulsion and duration of freezing.

The number of faecal pellets and duration of freezing was found to be significantly reduced when tested on 15th day of drug administration which clearly indicates presence of significant anti-anxiety activity of the drug at this time period. However, this effect was not apparent on 30th day of testing but on 60th day significant decrease in duration of freezing was observed. This shows that anti-anxiety activity is retained when administered on long term basis. These observed effects may be due to the presence

of kaempferol- 3- o-rhamnosyl as a chemical constituent with other similar constituents.

Behavioural despair test

In this test the duration of immobility was considered as an index of depression. Data obtained when compared with the initial day and final day revealed a significant decrease in the immobility time and a very significant decrease in the frequency of immobility by the trial group rats compared to the control group. This indicates the anti-depressant property of the *Sankhapushpi*.

DISCUSSION ON HISTOLOGY

Histological examination revealed some changes in one rat in the test formulation administered group in the form of hemorrhage and infarct formation in parts of brain and some disturbance in the organization of gray matter in spinal cord. Since it was observed only in one rat not in other rats indicates that it is not linked to the test formulation administration.

Sankhapushpi, a well-known *medhya* drug used is for obtaining different central nervous system (CNS) effects especially memory enhancement and anti-anxiety activity. Different plants are used under the name *Sankhapushpi* in different regions of India. Commonly used source plants are - *Convolvulus pluricaulisChois*, *Evolvulusalsinoides Linn*, both from *Convolvulaceae*, and *Clitoriaternatea Linn*. (*Leguminosae*). Further it is administered on repeated doses and in classical forms. Most of the studies are on single dose administration conducted with extracts and active principles which is not the form used in *Ayurveda*. In the present study, the effect of *Sankhapushpi* form one of the sources *Clitoriaternatea* was studied in rats on chronic dose administration. The results obtained confirm the presence of significant anti-depressant and anti-anxiety activities on both short and long duration of administration. However, the duration of administration has to be carefully monitored as per specific clinical requirements, since some of the effects may taper off at certain periods.

CONCLUSION

All the above said points are concluded in the section of conclusion on the basis of available *Ayurveda* and modern literatures and the data of present study on the completion of any work the achievements as well as the drawbacks have to be narrated these can be placed under the umbrella of conclusion.

- In the present study *Sankhapushpi* has shown better results compared to control group.
- Behavioural assessments done substantiate that *Sankhapushpi* was found to possess, anxiolytic, antidepressant, and antistress activity. *Sankhapushpi* is an herbal drug which has the potential to modulate CNS activities.
- Histological examination didn't show any significant changes.
- Hence behavioural changes noticed but no structural changes were noticed on administering *Sankhapushpi Swarasa*.

REFERENCES

1. Dr S Nesamony, Oushadha Sasyangal, revised 12th edition, The State Institute of Languages Kerala, p 472
2. Dr Ram Karan Sharma, Vaidya Bhagwan dash, Agnivesa's Charaka Samhitha Chikitsasthana, Chowkhambha press Varanasi 2007, p 46.
3. Richard L drake, Gray's Anatomy for Students, Churchill Livingstone, p 16.
4. Acetylcholinesterase inhibitor from Clitoria ternatea V Kumar[1], K Mukherjee [1], BC Pal [2], PJ Houghton [3], PK Mukherjee [1]
5. PHARMACOGNOSTICAL, PHYTOCHEMICAL AND PHARMACOLOGICAL REVIEW ON *CLITORIA TERNATEA* FOR ANTIASTHMATIC ACTIVITY Neelmani Chauhan*, Sourabh Rajvaidhya and B. K. Dubey Department of Pharmacognosy, T.I.T. College of Pharmacy, Bhopal-462 021, Madhya Pradesh, India
6. Taranalli AD and Cheeramuzhy TC. Influence of

Clitoria ternatea extracts on memory and central cholinergic activity in rats. Pharm Niol 2000;38(1):51-56.

7. SiraDíaz-Morán, CelioEstanislau, Toni Cañete, Gloria Blázquez, Andrea Ráez, Adolf Tobeña1, Albert Fernández-Teruel. Relationships of open-field behaviour with anxiety in the elevated zero-maze test: Focus on freezing and grooming. World Journal of Neuroscience. 2014; 4 : 1-11

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