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EFFECTS OF SWARNAPRASHANA ON HIPPOCAMPUS OF ALBINO RATS - AN EXPERIMENTAL STUDY

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ABSTRACT

Introduction: According to ***Achraya Kashyapa***, by the intake of gold for 1month starting from Pushyanakshatra increases memory and the child becomes ***"Parama medhavi".*** For an experience to become part of memory, it must produce persistent structural and functional changes that represent the experience in brain. So this study was undertaken by administering ***Swarnaprashana*** with an intension to observe the structural changes in various parts of brain. **Methods:** 18 albino rats of either sex was randomly selected and grouped into 3 different groups. All the rats were weighed on the first day of experiment and the dose of *Swarnaprashana* was fixed and was administered for 1 month daily. At the end of 30th day all the animals were sacrificed by ether anesthesia and the parts of CNS were collected and processed for histological study. **Result:** Histologically Control group showed normal cellularity, Group1 showed moderate increase in cellularity- normal cytoarchitecture. Group2 showed increased cellularity in Dentate gyrus.

Keywords: *Swarnaprashana, SwarnaBhasma,* Memory, Hippocampus

INTRODUCTION

Human nervous system is the chief controlling and coordinating system of our body. It is responsible for judgment, intelligence and memory. Memory is one of our major mental activities. The brain is capable of storing and receiving both short term and long term memories.[1] **The hippocampus** is essential (for learning new information) to the consolidation of information from short-term to long-term memory, although it does not seem to store information itself.[2] It is widely accepted that brain consists of some kind of permanent changes in the synapse in a specific circuit of neurons when the memory centers are triggered repeatedly.

It may be due to two reasons:

1. Increase in the number of pre-synaptic axon terminals or increase in number of receptors in postsynaptic neurons.[3]
2. Changes in the concentration of neurotransmitters or functions of astrocytes.

Kaashyapa has given much importance to Child's health. *SwarnaPrashana* has a tremendous effect on prevention of disease and intelli-

gence of a child, according to him *Swarnaprashana* can be started from day one of life. It has been further mentioned that if it is taken daily for a month, child becomes extremely intelligent *(parama Medhaavi)* and occurrence of disease is not observed ("*Vyadhibi Na Cha Drushyate*") *SwarnaPrashana* has been traditionally practiced across India as a recipe for child growth and memory enhancement and also to promote longevity.[4]

Swarna prashana contains *Swarnabhasma, Ghrita and Madhu* which were proved to be having memory (*medhya*) effect by various studies conducted in the field.

In experimental studies *Swarnaprashana* is proved to have good behavioral, spatial memory and reference memory changes, along with weight gain and immunomodulatory effects in albino rats. But whether *Swarnaprashana* produces any structural changes in brain either cytostructure or the synaptic changes is still unknown.

For an experience to become part of memory, it must produce persistent structural and functional changes that represent the experience in brain, so present clinical study was undertaken entitled **"Structural Changes In Cns Wsr To *Medhya* Effect Of *Swarnaprashana* An Experimental Study"** to prove its effect as *Medhyarasayana*.

AIM

- To study the histological changes in various parts of brain of albino rats after the administration of *Swarnaprashana.*

METHODOLOGY

MATERIALS AND METHODS:

Healthy young wistar albino rats of either sex weighing about 100-150gms were selected and divided into 3 groups. The animals were obtained from the animal house attached to S.D.M centre for research in Ayurveda and allied sciences .The selected animals were maintained under prevailing husbandry conditions. They were fed Sai durga feed Bangalore 'Amrut' brand rat feed and water given *adlibitum*. The experiments were undertaken after obtaining permission from the institute's animal ethics committee permission (SDMCRA/IAEC/MB-SR-01.) And as per CPSEA guidelines.

Table 1: Animal grouping: Each group had 8 albino rats and were kept in separate cages.

GROUP	DRUG USED	METHOD	NUMBER OF RATS
G-1	NORMAL DIET AND WATER	CONTROL GROUP	8
G-2	*GHRITA* AND *MADHU*	TRIAL GROUP-1	8
G-3	*SWARNAPRASHANA (SWARNABASMA+GHRITA+MADHU)*	TRIAL GROUP-2	8

Test drug-1 : *Ghrita + Madhu*

Test drug -2: *Swarna bhasma* (purchased from Shree Dhoot paapeshawar Pharmacy (Batch Number P16040035 with *Ghrita* and *Madhu*

Dose fixation

The dose of *Swarna Bhasma* 1/4 ratti (31.25mg) selected according to *Rasaratnasamuchaya.* The dose for experimental study was calculated by extrapolating the human dose to animal dose based on the body surface area ratio by referring Puget's and Barnes (1964) chart.

Rats Dose: Therapeutic human dose x surface area ratio (convertibility factor)

- single dose *Swarnaprashana*

= Therapeutic human dose x 0.018x50(per kg body weight)
= 31.25 x 0.0026 x 5
= 2.8125÷1000=0.0028mg/gm

- single dose *Ghrita Madhu* group
  8ml *Ghrita* was mixed with 6ml *Madhu*
  = 1ml/100gm

g) Drug Preparation:

For test group 3, 3milli gram *Swarna bhasma* was taken and was mixed with 6ml of *Ghrita* and 4ml *Madhu*. For test group 2, 9ml of *Ghrita* was taken and was mixed with 6ml of *Madhu.*

h) Drug Administration:

Control, group2 and test group3 were administered for 30 days including experiment day in the morning session between 9-10 AM orally.

EXPERIMENTAL PROTOCOL:

The test formulation was administered orally once a day for 30 consecutive days. Assessment of behavioral changes was done weekly.

Weight of rats was calculated on the last day. Rats of test group II and III were subjected to Digital cook's pole test and Morri's water maze tests for last 5 consiquitive days.

On 31st day, all animals were scarified under over dose of ether anesthesia. The head was opened through midline incision to record the autopsy changes followed by dissecting out the brain, spinal cord and weighed. The brain was transferred to bottles containing 10% formalin for the purpose of Histological study.

OBSERVATION AND RESULTS

HISTOLOGICAL EXAMINATION:

Mid brain /Hippocampus

Microscopic examination of the mid brain sections from control group was carried out and the profile was compared with the microscopic profile of sections from test group. Remarkable difference between control group sections and sections from group 2-3 test groups was identified.

Table 2: Control group:

Rat number	DG	Hilus	CA1	CA2	CA3 and CA4	Over all inference
1	N	N	N	N	N	Less cellulaity
2	N	N	N	N	N	Normal cellularity
3	N	N	N	N	N	Less cellularity
4	N	N	N	N	N	Less cellularity

N- normal, DG (Dentate Gyrus): granular layer, molecular layer, polymorphic layers

Rat 1: pyramidal cells normal cells more than deeply staining cells; cellularity less, no degenerative changes; DG: GL, ML and PL exhibit less cytoarchitecture

Rat 2: pyramidal cells normal cells more than deeply staining cells; cellularity normal, no degenerative changes; DG: GL, ML and PL exhibit normal cytoarchitecture

Rat 3: pyramidal cells normal cells more than deeply staining cells; cellularity low, no degenerative changes; DG: GL, ML and PL exhibit normal cytoarchitecture with comparatively less cellularity

Rat 4: pyramidal cells normal cells more than deeply staining cells; cellularity low, no degen-

erative changes; DG: GL, ML and PL exhibit normal cytoarchitecture and cellularity

Table 3: Group 2 (*Ghrita+Madhu*)

Rat number	DG	Hilus	CA1	CA2	CA3 and CA4	Over all inference
1	N	N	N	N	N	Normal cellulaity
2	N	N	N	N	N	Normal cellularity
3	N	N	N	N	N	Less cellularity
4	N	N	N	N	N	Less cellularity

Group 2:

Rat 1: pyramidal cells normal cells more than deeply staining cells; cellularity normal, no degenerative changes; DG: GL, ML and PL exhibit normal cytoarchitecture

Rat 2: pyramidal cells normal cells more than deeply staining cells; cellularity normal, no degenerative changes; DG: GL, ML and PL exhibit normal cytoarchitecture

Rat 3: pyramidal cells normal cells more than deeply staining cells; cellularity low, no degenerative changes; DG: GL, ML and PL exhibit normal cytoarchitecture with comparatively less cellularity

Rat 4: pyramidal cells normal cells more than deeply staining cells; cellularity normal, no degenerative changes; DG: GL, ML and PL exhibit normal cytoarchitecture and cellularity

Table 4:- Group 3(*Swarnaprashana*)

Rat number	DG	Hilus	CA1	CA2	CA3 and CA4	Over all inference
1	N	N	N	N	N	Good cellulaity
2	N	N	N	N	N	Good cellularity
3	N	N	N	N	N	Good cellularity
4	N	N	N	N	N	Normal cellularity

Rat 1: pyramidal cells normal cells more than deeply staining cells; cellularity normal, no degenerative changes; DG: GL, ML and PL exhibit normal cytoarchitecture. Overall cellularity is good

Rat 2: pyramidal cells normal cells more than deeply staining cells; cellularity normal, no degenerative changes; DG: GL, ML and PL exhibit normal cytoarchitecture; Overall cellularity is good

Rat 3: pyramidal cells normal cells more than deeply staining cells; cellularity low, no degenerative changes; DG: GL, ML and PL exhibit normal cytoarchitecture with; cellularity is good.

Rat 4: pyramidal cells normal cells more than deeply staining cells; cellularity normal, no degenerative changes; DG: GL, ML and PL exhibit normal cytoarchitecture and cellularity

Microscopic examination of the hippocampal sections from control group was carried out and the profile was used to compare the microscopic profile of sections from test group. Group 2; moderate cellularity- normal cytoarchitecture. Group 3 increased cellularity in Dentate gyrus.

Fig-4 shows photomicrographs of representtive sections from different group

DRUG PREPERATION

FIG 1: *Swarna bhasma* weighed

FIG 2*: Swarnaprashana(Swarna bhasma+Ghritha+Madhu)*

DISSECTED PICTURES

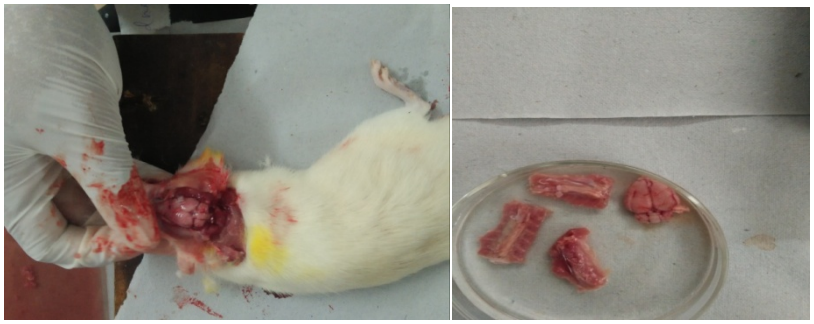
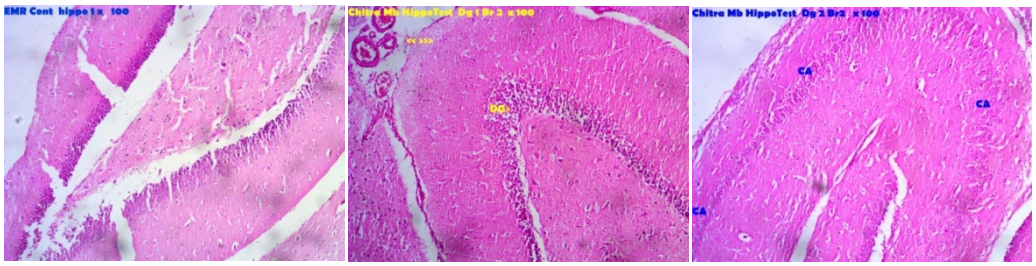


FIG 3: Brain and spinal cord

Fig4. HIPPOCAMPUS (HISTOLOGY)

GROUP1 GROUP2 GROUP3



DISCUSSION

Neuroanatomy can be studied under two different headings:-

a) Gross anatomy-the study of the surface features and internal structure of Brain and Spinal cord that can be seen with the naked eye

b) Microanatomy- Studying of minute structures of Brain and Spinal cord with the help of Microscope which cannot be visible through naked eye.[5]

Histology is the study of microscopic anatomy of cells and tissues of animals. *Swarnaprashana* is considered to be the *Medhya* drug having nootropic activity, so in the present study histology of Brain and Spinal cord was done to observe whether *Swarnaprashana* is potent enough to produce cyto- structural changes in CNS especially in the Hippocampus which is the main region for memory and learning.

CEREBELLUM:

Cerebellum from the control group was found to have normal cytoarchitecture with clear distinction of different layers viz., outer white matter, purkinje layer comprising of large purkinje layer, granular layer with numerous thickly populated cells and molecular layer. The cerebellar sections from the test Groups from Group 2 and Group 3 were found to have a cytoarchitecture similar to the control group. No gross or remarkable change could be observed

FOREBRAIN

Microscopic examination of fore brain sections from both normal control and test groups carried was out at different magnifications. The focus was on neurofil proportion, outer granular layer, pyramidal layer and blood vessels. Control group sections exhibited normal profile- with distinct outer granular layers, blood vessels and pyramidal layers. The cytoarchitectural profile of control group was taken as reference for interpreting the profile of sections from test drug administered groups. In group2 the fore brain profile was similar to the control group profile. In fore brain sections from group3 cellularity in both outer granular and pyramidal layers was comparatively higher in comparison to the control group

HIPPOCAMPUS:

The hippocampal formation occupies venteroposterior and ventrolateral part of the cerebrum. The hippocampal formation is subdivided into hippocampus propius, dentate gyrus and subiculum. Pyramidal cells are the dominant cell types in hippocampus propius and subiculum; granular neurons are the major cell type in DG*-dentate gyrus. The hippocampus proius is divided into different sections called CA*- (Cornu Ammonis or Ammon's Horn) viz- CA1, CA2, CA3 and sometimes CA4

Microscopic examination of the hippocampal sections from control group was carried out and the profile was used to compare the microscopic profile of sections from test group. Group 2 and Control group showed moderate cellularity-normal cytoarchitecture, whereas Group 3 showed increased cellularity in Dentate gyrus.

SPINAL CORD:

In Human being Spinal cord has less role to play with respect to Memory, but in lower animals like wistar albino rats spinal cord contributes to memory with memory centres being triggered during various learning activities.[6] So in the present study along with Brain, Spinal cord sections were observed histologically. The Spinal cord sections of the Groups2 and Group 3 were found to have a cytoarchitecture similar to the control group. No gross or remarkable change could be observed.

Toxicity:

Clinical tests revealed that *Swarnaprashana* is absolutely free from any type of toxicity as the ingredients used for its preparation are used only after their non-toxic certification. In an experimental model, it was observed that, acute oral administration of *Swarna Bhasma* showed

no mortality in mice (upto 1 ml/20 g body weight of *Swarna Bhasma* suspension containing 01 mg of drug). Moreover, chronic administration of *Swarna Bhasma* also showed no toxicity as judged by SGOT, SGPT, serum creatinine and serum urea level and histological studies.[7]

CONCLUSION

Swarna bhasma is a nanoparticle, *Ghritha* has a *"Samskarasyaanuvartana" Guna* and is fat soluble, so they both cross the blood brain barrier. Whereas *Madhu* has *Yogavaaahi Guna* and acts as catalyst.[8] In the present study *Swarnaprashana* has shown better results compared to plain *Ghritha* and *Madhu* combination. Microscopic examination of the hippocampal sections from control group was carried out and the profile was used to compare the microscopic profile of sections from test group. Control group showed normal cellularity, *Ghrita+Madhu* group showed moderate increase in cellularity- normal cytoarchitecture and *Swarnaprashana* group showed increased cellularity in Dentate gyrus.

Thus the test formulations *Swarnaprashana* and *Ghritha Madhu* exhibited good learning and memory enhancing effect and may contribute to the neuro-protective activity. This is an important finding since learning and memory are major issues now. Based on the result obtained in this study the test formulations have good potential in neurodegenerative disorders characterized by cognitive and memory deterioration. Their administration for memory effect in growing children seems to strongly justified.

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