Study Protocol

A Comparative Pharmaceutico-Analytical Study of *Saindavadhi Ghrita*Prepared with*Murchchhit and Amurchchhit Ghrita*and Evaluation of Its Antiepileptic Efficacy in Swiss Albino Mice – A Study Protocol

ABSTRACT

Background: Epilepsy accounts for a significant proportion of the world's disease burden, affecting around 50 million people worldwide. With the increased incidence rate of epilepsy, a few treatment modalities as well as formulations are being developed in recent years, but the complete recovery from the symptoms of epilepsy is not recorded till today. However, there is a search fora formulation thatshows quick and longstanding efficacy on epilepsy. *Saindavadhi Ghrita*is one such formulation mentioned in *Yogaratnakara Apasmara Chikista*.

Aim:A comparative Pharmaceutico-analytical study of *Saindavadhi Ghrita*prepared with *Murchchhit and Amurchchhit Ghrita (*MSG & ASG) and evaluation of its antiepileptic efficacy in Swiss albino mice.

Methodology: Preparation of *Saindavadhi Ghrita*will be carried out with*Murchchhit and Amurchchhit Ghrita and* an analytical study will be done.Prepared *Saindavadhi Ghrita*(MSG & ASG) will be evaluated for its antiepileptic action on Swiss albino mice.

Observations & Results:Analysis and Experimental study shall be documented and presented in the form of data, photographs, tables, charts, etc. as applicable. The data obtained will be analyzed by using a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison t-tests as a post hoc test for determining the level of significance of the observed effects.

Conclusion:MSG is expected to be to show significant antiepileptic efficacy in Swiss albino mice as compared to ASG

Keywords: *Sneha Kalpana, Ghrita Murchchhana,Saindavadhi Ghrita,* Antiepileptic study

1.INTRODUCTION

Epilepsy is defined as a condition in which a person has recurrent seizures. Seizures are episodes of disturbed brain functions that cause changes in attention or behavior. It is a chronic neurological condition caused by abnormal cerebral nerve cell activity. More than 10 million people in India and over 50 million people worldwide suffer from epilepsy. The incidence of epilepsy is between 0.3-0.5 percent in different populations throughout the world and the prevalence of epilepsy is roughly in the range of 5-10 persons per 1000[1].Epilepsy is a chronic neurological disorder and requires treatment with antiepileptic medication. While most patients with epilepsy can be treated with medication, about one-third will fail on medical treatment [2].For 70% of patients with epilepsy, drugs can control seizures. However, they can't cure epilepsy, and most people will need to continue taking medications. Adverse effects of antiepileptic drugs (AEDs) are common, can have a considerable impact on quality of life, and contribute to treatment failure in up to 40% of patients. The most common adverse effects are dose-dependent and reversible. Cognitive impairment is of particular concern, especially for patients who work or study. Idiosyncratic effects, such as skin rashes, and chronic effects, such as weight gain, can lead to high rates of treatment discontinuation and complicate clinical management. Nearly all conventional AEDs increase the risk of congenital malformations when taken during pregnancy, with valproate posing a potentially greater risk, whereas the potential teratogenicity of new-generation AEDs is largely unknown. However, further research is needed for the complete recovery of epilepsy.

According to Ayurveda principles, loss of *smriti* and loss of consciousness has been described to be the cardinal feature of the disease *Apasmara*and other neuro-developmental disorders [3-5] Ayurveda described many drug preparations to combat *Apasmara* in the form of Ghee/Oil (*Snehakalpana*); the *Ghrita Kalpana* (Ghee boiled with various medicinal plants). Various *Ghrita*formulations are used to combatingseizures along with the adverse effects of synthetic antiepileptic drugs (AEDs); which showed positive results as well as maintenance of good general health. Intractable or refractory epilepsy is defined by inadequate control of seizures despite optimal treatment with conventional medications.

Ayurveda's basic principles of disease management include dietary measures along with the medication; fasting (*Langhan*) and *Ghrita Kalpana* (medicated Ghee preparations) are one of the Management of Seizure disorder with AEDs along with *ApasmaraghnaGhrita Kalpana* as adjuvant seems to be more beneficial. In Ayurveda, many *Ghrita* formulations areavailable

in the treatment of epilepsy. However, there is a search for formulations that show quick and longstanding efficacy on epilepsy.

1.2 RESEARCH GAP

1. Epilepsy is one of the most common neurological diseases and approximately 23.4 million people are diagnosed with epilepsy each year globally[1]
2. With increased incidence rate of epilepsy, number of treatment modalities as well as formulations are being developed in recent years. In Ayurveda also many formulations are available in the treatment of epilepsy (*Apasmara*). However, there is a search for formulations which show quick and longstanding efficacy on epilepsy.
3. *Saindavadhi Ghrita*is mentioned in *Yoga Ratnakara Apasmara Chikitsa*having only 5 ingredients, are easily available drugs. The cost of *Ghrita* will also be viable, as the pharmaceutical processing of it is also easy.
4. Ingredients of the Saindavadhi *Ghrita* like *Pippali, Gomutra, Hingu are* already proved the action related to neurological disorders[6-8] However, there is no substantial evidence to prove the antiepileptic activity of compound formulation *SaindavadhiGhrita*. Hence the present study is proposed to experimentally evaluate the efficacy of *Saindavadhi Ghrita*in epilepsy.

1.3 AIM OF THE STUDY

A comparative Pharmaceutico-analytical study of *Saindavadhi Ghrita*prepared with *Murchchhit and Amurchchhit Ghrita and* evaluation of its antiepileptic efficacy in Swiss albino mice.

1.4 OBJECTIVES OF THE STUDY

1. To prepare *Saindavadhi Ghrita*with *Murchchhit and Amurchchhit Ghrita (*MSG & ASG) as per standard operative procedure.
2. To compare the quality control parameters of *Saindavadhi Ghrita*prepared with *Murchchhit and Amurchchhit Ghrita (*MSG & ASG).
3. To compare and evaluate the anti-epileptic efficacy of *Saindavadhi Ghrita*prepared with *Murchchhit and Amurchchhit Ghrita experimentally* in Swiss albino mice.

1.5 RESEARCH QUESTION

Whether *MurchchhitSaindavadhi Ghrita*is as efficacious as *AmurchchhitSaindavadhi Ghrita*in Epilepsy-induced Swiss Albino Mice?

2.MATERIALS & METHODS

2.1 MATERIALS

- Then the raw material will be authenticated with the help of chemical and instrumental analysis.
- Well-established*Rasashastra&BhaishajyaKalpana* laboratory for doing pharmaceutical work.
- Well-equipped drug analysis laboratory or source for drug analysis work
- The prepared *Saindavadhi Ghrita*(MSG & ASG) will be evaluated for its antiepileptic action on Swiss albino mice. The study would be carried out at a central Animal Research facility.

2.2 PLAN OF STUDY

2.3 PHARMACEUTICAL STUDY:

- Pharmaceutical study deals with the process of preparation of medicine starting from the collection of drugs till attaining the final product. It is divided into the following sections:

A. Collection of Raw drugs according to classical reference.

B. Authentication of the raw drugs

C. *GhritaMurchchhana*

D. Preparation of *SaindavadhiGhrita*

Ingredients	Latin Name	Part Used	Quantity
Amalaki	Emblica officinalis Gaertn	Pericarp	48 gm
Haridra	Curcuma longa Linn.	Rhizome	48 gm
Mustaka	Cyperus rotundus Linn Pennel	Rhizome	48 gm
Haritaki	Terminalia chebula Retz	Pericarp	48 gm
Bibhitaki	Terminalia bellericaRoxb	Pericarp	48 gm
Matulunga	Citrus medica L. Var	Swarasa	48 gm
GoGhrita	Cows' ghee		768 ml
Jala	Distilled water		3072 ml

Table 1: INGREDIENTS FOR *GHRITA MURCHCHHANA* [9]

2.3.1 PROCESS OF *SNEHAMURCHCHHANA* [10-12]

Kalkadravya is added slowly and gently to the vessel containing *Ghrita*.

According to quantity of *Ghrita* four parts water is added and mixed well.

*Ghrita*paka is carried out over *Madhyamagni* till appearance of *Siddhi Lakshana*of *MadhyamaPaka.*

After *Ghrita*paka*siddhi, Ghrita* is filtered and collected.

Table 2: INGREDIENTS OF *SAINDAVADHI GHRITA*[13]

Ingredients	Latin Name	Part Used	Quantity
Saindavalavana	Sodiichloridum	--	1 part
Hingu	*Ferulafoetida* Regel	Resin	
Pippali	*Piper longum* Linn	Fruit	
Go Ghrita	Cow's Ghee	--	4 parts
Gomutra	Cow's urine	--	8 parts

2.3.2 PREPARATION OF *SAINDAVADHIGHRITA*

Saindavalavana,Pippali and Hingu are pounded separately and filtered to obtain them in fine powder form and *kalka* is prepared.

Kalkadravya is added slowly and gently to the vessel containing *Ghrita* and *Gomutra*.

Ghritapaka is carried out over Madhyamagni till appearance of *Siddhi Lakshana*of *MadhyamaPaka*.

After *Ghritapakasiddhi, Ghrita* is filtered and collected.

2.4 ANALYTICAL STUDY [14]

Assessment oforganoleptic characters of *SaindavadhiGhrita*. Physico-chemical evaluation of *Saindavadhi Ghrita*on the following parameters:

- Refractive index
- Specific gravity
- Acid value
- Saponification value
- Iodine value
- Unsaponifiable matter (%)
- pH
- HPTLC

2.5 EXPERIMENTAL STUDY

Test drug: *MurchchhitSaindavadhi Ghrita*(MSG) &*AmurchchhitSaindavadhi Ghrita*(ASG)

Standard drug*: Maha-PanchaGavyaGhrita*[10]

Control: Plain *GoGhrita*

2.5.1 DOSE CALCULATION FOR TEST DRUG [15]

Mice dose was calculated based on the Human dose by using Standard Conversion Method on the bases of the body surface area ratio.

2.5.2 HUMAN DOSE

48 ml TED (Therapeutic effective dose)

2.5.3 MICE DOSE

HD(Human dose) ×0.0026×60 kg body weight

48×0.0026×60 kg body weight = 7.48 ml/Kgbodyweight.

Same formula used for calculating TED 2 & TED 1/2

2.5.4 Dose calculation for chemical inducing seizure (Pentylenetetrazol)

For PTZ general kindling method 80 mg /kg

2.5.5 Inclusive Criteria:

(1) Healthy Swiss albino mice of either sex of 4–5-week age will be considered

(2) Weighing about 30-40g

2.5.6 Exclusive Criteria

1. < 30gm > 40gm.
2. Pregnant and diseased mice
3. Mice that are under trial in other experiments

2.5.7 PROCUREMENT AND SELECTION OF ANIMALS: Required healthy Swiss albino mice,30-40 gm in weight, both male &female will be selected from the animal house for study.

2.5.8 GROUPING: A Day prior to dosing, the selected animal would be divided into different groups by randomization method. (Randomized block methods would be adopted for dosing and testing schedule-EFSA(European Food Safety Authority) Scientific Committee: 2011.

Table 3: PENTYLENETETRAZOLE INDUCED SEIZURE METHOD

S.no	Groups	Drugs	Dose	No. of Animals	Route	Duration
1	Group I- Standard	Maha-PanchaGavya*Ghrita*	7.48 ml /kg body weight	6 (3 Male & 3 Female)	Oral route	11 days
2	Group 2 - Control	Plain Go *Ghrita*	7.48 ml /kg body weight	6 (3 Male & 3 Female)	Oral route	11 days
3	Group 3- TED	MSG	7.48 ml /kg body weight	6 (3 Male & 3 Female)	Oral route	11 days
4	Group 4- TED 2	MSG	14.9 ml/ kg body weight	6 (3 Male & 3 Female)	Oral route	11 days
5	Group 5- TED 1/2	MSG	3.74 ml/ kg body weight	6 (3 Male & 3 Female)	Oral route	11 days
6	Group 6- TED	ASG	7.48 ml /kg body weight	6 (3 Male & 3 Female)	Oral route	11 days
7	Group 7- TED 2	ASG	14.9 ml/ kg body weight	6 (3 Male & 3 Female)	Oral route	11 days
8	Group 8- TED 1/2	ASG	3.74 ml/ kg body weight	6 (3 Male & 3 Female)	Oral route	11 days

TABLE 4: MAXIMAL ELECTROSHOCK SEIZURE (MES) METHOD

S.no	Groups	Drugs	Dose	No. of Animals	Route	Duration
1	Group I- Standard	*Maha-PanchaGavyaGhrita*	7.48 ml /kg body weight	6 (3 Male & 3 Female)	Oral route	7 days
2	Group 2 - Control	Plain *GoGhrita*	7.48 ml /kg body weight	6 (3 Male & 3 Female)	Oral route	7 days
3	Group 3- TED	MSG	7.48 ml /kg body weight	6 (3 Male & 3 Female)	Oral route	7 days
4	Group 4- TED 2	MSG	14.9 ml/ kg body weight	6 (3 Male & 3 Female)	Oral route	7 days
5	Group 5- TED 1/2	MSG	3.74 ml/ kg body weight	6 (3 Male & 3 Female)	Oral route	7 days
6	Group 6- TED	ASG	7.48 ml /kg body weight	6 (3 Male & 3 Female)	Oral route	7 days
7	Group 7- TED 2	ASG	14.9 ml/ kg body weight	6 (3 Male & 3 Female)	Oral route	7 days
8	Group 8- TED 1/2	ASG	3.74 ml/ kg body weight	6 (3 Male & 3 Female)	Oral route	7 days

2.6 METHODOLOGY:

Antiepileptic activity will be assessed by two different methods:

2.6.1 PENTYLENETETRAZOLE-INDUCED SEIZURE METHOD[16]

Pentylenetetrazole (PTZ) –induced seizures in mice is an accepted in-vivo model for the screening of antiepileptic drugs. Seizures are induced by the administration of 80 mg/kg, s.c. PTZ and the mice are then observed for a 60-minute period. Anticonvulsant drugs are usually tested in this model but other treatments could also be considered.

The animals would be administered Control, Standard and Test drugs to respective groups by oral route for 10 consecutive days. On the 11^{th} day one hour after drug administration, they will be subjected to chemo convulsion by injecting pentylenetetrazole (PTZ) intraperitoneally in the dose of 80mg/kg.

The effect of different treatments on the PTZ convulsion profile would be noted down. The parameters to be measured would bethe latency of onset of clonic and tonic convulsions, recurrence of the chronic or tonic convulsion, myoclonic twitches, mortality, and any other abnormal changes in the behavior. Abolishing the clonic convulsion would be considered as the index of anticonvulsant activity.

2.6.2 MAXIMAL ELECTROSHOCK SEIZURE (MES) METHOD[17]

The maximal electroshock seizure (MES) test was used to show efficacy of antiepileptic agents against partial and generalized seizure type epilepsy among therapy-resistant epileptic patients. The animals would be administered Control, Standard and Test drugs to respective groups by oral route and treated with Electro-convulsive shock 33mA, 0.2sec., using ear electrodes. A pre-test was done using the above-mentioned strength and animals showing the occurrence of Tonic Hind Limb Extension (THLE) were selected for the study. The first observation was taken after 24 hours of dosing for control and test drug groups i.e., on 2nd day. For the standard group dosing was done just 1 hour prior to observations. 2 nd day dosing was done after 60 minutes of first observations in the case of control and test drug groups. Dosing was continued for the next 5 days for all animals in each group. The last

observation was taken after 1 hour of dosing of each animal from all groups on the 7th day Duration of Tonic Hind Limb Extension (THLE) was considered as the assessment parameter.

3.OBSERVATIONS & RESULTS:

The observations noted while preparation of drug, Analysis and Experimental study shall be documented and presented in the form of data, photographs, tables, chart, etc. as applicable. The data obtained were analyzed by using one way analysis and variance (ANOVA) followed by Dunnett's multiple comparison t test as post hoc test for determining the level of significance of the observed effects.(Dunnett's multiple comparison test a statistical method for assessing whether a control group is significantly different from each of several treatment groups.)

4.DISCUSSION:

Epilepsy accounts for a significant proportion of the world's disease burden, affecting around 50 million people worldwide [1] The estimated proportion of the general population with active epilepsy (i.e., continuing seizures or with the need for treatment) at a given time is between 4 and 10 per 1000 people. Globally, an estimated 5 million people are diagnosed with epilepsy each year. In high-income countries, there are estimated to be 49 per 100 000 people diagnosed with epilepsy each year. In low- and middle-income countries, this figure can be as high as 139 per 100000. Epilepsy accounts for more than 0.5% of the global burden of disease, a time-based measure that combines years of life lost due to premature mortality and time lived in less than full health. Epilepsy has significant economic implications in terms of healthcare needs, premature death, and lost work productivity. With an increased incidence rate of epilepsy, several treatment modalities as well as formulations are being developed in recent years, but the complete recovery from the symptoms of epilepsy is not recorded till today and due to continue taking antiepileptic drugs there is a chance of getting many adverse effects.

*SnehaKalpana*is the protuberant *Kalpana* when it comes to the management of *Manasikavyadhis* like *Apasmara, Unmada* etc. It has been clearly quoted by *AcharyaChakrapani* that the therapeutic attribute of a drug can be modified by subjecting the

drug to various procedures and converting it into a desirable *Kalpana*. The main aim of these procedures is to bring about a change in the properties of the *dravya* or transformation of a crude drug into pharmacologically active compound.

In Ayurveda also many formulations are available in treatment of epilepsy (*Apasmara*). However, there is a search of formulations which show quick and longstanding efficacy on epilepsy.*Saindavadhi Ghrita*is mentioned in *Yoga Ratnakara ApasmaraChikitsa*having only 5 ingredients, are easily available drugs. The cost of *Ghrita* will also be viable, as the pharmaceutical processing of it is also easy.Ingredients of the *Saindavadhi Ghrita*like *Pippali, Gomutra, Hingu are* already proved the action related neurological disorders, so there is no substantial evidence to prove the antiepileptic activity of compound formulation *SaindavadhiGhrita*. Hence the present study is proposed to experimentally evaluate antiepileptic efficacy of *SaindavadhiGhrita*.

5.CONCLUSION:

A comparative data ofPrepared *Saindavadhi Ghrita*(MSG & ASG) with respect to pharmaceutical work, analysis and anti-epileptic study will be generated.

6.SCOPE OF THE STUDY:

- Doses forms will be used in a clinical study.
- Positive outcome of the study will facilitate the commercialization of product.

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