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# PHARMACEUTICO-ANALYATICAL STUDY OF *KUSHMANDA GHRITHA* AND ASSESSMENT OF ITS ANTI CONVULSANT ACTIVITY ON ALBINO MICE

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## ABSTRACT

*Sneha Kalpana* are the formulations where *Ghrita* and *Taila* are used as base to extract water soluble and lipid soluble active principles. *Ghrita* is considered as the best *Sneha Dravya* because of its speciality ie, "*Samskarasyaanuvartanam*" means Gritha carries the properties of Drug without leaving its own natural properties .*Ghrita* is also having Anti-oxidant property and ability to cross Blood Brain Barrier because of which *Ghrita* preparations are effectively used as Medya as well as in many psycho-somatic disorders. *Kusmanda Ghrita* is one of the such preparation, which is advised in *Apasmara* and acting as Medya. It is mentioned in *Chakradatta* in the context of *Apasmara chikitsa*[1].

**Keywords:** *Kushmanda Ghrita, Sheha kalpana ,Kushmanda Ghrita* Analytical and Pharmaceutical study, *Apasmara* ,experimental Study of Apasmara, *Epilepsy*.

## INTRODUCTION

*Sneha kalpana* medicines commonly prescribed internal and external use of a patients. Sneha preparations have better pharmacokinetic action in comparison to other dosage forms because of the lipoid nature of the bio-membranes, as lipid soluble substances readily permeate into the cells. *Apasmara* is the disease, which is third most common Neurological disorder affects 1% of the general population all over

the world. The recurrence rate of seizures after drug withdrawal is about 40%, so there is need of better management strategies for which *Ayurvedic Ghrita* preparations are needed because of *Ghrita* Preparations having capacity to Cross Blood Brain Barrier. *Kushmanda Ghrita* one such preparation mentioned by Acharya *Chakradatta* in the context of *Apasmara.* In Ayurveda also many formulations are available for treatment of *Apasmara*, however there is a search of formulations which shows quick and long lasting efficacy on epilepsy, so *Kushmanda Ghrita* is having good efficacy to treat the *Apasmara* mentioned by acharya *Chakradatta*. In this regard Pharmaceutico-analytical study of *Kushmanda Ghrita* and assessment of its anticonvulsant activity on Albino Mice was taken for study. The efficacy of *Kushmanda Ghrita* against MES (maximum electrical shock induced seizures) seizure as indicated by measuring Onset of tonic extensor seizure(sec) and Duration of tonic hind limb extension seizure (sec). These results are comparable to the effects produced by the Standard drug phenytoin.

**Pharmaceutical Preparation of *Kushmanda Ghrita* : Drugs Used for MG and KG**

| Main drugs for KG | Associated drugs for MG |
|---|---|
| *Murchita Ghrita* | *Hareetaki* |
| *Yashtimadhu kalka* | *Amalaki* |
| *Kushmanda Swarasa* | *Vibhitaki* |
| | *Haridra* |
| | *Musta* |

The Ratio 1:4:18 part of *Yastimadhu kalka* :*Murchita* Ghrita :Kushmanda Swarasa is taken respectively

**Ingredients in KG and its Quantity[1]:**

| ***Kalka*** | ***Ghrita*** | ***Swarasa*** |
|---|---|---|
| *Yastimadhu* | *Murchita Ghrita* | *Kushmanda swarasa* |
| 1 part | 4 part | 18 part |
| 250 gm | 1 litre | 18 litre |

**Murchana of Ghrita[2]** was Prepared as per Classics with *kwatha churna* of *Haritaki*, *Amalaki*, *Vibhitaki*, *Musta*, *Haridra* .Then MG was used to prepare KG.

**Method of preparation of KG** 1 litre of *Murchita Ghrita* was taken in wide mouthed stainless steel vessel, it was kept over gas stove and fire was ignited and allowed to heat over *mandagni*, till appearance of foam and later fire was put off, after cooling , *kalka-dravyas* was mixed with *matulunga* swarasa and made it into *kalka*, then added it into Ghrita and continuous stirring was done, it was followed by adding of mentioned quantity of *Kushmanda Swarasa*, then Start giving *mandagni* continuously. Frequent stirring was done to allow proper mixing of *kalkadravyas* and Ghrita, Procedure was carried out for span of 3 days till obtaining Sheha siddhi lakshana's[3] .After *Sneha Siddi lakshana* ,*Ghrita* was squeezed out of *kalka dravya* when it was luke warm with the help of cloth to avoid much loss. Now the obtained filtrate is called Kushmanda Ghrita .

**DEFINITION OF APASMARA:** *Apasmara* is defined as *Apagama* of *Smrti* associated with *Bibhatsa Cesta* due to derangement of *Dhi* and *Sattva*[4] *Apasmara* is *Apaya* of Smrithi(loss of memory)where the *Chitta*(mind)gets deranged by the aggravated *Doshas*, and gets localized in the *Hridaya*(mind) and *Deha*. When the mind gets deranged by the obstruction of *Sanjna Vahasrotas t*he following symptoms are seen. Patient enters into darkness (loss of consciousness) with his mind becoming inactive.

**SAMPRAPTI GHATAKA OF APASMARA**

The involvement of both the *Sharirika* and *Manasika Doshas* is vital for the *Samprapti. Sharirika Dosha:*

***Dosha** : Vata and Pitta – Sharirika Dosha (Ch, Chi, 10/6)*

*Raja and Tama – Manasika Dosha*

***Dushya :** Rasa Dhatu*

***Agni :** Jatharagni*

***Agnidusti** : Mandata*

***Srotas** : Samjna vaha ,Rasavaha Srotas .*

**Physio-chemical parameters of *Kushmanda Ghrita* and *Murchita Ghrita***

**Organoleptic parameters : Analytical Study**

| **Parameters** | ***Kushmanda Ghrita* Results** | ***Murchita ghrita* Results** |
|---|---|---|
| **Form** | **Liquid** | **Liquid** |
| **Colour** | **Greenish Brown** | ***Yellowish*** |
| **Odour** | **Characteristic** | **Characteristic** |
| **Taste** | ***Madhura Tikta*** | ***Madhura*** |

**Physio-chemical parameters of *Kushmanda Ghrita* and *Murchita Ghrita*[5-13]**

| Parameters | Results<br>*Kushmanda ghrita* | Murchita Ghrita |
|---|---|---|
| Specific gravity | **0.92 g/cm$^3$** | **0.91g/cm$^3$** |
| Weight | **0.95gm/ml** | **0.89gm/ml** |
| Density | **0.95kg/m$^3$** | **0.91kg/m$^3$** |
| Refractive index | **1.461** | **1.460** |
| Viscosity | **25.7mPa** | **26.5mPa** |
| Acid value | **2mg** | **0.4mg** |
| Iodine value | **15.5mg** | **22.8mg** |
| Saponification Value | **189.89mg** | **305.25mg** |
| Peroxide value | **3.8meq/kg** | **3.4meq/kg** |
| Rancidity | **Negative** | **Negative** |

**TLC of *Kushmanda ghrita***

| **Particulars** | **Steroids** | **Terpenoids** | **Flavonoids** | **Alkaloids** |
|---|---|---|---|---|
| Solvent front | 7 | 5.1 | 8 | 8 |
| Sample | 1.8&3.1 | 0.9,1.9 & 4.3 | 1 , 6.2 &7.2 | 7.1 |
| Rf values | 0.257 &0.442 | 0.176 , 0.372 &0.843 | 0.125, 0.775 & 0.9 | 0.887 |
| Reports | + | + | + | + |

**TLC of *Murchita ghrita***

| **Particulars** | **Steroids** | **Terpenoids** | **Flavonoids** | **Alkaloids** |
|---|---|---|---|---|
| Solvent front | 7.7 | 6.7 | 7.6 | 7.3 |
| Sample | 2.2 | 0.8 | 2.1 | 6.4 |
| Rf value | 0.285 | 0.119 | 0.276 | 0.876 |

| | | | | |
|---|---|---|---|---|
| Reports | + | + | + | + |

**EXPERIMENTAL STUDY.**

In the present study an In-Vivo method is used to screen the Anti-convulsant activity of *Kushmanda Ghrita* by Maximal electro shock(MES) induced seizures method by using Electro Convulsiometer.

**Experimental Methodology: Preparation of drugs according to group : Shows drugs according to groups.**

| Group | Number of Mice | Drugs | Purpose |
|---|---|---|---|
| **Group 1** | **6** | given with 2%w/v gum acacia served as receive MES (60 mA for 0.2 Sec), | **To serve as Control** |
| **Group 2** | **6** | **Phenytoin** | **To serve as Standard** |
| **Group 3** | **6** | ***Kushmanda Ghrita* Low dose** | **To serve as Trial** |
| **Group 4** | **6** | ***Kushmanda Ghrita* High dose** | **To serve as Trial** |

**METHODOLOGY:**

**Maximal electro shock (MES) method induced Seizures in mice:**

Albino mice of either sex with a body weight 22-25g were divided into 4 groups of 6 animals in each . Group 1 will serve as normal and will receive MES (60 mA for 0.2 Sec), Group 2 will be administered phenytoin (25mg/kg p.o) and serve as standard. Group 3 - 4 will be administered(1200mg/body kg wt and 2400mg/body kg wt) oral with compound *Kushmanda Ghrita* with low dose and high dose respectively for seven consecutive days. On the eighth day, one hour after oral administration of the compound/vehicle/standard drug MES seizures will be induced by electro-Convulsiometer. A 60mA current will be delivered trans auricularly for 0.2sec in mice. This current intensity should elicit complete tonic extension of the hind limbs in control mice. For recording various parameters, mice will be placed in a clear rectangular plastic cage with an open top, permitting full view of the animal's motor responses to seizure.

This current intensity should elicit complete tonic extension of the hind limbs in control mice. The onset time of seizures and duration of tonic hind limb extension and mortality for each animal was observed. Decrease in duration of hind limb extension was considered as a protective action[14,15].and Duration of tonic hind limb extension (sec.)seizures parameters were observed as end point.

**Results:-**

Table: 51 Effect of Different groups on MES induced convulsions in mice.

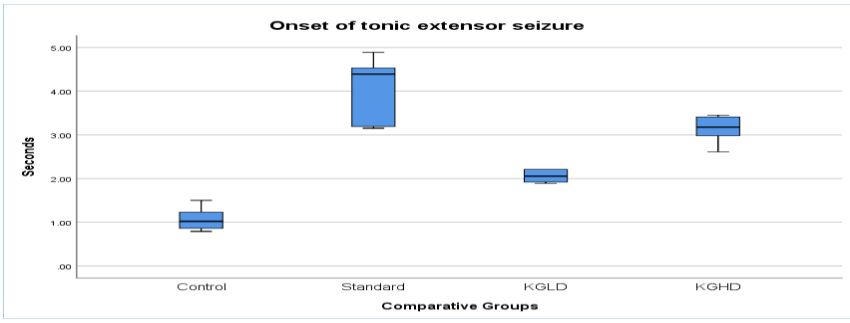
| Different time intervals comparisons | Descriptive | | | | | ANOVA | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Treatment Groups | N | Mean | ±SD | ±SE | Variance | Sum of Squares | Df | Mean Square | F | P | Remarks |
| Onset of tonic | Control (Gum of acacia) | 6 | 1.07 | 0.268 | 0.109 | Between Groups | 30.3 | 3 | 10.1 | 50.3 | <0.001 | HS |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| extensor seizure (sec) | Standard (Phenytoin) | 6 | 4.09 | 0.738 | 0.301 | | | | | | | |
| | *Kusmanda* Ghrita (Low Dose) | 6 | 2.14 | 0.304 | 0.124 | Within Groups | 4.0 | 20 | 0.2 | | | |
| | *Kusmanda* Ghrita (High Dose) | 6 | 3.13 | 0.310 | 0.127 | | | | | | | |
| Dura-tion of tonic extensor seizure (sec) | Control (Gum of acacia) | 6 | 23.47 | 1.228 | 0.501 | Between Groups | 646.6 | 3 | 215.5 | 102.86 | <0.001 | HS |
| | Standard (Phenytoin) | 6 | 10.40 | 1.263 | 0.516 | | | | | | | |
| | *Kusmanda Ghrita* (Low Dose) | 6 | 17.55 | 1.618 | 0.660 | Within Groups | 41.9 | 20 | 2.1 | | | |
| | *Kusmanda* Ghrita (High Dose) | 6 | 11.72 | 1.632 | 0.666 | | | | | | | |
| IS - Insignificant; MS - Moderately Significant; S - Significant; HS - Highly significant. | | | | | | | | | | | | |

According into statistical analysis of onset of time in tonic extensor seizure ,which is having F value 50.3 ,which is highly significant at P < 0.001 between all the groups and within the group.

In Duration of time in tonic extensor seizure ,which is having F value 102.86 ,which is highly significant at P< 0.001 between all the groups and within the group.

**Pair wise comparison According to Scheffe in homogenous subset of Onset of tonic extensor seizur**e:
**Onset of tonic extensor seizure (sec)**

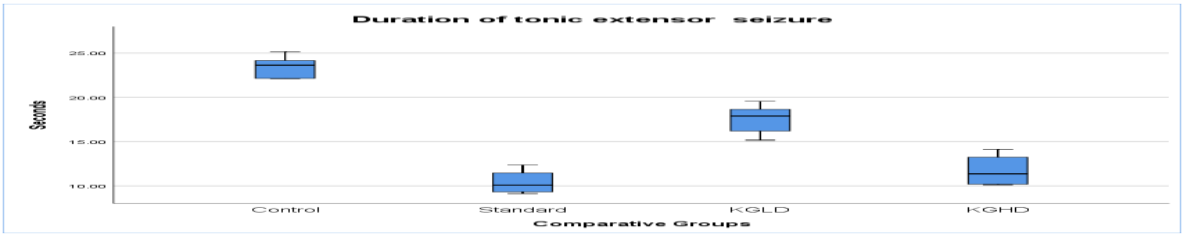



- ❖ It shows Lower dose of KG is better than control group .
- ❖ Higher dose of KG is better than lower dose (1200 mg/kg of wt)
- ❖ Phenytoin Standard group is better than higher dose of KG(2400mg/kg of wt)

❖ **Pair wise comparison According to Scheffe in homogenous subset of Onset of Duration of tonic extensor seizure:**

| **Table no: 53 Duration of tonic extensor seizure (sec)** | | | | |
|---|---|---|---|---|
| Homogeneous Subsets: Scheffe | | | | |
| Treatment groups | N | Subset for alpha = 0.05 | | |
| | | 1 | 2 | 3 |
| Standard | 6 | 10.40 | | |
| KGHD | 6 | 11.72 | | |
| KGLD | 6 | | 17.55 | |
| Control | 6 | | | 23.47 |
| Sig. | | 0.49 | 1.00 | 1.00 |
| Means for groups in homogeneous subsets are displayed. | | | | |

❖ **Duration of tonic extensor seizure (sec)**

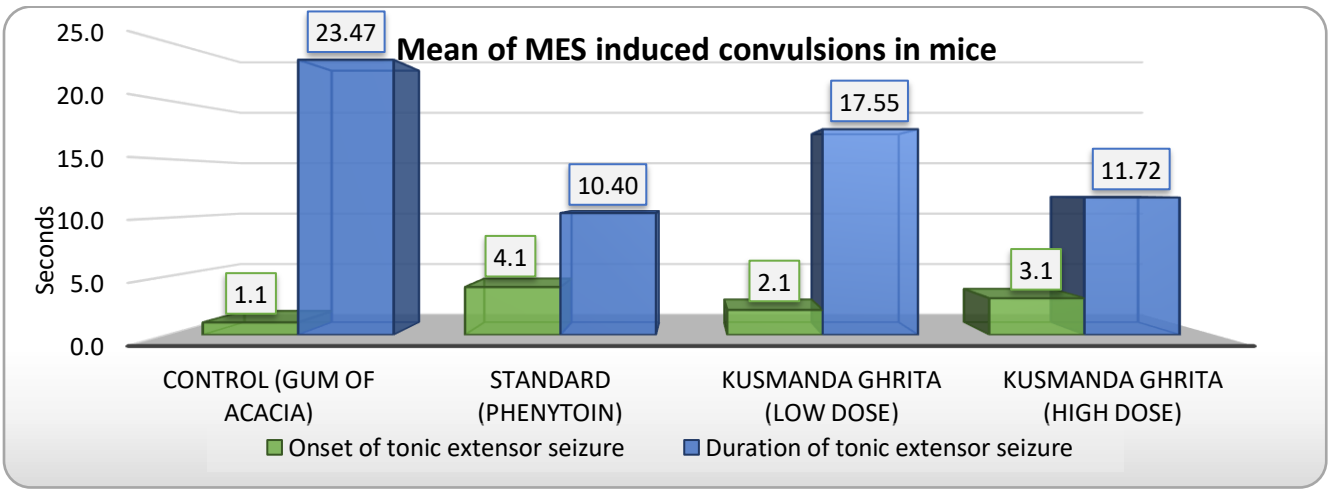



❖ **It shows** Lower dose Group of KG is better than control group

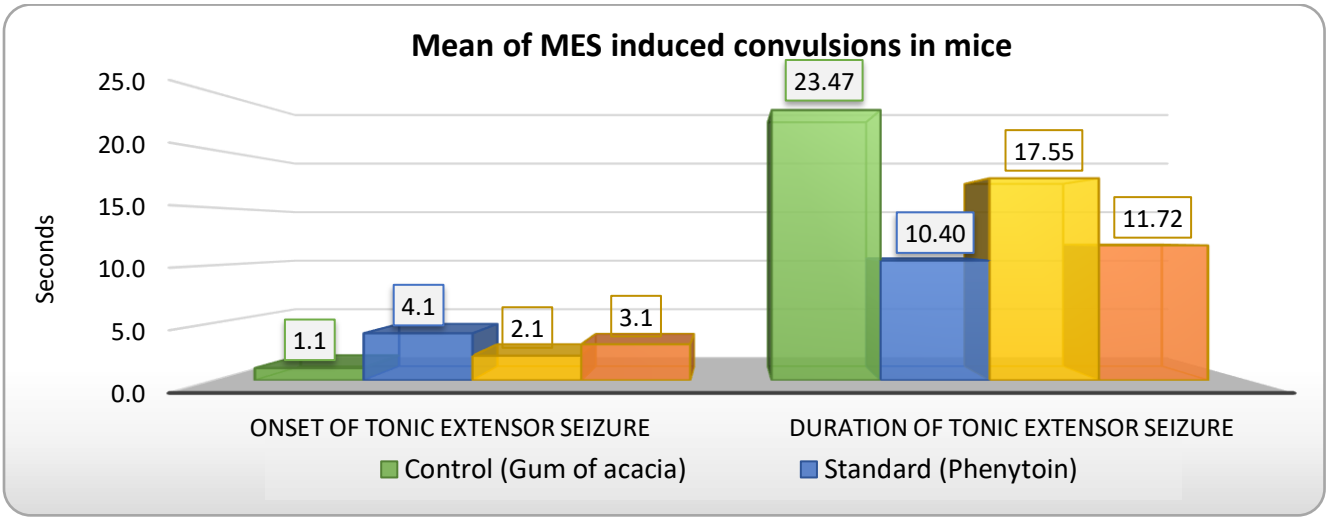
❖ Lower dose Group of KG is not equal to standard group

❖ There is no significant difference between higher dose Group and standard group .both are coming under same subset ,there is no difference between these two groups ,so it is statistically prove that Higher dose of KG is equal to Standard group in duration of tonic extensor seizure.

**Mean of MES induced convulsions in mice**



**Mean of MES induced convulsions in mice**




**Mean data of effect *Kusmanda Ghrita* on MES induced convulsions in mice**

| Sl. No. | Treatment | Dose | Onset of tonic extensor seizure (sec) | Duration of tonic extensor seizure (sec) | Death /Recovered |
|---|---|---|---|---|---|
| 1 | Control (2% Gum acacia p.o.) | 5mg/kg(p.o) | 1.080±0.1092 | 22.50±2.040 | Recovered |
| 2 | Standard (Phenytoin) | 25mg/kg (p.o.) | 4.080±0.3011*** | 10.80±4.128*** | Recovered |
| 3 | *Kusmanda* Ghrita (KGLD) | 1200mg/kg (p.o.) | 2.160±0.1240** | 14.65±1.404* | Recovered |
| 4 | *Kusmanda Ghrita* (KGHD) | 2400mg/kg (p.o.) | 3.153±0.1265*** | 12.82±1.548** | Recovered |

It was observed that, The test sample *Kusmanda Ghrita* was shown significant anticonvulsant in a dose dependent manner effect as compared to control by increasing onset time of seizures and reducing the duration of tonic extensor phase. The anticonvulsant activity was shown significant in high dose of test sample as compared to low dose of test sample. In case of onset of seizures Standard Drug is better than high dose of KG. In case of duration of tonic extensor seizure high dose(2400mg/kg of wt) of KG is equal to standard Drug Phenytoin.

## DISCUSSION

KG having *medhya* property produces good quality of Sadhaka pitta responsible for comprehension and data analysis. It uplifts the *Sattva guna* and counteracts the aggravated *rajas* and *tamas.* It acts on *agni* especially the *Sadhakagni* responsible for nutrition to brain cells by improving the process for transformation and assimilation.

KG is Agni Dipana, Dhatwagni Dipana property - Nourishes All Dhatus, By Snigdha ,Guru Guna ,Madhura Vipaka –Controls Chala And Laghu Guna Of Aggravated Vata, Due To Madhura Rasa ,Madhura Vipaka ,Madhura Veerya Pacifies Pitta And It Will Also Retaining Avalambaka Kapha Also, Elevation Of Ojus, Upliftment Of Satva Guna, Re-Establishment Of Dhee, Dhriti, Smruti, Correcting Vitiated Rajas And Tamas By Eliminating The Srotorodha, Helps In Reliving Sigh And Symptoms Of Apasmara.

## CONCLUSION

- KG is best in Vata Pittaja Apasmara
- Rf values of TLC states that the components seen in the KG was shown different places were travelled in glass slide which shows more phyto chemicals are there in KG comparatively in the MG .
- The anticonvulsant activity was shown significant in high dose of test sample as compared to low dose of test sample. In case of onset of seizures Standard Drug is better than high dose of KG. In case of duration of tonic extensor seizure high dose(2400mg/kg of wt) of KG is equal to standard Drug Phenytoin.

***GHRITA MURCHANA***

**FIG 1- *Murchana drugs* FIG- 2- Course powder of MG FIG:3 -*Nimbu Swarasa* FIG:4 *Murchana Dravya Kalka***

**FIG :5 Process of Boiling FIG:6 Murchita Ghrita**

***KUSHMANDA GHRITA* PREPARATION**

**FIG:7 *KUSHMADA PHALA* FIG:8 K PHALA JUICE FIG :9 *YASTIMADHU KALKA* FIG :10 KG BOILIG PROCESS**

**FIG: 11, *KUSHMANDA GHRITA* END PRODUCT . KG: *SNEHA SIDDI LAKSHANA* FIG:12 .*VARTIVATH KALKA* . FIG:13. *SHABDA HINA***

**EXPERIMENTAL STUDY: MES METHOD**

**FIG: 14,ANIMAL HOUSE FIG:15 : DRUG LOADING IN SYRINGE FIG:16: DRUG ADMINISTRATION FIG:17: MES STIMULUS .**

**FIG :18: TONIC EXTENSOR SEIZURE FIG:19: TONIC FLEXION SEIZURE FIG:20 : STATE OF RECOVERY**

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