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Pharmaceutical and Analytical Evaluation of *Suta Bhasma Yoga* w.s.r. to its Antiepileptic Activity

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ABSTRACT

Introduction: *Rasayogas* are frequently divided on the basis of *Samskaras* given to them has led to the evolution of *Kharaleeya*, *Parpati, Pottali* and *Kupipakwa Rasayana Yogas. Sutabhasmayoga* is a unique herbomineral compound indicated in *Apasmar*. Age matched young albino rats weighing about 200-250 g were employed in the present study. Rats were fed on standard diet and water. The experimental protocol used in the present study was approved by the Institutional Animal Ethical Committee. They were acclimatized in institutional animal house and were exposed to normal cycles of day and night. The Anti-Epileptic activity is evaluated in convulsions induced by MES Method protocol. **Aims & Objectives:** Pharmaceutical and Analytical Evaluation of Suta Bhasma Yoga w.s.r. to its Antiepileptic Activity. **Materials and Methods:** Preparation of *Sutabhasma Yoga* was done as per classics. *Parada Shodhana* was done acc to *Rasa Tarangini,Gandhaka Shodhana* was done acc to *Rasatarangini*. Then preparation of *Suta Bhasma Yoga* was done as per the reference in *Rasendra Sara Sangraha*. Prepared *Suta Bhasma Yoga* was subjected to various Analytical tests. Its Anti-Epileptic evaluation was done by the convulsions induced by MES method in albino rats. **Results:** *Suta Bhasma Yoga* has pH 8, Total ash 0.79%, Acid soluble ash 0.00%, Loss on drying less than 1% and SEM EDX. In Experimental Study Trial group shown highly significant Anti-epileptic activity against Control and Standard group result with $p < 0.05$. **Conclusion:** *Suta Bhasma Yoga* can be prepared easily. Its composition and properties make it potent Anti-Epileptic drug.

Key words: *Apasmar, Convulsions, Kupipakwa Rasayana, MES method, Suta Bhasma Yoga*

INTRODUCTION

Suta Bhasma Yoga, is a unique mode of preparation which comes under *Kupipakwa Rasayana*, it includes chiefly the *Rasasindoor*, and associated drugs *Shankhapushpi, Bhrahmi, Vacha, Kushta*, *Ela. Rasasindoor* is prepared by using *Samaguna Balijarita Parada*, and the *Kashaya* is prepared by above mentioned drug both are mixed, and indicated in *Apasmara*. They are possessing *Balya*, *Brumhaniya, Medhya, Rasayanaproperty* and *Apasmara* is treated with *Medhya, Balya, Rasaynas*.[1] *Apasmara* is a condition where *Asthirata* of body, *Chitta Nasha, Dhee, Dhruti*, *Smrutinasha*, *Akshepaka Laxanas* exists due to vitiation of *Vatadi Doshas* and upward moment of these vitiated *Doshas* causes *Vikruti* of *Manovaha Srotas* which leads to *Apasmar*.[2] Epilepsy is a neuropsychological disorder. It is the consequence of a paroxysmal uncontrolled discharge of neuron within the central nervous system. Epilepsy is a collection of diverse disorders that together affect approximately 1% of the general population. Around 75-80% of epileptic patients may be provided with adequate seizure control with the help of conventional antiepileptic drugs.[3] In modern medical science Phenytoin, Sodium Valproate, Clonazepam, Gabapentin etc. Are the often-used drugs in the management of epilepsy which cause mental suppressions i.e., they suppress the brain activity and causes many adverse effects like difficulty in breathing,

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many severe skin diseases, Osteomylesia, Gum disease swelling of face, eyes, lips, tongue, allergic reaction &may reduce immunity.[4]

Various formulations have been explained in our classics to treat the *Apasmar* those are *Bhrahmi Ghruta*, *Smrutisagar Rasa*, *Vatakulantaka Rasa*, *Tantupashana*, *Chaturbaddha Rasa*, *Shatavari*, *Mandukaparni*, *Bhutabhairava Rasa*. On going through many Ayurveda classics, *Suta Bhasma Yoga* is found to be explained in *Rasendra Sara Sangraha* & *Bruhat Bhaishajya Ratnavali* found to be a most Novel Herbo Mineral Product explained to treat all types of *Apasmar*.

In present research work we have selected *Suta Bhasma Yoga* as our test drug on virtue of its vital importance in therapeutic as well as in pharmaceutical preparation. Keeping therapeutic indication mentioned in our classics one may conclude that *Suta Bhasma Yoga* is effective in neuro muscular disorders so we had decided to conduct an experimental study to find out the effect of *Suta Bhasma Yoga* in epilepsy (nervous system) on suitable models.

Materials and Methods

Drugs and Chemicals

Suta Bhasmayoga, Inj Phenytoin were used in this study

Physico -Chemical Analysis

Table 1: Organoleptic Character of *Suta Bhasma Yoga*[5]

SN	Parameter	*Suta Bhasma Yoga*
1.	Colour	Brownish colour
2.	Odour	Odourless
3.	Touch	Smooth
4.	Appearance	Fine powder

Table 2: Physico-chemical analysis of *Suta Bhasma Yoga*[6]

SN	Parameter	*Suta Bhasma Yoga*
1.	Ph	8.00
2.	Ash value	0.79%
3.	Acid insoluble ash	0.00%
4.	Water soluble test	Soluble
5.	Chloroform soluble test	Insoluble
6.	Alcohol soluble test	Soluble
7.	Ether soluble test	Soluble
8.	Loss on drying	Less than 1%

Table 3: Showing Inorganic element Analysis *Suta Bhasma Yoga*[7]

SN	Inorganic Element	Presence
1.	Calcium	Absent
2.	Magnesium	Absent
3.	Sodium	Present
4.	Potassium	Absent
5.	Iron	Present
6.	Sulphate	Present
7.	Phosphate	Absent
8.	Chloride	Present
9.	Carbonate	Absent
10.	Nitrate	Absent

SEM /EDAX[8]

Table 4: Showing the result of EDX analysis of *Suta Bhasma Yoga*.

Element	Weight %	Atomic %
CK	2.29	23.49
O K	2.34	18.07
As L	0.06	0.09
Hg M	67.10	41.30
Tl K	28.22	17.05
Total	100	

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Result

SEM/EDX spectra shows the presence of Carbon, Oxygen, Arsenic, Mercury, Thalium in all the spectras. Mercury has found to be closely associated with Thalium very the quantity of mercury is around 65 weights % on an average and Thalium around 26 weight %, oxygen is found to be around 2.5 weight % in negligible point. Arsenic, Silica, Calcium are the other elements with minimum places & negligible weight %. The atomic no. of mercury is 80, & atomic no. of Thalium is 81 in the Periodic Table. The presence of Thalium in the Mercury difficult to be traced out. The peak of Thalium is repeatedly seen along with Mercury which may suggest that Thalium could be the inclusion from the source of Parada which is easily amalgamated in Mercury could have been used as adulterant. The source of inclusion of Thalium could not be traced.

Zeta Potential[9]

Table 6: Showing the result of zeta potential

Peak no	Zeta potential	Electrophoretic mobility
1	-48.2 mV	-0.000373 CM2/vS
2	-mV	-CM2/Vs
3	-mV	-CM2/vS

Zeta potential (mean): -48.2 mV

Electrophoretic mobility: -0.000373 CM2/vS

Particle Size Assessment

Z - Average: 244.4 nm

PI: 0.384

Determination of Mercury/Sulphur

Mercury: 1.72%

Sulphur: 0.44%

TLC (Thin layer chromatography)[10]

Results

Description macroscopy

Part - *Churna*

Colour - Brownish

Odour - Aromatic

Extract - Alcohol extract

Mobile phase - Tolune: Ethyle acetate-7:3

Rf values- Long wave length - 0.05, 0.1, 0.28, 0.35, 0.41, 0.49, 0.52, 0.60, 0.65

Short wave length - 0.30, 0.41, 0.45, 0.50, 0.55, 0.58, 0.65

HPTLC (High performance thin layer chromatography)[11]

Results

Max Rf value of 0.30 of Convolvine Aalkoloid of *Shankapushpi*

Max Rf value of 0.6 are bacoside A, bacoside of *Brahmi*

Max Rf value of 0.26,0. 19, 0.60, 0.6I, mystric acid, L-serine of *Vacha* and in TLC long wave 0.35 is identified to be of the same component.

Max Rf value of 0.30 Alanolactone of *Kushta*.

Max Rf value of 0.91 and 0.51 as β-terpeneols of Ela were found in HPTLC & TLC hence all the ingredients of *Medhya Kashaya* were identified hence it is genuine one.

Experimental Result[12]

Suta Bhasma Yoga is subjected to Anti-epileptic activity in Wister albino rats compared with Control Group Standard Group.

Group 1 - Control Group, Normal Saline.

Group 2 - Standard Group, Inj. Phenytoin.

Group 3 - Test Group, *Suta Bhasma Yoga.*

Table 7: Showing the results of anti-epileptic activity in Control Group[11]

Group		SN	Flexion In sec	Extension In sec	Clonus In sec	Stupor In sec	Recovery / death In sec
Control	H	1	10	12	12	110	Recovered

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	N	2	8	10	6	121	Recovered
	B	3	6	12	8	140	Recovered
	T	4	10	7	9	186	Recovered
	RL	5	6	20	7	90	Recovered
	LL	6	7	8	4	86	Recovered

Table 8: Showing the results of anti-epileptic activity of test drug[13]

Group		SN	Flexion In sec	Extension In sec	Clonus In sec	Stupor In sec	Recovery / death In sec
Test Group	H	1	6	9	9	120	Recovered
	N	2	7	12	13	118	Recovered
	B	3	6	14	3	0	Recovered
	T	4	5	0	2	98	Recovered
	RL	5	4	0	0	134	Recovered
	LL	6	6	14	2	126	Recovered

Table 9: Showing the result of Anti -epileptic activity[14]

Parameters	Control	Standard	Treated	One-way Annova	Level of significance
	Mean S.E	Mean S.E	Mean S.E	d.f. F value	P value
Tonic hind limb flexion	7.833 +/- 0.168	4.833 +/- 0.083	5.666 +/- 0.088	2, 15 137.36	<0.05
Tonic hind limb extension	11.50 +/- 0.137	5.166 +/-0.085	8.166 +/- 0.112	2, 15 327.5	<0.05
Clonus	7.666 +/- 0.172	3.666 +/- 0.072	4.833 +/- 0.046	2, 15 210.87	<0.05
Stupor	122.16 +/- 0.958	87.166 +/- 0.571	99.33 +/- 0.411	2, 15 658.41	<0.05
Recovery/ Death	R=6 D=0	R=6 D=0	R=6 D=0		

As the readings indicates when One Way Annova variance test applied. Each group shows significance at $p<0.05$. The differences in the mean value among the treated groups are the differences are greater than that of the table F value at 95% of level of significance statistically. ($P<0.05\%$).

By the above findings null hypothesis rejected and alternate hypothesis is accepted. Before the study the null hypothesis presumed was

H_0 - The drug is not effective in epilepsy.

H_1 - The drug is effective in epilepsy.

After Annova test were conducted further the readings were subjected Dunnet's test of multiple comparison and the values of Treated group were compared with Standard and Control group with respect to each parameter and the comparison is as follows

Tonic hind limb Flexion:

Table 10: Showing the Dunnet's test for Tonic hind limb Flexion.

Comparison	Difference of means	q	P value
Treated Vs Standard	1.062	6.327	<0.05
Treated Vs Control	-1.62	10.060	<0.05

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Tonic hind limb Extension:

Table 11: Showing the Dunnet's test for Tonic hind limb Extension.

Comparison	Difference of means	q	P value
Treated Vs Standard	-8.122	53.300	<0.05
Treated Vs Control	-11.200	276.134	<0.05

Clonus:

Table 12: Showing the Dunnet's test for Clonus.

Comparison	Difference of means	q	P value
Treated Vs Standard	1.000	5.841	<0.05
Treated Vs Control	-2.422	14.216	<0.05

Stupor:

Table 13: Showing the Dunnet's test for Stupor.

Comparison	Difference of means	q	P value
Treated Vs Standard	12.023	12.142	<0.05
Treated Vs Control	-22.422	23.216	<0.05

In all the above tables the study was significant at the level of $p < 0.05$ by applying Dunnet's multiple comparison tests. From the all above calculation it is stated that *Suta Bhasma Yoga* showed a considerable protection against convulsions.

DISCUSSION

The *Apasmra* is the cardinal symptoms of the *Vyadhi*. It is a combination of two words viz. *Apa* and *Smara* i.e., impairment in memory or awareness. Even though it is considered most of the times under *Manasika Rogas*, but it is not a *Manasikaroga*. *Apasmara* is one of the diseases, which effects both *Shareera* and *Manas*. Both *Shareerikadoshas* i.e., *Vata, Pitta* and *Kapha*; as well as *Manasikadoshas* i.e., *Rajas* and *Tamas* plays equal role in the manifestation of the disease *Apasmara*.

Epilepsy is a neuropsychological disorder. It is the consequence of a paroxysmal uncontrolled discharge of neuron within the central nervous system. Cardinal symptoms are Tonic clonic seizures, Complex partial seizures, Absence seizures, Atonic seizures, Partial motor seizures, Partial sensory seizures, Versive seizures, Partial visual seizures. Under normal circumstances, recurrent and collateral inhibitory circuits in the cerebral cortex limit synchronous discharge of neighbouring groups of neurons. The inhibitory transmitter gamma-amino butyric acid (GABA) is particularly important in this role, & drugs that block GABA receptors provoke seizures conversely, excessive stimulation by excitatory neurotransmitters. *Suta Bhasmayoga* is considered as one of the best remedy for *Apasmara* in *Ayurveda*. Experimental evaluation of Anti-epileptic activity of *Sutabhasma Yoga* has been carried out on Wister strain albino rats. Maximum electric shock induced convulsions model was selected because of its wide acceptability. Phenytoin was a standard anti-epileptic drug used to compare the anti-epileptic property in experimental rats. For Anti-epileptic study rats were equally divided into 3 groups consisting of six rats each.

Group 1 - Control group

Group 2 - Standard group (phenytoin)

Group 3 - Trial group (*Sutabhasma Yoga*) 5mg/0.2kg

During the study in control group hind limb bleeding is seen,

In standard group all the limb bleeding as well as the orbital bleeding is observed and in both groups some animals were making some kind of noise during initial period of convulsions. In test group no side effects were seen & also skip of extension phase in some animals noted & clonus phase in some animals were observed.

Data Analysis

Data were analyzed for statistical significance by one-way Annova. The value of F in calculation is greater

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than that of the table value. Hence Ho i.e., *Suta Bhasma Yoga* is not effective in epilepsy is rejected, & H_1 i.e., *Sutabhasmayoga* is effective and accepted i.e., the *Sutabhasma Yoga* will act as a anti-epileptic at P<0.05 was considered as significant.

Conclusion

Classics of *Rasashastra* have given emphasis on *Apasmara Nashaka* property of *Sutabhasma Yoga*. The *Suta Bhasma Yoga* is given with the *Medhya Kashaya* according to *Rasendra Sara Sangraha* text. *Suta Bhasma Yoga* was *Apasmara Nashaka* due to its *Ushna Veerya* property, may pacify the *Vatadi Doshas* and *Rajasa Tamas* of *Manasika Doshas* which reduces the obstruction in *Manovaha Srotas*. It's *Balya*, *Medhya* and *Rasayana*, properties give *Sthairya* to the *Dhatus* which diminishes the *Akshepaka*.

Rasasindoor is having *Shadrasa, Guru Snigdha Guna, Ushna Veerya, Madhura Vipaka, Rasayana*, *Balya* property along with this it is having the *Madhura* rasa which pecify the *Vata Dosha* hence providing in the control over the *Vata Dosha*. In the *Medhya Kashaya*, *Shankapushpi* is having *Tikta Katu, Kashaya Rasa Sara Guna Sheeta Virya* having *Medhya, Balya* and *Rasayana* property and having convolvine a phytochemical, *Brahmi* is having *Tikta Kashay Madhura Rasa, Madhura Vipaka, Medhya Rasayana Balya* and *Vata Kaphahara* property and having bacoside a phytochemical. *Vacha* is categorized under *Sanjnashtapaka Gana*, *Shirovirechaneeya Gana, Lekhaneeya Gana, Sheeta Prashamana Gana* having *Tikta Katu Rasa, Laghu Teekshna Guna, Katu Vipaka, Tridosha Shamaka* having *Medhya, Balya, Rasayana* property and also having mysteric acid as a phytochemical. *Kustha* is having *Tikta Madhura Rasa, Ushna Veerya, Katu Vipaka, Lekhaniya Vatakaphahara* property and also having a Alanolactone as a phytochemical. *Ela* is having *Madhura Katu Rasa, Sheeta Virya, Katu Vipaka, Vata Kaphahara* property and also having a β-terpeneols a phytochemical. *Suta Bhasma* is being a very good *Yogavahi* drug helps in carrying all the properties of *Medhya Kashaya* to the targeted tissues and there by the efficacy of the drug is fortified. So, this *Tridoshashamaka, Rasayana Medhya Balya* property of *Suta Bhasma Yoga* helped to correct the vitiated *Vatadi Dosha* and *Sanjnashtapana* was re-established & thus control over convulsions was achieved as evident by experimental study. Thus, we can conclude that, *Suta Bhasma Yoga* is a typical synergistic combination, which has anti -epileptic property.

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