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Experimental study on antiepileptic action of *Kousheyashma Bhasma*

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Epilepsy is one of the most common neurological diseases. It is characterised by recurrent episodes of disturbance in movement, sensation and consciousness. With the increased incidence rate of epilepsy, a number of treatment modalities as well as formulations are being developed in recent years. In Ayurveda also many formulations are available for the treatment of epilepsy. However, there is a search of formulations which can show quick and longstanding efficacy on epilepsy. *Kousheyashma* is a mineral drug used in Ayurveda pharmaceutics. It is yellowish white and is identified as asbestos. Magnesium, calcium and silicate are the chief chemical entity of this drug. It is an easily and abundantly available mineral in India. The *Bhasma* is economic as the pharmaceutical processing of it is easy. In the pharmacological and therapeutic properties of this drug, it is mentioned that it has anti-epileptic activity. However, there is no substantial evidence to prove the antiepileptic activity of *Kousheyashma*. Hence, the present study was undertaken to experimentally evaluate the efficacy of *Kousheyashma Bhasma* in epilepsy. Antiepileptic activity was evaluated in Swiss albino mice by two methods namely Pentylenetetrazol (PTZ)-induced seizure method and kindling PTZ-induced seizure method. Mice selected based on exclusion and inclusion criteria were randomly allocated into five groups. Mice were subjected to chemo convulsions by injecting PTZ intraperitoneally and observed for 35 min to analyse convulsion behaviours. *Kousheyashma Bhasma* has shown statistically significant results in PTZ-induced epileptic symptoms in Swiss albino mice.

Keywords: Antiepilectic activity, Epilepsy, *Kousheyashma Bhasma*

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Kousheyashma is a yellowish-white stone which looks like compressed fibers attached to one another[1]. *Kousheyashma* is enlisted under a separate group called *sikata varga*[2]. It is a naturally occurring mineral in abundance in all parts of India. It is used as an ingredient in a few formulations mentioned in books of *Ayurveda*. It is also a drug mentioned in the list of 120 *uparasa* in *Siddha* system of medicine. It is termed as *Kalnar* in Tamil, *Kannaram* and *Hiravi*[3] in Malayalam, *Ratinara* in Telugu and *Kalnaru* in Kannada. Mineralogical features like hardness, specific gravity and appearance suggest that it is a drug resembling asbestos. The studies done earlier have proved that it is a variety of asbestos exhibiting the property of silicate of magnesium and calcium[4,5].

Epilepsy is one of the most common neurological diseases and approximately 23.4 million people are diagnosed with epilepsy each year globally[6]. Epilepsy is characterized by recurrent episodes of disturbance of movement, sensation and consciousness. With the increased incidence rate of epilepsy, a number of treatment modalities as well as formulations are being developed in recent years. In Ayurveda also many formulations are available for the treatment of epilepsy.

However, there is a search of formulations which show quick and longstanding efficacy on epilepsy. In recent books of *Rasashastra*, it is mentioned that *Kousheyashma Bhasma* has anti-epileptic activity[7]. The present study was conducted to evaluate the efficacy of *Kousheyashma Bhasma* in epilepsy experimentally.

Methodology

The study was performed on Swiss albino mice weighing 20-40 g. They were found in a zoo attached to the SDM Center for Research in Ayurveda and Allied Sciences, Udupi, Karnataka. They were randomly assigned to five groups of six mice each.

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Drug preparation

Kousheyashma Bhasma was prepared according to standard guidelines in the Department of Rasasashtra and Bhaishajya Kalpana, Sri Dharmasthala Manjunath-eswara College of Ayurveda and Hassan Hospital.

The TED (therapeutic dose) drug test solution was prepared by mixing 250 mg *Kousheyashma Bhasma*, 10 mL of distilled water with carboxy methylcellulose sodium salt (CMC) 50 mg and the same method was followed for preparing TED 1/2 - 125 mg and TED 2-500 mg solution. HD × 0.0026 × 50 kg body weight/1000 was calculated on the basis of human volume using the Standard conversion method on the basis of body mass index[8]. The dose of diazepam was approximately 0.05 mg/100 g body weight of mice. Similarly the dose of the aesthetic agent was also calculated as 80 mg/kg and 40 mg/kg of the PTZ method and the PTZ ignition method, respectively (Table 1).

Antiepileptic activity was tested in two different ways:

Pentylenetetrazol-induced seizure method

The animals were given a vehicle, test drugs and a reference level to the appropriate groups by oral route for 10 consecutive days. On the eleventh day, one hour after drug administration, they developed a chemo disorder after injecting Pentylenetetrazol (PTZ) at a dose of 80 mg/kg. The effect of a different treatment on the PTZ seizure profile is described below. Parameters such as delayed onset of clonic and tonic tremors, recurrence of chronic or tonic convulsions, myoclonic fluctuations, death and other abnormal changes in behaviour were recorded. Elimination of clonic convulsions is considered to be an indication of anti-convulsive activity.

Pentylenetetrazol kindling method in mice

For implantation, Pentylenetetrazol (PTZ) dissolved in standard saline and a dose less than 40 mg/kg, intraperitoneal was administered daily up to 11 injections. After each sub-convulsive dose of PTZ, mice in different groups were observed for 35 min and PTZ-induced seizures were recorded with Fischer and Kittner's scoring program (1998)[9].

- Ear and face tingling, nodding of the head;
- Myoclonic Jerks.
- Increasing general tremors, climbs, and falls,
- Clonic-tonic tremor, enlargement of posterior tonic organs.

Measurement levels were calculated for all groups after each PTZ injection. Scores from test construction and general comparison groups were compared to vehicle controls

Histology study

At the end of the study the mice were sacrificed and the brains were carefully removed, purified and transferred to 10% formalin before being analyzed for histological examination by taking their stage using standard laboratory procedures. The slides with the found parts were scanned with a Trinocular Carl Zeiss microscope under various magnifications. Changes in the structure of histoarchitecture were noted.

Results

Experimental study was done to evaluate the antiepileptic action of *Kousheyashma Bhasma* using two methods, Pentylenetetrazol-induced convulsion and kindling Pentylenetetrazol-induced convulsion. Observations are listed below (Tables 2-6).

Histopathological study

Histopathological analysis of section of brain was done to evaluate the changes in brain of mice after

Table 1 — Grouping of animals for the experimental study

Group I	Standard	Diazepam orally 0.05 mg/100 g
Group II	Control	Normal control
Group III	Study group TED	250 mg
Group IV	Study Group TED ½	125 mg
Group V	Study Group TED 2	500 mg

Table 2 — Effect of *Kousheyashma Bhasma* on Pentylenetetrazol-induced convulsion

Groups	Latency of onset (in sec)	No of Myoclonic convulsions	No of Clonic convulsions
Control	42.4±9.81	41 ± 13.598	7 ± 0.7071
Standard	209	01	0
Ted	85.83± 14.23*	43.8 ± 5.894	2.2 ± 0.3742**
Tedx2	46.6 ± 7.18	12 ± 3.240*	3.2 ± 0.4899**
Tedx1/2	41.08 ± 3.69	13.2 ± 1.772	2.4 ± 0.6782**

Data: MEAN ± SEM, **p<0.01, * p < 0.05

Table 3 — Effect of *Kousheyashma Bhasma* on Pentylenetetrazol-induced convulsion (Death and Recovery)

Groups	Recovery (in sec)	Death (in sec)
Control	2663.5 ± 36.50	292.33 ± 25.44
Standard	2700 ± 0.00	0
Ted	2581.5 ± 66.43	1210± 644.00**
Tedx2	0	353.83 ± 82.4
Tedx1/2	0	416.16 ±63.79

Data: MEAN ± SEM, **p<0.01, * p < 0.05

Table 4 — Effect of *Kousheyashma Bhasma* on Pentylenetetrazol-induced kindling convulsion

Groups	No of Face twitching / head nodding	No of Myoclonic jerks	No of generalized clonic convulsions	No of clonic-tonic convulsions, tonic hind limb extensions
Control	87.8 ± 14.168	313 ± 88.648	6.2 ± 1.241	3.4 ± 0.2449
Standard	61.428 ± 10.869	6.1428 ±1.223**	0 ±00**	0.1428 ± 0.1429**
Ted	13 ± 2.302**	37.5 ± 10.115**	2.166 ± 0.6540**	0.5 ± 0.3416**
Tedx2	7.6 ± 1.208**	66.166 ±20.676**	4.833 ± 0.8724	0.5 ± 0.22**
Tedx1/2	19.6 ± 4.411**	40.33 ± 9.249**	4.66 ± 1.202	0 ± 00**

Data: MEAN ± SEM, **p<0.01

Table 5—Effect of *Kousheyashma Bhasma* on Pentylenetetrazol-induced total aggregation kindling convulsion

Groups	Total kindling
Control	397.8 ± 84.751
Standard	67.714 ± 12.047 **
Ted	50 ± 6.221 **
Tedx2	68.2 ± 21.264 **
Tedx1/2	62.6 ± 8.238 **

Data : MEAN ± SEM, **p<0.01

administration of *Kousheyashma Bhasma* and based on results observations are made (Table 7).

Discussion

Objective of the study was to evaluate the anti-epileptic action of *Kousheyashma Bhasma* by two methods i.e.; pentylenetetrazol convulsion method and kindling convulsion method. Evaluations were done by making 5 groups: control, standard, TED (therapeutic effective dose), TED ½ and TED 2, respectively.

Pentylenetetrazol (PTZ) is a CNS stimulant that acts as epileptogenic causing seizures. Therefore it is used as a drug to induce epileptic development in experimental model and to spot drugs that will manage seizure susceptibleness. As a non-competitive GABA (gamma- amino saturated fatty acid) antagonist, PTZ is specifically employed in seizure assays as a way of assessing the excitability of the central system and GABA activity. Kindling could be a chronic animal model of brain disorder that has been comprehensively studied to grasp the method of epileptogenesis and see novel anti-epileptic compounds. The impact of test/reference compounds may be tested by administering them either before the initiation of kindling (pre-kindling phase) or once animals are totally ignited (post kindling phase) [10].

After the observation of both methods (general & kindling) including all grades, effects of *Kousheyashma Bhasma* on PTZ-induced convulsions are mentioned below based on statistical reports.

Latency of onset

Duration of latency of onset of seizures was found to be increased in TED and TED 2 groups when compared to the control group. The observed increase was found to be statistically significant with p value <0.05 and non-significant respectively. There was a decrease in duration of latency of onset of seizures in TED 1/2 group when compared to the control group. The observed decrease was found to be statistically non-significant (Table 2). Epilepsy is a physical condition that occurs due to a sudden, brief change in the normal working of brain. At this time, the brain cells are unable to function properly and the level of consciousness was very poor[10]. So the increased latency of onset of seizures indicates reduced chances of getting epilepsy.

Myoclonic convulsion

There was a decrease in number of myoclonic convulsion in TED 2; the observed decrease was found to be statistically significant with p value < 0.05. Increase in number of myoclonic convulsion was in TED group when compared to the control group. The observed increase was found to be statistically non-significant. And there was a decrease in number of myoclonic convulsion TED ½ group when compared to the control group statistically shows non-significant in TED ½ (Table 2). Myoclonic seizures are brief, shock-like jerks of a muscle which occurs because of rapidly alternating contraction and relaxation, jerking or twitching of a muscle[11]. It is one of the major symptoms of epilepsy. Decrease in number of myoclonic seizures is a positive indication towards efficacy of the study drug.

Clonic convulsion

Number of clonic convulsions were found to be decreased in TED, TED 2 & TED ½ groups when compared to the control group. The observed decrease was found to be statistically significant with p value <0.01. Clonic convulsions occur in epilepsy because

Table 6 — Effect of *Kousheyashma Bhasma* on Pentylenetetrazol-induced kindling convulsion peak duration (10 to 20 min)

Groups	Face twitching /head nodding	No of Myoclonic jerks	No of Generalized clonic convulsion	No of clonic-tonic convulsions, tonic hind limb extension
Control	18.8 ± 3.541	83 ± 31.646	1.2 ±0.7348	0.666 ± 0.2562
Standard	17.571± 3.760	0.8571 ± 0.2608 **	0 ± 00 *	0 ±00
Ted	3 ± 0.3162 **	2.8 ± 0.3742 **	0 ± 00	0.1428 ± 0.1429
Tedx2	0.8± 0.3742**	13 ± 6.017**	0 ± 00	0.1428 ± 0.1429
Tedx1/2	5.2 ± 1.319 **	4.2 ± 1.497 **	0.2 ± 0.2000	0 ± 00

Data: MEAN ± SEM, **p<0.01, * p < 0.05

Table 7 — Histopathological observations of all groups

Groups	Changes observed	Remarks
Ctl	All sections showed many degenerated and necrosed neurons in cerebrum and hippocampus. Reduced Purkinje cells in cerebellum.	Moderate to severe degenerative features seen
STD	Degenerated neurons have decreased in cerebrum, hippocampus in all sections compared with C group. There is an increase in Purkinje cells of cerebellum compared to C group.	No to mild degenerative features. Protection seen
TED	Degenerated neurons have decreased in cerebrum, hippocampus in all sections compared with C group. There is an increase in Purkinje cells of cerebellum in some sections.	No to mild degenerative features. Good protection seen.
TEDx2	Degenerated neurons have decreased in cerebrum, hippocampus in all sections compared with C group. There is an increase in Purkinje cells of cerebellum in most of the sections.	No to mild degenerative features. Good protection seen.
TED-half	Few sections showed more degenerated neurons compared to SDA and TED group. There was a reduction in Purkinje cells also.	Mild to moderate degenerative features. Protection seen but less compared with TED and SDA groups

of fast stiffening and relaxing of a muscle that happens repeatedly. Clonic seizures can start in the motor area on one side of the brain. The jerking movements would affect just one side or part of the body or face[11]. The significant results suggest that the study drug acts on muscles.

Recovery/ Death

Duration of recovery shows there was a non-significant result in all groups when compared to the control group. Duration of death was increased in TED group when compared to the control group. The observed increase was found to be statistically significant with p value < 0.01. Data shows there was increase in duration of death in TED 2 and TED ½ group when compared to the control group. The observed increase was found to be statistically non-significant (Table 3).

Face twitching / head nodding

Number of face twitching / head nodding was decreased in TED, TED 2 & TED ½ groups when compared to the control group. The observed decrease was found to be statistically significant with p value < 0.01 (Table 4).

Number of clonic-tonic convulsions, tonic hind limb extensions

Number of clonic-tonic convulsions, tonic hind limb extensions shows there was decrease in TED, TED 2 & TED ½ groups when compared to the control group. The observed decrease was found to be statistically significant with p value < 0.01 (Table 4).

These show the combined characteristics of tonic and clonic seizures. Tonic means stiffening, and clonic means rhythmical jerking[11]. Significant changes in the parameter may be considered for the action of study drug on muscular skeletal and neurological symptoms. It is pertinent to note that the better results were seen in 10-20 min suggesting the bioavailability or concentration with which the medicine acts.

Number of total aggregation kindling convulsion

The total number of combined comorbidities shows a decrease in the TED, TED 2 & TED groups when compared to the control group. The decrease was found to be statistically significant in the value of p <0.01 (Table 5).

Histopathological changes

In the cerebrum, all doses of treatment showed fewer destructive changes while compared to the control group. Positive changes indicate the protective effect of *Kousheyashma Bhasma* on cerebrum tissue. In the cerebellum, an increase in Purkinje cell was observed in TED & TED 2 compared to the control group. The changes may be due to reduced sensory transmission that promotes motor control.

While comparing histopathological changes in the hippocampus, very few destructive detectors were observed in the TED and TED groups 2. *Kousheyashma Bhasma* probably reduces cell damage due to the presence of essential micronutrients such as calcium, iron and magnesium.

Complete examination of histopathological reports in all study drug groups shows a slight change in cerebral palsy. The protective and anti-inflammatory action of *Kousheyashma Bhasma* can be understood in that way. The chances of getting epilepsy are reduced by the action of *Kousheyashma Bhasma* by using a defence mechanism for brain cells, controlling sudden sensory nerves and reducing inflammation.

Possible action mode

Kousheyashma Bhasma was widely used in the treatment of epilepsy, in the ancient tradition and Rasashastra's latest textbook is described in the treatment of Apasmara. *Kousheyashma* is mineralogically identified as asbestos and studies proved that it contains magnesium, silicate, calcium, and iron as chemical elements[7,8] and Haritamanjari (*Acalypha indica*) used as a bhavana dravya in process for bhasma preparations for the antiepileptic process[12].

Magnesium is one of the essential nutrients in a normal diet. It has been reported that people with epilepsy have lower levels of magnesium than people without epilepsy[13]. Removal of Mg can produce spontaneous events with a tonic shooting phase and a clonic release phase. Such tonic clonic events were also recorded from the entorhinal cortex of rat cortico hippocampus preparations to the Mg-free per-perfusate. Magnesium is a potential modem of the binding activity due to its ability to counteract calcium -flux-stimulating N-methyl-D-aspartate (NMDA) receptor. A single dose of Mg organic oral salt can prevent NMDA-induced convulsions in mice in a dose-dependent manner, similar to NMDA receptor blocker[14]. The presence of 15.30% of magnesium in *Kousheyashma Bhasma* could have an impact on colonic and tonic bursts as a result of which the results have been observed in the present study.

Calcium is essential for all neurotransmitter release and muscle contraction. Given these important physiological processes, it seems reasonable to assume that hypocalcaemia can lead to a reduction in neuromuscular pleasure. However, clinical observations often record the role of hypocalcemia in the formation of seizures[15].

Iron deficiency (ID) is most prevalent during pregnancy and adolescence, which is of great concern to them because its impact on the developing nervous system is associated with an increased risk of various psychiatric disorders later in life. Disruption of the balance of interest and inhibition within the brain can be detected by the brain's response to taking extreme insults[16]. Presence of 4.45% calcium and presence of 11.93% of iron in *Kousheyashma Bhasma* may reduce the risk of seizures.

In this study the preparation of Kousheyashma Bhasma haritamanjari (*Acalypha indica*) was used as bhavana dravya in the marana process. In a study examining the antiepileptic efficacy of this drug, the methanolic extract of *Acalypha indica* leaves was orally administered at doses of 200 mg / kg Sprague Dawlyrats. Performance results showed that the methanolic extract of *Acalypha indica* leaf extract had a potent anticonvulsant activity[12]. This further adds to the description of the possible functioning of *Kousheyashma Bhasma* in epilepsy.

Conclusion

Kousheyashma Bhasma has shown significant result on reducing the duration of latency of onset, clonic convulsion & duration of death in animal model of epilepsy induced by PTZ at a therapeutic dose. Study drug has shown reduction in number of clonic convulsions, result on myoclonic convulsions when consumed in double the therapeutic dose. Study drug has shown significant result on almost all parameters of epilepsy in kindling method. Histopathological reports are suggestive of protective action of *Kousheyashma Bhasma* on brain cells. Based on all above observations & results it can be concluded that *Kousheyashma Bhasma* has an effective result on epilepsy.

Conflict of Interest

All the authors declare that they have no conflict of interest.

Author Contributions

M M Nidhin: Principal Investigator, data collected and documented; G Sharma K: Guide for the study, document corrected and edited; S Bhat: Co- Guide for the study, statistical analyser; G G Chaitra: review of literature.

References

1 Joshi D, Text book of rasashastra, 2nded.Trivandrum: Ayurveda college publication;1997; p.330

2 Murthy P H C, Rasashastra the mercurial system, 1sted. Varanasi: Choukambha Sanskrit Series Office; 2008; p.418

3 Joshi D, Text book of rasashastra, 2nd ed. Trivandrum: Ayurveda college publication;1997; p.330

4 Sharma G K, A Study on the Analytical Evaluation of the Mineral Drug Kousheyashma. PG thesis, Govt. Ayurveda College Thiruvanathapuram, Kerala University; 2006

5 Saranya S, Pharamaceutico-Analytical and toxicity study of *kousheyashma bhasma*, Sri Dharmasthala Manjunatheswara College of Ayurveda and Hospital Hassan, pg thesis, RGUHS Bangalore, Karnataka-2018

6 http://www.who.int/mediacentre/factsheets/fs999/en/ accessed on 14/2/2019, 9.15 am.

7 Dole A V & Prakash P, A text book of Rasashastra, 1sted.Varanasi:Chaukhamba Surbharati Prakashan; 2004; p.384

8 Paget G E & Barnes J M, Evaluation of drug activities, In: Pharmacometrics, edited by D R Lawrence & A L Bacharach, New York: Academic Press; 1964, p.161.

9 Zhuk O & Zinkovsky V G, Golovenko N Y, The pharmacodynamics of anticonvulsant and subconvulsant effects of ethanol in CBA and C57BL/6 mice, Alcohol (Fayetteville, N.Y.), 23 (2001) 23-8. 10.1016/S0741-8329(00)00136-1.

10 http://www.psychogenics.com/pentylenetetrazole.html assessed on 16/02/2019 10 am

11 Khan Y U, Farooq O, Sharma P & Rafiuddin N, Latency study of seizure detection. In: Advances in Computer Science, Engineering & Applications. Advances in Intelligent and Soft Computing, edited by D Wyld, J Zizka & D Nagamalai, Vol 166, Springer, Berlin, Heidelberg, 2012.

12 https://www.epilepsy.com/learn/types-seizures/myoclonic-seizures assessed on 19/08/19 10 am

13 Kumar P, Chakrapani P, Arunjyothi B & Rojarani A, Anti-epileptic activity of *Acalypha indica* methanolic leaves extract with animal experiment, *Int J Pharmacogn Phytochem Res*, 8 (9) (2016) 1560–1565.

14 Yuen A W C, Sander J W, Society E & Peter C S, Can magnesium supplementation reduce seizures in people with epilepsy ? A hypothesis, *Epilepsy Res*, 100 (1–2) (2012) 152–6, http://dx.doi.org/10.1016/j.eplepsyres.2012.02.004

15 Fawcett W J, Haxby E J & Male D A, Magnesium : physiology and pharmacology, *Br J Anaesth*, 83 (2) (1999) 302–20.

16 Griffiths G M, Calcium in epilepsy, *J Neurol Neurosurg Psychiatry*, S1-15 (57) (1934) 29–38.

17 Rudy M & Mayer-Proschel M, Iron deficiency affects seizure susceptibility in a time- and sex-specific manner, *ASN Neuro*, 9 (6) (2017) 1-13. DOI: 10.1177/1759091417746521