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A comparative experimental study to evaluate the anti-epileptic activity of Kalyanaka Ghrita and Ksheera Kalyanaka Ghrita by PTZ induced generalised seizure method

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Abstract

Background: One of the major concerns about Epilepsy is the psychological and cognitive effects of commonly used Anti-epileptic drugs. In the treatment aspects related to psychological or psychosomatic diseases, Ayurvedic drug has stood the test of time as they don't produce any undesirable side effects. Kalyanaka Ghrita is widely used to treat the conditions like Unmada, Apasmara etc. Along with the reference of Kalyanaka Ghrita, Acharya Chakrapanidatta has explained Ksheerakalyanaka Ghrita. They only differ in the ratio of water added as well as the addition of four parts of Ksheera in case of Ksheerakalyanaka Ghrita. The current study aims to evaluate the anti-epileptic activity of Kalyanaka Ghrita and Ksheerakalyanaka Ghrita by Pentylenetetrazole(PTZ) Induced Generalised Seizure method in Swiss Albino Mice. **Aims and objectives:** To evaluate the antiepileptic activity of Kalyanaka Ghrita and Ksheerakalyanaka Ghrita by PTZ Induced Generalised Seizure Method. **Methodology:** Group specific drugs were administered for 21 consecutive days by oral route. Diazepam was taken as reference standard drug. On 22nd day, a single dose of Pentylenetetrazole 80mg/ kg body weight was injected intra peritoneal to all the groups. The effect of different formulations on Pentylenetetrazole induced generalised convulsions were noted down. The results were expressed as Mean ± SEM. The data was analysed by one way Anova followed by Dunnet's multiple 't' test as post HOC using Graph pad Inst 3. **Results:** There was an increase in the duration of latency of onset of seizures in Kalyanaka ghrta group and Ksheerakalyanaka ghrita group. There was a decrease in the occurrence of number of myoclonic convulsions in Kalyanaka ghrta group and Ksheerakalyanaka group. There was an increase in the number of clonic convulsions in Kalyanaka ghrta group and Ksheerakalyanaka ghrita. There was an increase in the number of straub tail occurrence in Kalyanaka ghrta group and a decrease in the number of straub tail occurrence in Ksheerakalyanaka Ghrta group. The latency of occurrence of death was reduced in Kalyanaka ghrta and Ksheerakalyanaka ghrita group. There was a decrease in the number of recurrent clonic jerks in Kalyanaka ghrta group and an increase in the number of recurrent clonic jerks in Ksheera Kalyanaka ghrta group. **Conclusion:** Kalyanaka Ghrita and Ksheerakalyanaka Ghrita showed statistically non-significant improvement in the management of symptoms of PTZ Induced Generalised Seizure.

Keywords: Kalyanaka Ghrita, Ksheerakalyanaka Ghrita, PTZ Induced Generalised seizure, Antiepileptic activity.

INTRODUCTION

Among the Chikitsachatushpada, Bhishak, Dravya, Upasthata and Rogi, Bhesaja possesses its own importance because the success of Bhishaka and Paricharaka depends on the properties of Bhesaja. Bhesaja can be taken into consideration with the trifold application i.e. pharmacognostic, pharmaceutical and therapeutic management.

Epilepsy [1] is the one of most common neurological diseases. It has the debatable distinction of affecting all the walks of life of an individual suffering from the disease. The ambiguity regarding the aetiology, pathogenesis and the therapy still exists. Though modern science boasts of many innovations in the field of Research, the knowledge related to Epilepsy is still dubious.

The concepts of Ayurveda regarding Apasmara [4] seem to controvert the presently held outlook but it

has to be understood that these principles have stood the test of time and have offered relief to the ailing mankind through centuries. Kalyanaka ghrita [2] is widely used in clinical practice to treat Apasmara, Unmada and other broad spectrum of diseases. It is usually prescribed as Medhya Rasayana in chiravyadhi like Apasmara. Along with the reference of Kalyanaka Ghrita, Acharya Chakrapanidatta has explained Ksheerakalyanaka Ghrita [3]. They only differ in the ratio of water added as well as the addition of Ksheera in case of Ksheerakalyanaka Ghrita.

Aims and Objectives

To evaluate and compare the antiepileptic activity of Kalyanaka Ghrita by PTZ Induced Generalised Seizure Method.

MATERIALS AND METHODS

Test Drugs

The sample of *Kalyanaka Ghrita and Ksheerakalyanaka Ghrita* was prepared as per standard references in Department of Rasasashtra and Bhaishajya Kalpana, Sri Dharmasthala Manjunatheswara College of Ayurveda and Hospital, Udupi.

Experimental Animal

24 Swiss albino mice were randomly selected and divided into 4 groups.

Inclusion Criteria

a) Animals will be selected are adult Swiss albino mice having weight from 30- 40 g.
b) Animals selected will be of both sexes.
c) Active and healthy mice.

Exclusion Criteria

a) Weight range below 30g and above 40g.
b) Mice, which were used for other studies previously.
c) Pregnant and diseased mice.

Mice Maintenance

Swiss Albino mice were obtained from animal house attached to S.D.M Centre for Research in Ayurveda and Allied Sciences, Udupi, Karnataka. They were maintained on feed of "Sai Durga feed and food, Bangalore" and tap water was given *ad-libitum*. The temperature and humidity were kept at optimum and animals were exposed to natural day-night cycles. The experiment were carried out in conformity with guidelines of the Institutional Animal Ethical Committee (SDMCRA/IAEC/UD/RS-01) after obtaining its permission.

Examination of the animal prior to the experiment

All the Swiss Albino mice were subjected to general check for weight. Weight of each animal was checked by using weighing machine and the dose was calculated according to Paget and Barne's formula(Paget and Barne's 1964) involving body surface area ratio and human dose. The cages were labelled with name of the group and drug.

Reference Standard Drug The reference standard drug used for antiepileptic activity evaluation was Diazepam. It was purchased from the market with the trade name Paciquil 5mg/ml, Mfd- 12.06.16, Exp-12.06.20 Manufactured by Stadmed pvt. Ltd, Kolkata, India.

Chemical for inducing seizure: Pentylenetetrazole was used as a chemical for inducing the seizures in mice for evaluating the anti-epileptic activity experimentally. Chemicals were procured from HiMedia laboratories Pvt.Ltd, Mumbai, India with the name Pentamethylene tetrazole (5 gm),PKD:04/2011 and is of analytical grade regularly used in the laboratory.

Grouping AF Animals

A day prior to dosing, the selected animals were divided into different groups by randomization method. Each group consisted of 6 albino mice each.

- Group 1-Positive control Group
- Group 2-Reference Standard Group (Diazepam)
- Group 3-Test Drug Group-KG group
- Group 4-Test Drug Group-KKG group

Dose Fixation [5]

Dose calculation of trial drug for mice = 0.0026*human dose*50/Kg body weight.

Here the dose of *Kalyanaka Ghrita and Ksheerakalyanaka Ghrita* is 48 ml (1 pala).

Therefore, Dose= Human dose * 0.0026*50/ Kg body weight

= 48* 0.0026*50/ Kg body weight

= 6.24 ml/Kg body weight.

Dose Calculation for Reference Standard (Diazepam)

Mice dose = 8 mg/Kg body weight

Dose calculation for Generalized PTZ induced seizure **(**Pentylenetetrazole)

Mice dose = 80mg/Kg body weight

PTZ Solution

A stock solution containing 80mg of PTZ and 10 ml of distilled water was prepared.

Methodology [6]

Antiepileptic activity will be assessed by two different methods:

a) Pentylenetetrazole Induced Seizure Method

Drug: The animals was administered test drug and reference standard drug to respective group by oral route for 21 consecutive days. On the 22nd day, one hour after drug administration, they were subjected to chemo-convulsion by injecting Pentylenetetrazole (PTZ) intra peritoneal in the dose of 80mg/kg. The effect of different treatment on PTZ convulsion profile was noted down. The parameters to be measured would be-

- latency of onset of clonic and tonic convulsions
- recurrence of the chronic or tonic convulsion
- myoclonic twitches, mortality
- Any other abnormal changes in the behaviour.

Abolishing of the clonic convulsion was considered as the index of anti-convulsion activity.

Statistical Analysis

All the values were expressed an MEAN ± SEM (Standard error of mean). The data was analysed by one-way ANOVA followed by Dunnet's multiple 't' test as post hoc test. Graph pad Inst 3 was used for this purpose. A level of $p \leq 0.05$ was considered statistically significant. Level of significance was noted and interpreted accordingly

RESULTS

The effect of Kalyanaka ghrita and Ksheerakalyanaka ghrita in PTZ induced generalised convulsions on the latency of onset, Number of Myoclonic jerks, Number of Clonic convulsions, Number of Straub Tail occurrence, Latency of Recovery, Latency of death, Number of recurrent clonic jerks is depicted in Table 1,2,3,4,5,6 and 7 respectively.

Table 1: Effect of kalyanaka ghrita and ksheerakalyanaka ghrita in PTZ induced convulsions- latency of onset

Groups	Latency of Onset of Seizures (SEC) Mean±S.E.M	Percentage Change
Positive Control(A)	61± 12.502	-
Standard (B)	0	100↑
K.G (C)	122.5±43.869	100.8↑
K.K.G(D)	64.6±2.542	5.90↑

Data: Mean±S.E.M **p<0.01, *p<0.05.

Table 2: Effect of kalyanaka ghrita and ksheerakalyanaka ghrita in PTZ induced convulsions-number of myoclonic jerks

Groups	Number of Myoclonic Jerks	Percentage Change
Positive Control (A)	33.8±15.262	-
Standard (B)	0	100↓
K.G (C)	15.2±3.367	55.02↓
K.K.G(D)	12±2.608	64.49↓

Data: Mean±S.E.M **p<0.01, *p<0.05.

Table 3: Effect of kalyanaka ghrita and ksheerakalyanaka ghrita in PTZ induced convulsions-number of clonic convulsions

Groups	Number of Clonic Convulsions	Percentage Change
Positive Control (A)	1±0.3162	-
Standard (B)	0	100↓
K.G (C)	3.4±0.6000**	240↑
K.K.G(D)	3.2±0.3742**	220↑

Data: Mean±S.E.M **p<0.01, *p<0.05.

Table 4: Effect of kalyanaka ghrita and ksheerakalyanaka ghrita in PTZ induced convulsions-number of straub tail occurrence

Groups	Number of Straub Tail Occurence	Percentage Change
Positive Control (A)	1.2±0.4899	-
Standard (B)	0	100↓
K.G (C)	2.2±0.2000	83.33↑
K.K.G(D)	1	16.66↓

Data: Mean±S.E.M **p<0.01, *p<0.05.

Table 5: Effect of kalyanaka ghrita and ksheerakalyanaka ghrita in PTZ induced convulsions-latency of recovery

Groups	Recovery latency (Sec)	Percentage change
Positive Control (A)	2850±150.00	-
Standard (B)	2700±0.00	52↓
K.G (C)	2325.5±208.50	18.40↓
K.K.G(D)	-	-

Data: Mean±S.E.M **p<0.01, *p<0.05.

Table 6: Effect of Kalyanaka Ghrita And Ksheerakalyanaka Ghrita In PTZ Induced Convulsions-Latency of Death

Groups	Death Latency (Sec)	Percentage Change
Positive Control (A)	1435.25±310.30	-
Standard (B)	-	-
K.G (C)	689.8±208.98	51.93↓
K.K.G(D)	693.2±209.47	51.70↓

Data: Mean±S.E.M **p<0.01, *p<0.05.

Table 7: Effect of kalyanaka ghrita and ksheerakalyanaka ghrita in PTZ induced convulsions-number of recurrent clonic jerks

Groups	Number of Recurrent Clonic Jerks	Percentage Change
Positive Control (A)	1.5±0.2887	-
Standard (B)	0	100↓
K.G (C)	1.2±0.2000	20↓
K.K.G(D)	2±0.3162	33.3↑

Data: Mean±S.E.M **p<0.01, *p<0.05.

DISCUSSION

On evaluating the anti-convulsant activity by PTZ induced convulsions it was observed that in the positive control group the survival rate was zero and duration of survival was considerably less compared to K.K.G group. The standard drug Diazepam exhibited remarkable prolongation in the latency period which was statistically extremely significant. Other parameters such as number of Myoclonic jerks, clonic convulsions, Recurrent convulsions, straub tail occurrence etc. were also reduced considerably which was found to be statistically significant. The survival rate in this group was 100%.

The test group K.G exhibited latency of onset of seizures similar to the positive control group and decreased the number of myoclonic jerks but exhibited an increase in the number of clonic convulsions which was statistically very significant and also the number of straub tail occurrence was increased. The latency of recovery of K.G was reduced compared to positive control group and standard group. The number of recurrent clonic jerks were decreased slightly in K.G group.

In the test group K.K.G, the latency of onset of seizures was similar to the positive control group. The number of myoclonic jerk occurrence were reduced considerably in this group. The number of clonic convulsions was increased significantly when compared to positive control group.The number of straub tail occurrence was also reduced when compared to Positive control group. The survival rate in the group was 0%.There was an increase in the number of recurrent clonic jerks in K.K.G group when compared to positive control group.

On assessing the results Kalyanaka Ghrita was effective in controlling some of the parameters even though the results were not statistically significant. Survival rate of K.G group was better when compared to that of Positive control group as well as K.K.G group. This indicates the presence of mild to moderate anti-seizure activity in K.G group.

CONCLUSION

Both Kalyanaka Ghrita group and Ksheerakalyanaka Ghrita group showed statistically non-significant improvement in the management of symptoms of PTZ induced generalised seizures Thus, Kalyanaka Ghrita and Ksheera Kalyanaka Ghrita can be administered as an adjuvant for main stream Anti-epileptic drugs to cope up with the cognitive and psychological side effects of commonly used anti-epileptic drugs.

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