



Research Article

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ACTIVITY OF PANCHAGAVYA GHRITA IN ANIMAL MODELS OF EPILEPSY AND COGNITION

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ABSTRACT

Panchyagavya Ghrita [PGG] is used in traditional Indian medicine to treat neurological disorders like epilepsy, schizophrenia, anxiety, depression, insomnia etc. PGG alone (in different dose) and as Polyherbal formulations has been studied for its antiepileptic activity but with variable results. As anti epileptics are known to cause memory impairment, the effect of on animal memory also needs to be studied. Methodology: Antiepileptic activity of PGG in 3 doses (2.5, 5 and 10 gm/kg) was estimated using ICES (Increasing current electro shock seizure) animal model wherein mice were treated with drugs for seven days. Animals were observed for occurrence of the THLE (tonic hind limb extension) at particular STC (seizure threshold current) in ICES test. PGG in 3 doses (1.8, 3.6 & 7.2 mg/kg) was also evaluated for its effect on memory after its administration for 21 days orally using Cook's pole avoidance rat model. Results: PGG at 5 gm/kg dose showed comparable results to that of standard drug Phenytoin in ICES model by increasing the STC. Avoidance responses were significantly reduced by Phenytoin ($p < 0.05$) after 21 days treatment as compared to all 3 doses of PGG. Conclusion: 7 days treatment with PGG in 5 gm/kg dose has shown anti epileptic activity in ICES animal model. PGG did not show deleterious effect on animal memory in Cook's pole climbing apparatus on 21 days of treatment as compared to Phenytoin.

KEY WORDS: Panchagavya Ghrita, Epilepsy, ICES, Memory

INTRODUCTION

Epilepsy is a neurological disorder characterized by recurrent seizures. Seizure is a paroxysmal event due to abnormal excessive hyper synchronous discharges from an aggregate of CNS neurons. About 50 million people worldwide have epilepsy and in India approximately 5.5 million people have been suffering from epilepsy¹. In existing modern practice, antiepileptic and CNS depressant drugs are available to manage epilepsy and convulsions. Although with effective results, these medicines precipitate certain adverse drug effects like drowsiness, osteomalacia, anaemia, teratogenic effects and memory impairment.²

Unmad and Apasmar are the Manovaha Strotas [psycho-neurological] disorders stated in Ayurvedic classical texts. According to ayurved, Apasmar is a neurological disorder occurs due to vitiation of Tridosha [Vata, Pitta, Kapha] entities and derangements of intellect and mind characterized by loss of memory, loss of consciousness and convulsive movements of the body.³ Traditional medicinal systems have documented use of several medicines for CNS disorders. In management of Apasmar, Shodhan followed by Shaman chikitsa [treatment] is advocated where specific herbal and herbo-mineral medicines are utilized.⁴ Although, these systems are in practice for centuries, systematic documentation of efficacy profile of their medicines is still not available with probable mode of actions. Hence it becomes imperative to research for their potentials using modern research tools and methods. It is also essential to search safe and better alternative to contemporary treatments for epilepsy from traditional practices.

Siddha Ghrita [Medicated ghee] is the foremost choice of ayurvedic fraternity which exhibits specific actions on CNS as

antidepressant, anti-psychotic, antiepileptic and is suggested to be used in cognitive disorders. Panchagavya Ghrita (PGG) is a lipid base formulation containing five products from 'Cow' viz Cow dung juice, Sour Curd, Cow urine, Cow milk and Cow ghee. It is routinely prescribed in ayurvedic practice to treat CNS disorders viz. Apasmara (Epilepsy), Unmad (Schizophrenia), Bhaya (Anxiety), Anidra (Insomnia), Jwar (Fever) and Kamala (Hepatitis)⁵.

Single drug of PGG possesses Medhya (intellect and memory enhancing), rejuvenating and aphrodisiac activities.^{6, 7,8,9} Researchers have studied antiepileptic and anticonvulsant activities of PGG given in different doses and as a polyherbal formation in MES and PTZ animal models. Hepatoprotective activity against Carbon tetrachloride (CCl₄) induced hepatotoxicity of PGG in PTZ was also estimated.^{10,11,12}

However, antiepileptic activity of PGG prepared with five cow products has not been assessed till date in models for Tonic-clonic seizures along with its effect on memory. Thus present study was planned to find out antiepileptic activity of PGG using ICES model along with its long term effect on memory.

MATERIAL AND METHODS

Fresh Cow dung juice, Cow urine, Cow ghee, Cow milk and Cow curd were procured from the authentic source where brownish colored cows of 'Gir' breed were cradled in open yard. The natural environment during cradling and organic source for feed was maintained for these animals.

Preparation of PGG: (Ayurvedic Formulary of India, Part-I, 6:25)

Standard operating procedure was followed throughout the study. All cow products were authenticated with Ayurvedic Pharmacopeia of India parameters. 600 ml cow dung juice was prepared by mixing fresh cow dung (750gm) and water (750ml). Homogeneous mixture was then filtered through four fold muslin cloth to get clear liquid. 500ml cow ghee was heated on low flame till fumes were appeared and then it was allowed to cool. After a while, Sour curd [500 ml], Cow milk [500ml], Cow urine [500ml] and Cow dung juice [500 ml] were added in 500ml Cow ghee and mixed thoroughly. Whole mixture was boiled on low flame till the total water gets evaporated.

Experimental study design

Swiss albino mice (18-20gm) and wistar rats 180-200 gm of either sex was obtained from CPCSEA registered (Reg.No.258) animal house of BVDU Medical College, Pune. All the animals were housed in a room maintained at $22 \pm 1^\circ\text{C}$ with a relative humidity of 50-60% and a 12-hr light-dark cycle. They were allowed to acclimatize for a week prior to experiment and had free access to standard pellet diet and potable water. Experiments were carried out with strict observance to ethical guidelines and were conducted as per the norms of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Approval was taken from the Institutional Animal Ethics Committee (IAEC), BVDU Medical College and vide approval number was IAEC/BVDUMC/08/11-12.

Drug dose and duration: The human dose of medicated ghee as per Ayurvedic classics is 40 gm per day.¹³ This extrapolated dose was treated as X dose for PGG and cow ghee groups. It was used for both mice and rats. Adult human dose of standard drug Phenytoin was also extrapolated to obtain its dose in mice & rats.

As per Ayurvedic principles, efficacy of medicated ghee might be seen in seven days and more than 20 days¹⁴ Considering this theory, all drugs were administered for seven days in ICES test to assess efficacy of drugs however to find out effect on animal memory all drugs were administered for twenty one days in Cook's Pole Climb Avoidance model

Model I: Increasing Current Electroshock Seizure (ICES) Test]¹⁵

Swiss albino mice 18-20 gm, were divided into five groups of eight animals in each group. Group-I received cow ghee [5gm/kg], Group-II received standard drug Phenytoin [39 mg/kg] Group-III, IV and V received test drug PGG in 3 different doses- 2.5, 5 and 10 gm/kg respectively. The drugs were administered orally to overnight fasted animals. Study mice were screened initially. Electrical current of 2 mA was given to each mouse via electrodes as a single train of pulses [for 0.2 sec] with linearly increasing intensity of 2 mA/2 sec using an electroconvulsimeter. The current at which Tonic Hind Limb Extension [THLE] occurred in animals was recorded as the seizure threshold current. [STC]

These STC readings were taken before drug treatment and recorded as baseline readings. Then animals were treated with respective drugs for 7 days and observed for occurrence of THLE at particular STC on eighth day.

Model II: Cook's Pole Climb Avoidance in Rat¹⁶

In this model, rats were grouped in five, 6 animals in each group and treated orally with cow ghee in 3.6 gm /kg, Phenytoin 27 mg/kg dose and three doses of PGG [1.8, 3.6, 7.2, gm/kg] respectively for 21 days

Each rat was placed in the chamber and allowed to explore the chamber for 2 minutes. Conditioned stimulus (CS) i.e. buzzer signal was turned on for 10 sec. followed by unconditioned stimulus (US) i.e. electric shock was delivered through grid floor for 10 sec. After 2 or 3 exposures to this situation, rat was capable of avoiding the foot shock by climbing the pole which is the safety area. This was noted as Avoidance Response (A.R.). Animals climb the pole after providing shock was noted as Escape Response (E.R).

Training was completed when three consecutive A.R. were obtained. Such 20 to 30 trials were given to animals for complete training. After 24hrs of completion of training sessions, 10 relearning trials were given to animals and number of A.R. was noted as baseline reading. Then all animals were treated with respective drugs for 21 days. On 22nd day, animals were exposed to apparatus again to assess the effect of all drugs on memory.

Statistical Analysis

Data was expressed as mean \pm S.E.M values. Paired t- test was used to compare the pre and post treatment values in the groups of ICES model. Intra-group comparison for AR obtained in Cook's pole climbing mice model was done using paired 't' test and intergroup comparison by one-way ANOVA followed by Tukey's test. $p < 0.05$ was considered statistically significant.

RESULTS

Table 1 indicates that PGG at 2.5 gm/kg ($p < 0.01$) and 5 gm/kg ($p < 0.001$) dose level showed significant increase in Seizure threshold current (STC) when compared with their baseline readings. The result with PGG 5 gm/kg dose was similar to that seen in standard drug group Phenytoin ($p \leq 0.001$). PGG 10 gm/kg dose did not show any significant change in the seizure threshold. Surprisingly, even in control group which was given the vehicle drug [cow ghee], showed significant increase in STC on 7th day which was comparable to PGG 2.5 gm/kg dose group.

Table 2 and Figure 1 depict the change in conditioned avoidance responses in study rats after 21 days drug treatment using Cook's pole climbing apparatus. It shows that number of avoidance responses were significantly ($p \leq 0.001$) reduced in Phenytoin group compared to the groups treated with three different doses of PGG.

Table 1: Change in the seizure threshold in ICES model with 7 days treatment of Panchagavya ghrita in mice

Group (n = 8)	Seizure Threshold Current (STC)	
	Baseline reading	After 7 days of drug treatment
Cow ghee	13.25 ± 0.83	16.50±0.5 **
Phenytoin	15.00±1.25	30.00 ***
PGG [2.5 gm/kg]	17.88±1.95	25.25±0.70**
PGG [5 gm/kg]	19.25±1.06	27.75±0.75 ***
PGG [10 gm/kg]	21.25±2.13	24.13±1.43

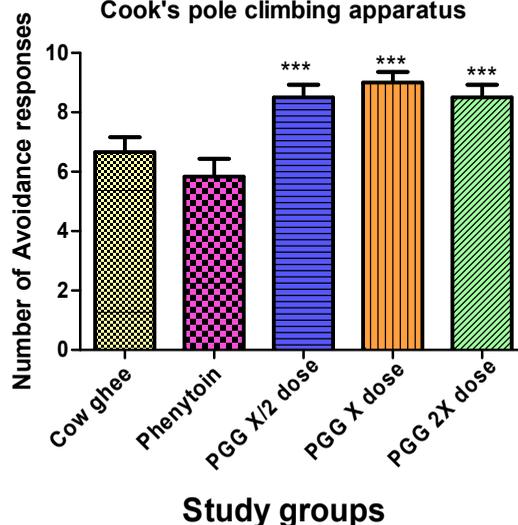
Values are expressed as mean ± SEM, Students' paired 't' test was used to compare the intragroup readings **p≤ 0.01,***p≤ 0.001 in comparison with the baseline readings; PGG= Panchagavya Ghrita

Table 2: Effect of Panchagavya ghrita and Phenytoin on Conditioned Avoidance Response in Cook's pole climbing apparatus in rats

Group (n=6)	Number of Avoidance responses	
	Baseline reading	After 21 day of Treatment
Cow ghee	8.16±0.30	6.66±0.49
Phenytoin	8.50±0.22	5.83±0.60 *
PGG [1.8gm/kg]	8.83±0.54	8.50±0.42 \$\$\$
PGG [3.6gm /kg]	8.83±0.40	9.00±0.36 \$\$\$
PGG [7.2 gm/kg]	8.66±0.42	8.50±0.42 \$\$\$

Values are expressed as mean ± SEM, Intra-group comparison was done using paired 't' test and intergroup comparison by One-way ANOVA followed by Tukey's test. *p<0.05 in comparison with baseline values; \$\$\$p≤ 0.001 in comparison with post treatment readings of Phenytoin group; PGG= Panchagavya Ghrita

Figure 1. Graph showing avoidance responses in rats after 21 days of drug treatment using Cook's pole climbing apparatus



***p≤ 0.001 in comparison with Phenytoin group using One-way ANOVA followed by Tukey's test; PGG= Panchagavya Ghrita

DISCUSSION

In the present scenario of epilepsy disorders, there is a need for medications which are helpful in controlling and preventing the episodes of seizures without disturbing the cognitive abilities of the epileptic patients. Currently available antiepileptic drugs are efficacious but do not adequately address beneficial effects on cognitive domains. Despite treatment advances with conventional antiepileptic drugs over the past decades; epilepsy still remains a challenge for the medical professionals.

ICES model was established for assessment of anti- and pro-convulsant activities of drugs in mice. In this method, a single strain of pulses (square wave, 5 msec, 20 Hz) of linearly increasing intensity from 5 to 30 mA (increment of 0.1 mA/0.1 sec, i.e., 5-30 mA in 25 sec) were applied via ear electrodes. The current at which tonic hind limb extension occurred was

recorded as the seizure threshold. Thus, this method allowed determination of the seizure threshold current for individual animals.¹⁷

There is considerable evidence that Na⁺ current is important for neuronal functions. Induction of epileptiform activity can also be correlated with changes in Na⁺ channel function. For instance, temporal lobe neurons exhibit larger-than-normal I_{NaP} in both the pilocarpine¹⁸ and kindling animal models¹⁹ of epilepsy and in the human temporal lobe surgically resected from patients.²⁰ Blockade of voltage-gated sodium channels is the most common mechanism of action among currently available AEDs. Antiepileptic drugs which have such sodium channel blocking activity have highest affinity for this channel when it is in the inactivated state. Binding of these antiepileptic drugs to the sodium channels in inactivated state, slows the conformational recycling process of the channel. As a result,

these drugs produce a characteristic reduction in channel conductance which is voltage- and frequency-dependent, resulting in a limitation of repetitive neuronal firing.²¹

In the present work, 7 day treatment with PGG given in 2.5 gm/kg ($p \leq 0.01$) and 5 gm/kg ($p \leq 0.001$) doses has shown significant increase in seizure threshold in the ICES model [Table 1]. PGG in 5 gm/kg dose exhibited a significant antiepileptic effect which was found to be comparable to the effect of standard drug Phenytoin ($p \leq 0.001$). Thus, it can be postulated that antiepileptic action of PGG in mice induced by increasing current might be due to the stabilization of sodium ion channel.

In Cook's pole climbing model, treatment with PGG and Phenytoin was carried out for 21 days in wistar rats to determine the long-term effect of PGG on cognitive function. In this test three doses of PGG showed a significant ($p \leq 0.001$) effect on preservation of memory as compared to the standard drug Phenytoin. However rats treated with Phenytoin for 21 days had a considerable deterioration of memory ($p < 0.05$) as evidenced by the reduction in avoidance responses [Table 2 & Figure 1].

In Ayurveda, combination of drugs prepared from herbs and animal products are advocated so that they break the Samprapti (pathogenesis) of epilepsy. These drug combinations are predicted to provide synergistic action which facilitates the target action of final formulation. In earlier research works, the components of PGG showed specific activities towards CNS. The omega-6 essential and omega-3 fatty linoleic acid of cow milk showed important role in prevention of brain diseases viz Alzheimers disease.²² Probiotics of cow curd when used in particular way reduce several deleterious effects of allergens and resistance of infections.²³ It is proved that the antioxidant action is attributed to the free radical scavenging activity of cow's urine components and these components may prevent the process of aging.²⁴ It is well said that consumption of cow ghee in required amount supports to maintain longevity of human body and helps to keep normal function of physical entities and mental factors i.e. intellect and memory.²⁵ Therefore PGG might be cumulatively showing antioxidant, neuro-protective activities and positive effects on memory owing to combination of five cow products. The antioxidant and nootropic activities of PGG have also been reported showing positive effect on animal learning and memory.^{26,27}

Even in present study, PGG has shown anti epileptic and memory improving actions in ICES and Cooke's pole climbing models respectively. These actions of PGG may be perceived due to lipophilic action of ghee which facilitates carrying of bio-components to target organ and delivery inside the cell as the cell membrane contains lipids.²⁸ The bio-components of PGG may be interacting with certain receptors that produce antiepileptic and memory enhancing activities. However, further studies are required to explore the mechanism of action of PGG as an antiepileptic and cognition enhancer.

CONCLUSION

The results of this study give scientific evidence of antiepileptic effect of PGG in ICES model with favorable effects on cognitive function of study animals as compared to standard drug Phenytoin.

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