Journal Homepage: - www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/12919
DOI URL: http://dx.doi.org/10.21474/IJAR01/12919

RESEARCH ARTICLE

PROMISING ANTI CONVULSANT EFFECT OF A HERBAL DRUG IN WISTAR ALBINO RATS

Dr. Reshma P. John[1] and Dr. A Shahul Hameed[2]

1. M D Scholar. Department of Dravyaguna Vijnana, Government Ayurveda College, Thiruvananthapuram 695001, Kerala, India.
2. Guide, Professor & HOD, Department of Dravyaguna Vijnana, Government Ayurveda College, Thiruvananthapuram 695001, Kerala, India.

Manuscript Info

Manuscript History
Received: 25 March 2021
Final Accepted: 29 April 2021
Published: May 2021

Key words:-
Anticonvulsant, Guchapatra, Ruta Graveolens, Ghritam, MES, Phenytoin, Aruta

Abstract

Epilepsy is one of the most common chronic disease affecting human beings.This brain disorder is characterized by tendency for recurrent seizures or fits. The seizures can leads to loss of consciousness, disturbance of movement, muscle spasms, autonomic and mental functions. In Ayurveda the epilepsy is correlated to Apasmara by classical symptomatology especially under generalized tonic-clonic seizure.Treatment of epilepsy is a long-term process and usage of conventional antiepileptic drugs Carbamazepine, Valproic acid, Ethosuximide, Phenobarbital, Benzodiazepine and Phenytoin produce unpleasant side effects in long run. So a safe herbal medicine in this condition is a necessity. The traditional healers of kerala, in their practice they widely use one herbal drug called Guchapatra (Ruta graveolens L) commonly known as “Aruta” in Malayalam. The plant is administered as ghrita (a preparation with ghee as the main base) for the effective management of Apasmara (Epilepsy). Guchapatra (Ruta graveolens L) is a strongly odoriferous perennial herb belonging to the family Rutaceae. The claim of anti epileptic effect of Ghee prepared with Ruta graveolens is not proven scientifically till date. The aim of present study is to test Guchapatra (Ruta graveolens .L) for its anti-convulsant effect by Maximal electroshock seizure (MES) method in Albino rats. The drug was administered in the form of Ghrita. The experiment was carried out in 3 groups having 6 Wistar albino rats per group. ‘Phenytoin’ was the standard drug. Group 1 (control – distilled water), Group 2 (standard drug - Phenytoin), Group 3 (Normal dose of Guchapatra ghritam (Ghee prepared with Ruta graveolens. L). Reduction in duration of Tonic Hind Limb Extension (THE) in seconds or the complete absence of tonic extensor phase of MES convulsions was taken as the assessment criteria for Anti-convulsant effect. The observations statistically analyzed using one way ANOVA and Post Hoc Tukey’s multiple comparison tests. The in-vivo experiment revealed that the Guchapatra (Ruta graveolens. L) as a ghrita (Ghee) preparation posses equal anti- convulsant effect in comparison with standard drug Phenytoin.

Corresponding Author:- Dr. Reshma P. John
Address:- M D Scholar. Department of Dravyaguna Vijnana, Government Ayurveda College Thiruvananthapuram 695001. Kerala, India.

Introduction:-

Epilepsy is one of the most common chronic disorders of CNS, characterized by recurrent seizures that affects not only the individual, but also has impact on family and the society in general. Epilepsy is a symptom of brain disease rather than disease itself. It is second most common neurologic disorder after stroke. Causes of epilepsy are extremely diverse, which are genetic, developmental or acquired ones. This condition affects human populations involving people at any age, any time. Epileptic seizures are finite episodes of brain dysfunction, resulting from abnormal discharge of cerebral neurons that occur suddenly without warning, have short duration that is up to few seconds or minutes and stop by themselves. Epilepsy can be equated to Apasmara in Ayurveda, by classical symptomatology especially under generalized tonic-clonic seizure. Treatments for seizures are broadly-based on long term usage of anticonvulsant medications such as Carbamazepine, Valproic acid/ Valproate semisodium, Phenytoin, Phenobarbital, or more recently with Gabapentin, Oxcarbazepine, Lamotrigine or Topiramate. Pharmacodynamics of Anti- epileptic drugs include inhibition of voltage gated Na, Ca channels, potentiate GABA mediated inhibition and decrease of Glutamate mediated excitation. Most of patients do not respond completely towards current therapeutic agents due to unpleasant side effects like cognitive impairment, dose-related neurotoxicity and a spectrum of other systemic ill effects in long run. Therefore it is necessary to search for new drugs with fewer or no side effects and predictable pharmacological action. Here arises the need of a safe herbal substitute. A wide variety of medicinal plants are used in the treatment of Epilepsy by traditional healers across the world.

The plant **'Guchapatra**' (**Ruta graveolens. L)** is a strongly odoriferous perennial herb belonging to the family Rutaceae. The plant is mentioned in Ayurveda lexicons like Brihat Nighantu Ratnakara and Nighantu Adarsh. In 'Nighantu Adarsh' it is explained as 'Sitab' under Bheejapoorakadi varga and having very potent medicinal properties. The drug being used as analgesic, anti-pyretic, anti-convulsant, emmenagogue, anti-helminthic, anti-fungal, anti-fertility and for the treatment of mental disorders. In Kerala it is commonly known as 'Aruta' and the ghrita (Ghee) prepared with it is widely used as a remedy for Apasmara (Epilepsy) by the traditional healers of Kerala. The efficacy of this plant 'GUCHAPATRA' (Ruta graveolens. L) in the classical dosage form of 'Ghrita' (Ghee) as an anticonvulsant has not been tested using scientific means, so as an initial step to authenticate this claim and in finding a safer drug for Epilepsy the present study is taken up.

The drugs given in the form of ghee is likely to be rapidly absorbed and distributed in the target areas of the body such as nervous system. The main reason behind this is the molecular structure of blood brain barrier (BBB), so this dosage form is selected to evaluate the anti convulsant effect of 'GUCHAPATRA' (Ruta graveolens. L) Experimentally in Wistar albino rats using Maximal Electro shock seizure (MES) test. The conservation of traditional knowledge, its validation through scientific procedure is the necessity of the day. If it is proven that Guchapatra ghrita (Ghee prepared with Ruta graveolens. L) has anti- convulsant effect it will be beneficial not only to the suffering community but will also help in broadening the horizons of ayurvedic wisdom.

Materials and Methods:-

Composition of Study Drug

Paste of Ruta graveolens .L	- 35g
Cows Ghee	- 125 ml
Coconut oil	- 125 ml
Fresh juice of Ruta graveolens .L	- 1L
Final quantity of drug obtained	- 250 ml (Ruta ghee – Guchapatra ghrita)

Guchapatra (Ruta graveolens .L) was collected personally from Jawaharlal Nehru Tropical Botanic Garden & Research Institute (JNTBGRI) Palode, Kerala and the drug were authenticated by Pharmacognosy unit and checked for purity in Drug Standardisation Unit, Government Ayurveda College, Thiruvananthapuram, Kerala. Collected whole plant were cleaned thoroughly and used for ghrita preparation.

Ghee (ghrita) of Agmark standard (Amul cow ghee, manufactured by Valsad district co-operative milk producers union LTD, Vasundhara Dairy Alipur, India, Batch no IAB2541) was used. Coconut oil (Kera taila) of Agmark

standard (Brahmins coconut oil, manufactured by K M oil industries, Kannur, Kerala, India. Batch no CN O60220) were used.

Preparation of Fresh Juice of Ruta graveolens .L
Fresh juice of drug was prepared according to the standard procedure. Drug collected was washed and cut into small pieces and then the juice will be extracted and filtered to get the required amount of juice.

Preparation of paste of different parts of plant Ruta graveolens.L
Kalka were prepared by crushing the whole plant in a mixer grinder.

Preparation of Medicated ghee using Ruta graveolens.L (Guchapatra Ghrita)
Ghrita (Ghee) was prepared with Guchapatra (Ruta graveolens. L) according to the standard procedure mentioned in Sharangadhara Samhitha Madhyama Khanda with Madhyama paka.

Standard drug: Phenytoin Sodium Injection (Eptoin), 2 ml ampule of Abbot health care Pvt Ltd. The medicine was procured from a local pharmacy near General Hospital, Thiruvananthapuram.

Animals:
18 adult Wistar albino rats of either sex weighing between 150-200 g were used for the experimental study. The animals were obtained from Sree Chitra Tirunal Institute of medical Science and Technology, Satelmond Palace, Poojappura, Thiruvanthapuram, Kerala. (98/GO/RBiBt/SL/99/CPCSEA 28.04.1999) guidelines were always followed and prior approval of Institutional Animal Ethical Committee (No 02/05/2019) of Govt. Ayurveda College was obtained as per letter no IEC 399/2.05.2019 before commencing experiments.

Housing and feeding conditions
All the animals were maintained in appropriate environmental and nutritional circumstances through of the experiment. The rats were maintained under standard laboratory conditions with natural dark and light cycle. They were provided with free supply of standard dry rat diet and water. Animals were acclimatized for 14 days prior to experimentation. 3 animals were housed in each cage made from polypropylene with chrome steel top grill. Adequate bedding was provided in rat cages which were periodically changed.

Grouping and posology
The acclimatised animals were weighed and randomly divided into 3 groups having 6 animals each. The random selection ascertained un-biased distribution of animal with regard to sex age, weight etc. in each group. The animals were marked for correct identification and kept in separately labelled cages. The dose calculation was according to the body weight of each animal using the table constructed by Paget G. E & Barnes T .M considering the normal human dose of ghrita as 48ml and the animal dose were taken as 0.864ml/ 200g animal. Route of Administration - oral

Group I - Negative Control group were no active treatment received. The animals were on normal rat diet and water. Group II - Standard group /positive control – Phenytoin (20 to 25 mg/kg) Group III -Normal dose of Ruta ghee (Guchapatra ghrita 0.864ml).

Experimental Design
Maximal Electroshock Seizure Test (MES)
The anticonvulsant effect was evaluated by Maximal Electroshock seizure test (MES). MES test represents Grand-mal type of epilepsy

Methods and timing for assessing, recording and analysing the parameters.
All animals were kept under standard laboratory condition and provide with standard diet and water. 14 days acclimatized animals were deprived of food overnight before the test. On the day of test (15^{th} day) the control group animals are subjected to administered distilled water and the standard group animals were administered Phenytoin. Then the animals are subjected to MES test after 2 hours of administration of drug.

The study drug will be administered every day to the group III for 15 days in the same time between 8 am to 10 am every day. Animals will be deprived of food overnight before the test. On the fifteenth day of regular medication, 2

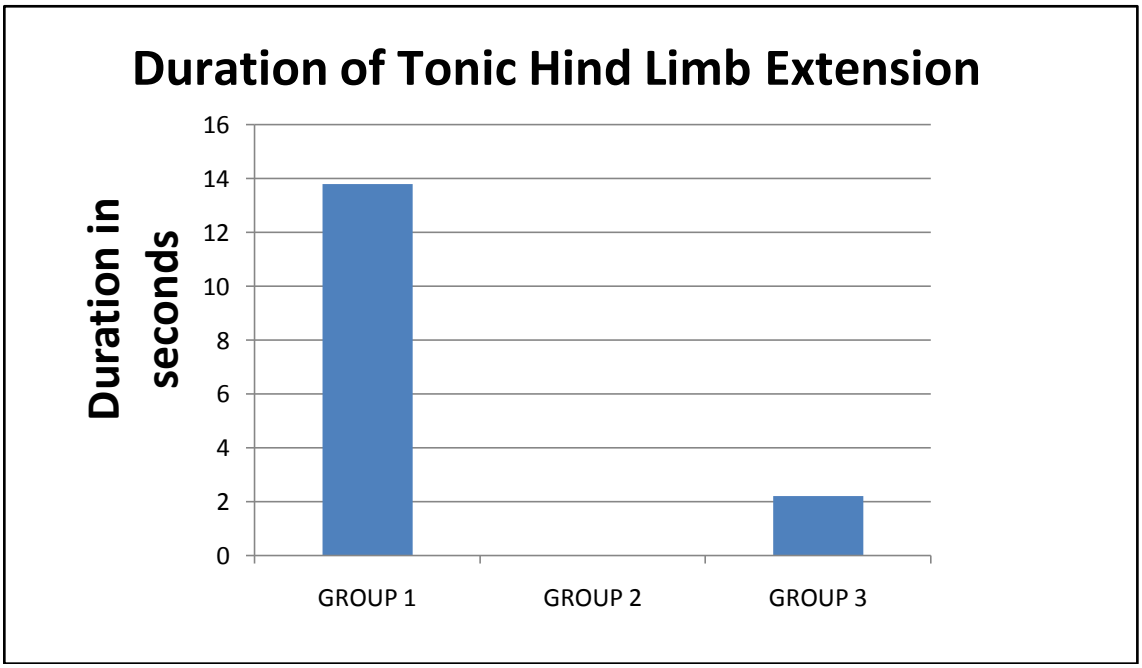
hour after the drug administration the rats are subjected to MES test and the time spent in each phase was noted. The Albino rats were restrained by hand and subjected to electroshock through their pinna using Electro convulsant unit. Before applying the electrodes Lignocaine gel was applied on the ear pinna of rats. Then the rats were immediately released following electrical stimulation (150mA alternating current of 100Hz frequency for 0.2 seconds) to permit observation of maximal seizure. The maximal seizure typically consists of a short period of tonic extension followed by clonic convulsions and stupor. Protection is defined as complete abolition of hind limb extension. The results were compared with normal control (Group I) and standard (Group II). Then the results is analysed statistically.

Results:-

The drug is meant to possess anti-convulsant effect if it reduces the duration of Tonic Hind limb Extension (THE) or abolishes the same. The statistical analysis of time (in seconds) over the tonic extensor phase of MES convulsions in each group was carried out to establish the effect of the study drug in each group. Data and percentage change of average Tonic Hind Limb Extension (THE) time in seconds among the various groups is given in Table no. 1. The Significance of the data across all groups were analysed by ANOVA. As ANOVA showed significance at $p<0.001$, post hoc analysis (Tukey's multiple comparison test) was then applied for finding the pair of group having statistical significance

Table 1:- Data and percentage change of average Tonic Hind Limb Extension (THE) the various groups.

	N	DURATION OF TONIC HIND LIMB EXTENSION IN SECONDS			
		Mean	sd	se	95% CI of mean
Group I	6	13.8	1.2	0.5	12.6 -15.1
Group II	6	0.0	0.0	0.0	0.0-0.0
Group III	6	2.2	2.5	1.0	-0.4-4.8



After 15 days of drug administration, the Experimentally Drug Treated Group displayed significant anti convulsant effect. Data and percentage change of average Tonic Hind Limb Extension (THE) time in seconds among the various groups is given in Table no. 2

Table 2:- Data and percentage change of average Tonic Hind Limb Extension (THE) time in seconds among the various groups.

Post hoc analysis (Tukey's multiple comparison test)	Mean Diff.	SE of diff.	95.00% CI of diff.	P Value	Significant
Group I vs. Group II	13.83	0.729	11.62 to 16.05	<0.001	Yes
Group I vs. Group III	11.67	0.729	9.449 to 13.88	<0.001	Yes

Group II vs. Group III	-2.167	0.729	-4.384 to 0.05074	0.059	No

The standard drug (Phenytoin) treated group showed 100% abolishment of THE time, exhibiting most anti-convulsant effect. In chronic dosing the normal dose or therapeutic dose of Guchapatra ghrita (Ruta ghee) posses equal anti- convulsant effect compared with standard drug Phenytoin. P-value is 0.059 and is not significant. In view of all these findings, it can be stated that Ghee prepared with Guchapatra (Ruta graveolens. L) posses significant anti- convulsant effect on long term use as showcased by Maximal electro-shock seizure test in albino rats. So the efficacy of this plant 'GUCHAPATRA' (Ruta graveolens. L) in a classical dosage form as an anticonvulsant can be claimed as equal to standard drug Phenytoin. Guchapatra (Ruta graveolens.L) a herb in use for centuries in India and in other countries, this will be a safer alternative to modern anti-epileptic agents.

Discussion:-

'Guchapatra' identified as **Ruta graveolens. L,** is a medicinal plant mentioned in Ayurveda lexicons. Description regarding the plant can be seen in Brihat Nighantu Ratnakara and Nighantu Adarsh. In 'Nighantu Adarsh' it is explained as 'Sitab' under Beejapoorakadi varga and having very potent medicinal properties. This plant is not mentioned in the classical Ayurvedic literature. In Kerala it is commonly known as 'Aruta'. However its usage in various ailments including Apasmara is much established and is vogue in traditional medicine and the ghrita prepared by this widely used as a remedy for epilepsy by the traditional healers of Kerala. Records of indigenous knowledge through scientific studies are very important for conservation and utilization of biological sources. So conservation of traditional knowledge, its validation through scientific procedure is the necessity of the day.

By considering the wide range of classical symptoms of Apasmara and the modern description of Epilepsy, they are widely equated to one another, especially with respect to tonic-clonic / generalized seizure. Although various drugs are available in market their clinical use are limited due to neuro toxicity and side effects. On the other hand herbal medicines are being used due to applicability and efficiency coupled with least side effects which in turn accelerated the scientific research regarding antiepileptic activity of indigenous plants.

The pharmacological activity of ghee prepared with Guchapatra (Ruta graveolens. L) was decided to be assessed by chronic Anticonvulsant experimental study using Maximal Electroshock seizure method. The plant is a native of Mediterranean region and distributed in South America, China, Middle East, South Africa and cultivated throughout India - Westwards to the canaries. The pharmacological evaluation of this drug was undertaken for ascertaining whether the drug pharmacologically active in the indicated condition or not. The experimental study was preceded by the literature review. The preliminary pharmacognostic, physical and phytochemical screening of Ruta graveolens was carried out. The physicochemical analysis of Guchapatra ghrita (Ruta ghee) was done.

MES-induced convulsion model causes movement of Ca2+ and another positive ion like Na+ into the cells, and their blockage can prevent MES-induced tonic extension. The potentiation of GABA receptor may offer protection against MES-induced seizures. MES-induced seizure can be prevented either by drugs that inhibit voltage-dependent Na+ channels such as phenytoin and valproate or by drugs that block glutamatergic receptor such as felbamate. Guchapatra ghrita shows significant Anti-convulsant effect in MES test which is probably achieved by either of these mechanisms.

The phytochemical qualitative analysis of Ruta graveolens.L revealed the presence of the following chemical components: saponins, tannins, cardiac glycosides, alkaloids, triterpene steroids and flavonoids. From various studies alkaloids & flavonoids as well as their glycosides, exert anxiolytic, sedative and anticonvulsant effects on the central nervous systems (CNS) . According to the research studies saponin and triterpene steroids have anticonvulsant effects. Since Ruta graveolens.L have alkaloids, flavonoids, saponins and triterpene steroids amongst other chemical components, it is possible that these secondary metabolites may be contributing to the anticonvulsant effect by non- competitive inhibition of the GABA receptors.

The oxidative stress is the most prominent mechanism in the development and progression of epilepsy and other diseases, including Alzheimer's disease, chronic degenerative diseases, stroke, rheumatoid arthritis, diabetes, and cancer. The constituents in this medicine that is cow's ghee (Go- ghrita) and coconut oil (Kera taila) are well demonstrated anti-oxidants. The presence of anti-oxidants prevents the possible damage of neurons occurring from

repeated seizures. Ghrita (Ghee) and Kera taila (Coconut oil) posses established effect on emolliate oxidative stress in rat brain.

Hence it can be stated that Guchapatra ghrita (Ruta ghee) possesses anti-convulsant activity. The anticonvulsant action of the medicine could be by the synergistic action of the phytochemicals like flavonoids and phenols that act by inhibition of GABA receptors. The formulation could also have the ability to prevent the occurrence of further seizures. The antioxidant potential of the preparation, they have significant role in its therapeutic efficacy as an anticonvulsive drug.

Conclusion:-

The trial drug Guchapatra ghrita (Ruta ghee) have a definite anticonvulsant action in chronic dosing of 15 days as described by the experimental study conducted on Wistar albino rats by MES test. The standard drug Phenytoin abolished the Tonic Hind Limb Extension (THE) phase, while the trial drug also posses equal anti- convulsant effect by showing protection against Tonic Hind Limb extension in MES induced seizures. From this point of view Ghee prepared with Ruta graveolens (Guchapatra ghrita) is equally effective pharmacologically. Thus the study revealing the anticonvulsant effect of Aruta as Guchapatra ghrita (Ruta ghee) that is widely used as a remedy for Apasmara (epilepsy) by the traditional healers of Kerala.

References:-

1. Bapalal G. Vaidya, Nighantu Adarsh vol.2, reprint edition 2005, Chaukhambha Bharati Academy Varanasi.
2. Dr. K R Raman Namboothiri. Athbutha Oushadhachedikal. Kottayam: H&C; 2009.
3. Indradev Tripady (n.d.) Brihat Nighantu Ratnakara, Edited by Indradev Tripady, Mumbai: khemaraj Publishers.
4. Prof.KSrikantha Murthy; Sarngadhara Samhitha; Chaukamba Orientalia; Reprint Edition 2012;Chapter 9,.p.116
5. S. Shrestha, J.-H. Park, D.-Y. Lee et al., “Rhusparviflora and its biflavonoid constituent, rhusflavone, induce sleep through the positive allosteric modulation of GABAAbenzodiazepine receptors,” Journal of Ethnopharmacology, vol. 142, no. 1, pp. 213–220, 2012.
6. Seneff S, Glyn W, Luca M. Nutrition and Alzheimer’s disease: The detrimental role of a high carbohydrate diet. Eur J Intern Med 2011; 22, 2; 134-40.
7. Vaidya Yadavji Trikamji Acharya editor. Charaka samhitha by Agnivesa with Commentary by Chakrapanidatta Nidana sthana 8/11, Reprint ed. Varanasi: Choukahamba Sanskrit Sansthan; 2014 .p.227
8. Waheed A, Pathak S. Mirza R; Epilepsy: A brief review; Pharma Tutor; 2016; 4(9); 21-28
9. Warrier P K, Nambiar V P, Ramankutty C.Indian Medicinal Plants a Compendium of 500 Species. Vol 5, Aryavaidya sala Kottakkal, Hyderabad: Orient Longman Ltd; 1994.