

FLAXSEED OIL ALONE AND AS AN ADJUVANT WITH PHENYTOIN IN MES-INDUCED SEIZURES IN ALBINO RATS

RAHUL H DAMODAR, SUNEEL KUMAR REDDY*, MALVIKA GOYAL, PRADEEP B E

Department of Pharmacology, JJMMC, Davanagere, Karnataka, India. Email: dr.sukure76@gmail.com

Received: 13 September 2017, Revised and Accepted: 20 November 2017

ABSTRACT

Objective: The objective of this study to evaluate the anticonvulsant activity of flaxseed oil alone and as an adjuvant to phenytoin sodium.

Methods: A total of 24 albino rats were used for this study. Four groups - control, standard (phenytoin sodium), test (flaxseed oil), and flaxseed oil along with phenytoin were made with six rats in each group. Maximal electroshock seizures 60-Hz AC of 150 mA intensity for 0.2 s were induced using an electroconvulsimeter with ear electrodes 60 min after oral drug administration. Duration of tonic hind limb extension (THLE) in seconds was used as a measure of seizures induced.

Results: The mean duration of THLE in 4 groups was 11.66 (Group I), 5.67 (Group II), 3.85 (Group III), and 2.69 (Group IV). Duration of THLE was reduced in flaxseed oil group ($P < 0.000$) compared to both control and standard. Other parameters such as regain of righting reflex and recovery time also showed improvement. The group where flaxseed oil was used as an adjuvant to phenytoin also showed significant anticonvulsant activity. It showed a greater reduction in the parameters compared to either drug alone.

Conclusion: The study showed that flaxseed oil possesses marked anticonvulsant activity when used alone and as an adjuvant to phenytoin. This study shows the potential of flaxseed oil in generalized tonic-clonic seizure.

Keywords: Anti-epileptic, Flaxseed oil, Maximal electroshock seizure, Omega-3 fatty acids.

© 2018 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2018.v11i2.22545>

INTRODUCTION

Epilepsy refers to a disorder of brain function characterized by the periodic and unpredictable occurrence of seizures.

This generally occurs because of unwarranted neuronal discharge in the different areas of the brain. The characteristics of seizures differ and are dependent on where the aberrant activity first begins and its extension into other areas of the brain [1].

Approximately 1% of the world's population is said to suffer from epilepsy with more than 40 varieties characterized [2]. Among these, the most common is the generalized tonic-clonic seizures (GTCS) also called grand mal seizures. In this type, the entire brain gets involved with seizure episodes that last from several seconds to minutes and associated with loss of consciousness.

Currently, a number of antiepileptic drugs belonging to various classes are available for the treatment of epilepsy. When monotherapy does not control seizures, adequately drug combination therapy with adjuvant drugs is employed. Although currently available drugs are able to control epilepsy in many patients, there is still a need for better drugs. About one in three patients are resistant to currently available anti-epileptic drugs [3], and they are also associated with significant side effects. Hence, there is a need to search for new drugs that have greater efficacy and with fewer side effects. Naturally, obtained products, especially plants, have been a good source for the discovery of new drugs.

The supplementation of diet with fatty acids has been suggested to have a beneficial role in the management of seizures in patients with epilepsy [4]. The Omega-3 fatty acids also termed n-3 polyunsaturated fatty acids (PUFAs) are essential for normal development of the brain with their deficiency causing neurologic dysfunction. The n-3 PUFAs can

raise the threshold of epileptic seizures [5]. It is also believed that the essential fatty acid alpha-linolenic acid (ALA) may have anticonvulsant effects [6].

Flaxseed is obtained from the flax plant. The flax plant (*Linum usitatissimum*) also known as common flax/linseed is a food and fiber crop cultivated in the cooler regions of the world. Flaxseed oil is a colorless to yellowish oil obtained from the dried, ripened seeds of the flax plant by cold pressed extraction method. It is a source of healthy fat, antioxidants, and fiber and is claimed to lower the risk of diabetes, cancer, and heart diseases as well as aid in neurotransmission. Flaxseed is believed to be one of the most substantial sources of omega-3 α -linolenic fatty acids [7]. It is also rich in ALA which endogenously gets converted to longer chain omega-3 fatty acids, eicosapentaenoic acid, and docosahexaenoic acid (DHA). Flaxseed oil delivers a greater amount of omega-3s compared to ground flaxseed.

Maximal electroshock seizure (MES) induction is the most frequently used screening model for the identification of anticonvulsant activity of any drug against GTCS. In this model, tonic hind limb extension (THLE) is evoked by the electrical stimuli administered through corneal or ear electrodes. Ear electrodes are commonly preferred as it is easier to use and less harmful compared to corneal electrodes [8]. Agents screened through this model are considered to show an anticonvulsant activity if they correct or suppress THLE in rats.

Whereas earlier studies were done using flaxseed oil as dietary supplements, this study was done with the aim of evaluating the direct anticonvulsant activity of flaxseed oil when used alone and as an adjuvant to phenytoin.

Objectives

The objectives of this study were as follows:

- To evaluate the anticonvulsant activity of flaxseed oil
- To evaluate the anticonvulsant activity of flaxseed oil as an adjuvant to phenytoin sodium.

METHODS

The study was initiated after obtaining the necessary approval of the Institutional Animal Ethical Committee (IAEC) of JJM Medical College, Davanagere.

Approval No. JJMMC/IAEC/08-2016.

Chemicals and drugs

The dose for rat was calculated by extrapolating the human dose to animals based on body surface area ratio by referring standard table of Paget and Barnes (1964) [9].

- Flaxseed oil: 1.8 ml/kg rat.
- Phenytoin sodium: 25 mg/kg rat.

Flaxseed oil was purchased from commercial dealer (sattvic foods and cold pressed flaxseed oil). It is a colorless to yellowish oil obtained from dried, ripened seeds of the flax plant. The oil was identified and authenticated by pharmacognosist of our institute. Milk was used as the vehicle for flaxseed oil and was purchased on the day of experiment.

Selection of animals

White strain albino rats were obtained from the animal house attached to Pharmacology Department of JJM Medical College. A total of 24 rats aged 8–10 weeks of either sex were taken. The animals were kept fed on a standard pellet diet and water. They were acclimatized for 7 days before commencement of the study in standard laboratory conditions of 12 h day and night rhythm, maintained at $25\pm 30^{\circ}\text{C}$ and 50-70% humidity as per the CPCSEA guidelines.

Inclusion criteria

The following criteria were included in the study:

- Healthy 24 albino rats weighing 100–150 g
- Rats previously unused for any experiments
- Rats that showed hind limb tonic extension when pre-tested 24 h prior for sensitivity to electric shock.

Exclusion criteria

The following criteria were excluded from the study:

- Rats that failed to give hind limb tonic extension when pre-tested 24 h prior for sensitivity to electric shock
- Pregnant and diseased animals

Duration of study

The duration of this study was 2 months.

Instruments

- Electroconvulsimeter - H.L. Scientific Industries was used.

Procedure

A total of 24 animals (n=24) were used.

The screened animals were separated into 4 Groups of 6 animals each. Each group received drugs as shown below.

- Group I: Control rats (normal saline 10 ml/kg)
- Group II: Standard (phenytoin sodium 25 mg/kg).
- Group III: Flaxseed oil (1.8 ml/kg) with milk as vehicle.
- Group IV: Flaxseed oil (1.8 ml/kg) with milk and phenytoin (25 mg/kg).

MES model

The drugs were administered orally with the help of gastric catheter sleeved to syringe. The MESs were induced 60 min after drug administration. The electroconvulsimeter (Fig. 1) was set to deliver current at a frequency of 60-Hz AC and intensity of 150 mA for 0.2 s through ear electrodes.

Parameters observed

1. THLE: Tonic hind limb extension (Fig. 2) and its duration recorded after each shock.
2. The time for regaining the righting reflex.
3. Recovery time - time taken to begin voluntary wandering movements.



Fig. 1: Electroconvulsimeter



Fig. 2: Tonic hind limb extension

Statistical analysis

The statistical analysis was carried out with SPSS version 20 for Windows. The mean and standard deviation were calculated for the variables. Comparison of the four groups was done using One-way analysis of variance. Multiple comparisons were done with Tukey's *post hoc*. $p < 0.05$ was considered to be statistically significant and $p < 0.01$ as highly significant.

RESULTS

Both flaxseed oil and phenytoin sodium significantly decreased the convulsion phase in the rats. The duration of extensor phase of THLE in the control group was 11.66 ± 2.42 s (Table 1). Pre-treatment with flaxseed oil showed significant anticonvulsant activity by decreasing the duration of THLE to 3.85 ± 0.98 s ($p < 0.05$). Flaxseed oil with standard drug phenytoin showed still greater reduction in THLE duration to 2.69 ± 0.32 s.

With regard to the "regain of righting reflex," both flaxseed oil and phenytoin sodium showed a decrease in time when compared to control, but the reduction was not statistically significant (Table 2).

Similarly, with regard to the "recovery time," both flaxseed oil and phenytoin sodium showed a decrease in the recovery period when compared to control, but the reduction was not statistically significant (Table 3).

Mean duration of all the 3 parameters are depicted in Graph 1. When compared to the control Group I, the 3 drug Groups II-IV showed statistical significant reduction in the THLE (Table 4). The combination of flaxseed oil with phenytoin (Group IV) showed protection which was superior to either drug alone (Tables 1-3).

DISCUSSION

The results of the study demonstrate that flaxseed oil has anticonvulsant activity against GTCS. The results correlate with an earlier study

conducted by Tanna *et al.* showing anticonvulsive effect of flaxseed oil [10]. However, this study in addition shows the beneficial role of flaxseed oil as an adjuvant to phenytoin.

The fact that flaxseed oil being a rich source of n-3 PUFAs it can be supposed that n-3 PUFAs have been responsible for the anticonvulsive effect. This study therefore correlates a protective role for n-3 PUFAs present in flaxseed oil and confirms their protective effect against the seizures [11].

Attempts have been made to understand the precise role of omega-3 fatty acids in neurological disorders. Chronic treatment with omega-3 promotes neuroprotection and positive plastic changes in the rat brain with epilepsy [12], with a decrease in neuronal death in CA1 and CA3 subfields of hippocampus. This is a cause of n-3 PUFAs ion channel modulation [13-15] and anti-inflammatory action. *In vitro* studies from rat neural tissues have shown that DHA inhibits epileptiform activity and synaptic transmission predominantly through the frequency-dependent blockade of Na⁺ channels in the rat hippocampus [11,13], in addition to stabilizing the neuronal membrane by suppressing voltage-gated Ca²⁺ currents and Na⁺ channels [11].

As far as humans are concerned, flaxseed oil is consumed as a part of diet, especially in India. It is not known to be associated with any significant adverse effect. A protective role for fatty acid supplementation against epilepsy has been also seen in humans [4]. Thus, flaxseed oil shows potential both as monotherapy and in combination with phenytoin in the treatment of epilepsy associated with GTCS. Further studies may be undertaken either as a dietary supplement or as pharmacotherapy with the specific isolates of flaxseed oil.

ACKNOWLEDGMENT

The authors are thankful to Principal and Pharmacology Department HOD, JJM Medical College, Davanagere for providing with all the facilities required in this work.

AUTHORS CONTRIBUTION

Conception and design of study: Dr Rahul H Damodar, Dr Suneel Kumar Reddy. Analysis/ Interpretation of data: Dr Malvika Goyal, Dr Pradeep B E, Dr Rahul H Damodar. Revising the manuscript for important intellectual content: Dr Suneel Kumar Reddy, Dr Rahul H Damodar

CONFLICT OF INTEREST

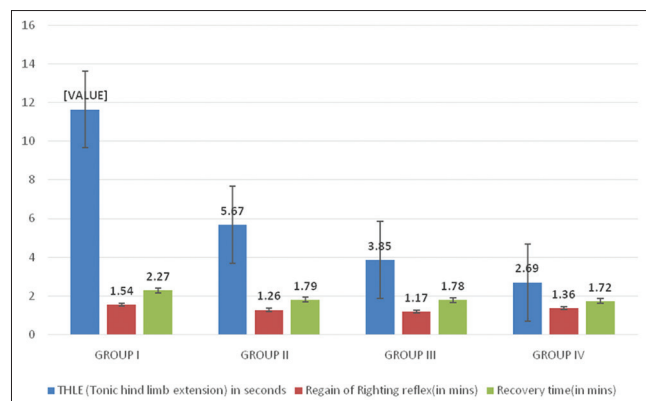
Nil

CONCLUSION

- Flaxseed oil has anticonvulsant action when given alone
- Flaxseed oil has adjuvant anticonvulsant action to phenytoin
- Flaxseed oil can be tried as a dietary supplement in epileptic patients.

REFERENCES

1. Deka D, Chakravarty P, Purkayastha A. Evaluation of the anticonvulsant effect of aqueous extract of *Centella asiatica* in albino mice. *Int J Pharm Pharm Sci* 2017;9:312-4.
2. McNamara JO. *Pharmacotherapy of the Epilepsies: Goodman and Gilman's the Pharmacological Basis of Therapeutics*. 12th ed. New York: McGraw Hill; 2011. p. 583.
3. Reetesh KM, Papiya B, Sunny S, Sonam J. Medicinal plants used in the treatment of epilepsy. *Int Res J Pharm* 2011;2:32-9.
4. Mostofsky DI, Rabinovitz S, Yehuda S. The use of fatty acid supplementation for seizure management. *Neurobiol Lipids* 2004;3:17-23.
5. Schlanger S, Shinitzky M, Yam D. Diet enriched with omega-3 fatty acids alleviates convulsion symptoms in epilepsy patients. *Epilepsia* 2002;43:103-4.
6. Dell CA, Likhodii SS, Musa K, Ryan MA, Burnham WM, Cunnane SC. Lipid and fatty acid profiles in rats consuming different high fat ketogenic diets. *Lipids* 2001;36:373-8.
7. Okhti ZA, Muthanna I, Al-Ezzi, Abdulmahdi R. The protective role of



Graph 1: Group wise comparison of the parameters

Table 1: Mean duration of THLE after MES

Groups	THLE in seconds		
	Mean±SD	ANOVA	p value
I	11.66±2.42	45.49	p<0.000**
II	5.67±1.21		
III	3.85±0.98		
IV	2.69±0.32		

THLE: Tonic hind limb extension, MES: Maximal electric seizure. **Highly significant, ANOVA: Analysis of variance, SD: Standard deviation

Table 2: Mean duration in the regain of righting reflex after MES

Groups	Regain of righting reflex (in minutes)		
	Mean±SD	ANOVA	p value
I	1.54±0.44	0.997	0.414
II	1.26±0.18		
III	1.17±0.33		
IV	1.36±0.13		

MES: Maximal electric seizure. p<0.414, statistically insignificant, ANOVA: Analysis of variance

Table 3: Mean duration of recovery time (postictal depression) after MES

Groups	Recovery time (in minutes)		
	Mean±SD	ANOVA	p value
I	2.27±0.54	1.74	0.194
II	1.79±0.42		
III	1.78±0.44		
IV	1.72±0.47		

SD: Standard deviation, ANOVA: Analysis of variance, MES: Maximal electric seizure

Table 4: Tukey's post hoc multiple comparisons for THLE

Groups compared	Significance
Group I versus II	p<0.000**
Group I versus III	p<0.000**
Group I versus IV	p<0.000**
Group II versus IV	p<0.01*

*Significant, **highly significant, THLE: Tonic hind limb extension

- flaxseed Lignan in male rabbits with high-fat diet: Histopathological study. *Int J Pharm Pharm Sci* 2016;8:90-4.
8. Animal Experiment on Central Nervous System, CNS. Practical Manual of Experimental and Clinical Pharmacology. 1st ed. New Delhi: Jaypee Brothers Medical Publishers Ltd.; 2010. p. 195.
 9. Paget GE, Barnes JM. Evaluation of drug activities. In: Lawrence DR, Bacharach AL, editors. *Pharmacometrics*. Vol. 1. New York: Academic Press; 1964. p. 161.
 10. Tanna IR, Aghera B, Ashok BK, Chandola HM. Protective role of Ashwagandharishta and flax seed oil against maximal electroshock induced seizures in albino rats. *Ayurvedic* 2012;33:114-8.
 11. Cysneiros RM, Ferrari D, Arida RM, Terra VC, de Almeida AC, Cavalheiro EA, et al. Qualitative analysis of hippocampal plastic changes in rats with epilepsy supplemented with oral omega 3 fatty acids. *Epilepsy Behav* 2010;17:33-8.
 12. Ferrari D, Cysneiros RM, Scorza CA. Neuroprotective activity of omega-3 fatty acids against epilepsy-induced hippocampal damage: Quantification with immunohistochemical for calcium-binding proteins. *Epilepsy Behav* 2008;13:36-42.
 13. Young C, Gean P, Chiou LC, Shen Y. Docosahexaenoic acid inhibits synaptic transmission and epileptiform activity in the rat hippocampus. *Synapse* 2000;37:90-4.
 14. Xiao YF, Kang JX, Morgan JP, Leaf A. Blocking effects of polyunsaturated fatty acids on Na⁺ channels of neonatal rat ventricular myocytes. *Proc Natl Acad Sci USA* 1995;92:11000-4.
 15. Xiao Y, Li X. Polyunsaturated fatty acids modify mouse hippocampal neuronal excitability during excitotoxic or convulsant stimulation. *Brain Res* 1999;846:112-21.