

# Pharmacological Investigations To Assess And Compare In-Vivo Anti-Epileptic Activity Of *Zingiber Officinale* (Adrak) And *Linum Usitatissimum* (Flaxseeds) In Wistar Rats

Shilpi Goyal

M.Pharm, IEC College of Engineering and Technology

**Abstract :** **Background:** Epilepsy is group of related disorders that causes unprovoked , recurrent seizures. Epilepsy has a high prevalence among people of all ages and it has been estimated that 70 million people worldwide live with epilepsy and nearly 80% of people live in low-and middle -income countries. Here, I have used plant sources to treat epilepsy because they comparatively do not have side effects and herbal formulations present in the market to treat epilepsy are very limited .Although there so many allopathic medicines to treat epilepsy but all are having numerous side effects .

**Results :** Extensor phase duration in control was  $8.86 \pm 0.38$  sec. EEZOR-B extract (Group IV) was found to be effective ( $P < 0.05$ ) in epileptic seizures like standard drug Phenytoin / Saraswat Syrup. Both EEZOR-B(Group-IV) and EELUS-B(Group-VI) extracts induced dose dependent anti-convulsant effect and was found to be more effective than and EEZOR-A(Group-III) and EELUS-A(Group-V) but less effective than standard drug Phenytoin / Saraswat Syrup. The present study reveals that both EEZOR and EELUS are having antiepileptic activity; besides, they are having excellent anti-post-ictal depression effect.

**Conclusion:** In this, an attempt was made to perform research on pharmacological investigations to assess and compare anti-epileptic activity of *Zingiber officinale* and *Linum usitatissimum* in Wistar albino rats. Finally, in the present study, EEZOR-B significantly reduced the duration of THLE as compared to EEZOR-A, EELUS-A and EELUS-B but further, pre-treatment with combination of EEZOR-A and EELUS-A extracts very significantly shortened the flexion phase of MES-induced seizure and very significantly decreased the duration of convulsion and concluded that both EEZOR and EELUS in combination induced effective antiepileptic effects as compared to single dose dependent anticonvulsant effect but less effective than standard drugs.

**Keywords:** EEZOR-A, EELUS-A, EEZOR-B, EELUS-B, *Zingiber officinale*, *Linum usitatissimum*, MES-induced seizure, anti-post-ictal depression effect, unprovoked, THLE.

## Introduction

The epilepsy disease results from abnormal activities in the brain caused due to an inherited genetic condition, trauma to head, or diseases and developmental disorders of the brain [1,2,3]. It revealed that epilepsy has a high prevalence among people of all ages and it has been estimated that 70 million people worldwide live with epilepsy, and that >85% of this disease occurs in low-income and lower middle-income countries [4,5,6,7].

The patients treated with antiepileptic drugs (AED) like Carbamazepine · Valproate · Lamotrigine and ADRs up to 81.3% [8,9]. It revealed that there are so many allopathic medicine available to treat epilepsy but all are having numerous side effects [10]. Plant sources may be a good substitute to treat epilepsy because they comparatively do not have side effects. Herbal formulations present in market to treat epilepsy are very limited [11].

Seizure medicines may cause unwanted side effects in some people. Most of the time, the effects are mild and don't last long. The use of herbal medicine is restricted to extract level. Standardized protocols regarding the phytopharmaceuticals, their doses, dosing schedule, special precaution etc. have not been developed.

## Advantages of Herbal Medicine

- (i) The low cost of the herbal products make it economically feasible;
- (ii) Easy availability of herbal medicine products for consumption
- (ii) Herbal medicines are relatively harmless, digest easily, with minimum or no side effects and safe.

## Methods

### Collection of Plant Raw Materials

Fresh Rhizome of *Zingiber officinale* Roscoe (*Zingiberaceae* family) and Seeds of *Linum usitatissimum* Linn. (flaxseeds; family: Linaceae) were procured locally from reliable commercial sources and authenticated by pharmacognostical analysis and also by a Botanist / Pharmacognocist.

**Authentication of Plant Raw Materials** Procured fresh rhizomes of *Zingiber officinale* (Roscoe.; Adrak) and Seeds of *Linum usitatissimum* (Linn.; flaxseeds) were assessed pharmacognostically (macroscopical, microscopical and phytochemical methods) for their scientific authentication. Phyto-chemical screening were also undertaken to assess the presence of secondary plant metabolites (SPMs).

### Morphology of Rhizomes of *Zingiber officinale*

□ tuberous rhizome with irregular branched or palmate shape (polyhedral/rounded/oval) and size;

- fracture Short, starchy, fibrous
- surface longitudinally striated with occasional projecting fibers.
- aromatic odour and pungent / acidic taste (pH of 5.6 to 5.9);
- "completely scraped", "partially scraped," or "unpeeled" rhizomes;

#### Microscopy of Rhizomes of *Zingiber officinale* (Roscoe)

- fibres occur in groups, fairly large, wall is frequently dentate; give faint reaction for lignin;
- parenchyma cells (wrinkled; filled with starch granules or oleoresin) are associated with thin walled tissue (several rows of collapsed cells);
- endodermis consists of tangentially elongated cells;
- ring of vascular bundles without fibres (below endodermis);
- indistinct phellogen;
- cork consists of irregularly arranged cells (outer zone) and cells arranged in radial rows (inner zone);
- cork absent in Jamaica ginger;
- cortex contains collateral fibro-vascular bundles (closed);
- starch granules are polyhedral/rounded/oval (dried form);
- granules are irregular/orbicular/oval/reniform (freeze dried);

#### Morphology of Seeds of *Linum usitatissimum* (Linn.)

- small, brown, glossy with pitted surface;
- elongated-ovoid, flattened, rounded (at one end and obliquely pointed at the other);
- hilum and micropyle;
- embryo contains two yellowish-white, flattened planoconvex cotyledons;
- whitish endosperm;
- both endosperm and embryo oily;
- testa mucilaginous when soaked in water;
- odour, characteristic, taste, oily when chewed;
- mucilaginous sweetish in taste, whitish yellow in colour and bland odour;

#### Microscopy of Seeds of *Linum usitatissimum* (Linn.)

- testa contains isodiametric cells (with mucilaginous outer walls);
- rounded collenchymatous cells (in hypodermis)
- abundant globule of fixed oil;
- yellow coloured fibre, pitted walls, lignified sclerides;
- polygonal epidermis cells filled with mucilage;
- fibres, lignified sclerides;

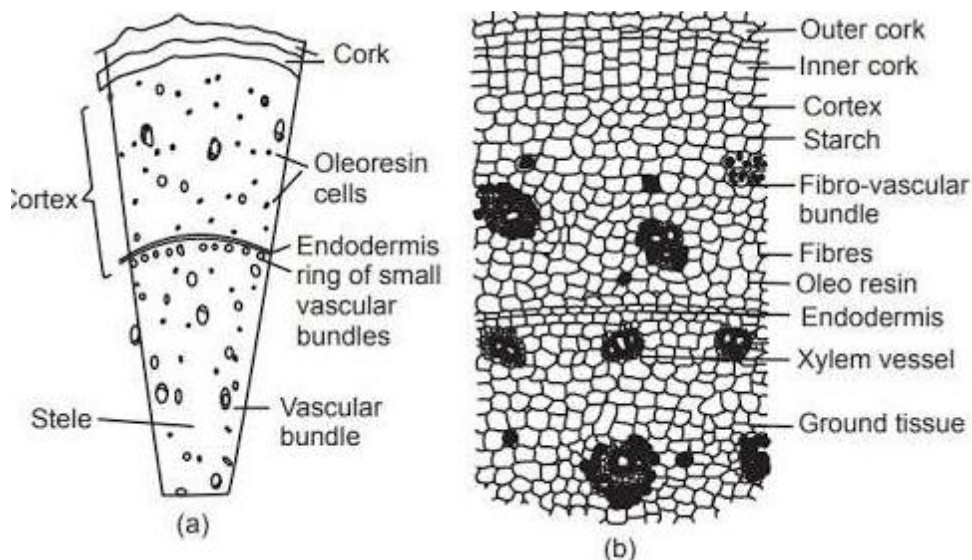


Figure 1: Microscopical features of Rhizome of *Zingiber officinale*. [12]

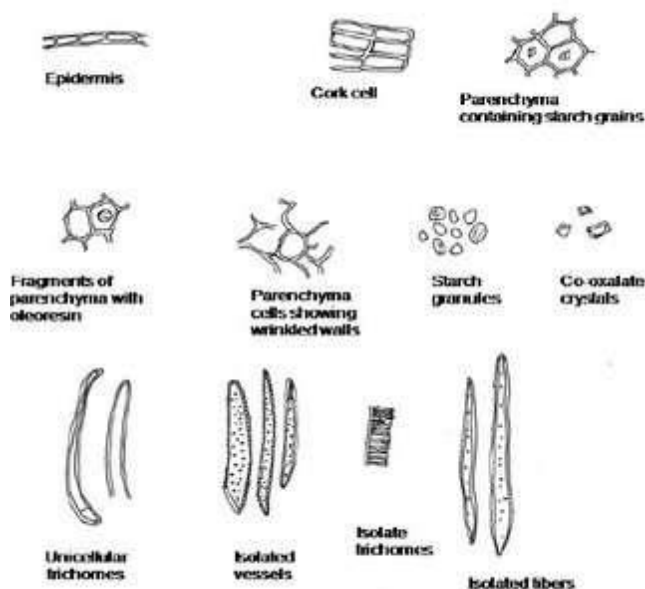
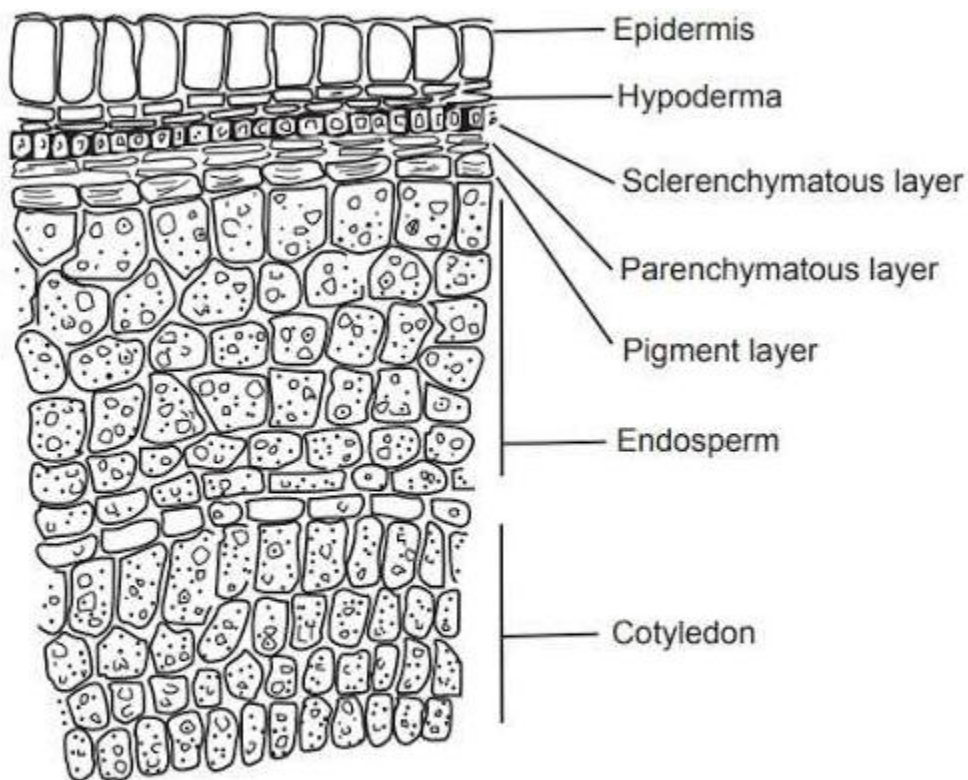


Figure 2: Microscopic characteristics of *Zingiber officinale* Rhizome.[13]



Transverse section of Linseed seed

Figure 3: Transverse Section (TS) of *Linum usitatissimum* seed.[14]

Table 1: Phytochemical analysis of *Zingiber officinale* & *Linum usitatissimum*.

S. No.	SPMs	<i>Zingiber officinale</i> Rhizomes	<i>Linum usitatissimum</i> Seeds
1.	Alkaloid	++	-
2.	Anthraquinones	+	-
3.	Phenolic Compounds	++	++
4.	Flavonoids	++	+
5.	Resins	++	+
6.	Steroids	+	+
7.	Amino acids	+	+

8.	Saponins	+	+
9.	Glycosides	+	++
10.	Tannins	+	-
11.	Terpenes	++	++
12.	Fatty Acid	-	++

- = absent; + = present

#### Preparation of EEZOR Extracts

- Zingiber officinale* rhizomes (required quantity) was cut into small pieces, dried under shade, pulverized into coarse powder;
- Air-dried and pulverized rhizomes (sieved through a 300 µm sieve)
- Extracted (ethanol) by continuous extraction.
- Extract filtered, solvent removed by rotary evaporator, concentrated.
- Dried under vacuum, lyophilized.

#### Preparation of EELUS Extracts

- Linum usitatissimum* seeds were taken.
- Air-dried and pulverized seeds.
- Extracted with crude ethanol (80%) by percolation method thrice.
- Extract fractions were pooled up, filtered, concentrated to dryness, lyophilized, yield : 10% w/w.

#### Collection and management of animals

Finally, 54 Albino Rats (Either Sex/6-8 weeks/180-200 g) were used. Animals were procured from Dr. Dinesh Kanwar Yadav, Head, Central Laboratory Animal Resources, Jawaharlal Nehru University (JNU), New Delhi. Animals were acclimatized, housed in animal house at temperature 25±2°C, 55±5% humidity and 12 hrs day-night cycle, and given pellet diet (Chaudhary Feeds, Delhi) with water *ad libitum*.

#### Animal grouping

After acclimatization, animals were randomly divided into nine groups (Group I, II, III, IV, V, VI, VII, VIII and IX; 06 animals / group) MES (Maximal Electroshock Seizures) Induced Seizures Model was used [15]

**Table: 2 Grouping of Animals.**

Group	Treatment / Dose	Animals Required
I	Normal Control / Normal saline (25 ml/kg)	6
II	Electroshock Group (Toxic Control Group)	6
III	EEZOR-A (100 mg/kg) + Electroshock	6
IV	EEZOR-B (200 mg/kg) + Electroshock	6
V	EELUS-A (100 mg/kg) + Electroshock	6
VI	EELUS-B (200 mg/kg) + Electroshock	6
VII	Combination of EEZOR-A Extract (100mg/kg) + EELUS-A Extract (100mg/kg) + Electroshock	6
VIII	Standard "Phenytoin sodium" (25 mg/kg) + Electroshock	6
IX	Standard "Saraswat Syrup" (Baidyanath Formulation) (5 ml/kg). + Electroshock	6

#### Evaluation of Anti-epileptic activity by MES Model

MES (Maximal Electroshock Seizures) Induced Seizures Model was used. In the present investigation, seizures will be induced in wistar albino rats by maximal electroshock method (MES). Seizures will be induced by using an electro convulsimeter and elicited by a 60-Hz alternating current of 150 mA intensity for 0.2 second. Once the epilepsy is induced, the animals will be given treatment with distilled water (Group-I), Toxic Control group (Group-II), *Zingiber officinale* extract treated groups (Group III & IV), flax seed extract (Group V & VI), *Zingiber officinale* and Flax seed (Group VII), Phenytoin - Standard Drug (Group VIII) and Saraswat syrup-Standard drug (Group IX).

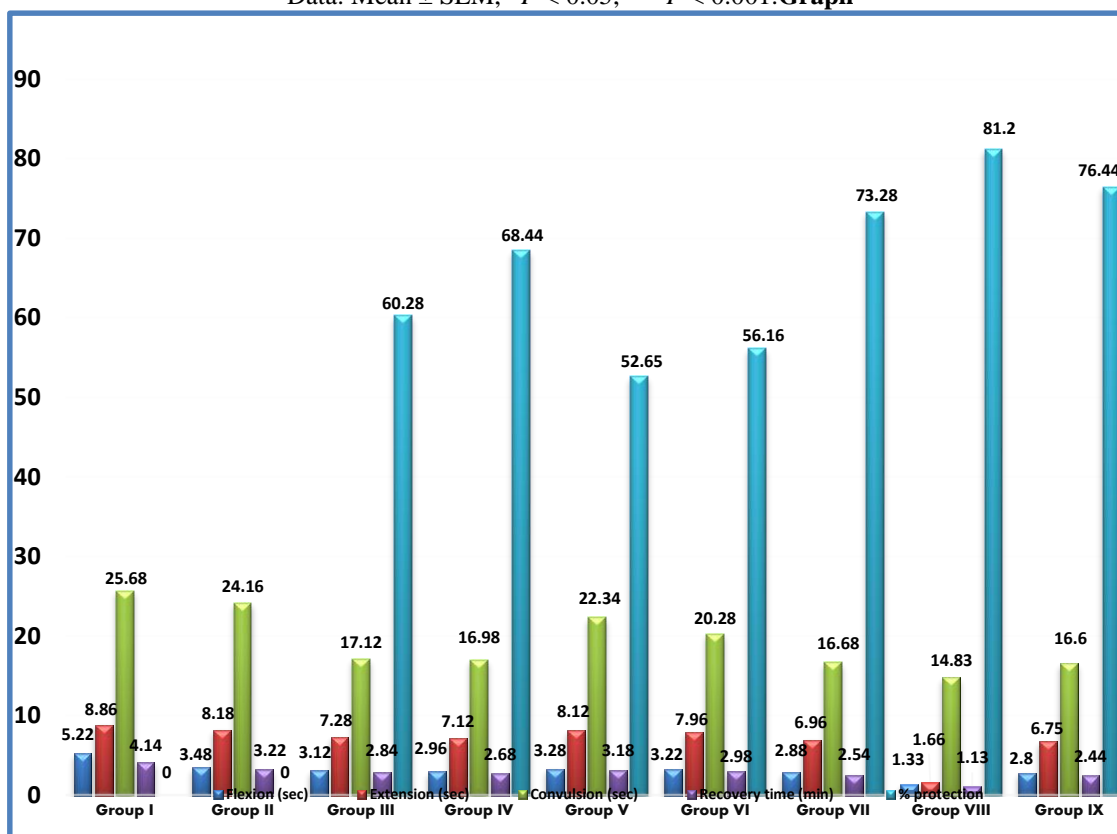
**Table-3 Effect on MES-induced seizures in rats**

Groups	Flexion (sec)	Extension (sec)	Convulsion (sec)	Recovery time (min)	% protection
Group I	5.22 ± 0.32	8.86 ± 0.38	25.68 ± 4.12	4.14 ± 0.56	-
Group II	3.48 ± 0.32*	8.18 ± 0.52	24.16 ± 3.68	3.22 ± 0.46	-
Group III	3.12 ± 0.42**	7.28 ± 0.36	17.12 ± 3.26*	2.84 ± 0.20	60.28
Group IV	2.96 ± 0.24**	7.12 ± 0.28*	16.98 ± 3.16	2.68 ± 0.16*	68.44
Group V	3.28 ± 0.14*	8.12 ± 0.52	22.34 ± 3.72	3.18 ± 0.16	52.65
Group VI	3.22 ± 0.12*	7.96 ± 0.42	20.28 ± 3.62	2.98 ± 0.12	56.16
Group VII	2.88 ± 0.18***	6.96 ± 0.42*	16.86 ± 3.12	2.54 ± 0.18*	73.28
Group VIII	1.33 ± 0.05***	1.66 ± 0.55***	14.83 ± 1.28*	1.13 ± 0.04***	81.20



Group IX	2.80 ± 0.20***	6.75 ± 0.48*	16.60 ± 3.16	2.44 ± 0.16*	76.44
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Data: Mean ± SEM, \*P < 0.05, \*\*\*P < 0.001. Graph



**Summary**

In this dissertation an attempt was made to perform research on pharmacological investigations to assess and compare anti-epileptic activity of *Zingiber officinale* and *Linum usitatissimum* in Wistar albino rats.

Further, in *in-vivo* anti-epileptic activity of EEZOR (*Zingiber officinale*) and EELUS Extracts (*Linum usitatissimum* Linn.) individually / separately and in combination (EEZOR + EELUS), it was found that EEZOR-A / EEZOR-B extracts (Group III and IV) shortened the duration of THLE. EEZOR-B extract (Group IV) was found to be effective (P<0.05) in epileptic seizures. Pre-treatment with EELUS-A / EELUS-B (Group V & VI) extracts induced significant anticonvulsant activity (dose dependent). EELUS extracts significantly reduced the duration of THLE in rats. EELUS-B (Group VI) extract was found to be more effective than EELUS-A but less effective than standard drug Phenytoin / Saraswat Syrup. In the present study, standard drug phenytoin (Group VIII) and Standard

“Saraswat Syrup” (Group IX) abolished the extensor phase with 81.20 % and 76.44 % protection respectively (P<0.001; significant anticonvulsant effect) Finally, in the present study, EEZOR-B (Group IV) significantly reduced the duration of THLE as compared to EEZOR-A (Group-III), EELUS-A (Group-V) and EELUS-B (Group-VI). Further, pre-treatment with combination of EEZOR-A and EELUS-A (Group VII) extracts shortened the flexion phase and very significantly decreased the duration of convulsion (\*\*P<0.001).

**Result**

Extensor phase duration in control was 8.86 ± 0.38 sec. EEZOR-B extract (Group IV) was found to be effective (P<0.05) in epileptic seizures like standard drug Phenytoin / Saraswat Syrup. Both EEZOR-B(Group-IV) and EELUS-B(Group-VI) extracts induced dose dependent anti-convulsant effect and was found to be more effective than and EEZOR-A(Group-III) and EELUS-A(Group-V) but less effective than standard drug Phenytoin / Saraswat Syrup. The present study reveals that both EEZOR and EELUS are having antiepileptic activity; besides, they are having excellent anti-post-ictal depression effect.

**Discussion**

Plant sources may be a good substitute to treat epilepsy because they comparatively do not have side effects. Herbal formulations present in market to treat epilepsy are very limited. Ginger is a safe herbal medicine used as anti-oxidant with few insignificant adverse effects. It contains bioactive compounds which have shown various pharmacological activities. *Linum usitatissimum* (Linn.; family: Linaceae; Flaxseed / Linseed) is a rich source of essential fatty acids, 38–45% oil, linolenic acid (ALA), dietary lignans (phytoestrogens; lipid-lowering and antioxidant).

So far, pharmacological investigation for comparative and combination studies for anti-epileptic activity (synergistic / antagonizing / indifference) of EEZOR and EELUS against seizures induced in wistar albino rats by maximal electroshock method (MES) is not reported in literature. This is an attempt to perform research on pharmacological investigations to assess and compare anti-epileptic activity of *Zingiber officinale* and *Linum usitatissimum* in Wistar Rats.

**CONCLUSIONS**

Present work was envisaged to perform research on pharmacological investigations to assess and compare pharmacological activities of *Zingiber officinale* and *Linum usitatissimum* in Wistar albino rats. They have very wide safety margin and classified as

Safe / Non-Toxic. Finally, in the present study, EEZOR-B significantly reduced the duration of THLE as compared to EEZOR-A, EELUS-A and EELUS-B. Further, pre-treatment with combination of EEZOR- A and EELUS-A extracts very significantly shortened the flexion phase of MES-induced seizure and very significantly decreased the duration of convulsion and concluded that both EEZOR and EELUS were induced antiepileptic effects.

This project, would contribute in giving a new medicine against epilepsy and it would be cheap and affordable. Since it is a novel work, it would be useful in finding a new alternative for various drugs which have narrow therapeutic index and show a number of adverse effects like hepatotoxicity, hyperglycemia, anemia, etc.

#### Abbreviations

IAEC	:	Institutional Animal Ethical Committee
ML	:	Milliliter
SPM	:	Secondary Plant Metabolites
MES	:	Maximal Electroshock
TS	:	Transverse Section

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