

Protective Effects of Aqueous and Alcoholic Extracts of *Piper longum* in Experimental Rodent Models of Seizures

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ABSTRACT

Epilepsy affects 5 to 10 per 1000 of the general population. It is the third most common neurological disorder after stroke and Alzheimer's disease. Available anti-convulsant drugs effectively control epilepsy in about 50% of the patients. Many epileptic seizures are refractory to current anti-epileptic drugs and safety of the anti-epileptic drugs has always been a concern. Herbal drugs may serve as the alternative for some such patients. Many plants have been used for the treatment of epilepsy in traditional system of medicines have shown useful anti-seizure activity. Herbal remedies have become popular, due in part to the lower risk of adverse reactions. Thousands of plants have been used traditionally to treat various diseases. Among them, species of the genus *Piper* are important medicinal plants used in various systems of medicine. The *Piper longum* fruit has been used in traditional medicine, including the Ayurvedic system of medicine. Although there are numerous indications for its use and plant contains many active constituents, controlled trials are needed to determine its anti-epileptic efficacy in small animals. The present study was done to evaluate anti-seizure activity of fruits of *Piper longum* on animal models of seizures and also to find out effectiveness of aqueous and alcoholic extract.

Keywords: Anti-oxidant action, anti-seizure activity, fruits, piperine, traditional medicine

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INTRODUCTION

Details about *Piper longum* plant was first described by Hippocrates, who described it as a medicament rather than a spice [1]. The word pepper is derived from the Sanskrit word for long pepper (pippali). Long pepper (*Piper longum*), sometimes called Javanese, Indian, or Indonesian long pepper, is a flowering vine in the family Piperaceae cultivated for its fruit, which is usually dried and used as a spice. Long pepper is a close relative of *Piper longum*, which gives black, green, and white pepper and has a similar but generally hotter flavor. The fruits contain the alkaloid piperine, which contributes to their pungency. Another species of long pepper, *Piper. retrofractum*, is native to Java, Indonesia. When applied topically, it soothes and relieves muscular pains and inflammation. In ayurvedic medicine, it is said to be a good rejuvenator. *Piper longum* stimulates the appetite and dispels gas from the intestines. An infusion

of *Piper longum* root is used after birth to induce expulsion of the placenta [2].

The whole plants as well as plant parts such as the fruit are used traditionally. This plant is inexpensive, readily available, and effective for many diseases, including cancer, inflammation, depression, diabetes, obesity, and hepatotoxicity [3].

Scientific classification:

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Piperales

Family: Piperaceae

Genus: *Piper*

Species: *longum*

Botanical name: *Piper longum*

Principal Constituents [4-7]:

The fruit contains a large number of alkaloids and related compounds, the most abundant of which is piperine, followed by methyl piperine, pipernonaline, piperettine,

asarinine, pellitorine, piperundecalidine, piperlongumine, piperlonguminine, retrofractamide A, pergumidiene, brachystamide-B, a dimer of desmethoxy pipartine, N-isobutyl decadienamide, brachyamide-A, brachystine, piperide, piperderidine, longamide, dehydropiperonaline piperidine, and tetrahydro piperine. Piperine, piperlongumine, tetrahydropiperlongumine, trimethoxy cinnamoyl-piperidine, and piperlonguminine have been found in the root.

Lignans:

The main lignans present in the fruits are sesamin, pulvatiol, and fargesin.

Esters:

The fruits contain tridecyl-dihydro-p-coumarate, eicosanyl-(E)-p-coumarate, and Z-12-octadecenoicglycerol- monoester.

Volatile oils:

The essential oils of the fruit are a complex mixture. The volatile piperine, the three major components are caryophyllene, pentadecane, and bisabolene. Others include thujone, terpinolene, zingiberene, p-cymene, p-methoxyacetophenone, dihydrocarveol, and vitamin A and E.

Organic acids:

The major organic acids present are palmitic acid and tetrahydropiperic acid.

Pharmacological Profile:

The reported pharmacological activities include the following:

- Anticancer [8-9]
- Hepatoprotective [10-11]
- Anti-inflammatory [10-11]
- Immunomodulatory [9,12]
- Coronary vasodilation [13]
- Antimicrobial [14]
- Bioavailability-enhancing [14]
- Antiplatelet [15,16]
- Antifertility [17]
- Antihyperlipidemic [18]
- Antiobesity [19]
- Analgesic [20]
- Larvicidal [21,22]
- Adulticidal [23]
- Radioprotective [24]
- Melanin-inhibiting [25]
- Cardioprotective [26]
- Antidepressant [27-29]
- Antifungal [30]
- Antiamoebic [31]
- Antioxidant activity [32,33]

MATERIAL AND METHODS

Albino rats of either sex, weighing (120-150g) were procured from CCSAU, Hisar. Rats were housed in metallic cages in groups of 10 at controlled temperature and relative humidity of 45 to 55 %. They were provided with standard food pellets and water ad libitum but food was withdrawn 8 h before experiment. A 12:12 h dark:light cycle was followed during experiments. Care was taken to minimize suffering and pain to animals as per guidelines of the National Institute of Health 1996. . The Institutional Animal Ethical Committee approved the protocol of this study.

Preparation of aqueous and alcoholic extracts

Piper Longum fruits were purchased from commercial source. Fruits were crushed to a coarse powder. Powder was soaked in distilled water overnight. On the next day suspension was filtered through a Whatman no. 1 filter paper. Supernatant fluid was allowed to evaporate in glass petri dish. When completely dry, powder was collected and stored. Alcoholic extract was prepared using Soxhlet apparatus and ethyl alcohol as a solvent. Dry fruit powder was filled up in Soxhlet apparatus. When alcohol was boiled vapors of alcohol was soaked by powdered fruit and dissolved material which is alcohol soluble collected in the flask. Finally obtained solution was evaporated to get solid material. Oral doses of *Piper longum* fruit extracts 100mg/kg were used in this study and selected after a pilot study using 25, 50 & 100 mg/kg doses.

Audiogenic seizures

For induction of seizures "Techno-audiogenic test chamber" was used as described by Plotnikoff & Green [34]. Albino rats of either sex weighing 120-150g were subjected (individually) to auditory stimulus by placing in the test chamber for 90 seconds. Stimulus was produced by 2 door bells in the chamber and animals were selected in which seizures were produced within 10 sec. After an overnight rest animals were divided in to 3 groups of 20 each. Group I- Rats were pretreated with *Piper longum* aqueous extract (100mg/kg, po) 60 min before subjected to audiogenic stimulus. Group II- Animals were pretreated with *Piper longum* alcoholic extract

(100mg/kg, po) 60 min before audiogenic stimulus. Group III- Rats received Phenytoin (130mg/kg,ip) injection, served as standard for comparison. After 60 min of extracts administration animals were subjected to auditory stimulus as before. Animals were observed for convulsions or time of onset of seizures in seconds was noted for each animal. Effect of extract was evaluated by using each animal as its own control. Percentage protection/ delay in the onset of convulsions or mortality if any were recorded. Results were analysed statistically by using Student's "t" test.

Maximal Electroshock Seizures (MES)

Electroshock seizures were produced by delivering a current of 150 mA, 50 Hertz through corneal electrodes for a period of 0.2 seconds from a convulsimeter [35]. Rats which showed tonic hind limb extension were selected and given

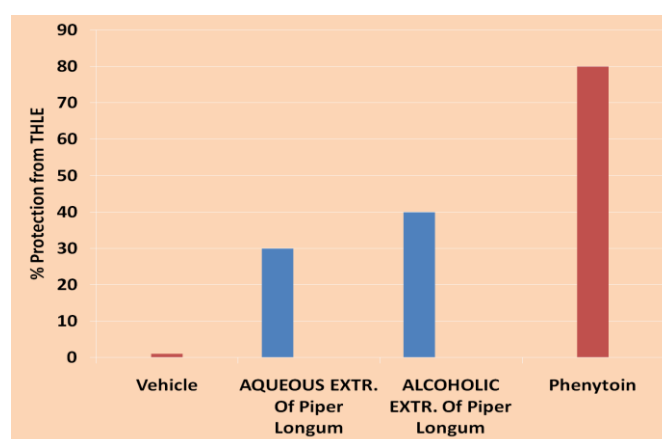
overnight rest. On the next day, rats were divided in to 4 groups of 10 each. Group I- Served as control, received vehicle. Group II and III - animals were pretreated with aqueous and alcoholic extracts (100mg/kg,po) of *Piper Longum* respectively, 60 min prior to electroshock. Group-IV- Received phenytoin (130mg/kg,ip) injection, as standard anticonvulsant drug for comparison. Rats were subjected to electroshock after 60 min of extracts/drug. The duration of hind limb extension (time in seconds), in control and drug treated was noted in each rat. Percentage protection and mortality within 24 h was also recorded. The difference between control and drug treated group was taken and used as the measure of protection afforded by the extracts/drug (% protection). Results were analysed statistically by using Student's "t" test.

RESULTS

Table 1: Effect of aqueous and alcoholic extracts of *Piper longum* (100mg/kg p.o.) on MES induced seizures in rats [n=10]

Group	Duration of hind limb extension [HLE] in seconds	% Protection	Mortality within 24 hours
Control	9.34 ± 0.42	----	1
Aqueous extract	4.28 ± 0.18*	[3] 30%	0
Alcoholic extract	3.34 ± 0.42*	[4] 40%	0
Phenytoin	0.70 ± 0.41*	[8] 80%	0

P < 0.001 when compared with control, Unpaired student "t" test



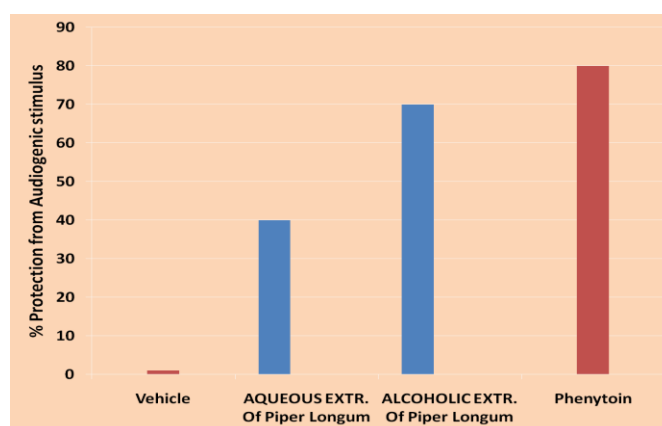
The duration of tonic hind limb extension in rats treated with vehicle (control) showed mean 9.34 ± 0.42 sec. *Piper longum* aqueous extract, reduced the duration of hind leg extension and the value was 4.28 ± 0.18*. Alcoholic extract of *Piper longum* was also effective in reducing the duration of hind

limb extension with values 3.34 ± 0.42*. 30% rats showed complete protection with aqueous extract and 40% rats with alcoholic extract of *Piper longum* pre-treatment in MES-model. Standard drug Phenytoin showed 80% protection in rats against MES.

Table 2: Effect of *Piper longum*, aqueous and alcoholic extracts (100mg/kg p.o.) on seizures induced by audiogenic stimulus in rats [n = 20]

Group	Time of onset of seizures		% Protection	Mortality within 24 hours
	Before	After		
Aqueous extract	8.50 ± 0.41	52.16 ± 1.72*	[8] 40%	0
Alcoholic extract	7.45 ± 1.00	69.30 ± 1.17*	[14] 70%	0
Phenytoin	8.50 ± 0.41	69.50 ± 2.04*	[16] 80%	0

P < 0.001 when compared with pre-drug values ; paired- " t " test



The time of onset of audiogenic seizures in rats before treatment was mean 8.50 ± 0.41 sec. After *Piper longum* fruit aqueous extract (100mg/kg,po) pre-treatment, out of 20 rats, 8 showed complete protection and remaining 12 rats showed significant delayed convulsions ie mean 52.16 ± 1.72 . Similarly, in alcoholic extract treated animals 10 rats showed complete protection (70%) and remaining 6 showed delayed convulsions i.e. mean 69.30 ± 1.17 . Both aqueous and alcoholic extracts of *Piper longum* were less potent as compared to phenytoin in protecting the rats against audiogenic seizures.

DISCUSSION

Aqueous and Alcoholic extracts of *Piper longum* protects rats from audiogenic and maximal electro-shock seizures.

Mechanisms postulated are:

1. Anti-oxidant action [36]: *Piper longum* contain Vitamin - A, E. Posttraumatic epileptogenesis is closely associated with the generation of ROS and RNS. Increased free radicals can lead to initiation of lipid peroxidation , protein oxidation and DNA damage. Free radical production act on seizure via inactivation of glutamine synthesis that

result in the enhancement of L-glutamate brain level.

ANTIOXIDANT ACTION:

2nd Mechanism:

There is an alteration in levels / concentration of CNS-neurotransmitters. It is well known that GABA and voltage-gated channels are involved in seizures induced by MES. Decrease in GABA transmission has been implicated in the excess excitation that is characteristic of epilepsy. Herbal extracts, may act by increasing the density of GABA-binding sites in certain brain areas and thereby increase [GABA] in brain. It may also act by reducing glutamate release in brain tissue and thus decreases extra-cellular [Glutamate] in brain.

3rd Mechanism:

Modulation of voltage-gated ion channels:

- A] Interaction with voltage dependent sodium channels and causing blockade of sodium-ion channels.
- B] Interaction with voltage-dependent calcium channels and act by inhibiting them.
- c] Potentiation of GABA-induced chloride currents.

4th Mechanism [*Piper Longum*]:

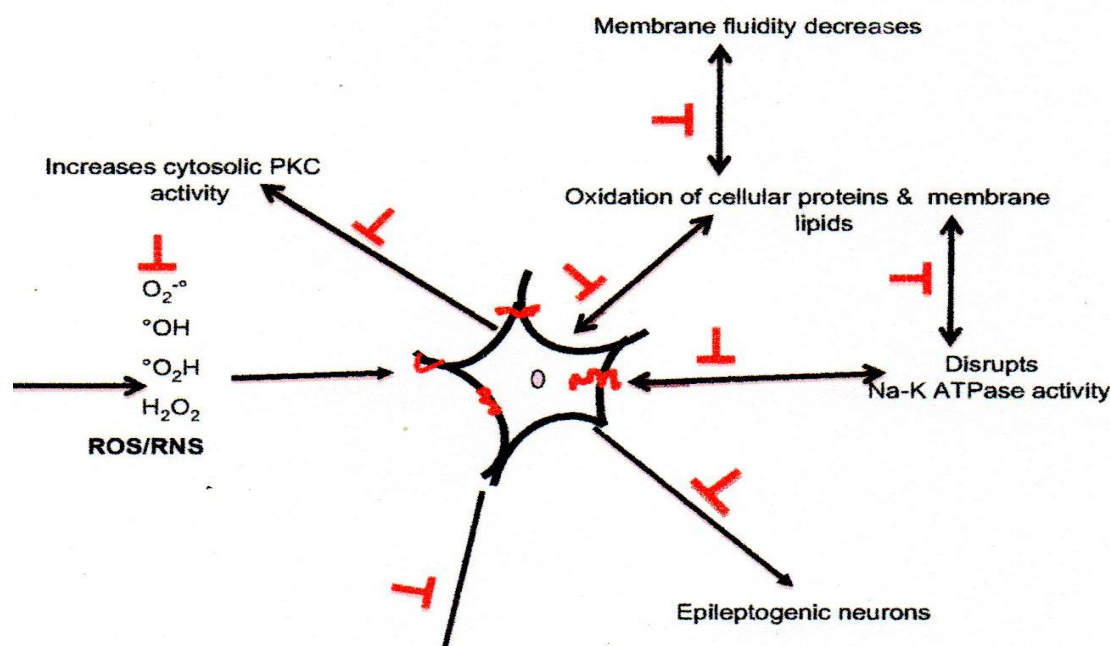
Piperine activate transient receptor

potential cation channel subfamily V member 1 (TRPV1) receptor that belongs to the vanilloid receptor family[37-40]. TRPV1 is highly expressed in the hippocampus, cortex and other regions of the brain such as the substantia nigra, hypothalamus and locus coeruleus. Piperine, administered at doses of 40 and 80mg/kg, significantly delayed the onset of myoclonic jerks and generalized clonic seizures, and decreased the seizure stage and mortality compared

with the vehicle-treated animals. Piperine also significantly reduced the incidence of MES-induced tonic hind limb extension (THE).

5th Mechanism:

Herbal extract may enhance endogenous adenosine levels in the CNS by reducing adenosine re-uptake, thereby increasing inhibitory adenosinergic tone to aid seizure suppression [41].



CONCLUSION

Aqueous and alcoholic extract of *Piper longum* protects rats in experimental model of seizures.

CARRY HOME MESSAGE:

This study suggest a further scope of evaluation of herbal extracts as an adjuvant treatment with conventional AEDs for controlling seizures. Future research is required to investigate the potential synergistic, additive or antagonistic effects of herbal extracts on AEDs.

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