

**Original Article**

**TO EVALUATE THE ANTICONVULSANT ACTIVITY OF HYDROALCOHOLIC EXTRACT OF LEAVES OF *ZIZIPHUS JUJUBE* IN SWISS ALBINO MOUSE**

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**ABSTRACT**

**Objective:** This study was aimed to investigate the anti-epileptic potential of Hydroalcoholic (30:70) Extracts of leaves of *Ziziphus jujube* (HELZJ) in Maximal Electroshock Seizure (MES) induced convulsions and to compare its efficacy with standard drug-phenytoin for MES method.

**Methods:** Different doses of HELZJ were separately administered orally to 4 groups (total 20 numbers) of Swiss albino mice (20±2 g). Phenytoin was used as positive control. After 30 min, tonic-clonic seizures were induced by MES method. Animals were monitored for 1 hour and different parameters, including decrease in duration of tonic hind limb flexion, tonic hind limb extension, clonus and postictal depression, were noted. Results were analyzed using Analysis of Variance (ANOVA) and Bonferroni's multiple comparison post hoc test. The significant difference was established when probability value (p-value) was less than 0.05.

**Results:** The HELZJ had shown a significant anticonvulsant effect against MES-induced convulsions in Swiss albino mice mean duration of tonic hind limb extension of both the test groups, T1(1.50±3.21) and T2 (0.17±0.41) were significantly reduced compared to the control group (12.50±3.54).

**Conclusion:** The HELZJ contains the phenolic compounds which may induce the Gamma Amino Butyric Acid (GABA) transmission that could be attributed to the anti-epileptic activity.

**Keywords:** *Ziziphus jujube*, Antiepileptic, MES induced seizure

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**INTRODUCTION**

Epilepsy is one of the most common chronic neurological disorders and is characterized by recurrent unprovoked seizures [1]. Epileptic seizures are recurrent paroxysmal events characterized by stereotyped behavioural alterations that reflect the underlying neural mechanism of the disease. Although an etiologic agent can be identified in some, still in about 50 % cases, the cause is unknown [2].

Epilepsy is one of the most common diseases of the Central Nervous System (CNS). With an estimated incidence of 34 to 76 new cases per year per 100,000 people, epilepsy affects about 70 million people worldwide. In low-and middle-income countries, estimates of epilepsy's prevalence are generally higher. Epilepsy is considered to be resolved for individuals who are seizure-free for the last 10 y, with no seizure medicines for the last 5 y [3].

A variety of factors contribute to cognitive impairment in patients with epilepsy, including anticonvulsant drugs, seizure type/frequency, structural lesions, and psychosocial stress [4]. Dose-related neurotoxicity, cognitive impairment, and range of systemic side effects are the major problems caused by antiepileptic drugs. There remains an unmet need for drugs that can suppress seizures effectively without affecting cognitive function.

Although the pathophysiology and the underlying disease processes are increasingly well understood in recent times, one-third of patients with epilepsy still do not become seizure-free with available anti-epileptic drugs (AEDs) [5].

At least 25% of the patients with epilepsy require the use of various AED combinations, which in turn further increases the risk of adverse events manifold [6]. Fourteen percent of the patients with drug-refractory epilepsy do not become seizure-free even with two or more AEDs in combination. Furthermore, the pharmacokinetic interaction between different AEDs in combination require more stringent therapeutic drug monitoring. Therefore, the pressing need

for newer and safer AEDs has to be met. Herbal drugs with proven anticonvulsant efficacy could be used as an adjuvant to standard AEDs, which in turn could lower the doses as well as the side effects of AEDs [7].

A lot of work has been done previously to establish anticonvulsive drugs from plant sources and these attempts will continue till a suitable option is obtained. Natural products from folk medicine have made important contributions in the innovation of modern drugs and can serve as an alternative option for the discovery of AEDs with novel structures and better tolerance [8].

The plant *Ziziphus jujube* (synonym-*Ziziphus mauritiana* lam.) belongs to the family Rhamnaceae and order Rosales [9]. Jujube is native to Australia, Bangladesh, China, Egypt, India, Indonesia, Malaysia, Nepal, Pakistan, Thailand, Vietnam etc [10]. Locally known as kul in Bengali, boroi, bogori in Assamese, Jujube is a shrub or small thorny tree that can grow to a height of 3-15 m. Deciduous or almost evergreen, jujube has an erect or spreading habit. The trunk is around 40 cm in diameter, covered with a dark grey or dull black, irregularly fissured bark. The branches are numerous and drooping. The flowers are pentamerous, greenish yellow in colour, hairy outside and about 5 mm wide. The fruit skin may be smooth or rough, glossy, yellowish to reddish or blackish. The flesh is white, juicy, slightly acid to sweet, turning mealy when fully ripe [11]. Many studies have suggested that various components of ber trees, such as fruit, seed leaves, roots, and flowers, include bioactive substances that demonstrate the potential for antioxidant activity and have anxiolytic effects, anticancer, antibacterial, and antidiabetic effects [12].

Ascorbic acid, vital minerals, and carbohydrates are abundant in ber fruit, which is often consumed freshly. Jujube includes a variety of components, including flavonoids, triterpenic acids, amino acids, cerebrosides, mineral components, phenolic acids, and polysaccharides, according to earlier research. Hence this study was

undertaken with the aim to evaluate the anticonvulsant activity of HELZJ on Swiss albino mouse.

## MATERIALS AND METHODS

### Plant

The *Ziziphus jujube* leaves were collected from premises of Jorhat Medical College and Hospital, Jorhat, Assam. The plant was then authenticated by Iswar Chandra Barua (Principal scientist, Department of Agronomy, Assam Agricultural University, Jorhat [AAU-WH-5491]); the leaves were then shade dried to retain its important phytoconstituents and then subjected to reduction in size and powder preparation for further extraction process.

### Animal

Swiss albino mice of either sex weighing between 20±2 g were procured from Chakraborty Enterprise, Kolkata, India (Regd no. 1443/PO/b/11/CPCSEA). Housing of the animals were done in ambient temperature of 25±1 °C and 12/12 h light-dark cycle in polypropylene cages in animal house of Jorhat Medical College and Hospital. Animals were acclimatized for 7 ds in laboratory conditions before starting the experiment [13]. Animals were fed mice chow and water ad libitum. All animals were handled in accordance to guidelines of Committee for the purpose of Control and Supervision of Experiments in Animals (CCSEA) and IAEC (Institutional Animal Ethics Committee) of Jorhat Medical College and Hospital (Permission letter Ref no. IAEC/JMCH/09/2023/007).

### Toxicity

According to Organisation for Economic Cooperation and Development (OECD) 420 guideline, the dry ethanolic leaf extract of *Ziziphus jujube* was placed in the Globally Harmonized System (GHS) category 5 (LD50>5000 mg/kg b. w., p. o.) [14].

### Preparation of plant extract

The leaves of the plant were room-dried and the dried leaves were grounded. The powdered form (50 gm) was dissolved in hydroalcoholic mixture (30:70) of 300 ml and then transferred to the thimble with filter paper of Soxhlet apparatus [15]. It is then allowed to boil for 5 cycles (each lasting for 6 h). The solid remains in the thimble was discarded. The concentrated extract remained in the boiling flask, which was filtered through Whatman filter paper (no. 1) and the filtrate is evaporated using a spirit lamp. The extract mass was weighed in a digital balance and the % yield was calculated. The extract was kept in a beaker covered with aluminium foil labelled and kept in freezer for future use.

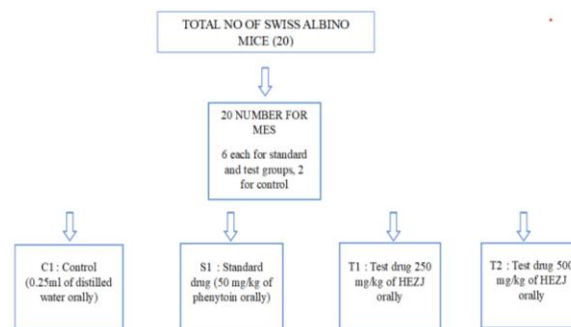
### Drugs

HELZJ (as test drug), Phenytoin (Abbott, Batch number: APJ0012) (as standard).

The mice were subjected to MES convulsions using an electroconvulsometer by applying a current of 50 mA for 0.2 sec via ear electrodes. The electrodes are moistened with saline solution before

application. The resultant seizure passes through various phases: phase of tonic limb flexion, tonic limb extension, and clonus and post-ictal depression followed by recovery or death. The mouse was considered protected if the drug prevented the appearance of hind limb extensor component of the seizure.

The design of the study is as follows:



Firstly, in the MES method, two mice received standard drug phenytoin 50 mg/kg orally. Six mice received 0.25 ml of distilled water orally [7]. Six mice were subjected to first test dose of HELZJ (250 mg/kg) and another six mice were given test drug HELZJ of dose 500 mg/kg body weight orally. Each mouse was pretreated with these drugs one hour before induction with MES seizure. Those animals that exhibited a convulsive response in the form of clonus, tonic limb flexion, tonic hind limb extension, post ictal depression followed by recovery or death were used for the experiment. Abolition of tonic hind limb extension was considered as protective criteria for the drug.

The mean duration of tonic hind limb flexion, tonic hind limb extension, clonus, post-ictal depression are recorded for different test doses of HELZJ (T1 (250 mg/kg and T2 500 mg/kg for MES method) and findings were compared with the mean duration of above-mentioned parameters recorded for the standard and the control groups.

### Statistical analysis

The results of the study were expressed as mean±Standard Deviation (mean±SD). Results were analyzed by ANOVA and post hoc test was done by Bonferroni's multiple comparison test [16]. P values<0.05 was considered as significant. The results were calculated with the use of Graph Pad Prism software version 5.0

## RESULTS

Mean duration of tonic hind limb extension of both the test groups, T1(1.50±3.21) and T2 (0.17±0.41) were significantly reduced compared to the control group (12.50±3.54). Since abolition of tonic hind limb extension was considered suggestive of protection against MES-induced convulsions and the standard antiepileptic drugs such as phenytoin, valproate, all abolish tonic hind limb extension in MES model, this finding was suggestive that HELZJ has anticonvulsant action against MES induced convulsions at doses of 250 mg/kg and 500 mg/kg (table 1).

**Table 1: It shows comparison of mean duration (in seconds) with control group of different parameters in MES method**

Parameters duration in seconds	Control	Standard	Test 1 (250 mg/kg)	Test 2 (500 mg/kg)
Tonic hind limb flexion	1.50±0.71	0.0±0.0*	0.33±0.52*	0.0±0.0*
Tonic hind limb extension	12.50±3.54	0.17±0.41*	1.50±3.21*	0.17±0.41*
Clonus	27.50±4.95	0.50±1.23*	0.50±0.84*	0.50±1.23*
Post ictal depression	175.0±7.07	1.67±4.08*	1.67±4.08*	0.0*

(Data are expressed as Mean±SD. \*p<0.05 (compared with control). One-way ANOVA followed by Bonferroni's multiple comparison test.), From table 1, it was evident that a decrease in mean duration of tonic hind limb extension for both the test dose (T1 250 mg/kg and T2 500 mg/kg) and it was highly significant for T1 and T2 as compared with control.

## DISCUSSION

The present study was conducted to investigate the anti-epileptic potential of HELZJ in MES induced convulsions and to compare its efficacy with standard drug-phenytoin. In this method, it was evident that HELZJ showed reduction of duration of tonic hind limb

extension at 250 mg/kg and 500 mg/kg and hence is suggestive of anti epileptic activity.

In a study by Nadia Anwar *et al.* (2020) it was found that there was a dose dependent significant delay in onset and decrease in seizure frequency as well as mortality was observed in animals treated with

plant extracts (*Ziziphus Vulgaris* and *Ferula Asafoetida*). Positive control (phenytoin) also showed significant delay in seizure onset and decreased the seizure frequency [13]. N Shiddique *et al.* (2020) also found a dosage-dependent reduction in all phases of an epileptic episode in their study using extracts of *Ziziphus mauritiana* [17].

The leaves of jujube plant has therapeutic potential for neurological disorders. Traditionally, jujube has been used as an anticonvulsant and for the treatment of anxiety and insomnia in folk medicine in India [18]. Pharmacologically active peptides, including cyclopeptide alkaloids, flavonoids, sterols, jujuboside A, jujuboside B, lauric acid, and triterpenoid saponins have been isolated and chemically identified from different species of *Zizyphus*, among which alkaloids, triterpenes, and flavones have been shown to possess central inhibitory activity [19]. The ethanolic fruit extract of *Z. jujuba* also might possess antioxidant and anticancer activities owing to the occurrence of bioactive compounds [20].

The jujube extract showed neuroprotective effects in animal cerebral ischemia models by attenuating oxidative stress in different brain regions in some studies. Jujube is involved in treatment of epilepsy, which is believed to be by alleviating oxidative stress and regulating cholinesterase synthesis and release. Jujube contains a variety of nutrients and bioactive substances, including polyphenols, polysaccharides, nucleotides, ascorbic acid, and triterpenoid acids. Phenolic compounds exert strong antioxidant effects against free radicals and Reactive Oxygen Species (ROS). Moreover, the oleamide extracted from jujube exhibits Choline Acetyltransferase (ChAT)-activating and neurotoxic inhibitory effects [16].

The limitation of our study is that is that the phytoconstituents which confer antiepileptic activity could not be assessed. Our study attempted to understand the use of herbal medication in epilepsy.

## CONCLUSION

Hence, from our study, we can conclude that HELZJ has shown efficacy in MES-induced convulsions in mice at dose of 250 mg/kg and 500 mg/kg. The antiepileptic potential may be attributed to the antioxidant property of phytochemicals as found in some studies. Further studies are required to establish the exact mechanism, active constituents and safety profile of the plant as a medicinal remedy for epilepsy.

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Nil

## AUTHORS CONTRIBUTIONS

The study protocol was designed by Dr. Pallavi Bordoloi. Data collection, analysis and preparation of manuscript were done by Dr. Nandita Deka. Editing and overall compilation was done by Dr. Dipjyoti Deka.

## CONFLICT OF INTERESTS

There are no conflicts of interest.

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