Effect of *A. indica* on the expression profiles of CNN3 and BDNF genes in epileptic rats

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Abstract

Introduction

Epilepsy is considered as the one of the serious neurological diseases and it is characterized by abnormal, excessive neuronal activity in the brain, related with many neurobiological, cognitive and psychological signs were noticed [1]. Several chemical drugs such as benzodiazepines also known as Diazepam, barbiturates, GABA (gamma amino butyric acid) analog, succinimides, hydantoins, carbamazepine were used in the management of epilepsy disease [2]. Currently in market, new drugs include levetiracetam, topiramate, zonisamide, lacosamide, and stiripentol are available and it is safe [3]. More importantly, these drugs cause mental slowing, mental confusion, ataxia, anorexia, aggression, sleep disturbance and impaired concentration [3].

Many reporters are focusing on herbal remedies for safe medicine for epilepsy. In this regard, several plant-based medicines such as *myrtus communis* [5], *melanthera scanden* [4], *abies webbiana Lindl* [6], *crocus sativus* [7] and *Dodonaea viscosa* [8] have been proved to possess potent antiepilepsy property in experimental models of epilepsy. Like-wise, traditional system of medicine many parts of *Acalypha indica* has been used to treat varieties of ailments, and its part are scientifically proved that diverse biological activities such as antioxidant [9] anti-

bacterial [10], wound healing [11]. Therefore, *A. indica* is one of the most common and potent plant-based medicine in the management of various ailments. Here, we considering the strong literature reports on antioxidant actions of *A. indica*, this study was undertaken to evaluate the antiepileptic activity of leaf extracts of *A. indica*.

MATERIALS AND METHODS

Drugs and Chemicals

Diazepam and 0.9% of normal saline containing 0.9% Nacl solution; Ferric chloride (Fecl3) were Ranbaxy Laboratories, New Delhi, India. All other solvents and chemicals used of analytical grade were purchased from Hi-media Laboratories Pvt., Ltd., Bengaluru, India.

Collections of plant parts

The leaves of *Acalypha indica* were collected from Tirupati (Andhra Pradesh) in the month of May-June 2021 and it was identified by Dr. Shashikant, Taxonomist, Department of Botany, Osmani University, Hyderabad, India.

Preparation of methanolic extract of Acalypha indica

The shade dried leaves of *Acalypha indica* were powdered, sieved and subjected to extraction process as follows. The plant material extracted with many organic solvents successively in the ascending order of their polarity petroleum ether, chloroform, ethyl acetate, ethanol, methanol extracts. Furthermore, solvent was removed from extraction, and then evaporator, yielding the extracted compound. The non-soluble portion was discarded and plant extract was dried under vacuum, stored at room temperature and protected from direct sunlight.

Experimental design

Male wistar rats with a body weight of 200±10 g (12-week-old) were purchased from an authorized vendor from Jeeva Life Science, Hyderabad, India. All rats were housed (4 per cage) in clean polypropylene cages containing paddy husk as bedding material and were provided with tap water *ad libitum* and standard rodent chow purchased NIN, Hyderabad, India. Rats were acclimatized for one week before being used for experimentation and maintained at temperature 22-25°C; 12:12 h light: dark cycle. The experiments were carried out in accordance with guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India, Ministry of Social Justice and Empowerment,

Government of India. All the procedures were approved by the Institutional Animal Ethical Committee.

Animals were divided into five groups and for each group six animals (n=6) were taken. The investigation was carried out for 4 weeks. The first two weeks were for the acclimation of rats.

Group-1 : Normal saline-treated rats as a Control rats

Group 2 : Normal saline-treated control rats

Group 3 : Fecl3 ((20µl)) induced epileptic rats

Group 4 : Fecl3 (20µl) plus induction of plant extract

Group 5 : Fecl3 plus induction of diazepam after adaptation.

Induction of epilepsy in male wistar rats

In this study, we incision made in midline scalp and peri-cranial muscles and fascia were retracted laterally. After that, a bone hole around 1 mm diameter was drilled 1 mm posterior and 2 mm lateral to bregma. Through this hole, 20µl of Fecl3 was injected over a period of 5 min to sensorimotor cortex using a microinjection syringe.

Acute toxicity study

We performed acute toxicity for plant extract according to acute toxic classic method of Lorke. The animals were divided into five groups containing six animals each. The plant extract of *A. indica* were administrated orally up to 200 mg/kg body weight as we were investigation in low doses. These animals were observed for mortality and toxicity for 14 days.

mRNA expression of CNN3 and BDNF genes

Statistical analysis

Results

Acute oral toxicity study for the plant extracts was performed as per OECD guidelines up and down method. The outcome of the study showed that methanolic extract of *A. indica* were safe up to 200 mg/kg p.o. Further, no signs of toxicity were observed during short-term and long-term observation period.

In the present study, we have found that *calponin-3* (*CNN3*) was significantly higher in the brain and CSF of rats with epilepsy. The elevation of CSF *calponin-3* (*CNN3*) was highly

correlated with disease duration. In a rat model of epilepsy, the cortical up-regulation of calponin-3 occurred at 24 h and thereafter, while the expression of hippocampal *calponin-3* only increased at 24 h and 1 week after seizures, respectively.

It was observed that the normalized fold expression of *calponin-3* (*CNN3*) increased in epilepsy induced rats ranging from 3.3 to 6.1 which were consistent with earlier reports. It was also found that *A. indica* treated rats (fold expression range: 1.5-2.6) and those treated with standard drug (fold expression range: 1.3-2.3) showed reduced *CNN3* levels compared to epilepsy induced rats. Moreover, *CNN3* expression levels of *A. indica* treated and those treated with standard drug were consistent with the expression in control rats (fold expression range: 0.6-2.5) (Table 2).

On the other side, we noticed the rat induced with ferric chloride showed that induced epileptic activity resulted in increased expression of BDNF. Rats treated with ferric chloride combined with plant extract resulted increased in mRNA expression in BDNF. Similarly, rats treated with ferric chloride with diazepam standard drug caused increase expression of mRNA (Figure 2).

Discussion

Epilepsy is one of the chronic and common neurological disorders, affecting approximately around 50 million people worldwide [12]. The basic and major mechanisms associated with epilepsy are increased synaptic connectivity of neurons (such as excitatory glutaminergic neurons), perturbance in synaptic receptors (suppressed GABA receptors, nicotinic receptors), channelopathies (weekening of K^+ channels and/or more persistent sodium channels, changes in voltage-gated ion channels), decrease in inhibitory neurotransmission (decreased GABA levels), enhanced excitatory neurotransmission (enhanced glutamate levels) [13].

In this study, antiepileptic activity of leaf extracts of *A. indica* on expression profiles were evaluated. Many studies have shown that BNDF (brain-derived neurotrophic factor) increases neuronal excitability and is localized and upregulated in areas implicated in epileptogenesis was noticed. In one study, seizure activity increases the expression of BDNF mRNA and protein and showed that interfering with BDNF signal transduction inhibits the development of the epileptic state *in vivo*. These results suggest that BDNF contributes to epileptogenesis. Similarly, out study also shown increase expression of BDNF mRNA level when compared to control group. On the other side, plant extract of A. indica resulted in decreased mRNA expression of BDNF in brain of rat model. These results were supported that cellular and

molecular mechanisms by which BDNF influences excitability and connectivity in adult brain could provide novel concepts and targets for anti-epileptogenic therapy.

Conclusion

These findings suggest that the *A. indica* possesses significant antiepileptic property. Further one of the possible mechanisms behind the antiepileptic activity may be due abnormal expression of *BDNF*, *CNN3* gene in the brain. Indeed, there is a scope for further studies to responsible for the epileptic activity.

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Conflict of interest

No conflict of interest exists

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Table 1. PCR primer design

S. No.	Genes	Sequences (5' to 3')
1.	*CNN3*	F: TCCTCAGCTTTTCAACTCAGCTCCT R: GGCTTCCTTCAACAAAGGTCCAGCC
2.	*BDNF*	F: TCCGGGTTGGTATACTGGGT R: CTGGTGGAACTTCTTTGCGG

F: Forward; R: Reverse; CNN-3 (calponin-3); BDNF (Brain-derived neurotrophic factor).

Table 2. Fold expression range values of rats under different treatment conditions ('n' represents number of rats).

Rats under study	Fold expression range
Control rats (n=6)	0.6 - 2.5
Epilepsy induced (n=6)	3.3 - 6.1
Treated (n=6)	1.5 - 2.6
Treated with standard (n=6)	1.3 - 2.3

Figure 1. Fold expression study of CNN3 in rats under different treatment conditions

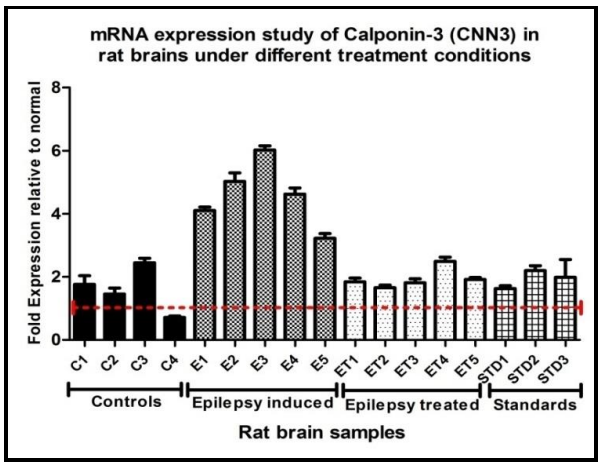
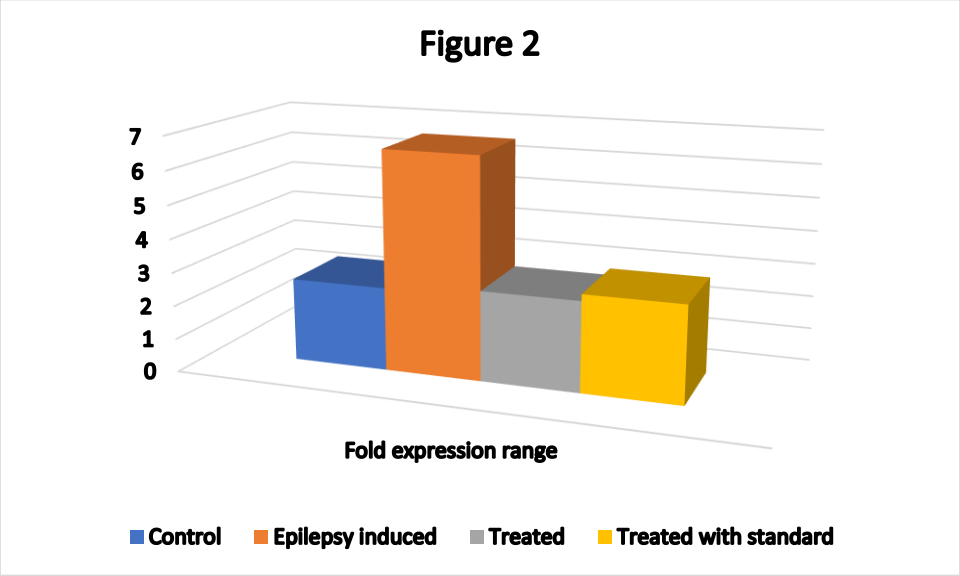


Figure 2. Fold expression study of BDNF in rats under different treatment conditions



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