Evaluation of Anticonvulsant Activity of Allyl Isothiocyanate in Experimental Animals

Patil N. A.[1], Shete R. V. [1*], Borate S. R.[1], Ghaisas M. M.[2], Savairam V. D.[1], Fajage D. V.[1], Dhekale P. B.[1], Gadhire S. K.[3], Gujar S. V.[4], Nagre B. S.[5],**

[1] Department of Pharmacology, Rajgad Dnyanpeeth's College of Pharmacy, Bhor, Tal Bhor, Dist Pune 412206.

[2] Department of Pharmacology, BVDU's Poona College of Pharmacy, Dist- Pune 411030.

[3] Department of Pharmacology, Aditya Pharmacy College, Nalwandi, Beed Dist- Pune 431122.

[4]Department of Pharmacology, Loknete Shri Dadapatil Farate College of Pharmacy, Mandavgan Farata, Shirur, Pune 412211.

[5]Department of Pharmaceutical Quality Assurance, Amrutvahini College of Pharmacy, Sangamner, Dist- Pune 422608.

***Corresponding author: Dr. Rajkumar V. Shete**,

Principal, Rajgad Dnyanpeeth's College of Pharmacy, Bhor, Pune. Email ID: rvshete09@gmail.com

Tel: + 91-9822336456

****Co-Corresponding author:**

Miss. Neha A. Patil

Research Scholar, Rajgad Dnyanpeeth's College of Pharmacy, Bhor, **Pune.** Email ID: nehapatil9816@gmail.com

Tel: + 91-8007487282

ABSTRACT

The present investigation aimed to evaluate the anticonvulsant effect of Allyl isothiocyanate (AITC) on chemically induced convulsions in experimental animals and compare the effect of AITC with the standard dose of Diazepam on Pentylenetetrazol (PTZ) and Strychnine-induced epilepsy model. Moreover, brain GABA levels were estimated to investigate the mechanisms underlying the anticonvulsant activity. In this study, AITC (5, 10, and 20 mg/kg, p.o.) showed significant and dose-dependent inhibition of PTZ and Strychnine-induced convulsions. Furthermore, administration of AITC showed significantly increased GABA levels in the brain. The parameters observed in PTZ and Strychnine-induced models were seizure latency, duration, and mortality. AITC showed significant anticonvulsant activity.

Keywords: Allyl isothiocyanate, AITC, Anticonvulsant, Pentylenetetrazol, Strychnine

INTRODUCTION

A group of neurons discharges excessively, synchronously, and continuously during epileptic convulsions. The continuous increase in neuronal excitability is the defining characteristic of all epileptic disorders [1]. Epilepsy affects around 50 million people globally [2]. Antiepileptic drug (AED) therapy for epilepsy is accompanied by side effects, dosages, and chronic toxicity which affects almost every organ system [3]. Anticonvulsant treatment during pregnancy carries risks, including teratogenic consequences [4]. However, the majority of anticonvulsant drugs have side effects that limit their long-term use, including drowsiness, ataxia, vertigo, hypersensitivity [5], cognitive impairment, anemia, and dizziness [2]. Thus, researchers are looking for a more effective and secure medicine to treat epilepsy.

In the last two decades, the usage of natural products and nutraceuticals as medicines has significantly increased [6]. Natural compounds can be used to treat a wide range of diseases and frequently lack the hazardous side effects associated with synthetic compounds [7]. Thus, researchers search for nutraceuticals that can treat neurodegenerative diseases. We concentrated on allyl isothiocyanate in this investigation. An aliphatic isothiocyanate called AITC is produced from sinigrin [8], which is a glucosinolate present in different vegetables of the brassica family [9] glucosinolate is found in various brassica vegetables, such as mustard, horseradish, wasabi, cabbage, and Brussels sprouts [10]. AITC has antioxidant, anti-inflammatory, neuroprotective [11], chemo-protective [12], antidiabetic, antimicrobial, antibacterial, antifungal, and hepatoprotective activity [13]. AITC, which is also used as a rubefacient, flavoring agent, preservative in food and pharmaceuticals, and a cat and dog repellent, is the chief constituent in natural mustard oil. AITC has possible neuroprotective advantages in its ability to pass the blood-brain barrier and influence the activation of numerous signaling pathways in the brain [14]. AITC serves as an anti-inflammatory and antioxidant agent by reducing oxidative stress and inflammation in the brain and avoiding neuronal damage. Moreover, AITC activates glutamate and GABA receptors, which could help prevent excitotoxicity. AITC was observed to have both neuroprotective and neurodegenerative properties in the brain. It has also been noted to enhance neurogenesis and lower pro-inflammatory cytokinesis. As a result of AITC, the concentration of neurotrophic factors increases, promoting the survival and growth of neurons [15]. Hence, the present study was designed to evaluate the anticonvulsant activity of AITC against convulsions induced by PTZ and Strychnine HCL in mice.

MATERIALS AND METHODS

Drugs and chemicals

Allyl isothiocyanate was purchased from Bharat Essentials Industries, Delhi. Pentylenetetrazol was purchased from Research-lab fine chem industries, Mumbai and Strychnine Hydrochloride from laboratory reagent, New Neetha Chemicals in Pune Diazepam Tablets from Abbot Healthcare, Pvt. Ltd was used. All other reagents and chemicals were of standard analytical grade.

Experimental Animals

Swiss albino mice (18-25 gm) male were purchased from the National Institute of Bioscience, Dhangawadi, Bhor, Pune. Animals were housed in standard conditions at temperature of 22 ± 2 ^{0}C and relative humidity of 45–55%. Under a 12:12-hour light–dark cycle. The animals had free access to purified water *ad libitum* and a standard pellet diet. All the experimental protocols were approved by the Institutional Animal Ethics Committee, and all procedures and techniques used in this study were by the CPCSEA Guidelines.

Evaluation of antiepileptic activity

Pentylenetetrazol-induced convulsions

Swiss albino mice (18-25gm) of the male sex were randomly allotted and divided into six different groups of six mice each. Group 1 received the suspending agent CMC (0.5%), Group 2 mice were administered Pentylenetetrazol (80 mg/kg, i.p.), Group 3 received the standard drug, Diazepam at the dose of 5 mg/kg, p.o. Groups 4, 5, and 6 received AITC at 5, 10, and 20 mg/kg, p.o. respectively. Treatment was given to each test group once daily for 14 days. On the fourteenth day, all groups received treatment an hour before Pentylenetetrazol was administered. Following the administration of Pentylenetetrazol (PTZ), animals were observed for 30 minutes. Mortality rates as well as seizure latency and duration were noted.

Strychnine-induced convulsions

Swiss albino mice (36) are divided into six groups, six mice in each group were used in this study. A suspending agent CMC (0.5%) was given to Group 1, strychnine (2 mg/kg, i.p.) was

administered to Group 2, and Diazepam (5 mg/kg, p.o.) was given to Group 3. The doses of AITC given to groups 4, 5, and 6 were 5, 10, and 20 mg/kg, p.o., respectively. Each test group received treatment once per day for 14 days. 1 hour before the administration of strychnine HCL on each of the 14 days, all of the groups received treatment. After administering strychnine HCL, the animals were observed for 30 minutes. Seizure latency and duration were measured, as well as mortality rates.

Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's test using Graph pad Prism Software version 9.5.1. The data are expressed as mean ± SEM. The level of $P<0.05$ was considered statistically significant.

RESULTS

Evaluation of anticonvulsant activity

In the current investigation, the anticonvulsant effect of AITC (5, 10, 20 mg/kg, p.o.) against PTZ and strychnine-induced convulsions in mice was evaluated.

Pentylenetetrazol (PTZ) -induced convulsions in mice

After administration of PTZ (80 mg/kg, i.p.), the mean seizure latency in the negative control group was 99.50± 3.92 Sec and the mean seizure duration was reported to be 43.33±1.99 Sec. When compared to the negative control group, the mean of seizure latency in Test 1 (5 mg/kg, p.o.) was delayed up to 116.3±6.56 Sec. not significant and the mean duration of the convulsion was significantly reduced to 26.50±1.76 Sec. ($p<0.001$). The AITC test 2 (10 mg/kg, p.o.) significantly reduced the duration of the convulsion and delayed the latency of seizure by up to 26.50±2.44 Sec. ($p<0.001$) and 149.7±14.23 Sec. ($p<0.05$) respectively. Test 3 (20 mg/kg, p.o.) showed a highly significant effect on seizure latency up to 170.3±16.39 Sec. ($p<0.001$) and a reduction in seizure duration up to 24.67±2.80 Sec. ($p<0.001$). The standard group showed highly significant increase in means seizure latency up to 241.2±15.77 Sec. ($p<0.001$) and significantly decrease mean seizure duration up to 22.83±2.68 Sec. ($p<0.001$) as compared to the negative control group. The normal control group, standard Diazepam-treated group (5 mg/kg, p.o.), Test 2 (10 mg/kg, p.o.), and Test 3 (20mg/kg, p.o.) all showed 100% protection against mortality. Mortality was 83.33% in the PTZ, 80 mg/kg, i.p. (negative control) group. Test 1 (5

mg/kg) in the AITC-treated group demonstrated 66.66% protection against mortality. The results are given in Table 1 and Figure 1.

Table 1 Effect of AITC on PTZ-induced convulsions in mice

Group	**Treatment**	**Seizure latency(sec)**	**Seizure duration (sec)**	**Mortality**	**% Protection against mortality**
Normal Control (NC)	CMC (0.5%) 1ml/kg p.o.	-	-	0/6	100%
Negative Control (NeC)	PTZ (80mg/kg i.p.)	99.50± 3.92	43.33±1.99	5/6	16.67%
Standard	Diazepam (5 mg/kg, p.o.)	241.2±15.77***	22.83±2.68***	0/6	100%
Test 1	AITC 1 (5 mg/kg p.o.)	116.3 ± 6.561^{ns}	26.50±1.76***	2/6	66.66%
Test 2	AITC 2 (10 mg/kg p.o.)	149.7±14.23*	26.50±2.44***	0/6	100%
Test 3	AITC 3 (20 mg/kg p.o.)	170.3±16.39***	24.67±2.80***	0/6	100%

Significance value expressed by mean ±SEM, n=6, ANOVA following multiple comparisons Dunnett's test *$p<0.05$, ***$p<0.001$ and ns as compared to the negative control group. (SEM: Standard error mean, ns: Non-Significant, ANOVA: One-way Analysis of Variance)

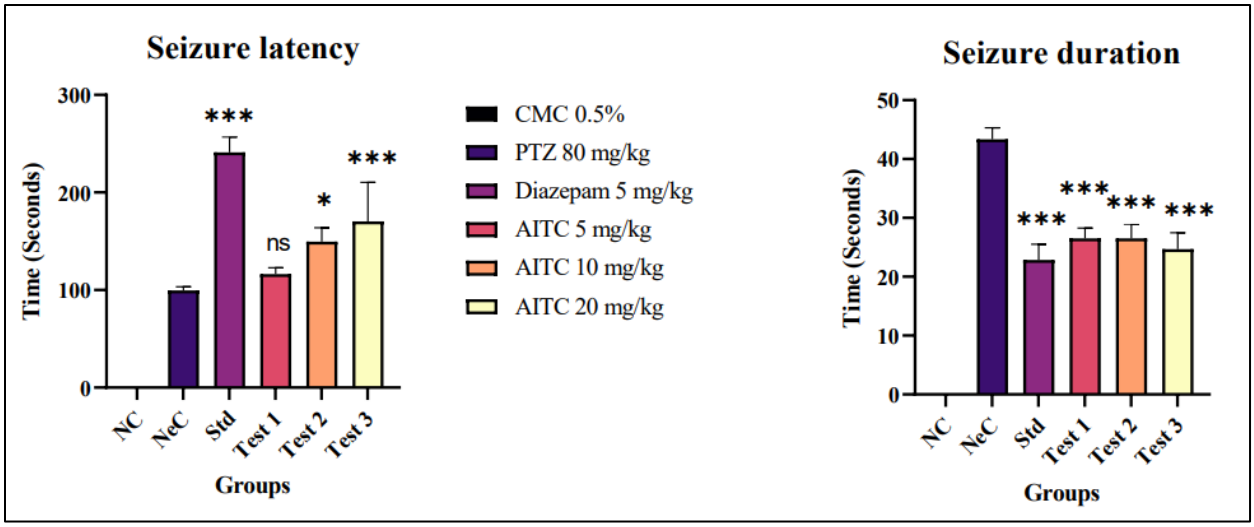

Figure 1 Effect of AITC (tests 1, 2, and 3) and Diazepam (5mg/kg, p.o.) on the seizure latency and duration of PTZ-induced convulsions in mice. Data are expressed as mean ± SEM (n=6). ns, *p<0.05, **p<0.01, and ***p<0.001 compared with negative control group. (One-way ANOVA followed by Dunnett's test).

Strychnine-induced convulsions in mice

After the administration of strychnine HCL (2 mg/kg, i.p.), the mean of seizure latency in the negative control group was 272.0±1.99 Sec and the mean duration from the end of a seizure was observed as 34.50±2.51 Sec. As compared to the negative control group, the mean of seizure latency in Test 1 (5 mg/kg, p.o.) was delayed by up to 285.2±3.14 Sec. not significant and the seizure duration was reduced by up to 26.50±2.446 sec. ns in a statistically non-significant manner. As compared to the negative control group, the mean of seizure latency in Test 2 (AITC 10 mg/kg, p.o.) was significantly delayed to 322.2±5.45 Sec. ($p<0.001$) and the mean duration of a seizure was significantly reduced to 24.67±2.80 Sec. ($p<0.05$). When compared to the negative control group, the test 3 group significantly reduced the duration of the convulsion to 22.83±2.68 Sec. ($p<0.001$) and delayed the seizure latency to 394.0±0.85 Sec. ($p<0.001$). The standard group showed highly significant increase in means seizure latency up to 429.7±12.36 Sec. ($p<0.001$) and significantly decrease mean seizure duration up to 20.50±0.99 Sec. ($p<0.001$) as compared to the negative control group. The standard Diazepam-treated group (5mg/kg, p.o.) resulted from 83.33% protection against mortality after receiving Strychnine (2 mg/kg, i.p.). The negative control group (Strychnine, 2mg/kg, i.p.) showed 100% mortality. The AITC-treated group Test

1(5 mg/kg), Test 2 (10 mg/kg, p.o.), and Test 3 (20 mg/kg, p.o.) simultaneously showed 33.34%, 50%, and 66.66% protection against mortality in a dose-dependent manner. The results are given in Table 2, and represented in Figure 2.

Table 2 Effect of AITC on Strychnine-induced convulsions in mice

Groups	**Treatment**	**Seizure latency (sec.)**	**Seizure duration (sec.)**	**Mortality**	**% Protection against mortality**
NC	CMC(0.5%) 1ml/kg, p.o.	-	-	0/6	100%
NeC	Strychnine HCL (2mg/kg, i.p.)	272.0±1.99	34.50±2.51	6/6	0
Standard	Diazepam (5mg/kg, p.o.)	429.7±12.36***	20.50±0.99***	1/6	83.33%
Test 1	AITC 1 (5mg/kg, p.o.)	285.2 ± 3.14^{ns}	26.50 ± 2.44^{ns}	4/6	33.34%
Test 2	AITC 2 (10mg/kg, p.o.)	322.2±5.45***	24.67±2.80*	3/6	50%
Test 3	AITC 3 (20mg/kg, p.o.)	394.0±0.856***	22.83±2.68**	2/6	66.66%

Significance value expressed by mean ±SEM, n=6, One-way Analysis of Variance (ANOVA) following multiple comparisons Dunnett's test. Standard, AITC (Low, medium, High) dose compared with a negative control group is ns, *P<0.05, **p<0.01, and ***p<0.001.

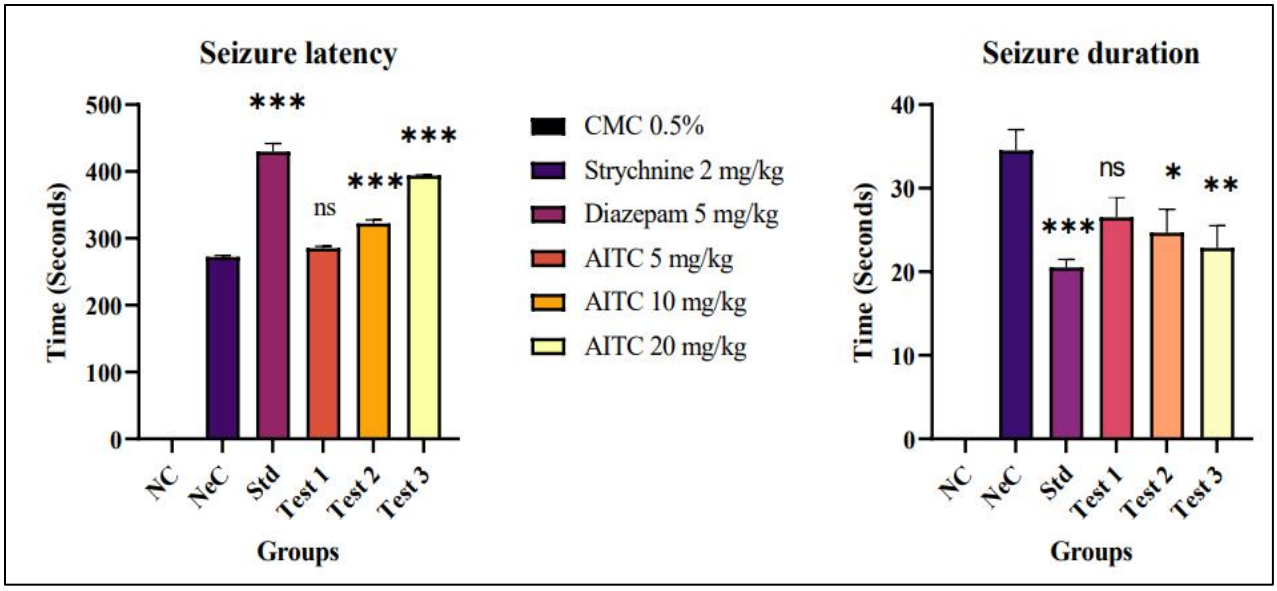

Figure 2 Significance value expressed by mean ±SEM, n=6, ANOVA following multiple comparisons of Dunnett's test. Standard, AITC (Low, medium, High) dose compared with a negative control group is ns, *P<0.05, **p<0.01, and ***p<0.001.

GABA estimation from the brain suspension of mice

PTZ-induced model

In table no. 3, the mean GABA level in the brain was observed to be 24.13±0.32 pg/mL at the dose of PTZ (80 mg/kg, i.p.) in the negative control group. In comparison to the negative control group, the mean GABA level increased significantly in Test 1 (5 mg/kg, p.o.), Test 2 (10 mg/kg, p.o.), and Test 3 (20 mg/kg, p.o.) to 37.47±0.318 pg/mL (p<0.001), 42.53±0.26 pg/mL (p<0.001), and 51.36±0.28 pg/mL (p<0.001). The administration of diazepam (5 mg/kg, p.o.) resulted in a highly significant increase in the mean GABA level of the Standard group, which was then 55.67±0.37 pg/mL (p<0.001) (Figure 3).

Table 3 Effect of AITC GABA level in PTZ-induced convulsions

Sr. no.	**Group**	**Treatment**	**Dose and Route**	**GABA level (pg/ml)**
1.	NC	CMC (0.5%)	1ml/kg p.o.	66.45±0.11
2.	NeC	PTZ	80 mg/kg i.p.	24.13±0.32$^{\#}$
3.	Standard	Diazepam	5 mg/kg p.o.	55.67±0.37***
4.	Test 1	AITC 1	5 mg/kg p.o.	37.47±0.31***
5.	Test 2	AITC 2	10 mg/kg p.o.	42.53±0.26***
6.	Test 3	AITC 3	20 mg/kg p.o.	51.36±0.28***

Significance value expressed by mean ±SEM (Standard error mean), n=6, One-way Analysis of Variance following multiple comparisons Dunnett's test. *p<0.05, **p<0.01 ***p<0.001 as compared with NeC, $^{\#}$p<0.001 as compared with NC. (One-way ANOVA followed by Dunnett's test).

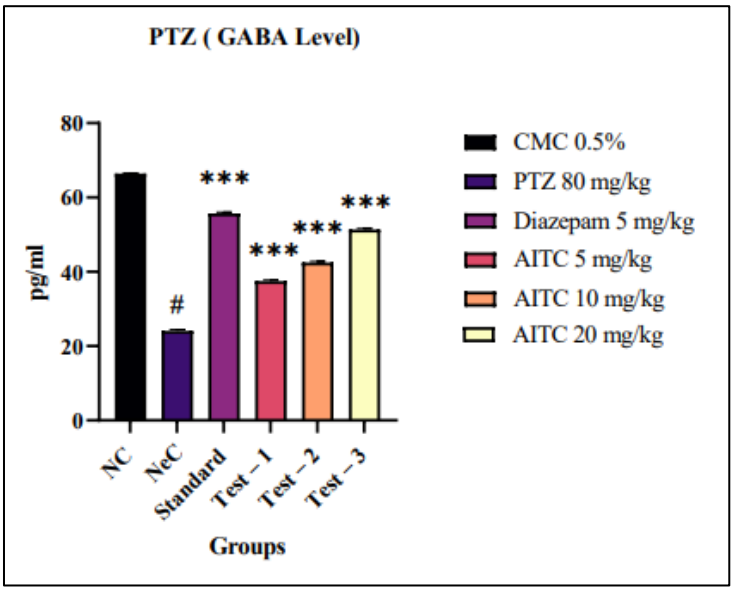

Figure 3 Effect of AITC (5, 10, 20 mg/kg, p.o.) and Diazepam (5mg/kg, p.o.) on the GABA level of PTZ-induced convulsions in mice. Data are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01 ***p<0.001 as compared with NeC, $^{\#}$p<0.001 as compared with NC. (One-way ANOVA followed by Dunnett's test).

Strychnine-induced model

Strychnine (2 mg/kg, i.p.) was administered to the negative control group, and the mean GABA level in the brain was reported to be 22.99±0.41 pg/mL that showed in Table no. 4. The mean GABA levels in Test 1 (5 mg/kg, p.o.), Test 2 (10 mg/kg, p.o.), and Test 3 (20 mg/kg, p.o.) were significantly higher at 36.97±0.29 pg/mL ($p<0.001$), 43.35±0.30 pg/mL ($p<0.001$), and 49.14±0.24 pg/mL ($p<0.001$), as compared to the negative control group. After being administered Diazepam (5mg/kg, p.o.), the mean GABA level of the standard group significantly increased to 52.42±0.32 pg/ml ($p<0.001$) it's represented in figure 4.

Table 4 Effect of AITC GABA level in Strychnine-induced convulsions

Sr. no.	**Group**	**Treatment**	**Dose and Route**	**GABA level (pg/ml)**
1.	NC	CMC (0.5%)	1ml/kg p.o.	65.52±0.10
2.	NeC	Strychnine	2 mg/kg i.p.	22.99±0.41$^{\#}$
3.	Standard	Diazepam	5 mg/kg p.o.	52.42±0.32***
4.	Test 1	AITC 1	5 mg/kg p.o.	36.97±0.29***
5.	Test 2	AITC 2	10 mg/kg p.o.	43.35±0.30***
6.	Test 3	AITC 3	20 mg/kg p.o.	49.14±0.24***

Significance value expressed by mean ±SEM (Standard error mean), n=6, ANOVA following multiple comparisons Dunnett's test *$p<0.05$, **$p<0.01$ ***$p<0.001$ as compared with NeC, $^{\#}p<0.001$ as compared with NC. (One-way ANOVA followed by Dunnett's test).

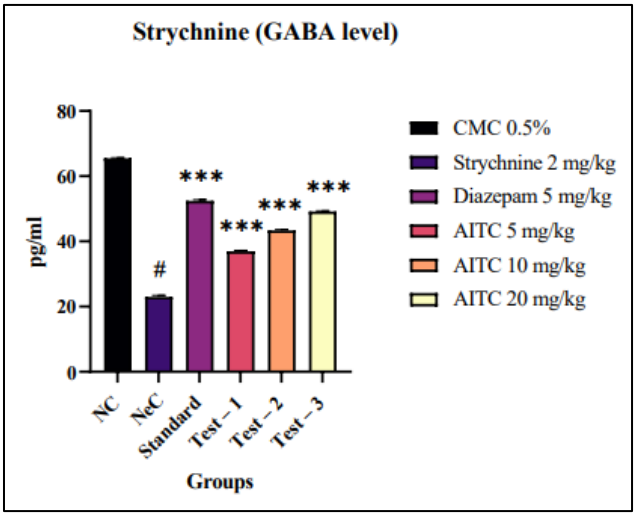

Figure 4 Effect of AITC (5, 10, 20 mg/kg, p.o.) and Diazepam (5mg/kg, p.o.) on the GABA level of Strychnine-induced convulsions in mice. Data are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01 ***p<0.001 as compared with NeC, $^{\#}$p<0.001 as compared with NC. (One-way ANOVA followed by Dunnett's test).

DISCUSSION

The present study assessed the anticonvulsant activity of Allyl isothiocyanate in male Swiss albino mice and compares its results with the standard drug Diazepam. AITC has an irritating smell and a pungent taste [8]. It is used as a food preservative, and rubefacient [16]. AITC can cross the blood-brain barrier and influence the activity of different signaling pathways in the brain. AITC may be modulates the activity of GABA receptors, which may aid in excitotoxicity prevention [8]. TRPA1 (Transient receptor potential Ankyrin 1) channels are expressed in a variety of cell types, including neurons and astrocytes, and have been linked to pain, sensation, and inflammation in the brain. AITC activates these TRPA1 channels, which improves GABAergic neurotransmission and may inhibit excitatory neurons, resulting in a reduction in seizure activity [17]. However, it might be seems AITC is effective by inhibiting the activity of certain ion channels, such as the N-methyl-D-aspartate (NMDA) receptor, which has been shown to have a role in the activity of seizures regulation. Taking into consideration the reported pharmacological action like neuroprotective, anti-inflammatory & antioxidant activity of AITC the present study is undertaken to evaluate the antiepileptic activity of AITC.

In this study, mice were given fourteen days of treatment with AITC (5, 10, and 20 mg/kg, p.o.) suspended in CMC 0.5% to reduce seizures which are compared with a group of standard drugs. This suggests that treatment with AITC reduces the seizure activity induced by PTZ and strychnine HCL. The current study indicates that administering AITC increased seizure latency and showed a more significant decreased duration in seizures induced by PTZ and Strychnine HCL, a convulsant widely used in the study of seizure generation, expansion latency, and also decreases seizure duration mechanisms, as well as the development and screening of new anticonvulsant compounds [18]. Diazepam was used as the standard drug in this study because it binds to the gamma subunit of the GABA-A receptor. As a result, this receptor is opened, and chloride ions enter the neuron. Furthermore, diazepam increases the frequency with which

the GABA receptor opens, causing more chloride influx than usual and, as a result, neuronal membrane hyperpolarization. This counteracts the depolarizing effect of and increases the postsynaptic resting period.

A study has reported that PTZ is a potent antagonist at the gamma-aminobutyric acid (GABA) receptor site, PTZ is a potent CNS stimulant whose action is similar to that of a direct producer of CNS depolarization, i.e., they directly trigger sodium channel opening and will generate action potential with the help of any neurotransmitter [19]. This modulation results in increased neuronal activity and generalized seizures in animals by suppressing the function of inhibitory synapses [20]. PTZ is being administered as a convulsant to the animal model to cause a chemically induced seizure. Absence seizures and myoclonic jerks are the two major symptoms of PTZ. Parameters evaluated in the PTZ model were reduced seizure duration, seizure latency, and mortality.

In the PTZ-induced model, the Test 3 group significantly increased the seizure latency & decreased seizure duration more than test 1, & test 2 groups as compared to the negative control group. Standard group had highly significant increase in latency and a decrease in the duration of seizure as compared to the negative control group. This study suggests that Test 3 (20 mg/kg) has a protective effect against the PTZ-induced model in mice. Additionally, we discovered that administration of AITC to the PTZ-induced model significantly increased GABA levels in the brain.

Strychnine is a neurotoxin that inhibits glycine receptor function [21]. In addition to playing a crucial role in peripheral and nerve tissue, glycine is an inhibitory neurotransmitter in the central nervous system. In the spinal cord, strychnine mostly inhibits postsynaptic glycine receptors [22]. When inhibitors are blocked, continuous neuronal excitability rises, sensory stimuli enhance the consequences of reflux, and strong muscular contraction results [23].

In the Strychnine-induced model, GABA level is increased at the dose of 20 mg/kg AITC more significantly than the other two test groups, also increased seizure latency and decreased seizure duration as compared to the negative control group. Also, AITC has been shown to increase GABA release in the brain, which can counteract the effect of strychnine-induced seizures. By regulating the balance between inhibitory and excitatory neurotransmitters in the brain [24], AITC may help to prevent or decrease seizures in the animal models of epilepsy. The result showed that administration of AITC 20 mg/kg, p.o. is a more effective dose in both PTZ and strychnine-

induced models of epilepsy. Overall, this study suggests that AITC shows the best effect for the treatment of epilepsy and other neurological disorders.

CONCLUSION

According to the results of the current investigation, it concluded that the Allyl isothiocyanate exhibited significant anticonvulsant activity. These findings showed that the AITC significantly reduced convulsive activity in PTZ & Strychnine-induced model due to possible mechanism linked with the modulation of GABAergic neurotransmitter system. However, there is need to evaluate the safety and efficacy of AITC in clinical studies.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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