ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4

Journal of Coastal Life Medicine

Effect of Xanthotoxin on Potentiating Anticonvulsant Activity of Rauwolfia Serpentina in Experimental Animals.

Received: 13 February 2023, **Revised:** 15 March 2023, **Accepted:** 19 April 2023

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Keywords

Rauwolfia serpentina, anticonvulsant, Raubasine, Xanthotoxin, Pentylenetetrazol, Strychnine HCL.

Abstract

The present investigation aimed to evaluate effect of xanthotoxin on potentiating anticonvulsant activity of Rauwolfia serpentina on the chemically induced convulsions in experimental animals and compare the effect of xanthotoxin on anticonvulsant activity of Rauwolfia serpentina with the standard dose of Diazepam on Pentylenetetrazol and Strychnine HCL model. Moreover, brain GABA levels were estimated to investigate the mechanisms underlying the anticonvulsant activity. In this study, results indicate that xanthotoxin (50 and 100 mg/kg, i.p.) significantly potentiated anticonvulsant activity of Rauwolfia serpentina. Similarly, xanthotoxin (100 mg/kg, i.p.) markedly enhanced the anticonvulsant action of Rauwolfia serpentina showed significant and dose-dependent inhibition of PTZ and Strychnine-induced convulsions. Furthermore, addition of xanthotoxin with Rauwolfia serpentina showed significantly increased GABA levels in brain as compared to Rauwolfia serpentina. The parameters observed in PTZ and Strychnine-induced models were seizure latency, duration, and mortality. Combination of xanthotoxin with Rauwolfia serpentina showed significant anticonvulsant activity in experimental animals.

1. Introduction

Epilepsy is a persistent neurological condition affecting people all over the world. According to the World Health Organization, epilepsy is defined by repeated seizures, which are short events of uncontrollable movement that may affect only a portion of the body (partial) or even the whole body (generalised) and are occasionally followed by unconsciousness and bowel or bladder control.(1)Epilepsy is a prevalent neurological disorder that affects 80% of persons in countries with low and middle incomes. Although it is predicted that up to 70% of people with epilepsy may live seizure-free if accurately recognised and examined, the risk of mortality from epilepsy is greater than in the general population. (1)

Antiepileptic drug (AED) medication treating epilepsy has been associated with side effects, doses, and chronic toxicities that affects almost every part of the systems. (2)To overcome these drawbacks, herbal drug treatment is becoming increasingly popular. Herb therapy is one of the most commonly used forms of balancing and alternative medicine therapy among patients. Traditional medicinal systems are prevalent in emerging nations, requiring an alternative agent

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CODEN: JCLMC4
Journal of Coastal Life Medicine

derived from natural sources.(3) Although 70% of Epileptic seizures can be controlled by monotherapy, a combination of two or more antiepileptic drug (AEDs) may be required to improve the efficacy and tolerability. As a result, researchers are looking for herbal drug that help cure neurological disorder. We focused on *Rauwolfia serpentina* an ancient traditional herb has been reported for various neurological diseases. *Rauwolfia serpentina* has antioxidant, anxiolytic(4), antihyperlipidemic(5), antifungal(6), antibacterial(6), antidiabetic(7), antihypertension(5), antivenom(8), antidiarrheal(9) and anticonvulsant(10). Xanthotoxin is a natural furocoumarins obtained from *Ammi majus* seeds(11). Xanthotoxin is reported as anticonvulsant(12), anticancer(13), osteoporosis(14), Alzheimer diseases(15), antianxiety(16). As their study on the screening of phytochemical study of *Rauwolfia serpentina* it has been observed that there are few alkaloids such as Raubasine(ajmalicine) responsible for anticonvulsant activity of *Rauwolfia serpentina*. (10)Here we tried to investigated the effect of xanthotoxin potentiating anticonvulsant activity of *Rauwolfia serpentina* in the experimental animals.

2. Methodology

Drugs and chemicals

Xanthotoxin was purchased from Prince scientific, Hyderabad, Pentylenetetrazol was purchased from Research-lab Fine chem industries, Mumbai and Strychnine Hydrochloride from laboratory reagent, New Neetha Chemicals in Pune, and Diazepam Tablets from Piramal Enterprises limited was used. All the chemicals used in the study were of standard analytical grade.

Experimental animals

Healthy Swiss albino mice of either sex weighing from 22-26gm were selected for study. The animals were purchased from National Institute of Bioscience, Dhangawadi, Taluka-Bhor Dist. Pune. The animals were housed in an animal housing and held at a temperature of 22°C ± 2°C and a relative humidity of 45- 55% under artificial lighting of 12-hour light and 12-hour dark cycle. The animals were given easy accessibility to a regular pellet feed and tap water ad libitum. The Institutional Animal Ethics Committee approved all experimental methods, and all procedures and techniques employed in this study followed the CPCSEA Guidelines.

Total Ash Content

The ash content is determined by weighing 2g of Ethanolic extract *Rauwolfia serpentina* roots, placed on asbestos, flattened, and heated until ash. Then the ash was weighed(17)

Calibration curve

The calibration curve was plotted for the range of 0.2 to 1mg/ml for linearity studies was performed on UV-spectrophotometer (Model- 1700, JASCO, India).

FT-IR studies

FT-IR study was carried out to assure the purity of the procured drug. Following FT-IR study was performed in Savitribai Phule Pune University on Model Tensor 37 Bruker.

Experimental protocol

Pentylenetetrazol-induced convulsion.

Healthy Swiss albino mice (22-26gm) of the either sex was randomly distributed and divided into six different groups of six mice each. Group 1 received the suspending agent CMC (0.5%) orally, 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 received Test 1 drug (EERS 200mg, p.o.), Group 5, 6 and 7 received EERS (200mg/kg, p.o.) + xanthotoxin (25, 50 and 100 mg/kg, i.p.) respectively. Each test group received treatment once per day for 14 consecutive days. Every single group received treatment for one hour before Pentylenetetrazol was delivered on the fourteenth day. Animals were monitored for 30 minutes after receiving Pentylenetetrazol (PTZ). onset of seizure and seizure duration, along with mortality rates, were all observed.

Strychnine-induced convulsion.

Healthy Swiss albino mice (22-26gm) of the either sex was randomly distributed and divided into six different groups of six mice each. Group 1 received the suspending agent CMC (0.5%) orally, Group 2 mice were administered Strychnine (2 mg/kg, i.p.), Group 3 received the standard drug, Diazepam at the

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

dose of (5 mg/kg, p.o.) Groups 4 received Test 1 drug (EERS 200mg p.o.), Group 5, 6 and 7 received EERS (200mg/kg p.o.) + xanthotoxin (25, 50 and 100 mg/kg, i.p.) respectively. Each test group received treatment once per day for 14 consecutive days. Every single group received treatment for one hour before Strychnine was delivered on the fourteenth day. Animals were monitored for 30 minutes after receiving Strychnine onset of Seizure and seizure duration, along with mortality rates, were all observed.

Statistical analysis

The data were analysed using one-way ANOVA, subsequently subjected to Dunnett's test in Graph pad Prism Software version 9.5.1 for figure on display. The data is shown as mean ± SEM.

3. Result:

Total Ash value (%)

The ash was obtained of ethanolic extract of *Rauwolfia serpentina* roots was 1.82 gm. So, the total ash value in percentage is 90%

Calibration curve of xanthotoxin in methanol

Xanthotoxin Maximum Concentration was reported to be at 200-400 nm in Methanol. In this investigation, the xanthotoxin calibration curve was plotted using a concentration range of 0.2-1mg/ml. The R^2 value was 0.9991.

Table 1 Absorbance Value of Xanthotoxin in Methanol

Concentration	Absorbance
0	0
2	0.0515
4	0.1032
6	0.1555
8	0.2082

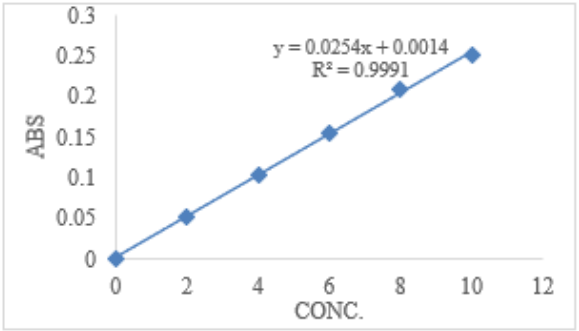

Figure 1 Calibration curve of xanthotoxin in methanol.

FT-IR spectroscopy

The primary goal of the IR multiphoton research is to patiently wait unless the practical clustering of this model is identified. On various occasions, various effective clusters engage IR fallout. IR spectrum evaluation using various sample attachments.

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

FT-IR spectrum data of xanthotoxin

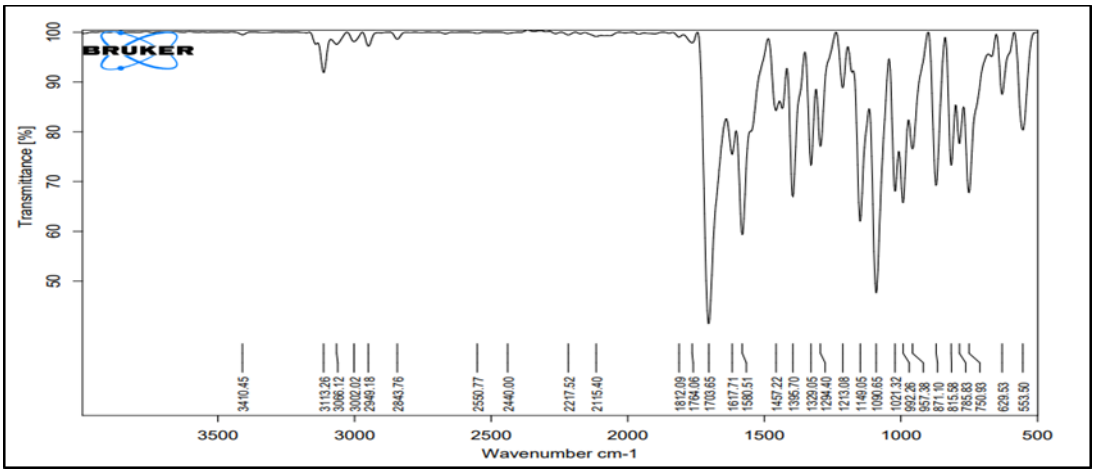

Figure 2 FT-IR data for xanthotoxin

Infrared spectroscopy revealed a reliable cluster and the standard value for xanthotoxin as follows:

FT-IR data for xanthotoxin

Table 2 FT-IR data for xanthotoxin

Sr. No.	Functional group	Standard range(cm^{-1})	Wave number (cm^{-1})
1.	C=O(ketone)	1725-1705	1703
2.	C-O	1300-1000	1149
3.	C-H (Aromatic)	1450-1375	1395

Evaluation of anticonvulsant activity

In the current study, the effect of xanthotoxin on potentiating anticonvulsant action against PTZ and strychnine-induced convulsions in mice were evaluated.

Pentylenetetrazol (PTZ) -induced convulsions in mice

The mean of seizure latency for negative control PTZ (80mg/kg i.p.) showed 89.33 ± 2.741sec. and mean seizure duration was observed 13.00 ± 1.653 sec. after administration of PTZ (80mg/kg, i.p.). The mean of seizure latency for standard drug diazepam (5mg/kg, i.p.) showed 224.2 ± 6.04 sec.($p<0.001$) as compared to negative control group and it showed statistically significant reduction in seizure up to 5.500 ± 0.4282 sec.($p<0.001$). The mean of seizure latency in Test 1 EERS (200mg/kg, p.o.) delayed 103.71 ± 2.011 sec.($p<0.05$) and significantly reduction in the seizure duration 9.333 ± 0.7601 sec. ($p<0.05$) in Test 1 as compared to negative control. The mean seizure latency in Test 2 (EERS 200 mg/kg, p.o. + xanthotoxin 25mg/kg, i.p.) significantly delayed to 118.8 ± 2.469 sec.($p<0.001$) and mean seizure

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

duration is significantly reduced to 8.667 ± 1.054 sec. ($p<0.05$) as compared to negative control group. The mean of seizure latency in Test 3 (EERS 200mg/kg, p.o. + xanthotoxin 50mg/kg, i.p.) is significantly delayed 147.2 ± 2.442 sec. ($p<0.001$) and significantly reduction in the seizure duration 7.167 ± 0.4773 sec. ($p<0.001$) as compared to the negative control group. The mean of seizure latency in Test 4 (EERS 200mg/kg, p.o. + xanthotoxin 100mg/kg, i.p.) is significantly delayed 179.00 ± 3.266 sec.($p<0.001$) and reduction in seizure duration 5.500 ± 0.7638 sec.($p<0.001$) as compared to negative control. The normal control group, Test 3 (EERS 200mg/kg, p.o.+ xanthotoxin 50mg/kg, i.p.), Test 4 (EERS 200mg/kg, p.o. + xanthotoxin 100mg/kg, i.p.) showed 100% protection against mortality. Negative control (PTZ 80mg/kg) showed 33.33% protection against mortality. The standard drug Diazepam (5mg/kg) and Test 3 (EERS 200mg/kg, p.o. + xanthotoxin 50 mg/kg, i.p.) showed 83.33% protection against mortality. The Test 1 (EERS 200mg/kg, p.o.) and Test 2 (EERS 200mg/kg, p.o. + xanthotoxin 25mg/kg) showed 66.66% protection against mortality.

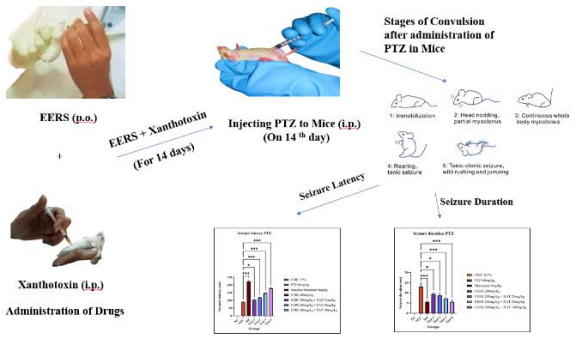

Figure 3 PTZ inducing seizure model

Table 3 Behavioral parameter of PTZ inducing seizure model.

Groups	Treatment & Route	Seizure Latency (sec)	Seizure Duration (sec)	% Mortality	% Protection against mortality
Normal Control (NC)	CMC 0.5% (1 ml/kg)	-	-	0/6	100%
Negative Control (NGC)	Pentylenetetrazol (80 mg/kg, i.p.)	89.33± 2.741	13.00± 1.653	4/6	33.33%
Standard	Diazepam (5mg/kg, i.p.)	224.2±6.041***	5.500±0.4282***	1/6	83.33%
Test 1	EERS	103.71±2.011*	9.333± 0.7601*	2/6	66.66%

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

	(200mg/kg, p.o.)				
Test 2	EERS (200 mg/kg p.o.) + XAT (25mg/kg, i.p)	118.8±2.469***	8.667± 1.054*	2/6	66.66%
Test 3	EERS (200mg/kg p.o.) + XAT (50 mg/kg, i.p.)	147.2±2442***	7.167±0.4773***	1/6	83.33%
Test 4	EERS (200mg/kg p.o.) + XAT (100mg/kg, i.p)	179.00±3.266***	5.500±0.7638***	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)

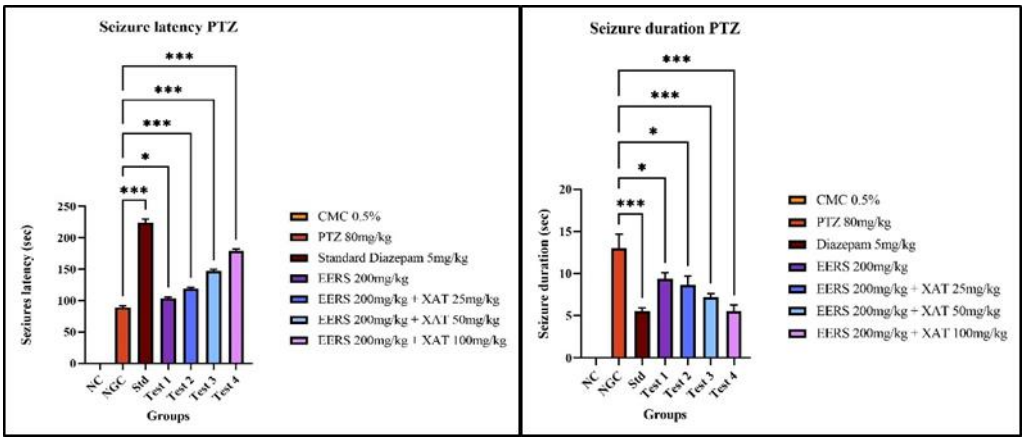

Figure 4 Effect of Test (1, 2, 3, 4) and standard Diazepam (5mg/kg p.o.) on the seizure latency and duration of PTZ-induced convulsions in mice. Data are expressed as mean ± SEM (n=6). ns, *p<0.05, **p<0.01 and ***p<0.001 compared with negative control group. (one-way ANOVA followed by Dunnett's test).

Strychnine-induced convulsions in mice

The mean of seizure latency for negative control showed 113.3 ± 4.074 sec. and mean seizure duration was observed 13.00 ± 1.653 sec. after administration of strychnine (2mg/kg, i.p.). The mean of seizure latency for standard drug diazepam (5mg/kg, i.p.) showed 169.8 ± 6.655 sec. (p<0.001) as compared to negative control group and it showed statistically significant reduction in seizure up to 5.500 ± 0.4282 sec. (p<0.001). The mean of seizure latency in Test 1 EERS (200mg/kg, p.o.) delayed 133.33 ± 4.616 sec. (p<0.01) and significantly reduction in the seizure duration 9.333 ± 0.7601 sec. (p<0.05) in Test 1 as compared to negative control. The mean seizure latency in Test 2 (EERS 200 mg/kg, p.o. + xanthotoxin 25mg/kg, i.p.) significantly delayed to 153.0 ± 2.219 sec. (p<0.001) and mean seizure duration is significantly reduced to 8.667 ± 1.054

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ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

sec. ($p<0.05$) as compared to negative control group. The mean of seizure latency in Test 3 (EERS 200mg/kg, p.o. + xanthotoxin 50mg/kg, i.p.) is significantly delayed 261.2 ± 3.114 sec. ($p<0.001$) and significantly reduction in the seizure duration 7.167 ± 0.4773 sec. ($p<0.001$) as compare to the negative control group. The mean of seizure latency in Test 4 (EERS 200mg/kg, p.o. + xanthotoxin 100mg/kg, i.p.) is significantly delayed 389.3 ± 3.490 sec. ($p<0.001$) and reduction in seizure duration 5.500 ± 0.7638 sec. ($p<0.001$) as compared to negative control.

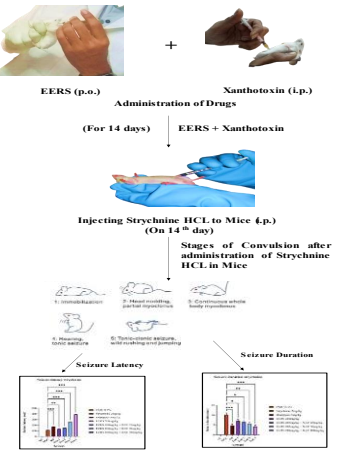

Figure 5 Strychnine inducing Seizure Model

Table 4 Behavioral parameter of strychnine inducing seizure model

Groups	Treatment	Seizure Latency(sec)	Seizure Duration(s)	% Mortality	% protection against mortality
Normal Control (NC)	CMC 0.5% (1ml/kg, p.o)	-	-	0/6	100%
Negative Control (NGC)	Strychnine HCL (2mg/kg i.p.)	113.3± 4.074	13.00±1.653	5/6	16.66%
Standard	Diazepam (5mg/kg, i.p.)	169.8±6.655***	5.500±0.4282***	4/6	33.33%
Test 1	EERS (200mg/kg, p.o.)	133.33±4.616**	9.333± 0.7601*	4/6	33.33%

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

Test 2	EERS (200mg/kg p.o.) +XAT (25mg/kg, i.p)	153.0±2.129***	8.667±1.054*	2/6	66.66%
Test 3	EERS (200mg/kg p.o.) +XAT (50mg/kg, i.p.)	261.2±3.114***	7.167±0.4773***	3/6	50%
Test 4	EERS (200mg/kg p.o.) + XAT (100mg/kg, i.p)	389.3±3.490***	5.500±0.7638***	2/6	33.33%

Significance value expressed by mean ±SEM (Standard error mean), n=6, One-way Analysis of Variance (ANOVA) following multiple comparisons Dunnett's test. Standard, Test (1, 2, 3, 4) compared with a negative control group, *P<0.05, **P<0.01 and ***p<0.001.

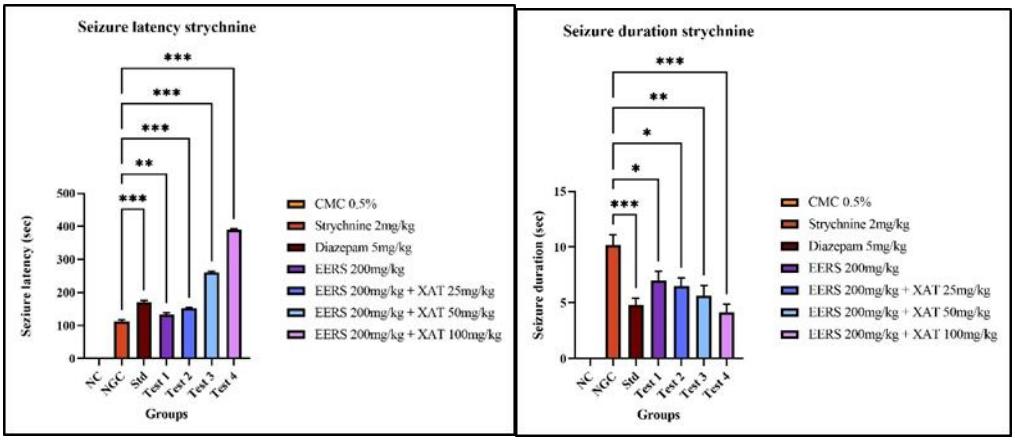

Figure 6 Effect of Test (1, 2, 3, 4) and standard Diazepam (5mg/kg p.o.) on the seizure latency and duration of Strychnine-induced convulsions in mice. Data are expressed as mean ± SEM (n=6). ns, *p<0.05, **p<0.01 and ***p<0.001 compared with negative control group. (one-way ANOVA followed by Dunnett's test).

GABA estimation from the brain suspension of mice

PTZ-induced convulsion model

The mean GABA level in brain was found 21.13 ± 0.7070 pg/ml in negative control group after the administration of PTZ (80 mg/kg, i.p.). The mean GABA level in standard drug diazepam (5mg/kg, i.p.) is highly significant increased to 60.28 ± 0.4100 pg/ml(p<0.001). The mean GABA level significantly increased to 23.77 ± 0.7869 pg/ml (p<0.01) in Test 1 EERS (200mg/kg, p.o.). The mean GABA level significantly increased to 29.86 ± 0.7345 pg/ml(p<0.001) for Test 2 (EERS 200mg/kg, p.o.+ XAT 25mg/kg, i.p.). The mean GABA level significantly increased to 39.87 ± 0.3932 pg/ml(p<0.001) for Test 3 (EERS 200mg/kg, p.o. +XAT 50 mg/kg, i.p.). The mean GABA level significantly increased to 55.88 ± 0.3918 pg/ml(p<0.001) for Test 4 (EERS 200 mg/kg, p.o. + XAT 100 mg/kg, i.p.).

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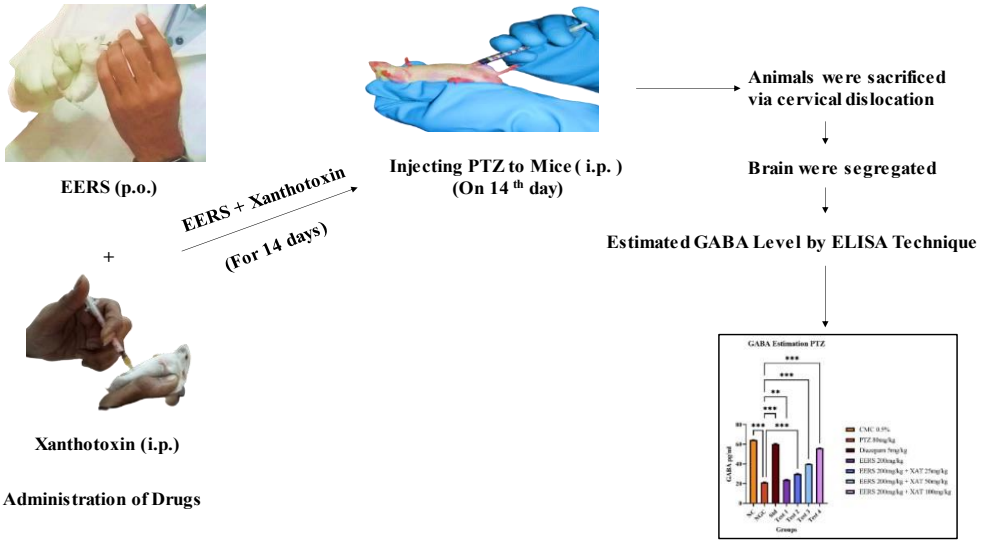

Figure 7 GABA estimation for PTZ induced Convulsion Model

Table 5 Effect of xanthotoxin on EERS GABA level in PTZ-induced convulsion model

Group	Treatment	GABA level (pg/ml)
Normal Control (NC)	CMC 0.5% (1ml/kg, p.o)	64.36 ± 0.2042
Negative Control (NGC)	Pentylenetetrazol (80 mg/kg, i.p.)	21.13 ± 0.7070#
Standard	Diazepam (5mg/kg,i.p.)	60.28 ± 0.4100***
Test 1	EERS (200mg/kg, p.o.)	23.77 ± 0.7869**
Test 2	EERS (200 mg/kg p.o.) + XAT (25mg/kg, i.p)	29.86 ± 0.7345***
Test 3	EERS (200mg/kg p.o.) + XAT (50 mg/kg, i.p.)	39.87 ± 0.3932***
Test 4	EERS (200mg/kg p.o.) + XAT (100mg/kg, i.p)	55.88 ± 0.3918***

Significance value expressed by mean ±SEM (Standard error mean), n=6, One-way Analysis of Variance (ANOVA) following multiple comparisons Dunnett's test **p<0.01, ***p<0.001 as compared to a negative control group.

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CODEN: JCLMC4
Journal of Coastal Life Medicine

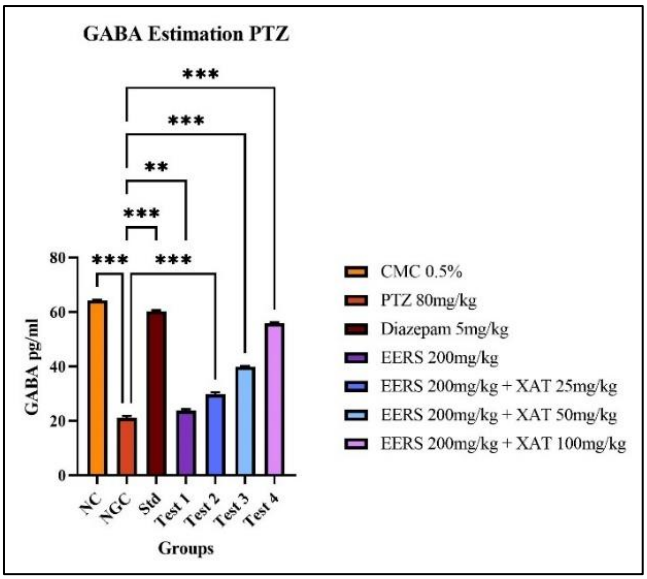

Figure 8 Effect of xanthotoxin on EERS and Diazepam (5mg/kg, p.o.) on the GABA level of PTZ-induced convulsions in mice. Data are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01 ***p<0.001 as compared with NGC, #p<0.001 as compared with NC. (One-way ANOVA followed by Dunnett's test).

Strychnine-induced model

The mean GABA level in brain was found 29.77 ± 0.3482 pg/ml in negative control group after the administration of strychnine (2mg/kg, i.p.). The mean GABA level in standard drug diazepam(5mg/kg) is significantly increased to 64.09 ± 0.0733 pg/ml (p<0.001). The mean GABA level significantly increased to 31.60 ± 0.3283 pg/ml (p<0.05) in Test 1 EERS (200mg/kg, p.o.). The mean GABA level significantly increased to 39.38 ± 0.4489 pg/ml (p<0.001) for Test 2 (EERS 200mg/kg + XAT 25mg/kg). The mean GABA level significantly increased to 56.08 ± 0.4489 pg/ml (p<0.001) for Test 3 (EERS 200mg/kg +XAT 50 mg/kg). The mean GABA level significantly increased to 60.89 ± 0.4489 pg/ml (p<0.001) for Test 4 (EERS 200 mg/kg + XAT 100 mg/kg).

Table 6 Effect of xanthotoxin on EERS GABA level in strychnine-induced convulsion model

Sr.no	Group	Treatment	GABA level (pg/ml)
1.	**Normal Control (NC)**	CMC 0.5% (1mg/kg, p.o)	66.06 ± 0.4749***
2.	**Negative Control (NGC)**	Strychnine Hydrochloride (2mg/kg)	29.77 ± 0.3482
3.	**Standard**	Diazepam (5mg/kg,i.p.)	64.09 ± 0.0733***

ISSN: 2309-5288 (Print)
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Journal of Coastal Life Medicine

4.	**Test 1**	EERS (200mg/kg, p.o.)	31.60 ± 0.3283*
5.	**Test 2**	EERS (200 mg/kg p.o.) + XAT (25mg/kg, i.p)	39.38 ± 0.4489***
6.	**Test 3**	EERS (200mg/kg p.o.) + XAT (50 mg/kg, i.p.)	56.08 ± 0.4489***
7.	**Test 4**	EERS (200mg/kg p.o.) + XAT (100mg/kg, i.p)	60.89 ± 0.4489***

Significance value expressed by mean ±SEM (Standard error mean), n=6, One-way Analysis of Variance (ANOVA) following multiple comparisons Dunnett's test **p<0.01, ***p<0.001 as compared to a negative control group

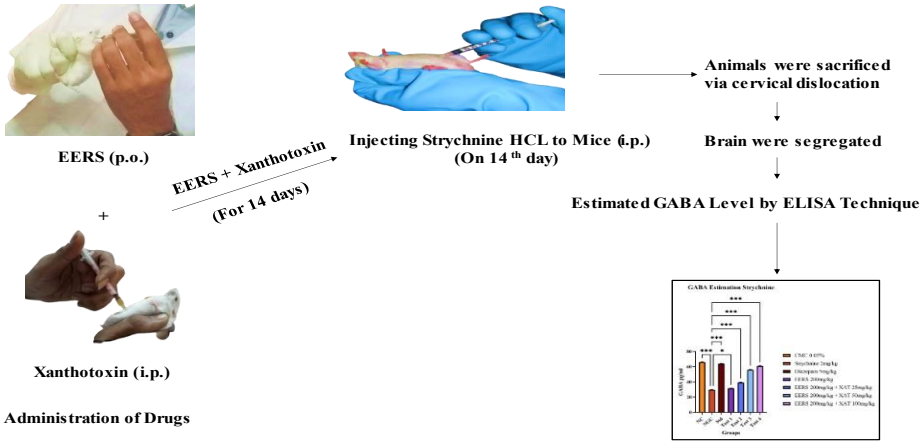

Figure 9 GABA estimation for Strychnine HCL induced Convulsion Model

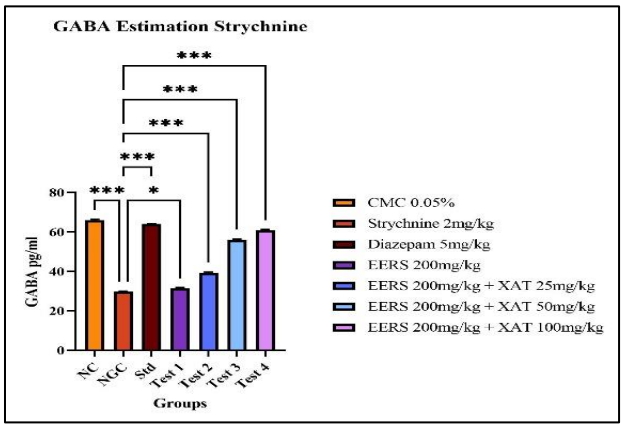

Figure 10- Effect of xanthotoxin on EERS and Diazepam (5mg/kg, p.o.) on the GABA level of strychnine-induced convulsions in mice. Data are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01 ***p<0.001 as compared with NGC, #p<0.001 as compared with NC. (One-way ANOVA followed by Dunnett's test).

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

4. Discussion

Epilepsy is a neurological condition that affects 1.00% of the world's population and is the third most common neurological disorder in the elderly. Epilepsy affects 5 million people worldwide, is more likely to cause physical difficulties and psychological diseases, and is more likely to cause premature death(1)

Herb therapy is a popular form of CAM therapy to treat epilepsy, but surgery has serious risks such as infections, stroke, paralysis, speech problems, and motor skills. A combination of two or more AEDs may be needed to improve efficacy and tolerability(2).

Rauwolfia serpentina is an ancient herbal drug used for a variety of conditions, including insomnia, high blood pressure, insanity, epilepsy, gastrointestinal problems, cardiovascular and liver diseases, constipation, and schizophrenia. It contains several alkaloids, such as Raubasine, which may be responsible for its anticonvulsant activity. A combination of two or more drugs can be used to control epileptic seizure, overcoming the drawbacks of monotherapy. This research aims to evaluate the effect of xanthotoxin on *Rauwolfia serpentina* anticonvulsant activity in suitable experimental models. Xanthotoxin is an furocoumarin obtained from *Ammi majus seeds*.

Raubasine is a benzodiazepines type of agonist that prolongs the opening of GABA receptors and increases the influx of chloride ions in post synaptic neurons, leading to anticonvulsant activity. Xanthotoxin inhibits the voltage gated sodium channel, blocks the depolarizing the state of presynaptic neurons and suppressing the epileptic seizures. It possesses anti-inflammatory and antioxidant properties, which may contribute to its anticonvulsant activities. *Rauwolfia serpentina* and xanthotoxin synergistically enhance anticonvulsants activity.

This study focuses on the GABA level in the brain of an experimental model to control the firing of seizures. The methods for induced convulsion include electrical stimulation and chemoconvulsants, such as pentylenetetrazol and strychnine HCL. The chosen chemoconvulsants are pentylenetetrazol and strychnine HCL.

PTZ is a GABA-A receptor antagonist that suppresses inhibitory synapses, leading to generalized seizure in animals. Strychnine is a neurotoxin that increases neuronal excitability and exaggerates reflux effects, resulting in muscle contraction.

The PTZ-induced convulsion model is popular for studying human generalized myoclonic and absence seizures. PTZ causes convulsions by activating glutamate receptors (NMDA) and promoting Ca^{+} ion entry into neurons(18). Diazepam is an efficacious drug for absence seizures due to its GABAergic facilitating effects.

In present study strychnine induces convulsions by blocking the inhibitory action of glycine in the spinal cord and brainstem, which leads to an increase in excitatory responses from neurons. Strychnine inhibits postsynaptic glycine receptors predominantly in the spinal cord(19). When inhibitors are blocked, ongoing neuronal excitability is increased & sensory stimuli exaggerated reflux effects thus producing powerful muscle contraction(20)

Diazepam was selected as the reference dosage in this investigation since the drug binds to the GABA-A receptor's gamma subunit. Therefore, an effect, this receptor is activated, allowing chloride ions to enter the cell's membrane. Finally, diazepam increases the frequency wherein the GABA receptor opens, resulting in greater chloride inflow and, as outcome, neuronal membrane hyperpolarization. This reduces the depolarizing impact of and extends postsynaptic resting time. Diazepam is an efficacious and widely used drug for absence seizures because to its GABAergic facilitating effects(21)

In this study, mice were given fourteen days treatment of EERS 200 mg/kg (p.o.) suspended in DMSO 0.05% and xanthotoxin (25, 50, 100mg/kg, i.p.) suspended in Tween 80 1% in combination to minimises seizures which are compared with a group of standard drugs. This study suggests that the treatment with the combination of EERS with xanthotoxin showed increases in seizure latency and decrease in seizure duration induced by PTZ and strychnine HCL. The present investigation showed that giving EERS with xanthotoxin increased seizure latency and decreased seizure duration in PTZ-induced myoclonic seizures a convulsant frequently used in seizure generation, development, and

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termination research. In PTZ model Test 4 (EERS 200mg/kg + XAT 100mg/kg) and Test 3 (EERS 200mg/kg + XAT 50mg/kg) showed significant increase in seizure latency and decreased in seizure duration. The GABA level of Test 4 was significantly increased in PTZ model as compared to the negative control.

In strychnine induced model Test 4 and Test 3 significantly increased in seizure latency and decreased in seizure duration. The GABA level for Test 4 is significantly increased as compared to the negative control. Raubasine, an alkaloid of *Rauwolfia serpentina*, is a benzodiazepines type of agonist that binds to GABA-A subunit and prolongs the opening of the chloride ions and increases the influx of chloride ions in post synaptic neurons. Xanthotoxin, like other anticonvulsant medications, may operate as a sodium channel blocker in epilepsy. It inhibits the voltage gated sodium channel, blocking the influx of sodium in the presynaptic neurons and suppressing the neurons. It also has anti-inflammatory and antioxidant properties, which may contribute to its anticonvulsant activities.

5. Conclusion:

Epilepsy is a chronic neurological disorder affecting nearly 5 million individuals globally. Ancient medical systems are popular in developing countries, necessitating the use of an alternative agent obtained from natural sources. The use of phytochemicals derived from nature has the potential to contribute to the creation of anti-epileptic medicines with novel structures and improved safety and effectiveness characteristics. according to the present investigation, the combination of xanthotoxin and *Rauwolfia serpentina* has demonstrated significantly Potentiating antiepileptic effects in PTZ and strychnine HCL inducing convulsion model. This suggests that xanthotoxin potentiates the anticonvulsant activity of Ethanolic extract of *Rauwolfia serpentina*.

6. Acknowledgment

The author is grateful to Honorable Shri. Sangramdada Thopte, Smt. Swarupa S. Thopte and the respected Principal of Rajgad Dnyanpeeth's College of Pharmacy, Bhor, Pune Dr. R.V. Shete Sir for providing an infrastructure facility for helping in the completion of a research article. The author is also extremely thankful to their teachers and friends for their continuous encouragement and support.

7. Conflict of Interest

Authors declare no conflict of interest.

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