Evaluation of anti-epileptic efficacy of combine herbal extract in an in-vivo model of epilepsy

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Abstract:

Background:

Epilepsy is a serious neurological disorder that affects 50 million people worldwide. Conventional antiepileptic drugs are coupled with several adverse effects and contraindications. Herbal agents that possess antiepileptic potential could be a better alternative to conventional medicine with minimal or no adverse effects. **Aims and objectives:** The aim of this study was to evaluate the antiepileptic effects of combined herbal extract of *Brassica nigra* and *Swertia chirata*, in comparison with standard drugs in pentylenetetrazole model of seizure. **Methodology:** Wistar albino rats (weighing 180-220 gram) were randomly divided into 6 groups having 6 animals in each group. Group 1: Control (DMSO 10%, 10ml/kg b.w, p.o), Group 2: Diazepam 10 mg/kgp.o; Group 3: Valproic acid 300 mg/kgp.o; Group 4: *Brassica nigra*(BN) 250 mg/kg and *Swertia chirata* (SC) 250 mg/kgp.o; Group 5: BN 250 mg/kg and SC 500 mg/kg p.o; Group 6: BN 500 mg/kg and SC 250 mg/kg p.o. **Results:** In pentylenetetrzole model; group 4, significantly delayed the onset (p=0.01) and duration of seizures (p=0.003) as compared to control. In group 5, the onset of seizure was significantly delayed (p=0.000, p=0.01) as compared to control and diazepam respectively; while, duration of seizure was increased (p=0.003) as compared to valproic acid. In group 6; the onset of seizure was significantly delayed (p=0.000, 0.000) as compared to control and diazepam; whereas, duration of seizure was significantly decreased (p=0.007) as compared to control. **Conclusion:** On the basis of these results, we can conclude that the herbal combinations possess antiseizure potential.

Key words: Epilepsy, Brassica nigra, swertia chirata, seizures, PTZ.

1. Introduction:

Epilepsy is a neurological disorder characterized by periodic seizures, presenting with episodes of motor, sensory or autonomic phenomenon with or without loss of consciousness(1,2). It is the second most frequently encountered neurological condition after stroke (3), that impose immense burden on individuals, families and as well as on health care system(4,5). In the year 2017, the World Health Organization (WHO) evaluated that epilepsy accounts for 0.75 percent, of global burden of disease and frequent in 50 million people worldwide. Nearly 80% of people having epilepsy live in low and middle income countries (6).

There are two neurotransmitters that are largely considered in relation to epileptic seizures: GABA (gamma-aminobutyric acid) and glutamate. GABA is an inhibitory neurotransmitter that binds to $GABA_A$receptors. While glutamte,an excitatory neurotransmitter that acts on three groups of *ionotropic* receptors i-e NMDA(N-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and kainate, are inborn cation permeable channels. It acts by increasing levels of glutamate which opens potassium and sodium channels and enhances depolarization state or by reducing GABA levels which result in decreasing chloride conductance in the brain could lead to hyperexcitability of neurons. Hence, the balance of these neurotransmitters is critical for the neuronal activity in the brain (7, 8,9).

Despite all the splendid advancements in conventional medicine, traditional medications have always been practiced. There are numerous herbs which have been used in the traditional system of medicine for epileptic seizures in China, Iran, India, Pakistan, North and South America, etc. Many people believe that traditional herbal treatment helps in controlling seizures and number of such people are increasing day by day(10). Unfortunately, anti-seizure drugs have several adverse effects which include drowsiness, hepatotoxicity, cognitive dysfunction, aplastic anemia, megaloblastic anemia, and teratogenicity etc (11). Due to these major adverse effects and lack of seizure control by standard drugs, patients consider herbal treatments for the cure and prevention of various disease(12).

Brassica nigra, in english it is called Black mustard, belongs to the family Brassicaceae. The phytochemical screening of *Brassica nigra* revealed that it consists of flavonoids, tannins, alkaloids(13), amino acids, sucrose and Glucosinates particularly sinigrin(14), phenolic compounds predominantly, galliac acid, ferulic acid, quercetin,rutin and caffeic acid(15), while other constituents are saponins, and glycosides(16). Another plant which is used in this study is Swertia Chirata, in english it is also known as Chirata, Kirataka, Chiratika, belongs to the family Gentinaceae. The major biological active compounds in crude extract of different parts of this plant are, mangiferin, amarogentin and swertimarin; secondary metabolites including glycosides, xanthones,secoirioid, phenolics, alkaloids, flavonoids, triterpenes, tannins, carbohydrates and sterols, these phytochemicalsare responsible for its pharmacological effects (17,18).

There is an intense urge to explore such alternative agents for the treatment of epilepsy which may prove to be more effective with minimal or no side effects and could be economical. Since epilepsy is a heterogeneous disorder, it is most likely that the combination of herbs could be effective in the management of epilepsy and a better alternative to conventional medicines in the management of this disorder.

2. Material and Methods:

Study design: Experimental animal study.

Collection and Identification of Plant:

The dried aerial part of *Swertia chirata* and seeds of *Brassica nigra* were procured from the local market of Karachi, Pakistan. The sample of the plant specimen was identified by Prof. Dr Mansoor Ahmad, Department of Pharmacognosy, University of Karachi and allocated a voucher specimen no MBN-20170302-1 for *Brassica nigra* and MSC-20170303-1 for *Swertia chirata*.

Drugs and chemicals used in this study:

The following chemicals and solutions were used in this study.

Chemical/Solution	Make
Diazepam	*TCI , Japan*
Valproic acid	*TCI , Japan*
Pentylenetetrazole	*Sigma, USA*
Methanol	*Merck, Germany*

DMSO (Dimethyl Sulfoxide)	*Merck, Germany*

Animals:

In this experimental research, adult male wistar albino rats weighing 180-220 gram, were used. Animals were acclimatized one week before the commencement of the experiment and kept under controlled room temperature 23 ± 2°C in 12/12 h light and dark cycles with free access to food and water. All the experiments were carried out between 09:00 am and 12:00 pm. The experiment was performed in accordance with the International standards for the Use and Care of Laboratory Animals set by National Institute of Health (US).

Extract Preparation:

Plant materials (1000 gram) of both plants were grounded into coarse powder separately, using an electrical grinder. The coarse powder of plants was soaked in methanol for 15 days at room temperature in a separate air-tight container, with occasional shaking and stirring. The resulting extract was then filtered using whatsman filter paper No 1 and evaporated under reduced pressure in rotary evaporator at 45 ℃ (19). The extract of each plant was then weighed and calculated the percentage yield. The obtain yield of *Brassica nigra* was 6.3 % (w/w) and *Swertia chirata*, 5.3% (w/w). The resultant thick extract was kept in a refrigerator for future use. Before administration, each extract was freshly reconstituted with 10% DMSO (Dimethyl Sulfoxide) to enhance the solubility of extract in distilled water.

Experimental design for pentylenetetrazole (PTZ)

Experimental protocol for PTZ-induced seizures model

Grouping of animals in PTZ model:

Total 36 rats were used in this model which was divided into six groups having six animals in each group.

Group 1: Control Received 10 % DMSO as placebo (10ml/kg body weight, p.o)

Group 2: Diazepam 10 mg/kg, p.o

Group 3: Valproic acid 300mg/kg, p.o

Group 4: *Brassica nigra* 250 mg + *Swetia chirata* 250 mg/kg (BN 250 + SC 250), p.o

Group 5: *Brassica nigra* 250 mg + *Swertia chirata* 500 mg/kg (BN 250 + SC 500), p.o

Group 6: *Brassica nigra* 500 mg + *Swertia chirata* 250 mg/kg (BN 500 + SC 250), p.o

Administration of drugs/extracts in PTZ induced seizures:

In this test, the experiment was conducted on overnight fasted rats and weighed prior to the experiment, then according to groups animals were treated with plain control, diazepam, valproic acid and plants extract in different combination doses. After One hour, the PTZ (70mg/kg,i.p) was injected to all groups(1).

Assessment of Antiepileptic efficacy:

After the administration of PTZ, the latency (the time prior to the onset of seizures) and duration of seizures was observed for the first one hour along with the percentage protection (percentage of deaths occurred within 24 hours). The ability of the plant extract to prevent or delay the onset of the seizure and decrease percentage of mortality in the animals was taken as an indication of anti-seizure activity(1,21).

Statistical Analysis:

Statistical analysis was carried out by using SPSS (Statistical Package for Social Sciences) version 20. Data is presented as mean and standard deviation. The difference amongst groups was measured by using one-way analysis of variance (ANOVA), followed by pair-wise comparison post hoc Tukey's test. $P < 0.05$ was taken to indicate the statistical significance.

Results:

Table 1 Effects of herbal combination on onset of seizure in comparison with control in PTZ model.

Comparison groups **Onset of seizure (Sec.)**		P-Value	95 % Confidence Interval	
			Lower	Upper
Control 31.33± 7.93	Diazepam 10mg 68.83 ± 61.97	0.7	-13.52	4.85
	Valproic acid 300 mg 192.17 ± 45.04	0.000*	-29.94	-11.55
	(Group-A) BN 250mg + SC250mg 122 ± 16.35	0.01*	-19.69	-1.30
	(Group-B) BN 250mg + SC500mg 165.33± 90.35	0.000*	-24.69	-6.30
	(Group-C) BN 500mg + SC250mg 385 ± 185.72	0.000*	-36.10	-17.72

*p-value<0.05 was considered significant, Data are expressed as Mean ± SD, n= 6, BN = Brassica nigra, SC = *Swertia chirata*, Dosages in mg/kg

Table 2 Effects of herbal combinations on onset of seizure in comparison with diazepam in PTZ model

Comparison groups **Onset of seizure (Sec.)**		P-Value	95 % Confidence Interval	
			Lower	Upper
Diazepam10mg 68.83 ± 61.97	Control 31.33 ± 7.93	0.7	-4.85	13.52
	(Group-A) BN250mg + SC 250mg 122 ± 16.35	0.3	-15.35	3.02
	(Group-B) BN 250mg + SC 500mg 165.33± 90.35	0.01*	-20.35	-1.97
	(Group-C) BN 500mg + SC 250mg	0.000*	-31.77	-13.39

	385 ± 185.72			

*p-value<0.05 was considered significant, Data are expressed as Mean ± SD, n=6, BN = Brassica nigra, SC = *Swertia chirata*, Dosages in mg/kg

Table 3 Effects of herbal combinations on onset of seizure in comparison with valproic acid in PTZ model.

Comparison groups **Onset of seizure (Sec.)**		P-Value	95 % Confidence Interval	
			Lower	Upper
Valproic acid 300mg 192.17 ± 45.04	Control 31.33± 7.93	0.000*	11.55	29.94
	(Group-A) BN 250mg + SC250mg 122 ± 16.35	0.02*	1.05	19.44
	(Group-B) BN 250mg + SC500mg 165.33± 90.35	0.5	-3.94	14.44
	(Group-C) BN 500mg + SC250mg 385 ± 185.72	0.3	-15.35	3.02

*p-value<0.05 was considered significant, Data are expressed as Mean ± SD, n=6, BN = Brassica nigra, SC = *Swertia chirata*, Dosages in mg/kg

Table 4 Effects of herbal combinations on duration of seizure in comparison with control in PTZ model.

Comparison groups Duration of seizures (Sec.)		P-Value	95 % Confidence Interval	
			Lower	Upper
Control 193.5 ± 112.18	Diazepam 10mg 35.83± 41.64	0.001*	6.56	31.43
	Valproic acid 300mg 19.83± 1.16	0.000*	12.73	37.59
	(Group-A) BN 250mg + SC250mg 61.33± 29.51	0.03*	0.59	22.23
	(Group-B) BN 250mg + SC500mg 67.33±18.42	0.401	-4.51	20.34
	(Group-C) BN 500mg + SC250mg 36.17 ±12.87	0.007*	3.31	28.18

*p-value<0.05 was considered significant, Data are expressed as Mean ± SD, n=6, BN =Brassica nigra, SC = *Swertia chirata*, Dosages in mg/kg

Table 5 Effects of herbal combinations on duration of seizure in comparison with diazepam in PTZ model.

Comparison groups Duration of seizures (Sec.)		P-Value	95 % Confidence Interval	
			Lower	Upper
Diazepam 10mg 35.83± 41.64	Control 193.5 ± 112.18	0.001*	-31.43	-6.56
	(Group-A) BN 250mg + SC250mg 61.33± 29.51	0.1	-19.65	1.98
	(Group-B) BN 250mg + SC500mg	0.1	-23.51	1.34

	67.33±18.42			
	(Group-C) BN 500mg + SC250mg 36.17 ±12.87	0.9	-15.68	9.18

*p-value<0.05 was considered significant, Data are expressed as Mean ± SD, n=6, BN = Brassica nigra, SC = *Swertia chirata*, Dosages in mg/kg.

Table 6 Effects of herbal combinations on duration of seizure in comparison with valproic acid in PTZ model.

Comparison groups Duration of seizures (Sec.)		P-Value	95 % Confidence Interval	
			Lower	Upper
Valproic acid 300mg 19.83± 1.16	Control 193.5 ± 112.18	0.000*	-37.59	-12.73
	BN 250mg + SC250mg (Group-A) 61.33± 29.51	0.001*	-26.56	-4.93
	BN 250mg + SC500mg (Group-B) 67.33±18.42	0.003*	-29.68	-4.81
	BN 500mg + SC250mg (Group-C) 36.17 ±12.87	0.2	-21.84	3.01

*p-value<0.05 was considered significant, Data are expressed as Mean ± SD, n=6, BN = Brassica nigra, SC = *Swertia chirata*, Dose in mg/kg

Protective Effect of different combinations against Mortality in PTZ -induced Seizure

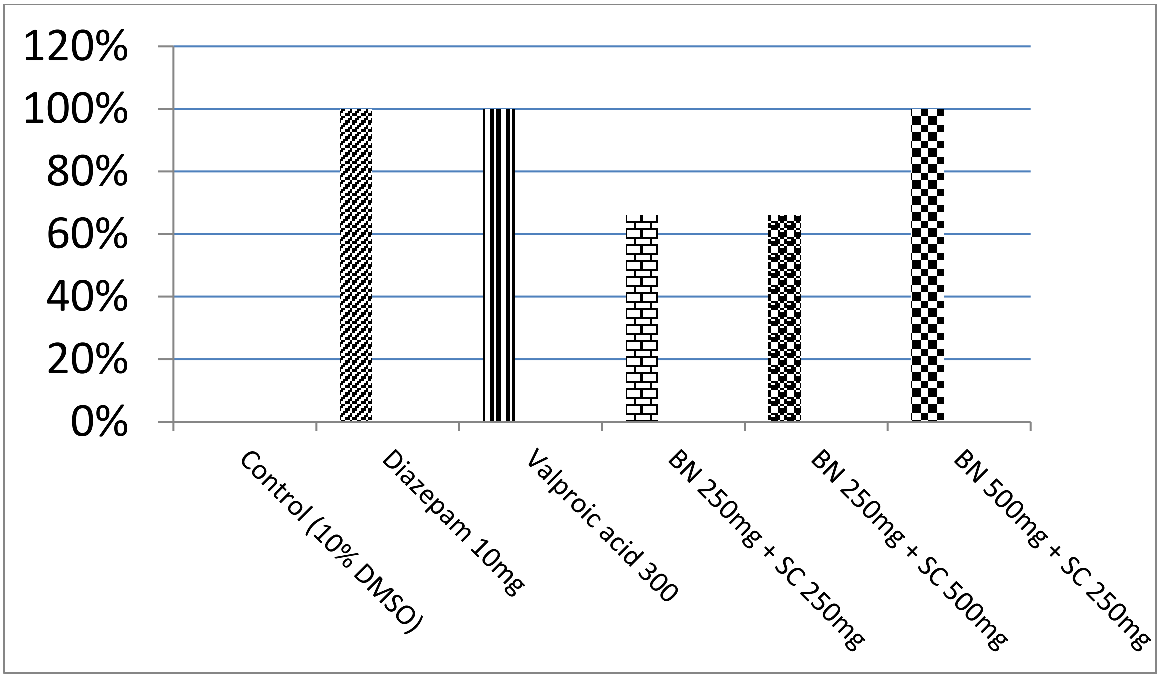

Figure 1. Percentage protections in PTZ model

Discussion:

The present study was proposed to assess the anti-epileptic effect of the methanolic extract of *Swertia chirata* and *Brassica nigra* in different combinations and analyzed in pentylenetetrazole (PTZ) animal model of seizure. As it is well documented that, the ability of a substance to delay or prevent the onset of clonic or tonic-clonic seizure induced by pentylenetetrazole (PTZ), is said to possess anti-seizure efficacy(22).The results of the current study indicates that the combination of *Swertia chirata* and *Brassica nigra* possess anti-seizure efficacy in different combinations by delaying the onset of tonic-clonic seizure in rats. In this model, three combinations were selected and then their onset and duration of seizures were compared with the control and standards drugs (diazepam and valproic acid) as well as percentage protection was noted in all groups. Different combination showed varied efficacy as compared to control and standard drug treatments.

In combined extract, when the low dose of both herbs (*Brassica nigra* 250mg/kg and *Swertia chirata* 250mg/kg) was administered, the latency or onset of seizure was delayed as compared to control (table 1). However, the onset of seizure was significantly reduced in this combination than the valproic acid group (table 3). While, no significant difference was observed as compared to the diazepam group (table 2). The same extract combination significantly decreased the duration of seizure as compared to control (table 4), but the duration of seizures were not significantly decreased as compared to standard drugs (diazepam and valproic acid) as shown in table 5 and 6 respectively.

In another combination, low dose of *Brasscia nigra* (250mg/kg) and high dose of *Swertia chirata* (500mg/kg)were administered, which significantly delayed the onset of seizure as compared to control and diazepam respectively (table 1,2); while, no significant difference in the onset of seizure was observed as compared to valproic acid (table 3). Although a reduction in duration of seizure was observed in this combination; but it was not significantly decreased as compared to valproic acid (table 6); similarly, no significant difference was found as compared to control and diazepam (table 4,5).

When a high dose of *Brassica nigra*(500 mg/kg) and low dose of *Swertia chirata* (250 mg/kg) were given to all rats in the PTZ model, a significant delayed of latency of seizures were noticed as compared to control and diazepam(table 1,2). The duration of seizures were significantly decreased as compared to control (table 4). However, duration of seizure was also decreased as compared to diazepam, but it was not significantly decreased. The results demonstrated, that the combined herbal extract exhibit better mean onset of seizure and similar duration of seizure as diazepam in PTZ-induced seizure.

PTZ is a GABA-A receptor antagonist and the most commonly used acute seizure model for the screening of new antiepileptic agents(23,24).The herbs that produce anti-seizure effects, might have profound effect on GABA-A receptors. GABA neurotransmitter has been documented to have a major role in epilepsy; All of the three combined herbal extract delayed the onset of seizure as compared to control, so most probable that our herbs enhancing the inhibitory activity of GABA by altering levels of GABA neurotransmitters in the central nervous system (CNS)(25).

Our results are similar to the previous study in which *Swertia chirata* (250mg/kg) was used in combination with *Cinnamomum zeylanicum* (500mg/kg) in PTZ model (26); however, in our study the combined extract of *Brassica nigra* 500 mg/kg and *Swertia chirata* 250mg/kg showed similar efficacy in PTZ model.

We can compare our results with *Swertia chirata* alone, the mean time of onset of seizure at 750 mg/kg was 447.0±37.86 seconds, which is more delayed than our combination (385 ± 185.72 sec.); however, the limitation of this study was, the toxicity at 750 mg/kg was not evaluated, it may possible that it may have toxic effects at 750 mg/kg.

We can also compare the mean onset of seizure of the herbal combination with the *Brassica nigra* used alone in PTZ model; the mean onset of seizure of *Brassica nigra* at 400 mg/kg was 84.6± 4.0(1); while in our study, the mean onset of combined herbal extract (*Brassica nigra* 500 mg/kg and *Swertia chirata* 250 mg/kg) was 385 ± 185.72. It proves that the combined herbal extract has better anti-seizure potential as compared to *Brassica nigra* alone, as the onset of seizure was much delayed in our herbal combination.

While analyzing the protective effect against mortality after PTZ-induced seizure, our findings demonstrated that all doses possess appropriate protection against PTZ. Standard drugs completely eradicate the seizures in rats and provided 100 % protection against mortality after PTZ-induced seizures. Similarly, one of the combinations (*Brassica nigra*500 mg/kg and *Swertia chirata* 250 mg/kg) also provided 100 % protection in the PTZ model (Figure 1).

Result:

Based on these facts, we can conclude that the methanolic extract of *Brassica nigra* and *Swertia chirata* in all combinations demonstrated antiseizure activity in the pentylenetetrazole (PTZ). However, the best antiseizure effect was observed in the combined herbal extract of *Brassica nigra* 500 mg/kg and *Swertia chirata* 250 mg/kg, and this combination delayed the onset of seizure better than diazepam in PTZ model.

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STATEMENTS AND DECLARATIONS:

Conflict of interest: None