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EVALUATION OF THE ANTI-EPILEPTIC ACTIVITY OF *VIOLA TRICOLOR L.* EXTRACT VS. CARBAMAZEPINE ON EXPERIMENTAL MODELS OF EPILEPSY IN MICE

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Abstract:
This study was carried out to compare the anticonvulsant effects of viola and those of carbamazepine on an animal model of seizure. Forty mice were divided into five groups. Carbamazepine and three experimental ones receiving viola extracts of 50, 100, and 200 mg/kg doses which were injected intraperitoneally one hour before the pentylenetetrazole administered. The factors under investigation included lack of animals' responding; duration of tonic, clonic and generalized convulsions; and mortality percentage. The results revealed that group receiving the viola extraction of 50 mg/kg dose were significantly different in tonic-clonic stage and total convulsion time whereas the mice with the 100 and 200 mg/kg dose injections showed significant differences from the control group in all stages. In general, the hydroalcoholic extract of the viola flower in 200 mg/kg dose can be recommended as an effective medication for preventing convulsion in an animal model.

Keywords: *Carbamazepine, Convulsion, Mice, Pentylenetetrazol (PTZ), Viola*

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INTRODUCTION:

Convulsion is the final event of impaired brain function which is due to abnormal electrical discharge of brain neurons. It starts from one region and spreads to other parts. Depending on the amount and manner of diffusion, different clinical manifestations may occur. Most of the convulsions last about 30 seconds up to two minutes. This disease is regarded as a chronic, progressive, unpredictable disorder which has been one of the general neurological issues in the world throughout the history.

Convulsion affects the ability and performance of individuals and is the third common neural disorder after brain attack and Alzheimer. The disorder is more common in children which leads to academic failure and daily unexplainable dreams [1]. Factors such as oxygen deficit, blood glucose deficit, blood calcium deficit, blood alkalosis, aggregation of liquids in the body, fluid retention in the body, lack of sleep, and certain drugs, which all intensify neural stimulation are among the causes of convulsion [2]. Currently, various types of chemical drugs are used to treat seizure and its following convulsions, which have many side effects.

Although various drugs have been manufactured, 30% of patients are resistant to all pharmacotherapy methods and their attacks cannot be controlled. The other patients are concerned with serious side effects of anti-seizure drugs; consequently, this reduces their cooperation and interferes the treatment [3].

Anti-convulsion drugs have a wide range of side effects such as liver damage, blood disorders, swollen lymph nodes, allergic reactions, memory weakness, headache, fever, fatigue, dizziness and drowsiness [4]. Carbamazepine is one of the most frequently used anti-seizure drugs. In theory, following the induction of hepatic enzymes, the administration of this drug can lead to metabolic changes such as increased body fats and the risk of premature atherosclerosis. Remarkably, developed epileptic spasms and hypsarrhythmia in electroencephalogram can be observed just after the administration of carbamazepine [5].

The drug's anticonvulsant activity may result from the use-dependent blockade of voltage-sensitive sodium channels [6]. Previous studies have shown that atherosclerosis starts from fetus or childhood stages; eliminating or changing resonator factors can prevent this pathological process [7]. Finally, one knows that the clinical condition of encephalopathy due to valproate or carbamazepine is accompanied by seizure increase. From the animal experiments, it is evident that especially carbamazepine and phenytoin may provoke generalized seizures as absences or myoclonic seizures [8]. Traditionally, nature is considered to be a rich source of plants which have been used to treat a variety of diseases. Medicinal plants have been used for centuries to control or treat diseases and their desirable therapeutic effects as well as their fewer side effects have been verified. Nowadays, medicinal plants are in frequent use and are recognized by World Health Organization [9]. One of these plants is the viola (*Viola tricolor L.*). This plant is used in India as diaphoretic and antipyretic [10]. According to Iranian Traditional Medicine, all viola parts including leaves, flowers, seeds, roots, and branches have medicinal properties. The nature of viola is wet, spicy, bitter and cold and has properties such as anti-inflammation, phlegm causing, stimulants, diuretics, anti-tumor, anti-rheumatic, laxative, antimicrobial, Diaphoretic, laxatives, anesthetics, sedatives, hypnotics, anticonvulsants, capillaries stabilizers and blood purifier [11].

Some reports established the content of *Viola tricolor* in saponins (5.98%), mucilages (14.20%) and total carotenoids (18.46 mg/100 g). In fresh vegetal drug determined by HPLC 8 carotenoids: violaxanthin (352 microg/100 g), antheraxanthin (711 microg/100 g), lutein (1575 microg/100 g), zeaxanthin (1488 microg/100 g), alpha-cryptoxanthin (66 microg/100 g), beta-carotene 5,6-epoxide (133 microg/100 g), beta-carotene (1678 microg/100 g) and 9Z-beta-carotene (312 microg/100 g) [12].
Considering the importance of this neural disease as well as absence of any similar study, this research study was carried out to compare anticonvulsant effects of viola vs. carbamazepine as a synthetic drug on an animal model of seizure.

MATERIAL AND METHODS:

Control vs. Experimental Groups

Forty mice (Balb/C race) prepared by Pasteur Research Institute (Karaj, Iran) were taken and kept in a room within the temperature of 22+ $2^{\circ C}$, natural photoperiod, and with free access to food as well as water. To adapt to the environment, mice were kept for two weeks before the experiment. Each mouse was used only for one test. All experiments were conducted in the time span of 9 a. m. to 5 p. m.
As the sample of the study, 40 mice were randomly divided into five groups each with eight members all kept in separate cages. The mice in the control group received no drug injection before the induced seizure while those in the Carbamazepine group received a dose of 0.5 mg/kg of the drug before the induced

seizure. In contrast, the mice in the three experimental groups received respectively 50, 100, and 200 mg/kg doses of extract in peritoneum before the induced seizure.

Inducing Seizures

All groups including the control group received intraperitoneal injections with a seizure-causing substance (PTZ 10 mg/kg). Seizure profiles were assessed through 30-minute post-PTZ administration [13]. After PTZ injections, the mice were studied for one hour and convulsion responses were classified as follows:

- Zero Stage: absence of response including a latency time where the animal did not show any reactions
- Stage One: tonic convulsion, muscle contraction observed in the whole body
- Stage Two: clonic convulsion as well as jumping muscles. In this stage, periods of muscle relaxation and increased saliva were observed. Convulsion wave was observed as muscle jumps, body rotation and head rotation.
- Stage Three: generalized as well as tonic-clonic convulsion. Convolution wave was expanded to the whole body, observed as sudden contraction and shaking of animal's body.

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Statistical Analysis

To study whether any differences can be found in the mortality rates, the data collected were analyzed using the Chi-square test through the SPSS 22.

RESULTS AND DISCUSSION:

According to results presented in table 1, an average time of absence of response was the highest (1841.37 sec) mice with 200 mg/kg dosage whereas those in the control group had the shortest time (26.25 sec). The average tonic convulsion time for the mice with 200 dosage (7.12 sec) was less than those in the other groups while those in the control and PTZ group had the highest tonic convulsion time (19.25 sec).

The clonic convulsion time for the mice with 200 mg/kg dosage was lower than those in the other groups (14 sec) whereas the mice in the control group and those with 50mg/kg dosage had the highest amounts (98.26 sec and 98.25 sec, respectively). The lowest average tonic-clonic convulsion time (29.12 sec) belonged to the mice with 200 mg/kg dosage while those in the control group had the highest time (65.37 sec). The total convulsion time for the mice with 200 mg/kg dosage was the shortest time (50.25 sec) whereas the longest time belonged to the mice in the control group (122.87 sec).

Table1: Means and standard deviation of treatment groups

groups	Zero stage (sec)		First stage(sec)		Second stage(sec)		Third stage(sec)		Total time(sec)	
	mean	Standard deviation	mean	Standard deviation	mean	Standard deviation	mean	Standard deviation	mean	Standard deviation
Carbamazepine	66.25	7.17	15.75	1.28	61.5	7.03	53.62	3.5	130.87	10.21
Control	26.25	5.75	19.25	2.25	98.26	6.69	65.37	5.52	182.87	4.64
50 mg/kg	44.12	4.99	17.87	1.12	98.25	4.68	60.12	2.85	176.25	4.2
100 mg/kg	89.87	11.66	13.87	1.45	52.75	5.17	50.25	1.03	116.87	3.83
200 mg/kg	1841.37*	65.72	7.12	1.45	14	1.51	29.12	1.81	50.25	1.83

*: Significant difference from control group ($p<0.05$)

Table 2 shows that the mice receiving the extract in 50 $_{mg/kg}$ dose did not show any significant differences with those in the control and carbamazepine groups in terms of response absence time. Moreover, the tonic and clonic convulsions for this group were different only from those in the carbamazepine group ($p<0.05$). For tonic-clonic convulsions and total convulsion time, the mice in the experimental group receiving the 50 $_{mg/kg}$ dose showed significant differences compared to those in the control and carbamazepine groups ($p<0.05$).

Table 2: Pair comparison of 50$_{mg/kg}$ group with other groups

Variables	treatment	Means difference	Standard deviation error	Significance	Insurance	
					lower	upper
Zero stage	Carbamazepine	-22.125	15.11	0.152	-52.79	8.54
	control	17.87	15.11	0.245	-12.79	48.54
First stage	Carbamazepine	2.12	0.78	0.01	0.53	3.7
	control	-1.37	0.78	0.087	-2.96	0.212
Second stage	Carbamazepine	36.75	2.72	0.001	31.21	42.28
	control	0.001	2.72	0.998	-5.53	5.54
Third stage	Carbamazepine	6.5	1.66	0.001	3.12	9.87
	control	-5.25	1.66	0.003	-8.62	-1.87
Total time	Carbamazepine	45.37	2.84	0.001	39.6	51.15
	control	-6.62	2.84	0.026	-12.39	-0.85

Significant difference from control group *: P<0.05, **: p<0.01

As shown in Table 3, in terms of non-responsiveness, the mice receiving the violet extract at a dose of 100 mg/kg were found to be significantly ($p<0.05$) different from those in the control group but not from those in the Carbamazepine group.

Table3: Pair comparison of 100$_{mg/kg}$ group with other groups

Variables	treatment	Means difference	Standard deviation error	Significance	Insurance	
					lower	upper
Zero stage	Carbamazepine	23.63	15.11	0.127	-7.04	54.29
	control	63.63	15.11	0.001	23.95	94.29
First stage	Carbamazepine	-1.87	0.78	0.022	-3.46	-0.287
	control	-5.37	0.78	0.001	-6.96	-3.78
Second stage	Carbamazepine	-8.75	2.72	0.003	-14.28	-3.21
	control	-45.5	2.72	0.001	51.03	-39.96
Third stage	Carbamazepine	-3.37	1.66	0.052	-6.75	-0.001
	control	-15.12	1.66	0.001	-18.5	-11.74
Total time	Carbamazepine	-14	2.84	0.001	-19.77	-8.22
	control	-66.2	2.84	0.001	-71.77	-71.77

The mice in the experimental group with the of $200_{mg/kg}$ dosage were found to be significantly different from those in the carbamazepine and control groups ($p<0.01$) for all factors under study. In other words, the injection of 200 $_{mg/kg}$ dose of the viola extract was found to be more effective for them compared to the mice in the carbamazepine (Table4).

Table4: Pair comparison of $200_{mg/kg}$ group with other groups

Variables	treatment	Means difference	Standard deviation error	Significance	Insurance	
					lower	upper
Zero stage	Carbamazepine	1775.125	15.11	0.001	1744.45	1805.79
	control	1815.125	15.11	0.001	1784.45	1845.79
First stage	Carbamazepine	-8.62	0.78	0.001	-10.21	-7.03
	control	-12.12	0.78	0.001	-13.71	-10.53
Second stage	Carbamazepine	-47.5	2.72	0.001	-53.03	-41.96
	control	-84.25	2.72	0.001	-89.78	-78.71
Third stage	Carbamazepine	-24.5	1.66	0.001	-27.87	-21.12
	control	-36.25	1.66	0.001	-39.62	-32.87
Total time	Carbamazepine	-80.62	2.84	0.001	-86.39	-74.85
	control	-132.62	2.84	0.001	-138.39	-126.85

Regarding the side effects of the synthetic drugs as well as lack of appropriate response to the existing treatments, currently herbal drugs are widely used to control convulsion.

The relative effectiveness of standard drugs to increase the PTZ seizure threshold for tonic extensor was found to be: triazolam > clonazepam > diazepam > rofecoxib > chlordiazepoxide > phenobarbital > carbamazepine > pentobarbital > pregabalin > phenytoin > progesterone > tiagabine > GABA > adenosine > gabapentin > ashwagandha > ethanol. The results of a lot of studies indicate that the intravenous PTZ seizure threshold may be useful for assessing the anticonvulsant effect of drugs which are effective in different stages of convulsions [14].
While in modern pharmacology, developing and introducing of new drugs are mainly dependent on our awareness about the pathophysiology of the disease, the exact pathophysiological origin of epilepsy is still unknown [15].

However, the excitatory glutamatergic system seems to play a key role in generating and spreading epileptic discharge [16]. Indeed, recent research studies have focused on the development of certain drugs that mainly counteract against the activity of this system.

A number of cognitive and psychomotor effects have been linked to carbamazepine [17]. A randomized, double-blind, placebo-controlled study involving 150 epilepsy patients on AED monotherapy (mainly carbamazepine or valproate) found that carbamazepine discontinuation significantly improved performance in tests that required complex cognitive processing under time pressure, but not in simpler tasks of attention and reaction time [18].

Viola is known in traditional medicine as a sedative, analgesic, laxative, and anticonvulsant medicinal plant with cold and wet nature. According to the traditional physicians, viola relieves thirst, blood acuity, fevers, and suffocation. Viola is claimed to be beneficial for catarrh, coughs, colds, upset stomach, liver and spleen as well as kidney pains [19].

An important point in using herbal drugs is determining the appropriate dosage of the drug. Accordingly, in the present study, the effects of three different dosages of viola extracts (50, 100 and 200 mg/kg) were investigated in treating convulsions caused by pentylenetetrazole injection. The results showed that the hydroalcoholic extract has therapeutic effects on the onset and duration of convulsion. The extract postponed convulsion onset and controlled all its variables. All in all, it can be concluded that the anti-convulsion effects of this

plant are verified. Main compounds of viola are saponins, rutin, flavonoids, coumarins, phenylpropanoid, and terpenoids. Flavonoids and coumarins react with $GABA_A$ receptor places, benzodiazepines and various ion canals which are targets of anticonvulsant synthetic drugs.

Most of herbal compounds have medicinal properties including acting as anti-inflammatory treatment, protecting the nervous system and strengthening the learning which are beneficial to seizure treatment [20].

Medicinal properties of this plant are related to macrocyclic peptides containing about 30 amino acids. Cytotoxic compounds have been identified in this plant which has anti-cancer properties. Moreover, as it has flavonoids, glycosides, alkaloids, steroids, saponins, and tannins, potentially viola can be used in developing new drugs of respiratory infectious diseases [11]. Existing flavonoids in viola plus its anti-inflammatory and pain-relieving effects may cause diuretic and diaphoretic effects [21].

One of the common convulsion-triggering substances in a laboratory is pentylenetetrazole. This substance is an antagonist of GABA receptors preventing neural control by governing GABA; consequently, the neural activity is increased and convulsion occurs [22]. In fact, PTZ causes convulsion by controlling messaging from this receptor [23]. Anti-convulsion drugs such as carbamazepine with agonistic effects on this receptor improve its messaging and suppress convulsion. In the current study, the viola extract controlled the PTZ convulsion effectively.

In practice, benzodiazepines bind to the gamma subunit of the GABAA receptor, for which a structural modification of the receptor results in an increase in GABAA receptor activity. Benzodiazepines do not substitute for GABA, which bind at the alpha subunit, but they increase the frequency of channel opening events, which leads to an increase in chloride ion conductance and inhibition of the action potential [24].

Flavonoids existing in viola bind to benzodiazepine receptors and probably increase chlorine entrance via chlorine canals which can be a probable anti-convulsion mechanism of viola against the PTZ convulsions (21). More studies are required to identify this mechanism.

CONCLUSION:

In view of the results of the present study, the hydroalcoholic extract of the viola in 200mg/kg dosage controlled the convulsion stages and the total convulsion time much better than the treatments administered to the other groups. The mortality rate for the mice in this group was also significantly different from that of other groups confirming the protective effects of the viola plant; accordingly, the 200mg/kg dose is recommended as the best one.

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CONFLICT OF INTEREST

The authors contributing to the present study and to this very manuscript have no conflict of interests to declare.

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