

Effect of a New Type of Drug Supplement on Epileptic Seizure Induced by Pilocarpine and the Amount of Nitric Oxide Produced by Vascular Epithelial Cells as a Trigger for Migraine Headache

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Drug Supplement; Biomaterial; Nitric oxide; Migraine; Epileptic; Seizure

1. Abstract

The present study aims to investigate the effect of a new type of dietary supplement on epileptic seizure induced by pilocarpine and the amount of nitric oxide produced by vascular epithelial cells as a trigger for migraine headache. New healthy and live drug supplement were synthesized by a green route. This organic biomaterial was named NBS. Concentrations of the drug supplement were prepared by dissolving dry powder in saline phosphate-buffered. In recent years, NO has been suggested as one of the factors involved in migraine headache. Nitrite concentration was also measured as an indicator of nitric oxide production by Griess method. In order to induce seizure, on 25th day after birth, each puppy was injected into control and treatment groups with healthy and live diet in 150 mg/kg subcutaneously. The behavior of each rat was recorded by a digital camera and two observers. It can be stated that this supplement causes a significant reduction in the level of 5% concentration of nitric oxide compared to the control group ($p \leq 0.05$). Also, 15 mg/kg of the Dapsone increases the duration of seizure from medication to onset of seizure, while, using the new NBS healthy and live diet significantly increases this time compared to the Dapsone recipient group. The new healthy and nutritious drug can significantly reduce the incidence of migraine headaches and would prevent the onset of a seizure.

2. Introduction

Headaches are common in childhood and adolescence [1]. The most common primary headache disorders, migraine and tension

headache are those that affect 80% of people worldwide [2]. Migraine is a severe disorder characterized by mild to severe headaches and is often associated with symptoms in the autonomic nervous system. Symptoms associated with it can include nausea, vomiting, light-headedness (increased sensitivity to light), noise (increase sensitivity to sound), and pain generally increase with increased physical activity. Migraine is a cerebrovascular disorder [3] that is debilitating, progressive and chronic, and has important implications for people's lives [4]. In migraines, the activity of a deep mechanism in the brain causes the release of inflammatory substances producing pain around the nerves and blood vessels of the head [5]. The main symptom of migraine attacks is a headache that may take a few hours or even 2 to 3 days [6] and is often severe, pulsating, and more unilateral [6]. Other symptoms include nausea, occasional vomiting, intolerance to light and tiredness [5], neck pain and muscle tension [7]. Migraines are almost twice as likely to increase the risk of ischemic attacks compared with non-migraine subjects [8]. Adult migraines are also associated with seasonal allergies, asthma, epilepsy, persistent nightmares, atopic disorders, stroke, cardiovascular disorders, sleep problems, travel sickness, nosebleeds and women of reproductive age with uterine bleeding and preeclampsia [1]. The most common causes are starvation or lack of adequate nutrition, which is especially important in young people [6]. Migraine is the 19th cause of disability in the world [9] that affects 10-20% of the population throughout life [10]. Recent data show that for every 4 adults in the United States, people suffer from frequent or severe headaches,

including migraine [1]. Women are more likely to have migraines than men, about 3 times more likely [6]. The prevalence of migraine in Turkey is 16.4% (8.5% for men and 24.6% for women) [11], in European adults 14.7% [2], but less for children [12]. In England, among women, 18.3% and among men, 6.6% [13]. In Germany, 13.4% [2], Africa (7-3%) [5] and Asia (3% in men and 10% in women) [5]. Migraine is one of the most common types of headaches in Iran [14].

In recent years, NO has been suggested as one of the factors involved in migraine headache [15-16]. The mechanism or molecular mechanism of migraine and molecule headaches involved in the formation of them are not known accurately. In this research, the NBS new healthy and live drug supplement were synthesized by a green route. The results showed that dietary supplement (healthy and alive) at 1000 concentrations reduced 10 units of nitric oxide concentration compared to the control group. Therefore, with this result it can be stated that this supplement causes a significant reduction in the level of 5% concentration of nitric oxide compared to the control group ($p \leq 0.05$). In studying the interactions of the studied concentrations with drug supplement (healthy and live), it can be stated that the maximum reduction effect is related to 1000 and the lowest is the concentration of 125 nutrients (healthy and alive).

On the other hand, epilepsy seizure refers to a series of chronic medical or long-term neurological disorders that are characterized by epileptic seizures. The attacks may be very mild and almost unidentifiable or vice versa, with prolonged and severe shaking. Epileptic seizures occur repeatedly, and there is no fixed cause, and no proper treatment for this disease has ever been seen. Accordingly, the present study aims to investigate the effect of healthy and healthy dietary supplement on the animal model of seizure induced by pilocarpine. Epilepsy as a major neurological disorder has a high incidence rate of 5 cases per 1,000 people. Epilepsy is an intermittent and reversible disorder in the activity of the neurons, which can be seen in the form of seizure-like behaviors and in the form of spikes and a spinal wave. The term epilepsy includes a number of different syndromes, the basic aspect of which all of them tend to be recurrent seizures without triggering [17-19] and can lead to multiple complications, including learning disabilities [20-21]. Past reports indicate that mental and emotional anomalies such as stress and events derived from it such as fear, anxiety, and impatience can affect seizures. Studies suggest that stress reducing or increasing adaptive methods can be useful in seizure control in patients with epilepsy [22-23]. On the other hand, there are conflicting reports that there is an effect of stress management on epilepsy. In this regard, empirical stresses such as swimming stress in animals have been shown to have anti-epileptic effects [24-25]. Other studies on live cerebrospinal fluid in rural and Syrian rats have shown that stress has weakened seizure activity. It has been mentioned that stress during pregnancy has reduced the number of

ectal attacks and reduced the duration of each activity on the hippocampus and entorhinal cortex [26]. Although the nature, cause, and mechanism of these opposite effects are not clearly known, and the mechanism involved in the relationship between stress and epilepsy is also in a state of ambiguity [27]. Based on the mentioned issues, necessity of the disease studying and the lack of proper treatment of this disease, this research was conducted with the aim of investigating the effect of healthy and live drug supplement on seizure induced by epilepsy in small experimental mice.

3. Materials and Methods

3.1. Migraine Headache

3.1.1. Extraction Methods

Concentration (125, 250, 500 and 1000 $\mu\text{g/ml}$) of healthy and fresh drug supplement were prepared by dissolving dry supplement in saline phosphate-buffered PBS.

3.1.2. Perform an Anti-Migraine Test

In this test, a measure of nitric oxide was used to evaluate the therapeutic effect of live and healthy drug supplement. For this purpose, mouse endothelioma F-2 cell line (Cellular Bank of Japan) was used. Availability, high reproducibility, and adequate knowledge of their growth and reproduction conditions were among the factors influencing the selection of this cell line. These cells were placed in 25 cm² flasks in DMEM (modified eagle Dulbecco medium) (sigma) with 10% fetal calf serum content (Roche) and 1-2% penicillin-streptomycin (sigma) CO₂ was multiplied by 5%. The cells were isolated in subconfluent using thripsin (sigma) at a concentration of 25% from the floor. The cells isolated from the flask floor were immersed in the medium. Then, centrifugation, cell deposition was immersed in the new culture. The cells were then transferred to the new flasks or into the container for testing.

3.1.3. Determine Cell Poisoning

To determine the survival of the cells, the test was carried out using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) MTT, which is based on the MTT-related mitochondrial-respiratory resuscitation. By performing this test, the maximum concentration that did not cause cell poisoning was determined (1000 $\mu\text{g/ml}$), several concentrations (500 250 125 $\mu\text{g/ml}$) of the less than maximum concentration were tested.

The concentration of 50 $\mu\text{g/ml}$ was the lowest concentration that affected NO, although its effect was not significant.

3.1.4. Measurement of Nitrite

Nitrite concentrations were measured as an indicator of nitric oxide production by Griess method. In this method, the sulfuryl amide and N-(1-naphthyl) ethylenediamine are added to the sample to measure the concentration of nitrate. Sodium nitrate was also used as standard. U1 100 supernatant medium was mixed with 100 μl grease containing 2% sulfonyl amide in 5% chloride oxide and 0.1% N-(1-naphthyl) ethylenediamine. The optical absorption was

then read by ELISA at 540 nm. The higher the concentration of nitrate in the sample, the greater the absorption in this wave, thus, it can be compared with the control method by reducing the grease or increasing the amount of nitric oxide in the samples.

3.1.5. Epileptic Seizure

In order to induce seizure, on 25th day after birth, each puppy was injected into control and treatment groups with healthy and live drug supplement in 150 mg/ kg subcutaneously. Pilocarpine was purchased from Sina Drug Company. Following injection, the behavior of each rat was recorded by a digital camera and two observers, and the degree of epileptic seizures in this study was categorized as follows.

Step 1: Immobility

Step 2: Lower the front or tail

Step 3: Repeated movements, head bobbing

Step 4: Stay on two feet and fall

Step 5: Getting up and falling steadily

Step 6: Tonic attacks and severe clonic

Other parameters, such as the duration of the first change in behavior and the duration of maximum seizure, were evaluated. Animals were also observed for 24 hours for lethal side effects of pilocarpine.

Signs of seizure (the six steps mentioned above), the latent time to start epileptic seizures, the time needed for maximum response and death and mortality of the rats up to 24 hours after pilocarpine injection in the control and stress groups were compared by Mann-Whitney test. Non parametric were compared. All results were presented as \pm Mean error and $p < 0.05$ was considered as meaningful.

Then the of treatment results of this disease were compared between groups receiving Dapsone at concentrations of 10 and 15 mg / kg and groups receiving healthy and live drug supplement at concentrations of 12.5, 25 and 50 mg / kg.

3.1.6. Statistical Analysis

In order to analysis the data in this study, we first make sure that the distribution of data is normal, using Kolmogorov-Smirnov test. Also, in this study, in order to evaluate the significance of the data, it is recommended to use ANOVA test. ANOVA test was used to investigate the differences between and within groups. $P < 0.05$ indicates statistical significance. In order to clarify this issue, by Scheffe post hoc test, this significance was tested one by one between groups.

4. Results

4.1. Migraine Headache

Table 1 (a-d), shows the results of Nitric Oxide concentrations of samples treated with a concentration of 125, 250, 500 and 1000 healthy and alive nutrients. The maximum reduction effect is relat-

ed to 1000 nutrients (healthy and alive). Compaction of the effects of various concentrations of healthy and live drug supplement on reducing the amount of nitric oxide are shown as Fig. 1.

In order to analysis the data in this study, we first make sure that the distribution of data is normal, using Kolmogorov-Smirnov test. In this regard, by using the information test obtained by observing Sig, it can be stated that these data have a normal distribution. Therefore, parametric tests should be used to analysis the differences between groups. In this study, in order to evaluate the significance of the data, it is recommended to use ANOVA test. ANOVA test was used to investigate the differences between and within groups. The results of this test showed that there was a significant difference between the groups with 5% probability level. In order to clarify this issue, by Scheffe post hoc test, this significance was tested one by one between groups (S2, not shown). NBS dietary causes a significant difference in nitric oxide levels.

4.2. Epileptic Seizure

Based on the comparison results of the Dapsone recipient groups, the difference in seizure frequency between the Dapsone receptor groups at concentrations of 10 and 15 mg / kg was significant. Also, there is a significant difference between the treatment groups and the control group in the 5% probability level. Overall, it can be stated that the Dapsone drug at concentrations of 10 mg / kg, has no effect on the duration of seizure, while the dose of 15 mg / kg significantly increases the duration of seizure from medication to onset of seizure. The results can be seen in Table 2-3.

The results of Kolomogorov-Smirnov test showed that in the three groups, control, Dapsone recipient with concentration of 10 mg/ kg and Dopson recipient with concentration of 15 mg/ kg the data are normal. Therefore, it is possible to analyze these three groups by parametric tests.

Then, the ANOVA and Scheffe's post hoc analysis was used to evaluate the significance of the differences between the three groups that based on the results, there is a significant difference between the data of Dapsone 15, Dapsone 10 and control groups, while the difference between the control and Dapsone 10 groups is considered meaningless (S3, not shown). Meanwhile, comparison of the timing of the acquisition of the drug and the seizure in the control and instance groups is shown as Fig. 2.

On the other hand, based on the results of the study and comparison of the healthy and live drug supplement recipients' groups (Table 4-5), the difference in time between the groups receiving healthy and live drug supplement with concentrations of 12.5, 25 and 50 mg / kg was meaningful. Generally, it was observed that with increasing dosage of the healthy and live drug supplement, the time to induce seizure significantly increased, and it was observed that 12.5 concentration had the least and 50 concentrations had the highest effect. Comparing the results with the control and control groups, it was observed that live and healthy drug supple-

ment had a better effect than the Dapsone drug.

The results of Kolomogorov-Smirnov test showed that in the three concentrations of the NBS compound (12.5, 25 and 50 mg/ kg), the data distribution is normal. Therefore, it is possible to analyze these three groups by parametric tests.

After making sure that the data were normal, using ANOVA and

Scheffe post hoc analysis, the significance of the differences between the three groups was evaluated. The data of the three NBS groups showed significant differences at concentrations of 12.5, 25 and 50 mg/ kg (S4, not shown). Meanwhile, the comparison chart obtained from the timing of the intake of healthy and live drug supplement and the observation of seizures in rats receiving doses of 12.5, 25 and 50 is shown as Fig.3.

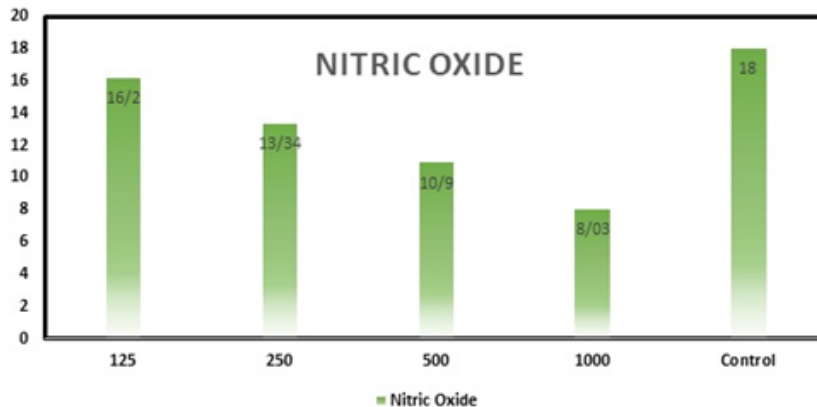


Figure 1: Compaction of the effects of various concentrations of healthy and live drug supplement on reducing the amount of nitric oxide.

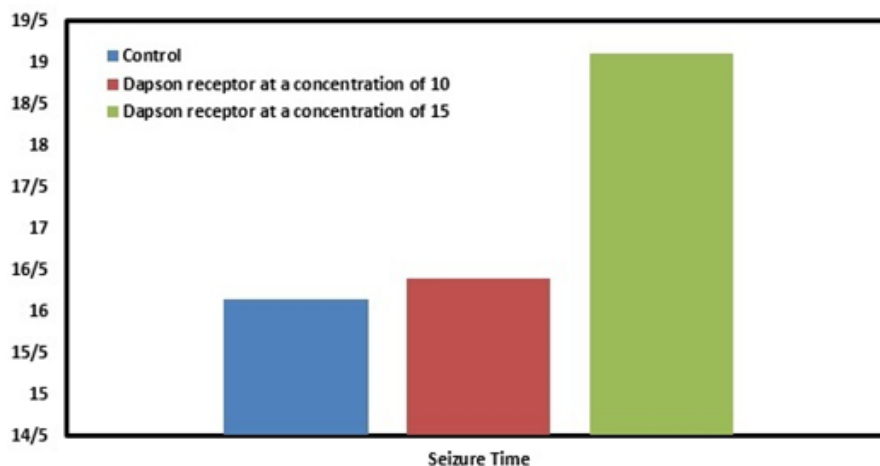


Figure 2: Comparison of the timing of the acquisition of the drug and the seizure in the control and instance groups.

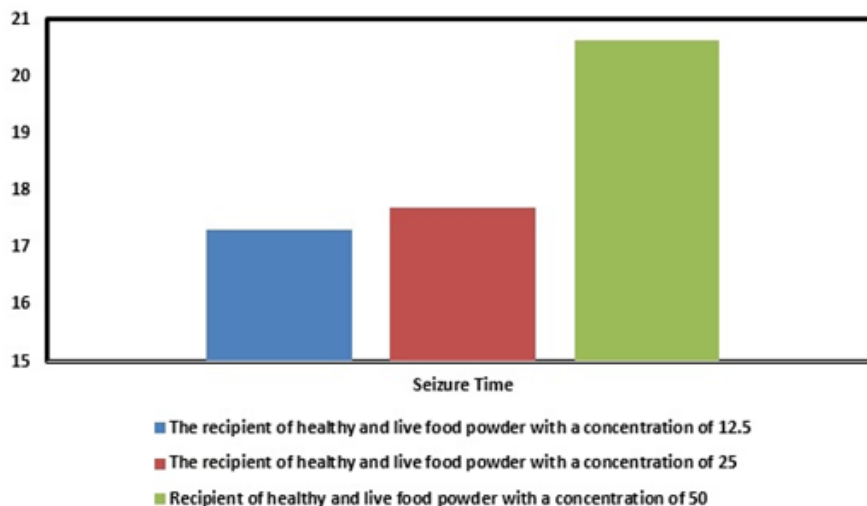


Figure 3: Comparison chart of the timing of the intake of healthy and live drug supplement and observation of seizure in mice in groups receiving doses of 12.5, 25 and 50.

Repeat	Treatment Group	Control Group
1	16.2 µM	18
2	16.4 µM	a
3	16 µM	
Average	16.2	
Standard deviation	0.026	
NO concentration	16.2 ± 0.026	

Repeat	Treatment Group	Control Group
1	13.8 µM	18
2	12.9 µM	b
3	13.3 µM	
Average	13.34	
Standard deviation	0.14	
NO concentration	13.34 ± 0.14	

Repeat	Treatment Group	Control Group
1	10.8 µM	18
2	11.3 µM	c
3	10.6 µM	
Average	10.9	
Standard deviation	0.086	
NO concentration	10.9 ± 0.086	

Repeat	Treatment Group	Control Group
1	7.8 µM	18
2	8.3 µM	d
3	8 µM	
Average	8.03	
Standard deviation	0.122	
NO concentration	8.03 ± 0.122	

a		NBS-125	NBS-250	NBS-500	NBS-1000
N		3	3	3	3
Normal Parametersa	Mean	16.2	13.3333	10.9	8.0333
	Std. Deviation	0.2	0.45092	0.36056	0.25166
Most Extreme Differences	Absolute	0.175	0.196	0.276	0.219
	Positive	0.175	0.196	0.276	0.219
	Negative	-0.175	-0.183	-0.203	-0.189
Kolmogorov-Smirnov Z		0.303	0.34	0.478	0.38
Asymp. Sig. (2-tailed)		1	1	0.976	0.999
a. Test distribution is Normal.					

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	191.996	4	47.999	549.607	0
Within Groups	0.873	10	0.087		
Total	192.869	14			b

Multiple Comparisons

(I) VAR00009	(J) VAR00009	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Ctrl c	Nbs-125	1.80000*	.24129	.000	.9000	2.7000
	Nbs-250	4.66667*	.24129	.000	3.7667	5.5667
	Nbs-500	7.10000*	.24129	.000	6.2000	8.0000
	Nbs-1000	9.96667*	.24129	.000	9.0667	10.8667
Nbs-125	ctrl	-1.80000*	.24129	.000	-2.7000	-.9000
	Nbs-250	2.86667*	.24129	.000	1.9667	3.7667
	Nbs-500	5.30000*	.24129	.000	4.4000	6.2000
	Nbs-1000	8.16667*	.24129	.000	7.2667	9.0667
Nbs-250	ctrl	-4.66667*	.24129	.000	-5.5667	-3.7667
	Nbs-125	-2.86667*	.24129	.000	-3.7667	-1.9667
	Nbs-500	2.43333*	.24129	.000	1.5333	3.3333
	Nbs-1000	5.30000*	.24129	.000	4.4000	6.2000
Nbs-500	ctrl	-7.10000*	.24129	.000	-8.0000	-6.2000
	Nbs-125	-5.30000*	.24129	.000	-6.2000	-4.4000
	Nbs-250	-2.43333*	.24129	.000	-3.3333	-1.5333
	Nbs-1000	2.86667*	.24129	.000	1.9667	3.7667
Nbs-1000	ctrl	-9.96667*	.24129	.000	-10.8667	-9.0667
	Nbs-125	-8.16667*	.24129	.000	-9.0667	-7.2667
	Nbs-250	-5.30000*	.24129	.000	-6.2000	-4.4000
	Nbs-500	-2.86667*	.24129	.000	-3.7667	-1.9667

*. The mean difference is significant at the 0.05 level.

S₂. (a) The Kolmogorov-Smirnov test to determine the data distribution normality, (b) results of ANOVA test between groups and data, and (c) the Scheffe post hoc test results to determine the differences between groups.

Table 1: The results of Nitric Oxide concentrations of samples treated with a concentration of (a) 125, (b) 250, (c) 500 and (d) 1000 healthy and alive nutrients.

Time Seizure View	Mouse weight studied	Number of mice
May-16	gr29	1
Mar-16	gr31	2
Apr-15	gr30	3
Jun-16	gr30	4
Sep-15	gr28	5
14/16	Average	a
.Time Seizure View	Mouse weight studied	Number of mice
Feb-16	gr31	1
Aug-15	gr30	2
16	gr30	3
Sep-15	gr29	4
Mar-16	gr31	5
Apr-16	Average	b
Time Seizure View	Mouse weight studied	Number of mice
Aug-17	gr30	1
Mar-19	gr29	2
Sep-18	gr28	3
Jan-20	gr30	4
Apr-19	gr31	5
Oct-19	Average	c

Table 2: Results from when the mice began to seizure and immediately after seeing the first symptom of seizure (a) in the control group mice, (b) in the dapstone receiving mice 10 mg/ kg, and (c) in the dapstone receiving mice 15 mg/ kg.

Time View the desired mode	View the state of mind	Considered mode a
Apr-16		motionless
Jul-15		Extending front or tail hands
Mar-15		Repeated movements, head bobbing
Apr-14		Fall on two feet and fall
07-Dec		Get up and fall continuously
Sep-15		Tonic attacks and severe clonic
Time View the desired mode	View the state of mind	Considered mode b
May-16		motionless
Apr-15		Extending front or tail hands
Apr-15		Repeated movements, head bobbing
Jun-14		Fall on two feet and fall
08-Dec		Get up and fall continuously
Jun-15		Tonic attacks and severe clonic
Time View the desired mode	View the state of mind	Considered mode c
Apr-16		motionless
Jul-15		Extending front or tail hands
Mar-15		Repeated movements, head bobbing
Apr-14		Fall on two feet and fall
07-Dec		Get up and fall continuously
Sep-15		Tonic attacks and severe clonic

a		ctrl	dap10	dap15
N		5	5	5
Normal Parametersa	Mean	16.34	16	19.1
	Std. Deviation	0.27019	0.27386	0.84558
Most Extreme Differences	Absolute	0.241	0.167	0.207
	Positive	0.168	0.137	0.161
	Negative	-0.241	-0.167	-0.207
Kolmogorov-Smirnov Z		0.539	0.374	0.462
Asymp. Sig. (2-tailed)		0.933	0.999	0.983

a. Test distribution is Normal.

ANOVA

VAR00012					
b	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	28.905	2	14.453	50.241	0
Within Groups	3.452	12	0.288		
Total	32.357	14			

Multiple Comparisons

(I) VAR00011	(J) VAR00011	Mean Difference (I-J)	Std. Error	c	95% Confidence Interval	
				Sig.	Lower Bound	Upper Bound
ctrl	dap 10	0.34	0.33921	0.617	-0.6056	1.2856
	dap 15	-2.76000*	0.33921	0	-3.7056	-1.8144
dap 10	ctrl	-0.34	0.33921	0.617	-1.2856	0.6056
	dap 15	-3.10000*	0.33921	0	-4.0456	-2.1544
dap 15	ctrl	2.76000*	0.33921	0	1.8144	3.7056
	dap 10	3.10000*	0.33921	0	2.1544	4.0456
* . The mean difference is significant at the 0.05 level.						

S₃. (a) The K-S test normal distribution of data in in the three groups of the control and Dapsone at concentrations of 10 and 15, (b) The ANOVA test results of the samples in three groups of the control and Dapsone at concentrations of 10 and 15, and (c) the Scheffe post hoc test results to determine the differences between groups.

Table 3: Average observation time of the different states of the mice body during epileptic seizures and their timing record (a) in the control group, (b) in the Dapsone receiving group 10 mg/ kg, and (c) in the Dapsone receiving group 15 mg / kg.

Time Seizure View	Mouse weight studied	Number of mice
17-Feb	gr29	1
16-Aug	gr31	2
16-Apr	gr30	3
16-Sep	gr30	4
17-Apr	gr28	5
17-Mar	Average	a
.Time Seizure View	Mouse weight studied	Number of mice
17-Jun	27 gr	1
17-Aug	32 gr	2
17-Apr	34 gr	3
17-Jun	30 gr	4
18-Jan	28 gr	5
17-Jul	Average	b
Time Seizure View	Mouse weight studied	Number of mice
20-Apr	gr30	1
21-Mar	gr29	2
20-Jun	32 gr	3
21-Feb	31 gr	4
19-Jun	34 gr	5
20/62	Average	c

Table 4: Results from when the mice began to seizure and immediately after seeing the first symptom of seizure in mice receiving the concentrations of (a) 12.5 (b) 25, and (c) 50 healthy and alive drug packs.

Time View the desired mode	View the state of mind	Considered mode a
17-Feb		motionless
16-Apr		Extending front or tail hands
16-Jan		Repeated movements, head bobbing
15-Jun		Fall on two feet and fall
14-Mar		Get up and fall continuously
16-Jun		Tonic attacks and severe clonic
Time View the desired mode	View the state of mind	Considered mode b
17-Jul		motionless
16-Aug		Extending front or tail hands
16-Jun		Repeated movements, head bobbing
16-Apr		Fall on two feet and fall
15-Sep		Get up and fall continuously
17-Jan		Tonic attacks and severe clonic
Time View the desired mode	View the state of mind	Considered mode c
20-May		motionless
19-Apr		Extending front or tail hands
19-Aug		Repeated movements, head bobbing
19-Jan		Fall on two feet and fall
18-May		Get up and fall continuously
19-Jul		Tonic attacks and severe clonic

a		NBS12.5	NBS25	NBS50
N		5	5	5
Normal Parametersa	Mean	17.02	17.7	20.82
	Std. Deviation	0.26833	0.26458	0.40249
Most Extreme Differences	Absolute	0.273	0.247	0.308
	Positive	0.273	0.247	0.308
	Negative	-0.206	-0.153	-0.227
Kolmogorov-Smirnov Z		0.61	0.553	0.688
Asymp. Sig. (2-tailed)		0.851	0.92	0.731

a. Test distribution is Normal.

ANOVA

VAR00019					
b	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	41.061	2	20.531	202.605	0
Within Groups	1.216	12	0.101		
Total	42.277	14			

Multiple Comparisons

VAR00019 Scheffe

(I) VAR00018	(J) VAR00018	Mean Difference (I-J)	Std. Error	c	95% Confidence Interval	
				Sig.	Lower Bound	Upper Bound
nbs 12.5	nbs 25	-.68000*	0.20133	0.018	-1.2412	-0.1188
	nbs 50	-3.80000*	0.20133	0	-4.3612	-3.2388
nbs 25	nbs 12.5	.68000*	0.20133	0.018	0.1188	1.2412
	nbs 50	-3.12000*	0.20133	0	-3.6812	-2.5588
nbs 50	nbs 12.5	3.80000*	0.20133	0	3.2388	4.3612
	nbs 25	3.12000*	0.20133	0	2.5588	3.6812

*. The mean difference is significant at the 0.05 level.

S_p. (a) The K-S test normal distribution of data in the three NBS concentrations of 12.5, 25 and 50, (b) The ANOVA test results of the samples in three groups of NBS with concentrations of 12.5, 25 and 50, and (c) the Scheffe post hoc test results to determine the differences between groups.

Table 5: Average time of observation of different states of the body of mice during epileptic seizures and their timing record in the group receiving the concentrations of (a) 12.5 (b) 25, and (c) 50 healthy and alive drug packs.

5. Discussion

The NBS healthy and live drug supplement has various vitamins, macro and micro molecules, and ingredients such as vitamin D, magnesium, and etc. A number of studies have shown a link between low levels of serum vitamin D with a higher incidence of chronic pain and headache [28-30]. Evidence has shown that vitamin D can be effective in headache, including migraine [16, 31-32]. Vitamin D also has an anti-inflammatory effect by regulating TNF and Tumor Necrosis Factor (TNF) and macrophage activity. Therefore, vitamin D supplement may have a beneficial effect on pain due to inflammation. Treatment with vitamin D reduced pain among neuropathy patients [30]. The factors that cause migraine are high and variable, including: alcohol (red wine) [33], drugs like chocolate, some types of cheeses, monosodium glutamate,

tyramine [33], starvation [33], delaying meals or not enough eating meals [34], dehydration [6], sleep (sleepiness and sleep deprivation) [34], psychological factors, emotional disturbances, rest after a stressful period [34], menstruation, hormonal replacement therapy or contraceptive therapy in women [34], organic aromatic compounds [33] extreme sports, long trips [34], staring at one spot, flashing lights [34], strong smells and remarkable climatic changes [34]. A higher latitude is one of the triggers for migraine [34]. The incidence of headache with lower latitude is lower because the level of serum vitamin D is higher in people who live in lower latitude [35]. Another possible mechanism of headache in patients with vitamin D deficiency is a lack of serum levels of magnesium in the blood [16]. Abnormal magnesium metabolism is involved in the pathogenesis of tension headache [16]. Magnesium defi-

ciency has been observed in the brain, blood, erythrocyte, monocyte and platelets in patients with a headache such as tension (and other type of headaches). Nearly 40-50% of patients with tension headaches have low levels of magnesium [16]. In various studies, approximately the same percentage of patients with tension-type headaches responded to magnesium therapy [16]. The absorption of drug magnesium through the intestine depends on vitamin D (1 and 25 dihydroxyvitamin D). Therefore, vitamin D deficiency is likely to cause tension-type headaches by limiting magnesium absorption [16]. Another mechanism, the presence of vitamin D receptors, alpha is a hydroxylase (the responsible enzyme for the formation of the active form of vitamin D) and the protein bound to vitamin D in the brain, in particular the hypothalamus [35].

The profile of epileptic seizure induced by pilocarpine on adult mice is described in details by Wang Lian et al., [36]. In this study, the time taken to initiate early epileptic seizures, such as repetitive movements of the head, on two legs, and falling, has been reported 20 minutes after injection, which is consistent with the estimated time in our study. In this study, the duration of symptoms or clonic tonic attacks was estimated to be 35 minutes, which is again similar to the corresponding time in our study, with 41 minutes. The minor differences in the type, species, and age of the tested animals are justifiable in two studies. Samland et al. [37], in a similar study reported that injecting 200 kg / mg pilocarpine in 60-day-old mice showed moderate epileptic symptoms, including recurrent motions and head shakes without the occurrence of clonic tonic attacks and mortality. Although the dose of pilocarpine used in this study was slightly higher than the dose used in our study, differences in the type and age of the animal under test and in vitro conditions could justify these differences [37]. In different models of experimental epilepsy, different types of stress can affect the animal's response to antiepileptic drugs [38-39]. The reports confirm that exposure to various types of acute stressors, such as painful threshold stimuli, reduces the induction of seizures in lithium-pilocarpine treated rats [40].

6. Conclusions

In conclusion the results of this study showed that dietary supplement (healthy and alive) at 1000 concentrations reduced 10 units of nitric oxide concentration compared to the control group. Therefore, with this result it can be stated that this supplement caused a significant reduction at 5% level, the concentration of nitric oxide is more controlled than the control group ($p \leq 0.05$). In studying the interactions of the studied concentrations with drug supplement (healthy and alive), it can be stated that the maximum reduction effect is related to 1000 and the lowest is the concentration of 125 nutrients (healthy and alive).

Meanwhile, based on the results, it can be stated that epileptic seizure induction was successful. In the study of the therapeutic effect of standard drug, the dose of 15 mg/ kg increases the duration of seizure from medication to onset of seizure, while, using a healthy

and healthy diet significantly increases this time compared to the Dapsone recipient group. Therefore, it can be stated that using a healthy and healthy dietary supplement will prevent the onset of seizures.

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8. Conflicts of Interest

The authors declare no conflict of interest.

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