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Effect of acute and chronic ellagic acid administration on penicillin induced epileptiform activity in rats

Ersin Beyazcicek[1], Seyit Ankarali[2], Ozge Beyazcicek[1] & Handan Ankarali[3]

[1]Department of Physiology, Faculty of Medicine, Duzce University, Duzce, Turkey

[2]Department of Physiology, Faculty of Medicine, Istanbul Medeniyet University, Turkey

[3]Department of Biostatistics and Medical Informatics, Faculty of Medicine, Istanbul Medeniyet University, Turkey

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One of the flavonoids found in some fruits and plants such as pomegranates, nuts, and apples is ellagic acid (EA). This compound has antidepressant, anxiolytic, antioxidant, and anti-inflammatory effects. Aim of this study was to investigate the effects of EA on experimental penicillin-induced epilepsy model electrophysiologically in rats. In this study, 70 adult male Wistar rats were divided into acute and chronic main groups. Only EA, and 10, 50 and 100 mg/kg doses of EA were the subgroups of the study. Sham and control groups were used as common groups for acute and chronic main groups. Substances were given to the acute group 30 min before the epileptiform activity started but for 21 days to the chronic group. Rats were anaesthetized with urethane. Electrodes were placed on the left somatomotor area. Electrocorticography (ECoG) recording was started and then penicillin was injected into the rats to induce epileptiform activity. 120 min more ECoG recordings were taken after penicillin was injected. In sham and only EA groups there was no epileptiform activity. Both acute and chronic groups of EA significantly increased the latency time to onset of the first spike-wave (P<0.05) and decreased the frequency and amplitude except for some time periods (P<0.05). Consequently, the administration of EA has an antiepileptic effect in penicillin-induced epilepsy in rats. Therefore it may be a potential anti-epileptogenic drug in the future.

Keywords: Antiepileptic, Electrocorticography, Neuroprotective, Onset of first epileptiform activity

Epilepsy, which is characterized by recurrent spontaneous seizures, is the fourth most common neurological disorder after migraine, stroke and Alzheimer's. Epilepsy is not only a disease but also a symptomatic condition. However, no reason can be determined in approximately 65% of the patients for epilepsy[1].

Today, there are approximately 65 million people with active epilepsy who need treatment and have persistent seizures[2]. 30% of these patients are robust to all antiepileptic drugs found by now[3]. Moreover, the repercussions of existing drugs used in epilepsy treatment are fairly vast. For this reason, trying to ascertain more efficient antiepileptic drugs with low side effect profiles and inexpensive and to elucidate the mechanisms of epilepsy are yet to continue intensively nowadays.

Recently, researchers and clinicians have sought to find new drugs isolated from medicinal plants or herbs that can suppress epileptic seizures. The herbal products play an important role in developing new antiepileptic drugs. A lot of herbs are known to have anticonvulsant efficacies. Various phytochemical, pharmacological, and electrophysiological research are carried out on these anticonvulsant herbals, which are rising daily.

Ellagic acid (2,3,7,8-tetrahidroksi-kromeno; $C_{14}H_6O_8$) is a polyphenolic compound found in many plants such as pomegranate, nuts and apples. This compound has been reported to have antidepressant[4], anxiolytic[5], anticancer[6], antioxidant[7], anti-inflammatory[8], anti-malarial[9], antiallergic[10], antiasthmatic[11], hepato-protective, cardioprotective[12] and antiproliferative[13] effects. Although there are some studies about effect of ellagic acid on human health, there are very few studies on its effects on the nervous system[14,15]. Epileptic seizures occur due to an increase in excitatory neurotransmitters (glutamate) or decrease in inhibitory transmitters (gamma aminobutyric acid; GABA). For this reason, antiepileptic drugs either strengthen GABA in the brain or act by reducing the level of glutamate in the brain or by suppressing glutamate receptors.

The aim of this study is to investigate the effect of acute and chronic administration of ellagic acid at the

*Correspondence:
Phone: +90 5432640653 (Mob.)
E-mail: beyazcicek13@gmail.com

doses of 10, 50 and 100 mg/kg on epileptiform activity induced by experimental penicillin in rats.

Material and Methods

Animals

The animals used in the study were obtained from Abant Izzet Baysal University Experimental Animal Application and Research Center, Bolu, Turkey. In the laboratory, male Wistar rats (n=70) weighing 250±30 g, were used. They were kept at constant room temperature (23°C) under a 12/12h light/dark cycle, and rats were given *ad libitum* access to food and water. Ethical approval for the study was obtained from Abant Izzet Baysal University Animal Research Local Ethics Committee.

Groups, drug and dose

The rats included in the study were divided into two main groups as chronic and acute before the experiments started. Then each of these main groups was divided into subgroups as shown in Table 1. In order to use less number of rats and in light of the findings from our previous studies, sham and control groups were used as common groups for both main groups. 7 rats were used in each group. The substances which specified in Table 1 were applied to the chronic EA groups between 08.00-10.00 am for 21 days. On the day after chronic application (22nd day), ECoG recording was taken. In order to determine whether the EA used in the study had any epileptic activity, the highest dose of 100 mg/kg EA was also applied. In the study, EA (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) was diluted with saline (1/1, v/v) after dissolving in ethyl alcohol. EA was applied intraperitoneally (i.p) as the doses of 10, 50 ve 100 mg/kg. Urethane (Sigma-Aldrich Chemical Co., St. Louis, Missouri, USA) in 1.25 g/kg dose was used as an anaesthetic and i.p was administered. For inducing epilepsy model, Penicillin G (IE Ulagay Turkey Pharmaceuticals Inc., Istanbul, Turkey) was applied as dose of 500 IU/2 µL intracortical (i.c). All substances were prepared daily.

Table 1 — Acute and chronic ellagic acid groups

Group	Substance	Dose	Route	n
Sham	-	-	-	7
Control (penicillin)	Saline	1mL/kg	i.p	7
Acute only EA	EA	100 mg/kg	i.p	7
Acute 10 mg/kg EA (AG_10_EA)	EA	10 mg/kg	i.p	7
Acute 50 mg/kg EA (AG_50_EA)	EA	50 mg/kg	i.p	7
Acute 100 mg/kg EA (AG_100_EA)	EA	100 mg/kg	i.p	7
Chronic only EA	EA	100 mg/kg/day	i.p	7
Chronic 10 mg/kg EA (CG_10_EA)	EA	10 mg/kg/day	i.p	7
Chronic 50 mg/kg EA (CG_50_EA)	EA	50 mg/kg/day	i.p	7
Chronic 100 mg/kg EA (CG_100_EA)	EA	100 mg/kg/day	i.p	7

Surgical procedure

Urethane in the dose of 1.25 g/kg was used to anaesthetize animals in all groups. Then rats were fixed in the stereotaxic frame (Harvard Instruments, South Natick, MA, USA) after being placed in the prone position. After the head area was shaved, the scalp was cut with a scalpel from front to back along the midline. The bone portion over the left cerebral cortex was carefully slenderized and removed with a drill (FST Rechargeable Microdrill, KF Technology, Rome, Italy).

Stimulation of epileptiform activity

Penicillin induced epileptiform activity model was created by using previously described method[16,17]. Briefly, epileptic activity was created by intracortical administration of 500 IU/2 µL penicillin with a Hamilton micro injector (701N, Hamilton Co., Reno, NV, USA) to 2 mm laterally, 1 mm in front of the bregma line and 1.2 mm in the cortex depth.

Electrophysiological recordings

Two silver-silver chloride (Ag-AgCl) ball electrodes were placed in the somatomotor cortex area which opened lateral to the Bregma line on the left hemisphere. The reference electrode was fixed on the right ear of the rats. The recording coordinates were set as follows; the first electrode was placed 1 mm in front of the bregma line and 2 mm lateral to the sagittal suture, and the second electrode was placed 5 mm posterior to the bregma line and 2 mm lateral to the sagittal suture. After the electrodes were placed, ECoG records were obtained with the PowerLab/8SP data acquisition recording system (PowerLab/8SP, ADInstruments Pty Ltd, Castle Hill, NSW, Australia). Signals conducted from the electrodes were digitized at 1024 Hz and filtered at 0.1–50 Hz intervals.Five minutes of basal activity was recorded before injecting substances into the acute EA groups. After basal activity recording, EA was given to the EA groups, and saline was given to the control (penicillin) group. Then ECoG was recorded for another 30 min.

After 30 min of the ECoG recording, penicillin was i.c. injected, and recording was taken for another 120 min. 100 mg/kg of EA was injected only to the EA group after five minutes of basal activity recording. A total of 155 min of ECoG recording was obtained from each animal.

Substances were administered between 08.00-10.00 am for 21 days to the chronic groups in which EA was applied. The ECoG recordings were obtained on the 22nd day of experiment. In order to take these recordings, after removing the skull bone on the left somatomotor area, as described in the surgical procedure section, two recording electrodes were placed in the left somatomotor area. Five minutes of basal activity was recorded after the electrodes were placed. After basal activity recording, intracortical penicillin was injected and recording was taken for another 120 min. A total of 125 min of ECoG recording was obtained from each animal. At the end of the study, rats were euthanized by cervical dislocation under urethane anaesthesia.

The signals received from the electrodes were filtered with a 0.1-50 Hz band-pass and recorded through an amplifier (BioAmp, AD Instruments, Australia). These were digitized at a sampling rate of 1024 Hz. The onset of first epileptiform activity, epileptiform activity spike-wave frequency and amplitude were evaluated. Records were analysed with the PowerLab Chart v.7.2.1 software program. Epileptiform activity in the form of bipolar spike and spike-wave complexes were examined, and the means of spike wave frequencies and amplitude in 5 min of the ECoG recordings for each animal were measured and used as data.

Statistical analysis

From the records obtained for each animal, the onset of the first epileptiform activity, epileptiform activity spike-wave frequency and spike wave amplitude were automatically calculated by using software (Chart v.7.2.1, ADInstruments Pty Ltd, Castle Hill, NSW, Australia). Epileptiform activity records were analysed after being divided into five-minute periods. The differences between the groups in terms of the time of onset of the first epileptiform activity and the spike-wave frequency and spike-wave amplitude measurements in each period were examined with the Kruskal-Wallis test, and homogeneous subgroups multiple comparison method was used to determine different groups. $P<0.05$ was accepted as the statistical significance level. Predictive Analytics SoftWare (PASW) program was used for analysis.

Results

Effect of EA applications in Sham and only EA groups

All substances used in the study were tested on the number of seven rats in each group, and it was investigated whether they had an effect on the ongoing basal activity (Fig. 1). It was determined that all doses of EA used in the study had no effect on basal activity. Epileptic activity discharge was not observed in the 120 min ECoG recordings which were taken from sham and only EA groups (Fig. 1B, 1C & 1D).

Onset of first epileptiform activity

Spike waves of epileptiform activity later in penicillin injection started to appear among the 3-20 min (Fig. 2). There was a significant difference between the groups in terms of onset of the first epileptiform activity ($P=0.028$). When the groups were examined in more detail, it was seen that the mean of the onset of first epileptiform activity of the acute groups and the mean of the onset of first epileptiform activity of the chronic groups were statistically higher than the control group ($P<0.05$). However, no statistically significant difference was found between the other groups ($P>0.05$).

Effect of EA on time-dependent spike wave frequency of epileptiform activity

There was no epileptiform activity in the basal activity recorded measurements taken just before the substance administration in the acute groups. Epileptiform activity was not found in the 30 min ECoG recordings which were taken from EA administration to penicillin administration in acute groups. In 24 different measurements taken in 5 min periods after penicillin application, certain numbers of spike-wave frequency values were obtained (Fig. 3 and Table 2). Any epileptiform activity was not found in the ECoG record measurements during the five-minute basal activity recording in the chronic groups. The mean number of spike waves in all acute and chronic groups between 6-110 min (except 56-70 and 76-80 min) after penicillin administration was found to be statistically lower than the control group ($P<0.05$). Moreover, the mean spike wave numbers of the AG_10_EA group were determined to be lower than AG_50_EA, AG_100_EA, and CG_50_EA groups between the 26 and 55 min ($P<0.05$). In

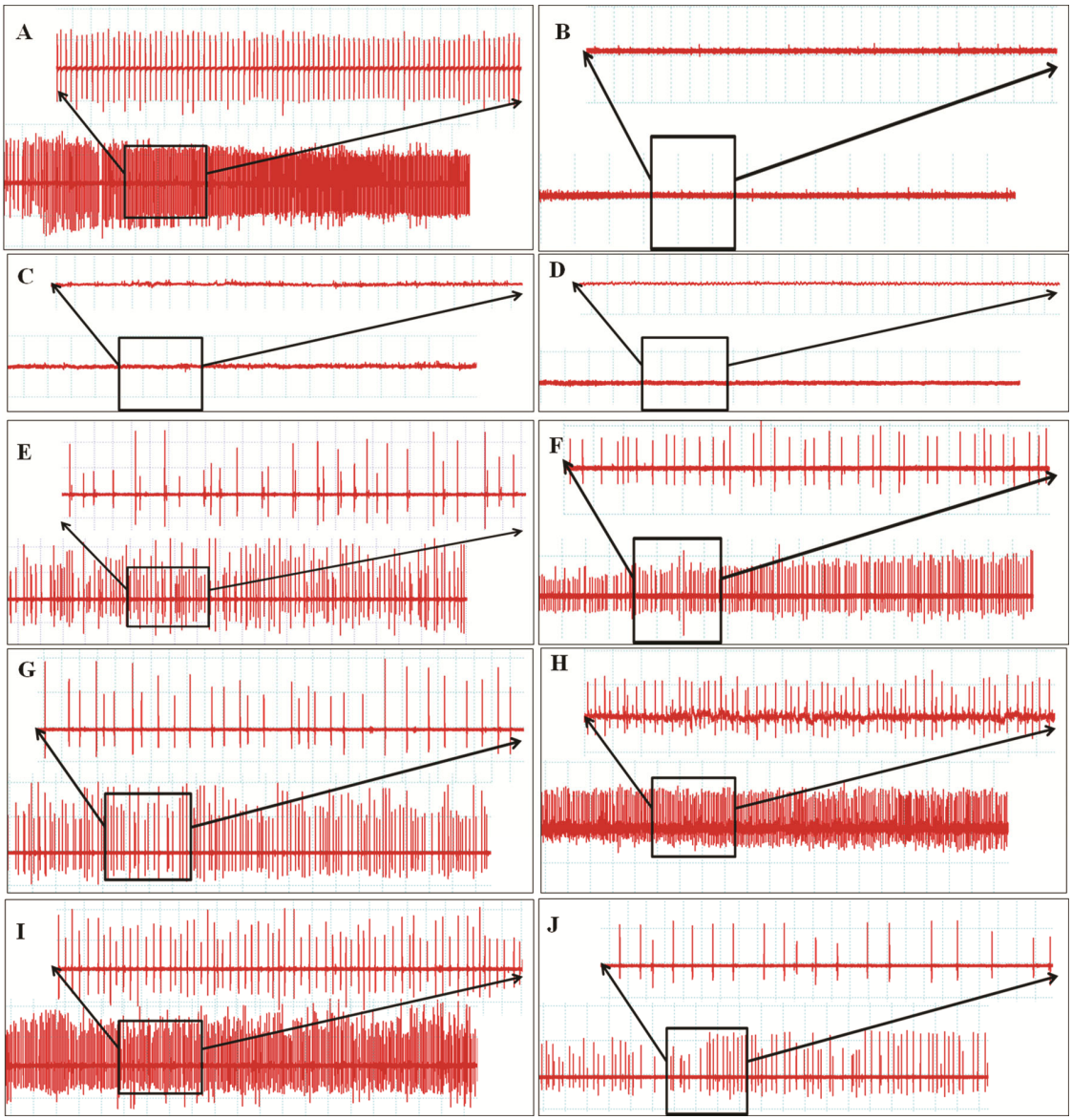

Fig. 1 — Representative samples of ECoG records from groups (A) Control, (B) Sham, (C) Acute only EA, (D) Chronic Only EA, (E) AG_10_EA, (F) CG_10_EA, (G) AG_50_EA, (H) CG_50_EA, (I) AG_100_EA, (J) CG_100_EA.

addition, the mean spike wave frequency of the CG_100_EA group between 71-110 min (except 76-80 min) was found to be statistically lower than all other groups (P<0.05). In the range of 0-5, 56-70, 76-80, and 111-120 were not any differences among the groups (P>0.05).

Effect of EA on total epileptiform activity spike wave numbers

The means of the total number of spike waves occurring during the 120 min ECoG recording after penicillin of the acute and chronic groups were compared. When the groups were compared in terms of total frequency means, a significant difference was determined among the groups (P=0.004) (Fig. 4). When the groups were examined in more detail, it was found that the mean frequency of the acute and chronic groups was significantly lower than the control group (P<0.05). In addition, the total frequency mean of the AG_100_EA group was found to be statistically higher than the other groups except the control group (P=0.05).

Effect of EA on the spike wave amplitude of epileptiform activity

In order to calculate the effects of the applied substances on the epileptiform activity's amplitude, 120 min spike wave amplitude (min-max) values were calculated at 5 min periods after penicillin

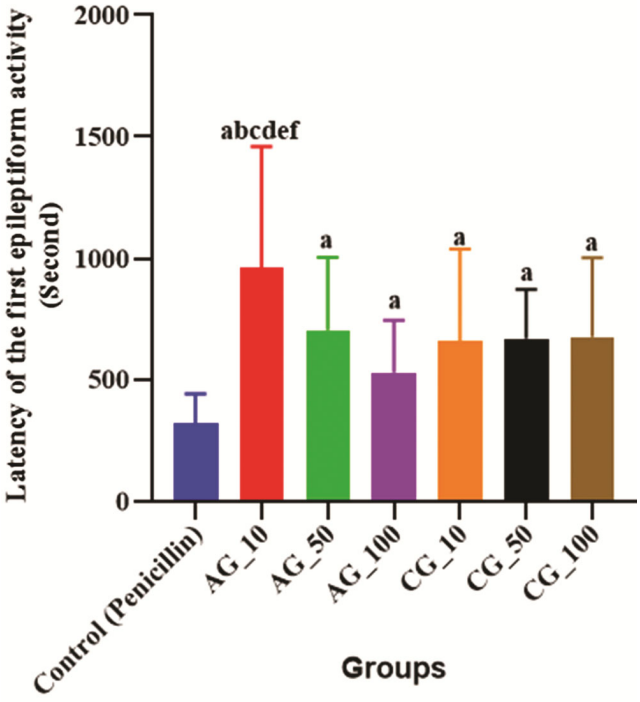

Fig. 2 — Latency of the first epileptiform activity. [[a]Significant compared to control group; [b]Significant according to AG_50_EA group; [c]Significant according to AG_100_EA group; [d]Significant according to CG_10_EA group; [e]Significant according to CG_50_EA group; [f]Significant according to CG_100_EA group]

application. Below, a summary of the findings including spike wave amplitude mean values obtained at each measurement interval of the groups and the differences between groups is given (Fig. 5 and Table 3). The mean spike wave amplitude of all acute and chronic groups between 6-30 min after penicillin administration was found to be statistically lower than the control group (P<0.05). In addition, the mean spike wave amplitudes of the AG_10_EA group between 6-50 min were found to be lower than the spike wave amplitudes of the other groups (P<0.05). There was no statistically significant difference between the groups for the 0-5, and 55-120 min time intervals (P>0.05).

Discussion

This research is the first study to examine the electrophysiological efficiency of ellagic acid upon epileptic seizures. In this study, the effect of 10, 50 and 100 mg/kg doses of acute and chronically administered ellagic acid on experimentally penicillin induced epileptiform activity was investigated in male Wistar rats. The epileptiform activity figures and patterns observed in the ECoG recordings are compatible with the literature[16,18,19]. EA which was administered acutely and chronically without inducing

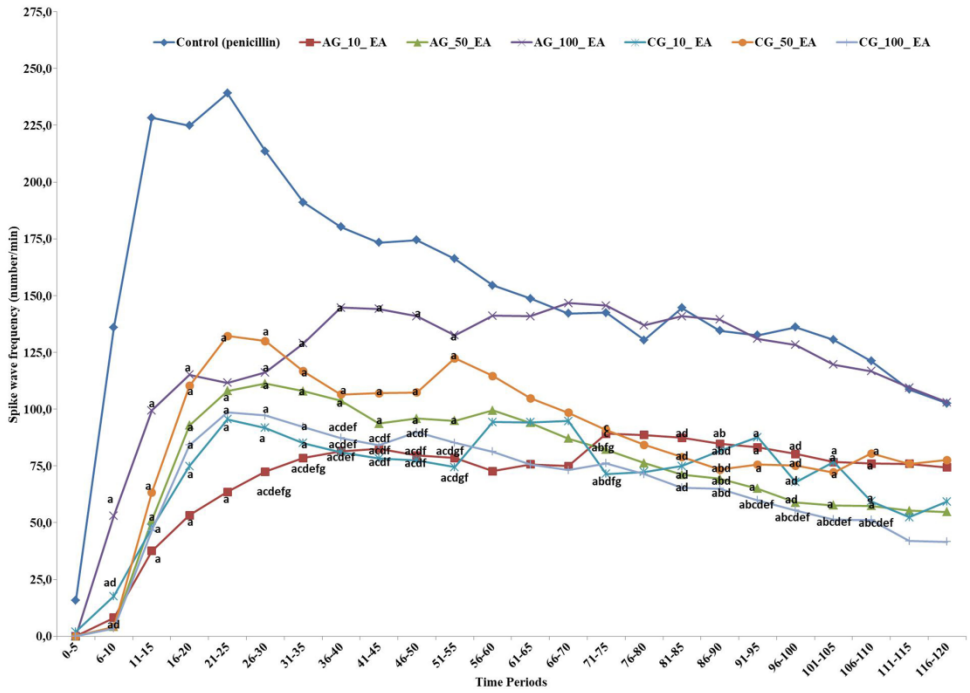

Fig. 3 — Time-dependent spike-wave frequency (number/min) values between 0-120 min obtained from after penicillin applied. [[a]Significant compared to control group; [b]Significant according to AG_10_EA group; [c]Significant according to AG_50_EA group; [d]Significant according to AG_100_EA; [e]Significant according to CG_10_EA group; [f]Significant according to CG_50_EA group; [g]Significant according to CG_100_EA group]

Table 2 — Descriptive values of epileptiform activity spike-wave frequency (number/minute) obtained from recordings between 0-120 min after penicillin in all groups and *P* value for comparison of groups

Period	CONTROL			AG_10_EA			AG_50_EA			AG_100_EA			CG_10_EA			CG_50_EA			CG_100_EA			*P* value
	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	
0-5	16±30	0	78	0±0	0	0	0±0	00	0	0±0	0	0	2±5	0	14	0±0	0	0	0±0	0	0	0.161
6-10	136±68	11	245	8±20^{ad}	0	53	4±6^{ad}	0	14	53±85^{ad}	0	187	18±31^{ad}	0	86	4±10^{ad}	0	27	3±7^{ad}	0	18	0.006
11-15	228±56	56	309	38±48^{a}	0	125	51±51^{a}	0	154	99±109^{a}	0	282	48±57^{a}	0	129	63±59^{a}	0	144	47±52^{a}	0	133	0.006
16-20	225±47	47	319	53±54^{a}	0	122	93±60^{a}	0	185	115±65^{a}	42	204	75±68^{a}	0	176	110±95^{a}	19	256	84±68^{a}	0	187	0.008
21-25	239±96	96	380	64±60^{a}	0	148	108±64^{a}	9	216	112±48^{a}	64	172	96±73^{a}	7	218	132±76^{a}	12	226	99±55^{a}	10	167	0.014
26-30	214±85	85	350	72±56^{acdefg}	0	155	111±46^{a}	29	158	116±38^{a}	75	168	92±82^{a}	0	211	130±72^{a}	16	229	97±56^{a}	12	164	0.034
31-35	191±63	63	320	78±52^{acdefg}	11	160	108±39^{a}	52	154	129±45^{a}	75	184	85±87^{a}	2	217	117±59^{a}	18	200	92±54^{a}	11	157	0.044
36-40	180±57	57	298	81±47^{acdf}	22	160	104±37^{a}	49	150	145±74^{a}	76	290	81±81^{acdf}	0	225	106±63^{a}	22	183	87±37^{acdf}	46	134	0.030
41-45	173±58	58	295	83±40^{acdf}	37	154	94±37^{a}	55	140	144±80^{a}	73	299	78±77^{acdf}	0	219	107±65^{a}	0	175	84±29^{acdf}	41	116	0.029
46-50	174±64	64	312	80±38^{acdf}	35	150	96±44^{a}	43	158	141±77^{a}	74	292	77±79^{acdf}	4	232	107±60^{a}	0	169	90±34^{acdf}	34	131	0.023
51-55	166±60	60	288	78±35^{acdfg}	35	140	95±47^{c}	51	179	133±61^{c}	59	236	74±65^{acdfg}	8	196	122±69^{c}	1	207	85±33^{c}	34	126	0.039
56-60	154±53	53	245	73±39	24	133	99±40	62	182	141±73	62	278	94±79	7	199	115±59	1	174	81±33	28	122	0.079
61-65	149±61	61	254	76±33	43	128	94±43	60	187	141±74	62	278	94±88	1	234	105±54	0	167	76±36	15	118	0.144
66-70	142±55	55	219	75±32	28	121	87±43	53	177	147±61	68	257	95±78	15	225	98±50	0	157	73±38	13	127	0.064
71-75	142±55	55	239	89±40c	33	146	82±50^{abfg}	50	192	146±60	73	253	71±69^{abdfg}	17	205	91±42^{c}	0	130	76±33^{abfg}	36	122	0.034
76-80	130±54	54	245	89±44	34	166	76±49	21	179	137±56	78	238	72±61	14	188	84±40	0	132	71±34	31	116	0.052
81-85	145±34	34	218	87±40^{ad}	42	158	71±52^{ad}	0	173	141±53^{a}	79	230	75±32^{ad}	28	114	79±37^{ad}	0	112	65±23^{ad}	41	99	0.002
86-90	135±50	50	204	85±38^{ab}	42	150	69±50^{abd}	0	168	139±51	79	227	82±46^{abd}	18	169	73±34^{abd}	0	100	65±25^{abd}	33	95	0.009
91-95	133±47	47	195	83±39^{a}	41	151	65±49^{a}	0	161	131±56^{a}	71	220	88±49^{a}	46	193	76±37^{a}	0	116	60±23^{abcdef}	30	95	0.022
96-100	136±55	41	210	80±35^{ad}	44	139	59±52^{ad}	0	158	128±51	73	217	68±21^{ad}	40	96	75±36^{ad}	0	112	55±24^{abcdef}	21	87	0.011
101-105	131±58	39	227	77±36^{a}	36	141	58±52	0	155	120±58^{a}	38	206	77±45^{a}	47	174	72±35^{a}	0	104	51±23^{abcdef}	19	87	0.046
106-110	121±62	27	226	76±36^{a}	35	139	57±51^{a}	0	153	117±54^{a}	61	199	59±16^{a}	40	80	80±40	1	122	51±25^{abcdef}	28	91	0.037
111-115	109±78	18	252	76±31	43	131	55±50	0	150	109±57	44	195	52±14	32	72	76±37	0	113	42±31	1	91	0.069
116-120	103±74	14	230	74±30	40	122	55±49	0	146	103±61	25	189	59±19	28	87	78±38	0	111	42±30	0	89	0.196

Kruskal-Wallis test was used to compare the groups. and the homogeneous subgroups multiple comparison method was used to determine the groups that were different. [^{a}Significant according to the contrc group; ^{b}Significant compared to the AG_10_EA group; ^{c}Significant according to the AG_50_EA group; ^{d}Significant according to the AG_100_EA group;^{e}Significant according to the CG_10_EA group ^{f}Significant according to the CG_50_EA group; ^{g}Significant according to the CG_100_EA group]

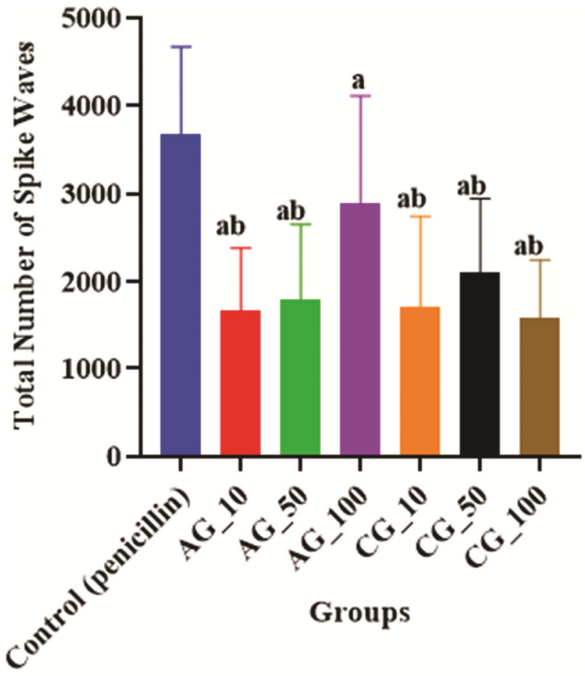

Fig. 4 — Means of total spike-wave numbers obtained from the 120 min recordings after the penicillin administration. [^{a}Significant compared to Control group; ^{b}Significant according to AG_100_EA group]

penicillin epilepsy did not cause any epileptiform activity in any animal. These data suggest that the use of EA in epileptic or healthy rats will not cause any epileptic effect.

In the ECoG records obtained from animals where epileptiform activity was stimulated with penicillin, the groups were compared in terms of the first epileptic activity onset time. It was found that the onset of the first epileptiform activity was longer than the Control group both in all acute and chronic groups. According to these results, it was observed that EA applied before penicillin in acute groups and chronic groups delayed the onset of the first epileptiform activity at least three times compared to the control group. In addition, it was determined that the AG_10_EA group prolonged the onset of the first epileptiform activity time most in acute groups, and the CG_100_EA group prolonged the first epileptiform activity onset time most in chronic groups. The results of the control group are consistent with other studies in the literature[16,19,20]. The mean onset time of the first epileptiform activity of the control groups in this study was determined as 3-10 min. The onset time of the first epileptiform activity for EA was tested in different epilepsy models, such as pentylenetetrazole (PTZ), picrotoxin and maximal electroshock (MES)[4,17]. Dhingra and Jangra reported in their study that EA prolonged the onset of the first epileptiform activity[4]. In their study, 20 and 40 mg/kg doses of acute and chronically administered EA were tested in PTZ and picrotoxin models. In acute groups, 40 mg/kg EA was found to delay the onset of first epileptiform activity, while in chronic groups,

Table 3 — Descriptive values of epileptiform activity spike-wave amplitude (mV) obtained from recordings between 0-120 min after penicillin in all groups and *P* value for comparison of groups

Period	CONTROL			AG_10_EA			AG_50_EA			AG_100_EA			CG_10_EA			CG_50_EA			CG_100_EA			*P* value
	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	
0-5	1.2±1.6	0.2	4.5	0.3±0.2	0.2	0.5	0.3±0.2	0.2	0.7	0.5±0.2	0.2	0.6	0.6±0.3	0.3	1.0	0.4±0.2	0.2	0.6	0.4±0.2	0.2	0.8	0.126
6-10	2.3±1.6	0.9	5.6	0.4±0.2[acde]	0.2	0.7	1.3±1.5[a]	0.2	4.1	1.7±1.5[a]	0.2	4.3	0.6±0.4[a]	0.2	1.3	0.5±0.3[acde]	0.2	1.1	0.6±0.5[acde]	0.1	1.3	0.027
11-15	2.7±1.6	1.6	6.0	0.7±0.3[acdf]	0.2	1.1	1.7±1.2[a]	0.5	3.2	2.2±1.3[a]	0.9	4.6	0.9±0.7[acdf]	0.3	2.2	1±0.7[acdf]	0.3	2.3	1±0.7[a]	0.2	2.0	0.009
16-20	2.9±1.7	1.4	6.4	0.9±0.3[acdefg]	0.3	1.3	2.1±1.5[a]	0.1	4.2	2.3±1.1[a]	1.1	3.9	1.3±1.0[a]	0.3	3.0	1.4±0.7[a]	0.4	2.6	1.2±0.6[a]	0.2	2.1	0.012
21-25	2.8±1.5	1.3	6.0	0.9±0.4[acdef]	0.3	1.4	2.8±1.2	1.2	5.0	2.5±1.1	1.1	4.2	1.7±1.1[acd]	0.4	3.6	1.5±0.9[acd]	0.5	2.8	1.3±0.5[acdef]	0.5	2.2	0.003
26-30	2.8±1.6	1.3	6.3	1.1±0.4[acdefg]	0.4	1.6	2.8±1.1	1.1	4.6	2.7±1.1[ac]	1.1	4.2	1.7±1.3[ac]	0.3	3.9	1.6±1.0[ac]	0.6	3.4	1.5±0.5[ac]	0.5	2.0	0.012
31-35	2.7±1.5[d]	1.2	6.0	1.4±0.8 [acdefg]	0.6	3.1	2.8±1.2[d]	1.2	4.6	2.8±1.1	1.1	4.4	1.8±1.3[d]	0.4	4.1	1.6±1[d]	0.7	3.6	1.5±0.6[d]	0.4	2.0	0.044
36-40	2.6±1.5	1.2	5.7	1.3±0.8	0.6	3.1	2.8±1.3	0.8	4.7	2.7±1	1.0	3.8	1.9±1.3	0.3	3.9	1.6±1	0.8	3.4	1.5±0.6	0.4	2.0	0.070
41-45	2.7±1.4[b]	1.2	5.5	1.5±1[acdefg]	0.7	3.6	3±1.4	1.1	5.2	2.7±1[c]	1.0	3.8	1.8±1.2[c]	0.3	3.7	1.6±0.8[c]	0.6	3.0	1.6±0.7c	0.4	2.3	0.046
46-50	2.6±1.4[d]	1.2	5.5	1.5±1.1[acdefg]	0.7	4.0	3.1±1.4	1.4	5.1	2.8±1.1[d]	1.1	3.9	1.9±1.3[d]	0.4	3.7	1.7±0.8[d]	0.6	2.8	1.6±0.6[d]	0.6	2.1	0.041
51-55	2.7±1.5	1.4	5.8	1.5±1.1	0.7	3.9	2.9±1.3	1.2	4.6	2.8±1	1.0	3.7	1.9±1.3	0.4	3.8	1.8±0.9	0.6	3.1	1.5±0.6	0.6	2.2	0.074
56-60	2.7±1.5	1.3	5.7	1.5±1.1	0.7	3.9	3.1±1.4	1.2	4.7	2.8±1	1.0	4.0	1.8±1.2	0.5	3.5	2±1.1	0.6	3.2	1.6±0.6	0.6	2.3	0.070
61-65	2.5±1.3	1.3	5.0	1.5±1	0.7	3.7	3±1.4	1.0	4.9	2.9±1.1	1.1	4.3	1.7±1.1	0.5	3.4	1.9±1.1	0.5	3.4	1.5±0.6	0.6	2.3	0.086
66-70	2.4±1.3	1.2	4.8	1.5±1	0.7	3.6	3±1.4	1.0	4.7	2.8±1.1	1.1	4.4	1.7±1	0.5	3.2	2.1±1.1	0.5	3.5	1.4±0.6	0.6	2.2	0.067
71-75	2.4±1.1	1.1	4.1	1.8±1	0.9	3.7	2.9±1.4	0.8	4.7	2.7±1.1	1.1	4.4	1.6±0.9	0.5	3.1	2.3±1.6	0.5	5.2	1.3±0.6	0.6	2.3	0.118
76-80	2.3±1	1.0	3.9	1.8±0.9	0.9	3.5	2.9±1.5	0.6	4.8	2.7±1.2	1.2	4.6	1.3±0.7	0.5	2.8	2.3±1.7	0.6	5.3	1.4±0.6	0.6	2.3	0.106
81-85	2.2±1	1.0	3.7	1.9±0.9	0.9	3.6	2.7±1.5	0.5	4.5	2.8±1.2	1.2	4.7	1.4±0.8	0.4	3.1	2.2±1.7	0.5	5.1	1.4±0.7	0.6	2.2	0.182
86-90	2.2±0.9	0.9	3.7	1.9±0.8	0.8	3.3	2.6±1.5	0.4	4.4	2.6±1.2	1.2	4.7	1.2±0.6	0.4	2.4	2.2±1.6	0.5	5.1	1.3±0.6	0.5	2.3	0.118
91-95	2±0.7	0.7	3.5	1.9±0.8	0.8	3.3	2.6±1.5	0.4	4.4	2.6±1.3	1.2	4.6	1.4±0.9	0.5	3.2	1.9±1.4	0.4	3.9	1.3±0.6	0.5	2.2	0.229
96-100	1.9±0.9	0.9	3.9	1.9±0.8	0.8	3.3	2.5±1.5	0.4	4.3	2.6±1.2	1.2	4.6	1.5±0.8	0.5	3.0	1.9±1.3	0.5	3.8	1.4±0.8	0.4	2.2	0.418
101-105	1.9±0.7	0.7	3.5	1.9±0.8	0.8	3.2	2.4±1.5	0.4	4.1	2.4±1.2	1.2	4.7	1.4±0.8	0.5	3.0	1.8±1.3	0.4	3.7	1.4±0.7	0.4	2.2	0.559
106-110	1.8±0.8	0.8	3.6	1.9±0.8	0.8	3.2	2.4±1.5	0.4	4.3	2.4±1.2	1.2	4.5	1.3±0.8	0.5	2.7	1.9±1.2	0.6	3.6	1.4±0.7	0.4	2.2	0.394
111-115	1.8±0.9	0.9	3.8	1.8±0.7	0.7	2.8	2.3±1.5	0.3	4.2	2.4±1.2	1.1	4.6	1.3±0.8	0.5	2.7	1.8±1.3	0.4	3.8	1.3±0.7	0.4	2.0	0.410
116-120	1.7±0.9	0.9	3.7	1.8±0.7	0.7	2.9	2.3±1.4	0.4	3.9	2.3±1.2	1.1	4.5	1.4±0.8	0.6	2.6	1.8±1.2	0.5	3.7	1±0.8	0.1	2.1	0.196

Kruskal-Wallis test was used to compare the groups. and the homogeneous subgroups multiple comparison method was used to determine the groups that were different. [[a]Significant according to the control group; [b]Significant compared to the AG_10_EA group; [c]Significant according to the AG_50_EA group; [d]Significant according to the AG_100_EA group;[e]Significant according to the CG_10_EA group; [f]Significant according to the CG_50_EA group; [g]Significant according to the CG_100_EA group]

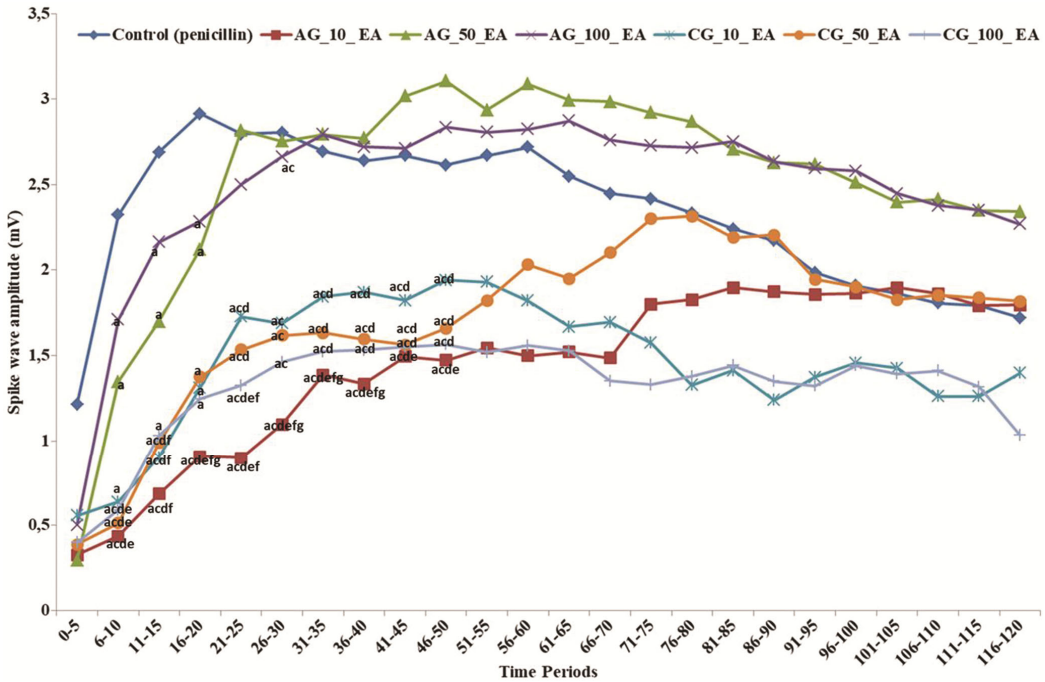

Fig. 5 — Time-dependent spike-wave amplitude (mV) values between 0-120 min obtained from post-penicillin recordings. [[a]Significant compared to control group; [b]Significant according to AG_10_EA group; [c]Significant according to AG_50_EA group; [d]Significant according to AG_100_EA group; [e]Significant according to CG_10_EA group; [f]Significant according to CG_50_EA group; [g]Significant according to CG_100_EA group.

both doses of EA prolonged the onset time of first epileptiform activity compared to the control groups. In the PTZ model, the 40 mg/kg dose of acute EA increased the onset time of first epileptiform activity from 87.4-106 second, while in the picrotoxin model, the EA's first epileptiform activity initiation time increased from 668.9-699.1 second. In the current study, all three doses of acute EA (10, 50 and 100

mg/kg) prolonged the onset time of first epileptiform activity from 320-961.4 second, 705.7 second and 527.4 second, respectively. In the PTZ model, the doses of 20 and 40 mg/kg EA which were administered chronically increased the onset time of first epileptiform activity from 86.11-102.33 and 555.9 second, respectively. In the picrotoxin model, the EA's onset time of first epileptiform activity increased from 669.44 to 701.33 and 707.55 second, respectively. In the present study, all three doses of chronically administered EA (10, 50 and 100 mg/kg) extended the first epileptiform activity onset time from 320-659.9 second, 672.6 second and 678.1 second, respectively. In contrast, Luszczki *et al*., in their study in mice, 300 mg/kg EA administered i.p. in 4 different time periods (15, 30, 60 and 120 min) before MES, and they had reported that 300 mg/kg EA was ineffective[21]. Another study was evaluated the effects of EA (doses of 25, 50, and 100 mg/kg) on both MES and PTZ models of acute seizures in mice[22]. In this study, it was reported that the latencies with EA were longer in groups treated with EA 25 mg/kg than with EA 50 and 100 mg/kg. Golechhave *et al*. had tested the hydroalcoholic extract of *Emblica officinalis* with a high EA content in a seizure model stimulated by PTZ[23]. In this study, 300, 500 and 700 mg/kg hydroalcoholic extract of *Emblica officinalis* were injected i.p into the rats for seven days. At the end of the study, 300, 500 and 700 mg/kg hydroalcoholic extract of *Emblica officinalis* (110.16 second, 207.16 second and 290 second, 16 second, respectively) prolonged the latency of myoclonic movement compared to the control (34.66 second) group.

In the present study, epileptiform activity was not found in ECoG recording measurements during the basal activity recording taken before penicillin in acute and chronic groups. In addition, epileptiform activity spike wave pattern was not observed during the entire recording period in sham and only EA groups. These data could not be compared due to the absence of any other electrophysiological study in the literature. However, data obtained only from EA groups suggest that acute or chronic use of EA does not cause any convulsions. This finding is important because of the absence of any other electrophysiological study in the literature.

In 24 different measurements taken in 5 min periods after penicillin administration, certain numbers of spike-wave frequency values were determined. The mean number of epileptiform activity spike waves obtained from both acute and chronic EA groups was found to be lower than the control group between 6-110 min (except 56 -70 and 76 -80 min). These data were obtained from the records in different periods depending on time. However, the obtained data could not be compared because of the absence of any literature showing the changes on epileptiform activity spike wave numbers depending on time. Nonetheless, when the groups were compared among themselves, it was determined that epileptiform activity spike wave numbers induced with penicillin decreased depending on time compared to the control group. In addition, it has been shown that 10 mg/kg EA reduced the spike wave frequency in acute groups more than other acute groups. In chronic groups, it was found that the dose of 100 mg/kg EA decreased the number of spike waves more than in other chronic groups. This shows that EA reduces the number of spike waves depending on the dose.

In the present study, the means of the total number of spike waves frequency occurring during the 120-min ECoG recording after penicillin administration in the acute and chronic groups were found that the total frequency means of both the acute and chronic groups were lower than the control group. In addition, it was determined that the total frequency mean of the AG_100_EA group was higher than the other groups except for the control group. These findings are consistent with the literature[4,17,23]. Dhingra and Jangra reported that in the PTZ model which they applied in mice, 40 mg/kg EA reduced the duration of tonic and clonic contractions in acute groups. However, both 20 mg/kg EA and 40 mg/kg EA decreased tonic-clonic contractions in chronic groups. Another model used in the same study was the picrotoxin model. In this model, it had been shown that only acute use of 40 mg/kg EA reduced tonic and clonic seizures, while chronically used doses of 20 and 40 mg/kg EA both reduced the duration of contractions[4]. In another similar study was evaluated the effect of EA on hippocampal cell damage in PTZ induced kindling model in rats. The results of this study showed that EA decreased the severity of seizures in kindling seizures[24]. In the PTZ model study conducted by Golechha *et al*, seizures were not observed in rats which given 500 and 700 mg/kg hydroalcoholic extract of *Emblica officinalis* containing EA. However, it had been reported that 300 mg/kg of *Emblica officinalis* hydroalcoholic extract shortens the duration of generalized tonic seizures[23]. In another study conducted in mice, it was reported that chronic administration of 300 mg/kg EA for seven days had

no effect on seizures induced by MES[17]. In a study investigating the antiseizure effect of EA in the pilocarpine-induced status epilepticus model, it was reported that EA decreased epileptiform activity and the severity of convulsive behavior[25].

Missiry *et al*. 2020 evaluated the neuroprotective and antiepileptic efficacy of EA encapsulated in calcium-alginate nanoparticles (Ca^{++}-ALG NPs) in PTZ induced seizures in male mice. They have reported that the newly formed Ca-crosslinked EA-ALG NPs showed anticonvulsion influence with remarkable effectiveness in improving the brain redox imbalance, disruption in neurotransmitters, downregulating inflammatory cytokines and inhibiting apoptosis in PTZ induced epileptic seizures[26].

In the present study, spike-wave amplitude values at certain voltages were determined in 24 different measurements taken in 5 minute periods after penicillin administration. The mean epileptiform activity spike wave amplitude obtained from both acute and chronic EA groups was found to be lower than the control group between 6-30 min. There was no similarity between the groups in the other periods 0-5, 36-40, 76-80 and 51^{t}-12. In addition, the obtained data could not be compared since there was no study in the literature showing the epileptiform activity spike wave amplitude changes depending on time.

Epileptic area potential and paroxysmal depolarization alteration can be created by various chemicals. Among these, the most commonly used are bicucullin and picrotoxin, which inactivate the $GABA_A$ receptor. According to this information, if the activity of GABA is reduced or destroyed, it will cause overstimulation of the excitatory neurotransmitter glutamate[27]. The epileptiform activity induced by penicillin also plays an active role in cortical pyramidal cells. In the epilepsy model induced with penicillin; potentials due to $GABA_A$ and $GABA_B$ receptors contribute to the sudden depolarization shifts observed in cells[28]. Penicillin, which is applied directly to the cortex causes inhibition of GABA receptors by acting similar to bicuculline thus suppressed GABA activity initiates the epileptiform activity that starts locally but continues to generalize by disrupting the inhibitory system of the brain. Studies show that penicillin reduces intracellular Cl-influx by binding to subunits of $GABA_A$ receptors[29]. In addition, other studies had reported that penicillin also blocks the opening of the canal by binding to the chlorine receptor[30]. In another study, it was suggested that penicillin causes convulsions by binding to the binding site of benzodiazepines[31]. The primary target of penicillin is the β-subunit of the $GABA_A$ receptor to which GABA binds. It is thought that penicillin prevents the binding of GABA to this region by binding to the GABA binding site with the β-lactam ring. This finding demonstrates the possible mechanism of penicillin in epileptogenesis[32,33].

In the study conducted by Dhingra and Jangra, it was reported that EA administered acutely and chronically prevented convulsions caused by PTZ and picrotoxin and reversed decreased brain GABA levels[4]. In addition, EA demonstrated its antiepileptic property by increasing the level of brain GABA[4]. This effect is supported by the study by Golechha *et al*.[23]. In the current study, brain GABA level was not investigated. However, it has been shown that different doses of EA administered in both acute and chronic groups can reverse the epileptiform activity induced by penicillin. This may suggest that the EA acts by increasing the decreased GABA level in the penicillin model as well as in the PTZ and picrotoxin models. It is likely that EA not only acts on $GABA_A$ receptors but also reduces the release of excitatory neurotransmitters from excitatory neurons by acting on $GABA_B$ receptors. However, there is no study has been found in the literature to verify this information.

Rahimi-Madiseh *et al*. showed that EA has an anticonvulsant effect and increases the threshold of PTZ-induced seizures in mice. In addition, they showed that EA decreased gene expression of Nr2a and Nr2b subunits of N-Methyl-D-aspartate (NMDA) receptors[34]. In a similar study by Mshelia *et al*., EA decreased the seizure intensity in all the treated groups. In addition, EA has also been reported to decrease glutamate concentration[35].

Conclusions

In terms of time-dependent spike wave frequency, all groups of EA decreased spike wave frequency of epileptiform activity except AG_100_EA group. However, the CG_100_EA group was more effective than the other EA groups. In addition, all EA groups reduced the total spike wave frequency. It has been shown that EA has a protective and reducing effect on the penicillin model epilepsy, albeit in a limited number, as in other experimental epilepsy models. In the presented study, the molecular and biochemical changes of ellagic acid in the brain were not analysed, but only electrophysiologically, its effect on

epileptiform activity was investigated. Conducting longer-term and multidisciplinary studies on this subject will shed light on the issue.

Conflict of interest

The authors declare that they have no competing interests.

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