DOI: 10.4274/jarem.galenos.2022.25238
J Acad Res Med 2022;12(2):87-98

The Effects of Acute and Chronic Metformin Treatment on Penicillin Induced Epileptiform Activity in Rats

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Cite this article as: Kılıç Ü, Demir Ş, Beyazçiçek E, Beyazçiçek Ö, Soytürk H. The Effects of Acute and Chronic Metformin Treatment on Penicillin Induced Epileptiform Activity in Rats. J Acad Res Med 2022;12(2):87-98

ABSTRACT

Objective: The aim of this study is to investigate acute and chronic administered metformin on epileptiform activity induced by penicillin and antioxidant activity in rats.

Methods: Eighty-four adult male Wistar albino rats were used in this study. The rats were divided into two large groups as acute and chronic groups, and later on each group was divided into different subgroups as control, sham, penicillin, metformin 100 mg/kg (Met_100), 200 mg/kg (Met_200) and only metformin 200 mg/kg (OMet_200) intraperitoneally. The substances were applied to the chronic groups for 21 days, while acute groups received them just before the initiation of epileptiform activity. In the present study, onset of first epileptiform activity, spike wave frequency and amplitude, and superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) parameters were evaluated.

Results: No epileptiform activity was observed in the control, sham, and OMet_200 groups. When metformin doses of 100 mg/kg, 200 mg/kg were compared with the penicillin group in both acute and chronic groups, the onset of first epileptiform activity was prolonged, spike wave frequency and spike wave amplitude decreased significantly. SOD, CAT and GPx levels were found to be significantly different in the acute and chronic metformin groups compared to the penicillin group.

Conclusion: In conclusion, this study shows that metformin can decrease epileptic seizures and increase the level of antioxidant enzymes and it can be used in the treatment of epilepsy in the future.

Keywords: Antioxidant, electrocorticography, epilepsy, epileptiform activity, metformin

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Received Date/Geliş Tarihi: 01.12.2021 **Accepted Date/Kabul Tarihi:** 27.07.2022

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1. INTRODUCTION

Epilepsy is a neurological brain disease that affects approximately 50 million people of all ages worldwide. It is characterized by recurrent seizures, transient occurrence of abnormal, excessive, and/or synchronous neuronal activity in the brain, and sometimes accompanied by loss of consciousness and poor control of bowel and bladder function. Of the people with epilepsy 80% live in low and middle income countries (1,2).

In general, epileptic seizures indicate the presence of an imbalance between inhibitory and excitatory neurotransmission. However, the mechanism of epileptogenesis is still unclear (3).

Until now, anti-epileptic drugs could not been developed to completely heal epilepsy. Therefore, the current therapeutic approach is symptomatic therapy to improve patients' lifetime and quality of life (4,5). The epileptogenesis process involves a variety of molecular and cellular changes that can be used as potential targets for the treatment and prevention of epilepsy, but most of the currently available drugs only work by suppressing the seizure without affecting the underlying pathological conditions (6).

Metformin is the most commonly prescribed oral antidiabetic drug in the world and has been used in the treatment of type 2 diabetes for many years. In addition, this drug is used in the treatment of prediabetes, polycystic ovary syndrome and insulin resistance. The positive effects of metformin on the cardiovascular system and its anti-cancer activity are positive aspects of the drug. Diabetes associations recommend the use of metformin at all levels of the national and global type 2 diabetes treatment algorithms (7).

Metformin is an inexpensive drug that is well tolerated, effective and easy to access. Besides its known antihyperglycemic effects, it has been shown to have positive effects on the cardiovascular system in patients with insulin resistance and diabetes. Increasing the studies on metformin, which is one of the oldest drug known, leads to an increase in its use in medicine (8).

It has been shown that metformin can decrease the symptoms of epileptic seizures as well as modifying the molecular and cellular changes, including oxidative stress, neuroinflammation, apoptosis, and neuronal loss. In experimental studies, the neuroprotective effects of metformin were investigated by using pentylenetetrazole (PTZ), pilocarpine, and kainic acid models that produced epileptiform activity. In the study of Mehrabi et al. (9), it was shown that metformin decreased brain-derived neurotrophic factor (BDNF) and TrkB expression, increased AMPK expression, and decreased mTOR protein expression in an animal model of temporal lobe epilepsy (TLE) created by pilocarpine administration. In the study conducted by Yang et al. (10), it was found that administration of metformin decreased mortality, increased AMPK levels and facilitated seizure termination compared to the control group in the kainic acid and PTZ epilepsy models in mice.

In this study, different from the studies which were mentioned above, the experimental epilepsy model was created with penicillin. In the present study, the onset of first epileptiform activity, spike wave frequency and spike wave amplitude were examined electrophysiologically to determine the chronic and acute effects of metformin on epileptiform activity induced by penicillin. In addition, in order to determine the antioxidant properties of metformin, it was aimed to investigate the enzyme values of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) in blood samples taken from rats.

2. METHODS

2.1. Animals Used in the Experiment

The ethical approval was obtained from Duzce University Animal Experiments Local Ethics Committee with the decision number 2019/6/1 (date: 02.07.2019), and the animals to be used in the experiment were obtained from Duzce University Experimental Animals Application and Research Center. Wistar albino male rats weighing 290±30 and 12-14 weeks old were used for the experiment. The animals were kept at 23 °C room temperature, 60±5% humidity and 12:12 light-dark cycle, and free to access food and water.

2.2. Experimental Groups

The animals were divided into two experimental groups as acute (n=42) and chronic (n=42) groups. Then, each of the acute and chronic groups was divided into 6 subgroups (Table 1, 2). The substances specified in Table 1 were administered to the rats in the acute group and the substances specified in Table 2 were administered to the rats in the chronic group intraperitoneally (i.p). Electrocorticography (ECoG) recordings were obtained on

Table 1. Acute metformin administered groups

Group no	Groups	Substances supplied	Amount given	Delivery method	Number of animals
1	Sham group	-	-	-	7
2	Control group	Saline	1 mL/kg	i.p	7
3	Penicillin group	Penicillin	500 IU/2 µl	i.c	7
4	AOMet_200	Metformin	200 mg/kg	i.p	7
5	AMet_100 group	Metformin	100 mg/kg	i.p	7
6	AMet_200 group	Metformin	200 mg/kg	i.p	7

i.c: intracortically, i.p: intraperitoneally

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the same day in the acute groups, and 22nd day in the chronic groups. Metformin used in the study (BioVision incorporated 155 S. Milpitas Boulevard, Milpitas, CA 95035 USA) was dissolved in saline. Metformin was administered at the doses of 100 mg/kg and 200 mg/kg i.p. to the rats. As an anesthetic, urethane (Sigma-Aldrich Chemical Co., St. Louis, Missouri, USA) was used at a dose of 1.25 g/kg i.p. To induce epilepsy, 500 IU/2µl penicillin (I.E. Ulagay Ilac Sanayi Turk A.S. Istanbul, Turkey) was injected intracortically (i.c.) to the rats. The substances used in the study were prepared daily.

2.3. Electrophysiological Study Procedure

2.3.1. Surgical Procedure

In all groups, rats were fixed to the stereotaxic frame (Harvard Instruments, South Natick, MA, USA) under the 1.25 g/kg urethane anesthesia, and bone on left cerebral cortex was precisely removed with tour motor (FST Rechargeable Microdrill, KF Technology, Rome, Italy).

2.3.2. Stimulation of the Epileptiform Activity

Epileptiform activity was induced by intracortical administration of 500 IU/2 µl penicillin with a Hamilton microinjector (701N, Hamilton Co. Reno, NV, USA) to 2 mm lateral, 1 mm in front of the bregma line and 1.2 mm cortex depth in the skull of rats.

2.3.3. Electrophysiological Recordings

For electrophysiological recordings, two Ag-AgCl ball electrodes were placed in the visible somatomotor cortex area by opening the lateral part of the bregma line on the left hemisphere. A reference electrode was placed to the right ear of the rats. The first electrode to take recordings was placed 1 mm in front of the bragma line and 2 mm lateral to the sagittal suture, the second was placed 5 mm posterior to the bregma line and 2 mm lateral to the sagittal suture. After the electrodes were placed, ECoG was recorded by the PowerLab/8SP (ADInstruments Pty Ltd. Castle Hill, NSW, Australia) data acquisition recording system.

Five minutes of basal activity was recorded before injecting the substances for both acute and chronic groups. In acute groups, metformin was given i.p after basal activity recording, and ECoG was recorded for another 30 minutes (Figure 1A). After 30 minutes of ECoG recording, intracortical penicillin was injected i.c. at the 35th minute. Then, another 120-minute recording was made. In the only metformin group without penicillin, after 5 minutes of basal activity recording, 200 mg/kg metformin was injected, and another 150-minute ECoG recording was made. Thus, a total of 155 minutes of ECoG recording was obtained from each animal (Figure 1A).

In the chronic metformin group, substances were given i.p at the same time intervals for 21 days. ECoG recording was made on the 22nd day in chronic groups. After removing the bone on the left somatomotor area as described in the surgical procedure, two recording electrodes were placed in this area. Five minutes of basal activity was recorded after the electrodes were placed. After basal activity recording, penicillin was injected i.c. and another 120-minute recording was made. A total of 125 minutes of ECoG recording was made in each animal (Figure 1B).

After 5 minutes of basal activity recording to both acute and chronic groups, penicillin was administered i.c and ECoG recording was made for another 120 minutes. These recordings were digitized at a sampling rate of 1024 Hz. The onset time of first epileptiform activity, general epileptiform activity, spike wave frequency and spike wave amplitude were evaluated. The data analyses were made with the PowerLab Chart v.8 software program. For each animal, the average spike wave frequency and amplitudes of the 5-minute time intervals of the ECoG recordings were used as data.

2.4. Determination of Antioxidant Activity

In order to determine the CAT, SOD and GPx levels in blood serum samples taken after ECoG from rats, samples were centrifuged at 4000 rpm for 15 minutes (Heraeus Labofuge 400, Thermo Scientific, Waltham, Massachusetts, USA). Serums taken after centrifugation were taken into eppendorf tubes and stored at -80 °C until the test day. SOD, CAT, GPx (Shanghai Sunred Biological Technology Co., Ltd, Shanghai, China) were used as ELISA kits in our study. Spectrophotometer (Bio-Rad model 680 microplate reader, Bio-Rad Labrotories, Inc, USA) at 450 nm light wavelength was used to perform plate reading, and optical densities were recorded.

2.5. Statistical Analysis

Analysis of epileptiform activity records were made in periods of five minutes. The differences between the groups in terms of the onset of the first epileptiform activity, the spike wave frequency and the spike wave amplitude in each period were examined with the One-Way ANOVA test. Homogeneous subgroups multiple

Table 2. Chronic metformin administered groups

Group no	Groups	Substances supplied	Amount given	Delivery method	Number of animals
1	Sham group	-	-	-	7
2	Control group	Saline	1 mL/kg	i.p	7
3	Penicillin group	Penicillin	500 IU/2 µl	i.c	7
4	COMet_200 group	Metformin	200 mg/kg	i.p	7
5	CMet_100 group	Metformin	100 mg/kg	i.p	7
6	CMet_200 group	Metformin	200 mg/kg	i.p	7

i.c: intracortically, i.p: intraperitoneally

comparison method was used to determine different groups. In the comparison of groups in terms of CAT, SOD, GPx values, the ANOVA test was used, and homogeneous subgroups multiple comparison method was used to determine the different groups. IBM SPSS program was used in the analyses of the data. P<0.05 was considered as statistically significant.

3. RESULTS

3.1. The Effects of Only Metformin Administration in Rats

It was found that the administration of different doses of metformin (100 and 200 mg/kg) had no effect on basal activity. Similarly, no epileptic activity was found in the control and sham groups.

3.2. Epileptiform Activity Recording in Rats

In the penicillin groups, epileptic discharges appeared with spike wave formations 3-5 minutes after penicillin injection.

3.2.1. The Effect of Metformin on the Latency of First Epileptiform Activity

The effect of metformin on the onset of first epileptiform activity induced by penicillin was compared with the penicillin group. Onset of first epileptiform activity was significantly prolonged in CMet_100, CMet_200, AMet_100, AMet_200 groups compared to penicillin group (P values respectively p=0.050, p=0.009, p=0.040, p=0.042) (*p<0.05 is significantly higher than penicillin group) (Figure 2, Table 3).

3.2.2. The Effect of Metformin on Spike Wave Frequency

Epileptiform activity as spike waves started within 3-5 minutes in penicillin groups. When the spike wave frequencies were compared in the first period (0-5 min) no significant difference was found (p>0.05) between acute, chronic and penicillin groups.

In the CMet_100 group, the spike wave frequency was significantly less than the penicillin group until the 5th period (20-25 min) (p<0.05). After the 5th period (20-25 minutes), the spike wave frequency of the CMet_100 group showed a tendency to decrease in parallel with the penicillin group without a significant difference until the end of the recording (p>0.05). The spike wave frequency in the CMet_100 group was statistically higher than AMet_100 and AMet_200 groups in the 6th-7th and 8th period (25-40 minutes) (p<0.05). In the 9th period (41-45 minutes), the spike wave frequency in the CMet_100 group was statistically and significantly higher than in the AMet_100 group (p<0.05). When CMet_100 and the other groups were compared, there was no significant difference between CMet_100 and CMet_200, AMet_100, and AMet_200 groups in terms of spike wave frequency starting from the 10th period (45-50 minutes) until the end of the 24th period (115-120 minutes) (p>0.05) (Figure 3). The spike wave frequency in the CMet_200 group compared to the penicillin group until the end of the 6th period except the 5th period was statistically higher (p<0.05). In the 8th period, the spike wave frequency in the CMet_200 group was statistically and significantly higher than the

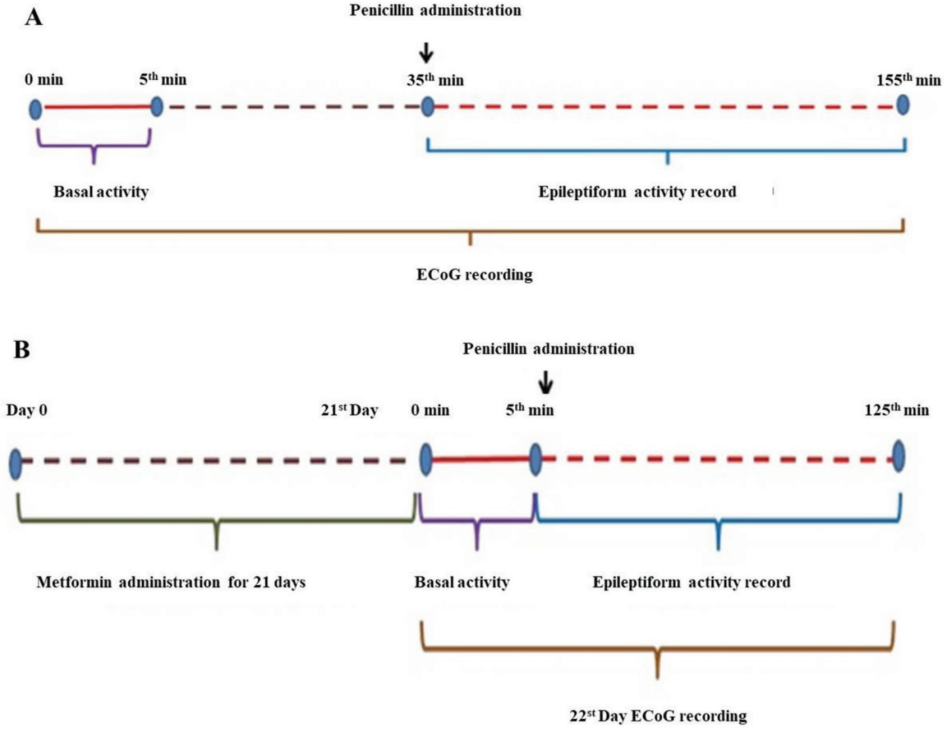

Figure 1. A) Experiment procedure and ECoG recording periods applied to acute groups. B) Experiment procedure and ECoG recording times applied to chronic groups
ECoG: electrocorticography

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AMet_100 and AMet_200 groups (p=0.004 and 0.005, respectively). In the 9th period, the spike wave frequency in the CMet_200 group was statistically and significantly higher than the AMet_100 group (p=0.050). When the spike wave frequency in CMet_200 group was compared with the other groups starting from the 10th period (55-60 minutes), there was no significant difference until the end of the 24th Period (115-120 minutes) (p>0.05) (Figure 3). When the spike wave frequency in the AMet_100 group was compared with the penicillin group up to the 10th period (except the 5th period), the spike wave frequency in the penicillin group was found statistically higher (p<0.05). The spike wave frequency in the AMet_100 group was statistically lower than the CMet_100 group in the 6th and 7th periods and the CMet_200 group in the 8th and 9th periods (p<0.05). When AMet_100 and other groups were compared, there was no significant difference between AMet_100 and CMet_100, CMet_200, and AMet_200 groups in terms of the spike wave frequency starting from the 10th period (45-50 minutes) to the end of the 24th period (115-120 minutes), (p>0.05), (Figure 3).

When the spike wave frequency in the AMet_200 group was compared with the penicillin group until the 9th period (except for the 5th period), the spike wave frequency in the penicillin group was statistically higher (except for the 5th period) (p<0.05).

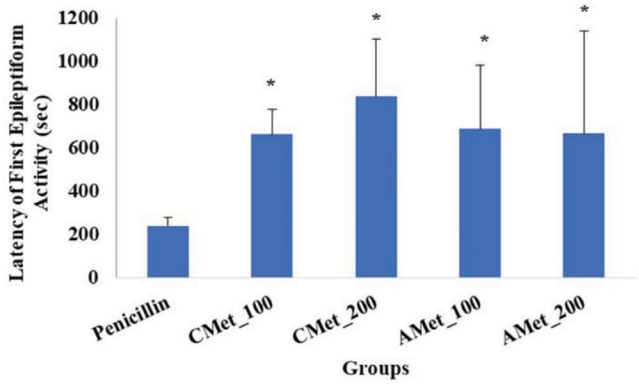

Figure 2. The effect of acute and chronic metformin administration on epileptiform activity onset time compared to penicillin group (mean values marked with * are statistically significant compared to penicillin group p<0.05)

Table 3. The effect of metformin on the latency of first epileptiform activity

Groups	n	Mean ± SD (second)	Min (ng/mL)	Max (ng/mL)	p
Penicillin	7	240±22	196.00	265.00	
AMet_100	7	688±111*	240.00	1114.00	
AMet_200	7	666±158*	0.00	1500.00	0.001
CMet_100	7	663±52*	544.00	793.00	
CMet_200	7	837±109*	494.00	1200.00	

The effect of acute and chronic metformin on the latency of first epileptiform activity compared to penicillin group (*Compared to penicillin group p<0.05). SD: standard deviation, min: minimum, max: maximum

The spike wave frequency of AMet_200 group was statistically and significantly lower than the spike wave frequency of CMet_100 group in the 6th and 7th periods, and the spike wave frequency of CMet_200 group in the 8th period (p<0.05). When the AMet_200 and other groups were compared, there was no significant difference between the AMet_200 and CMet_100 mg/kg, CMet_200, and AMet_100 groups starting from the 10th Period (45-50 minutes) to the end of the 24th Period (115-120 minutes) in terms of the spike wave frequency (p>0.05) (Figure 3, Table 4).

3.2.3. The Effect of Metformin on Spike Wave Amplitude

The effect of metformin on spike wave amplitude was compared with penicillin groups and between CMet_100, CMet_200, AMet_100, AMet_200 groups. Significance value was accepted as p<0.05. AOMet_200 group and COMet_200 group were not compared, since seizures did not occur.

In the 1st and 2nd periods (0-5 min and 6-10 min), the spike wave amplitudes in the penicillin group were significantly higher than the CMet_100, CMet_200, AMet_100, AMet_200 groups (p=0.000; p=0.000, respectively. p=0.000; p=0.000). In the 3rd and 7th periods, the spike wave amplitudes in the penicillin group were significantly higher than the CMet_200, AMet_100, AMet_200 groups (p<0.05). Between the 5th and 7th periods, the spike wave amplitudes in the CMet_100 group were significantly higher than the CMet_200, AMet_100 and AMet_200 groups (p<0.05). In the 8th period, the spike wave amplitude in the penicillin group was significantly higher than the amplitude of the AMet_100 group (p=0.026). The spike wave amplitude in CMet_100 group was significantly higher than in AMet_100 group (p=0.004). Spike wave amplitude in AMet_100 group was significantly higher than CMet_100 group (p=0.006). In the 9th period, the spike wave amplitude in the penicillin group was higher than the spike wave amplitude in the AMet_100 group (p=0.017). Spike wave amplitude in CMet_100 group was higher in AMet_100 group (p=0.025). The spike wave amplitude in the penicillin group was higher than the spike wave amplitude in the AMet_100 group (p=0.042). The spike wave amplitude in the CMet_100 group was higher than the AMet_100 group (p=0.005). In the 11th period, the spike wave amplitude in CMet_100 group was higher than the spike wave amplitude in AMet_100 group (p=0.025). In the 12th period, the spike wave amplitude in the CMet_100 group was higher than the AMet_100 group and the AMet_200 group (p=0.008; 0.048). In the 13th period, the spike wave amplitude in the CMet_100 group was higher than the AMet_100 group (p=0.008). In the 15th period, the spike wave amplitude in CMet_100 group was higher than AMet_100 group and AMet_200 group (p=0.042 and p=0.047). Between the 16th and 23rd periods, the spike wave amplitude in the CMet_100 group was higher than the AMet_100 group (p<0.05). In the 24th period, the spike wave amplitude in the CMet_100 group was higher than the AMet_100 group and CMet_200 group (p=0.027) (Figure 4, Table 5).

3.3. Determination of Blood Serum Antioxidant Activity of Metformin

3.3.1. Effect of Metformin Administration on CAT Level

Serum CAT value in COMet_200 group was statistically and significantly higher than penicillin, AMet_100, AMet_200, AOMet_200, CMet_100, CMet_200 groups (p=0.000; p=0.007; p=0.008; p=0.011; p=0.000; and p=0.000, respectively) (Figure 5, Table 6).

3.3.2. Effect of Metformin Administration on GPx Level

Serum GPx value in AOMet_200 group was statistically and significantly higher than CMet_100, CMet_200, COMet_200 and penicillin groups (p=0.049; p=0.007; p=0.005; and p=0.049, respectively) (Figure 6, Table 7).

3.3.3. Effect of Metformin Administration on SOD Dismutase Level

Serum SOD level in AMet_200 group was statistically and significantly higher than the CMet_200 and COMet_200 groups (p=0.009 and p=0.007, respectively). Although serum SOD level in AMet_200 group was higher than penicillin group, it was not statistically significant (p>0.05), (Figure 7, Table 8)

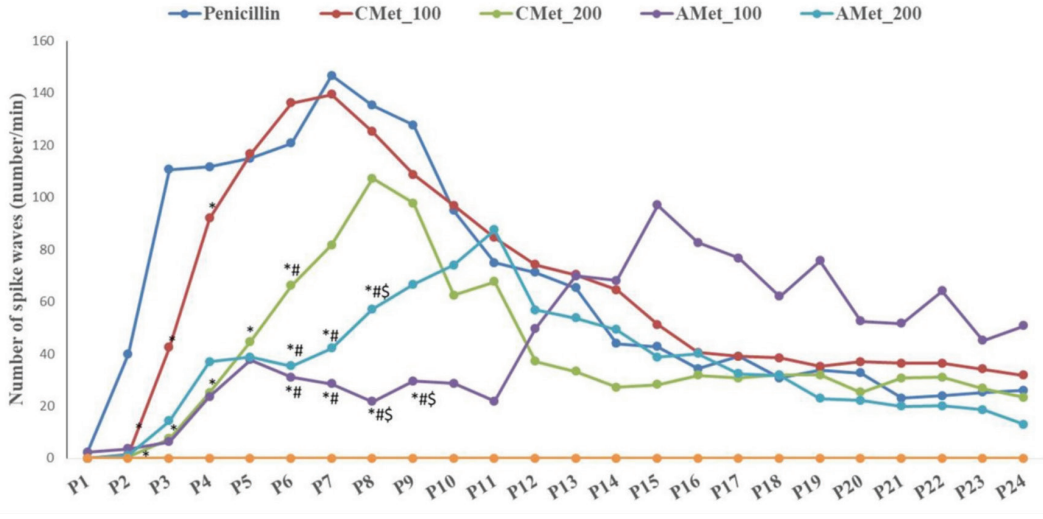

Figure 3. Number of spike waves in penicillin group and acute and chronic groups [mean values marked with * are statistically significant compared to penicillin group (p<0.05); mean values marked with # are statistically significant compared to CMet_100 group (p<0.05); and mean values marked with $ are statistically significant compared to the CMet_200 group (p<0.05)]

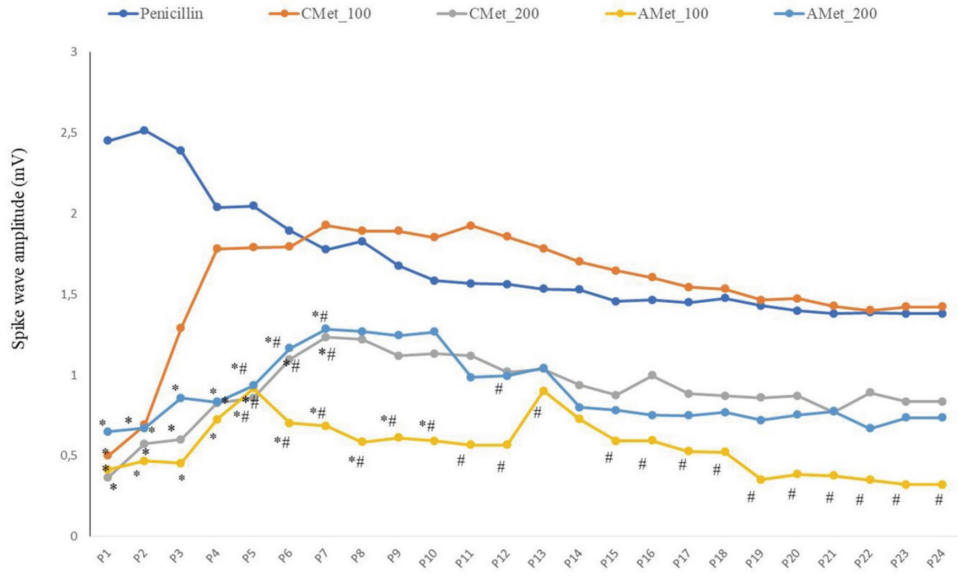

Figure 4. Spike wave amplitude [mean values marked with * are statistically significant compared to penicillin group (p<0.05); mean values marked with # are statistically significant compared to CMet_100 group (p<0.05)]

Table 4. The effects of penicillin, AMet_100 mg/kg, AMet_200 mg/kg, CMet_100 mg/kg, CMet_200 doses of metformin on frequency of penicillin-induced epileptiform activity

Time	Penicillin			AMet_100			AMet_200			CMet_100			CMet_200			p
	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	
0-5	2.33±1.4529	0.00	5.00	2.28±2.28	0.00	16.00	0±0	0.00	0.00	0±0	0.00	0.00	0±0	0.00	0.00	0.555
6-10	40.00±25.00	15.00	90.00	3.5714±1.82*	0.00	12.00	1.111±0.80*	0.00	7.00	1.40±0.87*	0.00	4.00	0.66±.66*	0.00	4.00	0.001
11-15	250.66±126.1	21.00	456.00	6.28±4.37*	0.00	32.00	14.33±9.27*	0.00	78.00	42.40±15.29*	3.00	76.00	7.50±6.716*	0.00	41.00	0.001
16-20	111.66±105.72	0.00	323.00	23.6±9.1*	2.00	69.00	37±16.2*	0.00	118.00	92±33.0	8.00	161.00	25.2±14.0*	0.00	75.00	0.026
21-25	84.00±55.00	28.00	194.00	37.8±17.0	0.00	113.00	39±20.0	0.00	140.00	117±38	19.00	202.00	45.0±21.4	0.00	120.00	0.068
26-30	121.0±36.0	58.00	182.00	31.1±16.4*#	2.00	126.00	35.4±21.2*#	0.00	169.00	136.2±36.8	33.00	218.00	66.2±28.1*#	0.00	145.00	0.007
31-35	146.7±42.0	78.00	223.00	28.6±17.5*#	1.00	131.00	42.2±22.0*#	0.00	185.00	139.4±25.8	49.00	200.00	81.7±22.1	10.00	150.00	0.001
36-40	135.3±46.4	84.00	228.00	21.7±17.0*#$	1.00	124.00	57.2±19.5*#$	0.00	175.00	125.2±26.1	28.00	185.00	107.2±17.0	51.00	168.00	0.001
41-45	127. 7±63.2	60.00	254.00	29.6±18.2*#	1.00	137.00	66.7±25.8*#	0.00	233.00	108.8±33.5	3.00	189.00	97.8±16.4	48.00	166.00	0.026
46-50	95±36.9	40.00	165.00	28.7±18.9	2.00	141.00	74±26.5	0.00	230.00	96.8±33.7	2.00	174.00	62.5±18.1	21.00	142.00	0.092
51-55	75±26.0	23.00	103.00	21.9±6.5	1.00	40.00	87.6±36.4	0.00	292.00	84.6±30.3	1.00	153.00	67.7±32.4	3.00	208.00	0.216
56-60	71.3±26.7	19.00	107.00	49.7±29.4	5.00	211.00	56.9±25.1	.00	206.00	74.2±30.5	2.00	160.00	37.2±15.4	2.00	99.00	0.445
61-65	65.3±24.2	18.00	98.00	69.8±49.3	2.00	360.00	53.7±24.24.9	.00	213.00	70.4±29.9	2.00	155.00	33.3±15.6	0.00	82.00	0.656
66-70	44±23.1	10.00	88.00	68.1±48.1	6.00	353.00	49.3±22.7	0.00	196.00	64.6±28.5	0.00	156.00	27.2±13.8	0.00	70.00	0.671
71-75	42.7±22.7	18.00	88.00	97±74.7	2.00	543.00	38.7±22.1	0.00	194.00	51.2±27.4	0.00	153.00	8.3±15.01	0.00	87.00	0.731
76-80	34.3±20.4	12.00	75.00	82.7±69.8	2.00	500.00	40.1±21.7	.00	193.00	40.6±27.3	.00	145.00	31.8±17.05	0.00	98.00	0.831
81-85	39±24	15.00	87.00	76.7±65.8	2.00	471.00	32.4±20.3	.00	186.00	39±25	0.00	132.00	30.8±17.2	0.00	106.00	0.844
86-90	30.7±23.7	7.00	78.00	62.1±56.0	0.00	398.00	31.8±17.5	0.00	159.00	38.4±27.1	0.00	139.00	32±18.0	0.00	113.00	0.887
91-95	33.7±25.7	6.00	85.00	75.7±55.0	0.00	386.00	22.9±15.0	0.00	138.00	35.2±25.2	0.00	129.00	32±18.3	0.00	111.00	0.705
96-100	32.7±23.4	4.00	79.00	52.6±42.3	0.00	300.00	22.1±16.9	0.00	155.00	37±26.7	0.00	137.00	25.3±13.3	0.00	69.00	0.844
101-105	23±19.6	1.00	62.00	51.7±40.3	.00	286.00	19.9±15.09	.00	138.00	36.4±25.2	0.00	128.00	30.8±16.4	0.00	93.00	0.816
106-110	24±22.5	0.00	69.00	64.1±42.4	0.00	271.00	20.1±16.1	0.00	147.00	36.4±24.9	0.00	126.00	31±17.0	0.00	96.00	0.682
111-115	25.3±20.9	1.00	67.00	45.1±34.4	0.00	243.00	18.6±15.4	.00	141.00	34.2±22.2	0.00	109.00	26.8±14.2	0.00	78.00	0.826
116-120	26±21.1	0.00	68.00	50.7±37.4	0.00	262.00	13±10.7	0.00	98.00	31.8±20.9	0.00	104.00	23.3 ±12.1	0.00	67.00	0.707

All values are number/minute. p<0.05 is considered statistically significant (*Compared to penicillin group, #Compared to CMet_100 mg/kg group, $Compared to CMet_200 mg/kg group)
SD: standard deviation, min: minimum, max: maximum

Table 5. The effects of penicillin, AMet_100 mg/kg, AMet_200 mg/kg, CMet_100 mg/kg, CMet_200 doses of metformin on amplitude of penicillin-induced epileptiform activity

Time (minute)	Penicillin			AMet_100			AMet_200			CMet_100			CMet_200			p
	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	
0-5	2.4±0.7	1.58	3.76	0.4±0.1*	0.26	0.75	0.6±0.1*	0.15	1.45	0.5±0.1*	0.31	0.71	0.4±0.1*	0.07	0.68	0.000
6-10	2.5±0.6	1.86	3.69	0.5±0.1*	0.16	1.10	0.7±0.1*	0.16	1.33	0.7±0.1*	0.32	1.06	0.6±0.2*	0.06	1.29	0.000
11-15	2.4±0.6	1.37	3.58	0.5±0.1*	0.11	1.09	0.9±0.2*	0.13	1.98	1.3±0.3	0.33	1.75	0.6±0.1*	0.14	0.89	0.000
16-20	2.2±0.5	1.47	3.27	0.7±0.2*	0.09	1.65	1.2±0.3*	0.12	2.60	1.5±0.3	0.39	2.35	0.7±0.2*	0.17	1.19	0.008
21-25	2.0±0.5	1.34	3.08	0.7±0.2*#	0.11	1.80	0.8±0.2*#	0.13	2.03	1.8±0.4	0.53	2.86	0.8±0.2*#	0.13	1.49	0.004
26-30	2.0±0.5	1.58	2.96	0.9±0.2*#	0.37	1.82	0.9±0.2*#	0.20	2.19	1.8±0.4	0.48	3.25	0.9±0.2*#	0.16	1.53	0.008
31-35	1.9±0.3	1.46	2.64	0.7±0.2*#	0.16	2.12	1.2±0.2*	0.18	2.27	1.8±0.5	0.38	3.16	1.1±0.2	0.25	1.50	0.018
36-40	1.8±0.3	1.35	2.38	0.7±0.2*#	0.17	1.94	1.3±0.08	0.35	2.33	1.9±0.5	0.43	3.49	1.2±0.2	0.32	1.98	0.012
41-45	1.8±0.4	1.24	2.63	0.6±0.2*#	0.13	1.86	1.3±0.3	0.28	2.37	1.9±0.5	0.38	3.55	1.2±0.3	0.34	2.27	0.015
46-50	1.7±0.3	1.26	2.25	0.6±0.2*#	0.09	1.95	1.2±0.2	0.27	2.40	1.9±0.5	0.32	3.71	1.1±0.3	0.34	2.29	0.025
51-55	1.6±0.3	1.06	2.19	0.6±0.2#	0.09	1.91	1.3±0.2	0.27	2.42	1.9±0.6	0.33	4.06	1.1±0.3	0.45	2.43	0.044
56-60	1.6±0.3	1.04	2.20	0.6±0.2#	0.10	1.69	1.0±0.3#	0.16	2.32	1.9±0.7	0.37	4.60	1.1±0.3	0.54	2.37	0.008
61-65	1.6±0.3	1.04	2.13	0.6±0.2#	0.10	1.66	1.0±0.2	0.15	2.26	1.9±0.7	0.46	4.46	1.0±0.3	0.52	2.27	0.008
66-70	1.5±0.3	1.08	2.09	1.0±0.2	0.25	1.68	1.0±0.2	0.17	2.26	1.8±0.7	0.40	4.53	1.0±0.2	0.61	2.10	0.140
71-75	1.5±0.4	0.97	2.20	0.7±0.2#	0.31	1.52	0.8±0.3#	0.18	2.08	1.7±0.7	0.32	4.42	0.9±0.2	0.46	1.97	0.042
76-80	1.5±0.3	0.97	2.01	0.6±0.2#	0.27	1.46	0.8±0.2	0.18	1.99	1.6±0.7	0.32	4.48	0.9±0.2	0.49	1.88	0.026
81-85	1.5±0.4	0.87	2.11	0.6±0.1#	0.20	1.22	0.75±0.2	0.17	1.94	1.6±0.8	0.37	4.50	0.9±0.2	0.47	1.83	0.033
86-90	1.4±0.4	0.85	2.11	0.5±0.1#	0.17	1.14	0.7±0.2	0.20	1.93	1.5±0.7	0.30	4.44	1.0±0.2	0.46	1.64	0.029
91-95	1.5±0.4	0.84	2.13	0.5±0.1#	0.16	0.87	0.8±0.2	0.18	2.08	1.5±0.8	0.18	4.68	0.9±0.2	0.43	1.61	0.042
96-100	1.4±0.4	0.69	2.18	0.4±0.1#	0.17	0.64	0.7±0.2	0.19	2.03	1.5±0.8	0.19	4.53	0.9±0.2	0.44	1.52	0.020
101-105	1.4±0.4	0.70	2.10	0.4±0.1#	0.15	0.99	0.8±0.2	0.16	2.38	1.5±0.8	0.19	4.67	0.9±0.1	0.47	1.50	0.031
106-110	1.4±0.4	0.65	2.07	0.4±0.09#	0.15	0.88	0.8±0.2	0.16	2.28	1.4±0.8	0.22	4.51	0.8±0.2	0.42	1.46	0.031
111-115	1.4±0.4	0.62	2.13	0.3±0.1#	0.13	0.67	0.7±0.2	0.16	2.22	1.4±0.8	0.18	4.47	0.8±0.2	0.43	1.77	0.031
116-120	1.3±0.4	0.69	2.04	0.3±0.2#	0.10	0.75	0.7±0.2	0.17	2.38	1.5±0.8	0.24	4.55	0.8±0.2	0.43	1.95	0.027

All values are number/minute. $p<0.05$ is considered statistically significant. (*Compared to penicillin group, #Compared to CMet_100 mg/kg group)
SD: standard deviation, min: minimum, max: maximum

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4. DISCUSSION

Epilepsy is a neurological disease that is characterized by changes in molecular, cellular and neuronal activity, and affects approximately 1% of the world's population (11). Although the mechanisms of epilepsy are better understood recently, drug resistance and side effects that limit the pharmacological treatment of epilepsy prevent the disease from cure completely. This reflects the need to develop new treatment strategies to end the progression of epilepsy and minimize associated comorbidities (12).

In this study, penicillin was used to induce epileptiform activity. After the intracortical administration of 500 IU penicillin, spike-wave activity was observed within 3-5 minutes. This value was determined as 8-14 minutes in the acute metformin groups and 10-16 minutes in the chronic metformin groups. It was observed that this activity reached the maximum frequency and amplitude in about 35th minute in penicillin groups. Cortical pyramidal cells play an active role in the development of epileptiform

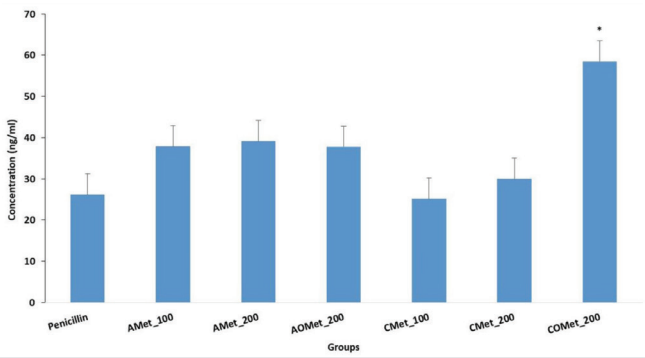

Figure 5. Showing the effects of acute and chronic metformin applied groups on serum catalase value in column graph (mean values marked with * are statistically significant from penicillin, AMet_100, AMet_200, AOMet_200, CMet_100, CMet_200 groups, p<0.05)

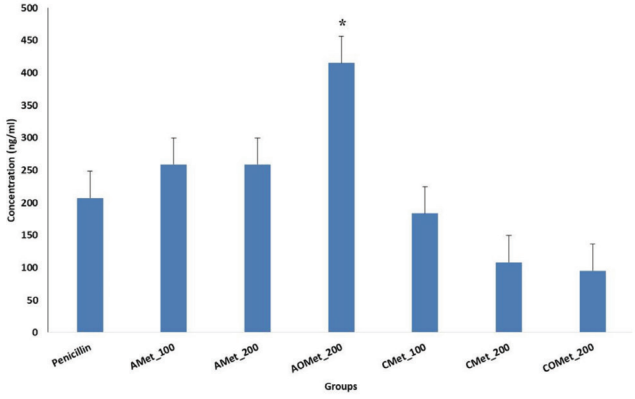

Figure 6. Showing the effect of acute and chronic metformin applied groups on serum GPx value (mean values marked with * are statistically and significantly higher than penicillin, CMet_100, CMet_200, COMet_200 groups, p<0.05)
GPx: glutathione peroxidase

Table 6. The effect of acute and chronic metformin applied on serum CAT level

Parameter	Groups	n	Mean ± SD (ng/mL)	Min (ng/mL)	Max (ng/mL)	p
CAT	Penicillin	7	26.2±3.8	14.00	37.00	0.001
	AMet_100	7	37.9±6.1	21.00	67.00	
	AMet_200	7	39.1±2.3	29.00	50.00	
	AOMet_200	7	37.8±2.0	32.00	43.00	
	CMet_100	7	25.1±4.1	15.00	45.00	
	CMet_200	7	30±4.1	17.00	49.00	
	COMet_200	7	58.5±10*	44.00	88.00	

p<0.05 is considered statistically significant, *compare to Penicillin, AMet_100, AMet_200, AOMet_200, CMet_100 and CMet_200 groups. SD: standard deviation, min: minimum, max: maximum, CAT: catalase

Table 7. The effect of acute and chronic metformin applied on serum GPx level

Parameter	Groups	n	Mean ± SD (ng/mL)	Min (ng/mL)	Max (ng/mL)	p
GPx	Penicillin	7	207±70.3	109.00	411.00	0.001
	AMet_100	7	258.7±46.5	83.00	409.00	
	AMet_200	7	258.6±55.6	4.00	441.00	
	AOMet_200	7	415±39.7*	336.00	461.00	
	CMet_100	7	183.3±130.3	39.00	443.00	
	CMet_200	7	108±34.3	13.00	165.00	
	COMet_200	7	94.8±35.1	14.00	178.00	

p<0.05 is considered statistically significant, *compare to Penicillin, CMet_100, CMet_200, COMet_200 groups. SD: standard deviation, min: minimum, max: maximum, GPx: glutathione peroxidase

activity induced by penicillin. Studies have shown that penicillin administration causes both focal and generalized seizures (13). The penicillin model is frequently used to answer questions about the mechanism of epilepsy (14).

Penicillin performs its activity through the GABAA receptor and blocks the inhibition of the GABAA receptor. There are many studies which claim that penicillin blocks the GABA receptor and induces epileptiform activity in rats. Arslan et al. (15) triggered epileptiform activity with penicillin (2.5 µl, 500 units, ic) in rats. Zhou et al. (16) also induced epileptiform activity with intraperitoneal injection of penicillin (10 million units/kg). In this study, we also obtained similar results which were compatible with previous studies and triggered epileptiform activity by administering penicillin. It was observed that only acute and chronic administration of metformin did not cause any epileptiform activity in rats. These data show that metformin does not have any epileptic effect in animals.

It has been shown in many experimental epilepsy studies that merformin, which is not used as an anti-epileptic drug, has anti-epileptic properties such as delaying the onset of seizures, decreasing the frequency and duration of seizures, as well as it causes behavioral and cognitive improvement, and it has antioxidant and neuroprotective effects (9,10,17,18).

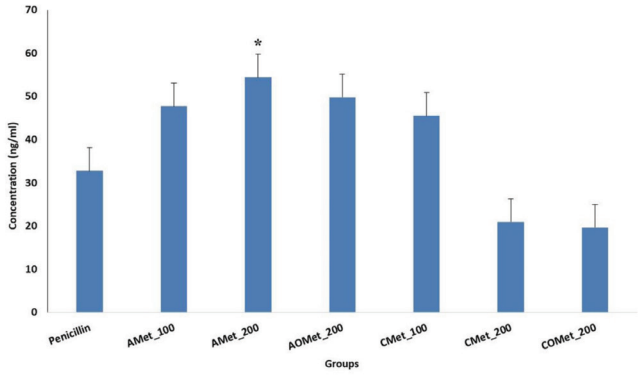

Figure 7. Showing the effect of acute and chronic metformin applied groups on serum SOD value in column chart (mean values marked with * are statistically and significantly higher than CMet_200, COMet_200 groups, p<0.05)
SOD: superoxide dismutase

Nevertheless, the mechanism underlying the anti-epileptic effect of metformin is not fully understood, but the antiepileptic effect of metformin is associated with its potential to improve brain oxidative damage, activate AMPK, inhibit mTOR and reduce apoptotic neuronal cell death, α'-synuclein, BDNF, and TrkB (19).

The AMPK activation may attenuate the generation of seizures by delaying the onset of epilepsy, reducing neuronal loss in the hippocampus, and preventing cognitive impairments in both acute and chronic epilepsy models. In the case of metformin, its antiepileptic effects could be attributed to an amelioration of oxidative brain damage, activation of the AMPK pathway, inhibition of the mTOR signaling, downregulation of BDNF, and TrkB levels or improvement of proteostasis (6,19).

Metformin has beneficial effects on several neurological disorders that could be attributed to both AMPK-dependent and AMPK-independent mechanisms of action. As a whole, metformin improves mitochondrial function [thereby reducing reactive oxygen species (ROS) production and oxidative stress], reduces the inflammatory response, reduces glucose production, and improves proteostasis (enhances the degradation of toxic aggregates) (20).

In our study, epileptogenesis was not examined molecularly, but it was shown electrophysiologically that it delayed seizures. We think that the anti-epileptic effect of metformin is through the pathways listed above.

Kainic acid and pilocarpine, which are used to induce TLE, status epilepticus, and other types of epilepsy in animals, have been the most widely used agents to provide a better understanding of the epileptogenesis process (21). In a study, abnormal neurogenesis is significantly increased in KA-induced status epilepticus, and metformin pretreatment has been shown to reduce this abnormal neurogenesis. In the same study, they reported that metformin significantly reduced spontaneous seizure activity and the number of epileptic spikes one month after KA administration, and that metformin was an antidiabetic drug with the potential to be used as a safe and well-tolerated antiepileptic drug (22).

Both acute and chronic epilepsy models induced by PTZ, which is an another agent to induce epileptiform activity, are frequently used in the studies (18,23). Results from these studies showed that

Table 8. The effect of acute and chronic metformin applied on serum SOD level

Parameter	Groups	n	Mean ± SD (ng/mL)	Min (ng/mL)	Max (ng/mL)	p
SOD	Penicillin	7	32.7±10.9	9.00	75.00	0.040
	AMet_100	7	47.7±8.9	22.00	77.00	
	AMet_200	7	54.4±7.1*	33.00	97.00	
	AOMet_200	7	49.8±18.5	8.00	114.00	
	CMet_100	7	45.5±5.1	25.00	61.00	
	CMet_200	7	21.0±8.1	0.78	47.00	
	COMet_200	7	19.7±12.7	6.00	45.00	

p<0.05 is considered statistically significant, *compare to CMet_200, COMet_200 groups. SD: standard deviation, min: minimum, max: maximum, SOD: superoxide dismutase

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metformin had a significant antiepileptic potential in experimental epilepsy models with PTZ, kainic acid and pilocarpine, by delaying seizures, reducing the frequency and duration of seizures, and reducing oxidative stress (9,10,18,23,24).

Experimental epilepsy model induced by penicillin was used in the present study. It was noticed in previous studies that metformin was not used in the penicillin model.

In addition, in our study, unlike other literature, the time periods in the acute and chronic models were different, and epilepsy was evaluated electrophysiologically and with antioxidant enzyme activity. In some literature, it has been reported that epileptiform activity may be mediated by behavioral scores (racine scoring) and the potential to activate AMPK and inhibit mTOR at the molecular level, reduced apoptotic neuronal cell death, downregulation of α'-synuclein, BDNF, and TrkB (19,25).

It was shown electrophysiologically in the present study that metformin in different doses (CMet_100, CMet_200, AMet_100, AMet_200) in both acute and chronic groups delayed the onset of seizures and decreased the frequency and amplitude of spike waves. The spike wave frequency in the CMet_200 group was significantly less than in the penicillin group until the end of the 6th period. The spike wave frequency in the AMet_100 group was significantly less than in the penicillin group up to the 10th period. The spike wave frequency in the AMet_200 group was significantly less than in the penicillin group until the 9th period. Epileptiform activity was significantly less in the CMet_200 and AMet_200 dose groups than the penicillin group, up to the 8th period, with spike wave amplitude. Spike wave amplitude in AMet_100 group wass significantly less than penicillin group until the end of the 10th period.

It is known that the risk of epilepsy is higher in diabetic patients (26). Therefore, metformin may be a potential pharmacological agent, especially for patients with diabetes and epilepsy. Seizures cause cognitive impairment in patients with epilepsy in the long term. In experimental epilepsy studies, metformin therapy improved epilepsy-related behavioral disorders (19). All these data demonstrate the potential role of metformin in preventing symptoms associated with epilepsy. Metformin is known to prevent cellular changes that cause epilepsy, such as neuronal cell loss, gliosis, and apoptosis (27,28). Metformin is also thought to inhibit oxidative stress, which plays a major role in the initiation and progression of epilepsy (29).

The role of oxidative stress in the initiation and progression of epileptic seizures has been widely recognized. Oxidative damage caused by neuronal hyper-excitability and excessive free radical production trigger the onset and progression of epilepsy (30). Metformin is known to exhibit antioxidant activity by reducing free radicals, including lipid peroxidation and enhanced glycation end products (18,23). Substantial evidence shows that metformin exerts antioxidant effects. Some of these can be attributed to the inhibition of mitochondrial complex I, which reduces production of ROS by the oxidative phosphorylation system respiratory chain (31). In addition, metformin has other functions related to the activation of the AMPK pathway: (1) reduction of ROS levels by upregulating the expression of antioxidant enzymes, such as thioredoxin, through the AMPK-FOXO3 pathway; (2) modulation of the expression of sirtuin 3 deacetylase, of which activity promotes antioxidant effects in the cell; (3) downregulation of NADPH oxidase, one of the main producers of cellular ROS; and (4) enhancement of mitochondrial biogenesis by enhancing the function of PGC1 alpha transcription factor (32). In addition, it has an antioxidant effect by increasing the levels of SOD, CAT and (GSH) glutathione, which are antioxidant enzymes (18,23). It is estimated that the anti-epileptic effect of metformin is partially due to its antioxidant properties (18). In a study, postnatal metformin administration to rats exposed to valproic acid in the uterus increased CAT activity by 1.87-fold in prefrontal cortex (PFC), by 1.55-fold SOD activity in the hippocampus, by 2.08-fold GSH in the hippocampus, by 1.68-fold in the hippocampus, and by 2.63-fold in PFC (33). Another study demonstrated that metformin inhibited apoptosis by decreasing caspase 3 and 9 expression in epileptic mice (34).

In the present study, blood serum samples were studied to determine antioxidant activity. Serum CAT value in COMet_200 group was statistically and significantly higher than penicillin, AMet_100, AMet_200, AOMet_200, CMet_100, CMet_200 groups. Serum GPx value in the AOMet_200 group was statistically and significantly higher than the CMet_100, CMet_200, COMet_200 and penicillin groups. Serum SOD level in the AMet_200 group was statistically and significantly higher than CMet_200 and COMet_200 group. Although serum SOD level in the AMet_200 group was higher than the penicillin group, it was not statistically significant. When we evaluate our blood serum antioxidant enzyme values and tissue antioxidant enzyme levels shown in the literature, we can say that metformin increases the antioxidant enzyme activities and partially delays the development of epilepsy.

4.1. Study Limitations

Molecular parameters were not evaluated due to budget constraints.

5. CONCLUSION

In our study, it was concluded that metformin prolonged the onset of seizure latency, decreased spike wave frequency and amplitude, and increased the level of antioxidant enzymes in the epilepsy model induced by penicillin. The good safety profile of metformin suggests that the drug can be used in the treatment of epilepsy, alleviating epileptic seizures, decreasing the level of free radicals, and strengthening the antioxidant system. However, more experimental, preclinical, and clinical studies are needed to determine the long-term efficacy and safety of metformin in epilepsy.

Ethics Committee Approval: The ethical approval was obtained from Düzce University Animal Experiments Local Ethics Committee with the decision number 2019/6/1 (date: 02.07.2019), and the animals to be used

in the experiment were obtained from Düzce University Experimental Animals Application and Research Center.

Informed Consent: Animal experiment study.

Peer-review: Externally and internally peer-reviewed.

Author Contributions: Surgical and Medical Practices - Ü.K., E.B.; Concept - Ü.K., Ş.D.; Design - Ü.K., Ş.D., Ö.B.; Data Collection and/or Processing - Ü.K., Ş.D., E.B.; Analysis and/or Interpretation - Ü.K., Ş.D., E.B., H.S.; Literature Search - Ü.K., Ö.B., H.S.; Writing - Ü.K., Ö.B., H.S.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: This research was supported as financial by Duzce University Scientific Research Coordination Office (Project number: 2019.04.01.1022).

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