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Effect of Cyclooxygenase Inhibition on P-Glycoprotein Expression and Phenytoin Level in Brain Tissue of Pilocarpine Induced Epilepsy in Rats

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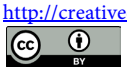


Abstract

Background: Increased brain P-glycoprotein (P-gp) expression may play important role in resistance to antiseizure drugs. The present work aimed to overcome the drug resistance that develop due to overexpression of P-gp with subsequent increase in brain phenytoin level in epileptic rats, using either non-selective (indomethacin) or selective (celecoxib) cyclooxygenase inhibitors. **Methods**: Fifty-six adult male albino rats were randomly divided into seven groups. Epilepsy was induced using the lithium pilocarpine model. Rats received indomethacin (2.5 mg/kg) or celecoxib (20 mg/kg), either alone or combined with phenytoin (50 mg/kg). Seizures were evaluated using Racine score. Motor coordination was assessed using open field and rotarod tests. Phenytoin brain level was measured using High Performance Liquid Chromatography (HPLC), glutamate expression was measured using Enzyme Linked Immunosorbent Assay (ELISA), ATP Binding Cassette Subfamily B Member 1 (ABCB1) gene expression was assessed using Real Time-Polymerase Chain Reaction (RT-PCR), and immunohistochemical analysis was done for P-gp expression. **Results**: Phenytoin combination with either indomethacin or celecoxib had improved the Racine score, motor coordination on rotarod apparatus, and open field test results. Also, phenytoin combination with either indomethacin or celecoxib decreased brain glutamate level, ABCB1 gene and P-gp expression, and increased brain phenytoin level compared to treatment with phenytoin alone. This indicated that both P-gp inhibitors indomethacin and celecoxib, increased the level of phenytoin that reached the brain of rats. However, brain uptake of phenytoin was significantly enhanced using celecoxib rather than indomethacin (CI 95%, 17.092: 32.808, P-value < 0.001). **Conclusion**: Cyclooxygenase inhibition using either celecoxib or indomethacin resulted in downregulation of P-gp expression, with subsequent increase

in brain phenytoin level in epileptic rats.

Keywords

P-Glycoprotein, Glutamate, Phenytoin, Indomethacin, Celecoxib, Epilepsy

Paper Highlights

- 30% of epileptic patients suffer from resistance to antiseizure drugs (ASD_S).
- P-glycoprotein (P-gp) over expression at the blood brain barrier (BBB) contributes to poor brain uptake of ASD_S.
- Cyclooxygenase (COX)-2 plays an essential role in post seizure inflammation and P-gp overexpression.
- COX inhibition using either celecoxib or indomethacin decreased P-gp expression at the BBB, with subsequent increase of brain phenytoin level in epileptic rats.

1. Introduction

Epilepsy is considered a chronic neurological disorder. In human, temporal lobe epilepsy (TLE) is the most common form of epilepsy [1]. Intracerebral or systemic administration of pilocarpine induces seizures resembling those of human complex partial seizures that can progress into status epilepticus (SE) [2]. The memory and cognitive impairment that are present in TLE patients are also found in pilocarpine treated rats. Antiseizure drugs (ASDs) that can treat complex partial seizures in human can also suppress spontaneous seizures in the pilocarpine model of epilepsy [3] [4].

The resistance to ASD_S represents a great health concern for the patients, their families, and the society [5]. The International League against Epilepsy (ILAE) had defined drug resistant epilepsy as failure of adequate trials of two tolerated and appropriately used ASDs, used either as monotherapy or in combination to produce sustained freedom from seizure [6]. Overexpression of adenosine triphosphate (ATP) Binding Cassette Subfamily B Member 1 (ABCB1) gene, which encodes P-glycoprotein (P-gp) expression at the blood brain barrier (BBB), is from the most considered theories to explain pharmacoresistance in epilepsy. Phenytoin is a P-gp substrate [7]. Consequently, the mechanism(s) that underlay P-gp over expression in neurons at the BBB, may constitute new therapeutic objective for controlling drug resistance in epilepsy. As a result, the co-administration of drugs that can prevent P-gp over expression or that are P-gp inhibitors can improve pharmacoresistance in epilepsy [8] [9]. Seizures mediate glutamate upregulation in the brain. Glutamate activates N-Methyl-D-Aspartate (NMDA) receptor giving the signal for arachidonic acid production, which is then oxidized by the activity of cyclooxygenase (COX)-2 to produce prostanoids, including prostaglandin (PG) E2. PGE2 activates the Prostaglandin E receptor 1 (EP1), leading to the expression of a second messenger system that increases the

transcription and the upregulation of P-gp at the BBB. Therefore, COX-2 plays an essential role in the hyperexcitability and post seizure inflammation during seizures, and the pharmacologic inhibition of COX-2 can provide neuroprotection, decreased P-gp expression, and greater uptake of phenytoin in brain [10] [11] [12].

Altogether, indicates that COX-2 inhibition can increase the response to antiseizure drugs in patients with P-gp mediated drug resistance. Both indomethacin (non-selective COX inhibitor) and celecoxib (selective COX-2 inhibitor) can decrease glutamate mediated P-gp induction in epileptic rats during seizures [13] [14].

In the present study, we determined the effect of indomethacin (non-selective COX inhibitor) or celecoxib (selective COX-2 inhibitor), on phenytoin brain level, glutamate level, ABCB1 gene expression, and P-gp expression in a model of pilocarpine induced epilepsy in rats.

2. Materials and Methods

2.1. Experimental Animals

Fifty-six adult male Wister rats (170 - 200 gm) were obtained from the animal house of the Ophthalmic Research Institute in Giza. Animals were housed in cages for acclimatization one week before the beginning of the study. Rats were kept under controlled laboratory conditions including normal day/night cycle, temperature 23˚C ± 3˚C, and humidity ranging from 50% - 55% with free access to standard rodent diet and tap water ad libitum.

2.2. Ethical Date of Approval

All experimental procedures were approved by the institutional animal care and use committee at Suez Canal University number 2554 on 11-11-2015, following the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals Publications No. 8023, revised 1978.

Every effort has been done to minimize suffering to the animals and to decrease the number of used animals.

2.3. Pharmacological Treatment

In the present study, we used pilocarpine 30 mg/kg intraperitoneal (*ip*), lithium chloride 127 mg/kg (*ip*), and scopolamine butylbromide1mg/kg (*ip*), to perform the model of SE in rats. Diazepam 10 mg/kg (*ip*) was used to terminate seizures [15]. Also, we used phenytoin (antiseizure drug) 50 mg/kg (*ip*) [16], indomethacin (non-selective COX inhibitor) 2.5 mg/kg (*ip*) [10], and celecoxib (selective COX-2 inhibitor) 20 mg/kg (*ip*) [17] to treat rats. Pilocarpine and lithium chloride were purchased as a white powder from (Sigma Aldrich Company, USA), and were dissolved in normal saline. Scopolamine butyl bromide was supplied in ampoules (20 mg/1ml) from Chemical Industries Development (CID) Company, Egypt. Diazepam was supplied in ampoules (5 mg/ml) from Memphis for Phar-

maceutical and Chemical Industries, Egypt. Phenytoin was supplied in ampoules (50 mg/ml) from Medical Union Pharmaceutics (MUP), Egypt. Indomethacin was supplied in ampoules (50 mg/2ml) from Nile Company, Egypt. Celexocib was purchased as white powder from Pfizer Laboratories, USA. Celexocib was dissolved in normal saline and filtered.

2.4. Induction of Status Epilepticus

The lithium pilocarpine model was used to induce epilepsy. Each rat was treated with lithium chloride 127 mg/kg (*ip*) 24 hours before the injection of pilocarpine. On the following day, each rat was injected with methyl scopolamine 1 mg/kg (*ip*) to reduce the peripheral effects of pilocarpine. Approximately after 30 minutes, the rats were injected with pilocarpine hydrochloride 30 mg/kg (*ip*) to induce seizures that progressed into SE. Diazepam was injected 10 mg/kg (*ip*) and administrated repeatedly up to three times for the suppression of continued seizure activity [15].

2.5. Study Groups

In the experiment, a total of fifty-six adult male albino rats had been used. The rats were randomly divided into 7 groups, 8 animals each.

Group (1) Control rats: Normal control rats received only 0.5 (ml) of normal saline (*ip*) as a solvent twice daily, without any medication [18].

Group (2) Epileptic non-treated rats: Rats received pilocarpine injection (*ip*) for the induction of epilepsy on the fourth day of the experiment [15].

Group (3) Phenytoin treated epileptic rats: Rats were given phenytoin on the fourth and fifth days respectively after the induction of epilepsy, in a dose of 50 mg/kg (*ip*) daily [10] [16].

Group (4) Indomethacin pre-treated epileptic rats: Rats received indomethacin 2.5 mg/kg (*ip*) daily during the first 3 days before pilocarpine injection and continued with the same dose after the induction of epilepsy on the fourth and fifth days respectively [10].

Group (5) Celexocib pre-treated epileptic rats: Rats received celecoxib in a dose of 20 mg/kg (*ip*) every 12 hours during the first 3 days before pilocarpine injection and celecoxib continued with the same dose after the induction of epilepsy on the fourth and fifth days respectively [10] [17].

Group (6) Pre-indomethacin/combined with phenytoin treated epileptic rats: Rats received indomethacin in a dose of 2.5 mg/kg (*ip*) daily, for 3 days before pilocarpine injection. After the induction of epilepsy, indomethacin dose continued in combination with phenytoin 50 mg/kg (*ip*) daily, during the fourth and fifth days respectively [10] [16].

Group (7) Pre-celecoxib/combined with phenytoin treated epileptic rats: Rats received celecoxib in a dose of 20 mg/kg (*ip*) every 12 hours for 3 days before pilocarpine injection. After the induction of epilepsy, celecoxib dose continued in combination with phenytoin 50 mg/kg (*ip*) daily, during the fourth and fifth days respectively [10] [16] [17].

2.6. Seizure Severity Score

Seizure activity was rated after 20 - 45 minutes following pilocarpine administration [15], according to the Racine scale, 1972 [19].

0 = No seizure response
1 = Immobility, eye closure, ear twitching, facial clonus
2 = Head nodding associated with more severe facial clonus
3 = Clonus of one forelimb
4 = Bilateral forelimb clonus without rearing
4.5 = Bilateral forelimb clonus with rearing
5 = Rearing and falling on back with generalized tonic-clonic seizures

2.7. Assessment of Motor Coordination and Exploratory Behavior

At the end of the experiment, on day five after the last phenytoin dose, motor coordination was evaluated as follows.

2.7.1. The Rotarod Test

The rotarod test was done for the evaluation of motor coordination. The test measured balance, coordination, and motor control. The rotarod apparatus consists of a suspended rod, which runs at constant speed. Each rat was placed on a rod (10 cm long and 5 cm in diameter). Rats were left on the rod for habituation about one minute. The rod was set to rotate at a rate of 6 rounds per minute over the course of 5 minutes. Normally the animals walk continuously forward to avoid falling from the apparatus. The falling time (time starting from putting the animal on the shaft of the rotarod, till it fell to the ground) was measured. The latency to fall is considered a measure of motor coordination and motor learning [20].

2.7.2. Open Field Test

Assessment was done in an open field chamber, measuring 115 × 115 × 44 cm. An arena was made of dark glass with painted white floor having black lines to form a 5 × 5 cm grid pattern. Rats were introduced individually in the center of the arena. The activity of the animal was recorded for 10 minutes. During this time, the exploratory behavior of rats was observed. The activity was recorded when the rat crossed the lines. Line-crossing was counted only when the rat crossed single line from one grid square into an adjacent grid with all four paws. Activity factor (A) (the number of squares crossed between two consecutive stops) = total number of squares divided by the total number of stops [21] [22].

2.8. Biochemical and Immunohistochemistry Measurement

On day 5, and one hour after the last phenytoin and COX inhibitor doses, rats from all groups were sacrificed, their brains were dissected on ice and divided into two lobes. The first lobe was frozen at −80˚C in liquid nitrogen and stored for the measurement of phenytoin brain level [17], glutamate expression [23], and analysis of ABCB1 gene expression [24]. The other lobe was immediately fixed in 10% buffered formalin for 24 hours, then embedded in paraffin, and

sectioned at a thickness of 5 μm thick for brain histopathological examination (hematoxylin and eosin staining) and immunohistochemistry staining for P-gp expression [25].

Determination of phenytoin level in brain: The level of phenytoin in brain tissue was measured using the HPLC technique described by [26].

Determination of glutamate expression in brain: ELISA technique was performed using Rat Glutamate (GLU) ELISA research kit from My Bio-Source.com (San Diego, USA), using the method described by [23].

Determination of ABCB1 expression in brain: RT-PCR was used to determine the expression of ABCB1 using Qiagen tissue extraction kit (Qiagen, USA) as described by [24].

2.9. Histopathology Analysis of Brain Tissues

The severity of neuronal damage was semi-quantitatively evaluated by a grading score: score 0, no obvious damage; score 1, slight lesions involving one-third of neurons; score 2, lesions involving two-thirds of the neurons; score 3, lesions involving more than two-thirds of the neurons. The neuronal loss must exceed 15 to 20%to be reliably detected by visual examination [10].

2.10. Immunohistochemistry and Image Analysis for P-Glycoprotein Expression

Sections of brain tissues were deparaffinized in xylene, rehydrated in graded ethanol, and pretreated with hydrogen peroxide for 15 minutes. Then, sections were soaked in a sodium citrate buffer (PH = 6.0) and heated in a microwave oven at 95˚C for about 20 minutes for antigen retrieval. The sections were then incubated in goat serum at room temperature for 20 minutes. After that, sections were incubated in the primary antibody [EPR10364-57] (Rabbit monoclonal antibody to P-gp, 1:50, Abcam, USA) at 4˚C overnight. Then sections were incubated in a secondary goat anti-rabbit antibody (Power-Stain™ 1.0 Poly HRP diaminobenzidine (DAB) Kit, Genemed, USA) at 37˚C for 30 minutes, and then incubated with the avidin-biotin complex (ABC) kit, Zhongshan Golden Bridge, at 37˚C for 30 minutes. Sections were then washed three times in phosphate buffer saline (PBS). After washing with PBS, the nickel intensified 3,3-(DAB) reaction was performed [0.05% DAB, 0.01%, nickel ammonium sulfate; both from Sigma, and 0.01% hydrogen peroxide (H_2O_2)] to visualize the sites of antibody binding. Counterstaining was carried out with Harris's hematoxylin stain for 30 seconds. PBS was used in place of the primary antibodies for negative controls [25].

P-gp staining of brain sections was done and assessed at magnification of 400x, using a computer-assisted image analysis system (Image J version 1.44 software) for obtaining %Area stained and integrated optical density (IOD). P-gp immunostaining was analyzed in the hippocampal and adjacent cortex [18] [25].

3. Statistical Analysis

All data were presented as mean ± SEM. One way analysis of variance (ANOVA)

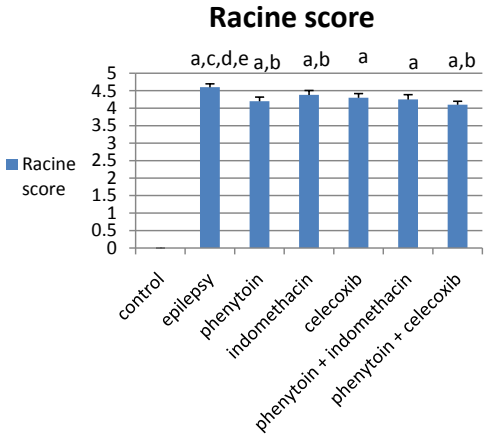
with Post-HocTukey test had been used for comparisons of data among the seven groups, with P-values < 0.05 considered statistically significant. ***Spearman correlation coefficients*** were used to assess the significant relation between two quantitative parameters in the same group.

4. Results

Effect of phenytoin combined with either indomethacin or celecoxib on Racine score in pilocarpine induced epilepsy in rats: as shown in Figure 1(a), Phenytoin administration improved the Racine score (CI 95%, 0.088: 0.712, P-value = 0.014) in comparison with epileptic non treated rats. Also, phenytoin combination with indomethacin (CI 95%, 0.019: 0.681, P-value = 0.039) and celecoxib (CI 95%, 0.188: 0.812, P-value = 0.003) improved the Racine score in comparison with the epileptic non treated rats.

Effect of phenytoin combined with either indomethacin or celecoxib on the latency time of falling from the rotarod apparatus in pilocarpine induced epilepsy in rats: Figure 1(b) showed that treatment with phenytoin (CI 95%, −127.133: −6.067, P-value = 0.032), phenytoin combined with indomethacin (CI 95%, −109.789: −25.711, P-value < 0.05), and phenytoin combined with celecoxib (CI 95%, −228.533: −107.467, P-value < 0.001) improved the latency of falling from the rotarod apparatus in comparison with epileptic non treated rats. The combination of phenytoin with celecoxib improved the latency of falling from the rotarod apparatus (CI 95%, −161.933: −40.867, P-value = 0.002) compared to phenytoin treated rats.

Effect of phenytoin combined with either indomethacin or celecoxib on the locomotor activity (open field test) in pilocarpine induced epilepsy in rats: Figure 1(c) showed that treatment with phenytoin increased the activity factor (CI 95%, −5.371: −2.224, P-value = 0.000) in comparison with epileptic non treated rats. Moreover, the combination of phenytoin with indomethacin (CI 95%, −4.280: −0.942, P-value = 0.004) or celecoxib (CI 95%, −4.829: −1.683, P-value = 0.000) resulted in increasing the activity factor in the open field test compared to epileptic non treated rats.



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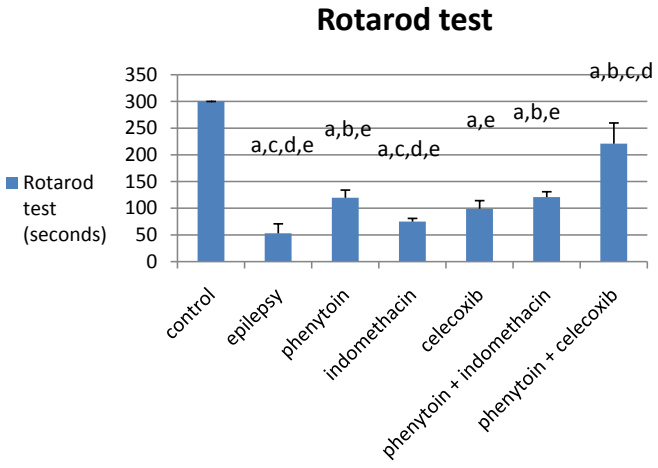

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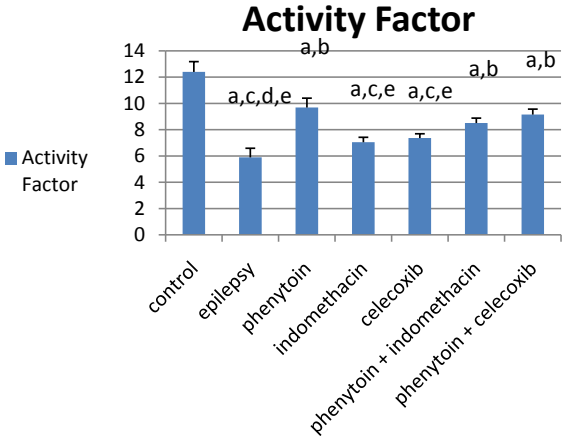


(c)

Figure 1. Effect of phenytoin combined with either indomethacin or celecoxib on pilocarpine induced changes in (a) Raine score, (b) the latency time of falling from the rotarod apparatus (seconds), (c) the activity factor (open field test) in rats. Values are means ± SE (n = 8); one-way ANOVA followed by with Post-Hoc Tukey test. [a,b,c,d,e]P < 0.05; [a]Compared with control rats. [b]Compared with non treated epileptic rats. [c]Compared with phenytoin treated rats. [d]Compared with phenytoin combined with indomethacin treated rats. [e]Compared with phenytoin combined with celecoxib treated rats.

Effect of P-glycoprotein inhibition with either indomethacin or celecoxib on brain level of phenytoin in pilocarpine induced epilepsy in rats: Figure 2 showed that combination of phenytoin with P-glycoprotein inhibitors like the non selective COX inhibitor, indomethacin (CI 95%, −23.108: −7.392, P-value = 0.001) and the selective COX 2 inhibitor, celecoxib (CI 95%, −47.608: −32.792, P-value < 0.001) increased the brain level of phenytoin in comparison with rats that received phenytoin only. There was a greater increase in the brain level of phenytoin in the group that received phenytoin combined with celecoxib (CI 95%, 17.092: 32.808, P-value < 0.001) compared to the group that received phenytoin combined with indomethacin. This indicated that both P-gp inhibitors

indomethacin and celecoxib, increased the level of phenytoin that reached the brain of rats. However, brain uptake of phenytoin was significantly enhanced using celecoxib rather than indomethacin (CI 95%, 17.092: 32.808, P-value < 0.001).

Effect of phenytoin combined with either indomethacin or celecoxib on brain glutamate level in pilocarpine induced epilepsy in rats: Figure 3(a) showed that treatment with phenytoin (CI 95%, 4.358: 30.842, P-value = 0.011), indomethacin (CI 95%, 1.147: 27.71, P-value = 0.03) and Celecoxib (CI 95%, 1.158: 27.642, P-value = 0.034) decreased the brain glutamate level in comparison with non treated epileptic rats. Also, the combination of phenytoin with either indomethacin (CI 95%, 11.105: 39.195, P-value = 0.001) or celecoxib (CI 95%, 16.758: −43.242, P-value < 0.001) reduced the brain glutamate level compared to non treated epileptic rats. The combination of celecoxib with phenytoin decreased the brain glutamate level to the extent that there was no significant difference from the values of normal control rats (P-value = 0.093).

Effect of phenytoin combined with either indomethacin or celecoxib on brain ABCB1 expression in pilocarpine induced epilepsy in rats: Figure 3(b) showed that treatment with phenytoin (CI 95%, 5.947: 8.853, P-value < 0.001), indomethacin (CI 95%, 7.209: −10.291, P-value < 0.05), and celecoxib (CI 95%, 6.947: 9.853, P-value < 0.001) decreased ABCB1 level compared to epileptic non treated rats. Also, the combination of phenytoin with indomethacin (CI 95%, 8.209: 11.291, P-value < 0.001) or celecoxib (CI 95%, 7.947: 10.853, P-value < 0.001) decreased the brain ABCB1 level in comparison with epileptic non treated rats. Moreover, the combination of phenytoin with indomethacin (CI 95%, 0.809: 3.891, P-value = 0.004) or celecoxib (CI 95%, 0.547: 3.453, P-value = 0.009) revealed more beneficial effects in reducing the ABCB1 level compared to rats treated with phenytoin only.

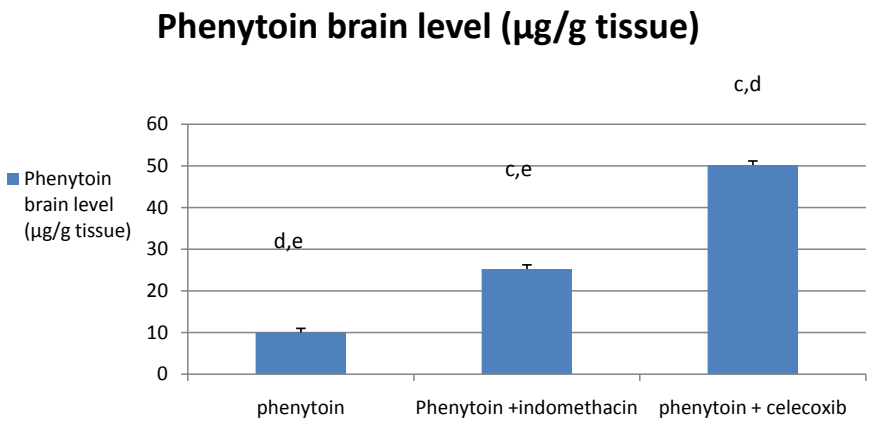
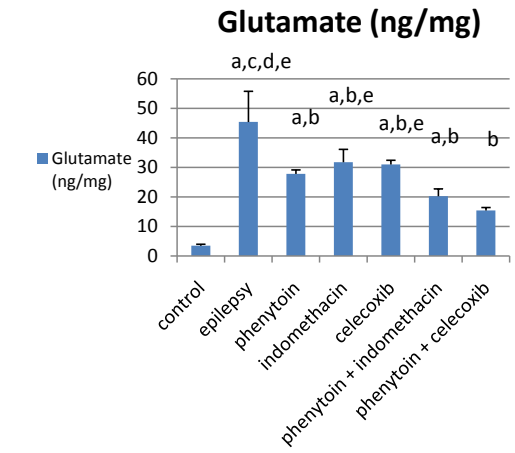
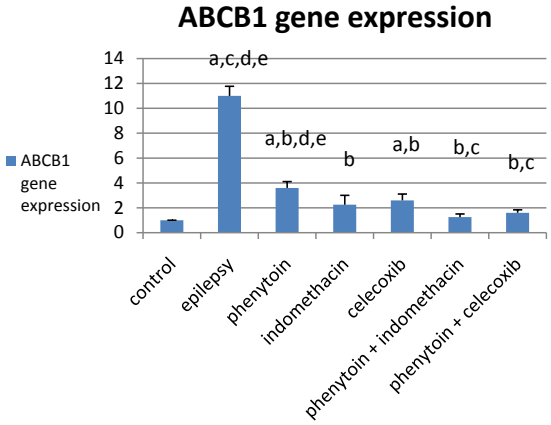


Figure 2. Effect of P-glycoprotein inhibition with either indomethacin or celecoxib on brain level of phenytoin in pilocarpine induced epilepsy in rats. Values are means ± SE (n = 8); one-way ANOVA followed by with Post-Hoc Tukey test. [a,b,c,d,e]P < 0.05, [a]Compared with control rats. [b]Compared with non treated epileptic rats. [c]Compared with phenytoin treated rats. [d]Compared with phenytoin combined with indomethacin treated rats. [e]Compared with phenytoin combined with celecoxib treated rats.



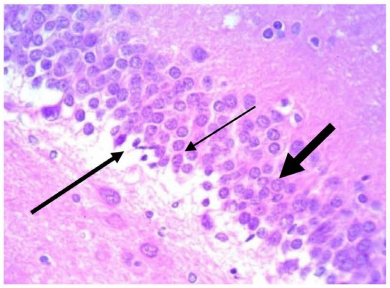
(a)



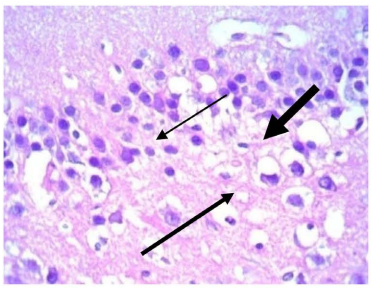
(b)

Figure 3. Effect of phenytoin combined with either indomethacin or celecoxib on pilocarpine induced changes in (a) Glutamate (ng/mg), (b) ABCB1 gene expression in rats. Values are means ± SE (n = 8); one-way ANOVA followed by with Post-Hoc Tukey test. [a,b,c,d,e]P < 0.05; [a]Compared with control rats. [b]Compared with non treated epileptic rats. [c]Compared with phenytoin treated rats. [d]Compared with phenytoin combined with indomethacin treated rats. [e]Compared with phenytoin combined with celecoxib treated rats.

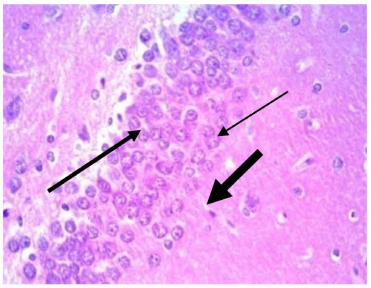
Effect of phenytoin combined with either indomethacin or celecoxib on the severity of neuronal damage in pilocarpine induced epilepsy in rats: Figure 4(a) and Figure 4(b) showed that treatment with phenytoin (CI 95%, 1.397: 2.603, P-value < 0.001), indomethacin (CI 95%, 0.411: 1.689, P-value = 0.002), and celecoxib (CI 95%, 0.597: 1.803, P-value < 0.001) reduced the degree of neuronal damage compared to epileptic non treated rats. Moreover, the combination of phenytoin with either indomethacin (CI 95%, 1.661: 2.939, P-value < 0.001) or celecoxib (CI 95%, 1.977: 3.203, P-value < 0.001) reduced the degree of neuronal damage in comparison with epileptic non treated rats. However, only the combination phenytoin with either indomethacin or celecoxib improved the degree of neuronal damage to the extent that there was no significant difference from normal control rats.



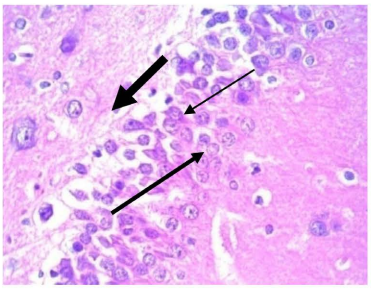
Control group stained with (H and E × 10)



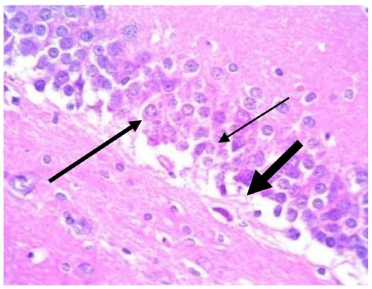
Epileptic non treated rats stained with (H and E × 10)



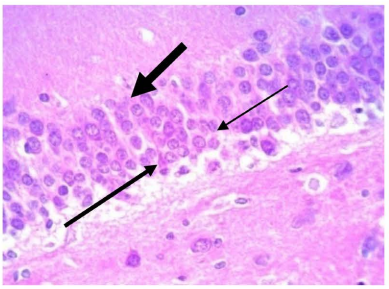
Phenytoin treated epileptic rats stained with (H and E × 10)



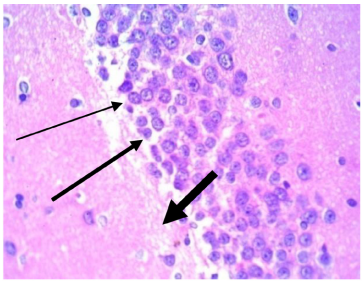
Indomethacin pre-treated epileptic rats stained with (H and E × 10)



Celecoxib pre-treated epileptic rats stained with (H and E × 10)



Pre-indomethacin/combined with phenytoin treated epileptic rats stained with (H and E × 10)



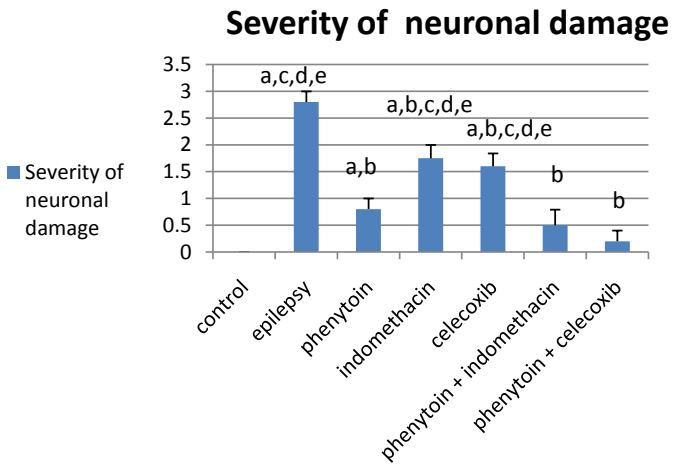
pre-celecoxib/combined with phenytoin treated epileptic rats stained with (H and E × 10)

→ restored layers of neurons

→ increased number of neurons showing normal vesicular nuclei

➡ residual mild degenerative changes

(a)



(b)

Figure 4. Effect of phenytoin combined with either indomethacin or celecoxib on pilocarpine induced neuronal damage in rats brain tissues. (a) Neuronal damage in the rat brain tissue among the studied groups. (b) Effect of phenytoin combined with indomethacin or celecoxib on the severity of neuronal damage in pilocarpine induced epilepsy in rats. Values are means ±SE (n = 8); one-way ANOVA followed by with Post-Hoc Tukey test. [a,b,c,d,e]$P < 0.05$; [a]Compared with control rats. [b]Compared with non treated epileptic rats. [c]Compared with phenytoin treated rats. [d]Compared with phenytoin combined with indomethacin treated rats. [e]Compared with phenytoin combined with celecoxib treated rats.

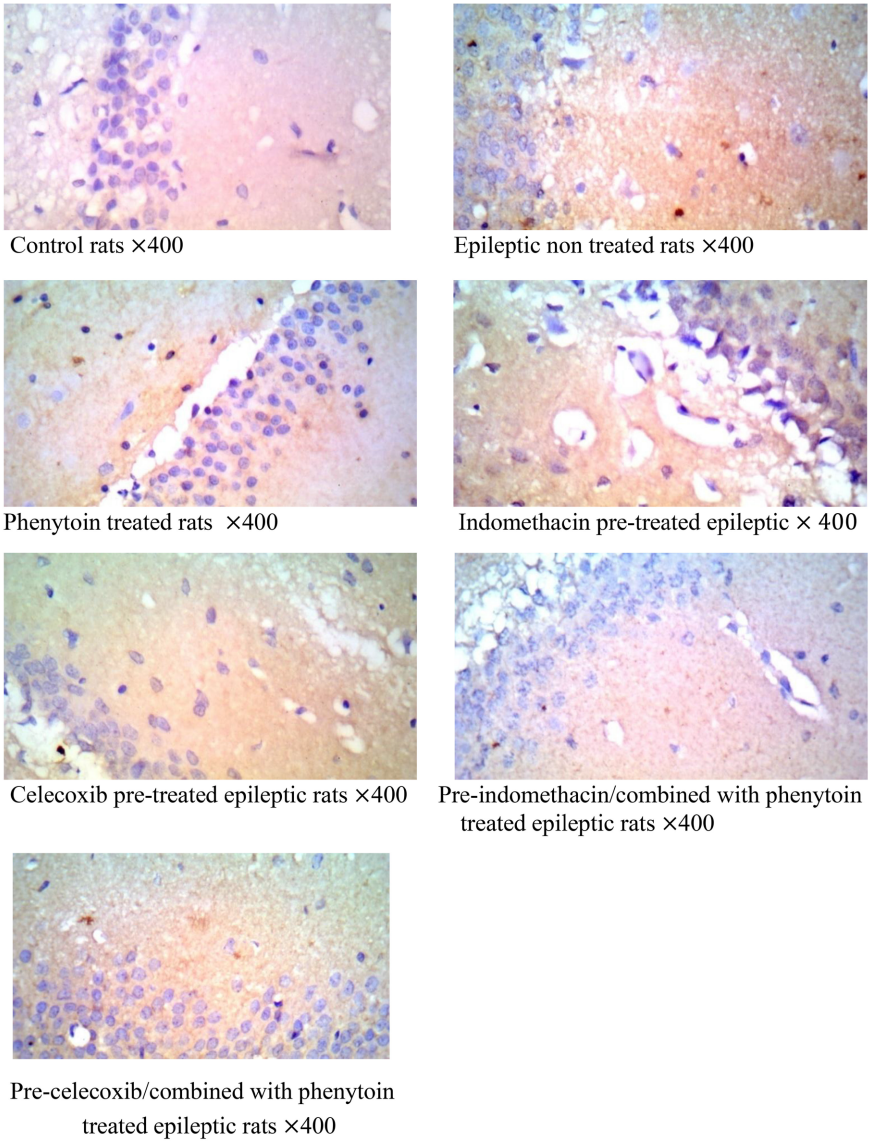
Effect of phenytoin combined with either indomethacin or celecoxib on P-glycoprotein expression (optical density) in pilocarpine induced epilepsy in rats: Figure 5(a) and Figure 5(b) showed that treatment with phenytoin (CI 95%, 0.003: 0.068, P-value = 0.033), indomethacin (CI 95%, 0.034: 0.102, P-value = 0.000) and celecoxib (CI 95%, 0.053: 0.118, P-value = 0.000) reduced the degree of P-glycoprotein expression (optical density) compared to epileptic non treated rats. Moreover, the combination of phenytoin with either indomethacin (CI 95%, 0.064: 0.133, P-value = 0.000) or celecoxib (CI 95%, 0.048: 0.113, P-value = 0.000) reduced the degree of P-glycoprotein expression (optical density) in comparison with epileptic non treated rats. However, treatment with celecoxib either alone (CI 95%, −0.082: −0.17, P-value = 0.004), or in combination with phenytoin (CI 95%, 0.013: 0.078, P-value = 0.008), and phenytoin combined with indomethacin (CI 95%, 0.029: 0.098, P-value = 0.001) had more beneficial effects in reducing P-glycoprotein expression in comparison with the phenytoin treated rats. only phenytoin combined with indomethacin treated rats showed a decrease in P-glycoprotein expression (optical density) to the extent that there was no significant difference with normal control rats.

Correlation between glutamate expression, ABCB1 gene expression, and P-glycoprotein expression (optical density): Figures 6(a)-(c) revealed that there was a positive correlation between glutamate expression P = 0.002, ABCB1 expression P = 0.001, and optical density (P-glycoprotein expression). This in-

dicated that the increase in brain glutamate level leads to an elevation in brain ABCB1 gene expression which is associated with P-gp upregulation and an increase in optical density.

5. Discussion

The present study aimed to determine the effect of the non-selective COX inhibitor indomethacin, and the selective COX-2 inhibitor celecoxib on P-gp expression and phenytoin brain penetration in a model of pilocarpine induced epilepsy in rats by studying their effects on ABCB1 gene expression and glutamate level. Treatment with phenytoin either alone or combined with either indomethacin or celecoxib improved the severity of seizures. However, the usage of indomethacin alone or celecoxib alone did not improve the Racine score. In accordance with our study, previous study showed that the pretreatment of rats with



Control rats ×400

Epileptic non treated rats ×400

Phenytoin treated rats ×400

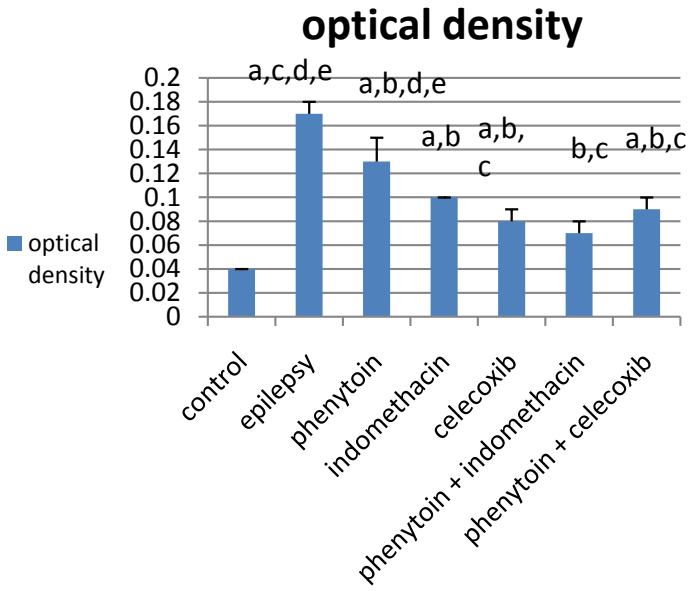
Indomethacin pre-treated epileptic × 400

Celecoxib pre-treated epileptic rats ×400

Pre-indomethacin/combined with phenytoin treated epileptic rats ×400

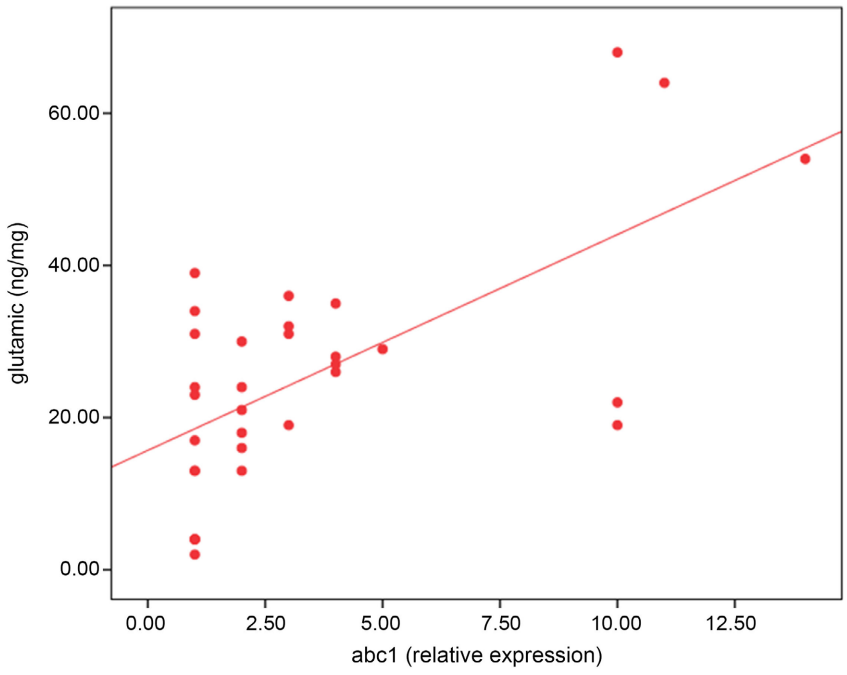
Pre-celecoxib/combined with phenytoin treated epileptic rats ×400

(a)

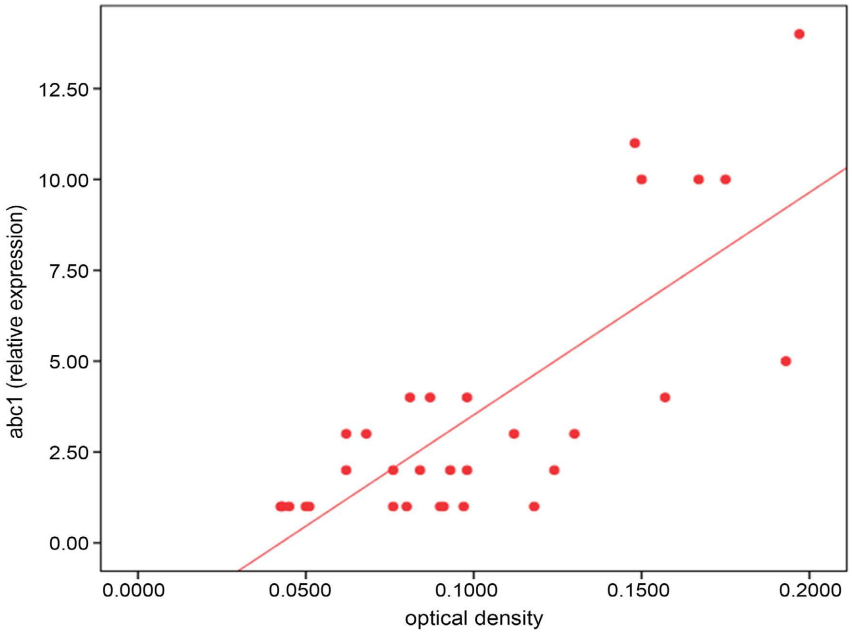


(b)

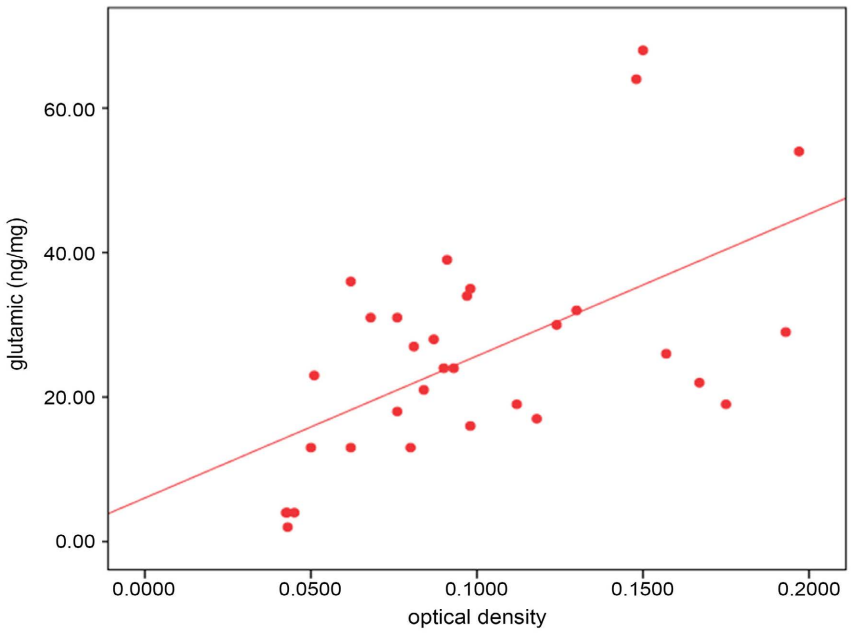
Figure 5. Effect of phenytoin combined with either indomethacin or celecoxib on pilocarpine induced changes in P-glycoprotein expression (optical denisty) in rats brain tissues. (a) Immunoreactivity of P-glycoprotein in the rat brain among the studied groups. (b) Effect of phenytoin combined with indomethacin or celecoxib on P-glycoprotein expression (optical denisty) in pilocarpine induced epilepsy in rats. Values are means ± SE (n = 8); one-way ANOVA followed by with Post-Hoc Tukey test. [a,b,c,d,e]$P < 0.05$; [a]Compared with control rats. [b]Compared with non treated epileptic rats. [c]Compared with phenytoin treated rats. [d]Compared with phenytoin combined with indomethacin treated rats. [e]Compared with phenytoin combined with celecoxib treated rats.



(a)



(b)



(c)

Figure 6. (a) Correlation between glutamate expression and ABCB1 gene expression; (b) Correlation between ABCB1 expression and P-glycoprotein expression (optical density); (c) Correlation between Glutamate expression and P-glycoprotein expression (optical density).

phenytoin reduced the average Racine score that was induced by pilocarpine injection [15], on the other hand, celecoxib alone did not affect the seizure severity during pilocarpine induced SE [10]. Also, using indomethacin alone did not have any effect in decreasing seizure score during SE [17].

Moreover, Temp *et al.* (2017) [27], studied two selective COX-2 inhibitors,

etoricoxib and celecoxib, and concluded that they did not change the Racine score in a pentylenetetrazole (PTZ) model of epilepsy. One possible explanation of these results might be that the proinflammatory cytokines such as interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, and interferon (INF)-ɣ are increased during seizures. Celecoxib decreased the levels of these cytokines but the reduction in the cortical and hippocampal regions was not sufficient to reduce seizure susceptibility.

Regarding the effect of pilocarpine injection on the latency of falling from rotarod apparatus, the present results revealed marked reduction in the falling time among pilocarpine injected rats, which was effectively ameliorated by phenytoin either alone or combined with either indomethacin or celecoxib. However, the combination of phenytoin with celecoxib showed a more beneficial effect in increasing the latency of falling from the rotarod apparatus. In accordance with the present study, Vyas *et al.* (2020) [28], reported that pilocarpine injection in mice reduced the latency to the first fall. However, in a pilocarpine model of epilepsy, treatment with carbamazepine, levetiracetam, and valproate showed no improvement in the motor coordination [28].

In the present study, the open field test proved that pilocarpine injection decreased the number of squares that are crossed by the rats after the induction of epilepsy, indicating remarkable decrease in the locomotor activity, which was improved by treatment with phenytoin either alone, or combined with either indomethacin or celecoxib. In accordance with the present results, a previous study proved that epileptic rats showed a reduction in the locomotor activity and an increase in the immobility periods in the open field test [29]. On the other hand, Zimcikova *et al.* (2017) [30], studied the effect of phenytoin, zonisamide, and carbamazepine on the open field test in rats, and did not find any important modifications in locomotion or exploratory behavior. This could be explained by the fact that the primary mode of action of phenytoin and carbamazepine, which is modulation of voltage-dependent sodium channels, did not have a significant anxiolytic effect.

Strong evidence exists that phenytoin, phenobarbital, levetiracetam, lamotrigine, and the active metabolite of oxcarbazepine are substrates of P-gp efflux at the human BBB [31], so the overexpression of P-gp at the BBB could reduce phenytoin brain uptake at specific limbic brain regions in chronic epileptic rats [20]. In the present study, brain phenytoin level was measured in the groups that received phenytoin either alone, or combined with either indomethacin or celecoxib, and it was obvious that the combination of phenytoin with either indomethacin or celecoxib increased the level of phenytoin that reached the brain of rats in comparison with rats that received phenytoin only, with a greater effect of celecoxib than indomethacin in enhancing the brain level of phenytoin.

In accordance with the present results, previous studies showed that direct P-gp inhibition with verapamil, markedly increased the concentration of phenytoin that reached the extracellular cortical fluid in a model of pharmacoresistance in epilepsy. Also, the selective COX-2 inhibitor SC-58236 had improved

the phenytoin brain penetration in epileptic rats following the development of SE, with associated downregulation of P-gp expression [16] [32].

Regarding the relation between epilepsy and the degree of P-gp expression, the present study revealed that pilocarpine injection was associated with upregulation of P-gp expression (optical density), which was effectively downregulated by treatment with phenytoin, indomethacin, celecoxib, phenytoin combined with either indomethacin or celecoxib. However, treatment with celecoxib either alone, or combined with phenytoin and indomethacin combined with phenytoin showed more beneficial effects in reducing P-gp expression than treatment with phenytoin alone.

In agreement with the present results, earlier studies revealed that P-gp expression at the brain capillaries was noticeably increased in the brain of rats after the induction of TLE [10] [26]. Moreover, Zibell *et al.* (2009) [17], showed that celecoxib treatment prevented the increase in P-gp expression in the brain of rats after the induction of SE. Furthermore, van Vliet *et al.* (2010) [16], reported that electrically or pilocarpine induced SE in rats was associated with an increase in brain P-gp expression. They used two highly selective COX-2 inhibitors NS-398, SC-58236 and found that both the two selective COX-2 inhibitors had the ability to suppress P-gp overexpression. Moreover, Schlichtiger *et al.* (2010) [13], reported that celecoxib suppressed P-gp expression and improved the brain penetration of phenobarbital, restoring the drug sensitivity in epileptic rats. The COX-2 role in P-gp upregulation can be explained as follow: seizures increase the extracellular glutamate, which signals through the NMDA receptor to increase COX-2 in the brain capillaries leading to P-gp expression at the BBB [33].

In consistence with this hypothesis, the present results revealed that pilocarpine injection was associated with an elevation in the brain glutamate level and treatment with phenytoin combined with either indomethacin or celecoxib succeeded to reduce this elevated brain glutamate level. Moreover, the combination of celecoxib with phenytoin ameliorated the brain glutamate level in this group to reach the values of normal control rats.

The present results were in accordance with a previous experiment in which exposing isolated rat brain capillaries to glutamate led to increase in P-gp expression, which was ameliorated by the non-selective COX inhibitor indomethacin and the selective COX-2 inhibitor celecoxib, while a selective COX-1inhibitor called SC-560 had no effect [10]. Although, the intermediate steps between COX-2 and P-gp expression are not fully understood. One of the theories is the upregulation of the mRNA levels of ABCB1 gene, which is the gene that encodes P-gp expression at the BBB [34].

In consistence with this hypothesis, the present study revealed that pilocarpine injection was associated with increased expression ABCB1 gene that was downregulated by treatment with either phenytoin, indomethacin, or celecoxib. Moreover, the combination of phenytoin with either indomethacin or celecoxib revealed more beneficial effects in reducing the ABCB1 gene level compared to treatment with phenytoin alone.

In accordance the present results, Tishler *et al.* (1995) [35]., were the first to report that ABCB1 gene expression was elevated 10-fold in the epileptic foci of drug resistant patients, proving its considerable role in drug resistant epilepsy. Furthermore, previous studies reported that ABCB1 gene expression was upregulated in the brain of rats after the induction of epilepsy by using different models of epilepsy like the kainate model, the pilocarpine model, and the electrical amygdala kindling [32] [36], and played a role in the reduced uptake of ASDs [6]. Another study reported that the brain level of the active metabolite of oxcarbazepine was inversely proportional to the brain tissue mRNA expression of ABCB1 gene in drug resistant epileptic patients [37].

Strong association is present between neuroinflammation, epilepsy and increased seizure susceptibility [28]. So, the present study assessed the degree of neurodegeneration in the brain of rats following the induction of SE. The present results showed that pilocarpine injection induced marked neuronal damage that was ameliorated by treatment with phenytoin, indomethacin, and celecoxib. Also, the combination of phenytoin with either indomethacin or celecoxib revealed marked improvement in the degree of neuronal damage. In consistence with the present results, previous studies reported that COX inhibition in epileptic rats using either indomethacin [10], celecoxib [15], or the selective COX-2 inhibitor NS398 [38], showed valuable neuroprotective effects, with decreased neuronal death and abnormal neurogenesis [39].

6. Conclusion

In conclusion, the present study revealed that the combination of either indomethacin or celecoxib with phenytoin inhibited P-gp expression leading to a decrease in glutamate level and ABCB1 gene expression. The decrease in P-gp expression was associated with increased phenytoin brain level, improved resistance to the drug and allowed higher drug levels to reach the brain without increasing the dose of phenytoin, which in turn could decrease its dangerous side effects. Also, both indomethacin and celecoxib showed marked neuroprotective effects. However, the brain uptake of phenytoin was significantly enhanced using celecoxib rather than indomethacin. The present results suggest that celecoxib should be further studied as an effective adjuvant to phenytoin therapy in epilepsy, both to ameliorate seizure severity, and to protect against the drug resistance that develops with the chronic use of phenytoin, taking into consideration that celecoxib usage should be used with cautious in patients at risk of cardiovascular or cerebrovascular diseases.

Declarations

Ethical Approval

All experimental procedures were approved by the institutional animal care and use committee at Suez Canal University number 2554 on 11-11-2015, following the National Institutes of Health (NIH) Guide for the Care and Use of Labora-

tory Animals Publications No. 8023, revised 1978.

Authors' Contributions

Each author has contributed to the following aspects:

Substantial contributions to the design of the work, analysis, and interpretation of data.

Drafting the work and revising it critically for important intellectual content.

Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Reham M. ELSAYED wrote the main manuscript text and prepared all figures

Amira S. MOHAMED Drafting the work and revising it critically for important intellectual content

Mona K. TAWFIK Final approval of the version to be published

Magda M. HAGRAS Final approval of the version to be published

Availability of Data and Materials

All data and materials can be accessed.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Keselman, I., Wasterlain, C.G., Niquet, J. and Chen, J.W.Y. (2017) Status Epilepticus—Lessons and Challenges from Animal Models. In: Varelas, P. and Claassen, J., Eds., *Seizures in Critical Care. Current Clinical Neurology*, Humana Press, Cham, 3-17. https://doi.org/10.1007/978-3-319-49557-6_1

[2] Rocha, L. and Cavalheiro, E.A. (2013) Pharmacoresistance in Epilepsy: From Genes and Molecules to Promising Therapies. Springer, New York. https://doi.org/10.1007/978-1-4614-6464-8

[3] Kandratavicius, L., Balista, P., Lopes-Aguiar, C., Ruggiero, R., Umeoka, E., Garcia-Cairasco, N., Bueno-Junior, L. and Leite, J. (2014) Animal Models of Epilepsy: Use and Limitations. *Neuropsychiatric Disease and Treatment*, **10**, 1693-1705. https://doi.org/10.2147/NDT.S50371

[4] Löscher, W. (2017) Animal Models of Seizures and Epilepsy: Past, Present, and Future Role for the Discovery of Antiseizure Drugs. *Neurochemical Research*, **42**, 1873-1888. https://doi.org/10.1007/s11064-017-2222-z

[5] Engel Jr., J. (2014) Approaches to Refractory Epilepsy. *Annals of Indian Academy of Neurology*, **17**, S12-S17. https://doi.org/10.4103/0972-2327.128644

[6] Tang, F., Hartz, A.M.S. and Bauer, B. (2017) Drug-Resistant Epilepsy: Multiple Hypotheses, Few Answers. *Frontiers in Neurology*, **8**, Article 301. https://doi.org/10.3389/fneur.2017.00301

[7] Enrique, A.V., Di Ianni, M.E., Goicoechea, S., Lazarowski, A., Valle-Dorado, M.G., Costa, J.J.L., Rocha, L., Girardi, E. and Talevi, A. (2019) New Anticonvulsant Can-

didates Prevent P-Glycoprotein (P-gp) Overexpression in a Pharmacoresistant Seizure Model in Mice. *Epilepsy & Behavior*, **13**, Article ID: 106451. https://doi.org/10.1016/j.yebeh.2019.106451

[8] Klepsch, F., Vasanthanathan, P. and Ecker, G.F. (2014) Ligand and Structure-Based Classification Models for Prediction of P-Glycoprotein Inhibitors. *Journal of Chemical Information and Modeling*, **54**, 218-229. https://doi.org/10.1021/ci400289j

[9] Hartz, A.M.S., Pekcec, A., Soldner, E.L.B., Zhong, Y., Schlichtiger, J. and Bauer, B. (2017) P-gp Protein Expression and Transport Activity in Rodent Seizure Models and Human Epilepsy. *Molecular Pharmaceutics*, **14**, 999-1011. https://doi.org/10.1021/acs.molpharmaceut.6b00770

[10] Bauer, B., Hartz, A.M.S., Pekcec, A., Toellner, K., Miller, D.S. and Potschka, H. (2008) Seizure-Induced Up-Regulation of P-Glycoprotein at the Blood-Brain Barrier through Glutamate and Cyclooxygenase-2 Signaling. *Molecular Pharmacology*, **73**, 1444-1453. https://doi.org/10.1124/mol.107.041210

[11] Potschka, H. (2012) Role of CNS Efflux Drug Transporters in Antiepileptic Drug Delivery: Overcoming CNS Efflux Drug Transport. *Advanced Drug Delivery Reviews*, **64**, 943-952. https://doi.org/10.1016/j.addr.2011.12.007

[12] Dhir, A. (2019) An Update of Cyclooxygenase (COX)-Inhibitors in Epilepsy Disorders. *Expert Opinion on Investigational Drugs*, **28**, 191-205. https://doi.org/10.1080/13543784.2019.1557147

[13] Schlichtiger, J., Pekcec, A., Bartmann, H., Winter, P., Fuest, C., Soerensen, J. and Potschka, H. (2010) Celecoxib Treatment Restores Pharmacosensitivity in a Rat Model of Pharmacoresistant Epilepsy: COX-2 Inhibition and Pharmacoresistant Epilepsy. *British Journal of Pharmacology*, **160**, 1062-1071. https://doi.org/10.1111/j.1476-5381.2010.00765.x

[14] Lalitha, S., Minz, R.W. and Medhi, B. (2018) Understanding the Controversial Drug Targets in Epilepsy and Pharmacoresistant Epilepsy. *Reviews in the Neurosciences*, **29**, 333-345. https://doi.org/10.1515/revneuro-2017-0043

[15] Borham, L.E., Mahfoz, A.M., Ibrahim, I.A.A., Shahzad, N., ALrefai, A.A., Labib, A.A., Bin Sef, B., Alshareef, A., Khan, M., Milibary, A. and Al Ghamdi, S. (2016) The Effect of Some Immunomodulatory and Anti-Inflammatory Drugs on Li-Pilocarpine-Induced Epileptic Disorders in Wistar Rats. *Brain Research*, **1648**, 418-424. https://doi.org/10.1016/j.brainres.2016.07.046

[16] van Vliet, E.A., Zibell, G., Pekcec, A., Schlichtiger, J., Edelbroek, P.M., Holtman, L., Aronica, E., Gorter, J.A. and Potschka, H. (2010) COX-2 Inhibition Controls P-Glycoprotein Expression and Promotes Brain Delivery of Phenytoin in Chronic Epileptic Rats. *Neuropharmacology*, **58**, 404-412. https://doi.org/10.1016/j.neuropharm.2009.09.012

[17] Zibell, G., Unkrüer, B., Pekcec, A., Hartz, A.M.S., Bauer, B., Miller, D.S. and Potschka, H. (2009) Prevention of Seizure-Induced up-Regulation of Endothelial P-Glycoprotein by COX-2 Inhibition. *Neuropharmacology*, **56**, 849-855. https://doi.org/10.1016/j.neuropharm.2009.01.009

[18] Jung, K.-H., Chu, K., Lee, S.-T., Kim, J., Sinn, D.-I., Kim, J.-M., Park, D.-K., Lee, J.-J., Kim, S.U., Kim, M., Lee, S.K. and Roh, J.-K. (2006) Cyclooxygenase-2 Inhibitor, Celecoxib, Inhibits the Altered Hippocampal Neurogenesis with Attenuation of Spontaneous Recurrent Seizures Following Pilocarpine-Induced Status Epilepticus. *Neurobiology of Disease*, **23**, 237-246. https://doi.org/10.1016/j.nbd.2006.02.016

[19] Racine, R.J. (1972) Modification of Seizure Activity by Electrical Stimulation: II.

Motor Seizure. *Electroencephalography and Clinical Neurophysiology*, **32**, 281-294. https://doi.org/10.1016/0013-4694(72)90177-0

[20] Janahmadi, M., Goudarzi, I., Kaffashian, M.R., Behzadi, G., Fathollahi, Y. and Hajizadeh, S. (2009). Co-Treatment with Riluzole, a Neuroprotective Drug, Ameliorates the 3-Acetylpyridine-Induced Neurotoxicity in Cerebellar Purkinje Neurones of Rats: Behavioural and Electrophysiological Evidence. *NeuroToxicology*, **30**, 393-402. https://doi.org/10.1016/j.neuro.2009.02.014

[21] Eilam, D. (2003) Open-Field Behavior Withstands Drastic Changes in Arena Size. *Behavioural Brain Research*, **142**, 53-62. https://doi.org/10.1016/S0166-4328(02)00382-0

[22] Correa, M., Wisniecki, A., Betz, A., Dobson, D.R., O'Neill, M.F., O'Neill, M.J. and Salamone, J.D. (2004) The Adenosine A2A Antagonist KF17837 Reverses the Locomotor Suppression and Tremulous Jaw Movements Induced by Haloperidol in Rats: Possible Relevance to Parkinsonism. *Behavioural Brain Research*, **148**, 47-54. https://doi.org/10.1016/S0166-4328(03)00178-5

[23] Janik, R., Thomason, L.A.M., Stanisz, A.M., Forsythe, P., Bienenstock, J. and Stanisz, G.J. (2016) Magnetic Resonance Spectroscopy Reveals Oral *Lactobacillus* Promotion of Increases in Brain GABA, N-Acetyl Aspartate, and Glutamate. *NeuroImage*, **125**, 988-995. https://doi.org/10.1016/j.neuroimage.2015.11.018

[24] van Vliet, E., Aronica, E., Redeker, S., Marchi, N., Rizzi, M., Vezzani, A. and Gorter, J. (2004) Selective and Persistent Upregulation of mdr1b mRNA and P-Glycoprotein in the Parahippocampal Cortex of Chronic Epileptic Rats. *Epilepsy Research*, **60**, 203-213. https://doi.org/10.1016/j.eplepsyres.2004.06.005

[25] Huang, Y., Zhao, F., Wang, L., Yin, H., Zhou, C. and Wang, X. (2012) Increased Expression of Histone Deacetylases 2 in Temporal Lobe Epilepsy: A Study of Epileptic Patients and Rat Models. *Synapse*, **66**, 151-159. https://doi.org/10.1002/syn.20995

[26] van Vliet, E.A., van Schaik, R., Edelbroek, P.M., Voskuyl, R.A., Redeker, S., Aronica, E., Wadman, W.J. and Gorter, J.A. (2007) Region-Specific Overexpression of P-Glycoprotein at the Blood-Brain Barrier Affects Brain Uptake of Phenytoin in Epileptic Rats. *Journal of Pharmacology and Experimental Therapeutics*, **322**, 141-147. https://doi.org/10.1124/jpet.107.121178

[27] Temp, F.R., Marafiga, J.R., Milanesi, L.H., Duarte, T., Rambo, L.M., Pillat, M.M. and Mello, C.F. (2017) Cyclooxygenase-2 Inhibitors Differentially Attenuate Pentylenetetrazol-Induced Seizures and Increase of Pro- and Anti-Inflammatory Cytokine Levels in the Cerebral Cortex and Hippocampus of Mice. *European Journal of Pharmacology*, **810**, 15-25. https://doi.org/10.1016/j.ejphar.2017.05.013

[28] Vyas, P., Tulsawani, R.K. and Vohora, D. (2020) Loss of Protection by Antiepileptic Drugs in Lipopolysaccharide-Primed Pilocarpine-Induced *Status Epilepticus* Is Mediated via Inflammatory Signaling. *Neuroscience*, **442**, 1-16. https://doi.org/10.1016/j.neuroscience.2020.06.024

[29] Midzyanovskaya, I.S., Shatskova, A.B., Sarkisova, K.Yu., van Luijtelaar, G., Tuomisto, L. and Kuznetsova, G.D. (2005) Convulsive and Nonconvulsive Epilepsy in Rats: Effects on Behavioral Response to Novelty Stress. *Epilepsy & Behavior*, **6**, 543-551. https://doi.org/10.1016/j.yebeh.2005.03.005

[30] Zimcikova, E., Simko, J., Karesova, I., Kremlacek, J. and Malakova, J. (2017) Behavioral Effects of Antiepileptic Drugs in Rats: Are the Effects on Mood and Behavior Detectable in Open-Field Test? *Seizure*, **52**, 35-40. https://doi.org/10.1016/j.seizure.2017.09.015

[31] Stępień, K.M., Tomaszewski, M., Tomaszewska, J. and Czuczwar, S.J. (2012) The Multidrug Transporter P-Glycoprotein in Pharmacoresistance to Antiepileptic Drugs. *Pharmacological Reports*, **64**, 1011-1019. https://doi.org/10.1016/s1734-1140(12)70900-3

[32] Ma, A., Wang, C., Chen, Y., Yuan, W. and Hu, Z. (2013) P-Glycoprotein Alters Blood-Brain Barrier Penetration of Antiepileptic Drugs in Rats with Medically Intractable Epilepsy. *Drug Design, Development and Therapy*, **7**, 1447-1454. https://doi.org/10.2147/DDDT.S52533

[33] Kambli, L., Bhatt, L.K., Oza, M. and Prabhavalkar, K. (2017) Novel Therapeutic Targets for Epilepsy Intervention. *Seizure*, **51**, 27-34. https://doi.org/10.1016/j.seizure.2017.07.014

[34] Stasiołek, M., Romanowicz, H., Połatyńska, K., Chamielec, M., Skalski, D., Makowska, M. and Smolarz, B. (2016) Association between C3435T Polymorphism of *MDR*1 Gene and the Incidence of Drug-Resistant Epilepsy in the Population of Polish Children. *Behavioral and Brain Functions*, **12**, Article No. 21. https://doi.org/10.1186/s12993-016-0106-z

[35] Tishler, D.M., Weinberg, K.I., Hinton, D.R., Barbaro, N., Annett, G.M. and Raffel, C. (1995) *MDR*1 Gene Expression in Brain of Patients with Medically Intractable Epilepsy. *Epilepsia*, **36**, 1-6. https://doi.org/10.1111/j.1528-1157.1995.tb01657.x

[36] Volk, H.A., Burkhardt, K., Potschka, H., Chen, J., Becker, A. and Löscher, W. (2004) Neuronal Expression of the Drug Efflux Transporter P-Glycoprotein in the Rat Hippocampus after Limbic Seizures. *Neuroscience*, **123**, 751-759. https://doi.org/10.1016/j.neuroscience.2003.10.012

[37] Marchi, N., Guiso, G., Rizzi, M., Pirker, S., Novak, K., Czech, T., Baumgartner, C., Janigro, D., Caccia, S. and Vezzani, A. (2005) A Pilot Study on Brain-to-Plasma Partition of 10,11-Dyhydro-10-Hydroxy-5H-Dibenzo(b,f)Azepine-5-Carboxamide and MDR1 Brain Expression in Epilepsy Patients Not Responding to Oxcarbazepine. *Epilepsia*, **46**, 1613-1619. https://doi.org/10.1111/j.1528-1167.2005.00265.x

[38] Takemiya, T., Maehara, M., Matsumura, K., Yasuda, S., Sugiura, H. and Yamagata, K. (2006) Prostaglandin E2 Produced by Late Induced COX-2 Stimulates Hippocampal Neuron Loss after Seizure in the CA3 Region. *Neuroscience Research*, **56**, 103-110. https://doi.org/10.1016/j.neures.2006.06.003

[39] da Silva Fernandes,, M.J., da Graca Naffah Mazzacoratti,, M. and Cavalheiro, E.A. (2010) Pathophysiological Aspects of Temporal Lobe Epilepsy and the Role of P2X Receptors. *The Open Neuroscience Journal*, **4**, 35-43. https://doi.org/10.2174/1874082001004010035

List of Abbreviations

ABCB1: ATP Binding Casette Subfamily B Member 1
ANOVA: Analysis of Variance
ASDs: Antiseizure drugs
ATP: Adenosine triphosphate
BBB: blood brain barrier
CID: Chemical Industries Development
COX: Cyclooxygenase
ELISA: Enzyme Linked Immunosorbent Assay
EP1: Prostaglandin E receptor 1
GLU: Glutamate
HPLC: High Performance Liquid Chromatography
IL: Interleukin
ILAE: International league against epilepsy
INF: Interferon
IOP: Integrated optical density
ip: Intraperitoneal
MUP: Medical union Pharmaceutics
NIH: National Institutes of Health
NMDA: N-Methyl-D-Aspartate
PG: Prostaglandin
P-gp: P-glycoprotein
PTZ: Pentylenetetrazol
RT-qPCR: Quantitative real time polymerase chain reaction
SE: Status epilepticus
SPSS: Statistical package of social science
TLE: Temporal lobe epilepsy
TNF-*α*: Tumor necrosis factor α