

## PROTECTIVE EFFECT OF MONTELUKAST AGAINST PENTYLENETETRAZOLE-INDUCED ACUTE SEIZURES AND KINDLING IN MICE

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### ABSTRACT

**Background:** Montelukast, a selective reversible cysteinyl leukotriene 1 (CysLT1) receptor antagonist, is used in treatment of asthma, exercise induced-bronchospasm, allergic rhinitis and urticaria. It was shown to protect against cerebral ischemia/reperfusion injury. **Objectives:** To examine the possible anticonvulsant effect of montelukast, either alone or in combination with a known antiepileptic drug "valproate" against pentylenetetrazole (PTZ)-induced seizures. **Methods:** 112 adult male Swiss albino mice were used in the study and divided into two main groups, each containing 5 subgroups (Gp). The first main group "acute PTZ model (PTZ-a)" contains: Gp1 "vehicle-treated group" was injected with saline (10 ml/kg, intraperitoneally "i.p." in a single dose), Gp2 "acute PTZ-control" injected with a single dose of PTZ (60 mg/kg, i.p.), Gp3 injected with valproate (50 mg/kg, i.p.) 30 minutes before PTZ, Gp4 injected with a single dose of montelukast (10 mg/kg, i.p.) 30 minutes before PTZ and Gp5 injected with both montelukast and valproate 30 minutes before PTZ administration. The second main group "kindling model (PTZ-k)" contains: Gp1 was injected with saline (i.p.) every other day for 17 days, Gp2 "PTZ-kindled control" received 9 PTZ injections in a dose of (40 mg/kg, i.p.) on alternate days for 17 days, Gp3 injected with valproate (50 mg/kg, i.p.) for 17 days, 30 minutes before PTZ, Gp4 daily injected with montelukast (10 mg/kg, i.p.) for 17 days 30 minutes before PTZ and Gp5 received both montelukast (10 mg/kg, i.p.) and valproate (50 mg/kg, i.p.) daily for 17 days, 30 minutes before administration of PTZ. **Results:** Single and repeated PTZ administration produced stage 4 seizures associated with a significant reduction in the brain level of reduced glutathione (GSH), concomitant with significant elevation in the brain levels of malondialdehyde (MDA), interleukin1 $\beta$  (IL1 $\beta$ ), tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) and leukotriene D4 (LtD4). The use of valproate alone and its combination with montelukast suppressed the incidence of stage 4 seizures in acute PTZ induced convulsion while montelukast, valproate and their combination decreased the percentage of animals reaching stage 4 seizures in kindled mice. In both PTZ acute and kindled models, montelukast, valproate and their combined administration significantly elevated brain level of GSH and significantly decreased brain levels of MDA, IL1 $\beta$ , TNF $\alpha$  and LtD4 as compared to PTZ control groups. Interestingly, co-administration of valproate and montelukast resulted in a significant elevation in the brain level of GSH concomitant with a significant reduction in the brain levels of MDA, IL1 $\beta$ , TNF $\alpha$  and LtD4 as compared to each of the valproate and the montelukast groups in both models. **Conclusion:** Montelukast, either alone or in combination with valproate protects against PTZ induced seizures via blockade of leukotriene receptors & amelioration of oxidative stress and inflammatory cascades.

**Key words:** Montelukast, PTZ., Seizures, Valproate.

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### INTRODUCTION

Epilepsy is a chronic neurological disorder characterized by repeated seizures resulted from excessive neuronal discharge<sup>[1]</sup>. The currently used antiepileptic drugs provide a symptomatic antiseizure effect without effective prophylaxis or cure. Furthermore, a wide range of adverse effects are associated with their long term use and limit their compliance<sup>[2]</sup>. Therefore, a need for new antiepileptic drug with better compliance is a promising target. Montelukast, a selective reversible CysLT1 receptor antagonist was shown to reduce ischemia reperfusion (I/R)-induced oxidative damage in the kidney and intestine<sup>[3]</sup>. Several studies have also demonstrated the neuroprotective effects for montelukast in various neurodegenerative

conditions such as acute and chronic ischemic brain injury<sup>[4]</sup>.

Increased inflammatory mediators (IL1 $\beta$ , TNF $\alpha$ ) and LtD4 are observed following epileptogenic insult and are thought to be implicated in seizure development<sup>[5]</sup>, possibly by modulating GABA and glutamate homeostasis<sup>[6,7]</sup> and blood brain barrier (BBB) breakdown that leads to leakage of albumin and IgG into the brain. Albumin via activation of the transforming growth factor  $\beta$  (TGF)- $\beta$  pathway, leads to increased neural excitability through down-regulation of inward rectifier potassium channel (Kir4.1) and glutamate transporter<sup>[8]</sup>. Moreover, LtD4 receptor activation is reported to be associated with activation of phosphoinositide-3-kinase (PI-3-K), extracellular receptor kinase (ERK), mitogen-activated-protein-kinase (MAPK),

NF- $\kappa$ B, TNF $\alpha$  and matrix-metalloproteinases-9 (MMP-9) pathway. These pathways are suggested to participate in the pathophysiological seizure propagation<sup>[1]</sup>.

The current work aimed to study the possible protective effect of montelukast and its combination with valproate against PTZ-induced acute and kindled seizures, and to investigate the possible mechanism(s) of action.

## MATERIALS AND METHODS

### Animals

112 adult male Swiss albino mice 8 weeks old weighing 15–35 g were used in the current study. Animals were purchased from the Faculty of Veterinary Medicine, Zagazig University, Egypt. Mice were allowed standard pellet diet and tap water ad libitum before, during one week of acclimatization period, and through the whole experimental period. They were kept at a constant temperature (23  $\pm$ 2°C), humidity (60  $\pm$ 10%) and a light/ dark (12 h:12 h) cycle. The animals were randomly assigned to experimental groups. Each mouse was used only once and all tests were performed between 8.00 and 15.00 h. The experiment was performed in the pharmacology department laboratory, faculty of medicine, Zagazig University. Experimental design and animal handling were performed in accordance with protocols approved by the local experimental ethics committee guidelines of the Egyptian Society of Neuroscience, the Ethical Committee of the Faculty of Medicine, Zagazig University, for Animal Use and the guidelines of the US National Institutes of Health

on animal care.

### Experimental protocol

The animals were divided into two main groups, each containing 5 subgroups (Gp). In the first main group "acute PTZ model": Gp1 (n: 8) "vehicle-treated subgroup" was injected with saline (10 ml/kg, i.p.), Gp2 (n:12) "acute PTZ-control subgroup" injected with a single dose of PTZ (60 mg/kg, i.p.)<sup>[9]</sup>, Gp3 (n:12) "valproate subgroup" injected with a single dose of valproate (50 mg/kg, i.p.)<sup>[10]</sup> 30 minutes before PTZ, Gp4 (n: 12) "montelukast subgroup" injected with a single dose of montelukast (10 mg/kg, i.p.) 30

minutes before PTZ<sup>[11]</sup> and Gp5 (12 animals) "montelukast and valproate subgroup" injected with both montelukast (10 mg/kg, i.p.) and valproate (50 mg/kg i.p.) once 30 minutes before PTZ. In the second group "PTZ-induced kindling": Gp1 (n: 8) "vehicle-treated subgroup" was injected with saline (10ml/kg, i.p.) every other day for 17 days, Gp2 (n:12) "PTZ-kindled control subgroup" received nine PTZ injections in a dose of (40 mg/kg, i.p.) on alternate days for 17 days<sup>[12]</sup>, Gp3 (n: 12) "valproate subgroup" daily injected with valproate (50 mg/kg, i.p.) for 17 days 30 minutes before PTZ, Gp4 (n: 12) "montelukast subgroup" daily injected with montelukast (10 mg/kg/d, i.p.) for 17 days 30 minutes before PTZ and Gp5 (n: 12) "montelukast and valproate subgroup" injected with both montelukast (10mg/kg, i.p.) and valproate (50 mg/kg, i.p.) daily for 17 days 30 minutes before PTZ (Table 1). All drugs were dissolved in saline just before injection. After injections, the animals were observed for 20 minutes in Plexiglas cages. Five seizure stages were recorded: stage 0 "no response"; stage 1 "ear and facial twitching"; stage 2 "convulsive waves through the body without rearing"; stage 3 "myoclonic jerks, upright position"; stage 4 "clonic-tonic convulsions, turn over into side position" and stage 5 "generalized clonic-tonic convulsions, loss of postural control"<sup>[12]</sup>. The maximum response reached was recorded in each mouse. The animals that exhibited convulsions after the first three injections, as well as the dead animals were excluded from this study. Kindling was achieved when mice had stage 4 seizures on three sequential PTZ injections throughout the experimental period. The percentage of animals at each seizure stage was calculated in each group<sup>[13]</sup>.

**Table 1:** Experimental protocol showing the two main groups and subgroups

<i>Acute PTZ subgroups</i>	<i>No.</i>	<i>Drugs used</i>
Gp1	8	Saline (10ml/kg, i.p.)
Gp2	12	PTZ single dose (60mg/kg, i.p.)
Gp3	12	Valproate (50 mg/kg, i.p.) 30 min before PTZ.
Gp4	12	Montelukast (10mg/kg, i.p. 30 min before PTZ.
Gp5	12	Both montelukast and valproate 30 min before PTZ.
<i>Chronic PTZ subgroups</i>	<i>No.</i>	<i>Drugs used</i>
Gp1	8	Saline (10ml/kg, i.p.) every other day for 17 days.
Gp2	12	PTZ (40mg/kg, i.p.) on alternate days for 17 days
Gp3	12	Valproate (50 mg/kg, i.p.) every day for 17 days 30 min before PTZ.
Gp4	12	Montelukast (10 mg/kg, i.p) every day for 17 days 30 min before PTZ.
Gp5	12	Both montelukast and valproate every day for 17 days 30 min before PTZ.

### Biochemical assays

Eight mice from each group were euthanized by decapitation 24 h after the acute and the last PTZ administration<sup>13</sup>. Their brains were quickly removed in liquid nitrogen. The whole brain was homogenized in ice-cold saline for estimation of TNF- $\alpha$ , IL-1 $\beta$ , MDA, GSH, and LD4. Brain GSH was assessed using colorimetric assay kit according to Beutler et al.<sup>[14]</sup> and values were expressed as mMol per milligram (mg) of protein. MDA was measured as described by Okhawa et al.<sup>[15]</sup> Its values were expressed as (mmol/mg ptn). Brain IL-1 $\beta$ , TNF- $\alpha$ , and LtD4 were estimated utilizing enzyme linked immunosorbant assay (ELISA) kits purchased from (R&D system Quantitative USA), CUSABIO Bender Med Systems, USA), and results were expressed as picograms per milligram protein (pg/mg ptn) for IL-1 $\beta$  and TNF- $\alpha$ , as nanograms per milligram protein (ng/mg ptn), for LtD4<sup>[16-18]</sup>.

### Statistical Analysis

The obtained variables were tabulated as mean  $\pm$ SE (standard error of the mean). Comparison between different groups were made using one way analysis of variances test (one-way ANOVA) followed by Post-Hoc (least significant difference "LSD") tests as described by Armitage and Berry<sup>[19]</sup>. The categorical variables were expressed as a

number percentage. Percent of categorical (ordinal variables were compared using Chi-square test for trend). The differences were considered to be significant when  $p < 0.05$ . Statistical package of social sciences (SPSS) computer software (version 16) was used to carry out the statistical analysis.

## RESULTS

### Pharmacological results

In acute PTZ model, administration of valproate and its combination with montelukast protected 58.3% and 91.7% of mice respectively from reaching stage 4 seizures ( $p < 0.05$ ). (Table2). In kindled model, the animals experienced seizures starting from the 4<sup>th</sup> dose of PTZ (7<sup>th</sup> day of the experiment), but all mice in the PTZ kindled control subgroup reached stage 4/5 at the 6<sup>th</sup> injection of PTZ (11<sup>th</sup> day of the experiment) and lasted thereafter. The protective effect of valproate started from the 9<sup>th</sup> day of the experiment, where only 41.6% of the animals reached stage 4. This percentage decreased to 33.3% on the last day of the experiment. The protective effect of montelukast started from the 13<sup>th</sup> day of the experiment; where 66.7% of the animals reached stage 4 and this percentage lasted till the last day of the experiment. Interestingly, none of the animals in the combination group reached stage 4 during the course of the experiment (Table 3). On the last day, repeated administration of valproate, montelukast and their combination protected 66.7%, 33.3% and 100%, respectively, of the animals from reaching stage 4 ( $p < 0.05$ ). (Table 4).

### Biochemical results

Compared to normal animals, PTZ-acute (PTZ-a) and PTZ-kindling (PTZ-k) caused a significant reduction ( $p < 0.05$ ) in brain GSH by 59% and 67% respectively. Moreover, both PTZ dose regimens significantly ( $p < 0.05$ ) increased brain MDA by 312% (PTZ-a) and 460% (PTZ-k) respectively.

In PTZ-a, pretreatment with valproate, montelukast or their combination significantly ( $p < 0.05$ ) increased brain GSH by 38.2%, 73.4%, and 96.6%, respectively, as compared to PTZ control subgroup. Furthermore, valproate, montelukast and their combination significantly ( $p < 0.05$ ) decreased the elevated

brain MDA by 29.5%, 36.4%, 63.5% respectively, as compared to acute PTZ control group. In kindled mice, valproate, montelukast and their combination significantly ( $p < 0.05$ ) increased GSH by 78%, 95.5%, 176%, as compared to PTZ control group. They also significantly ( $p < 0.05$ ) decreased brain MDA by 38%, 41%, 58.8% respectively. (Figures 1,2).

Additionally, both PTZ dose regimens (acute and kindling) significantly ( $p < 0.05$ ) elevated brain IL1 $\beta$ , TNF $\alpha$  and LtD4 by 165%, 205%, 164%, 194%, 333% and 402% respectively as compared to normal group (Figures 3-5). In acute PTZ model, pretreatment with valproate, montelukast or their combination significantly ( $p < 0.05$ )

decreased the elevated brain levels of IL1 $\beta$  by 29.9%, 30.4%, and 57%, respectively, as compared to acute PTZ control subgroup. Valproate, montelukast and their combination further caused a significant ( $p < 0.05$ ) reduction in brain TNF $\alpha$  levels by 32%, 37%, and 56% as well as LtD4 by 31%, 33%, and 62% respectively, as compared to acute PTZ control group. In PTZ kindled model, valproate, montelukast or their combination significantly ( $p < 0.05$ ) decreased brain IL1 $\beta$  by 24%, 29%, and 39%, respectively, as compared to chronic PTZ control group. Moreover, the used drugs significantly ( $p < 0.05$ ) reduced the brain TNF $\alpha$  by 35%, 30%, and 45%, respectively, as well as LtD4 by 38.5%, 38.8% and 60%, respectively.

**Table 2:** Percentage of reduction of stage 4 seizures induced by single dose of valproate (50 mg/kg, i.p.), montelukast (10 mg/kg, i.p.) or their combination in acute PTZ model in mice

	Control	Valproate	Monteleukast	Combination
Percentage	0%	58.3%	16.5%	91.7%
	A	B	A	C

Values with different capital letters are significantly different ( $p < 0.05$ )

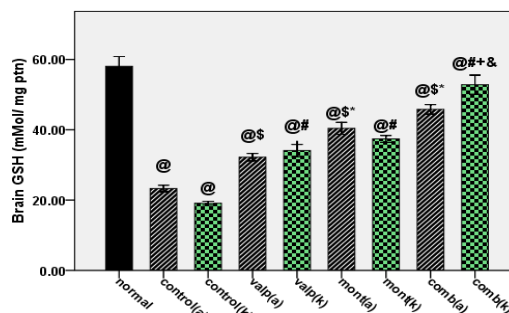
**Table (3):** Effects of valproate (50mg/kg, i.p.), montelukast (10mg/kg i.p), and their combination (every day for 17 days) on seizure stage 4, during the course of kindling (k) in mice (n=12)

	Control	Valproate	Monteleukast	Combination
4 <sup>th</sup> dose (day7)	50%	50%	50%	0%
5 <sup>th</sup> dose (day 9)	50%	41.6%	58.3%	0%
6 <sup>th</sup> dose (day11)	100%	41.6%	75%	0%
7 <sup>th</sup> dose (day13)	100%	41.6%	66.7%	0%
8 <sup>th</sup> dose (day 15)	100%	33.3%	66.7%	0%
9 <sup>th</sup> dose (day 17)	100%	33.3%	66.7%	0%

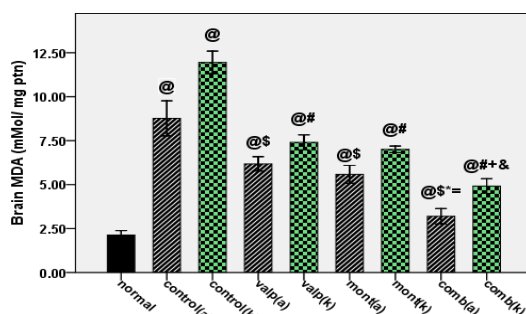
**Table 4:**Percentage of reduction induced by valproate (50mg/kg), montelukast (10mg/kg) or their combination (every day for 17 days) on stage 4 seizure in the last day of the experiment in PTZ kindling (k) model in mice (n=12)

Stage of seizure	Control	Valproate	Monteleukast	Combination
Percentage	0%	66.7%	33.3%	100%
	A	B	C	D

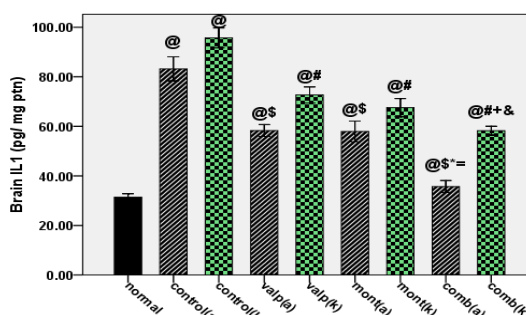
Values with different capital letters are significantly different ( $p < 0.05$ )



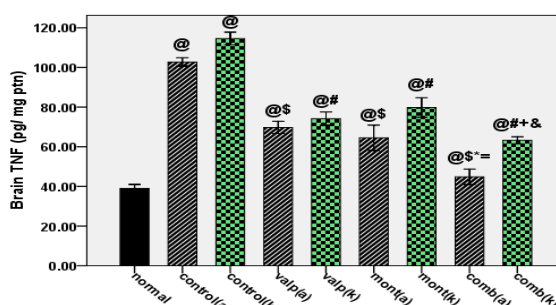
**Fig. 1:** showing comparison between different groups regarding brain GSH in PTZ-acute(a) and kindling (k) models. Values are means of 8 mice  $\pm$ SEM. @  $P < 0.05$  compared to normal group, \$  $P < 0.05$  compared to PTZ-acute control group, \* $P < 0.05$  compared to valproate group, = $P < 0.05$  compared to montelukast (a) group, # $P < 0.05$  compared to PTZ-kindled control, + $P < 0.05$  compared to valproate (k) group, &  $P < 0.05$  compared to montelukast (k).



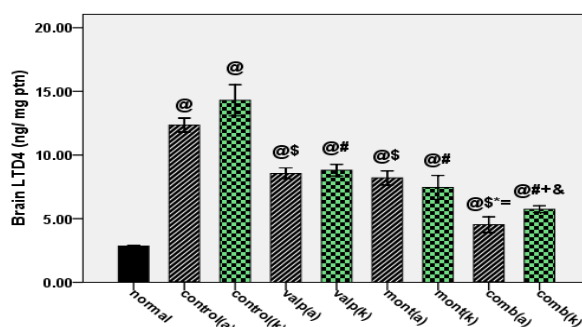
**Fig. 2:** showing comparison between different groups regarding brain MDA in PTZ-acute(a) and kindling(k) models. Values are means of 8 mice  $\pm$ SEM. @ P <0.05 compared to normal group, \$ P <0.05 compared to PTZ-acute control group, \*P <0.05 compared to valproate group, =P <0.05 compared to montelukast (a) group, #P <0.05 compared to PTZ-kindled control, +P <0.05 compared to valproate (k) group & P <0.05 compared to montelukast (k).



**Fig.3:** showing comparison between different groups regarding brain IL1- $\beta$  in PTZ-acute(a) and kindling(k) models. Values are means of 8 mice  $\pm$ SEM. @ P <0.05 compared to normal group, \$ P <0.05 compared to PTZ-acute control group, \*P <0.05 compared to valproate group, =P <0.05 compared to montelukast (a) group, #P <0.05 compared to PTZ-kindled control, +P <0.05 compared to valproate (k) group, & P <0.05 compared to montelukast (k).



**Fig. 4:** showing comparison between different groups regarding brain TNF- $\alpha$  in PTZ-acute(a) and kindling (k) models. Values are means of 8 mice  $\pm$ SEM; statistical comparisons are carried out using one-way ANOVA followed by post-hoc tests using LSD method. @ P <0.05 compared to normal group, \$ P <0.05 compared to PTZ-acute control group, \*P <0.05 compared to valproate group, =P <0.05 compared to montelukast (a) group, #P <0.05 compared to PTZ-kindled control, +P <0.05 compared to valproate (k) group, & P <0.05 compared to montelukast (k).



**Fig. 5:** showing comparison between different groups regarding brain LTD4 in PTZ-acute (a) and kindling (k) models. Values are means of 8 mice  $\pm$ SEM. @ P <0.05 compared to normal group, \$ P <0.05 compared to PTZ-acute control group, \*P <0.05 compared to valproate group, =P <0.05 compared to montelukast (a) group, #P <0.05 compared to PTZ-kindled control, +P <0.05 compared to valproate (k) group, &P <0.05 compared to montelukast (k).

## DISCUSSION

The present work revealed that all animals injected with PTZ (either a single or repeated doses) reached stage 4/5 clonic convulsions. These were associated with a significant reduction in brain GSH and significant elevation in brain MDA in both animal models as compared to normal vehicle-treated subgroups.

It is postulated that oxidative stress produced by reactive oxygen species (ROS) generation is likely implicated in the initiation and progression of epilepsy<sup>[20]</sup>. Our results coincide with Cárdenas-Rodríguez et al.<sup>[21]</sup> who stated that there is a significant elevation of lipid peroxidation in association with a significant decrease in the antioxidant enzymes and reduced glutathione (GSH) levels in whole brain of acute PTZ and PTZ-kindled animals.

One explanation how oxidative stress occurs during epileptogenesis is that seizures-associated glutamate excitotoxicity and N-methyl D-aspartate (NMDA) receptor overactivation result in elevation of intraneuronal  $Ca^{2+}$  and decrease of intracellular antioxidant GSH due to cystine deprivation caused by the binding of glutamate to cystine transporter<sup>[22]</sup>. ROS could be produced as a result of calcium-mediated activation of xanthine oxidase, protease and nuclease enzymes that disrupt the cellular antioxidant system<sup>[23]</sup>. Moreover,  $Ca^{2+}$ -dependent activation of phospholipase A2 releases arachidonic acid yielding free radicals through metabolism by lipoxygenases<sup>21</sup>. Likewise, calcium mediated mitochondrial damage plays a key role in ROS production. On the other hand, ROS are thought to be implicated in excitotoxicity and apoptosis that contribute to seizure-induced brain damage<sup>[24]</sup>. Free radicals in turn can further increase glutamate concentration and diminish GABA through inactivation of glutamine synthase, inhibition of glutamate decarboxylase and inactivation of glutamate transporter<sup>[25]</sup>. Additionally, ROS may activate NF $\kappa$ B, leading to production of pro-inflammatory cytokines such as IL-1, IL-6 and TNF- $\alpha$ , which in turn enhance inflammation and, therefore, the generation of other reactive species<sup>[26]</sup>.

In the current work, a single or chronic PTZ administration resulted in elevation of the brain levels of inflammatory cytokines (IL1 $\beta$  and TNF- $\alpha$ ). These findings are in agreement with those reported by Vezzani et al.<sup>[5]</sup> who found that increased levels of inflammatory cytokines are detected in animal models of epilepsy induced by chemoconvulsants or by electrical stimulation<sup>[13]</sup>. Moreover, Gómez, et al.<sup>[27]</sup> disclosed elevated cytokines level in the serum and cerebrospinal fluid of epileptic patients. The elevated levels of brain inflammatory mediators documented in the current work may indicate their role in seizure induction or propagation. In line with this view, IL-1 $\beta$  receptor antagonist inhibited PTZ-induced seizures, kindling development in rats<sup>[28]</sup>. IL-1 $\beta$  through binding to IL-1R1 and TNF $\alpha$  activate transcription factors such as NF- $\kappa$ B which regulates the synthesis of chemokines, cytokines, enzymes (for example, CoX-2) and receptors (for example, TLRS, IL-1R1, and TNF p55 and p75 receptors) that are involved in epileptogenesis<sup>[29]</sup>. NF- $\kappa$ B activation can also induce the generation of neurotoxic free radicals; resulting in neuronal apoptosis<sup>[30]</sup>. In addition, occupancy of IL-1R1 or TLRS results in activation of src tyrosine kinase, mitogen activated protein kinases (MAPK) system with subsequent induction of neuronal cyclic adenosine monophosphate (cAMP) response element-binding protein<sup>[31]</sup>. These pathways result in phosphorylation of voltage-dependent and receptor-coupled ion channels, and increasing the neuronal excitability<sup>[31]</sup>. For example, the proconvulsant activity of IL-1 $\beta$ /IL-1R1 complex is mediated by phosphorylation of the NMDA receptor 2B subunit via src, with subsequent neuronal calcium influx and excitotoxicity<sup>[5]</sup>. Furthermore, the phosphorylation of the NR2B subunit seems to prevent endocytosis and protect this subunit from calpain ( $Ca^{2+}$ -dependant pttease) degradation<sup>[32]</sup>.

Additional mechanisms of hyperexcitability induced by IL-1 $\beta$  and TNF- $\alpha$  include cytokine-mediated glutamate release from astrocytes, inhibition of glial glutamate reuptake (through inhibition of glutamate transporter GLUT-1)<sup>5</sup> and increasing the glutamate receptor subunit expression<sup>[29]</sup>. IL-1 $\beta$  and TNF- $\alpha$  can further decrease the GABA-

mediated inhibition in inflamed brain tissue through their ability to reduce GABA-A receptors expression or induce their endocytosis at neuronal membranes<sup>[33]</sup>.

The results of the current study showed that acute and chronic PTZ injection induced a significant elevation in the brain level of LtD4. Formation of leukotrienes is initiated by 5-LOX-mediated conversion of arachidonic acid to 5(S)-hydroperoxy-eicosatetraenoic acid, which is further transformed to LtA4. This unstable epoxide can be further hydrolyzed to LtB4<sup>[34]</sup>. Alternatively, LtA4 may be converted to LtC4 which get transformed by gamma glutamyl transpeptidase to LtD4 and LtE4<sup>[35]</sup>.

The implication of leukotrienes in epilepsy is supported by Shin, et al.<sup>[36]</sup> who elucidated that phenidone which decreased leukotriene production, protected against kainate-induced seizures. At the same time, phenidone dose dependently attenuated the KA-induced oxidative stress in hippocampal neurons. Similarly, Liu, et al.<sup>[37]</sup>, have shown that zileuton, a 5-LOX inhibitor, decreases pilocarpine induced seizures in rats.

In addition, Vezzani and Granata<sup>[31]</sup>, revealed that TNF- $\alpha$  stimulates arachidonic acid release and induces the transcription of inflammatory genes that increase LOX enzyme expression<sup>[13]</sup>. That effect was confirmed in the present work, where PTZ administration resulted in elevation of mice brain TNF- $\alpha$  and LtD4 in both models.

In the present study, valproate administration was observed to protect against PTZ-induced acute seizures and kindling, hampered brain oxidative stress and reduced the brain levels of inflammatory mediators. The anti-oxidant effect of valproate is attributed to its free radical scavenging ability<sup>[38]</sup>, enhancement of enzymatic as well as non-enzymatic antioxidants (Superoxide dismutase, catalase, vitamin E, and reduced glutathione)<sup>[39]</sup>. Furthermore, blockade of NMDA receptor and Na<sup>+</sup> channel-mediated glutamate release and excitotoxicity in cerebral nerve endings<sup>[27]</sup> may be an additional contributing factor in ROS production inhibition.

Moreover, by inhibiting histone deacetylase (HDACs), valproate has the ability

to activate the expression of genes targeting antioxidant enzymes (e.g. SOD), and induces several neuroprotective proteins such as angiogenin (ANG), brain-derived neurotrophic factor (BDNF), endothelial cell growth factor (ECGF1) and ganglia cell-derived neurotrophic factor (GDNF)<sup>[40]</sup>. Additionally, Ximenes et al.<sup>[41]</sup> referred the valproate-suppressing effect on inflammatory mediators to the inhibition of NF- $\kappa$ B.

The present work showed that montelukast alone failed to protect against PTZ-induced acute seizures which may explained on the basis that acute seizures are blocked mainly by agents either acting at the GABA<sub>A</sub> receptor or agents that reduce T-type Ca<sup>2+</sup> currents<sup>[42]</sup> rather than those affecting oxidative stress and inflammatory cascades as occur in kindling induced seizure<sup>[43]</sup> while repeated administration of montelukast, protected the animals against PTZ-induced kindling. The observed anti-seizure effect of montelukast could be attributed to its antioxidant property represented in elevation of GSH concomitant with reduction of the brain level of MDA. In line with our results, Ozturk, et al.<sup>[44]</sup> documented an anti-oxidant effect of montelukast in ischemia/reperfused liver, intestine, kidney, testes and bladder models.

In both PTZ models, montelukast administration suppressed the brain levels of inflammatory mediators; IL1 $\beta$ , TNF $\alpha$  and LtD4, that was in line with those of Al-Amran, et al.<sup>[45]</sup>. Such effect could be attributed to the blockade of LTs enhancement effect on TNF- $\alpha$  induced cytokines production via NF- $\kappa$ B<sup>[46]</sup> and the diminution of CysLTs-mediated calcium release and subsequently protein kinase C, MAP, and PI3K activation<sup>[47]</sup>. Interestingly, montelukast was reported to possess a range of secondary CysLT1R-independent, anti-inflammatory activities, apparently unrelated to antagonism of CysLT1Rs. These include (i) inhibition of 5-lipoxygenase (5-LOX) resulting in attenuation of production not only of cysLTs but also of LtB4<sup>48</sup>, (ii) inhibition of histone acetyltransferase (HAT) inhibiting inflammatory genes expression<sup>49</sup> and (iii) inhibition of cyclic nucleotide phosphodiesterases (PDEs) the accumulated

cAMP through cAMP-dependent protein kinase A (PKA) promoting restoration of  $Ca^{2+}$  homeostasis<sup>50</sup>.

The observed potentiating effect of montelukast on the anticonvulsant action of valproate in both models was associated with a significant attenuation in oxidative stress markers and inflammatory mediators release, as compared to each of the valproate and to the montelukast groups in both PTZ models. In parallel with our results, Fleck et al.<sup>11</sup> showed that montelukast synergistically enhances the anticonvulsant effect of phenobarbital in an acute PTZ-induced seizure model in mice. A clinical study performed by Takahashi et al.<sup>51</sup>, has shown that pranlukast (a CysLT receptor antagonist) add-on therapy in patients with intractable partial epilepsy reduced seizure frequencies.

### CONCLUSION

The results of the current study clarify the role of oxidative stress, inflammatory cytokines and leukotrienes in acute seizures, as well as in the process of kindling, and favor the protective effect of montelukast co-administered with valproate against experimentally induced seizures.

### ABBREVIATIONS

ANG: Angiotensin; BBB: Blood-brain barrier; BDNF: Brain-derived neurotrophic factor; cAMP: Cyclic adenosine monophosphate; COX: Cyclo-oxygenase enzyme; CysLT: Cysteinyl leukotriene; ECGF1: Endothelial cell growth factor; ERK: Extracellular receptor kinase; GABA: Gamma amino butyric acid; GDNF: Glial cell-derived neurotrophic factor; GSH: Reduced glutathione; I/R: Ischemia reperfusion; IL-1 $\beta$ : Interleukin1 $\beta$ ; IL-1R: IL-1 receptor; Kir4.1: Inward rectifier potassium channel; LOX: Lipo-oxygenase; MAPK: Mitogen activated protein kinase; MDA: Malondialdehyde; MMP-9: Matrix-metalloproteinases-9; NF $\kappa$  $\beta$ : Nuclear factor kappa; NMDA: N-methyl-D-aspartic acid; PI-3-K: Phosphoinositide-3-kinase; PKA, Protein kinase A; ROS: Reactive oxygen species; SOD: Superoxide dismutase; TGF- $\beta$ : Transforming Growth Factor- $\beta$ ; TLR: Toll like receptor; TNF- $\alpha$ : Tumor Necrosis Factor- $\alpha$ ; n: number of animals.

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