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# Enoxaparin pretreatment alleviates pentylenetetrazol–induced epileptic seizures in Wistar rats

## Pretratamiento con enoxaparina alivia las crisis epilépticas inducidas por pentilentetrazol en ratas Wistar

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### ABSTRACT

Epilepsy, is a prevalent neurological disorder characterized by recurring seizures. A low molecular weight heparin enoxaparin has multifaceted properties. In addition to its anticoagulant activity, enoxaparin has demonstrated anti–inflammatory, antioxidant and anti–apoptotic effects. Accordingly, the purpose of this study was to evaluate the protective effect of enoxaparin against seizures, oxidative stress, proinflammatory cytokines, apoptosis, brain–derived neurotropic factor (BDNF) concentrations and cognitive impairment in pentylenetetrazole (PTZ) induced kindling in Wistar rats. Twenty–four rats divided into 4 groups (Control, PTZ, $ENX_{250}$+PTZ, $ENX_{500}$+PTZ) were used. Enoxaparin (250 and 500 IU·kg$^{-1}$, intraperitoneal –ip–) or vehicle (saline) were given to rats for 5 days. On the fifth day, 30 min after drug administration, PTZ (45 mg·kg$^{-1}$, ip) was given to cause seizures. Behavioral seizure parameters were evaluated by video recording. A behavioral test, passive avoidance test was performed. PTZ administration decreased total antioxidant status (TAS) while increased total oxidant status (TOS) both in hippocampus and cortex. Furthermore, PTZ induced elevated levels of tumor necrosis factor alpha (TNF–α), interleukin–1β (IL–1β), BDNF, caspase–3, and caspase–9. Pretreatment with enoxaparin decreased the levels of these parameters and TOS, while increased TAS. Enoxaparin pretreatment significantly decreased the epileptic seizure scores according to the Racine scale, increased first myoclonic jerk (FMJ) time and the test trial time in passive avoidance test. These results indicate that enoxaparin (250 and 500 IU·kg$^{-1}$) at both doses has promising protective effect against PTZ induced epilepsy by improving memory impairment, inflammation, oxidative stress and apoptosis. This positive effect was more prominent at 500 IU·kg$^{-1}$ dose of enoxaparin.

**Key words:** Enoxaparin; epilepsy; antioxidant; anti–inflammatory; anti–apoptotic

### RESUMEN

La epilepsia, un trastorno neurológico prevalente caracterizado por convulsiones recurrentes, que centran la atención sobre las propiedades multifacéticas de la enoxaparina, una heparina de bajo peso molecular. Además de su actividad anticoagulante, la enoxaparina ha demostrado efectos antiinflamatorios, antioxidantes y antiapoptóticos. En consecuencia, el propósito de este estudio fue evaluar el efecto protector de la enoxaparina contra las convulsiones, el estrés oxidativo, las citoquinas proinflamatorias, la apoptosis, las concentraciones del factor neurotrópico derivado del cerebro (BDNF) y el deterioro cognitivo en el encendido inducido por pentilentetrazol (PTZ) en ratas Wistar. Se utilizaron veinticuatro ratas divididas en 4 grupos (Control, PTZ, $ENX_{250}$+PTZ, $ENX_{500}$+PTZ). Se administró a ratas enoxaparina (250 y 500 UI·kg$^{-1}$,intraperitoneal –ip–) o vehículo (solución salina) durante 5 días. El quinto día, 30 min después de la administración del fármaco, se administró PTZ (45 mg·kg$^{-1}$, ip) para provocar convulsiones. Los parámetros conductuales de las convulsiones se evaluaron mediante grabación de vídeo. Se realizó una prueba conductual, prueba de evitación pasiva. La administración de PTZ disminuyó el estado antioxidante total (TAS) mientras que aumentó el estado oxidante total (TOS) tanto en el hipocampo como en la corteza. Además, PTZ indujo niveles elevados de factor de necrosis tumoral alfa (TNF–α), interleucina–1β (IL–1β), BDNF, caspasa–3 y caspasa–9. El pretratamiento con enoxaparina disminuyó los niveles de estos parámetros y TOS, mientras que aumentó TAS. El pretratamiento con enoxaparina disminuyó significativamente las puntuaciones de las crisis epilépticas según la escala de Racine, aumentó el tiempo del primer tirón mioclónico (FMJ) y el tiempo de prueba en la prueba de evitación pasiva. Estos resultados indican que la enoxaparina (250 y 500 UI·kg$^{-1}$) en ambas dosis tiene un efecto protector prometedor contra la epilepsia inducida por PTZ al mejorar el deterioro de la memoria, la inflamación, el estrés oxidativo y la apoptosis. Este efecto positivo fue más prominente con una dosis de 500 UI·kg$^{-1}$ de enoxaparina.

**Palabras clave:** Enoxaparina; epilepsia; antioxidante; antiinflamatorio; antiapoptótico

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## INTRODUCTION

Epilepsy, which is caused by excessive electrical stimulation of certain cell groups in the brain, is a common and serious neurological disorder. It is estimated that there are approximately fifty million epilepsy patients worldwide (~ 1% prevalence). While the incidence of epilepsy is highest in childhood and old age, it is lower in early adulthood [1]. Based on clinical information and electroencephalography (EEG) changes, epilepsies can be broadly categorized into three types: generalized seizures (including tonic, clonic, myoclonic, absence seizures, etc.), partial seizures (such as simple partial, complex partial seizures, etc.) and unclassified seizures (including certain tonic-clonic seizures that occur during sleep) [2].

As the epileptic seizures created in animal models are very similar to those in humans, pentylenetetrazole (PTZ) is one of the commonly used agents in inducing primary generalized seizures [3]. PTZ is a tetrazole derivative (1,5-pentamethylene; 6, 7, 8, 9 tetrahydro-5 azetpotetrazole). Its mechanism of action is not fully understood. PTZ shows its central nervous system (CNS) stimulating effect by binding to the gamma amino butyric acid-A (GABA-A) / benzodiazepine (BZD) receptor complex and preventing the opening of chlorine ($Cl^-$) channels. PTZ reduces the activity of GABA synapses through the GABA receptor-BZD-chloride ionophore complex and facilitates the depolarization of neurons. It has been reported that the number of BZD receptors increases as a result of repeated PTZ injections [4]. Changes in the amount of extra and intracellular ions, increased excitatory or decreased inhibitory activity, impairments in specific membrane functions are observed with seizures provoked by PTZ administration [5].

Enoxaparin, categorized as a low molecular weight heparin, has been documented to demonstrate various pharmacological properties. These include anti-inflammatory, antioxidant anti-apoptotic, and neuroprotective effects, alongside its primary anticoagulant function [6, 7, 8, 9, 10, 11, 12]. Studies have shown that enoxaparin is effective in the treatment of many inflammatory disorders such as inflammation reaction after pediatric cataract surgery [6], ST-segment elevated myocardial infarction [7], mast cell mediated allergic inflammation [8], allergic asthma [8, 9] and acute colitis [10]. Enoxaparin has demonstrated efficacy in mitigating fibrosis progression in cirrhosis [11], as well as protecting against liver necrosis and apoptosis induced by $CCL_4$ [12], liver fibrosis induced by dimethyl nitrosamine (DMN) [13], liver toxicity induced by radiation [14], and experimental cholestatic liver injury [15].

In the literature, to our knowledge no protective effect of enoxaparin was found in PTZ-induced rat epilepsy model. In this study, we aimed to investigate the protective effect of enoxaparin on cognitive impairment, the oxidant-antioxidant balance, proinflammatory cytokines, apoptosis and brain-derived neurotropic factor (BDNF) concentrations at the level of the hippocampal and cerebral cortex in PTZ-induced rat epilepsy model.

## MATERIAL AND METHODS

### Drugs and Chemicals

Enoxaparin (Oksapar) was obtained from Kocak Pharma (Bagcilar, Istanbul, Türkiye), while PTZ was sourced from Sigma-Aldrich (St. Louis, MO, USA). The assay kits for total antioxidant status (TAS) and total oxidant activity (TOS) were acquired from Sunred Biological Technology Co. Ltd. (Shanghai, China). Assay kits for tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), BDNF, Caspase-3, and Caspase-9 were purchased from BT Lab, Technology Laboratory (Shanghai, China). All remaining chemical agents utilized in the study were of analytical grade purity.

### Animals

The research was conducted at the Experimental Animals Laboratory within the Faculty of Medicine at Sivas Cumhuriyet University. A total of twenty-four Wistar Albino rats *(Rattus norvegicus),* aged between 2 and 3 months and weighing an average of 220 g, were utilized in the research. The rats were housed in a specially designed room with sound insulation, maintained at a room temperature of 22 ± 1°C, and a humidity level of 55 ± 6%. The rats were exposed to a 12-hour light/dark cycle and provided *ad libitum* access to standard pellet chow and water throughout the experimental duration. Experiments were conducted from 9:00 to 16:00 hours, with vigilant monitoring of light and sound levels in the experimental setting. The study adhered to the ethical guidelines set by the Sivas Cumhuriyet University Local Ethical Committee (CUHAYDEK) (Number:65202830-050.04.04-396). Additionally, the research followed the guidelines outlined in the EU Directive 2010/63/EU pertaining to animal experimentation.

### Animal Groups and Treatment Protocols

Twenty-four rats were randomly divided into the control, PTZ, $ENX_{250}$ + PTZ, and $ENX_{500}$ + PTZ groups, 6 rats per group. Rats received enoxaparin (250 or 500 IU·kg$^{-1}$day), or vehicle (saline) intraperitoneal -ip- for 5 days at the same time (10:00 AM) and a single dose PTZ (45 mg·kg$^{-1}$, ip) was applied on the 5th day, 30 min after drug administration to induce epileptic seizure [16]. Enoxaparin doses of 250 IU·kg$^{-1}$day and 500 IU·kg$^{-1}$day were adopted from previous studies [17, 18, 19, 20]. Seizure behavior parameters were assessed using two criteria: the Racine convulsion scale (RCS) and time to first myoclonic jerk (FMJ), observed via video recording for 30 min post-administration of PTZ [21]. The RCS entails a 6-point scoring system for evaluating epilepsy in mice. The seizure stages were categorized based on RCS as follows: stage 0, indicating no seizure response; stage 1, characterized by twitching of the vibrissae and pinnae; stage 2, involving motor arrest accompanied by more pronounced twitching; stage 3, featuring motor arrest with generalized myoclonic jerks; stage 4, exhibiting tonic-clonic seizure while the animal retains an upright position; stage 5, presenting tonic-clonic seizure with loss of the righting reflex; stage 6, representing a lethal seizure [22]. A behavioral test, specifically the passive avoidance test, was conducted 24 hours after the administration of PTZ. The experimental procedure of the study is depicted in FIG. 1.

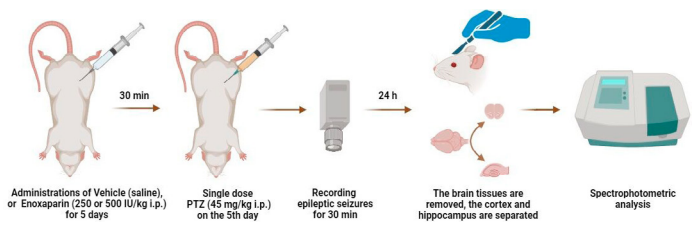


**FIGURE 1. Design of the experiment (Generated using BioRender)**

### Passive Avoidance Test

The passive avoidance test is a widely used, straightforward, and swiftly applicable memory assessment. It is conducted within a setup comprising two compartments—one light and one dark—measuring 20.3×15.9×21.3 cm in size. The floors of these compartments are constructed of stainless steel wire with a diameter of 3.175 mm at 8 mm intervals, overlaid with a grid connected to an electrical source, and separated by a door. The test spans two days: the first day serves as the learning phase, during which the rat is situated in the light chamber; once it fully enters the dark chamber, a 1 mA shock is administered to its feet via the electric grid for 5 seconds –s–. The subsequent day constitutes the testing phase. The test is concluded if the rat fails to transition into the dark chamber within 300 s. If, on the second day, the rat exhibits avoidance of the dark partition, it is indicative of passive avoidance resulting from learning. Typically, rats tend to prefer transitioning from the light chamber to the dark chamber.

### Preparation of Brain Tissue Homogenates

At the end of passive avoidance test, rats were euthanized by cervical dislocation method. The brain tissues were removed, the cortex and hippocampus were separated. The brain tissue samples were subjected to homogenization using a mechanical homogenizer (IKA T 25 Digital Ultra–Turrax, Germany). Then the homogenates were centrifuged (4000 rpm, 10 min, 4°C). The harvested supernatants were quickly frozen using liquid nitrogen and then preserved at -80°C for subsequent analysis.

### Total Oxidant Status and Total Antioxidant Status

The brain homogenate's total antioxidant status (TAS) and total oxidant status (TOS) were evaluated utilizing a microplate spectrophotometer (Thermo Scientific Multiskan GO Microplate Spectrophotometer, USA). We used commercially available standard enzymatic kits (Sunred, China) for the analysis, following the manufacturer's instructions. Absorbance measurements were taken at a wavelength of 450 nm. TAS and TOS activities were quantified as "U·mg protein$^{-1}$ for brain tissue."

### TNF–α and IL–1β Levels

We used ELISA kits from BT Lab (China) to measure the levels of TNF–α and IL–1β in the tissue samples. After centrifuging the brain tissue homogenates at 300 G for 10 min at 4°C, we collected the supernatant for further analysis. The absorbance of the samples was then measured at 450 nm using an ELISA reader. Levels of TNF–α were measured in ng·mg of protein$^{-1}$, while IL–1β levels were quantified in pg·mg of protein$^{-1}$.

### Caspase–3 and Caspase–9

Caspase–3 and caspase–9 activity were assessed employing commercial ELISA kits (BT Lab, China) following the manufacturer's guidelines. Brain tissue samples were homogenized in PBS using a tissue homogenizer, then centrifuged at 5000 G for 15 min at 4°C. The resultant supernatant was collected, and ELISA reagent was added to each well. Following a 1–hour incubation at 37°C, the samples were measured at 450 nm using an ELISA reader. Caspase–3 and caspase–9 levels were expressed as a percentage relative to the control. Furthermore, the levels of caspase–3 and caspase–9 were quantified as ng·mg of protein$^{-1}$.

### Measurement of Brain–Derived Neurotrophic Factor (BDNF)

Brain supernatants were used to measure BDNF levels utilizing a rat ELISA commercial kit (BT Lab, Shanghai, China), following the manufacturer's instructions. In brief, tissue samples and standards were added to the plate and then incubated at 37°C for 60 min (Boeco, PST–60 HL 4 Plus, Germany). After incubation, the plate was washed five times with washing solution, and staining solutions were added, followed by another incubation at 37°C for 10 min. Finally, stop solution was added, and the absorbance of all samples was read at 450 nm using an ELISA reader (Thermo Fisher Scientific, Altrincham, UK). Standard curves provided in the kit were used for calculations, ensuring accuracy. Coefficients of variation within groups were less than 10% for all analyzed samples. Total protein content was assessed using the method described by Bradford *et al*. [23].

### Statistical analysis

Racine scale data, myoclonic jerk time, behavioral evaluations and biochemical analysis obtained as a result of the experiments was converted into numerical values by first and SPSS statistics program (SPSS 25.0 for windows) was used for statistical analysis. The experimental findings were presented as mean ± standard error. Data comparison among groups was conducted through analysis of variance (One–way ANOVA), and the group originating from the intergroup difference was determined by Tukey HSD (post–hoc test). Statistical significance was defined at the $P<0.05$ level.

## RESULTS AND DISCUSSION

### Seizure stage and first myoclonic jerk

Epileptic parameters were assessed through video recordings following PTZ injection in rats. In comparison to the PTZ group, enoxaparin notably reduced epileptic seizure scores as per the Racine scale ($P<0.05$) at a dose of 500mg·kg$^{-1}$ (FIG. 2a). Moreover, delayed the FMJ time significantly both in $ENX_{250}$+ PTZ and $ENX_{500}$+ PTZ groups ($P<0.001$) (FIG. 2b). A tendency was observed towards reduced RCS scores with the administration of a higher dose (500 IU·kg$^{-1}$) of enoxaparin compared to a lower dose (250 IU·kg$^{-1}$) of enoxaparin. Moreover, the duration of FMJ (generalized myoclonic jerks) increased with the higher enoxaparin dose in contrast to the lower enoxaparin dose.

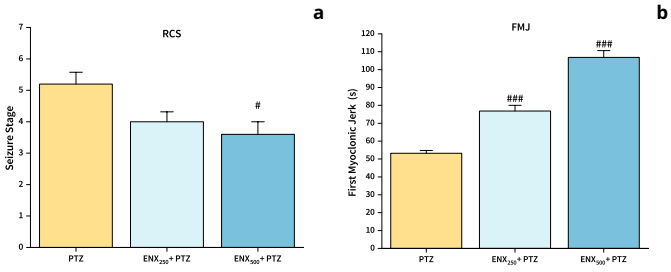


**FIGURE 2. Seizure stage (RCS) and first myoclonic jerk (FMJ). Effect of enoxaparin on (a) seizure stage and on (b) the first myoclonic jerk following PTZ–induced seizure in rats. PTZ: Pentylenetetrazol; ENX: Enoxaparin. # $P<0.05$, ### $P<0.001$ compared with the PTZ group**

### Passive Avoidance Test

To assess memory impairment subsequent to PTZ–induced seizures, we employed the passive avoidance test. During the training trials, no statistically significant differences were detected among the groups ($P$>0.05) (FIG. 3a). However, noticeable disparity was noted during the test trials, particularly within the PTZ group. ($P$<0.001) (FIG. 3b). Notably, the test trial duration in the $ENX_{250}$ + PTZ and $ENX_{500}$ + PTZ groups demonstrated a significant increase compared to the PTZ group ($P$<0.001) (FIG. 3b).

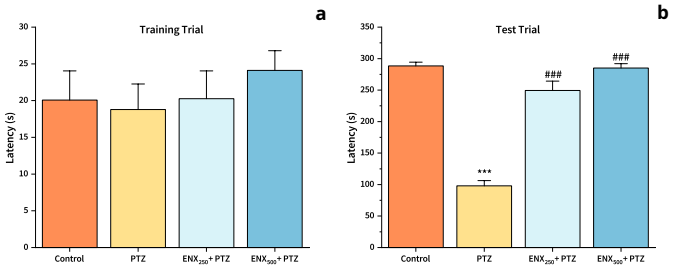


**FIGURE 3. Passive avoidance assessment. Effect of enoxaparin on (a) passive avoidance test during training 001 co trial and on (b) test trial following PTZ–induced seizures in rats. PTZ: Pentylenetetrazol; ENX: Enoxaparin. *** $P$<0.001 compared to the control group; ### $P$<0.001 compared to the PTZ group**

### TAS and TOS Levels

The levels of TAS and TOS in brain cortex and hippocampus tissue homogenates were assessed using commercially available kits. A notable reduction in TAS levels within brain tissue homogenates was observed in the PTZ–treated group compared to the control group ($P$<0.01). Treatment with enoxaparin at a dose of 500 IU·kg$^{-1}$ significantly improved TAS levels in cortex and hippocampus tissues following PTZ–induced neurotoxicity ($P$<0.05, $P$<0.01) (FIG. 4 a–b). Furthermore, enoxaparin treatment at doses of 250 IU·kg$^{-1}$ and 500 IU·kg$^{-1}$ significantly decreased TOS levels in cortex tissue homogenates in the PTZ–treated group ($P$<0.01, $P$<0.001). In hippocampus tissue homogenates, 500 IU·kg$^{-1}$ enoxaparin treatment significantly reduced TOS levels ($P$<0.05) (FIG. 4c–d).

### Inflammatory Markers (TNF–α and IL–1β)

Brain TNF–α was significantly higher in both cortex and hippocampus tissues ($P$<0.001, $P$<0.01) in the PTZ group relative to controls (FIG. 5 c–d). Enoxaparin treatment significantly lowered cortex TNF– α levels both in $ENX_{250}$ + PTZ and $ENX_{500}$ + PTZ groups (both $P$<0.001) (FIG. 5a), while in hippocampal tissues significant decrease was only seen in $ENX_{500}$ + PTZ group ($P$<0.05) (FIG. 5b). Again, IL–1β levels were significantly higher in both cortex and hippocampus tissues ($P$<0.01, $P$<0.001) in the PTZ group compared to controls (FIG. 5c–d). Enoxaparin treatment led to a significant reduction in IL–1β levels in both cortex ($P$<0.01, FIG. 5c) and hippocampal tissues ($P$<0.01, FIG. 5d) in $ENX_{250}$ + PTZ and $ENX_{500}$ + PTZ groups.

### Caspase–3 and Caspase–9

Administration of PTZ resulted in a significant increase ($P$<0.001) in hippocampal levels of caspase–3 and caspase–9 compared to the control group. Enoxaparin administration at both 250 IU·kg$^{-1}$ and 500 IU·kg$^{-1}$ doses reduced tissue caspase–3 and caspase–9 levels compared to the PTZ group ($P$<0.001) (FIG. 6a, b).

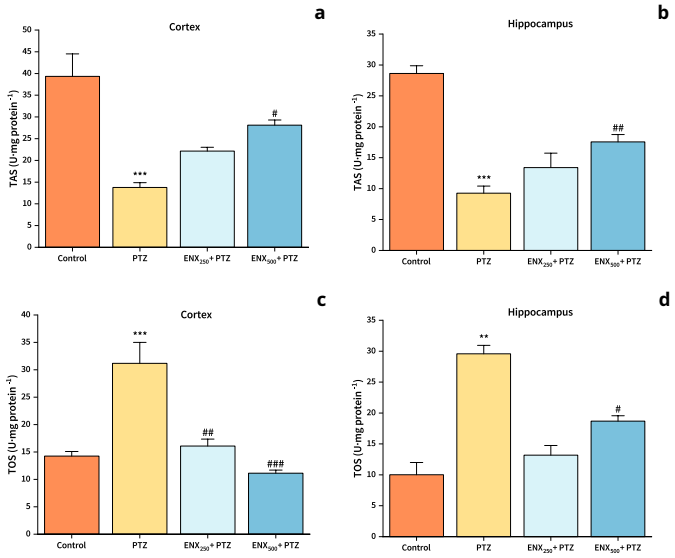


**FIGURE 4. Total antioxidant status (TAS) levels. Effect of enoxaparin on (a) TAS levels in the cortex and (b) in the hippocampus following PTZ–induced seizures in rats. *** $P$<0.001 compared to the control group; # $P$<0.05, ## $P$<0.01 compared to the PTZ group. Total oxidant status (TOS) levels. Effect of enoxaparin on (c) TOS levels in and (d) in the hippocampus after PTZ–induced seizures in rats. PTZ: Pentylenetetrazol; ENX: Enoxaparin. ** $P$<0.01, *** $P$<0.001 compared to the control group; # $P$<0.05, ## $P$<0.01, ### $P$<0.001 compared to the PTZ group**

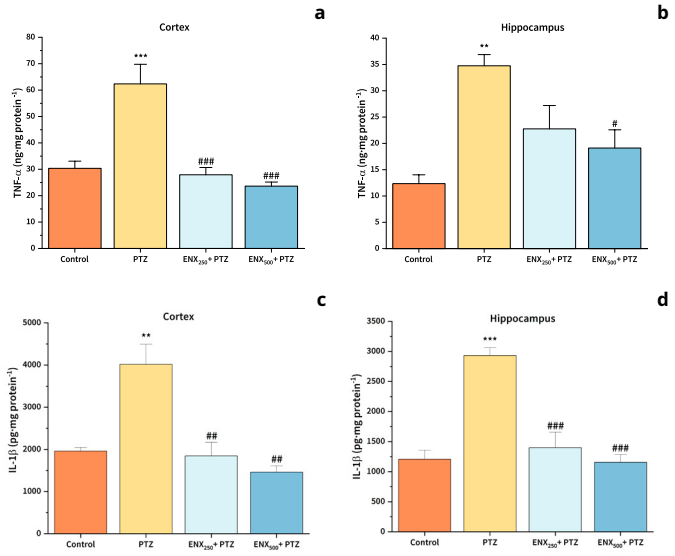


**FIGURE 5. Tumor necrosis factor alpha (TNF–α) levels. Effect of enoxaparin on (a) TNF–α levels in the cortex and (b) in the hippocampus following PTZ–induced seizures in rats. ** $P$<0.01, *** $P$<0.001 compared to the control group; # $P$<0.05, ### $P$<0.01 compared to the PTZ group. Interleukin–1β (IL–1β) levels. Effect of enoxaparin on (c) IL–1β levels in the cortex and (d) in the hippocampus following PTZ–induced seizures in rats. PTZ: Pentylenetetrazol; ENX: Enoxaparin ** $P$<0.01, *** $P$<0.001 compared to the control group; ## $P$<0.01, ### $P$<0.01 compared to the PTZ group**

### BDNF Levels in Hippocampus and Cortex

In the PTZ group, BDNF levels were observed to be heightened in both the cortex and hippocampus compared to the control group

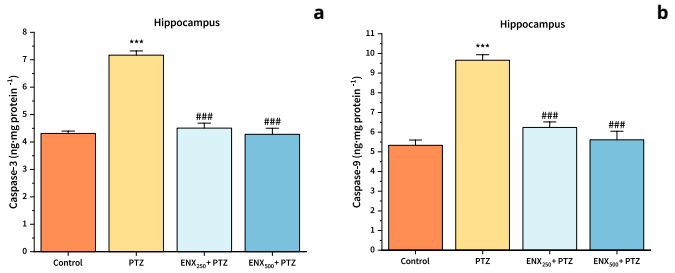


**FIGURE 6. Levels of caspase–3 and caspase–9. Effect of enoxaparin on (a) caspase–3 levels in the hippocampus and (b) caspase–9 levels in the hippocampus following PTZ–induced seizures in rats. PTZ: Pentylenetetrazol; ENX: Enoxaparin. *** *P*<0.001 compared to the control group; ### *P*<0.01 compared to the PTZ group**

(*P*<0.05, *P*<0.001). Nonetheless, administration of enoxaparin markedly reduced BDNF levels in both the cortex and hippocampus compared to the PTZ group (*P*<0.05 to *P*<0.001; FIG. 7a, b).

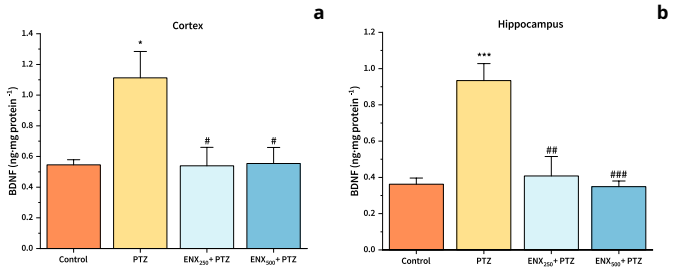


**FIGURE 7. Brain–derived neurotrophic factor (BDNF) levels. Effect of enoxaparin on (a) BDNF levels in the cortex and (b) in the hippocampus following PTZ–induced seizures in rats. PTZ: Pentylenetetrazol; ENX: Enoxaparin. * *P*<0.05, *** *P*<0.001 compared to the control group; # *P*<0.05 ## *P*<0.01, ### *P*<0.01 compared to the PTZ group**

In this study, we explored the potential protective role of enoxaparin, a low molecular weight heparin, against PTZ–induced epilepsy. Our results indicate that enoxaparin significantly mitigated the severity of seizure stages and extended the latency to the initial myoclonic jerk in rats with PTZ–induced epilepsy. Moreover, enoxaparin decreased the memory impairment occurring after epileptic seizures. Enoxaparin pretreatment reduced TOS levels while increasing TAS levels after PTZ–induced epilepsy. In addition, enoxaparin pretreatment caused decreased levels of TNF–α, IL–1β, BDNF, caspase–3 and caspase 9 after PTZ–induced epileptic seizures in rats.

Due to its high oxygen utilization and abundance of polyunsaturated fatty acids that are susceptible to lipid peroxidation, the brain is particularly vulnerable to oxidative stress [24]. Oxidative stress leads to functional cellular degradation and damage that can cause cell death by oxidation of biomolecules such as nucleotides, lipids and proteins. Therefore, oxidative stress–induced brain damage has a strong potential to adversely affect normal central nervous system functions [25]. Oxidative stress takes place in the pathogenesis of a number of neurodegenerative diseases including amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease and epilepsy [25, 26, 27, 28].

Heightened production of reactive oxygen species (ROS) and oxidative stress is a consistent finding in experimental models of epileptogenic insults and in surgical specimens obtained from epilepsy patients. Multiple sources, such as mitochondria, NOX enzymes, and other enzymes, can produce detrimental levels of ROS within crucial compartments. [27, 29]. Previous studies have demonstrated that pretreatment of rodents with antioxidants can decrease or delay seizure activity following the administration of convulsants like PTZ [16, 29]. The anticonvulsant effect is accompanied by reduction of oxidative stress indicators. Studies have demonstrated that PTZ induced epileptic seizures increase free radical production and oxidative damage. Following epileptic seizures, elevated levels of mitochondrial superoxide, inactivated iron– and sulfur–dependent enzymes, may contribute to oxidative damage to neurons [21, 22, 30]. In the present study, TOS levels increased while TAS levels decreased in PTZ applied rats both in the cortex and hippocampus. Pretreatment with enoxaparin reduced TOS levels following induction with PTZ. On the other hand, enoxaparin treatment significantly raised TAS levels in PTZ induced epilepsy rat model in the cortex and hippocampus. Previous studies have demonstrated that enoxaparin reduced oxidative stress and exerted antioxidant effect in various tissues [19, 31, 32]. Our study is in line with these studies. The action of enoxaparin may contribute to the reduction of oxidative stress associated with PTZ–induced epileptic seizures in rats.

Similar to oxidative stress, inflammation appears to be a cause and consequence of epileptic seizures. Inflammation and oxidative stress appear to be linked. ROS modulates inflammatory pathways and in turn, inflammation modulates ROS generation [29]. Therefore, oxidative stress and inflammation are distinct targets for therapeutics in epilepsy. Increased expression of pro–inflammatory cytokines is a consistent feature of the epileptic brain. Several inflammatory cytokines have been shown to exacerbate seizures, and seizures induce inflammation [27, 29]. Inflammatory cytokines TNF–α, IL–6 and IL–1β have been demonstrated to modulate directly or indirectly neuronal excitability [33] In a comparative study increased levels of IL–6 and elevated oxidative stress were seen in patients with drug resistant epilepsies compared to healthy patients [34]. After PTZ application to rats, TNF–α and IL–1β pro–inflammatory cytokine levels increased in this study indicating inflammation in line with similar studies [22, 30]. The anti–inflammatory effect of enoxaparin has been previously reported [6, 9, 35]. Enoxaparin has been shown to attenuate LPS–induced neuronal damage in a mice model indicating that inflammation and neuronal damage are linked [36]. To evaluate the anti–inflammatory protective effect of enoxaparin, we investigated the levels of TNF–α and IL–1β, recognized mediators of inflammatory disorders. Our findings indicate that pretreatment with enoxaparin resulted in decreased levels of PTZ–induced cytokines TNF–α and IL–1β. This observed effect of enoxaparin may contribute to the alleviation of inflammation associated with PTZ–induced epilepsy.

Apoptosis, a process of programmed cell death, can occur through intrinsic and extrinsic pathways. The initiation of the cell's death cascade is marked by the activation of caspase 3. Within neurons, epileptic seizures trigger apoptosis by activating caspase 3 in diverse brain regions, including the hippocampus, thalamus, and amygdala. Recent research suggests that attenuating the overexpression of caspase 3 could alleviate the initiation of seizures [37]. Elevated expression of caspase–3 and caspase–9 has been detected in tissue samples from patients with drug–resistant temporal lobe epilepsy [38]. Furthermore, previous studies have demonstrated that the downregulation of miR–145 enhances the apoptosis of hippocampal neurons in epileptic rats by reducing the expression of caspase 9 [39].

Enoxaparin has been reported to have protective effect by decreasing apoptotic scores in cardiac cells and lung tissue in vivo studies [40, 41]. In the present study, caspase3 and caspase 9 both increased in the cortex and hippocampus after PTZ inducement. However, enoxaparin reduced the elevated caspase3 and caspase 9 levels after PTZ inducement in rats. Additionally neuronal damage in the hippocampus that is known to be involved in memory processes, leads to learning and memory impairment. Several studies have reported that PTZ induced epilepsy led to impairment in passive avoidance memory and caused spatial memory impairment, which is consistent with our study [42, 43]. Enoxaparin treatment has been shown to improve cognition at both early stage and late stage of amyloid β accumulation in mice [44]. Enoxaparin has been shown to improve cognitive functional recovery and reduce brain edema and lesion size in different in vivo models [45]. In our study enoxaparin, significantly improved memory impairment due to PTZ induced epileptic seizure.

BDNF is known to critically regulate synaptic plasticity associated with the cellular learning and memory pattern. The BDNF–TrkB signaling pathway is also known to play a very important role in epileptogenesis. It has been reported that epileptic conditions up-regulate BDNF during the occurrence of epileptic seizures in brain areas such as cortex and hippocampus [46]. It has been observed that in epileptic patients serum BDNF levels increase and BDNF may be a biomarker for epilepsy [47]. Consistent with these studies, PTZ induction resulted in increased levels of BDNF, both in the cortex and hippocampus in rats after epileptic seizures. Furthermore, following PTZ-induced epileptic seizures, the administration of enoxaparin resulted in reduced BDNF levels in both the cortex and hippocampus. Enoxaparin has the capability to enhance BDNF expression in these brain regions, potentially contributing to the suppression of seizures. This mechanism could be a potential pathway through which enoxaparin exerts its neuroprotective effects against PTZ-induced comorbidities related to learning and memory. These findings align well with the results obtained from the passive avoidance test, further supporting the role of enoxaparin in modulating cognitive impairments associated with PTZ-induced seizures.

Efforts directed towards the development of therapeutic approaches aimed at mitigating the deleterious effects of inflammation, oxidative stress, and apoptosis hold the potential to impede the progression of epilepsy. In this study enoxaparin significantly improved PTZ-induced epileptic seizure-related memory impairment. Thus, enoxaparin-mediated reductions in neuronal loss and seizure scores may have contributed to better cognitive abilities.

The current recommendation for thromboembolic complications is reportedly 3.5 mg·kg$^{-1}$ [48]. Additionaly, according to Kobbi *et al.* [17], a dose of 20 mg·kg$^{-1}$ is considered to be the toxic upper limit in rats. The doses of 250 IU·kg$^{-1}$ and 500 IU·kg$^{-1}$ exhibited no negative effects, and as a result, they are recommended for further pharmacodynamic studies [17, 18, 19, 20]. Pharmacokinetic and pharmacodynamic analyses are essential to determine the optimal dose and duration of enoxaparin's effects in this model. Although enoxaparin showed no signs of causing bleeding, further investigation involving non-anticoagulant heparin oligosaccharides may help mitigate potential bleeding risks.

## CONCLUSION

The current study has a limitation that it has not elucidated the molecular mechanism underlying the protective effect of enoxaparin in PTZ-induced epileptic seizures in rats. Another limitation is that it has not clarified the dosage regimens for the neuroprotective effect of enoxaparin. Additional study is needed to unravel the underlying molecular mechanisms and investigate other potential effects of enoxaparin in experimental epilepsy.

In conclusion the outcomes of this study provided persuasive evidence for the neuroprotective efficacy of enoxaparin, low molecular weight heparin against PTZ-induced epilepsy in rats. The findings suggest that enoxaparin's neuroprotective effect may stem from its anti-inflammatory and antioxidant properties, as well as its ability to reduce the expression of caspase-3 and caspase-9. We believe that enoxaparin, as an adjunct agent, has the potential to contribute to epilepsy therapy. Therefore, further investigations are warranted to explore enoxaparin as a promising candidate for the treatment of epilepsy.

### Author Contributions

The study design, material preparation, data collection, and analysis were conducted by [Huseyin Gungor] and [Nergiz Hacer Turgut]. The initial draft of the manuscript was written by [Nergiz Hacer Turgut].

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### Conflicts of interest

The authors declare no conflicts of interest.

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## BIBLIOGRAPHICS REFERENCES

[1] Zupec-Kania BA, Spellman E. An overview of the ketogenic diet for pediatric epilepsy. Nutr. Clin. Pract. [Internet]. 2008; 23(6):589–596. doi: https://doi.org/bhnjc5

[2] Koutroumanidis M, Arzimanoglou A, Caraballo R, Goyal S, Kaminska A, Laoprasert P, Oguni H, Rubboli G, Tatum W, Thomas P. The role of EEG in the diagnosis and classification of the epilepsy syndromes: a tool for clinical practice by the ILAE Neurophysiology Task Force (Part 2). Epileptic Disord. [Internet]. 2017; 19(4):385–437. doi: https://doi.org/gt7bjx

[3] Dhir A. Pentylenetetrazol (PTZ) kindling model of epilepsy. Curr. Protoc. Neurosci. [Internet]. 2012; 58:9.37.1–9.37. 12. doi: https://doi.org/fzszjp

[4] Singh T, Mishra A, Goel RK. PTZ kindling model for epileptogenesis, refractory epilepsy, and associated comorbidities: relevance and reliability. Metab. Brain. Dis. [Internet]. 2021; 36(7):1573–1590. doi: https://doi.org/gqr6rk

[5] Speckmann EJ, Walden J, Bingmann D. Die funktionelle Bedeutung von Calcium-Ionen bei epileptischen Anfällen. Antiepileptische Wirkung organischer Calcium-Antagonisten [Functional

implication of calcium ions in epileptic seizures. Antiepileptic effects of organic calcium antagonists]. Arzneimittelforschung. 1989; 39(1A):149–156. German. PMID: 2655613

[6] Rumelt S, Stolovich C, Segal ZI, Rehany U. Intraoperative enoxaparin minimizes inflammatory reaction after pediatric cataract surgery. Am. J. Ophthalmol. [Internet]. 2006; 141(3):433–437. doi: https://doi.org/dxsg4h

[7] Nasiripour S, Gholami K, Mousavi S, Mohagheghi A, Radfar M, Abdollahi M, Khazaeipour Z, Mojtahedzadeh M. Comparison of the effects of enoxaparin and heparin on inflammatory biomarkers in patients with ST-segment elevated myocardial infarction: a prospective open label pilot clinical trial. Iran J. Pharm Res. [Internet]. 2014 [cited 10 Jan. 2024]; 13(2):583. PMID: 2523735. Available in: https://goo.su/anurf

[8] Baram D, Rashkovsky M, Hershkoviz R, Drucker I, Reshef T, Ben-Shitrit S, Mekori YA. Inhibitory effects of low molecular weight heparin on mediator release by mast cells: preferential inhibition of cytokine production and mast cell-dependent cutaneous inflammation. Clin. Exp. Immunol. [Internet]. 1997; 110(3):485–491. doi: https://doi.org/ctfpt9

[9] Shastri MD, Stewart N, Horne J, Zaidi STR, Sohal SS, Peterson GM, Korner H, Gueven N, Patel RP. Non-anticoagulant fractions of enoxaparin suppress inflammatory cytokine release from peripheral blood mononuclear cells of allergic asthmatic individuals. PLoS One [Internet]. 2015; 10(6):e0128803. doi: https://doi.org/gt7bjz

[10] Lean QY, Eri RD, Randall-Demllo S, Sohal SS, Stewart N, Peterson GM, Gueven N, Patel RP. Orally administered enoxaparin ameliorates acute colitis by reducing macrophage-associated inflammatory responses. PLoS One [Internet]. 2015; 10(7):e0134259. doi: https://doi.org/gt7bj2

[11] Assy N, Hussein O, Khalil A, Luder A, Szvalb S, Paizi M, Spira G. The beneficial effect of aspirin and enoxaparin on fibrosis progression and regenerative activity in a rat model of cirrhosis. Dig. Dis. Sci. [Internet]. 2007; 52:1187–1193. doi: https://doi.org/dbc93t

[12] Kukner A, Tore F, Firat T, Terzi EH, Oner H, Balaban YH, Ozogul C. The preventive effect of low molecular weight heparin on $CCL_4$-induced necrosis and apoptosis in rat liver. Ann. Hepatol. 2010; 9(4):445–454. PMID: 21057164

[13] Lee JH, Lee H, Joung YK, Jung KH, Choi JH, Lee DH, Park KD, Hong SS. The use of low molecular weight heparin-pluronic nanogels to impede liver fibrosis by inhibition the TGF-β/Smad signaling pathway. Biomaterials [Internet]. 2011; 32(5):1438–1445. doi: https://doi.org/cnwdqj

[14] Seidensticker M, Seidensticker R, Damm R, Mohnike K, Pech M, Sangro B, Hass P, Wust P, Kropf S, Gademann G. Prospective randomized trial of enoxaparin, pentoxifylline and ursodeoxycholic acid for prevention of radiation-induced liver toxicity. PloS One [Internet]. 2014; 9(11):e112731. doi: https://doi.org/gkpjh4

[15] Abdel-Salam OM, Baiuomy AR, Ameen A, Hassan NS. A study of unfractionated and low molecular weight heparins in a model of cholestatic liver injury in the rat. Pharmacol. Res. [Internet]. 2005; 51(1):59–67. doi: https://doi.org/fd2b62

[16] Koyuncuoğlu T, Vızdıklar C, Üren D, Yılmaz H, Yıldırım Ç, Atal SS, Akakın D, Demirci EK, Yüksel M, Yeğen BÇ. Obestatin improves oxidative brain damage and memory dysfunction in rats induced with an epileptic seizure. Peptides [Internet]. 2017; 90:37–47. doi: https://doi.org/f9475w

[17] Kobbi Z, Kraiem H, Benlasfar Z, Marouani A, Massoud T, Boubaker S, Bouhaouala-Zahar B, Fenina N: Comparative subcutaneous repeated toxicity study of enoxaparin products in rats. Regul. Toxicol. Pharmacol. [Internet]. 2017; 84:9–17. doi: https://doi.org/f9xd55

[18] Sandri ASS, Rodriguez R, Costa MM, Porto SM, Schwingel D, Vieira MIB. High-dose enoxaparin in the treatment of abdominal angiostrongyliasis in Swiss mice. J. Helminthol. [Internet]. 2018; 92(6):662–667. doi: https://doi.org/gbzs59

[19] Shaker RA, Abboud SH, Assad HC, Hadi N. Enoxaparin attenuates doxorubicin induced cardiotoxicity in rats via interfering with oxidative stress, inflammation and apoptosis. BMC Pharmacol. Toxicol. [Internet]. 2018; 19:3. doi: https://doi.org/nc8d

[20] Aboyoussef AM, Abdel-Sattar AR, Abdel-Bakky MS, Messiha BA. Enoxaparin prevents CXCL16/ADAM10-mediated cisplatin renal toxicity: Role of the coagulation system and the transcriptional factor NF-κβ. Life Sci. [Internet]. 2021; 270:119120. doi: https://doi.org/gt7bj3

[21] Erdogan MA, Yusuf D, Christy J, Solmaz V, Erdogan A, Taskiran E, Erbas O. Highly selective SGLT2 inhibitor dapagliflozin reduces seizure activity in pentylenetetrazol-induced murine model of epilepsy. BMC Neurology [Internet]. 2018; 18(81):1–8. doi: https://doi.org/gdqx3b

[22] Erdogan MA, Yusuf D, Erdogan A, Erbas O. Levodropropizine suppresses seizure activity in rats with pentylenetetrazol-induced epilepsy. Epilepsy Res. [Internet]. 2019; 150:32–37. doi: https://doi.org/gt7bj4

[23] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 1976; 72(1–2):248–254. doi: https://doi.org/bv9drn

[24] Salim S. Oxidative stress and the central nervous system. Journal of Pharmacology and Experimental Therapeutics. J. Pharmacol. Exp. Ther. [Internet]. 2017; 360(1):201–205. doi: https://doi.org/gjfxcr

[25] Hayashi M, Miyata R, Tanuma N. Oxidative stress in developmental brain disorders. In: Ahmad SI, editor. Neurodegenerative Diseases. Vol. 724 [Internet]. New York: Springer; 2012. p. 278–290. (Advances in Experimental Medicine and Biology Series). doi: https://doi.org/gt7bj5

[26] Trist BG, Hare DJ, Double KL. Oxidative stress in the aging substantia nigra and the etiology of Parkinson's disease. Aging Cell [Internet]. 2019; 18(6):e13031. doi: https://doi.org/gn6fn2

[27] Eastman CL, D'Ambrosio R, Ganesh T. Modulating neuroinflammation and oxidative stress to prevent epilepsy and improve outcomes after traumatic brain injury. Neuropharmacology [Internet]. 2020; 172:107907. doi: https://doi.org/ggqgrd

[28] Xiong L, McCoy M, Komuro H, West XZ, Yakubenko V, Gao D, Dudiki T, Milo A, Chen J, Podrez EA, Trapp B, Byzova TV. Inflammation-dependent oxidative stress metabolites as a hallmark of amyotrophic lateral sclerosis. Free Radic. Biol. Med. [Internet]. 2022; 178:125–133. doi: https://doi.org/gt7bj6

[29] Parsons ALM, Bucknor EMV, Castroflorio E, Soares TR, Oliver PL, Rial D. The Interconnected Mechanisms of Oxidative Stress and Neuroinflammation in Epilepsy. Antioxidants [Internet]. 2022; 11(1):157. doi: https://doi.org/gt7bj7

[30] Amin F, Tabassum S, Sarwar S, Qureshi R, Sohaib Khalid M, Riaz N, Al-Qahtani WH, Murtaza I. Neuroprotective Effect of *Otostegia limbata* Against PTZ-Induced Mice Model of Epilepsy by Attenuated Expression of p-NFκB and TNF-α. Front. Neurosci. [Internet]. 2022; 16:779681. doi: https://doi.org/gt7bj8

[31] Köksoy FN, Yankol Y, Şen Oran E, Özkan Gürdal S, Yüksel M, Akyildiz Iğdem A, Yildirim Yazgan N, Soybir GR. Preventive effects of enoxaparin and hesperidin in cerulein-induced acute pancreatitis in rats. Turk. J. Gastroenterol. [Internet]. 2013; 24(6):495–501. doi: https://doi.org/gt7bj9

[32] Ozcil MD, Ozcan O, Hakverdi S, Bayraktar H, Dirican E, Kacmaz F. Impact of Enoxaparin +α Lipoic acid combination on oxidative stress, follicle development and apoptotic damage in ovarian ischemia reperfusion model. Eur. Rev. Med. Pharmacol. Sci. [Internet]. 2022; 26(18):6583–6592. doi: https://doi.org/gt7bkb

[33] Kamali AN, Zian Z, Bautista JM, Hamedifar H, Hossein-Khannazer N, Hosseinzadeh R, Yazdani R, Azizi G. The potential role of pro-inflammatory and anti-inflammatory cytokines in epilepsy pathogenesis. Endocr. Metab. Immune. Disord. Drug. Targets. [Internet]. 2021; 21(10):1760–1774. doi: https://doi.org/gt7bkc

[34] Ethemoglu O, Ay H, Koyuncu I, Gönel A. Comparison of cytokines and prooxidants/antioxidants markers among adults with refractory versus well-controlled epilepsy: A cross-sectional study. Seizure [Internet]. 2018; 60:105–109. doi: https://doi.org/gfcbhd

[35] Grosskopf I, Shaish A, Ray A, Harats D, Kamari Y. Low molecular weight heparin-induced increase in chylomicron-remnants clearance, is associated with decreased plasma TNF-α level and increased hepatic lipase activity. Thromb. Res. [Internet]. 2014; 133(4):688–692. doi: https://doi.org/f5vzcw

[36] Berkowitz S, Gofrit SG, Aharoni SA, Golderman V, Qassim L, Goldberg Z, Dori A, Maggio N, Chapman J, Shavit-Stein E. LPS-Induced Coagulation and Neuronal Damage in a Mice Model Is Attenuated by Enoxaparin. Int. J. Mol. Sci. [Internet]. 2022; 23(18):10472. doi: https://doi.org/gt7bkd

[37] Lei J, Feng F, Duan Y, Xu F, Liu Z, Lian L, Liang Q, Zhang N, Wang F. Intranasal nerve growth factor attenuating the seizure onset via p75R/Caspase pathway in the experimental epilepsy. Brain Res. Bull. [Internet]. 2017; 134:79–84. doi: https://doi.org/gb2qs2

[38] Vega-García A, Orozco-Suárez S, Villa A, Rocha L, Feria-Romero I, Vanegas MA, Alonso-Vanegas MA, Guevara-Guzmán R. Cortical expression of IL1-β, Bcl-2, Caspase-3 and 9, SEMA-3a, NT-3 and P-glycoprotein as biological markers of intrinsic severity in drug-resistant temporal lobe epilepsy. Brain. Res. [Internet]. 2021; 1758:147303. doi: https://doi.org/gt7bkf

[39] Zhao Q, Yin C, Yuan Y, Zhang H, Teng L. Down-regulation of Mir-145 improves learning and memory abilities in epileptic rats by regulating hippocampal neuron apoptosis. World Neurosurg. [Internet]. 2019; 122:e1432–e1438. doi: https://doi.org/gt7bkg

[40] Findik O, Yilmaz MY, Yazir Y, Rençber SF, Sarihan KK, Kunt AT. Investigation of the protective effect of enoxaparin and ticagrelor pretreatment against ischemia-reperfusion injury in rat lung tissue. Rev. Assoc. Med. Bras. [Internet]. 2019; 65(9):1193–1200. doi: https://doi.org/ghhdzc

[41] Findik O, Baris O, Yazir Y, Yilmaz MY, Rencber SF, Sarihan KK, Kunt AT. Changes in cardiac cells due to ticagrelor and enoxaparin in a rat ischemia/reperfusion model. Rev. Assoc. Med. Bras. [Internet]. 2021; 67(12):1764–1770. doi: https://doi.org/gt7bkh

[42] Koshal P, Kumar P. Neurochemical modulation involved in the beneficial effect of liraglutide, GLP-1 agonist on PTZ kindling epilepsy-induced comorbidities in mice. Mol. Cell. Biochem. [Internet]. 2016; 415:77–87. doi: https://doi.org/f8g2b7

[43] Karabulut S, Filiz AK, Akkaya R: Thiamine alleviates cognitive impairment and epileptogenesis by relieving brain inflammation in PTZ-induced kindling rat model. Neurol. Res. [Internet]. 2022; 44(10):902–909. doi: https://doi.org/gt7bkj

[44] Timmer NM, van Dijk L, van der Zee CEEM, Kiliaan A, de Waal RMW, Verbeek MM. Enoxaparin treatment administered at both early and late stages of amyloid β deposition improves cognition of APPswe/PS1dE9 mice with differential effects on brain Aβ levels. Neurobiol. Dis. [Internet]. 2010; 40(1):340–347. doi: https://doi.org/cvhd8r

[45] Stutzmann JM, Mary V, Wahl F, Grosjean-Piot O, Uzan A, Pratt J. Neuroprotective profile of enoxaparin, a low molecular weight heparin, in *in vivo* models of cerebral ischemia or traumatic brain injury in rats: a review. CNS Drug Rev. [Internet]. 2002; 8(1):1–30. doi: https://doi.org/bx63vf

[46] Hao F, Jia LH, Li XW, Zhang YR, Liu XW. Garcinol upregulates GABAA and GAD65 expression, modulates BDNF-TrkB pathway to reduce seizures in pentylenetetrazole (PTZ)-induced epilepsy. Med Sci. Monit. [Internet]. 2016; 22:4415. doi: https://doi.org/f9cfbc

[47] Binder DK, Scharfman HE. Brain-derived neurotrophic factor. Growth factors [Internet]. 2004; 22(3):123–131. doi: https://doi.org/bg73sd

[48] Quartermain D, Li YS, Jonas S. The low molecular weight heparin enoxaparin reduces infarct size in a rat model of temporary focal ischemia. Cerebrovasc. Dis. [Internet]. 2003; 16(4):346–355. doi: https://doi.org/ftsbxg