Archives of Epilepsy

Original Article

# Effects of S-Methyl Methionine Sulfonium Chloride on Lens Tissue in Pentylentetrazol-Induced Seizures in Rats

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**Cite this article as:** Bayram S, Türkyılmaz IB, Karaman GB, Yanardağ R. Effects of S-methyl methionine sulfonium chloride on lens tissue in pentylentetrazol-induced seizures in rats. *Arch Epilepsy.* 2022;28(3):98-105.

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**Received:** April 18, 2022 **Accepted:** September 13, 2022

DOI: 10.5152/ArchEpilepsy.2022.221527



## Abstract

**Objective:** Epilepsy is a serious neurologic disease characterized by spontaneous seizures. During epileptic conditions, the antioxidant system is affected in both the brain and other tissues/organs. Pentylenetetrazol is used to induce animal-sourced epilepsy models. S-methyl methionine sulfonium chloride is a novel antioxidant that ameliorates much toxicity via its property. This study aimed to investigate the protective effect of S-methyl methionine sulfonium chloride on pentylenetetrazol-induced seizures lens injury.

**Methods:** Male Sprague–Dawley rats were divided into 4 groups. The control group received 0.9% NaCl per day intraperitoneally for 1 week, S-methyl methionine sulfonium chloride group was given S-methyl methionine sulfonium chloride at a dose of 50 mg/kg per day orally for 1 week, pentylenetetrazol group was given 60 mg/kg of pentylenetetrazol as a single dose, and pentylenetetrazol + S-methyl methionine sulfonium chloride group treatments were administered at the same dose and time. At the end of the experiment, all the animals were sacrificed, and lenses were taken.

**Results:** Lens glutathione and total antioxidant capacity were decreased, while advanced oxidized protein products, catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase, aldose reductase, sorbitol dehydrogenase, and carbonic anhydrase were increased in pentylenetetrazol group. The administration of S-methyl methionine sulfonium chloride reversed these parameters.

**Conclusion:** S-methyl methionine sulfonium chloride ameliorated pentylenetetrazol-induced lens injury through its unique antioxidant activity.

**Key words:** Lens, s-methyl methionine sulfonium chloride, pentylentetrazol-induced seizures

## INTRODUCTION

Epilepsy is one of the most common diseases in the world characterized by spontaneous seizures.[1] According to the reports of the World Health Organization, 2.4 million new cases are reported per year. The imbalance in neurotransmission makes life conditions harder for patients and affects their psychiatric comorbidities.[2] Anticonvulsant therapy has been tried on animals for many years by developing some experimental models.[3,4] When the chemical alterations in terms of epilepsy are evaluated, oxidative stress shows a large spectrum via accompanying hyperexcitability following disturbances of antioxidant molecules and enzymatic systems.[5-7]

Pentylenetetrazole (PTZ)-induced chemical kindling model is one of the animal model studies for the development of epilepsy.[8] Pentylenetetrazole is a selective gamma amino butyric acid (GABA) receptor blocker with convulsing property.[9] This substance has bicyclic tetrazole structure, and therefore, it can easily penetrate through cell membranes and is known to have high bioavailability.[10,11] Its intraperitoneal injection accelerates its distribution to other organs. Besides, PTZ-induced kindling model has been associated with increased reactive oxygen species levels.[12,13]

Lens transmits and focuses the light on the retina.[14] Its primary duty is to filter harmful ultraviolet (UV) lights.[15] This process is necessary for healthy vision formation in the retina. However, since the lens contains high protein contents, it becomes vulnerable due to its filtering role.[15] Lens resists oxidative conditions via its thiol contents including glutathione and through antioxidant enzyme systems.[16] However, this resistance may not be effective, especially in certain conditions like oxidative stress or other diseases.[17,18] For this purpose, researchers have been searching for new solutions for supporting and maintaining antioxidant defense systems.

The relationship between epilepsy and lens has been explained by some researchers as an indication of the photosensitivity of lens tissue which occurs in the presence of epilepsy.[19,20] Moreover, they also emphasized that this situation was an unwanted consequence including anxiety who had possible reflex seizures. Furthermore, oxidative stress, either related to epilepsy models or diseases such as diabetes and hyperlipidemia, has been well documented to affect many tissues and organs including lens.[21-24]

S-methyl methionine sulfonium chloride (MMSC) is a methionine derivative and is also known as vitamin U. It has been declared very effective in protecting organs and tissues including skin,[25] liver,[26] lung,[27] and lens[24] due to its unique antioxidant property and sulfur moiety. This substance exists in green vegetables such as raw cabbage, spinach, garlic, and other vegetables like tomatoes[28] and it is highly recommended to be consumed.

So based on these reports, this present study was intended to investigate the possible protective effects of MMSC, a novel protective substance, against lens injury in PTZ-induced epilepsy.

## METHODS

### Animals and Ethic Statements

In this study, male Sprague Dawley rats, aged 4-5 months, were chosen. The experimental protocols were approved by the Istanbul University Animal Care and Use Committee (approval number: 2014/105; date of approval: November 28, 2014).

### Experimental Design and Pentylenetetrazole-Induced Kindling Epilepsy Model

The rats were split into 4 groups. The first group (n = 6) was control group animals that received 0.9% NaCl, intraperitoneally. The second group (n = 6) was the MMSC group, and these animals received 50 mg/kg per day of MMSC for 1 week orally. The third group (n = 8) was PTZ group, and the animals received a single dose of PTZ at 60 mg/kg intraperitoneally. The fourth group (n = 8) was PTZ + MMSC group, and these animals received MMSC and PTZ at the same doses and times. Pentylenetetrazole was injected 60 minutes after the last MMSC dose. After PTZ injection, the rats were put into a safety cage and convulsive and seizure behaviors were observed and detected for 30 minutes by Racine Scale method[29] which is given in Table 1. All the animals were sacrificed under anesthesia (ketamine hydrochloride and xylazine HCl were used). The lens tissues of all animals were collected in 0.9% NaCl. The tissue samples were kept at −80°C until the experiments were done. The doses of MMSC and PTZ were chosen according to previous studies.[26,30]

### Biochemical Analyses

The lens tissues were homogenized in 0.9% NaCl to make 10% (w/v) homogenate. The homogenates were centrifuged at 10 000 g at +4°C for 10 minutes, and clear supernatants were collected for analysis. The supernatants were used for determining the levels and activities of biochemical parameters.

**Table 1.** Racine Scale for Determining Epileptic Seizures and Behavioral Categorization[29]

| Racine Scale | |
|---|---|
| Phase 0 | No reaction |
| Phase 1 | Ears and facial twitching |
| Phase 2 | Convulsive waves |
| Phase 3 | Myoclonic jerks and rearing |
| Phase 4 | Clonic seizures and turning over onto on-side position |
| Phase 5 | Generalized tonic-clonic seizures and turning over onto back position |

Reduced glutathione (GSH) was determined by the reduction of Ellman's reagent by free thiol groups to form 5,5'-dithiobis (2-nitrobenzoic acid) with yellow color substance. The final product was measured at 412 nm.[31] Advanced oxidized protein product (AOPP) method is based on the determination of oxidized protein products by the presence of potassium iodide and the absorbance of the final product is measured at 340 nm.[32]

Catalase (CAT) catalyzed the transformation of hydrogen peroxide to water and the decreased absorbance levels were measured at 240 nm.[33] Superoxide dismutase (SOD) activity is a measure of the ability of riboflavin-sensitized o-dianisidine to increase the rate of photooxidation and was determined at 460 nm.[34]

In glutathione peroxidase (GPx) activity determination, glutathione peroxidase oxidizes GSH to oxidized glutathione (GSSG) in the presence of $H_2O_2$. Oxidized glutathione formed by the GPx reaction is converted back to GSH by the enzyme glutathione reductase, in which Nicotinamide-adenin dinucloetide phosphate (reduced) (NADPH) is used as a reducing substrate. This reaction is measured at 366 nm.[35] Glutathione reductase (GR) activity is a method of calculating the ratio of oxidized NADPH during the reduction of GSSG with GR.[36]

Glutathione-S-transferase (GST) activity determination is based on the spectrophotometric measurement of the product formed because of the conjugation of glutathione and 1-chloro-2,4-dinitro benzene at 340 nm.[37] Total antioxidant capacity (TAC) level determination is based on decolorization reaction of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid radical cation ($ABTS^{*+}$) by antioxidants. The alteration of color is measured at 660 nm.[38]

Aldose reductase (AR) activity method is based on transformation of glucose to sorbitol by the presence of $NADPH + H^+$ and sorbitol dehydrogenase (SDH) activity method is based on the transformation of sorbitol to fructose by the presence of $NAD^+$.[39,40]

The absorbance alteration is measured at 340 nm in both enzyme activity reactions. Carbonic anhydrase (CA) hydrolyzes p-nitrophenyl acetate to p-nitrophenol and the absorbance of this final product can be measured at 348 nm.[41]

All the levels and enzyme activities were expressed in the protein levels of the lens which were determined by referencing the method of Lowry.[42]

### Statistical Analyses

Statistical analysis of biochemical results was performed via GraphPad Prism 6.0 (GraphPad Software, San Diego, Calif, USA). All the data were expressed as means ± standard deviation. The results were evaluated via analysis of variance followed by Tukey's multiple comparison tests. $P < .05$ was considered to be statistically significant.

## MAIN POINTS

- The whole body, including the brain, is affected in epileptic conditions, and lens is also vulnerable to internal and external factors.
- Oxidative stress plays an important role during epileptic seizures and its deleterious effects spread to all the organs including lens.
- Pentylenetetrazol (PTZ) is a widely used agent for designing epilepsy models in animals and is a potent oxidative stress elevator.
- S-methyl methionine sulfonium chloride (MMSC) is a novel and potent antioxidant and capable of preventing oxidative stress by ameliorating antioxidant defense mechanisms and helping the regulation of cell metabolism functions.
- This present study showed the potential antioxidant property of MMSC on lens tissue which is affected in epilepsy induced by PTZ.

## RESULTS

Observation of Epileptic Phases in Group 3 (Pentylenetetrazole Group) and Group 4 (Pentylenetetrazole+S-Methyl Methionine Sulfonium Chloride Group)

Epileptic phases were indicated according to our previous study.[43] After PTZ administration, these phases were observed in PTZ-given group (Group 3) as mentioned below:

Phase 0: this phase was determined as too short.

Phase 1-2: the movements of ears and facial twitching and convulsive waves were observed throughout the body and these phases have been accelerated.

Phases 3-4: the movements of myoclonic jerks and rearing, clonic seizures and turning over onto on-side position were observed. These movements happened very fast in very short intervals.

Phase 5: the movements of generalized tonic-clonic seizures and turning over onto back position were observed and this phase was determined as the most severe part of epileptic seizure.

S-methyl methionine sulfonium chloride was applied to epileptic animals 1 hour before PTZ (to MMSC+PTZ group, group 4). We determined that MMSC diminished phase 2 duration and convulsive waves of animals were also observed as short in MMSC+PTZ group when compared to PTZ group. The phase 3-4, myoclonic jerks and rearing, clonic seizures and turning over onto on-side position, was observed very shortly as compared to the same phases of epileptic group. Group 4 animals could easily stop the movements of turning over onto their on-side position. S-methyl methionine sulfonium chloride decreased the tonic-clonic seizures at last stage and the number of repeated seizure attacks was also observed as decreased.

## BIOCHEMICAL RESULTS

The lens GSH levels are shown in Figure 1A. S-methyl methionine sulfonium chloride increased GSH levels of control group in a significant manner ($P < .0001$). Pentylenetetrazole administration significantly decreased GSH levels in PTZ group compared to control group ($P < .01$). The administration of MMSC in PTZ group reversed this level in PTZ+MMSC group compared to PTZ group ($P < .05$) (Figure 1A).

The lens AOPP levels are shown in Figure 1B. S-methyl methionine sulfonium chloride administration into control group increased AOPP levels compared to control group ($P < .05$). Pentylenetetrazole administration also significantly increased AOPP levels as compared to control group ($P < .01$). S-methyl methionine sulfonium chloride reversed these levels in PTZ administered group compared to PTZ given group ($P < .0001$, respectively) (Figure 1B).

The lens CAT activities of all groups are given in Figure 2A. S-methyl methionine sulfonium chloride significantly increased CAT activities of the control group ($P < .0001$). Catalase activities were found to be elevated in a significant manner after PTZ administration into control group ($P < .0001$). In PTZ+MMSC group, these activities were found to be significantly diminished as compared to PTZ group ($P < .0001$) (Figure 2A).

The lens SOD (Figure 2B) activities of all groups are given in Figure 2B. Pentylenetetrazole administration increased SOD activities in a significant manner compared to control group ($P < .01$). S-methyl methionine sulfonium chloride reversed this activity in PTZ+MMSC group significantly compared to PTZ group ($P < .001$) (Figure 2B).

The lens GPx activities of all groups are presented in Figure 3A. Pentylenetetrazole administration increased GPx activities of control group as compared to control group ($P < .0001$). S-methyl methionine sulfonium chloride significantly decreased GPx activities in PTZ+MMSC group when compared to PTZ group ($P < .0001$) (Figure 3A).

The lens GR activities of all groups are presented in Figure 3B. According to the results, MMSC caused a statistically significant elevation of GR activities of control group ($P < .01$). GR activities of control group were increased in a significant manner after PTZ administration ($P < .001$). S-methyl methionine sulfonium chloride significantly decreased these activities in PTZ group ($P < .01$) (Figure 3B).

The lens GST activities of all groups are presented in Figure 4A. Glutathione-S-transferase activities were found to be increased after

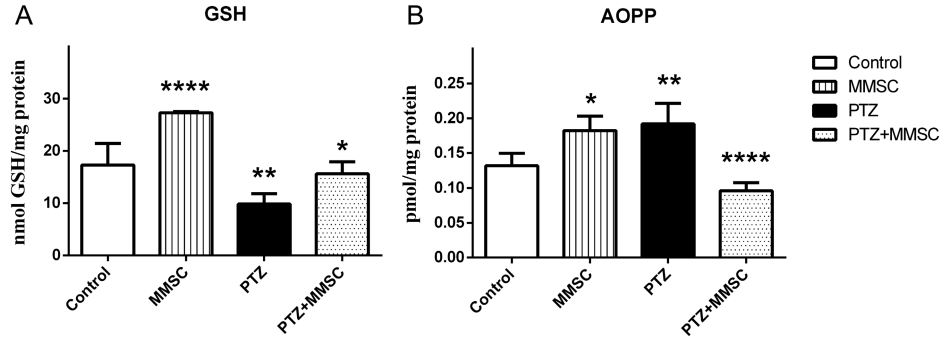


**Figure 1.** The lens glutathione and advanced oxidized protein product levels of all groups. (A). GSH, reduced glutathione; MMSC, S-methyl methionine sulfonium chloride; PTZ, pentylenetetrazole. Control group received 0.9% NaCl per day; MMSC group was given MMSC at a dose of 50 mg/kg per day; PTZ group was given PTZ at a single dose, 60 mg/kg; PTZ+MMSC group, substances administered at the same dose and time. Data are expressed as "mean ± SD." ****$P < .0001$ versus control group; **$P < .01$ versus control group; *$P < .05$ versus PTZ group; (B). AOPP, advanced oxidized protein product; MMSC, S-methyl methionine sulfonium chloride; PTZ, pentylenetetrazole. Control group received 0.9% NaCl per day; MMSC group was given MMSC at a dose of 50 mg/kg per day; PTZ group was given PTZ at a single dose, 60 mg/kg; PTZ+MMSC group, substances administered at the same dose and time. Data are expressed as "mean ± SD." *$P < .05$ versus control group; **$P < .01$ versus control group; ****$P < .0001$ versus PTZ group.

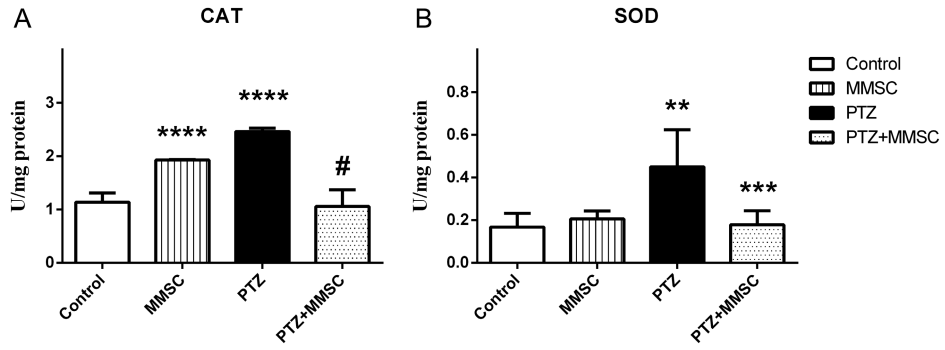


**Figure 2.** The lens catalase and superoxide dismutase activities of all groups. (A). CAT, catalase; MMSC, S-methyl methionine sulfonium chloride; PTZ, pentylenetetrazole. Control group received 0.9% NaCl per day; MMSC group was given MMSC at a dose of 50 mg/kg per day; PTZ group waas given PTZ at a single dose, 60 mg/kg; PTZ+MMSC group, substances administered at the same dose and time. Data are expressed as "mean ± SD." ****$P < .0001$ versus control group; #$P < .0001$ versus PTZ group. (B). SOD, superoxide dismutase; MMSC, S-methyl methionine sulfonium chloride; PTZ, pentylenetetrazole. Control group received 0.9% NaCl per day; MMSC group was given MMSC at a dose of 50 mg/kg per day; PTZ group was given PTZ at a single dose, 60 mg/kg; PTZ+MMSC group, substances administered at the same dose and time. Data are expressed as "mean ± SD." **$P < .01$ versus control group; ***$P < .001$ versus PTZ group.

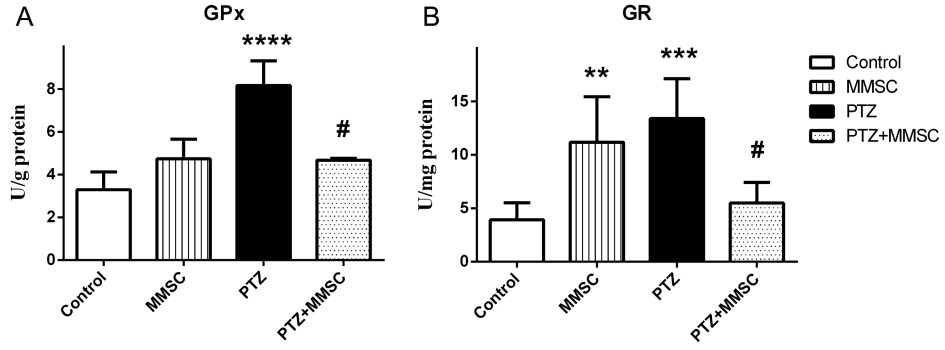


**Figure 3.** The lens glutathione peroxidase and glutathione reductase activities of all groups. (A). GPx, glutathione peroxidase; MMSC, S-methyl methionine sulfonium chloride; PTZ, pentylenetetrazole. Control group received 0.9% NaCl per day; MMSC group was given MMSC at a dose of 50 mg/kg per day; PTZ group was given PTZ at a single dose, 60 mg/kg; PTZ+MMSC group, substances administered at the same dose and time. Data are expressed as "mean ± SD." ****$P < .0001$ versus control group; #$P < .0001$ versus PTZ group. (B). GR, glutathione reductase; MMSC, S-methyl methionine sulfonium chloride; PTZ, pentylenetetrazole. Control group received 0.9% NaCl per day; MMSC group was given MMSC at a dose of 50 mg/kg per day; PTZ group was given PTZ at a single dose, 60 mg/kg; PTZ+MMSC group, substances administered at the same dose and time. Data are expressed as "mean ± SD." **$P < .01$ versus control group; ***$P < .001$ versus control group; #$P < .01$ versus PTZ group.

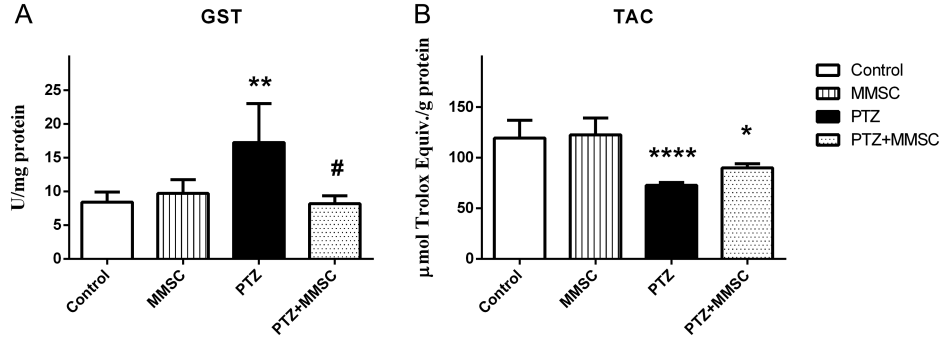


**Figure 4.** The lens glutathione-S-transferase activities and total antioxidant capacity levels of all groups. (A). GST, glutathione-S-transferase; MMSC, S-methyl methionine sulfonium chloride; PTZ, pentylenetetrazole. Control group received 0.9% NaCl per day; MMSC group was given MMSC at a dose of 50 mg/kg per day; PTZ group was given PTZ at a single dose, 60 mg/kg; PTZ+MMSC group, substances administered at the same dose and time. Data are expressed as "mean ± SD." **$P < .01$ versus control group; #$P < .01$ versus PTZ group. (B). TAC, total antioxidant capacity; MMSC, S-methyl methionine sulfonium chloride; PTZ, pentylenetetrazole. Control group received 0.9% NaCl per day; MMSC group was given MMSC at a dose of 50 mg/kg per day; PTZ group was given PTZ at a single dose, 60 mg/kg; PTZ+MMSC group, substances administered at the same dose and time. Data are expressed as "mean ± SD." ****$P < .0001$ versus control group; *$P < .05$ versus PTZ group.

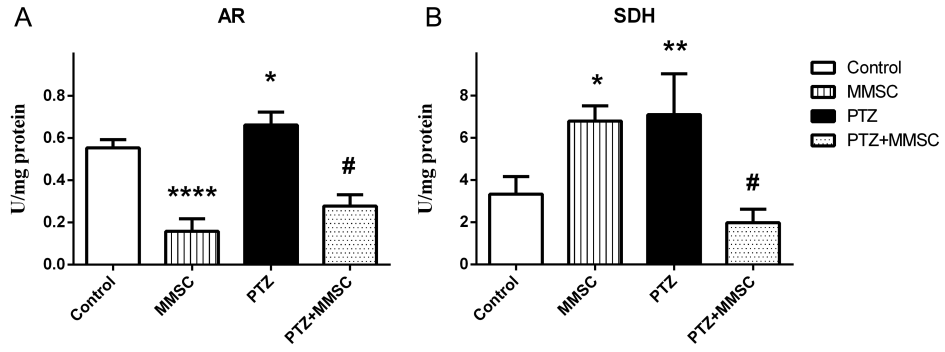


**Figure 5.** The lens aldose reductase and sorbitol dehydrogenase activities of all groups. (A). AR, aldose reductase; MMSC, S-methyl methionine sulfonium chloride; PTZ, pentylenetetrazole. Control group received 0.9% NaCl per day; MMSC group was given MMSC at a dose of 50 mg/kg per day; PTZ group was given PTZ at a single dose, 60 mg/kg; PTZ+MMSC group, substances administered at the same dose and time. Data are expressed as "mean ± SD." ****$P < .0001$ versus control group; *$P < .05$ versus control group; #$P < .01$ versus PTZ group. (B). SDH, sorbitol dehydrogenase; MMSC, S-methyl methionine sulfonium chloride; PTZ, pentylenetetrazole. Control group received 0.9% NaCl per day; MMSC group was given MMSC at a dose of 50 mg/kg per day; PTZ group was given PTZ at a single dose, 60 mg/kg; PTZ+MMSC group, substances administered at the same dose and time. Data are expressed as "mean ± SD." *$P < .05$ versus control group; **$P < .01$ versus control group; #$P < .05$ versus PTZ group.

PTZ administration into control group ($P < .01$). In PTZ+MMSC group, GST was decreased after MMSC administration as compared to PTZ group ($P < .01$) (Figure 4A).

The lens TAC levels of all groups are presented in Figure 4B. Total antioxidant capacity levels were significantly decreased after PTZ administration into control group ($P < .0001$). In PTZ+MMSC group, TAC was increased in a significant manner compared to PTZ group ($P < .05$) (Figure 4B).

The lens AR activities of all groups are shown in Figure 5A. S-methyl methionine sulfonium chloride administration showed statistically significantly diminishing AR activities in control group ($P < .0001$). Pentylenetetrazolesignificantly raised AR activities in the control group ($P < .05$). The administration of MMSC to PTZ group significantly decreased AR activities when compared to PTZ group ($P < .01$) (Figure 5A).

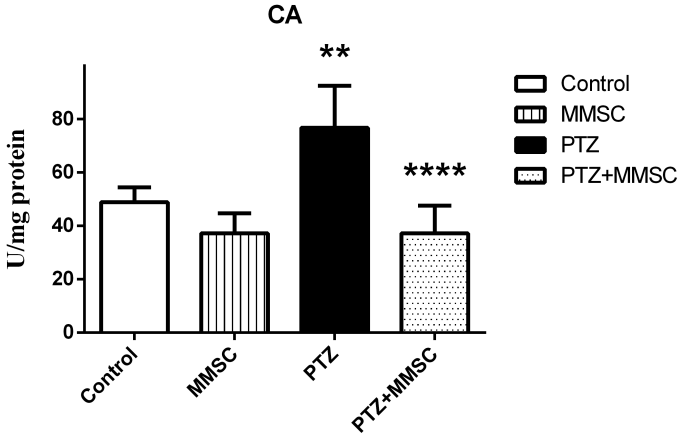


**Figure 6.** The lens carbonic anhydrase activities of all groups. CA, carbonic anhydrase; MMSC, S-methyl methionine sulfonium chloride; PTZ, pentylenetetrazole. Control group received 0.9% NaCl per day; MMSC group was given MMSC at a dose of 50 mg/kg per day; PTZ group was given PTZ at a single dose, 60 mg/kg; PTZ+MMSC group, substances administered at the same dose and time. Data are expressed as "mean ± SD." **$P < .01$ versus control group; ****$P < .0001$ versus PTZ group.

The lens SDH activities of all groups are shown in Figure 5B. S-methyl methionine sulfonium chloride administration increased SDH activities in control group ($P < .05$). Pentylenetetrazole significantly elevated SDH activity in the control group ($P < .01$). The administration of MMSC to PTZ group significantly decreased these activities ($P < .05$) (Figure 5B).

The lens CA activities of all groups are given in Figure 6. Pentylenetetrazole increased CA activity of control group in a significant manner ($P < .01$). In PTZ+MMSC group, the increased activity was significantly reversed after MMSC administration as compared to PTZ group ($P < .0001$) (Figure 6).

## DISCUSSION

Pentylenetetrazole, a bicyclic tetrazole derivative, has many deleterious effects, such as activation of N-methyl-D-aspartate receptors, elevation of glutamate concentrations, and alteration of membrane mechanism.[44] Moreover, PTZ has been associated with diminished mitochondrial respiration by inhibiting complex I (in the electron transport system).[45] Although these circumstances happen in the brain, researchers revealed that PTZ-induced epilepsy also affected other tissues (like the lung) by increasing oxidative conditions.[27] In this current study, possible deleterious effects of PTZ on lens tissue were investigated. The epilepsy model was evaluated according to the Racine scale as shown in Table 1.[29] As a protective agent, MMSC was used due to its well-known antioxidant property as described by various researchers on many toxicity models.[46-48]

Lens is continually exposed to many factors like UV lights and radiation because it is an organ located externally when considering the location of other organs.[4] Besides, coming across free radicals and oxidative stress-derived molecules is obviously inevitable. These may cause elevated vascular permeability, alterations of lens functions, and crystalline decay.[49,50] In addition, the lens can be affected due to the photosensitivity that can be caused by epilepsy.[19,20] There also exists another report which is related to congenital cataract in childhood times under epileptic conditions.[51]

Glutathione (composed of γ-glutamic acid, cysteine, and glycine) is abundantly present in the lens. The existence of GSH provides stabilization of crystalline structures (as protein chaperons), while excess loss

of GSH results in crystalline aggregation in the lens.[52,53] According to Berthoud and Beyer,[54] augmentations of lens proteins after oxidative damage exposure is also connected to GSH levels. Besides, advanced oxidation of amino acids (like methionine and cysteine) indicates protein oxidation, which in turn leads to insoluble aggregates after lens damage. For this reason, GSH, AOPP, TAC levels, and antioxidant enzyme activities like CAT, SOD, GPx/GR, and GST were assessed in the present study.

Superoxide anion formation is a constant procedure for systems like mitochondrial electron transport or cytochrome $P_{450}$ in every tissue and organ. Superoxide dismutase converts this molecule to $H_2O_2$ by capturing hydrogen ions in most tissues including lenses. Additionally, $H_2O_2$ can be directly transported via aqueous humor and plasma membrane of lens.[54] $H_2O_2$ is converted into other less toxic molecules with the help of GPx and CAT. Under these circumstances, GPx uses GSH for its activity, while GR converts the oxidized glutathione to its reduced form (GSH) for further utilization directly for antioxidant defense or as a co-substrate. Glutathione-S-transferase has a xenobiotic detoxification role, and it utilizes the thiol group of GSH for its nucleophilic addition to electrophilic compounds. Its absence and polymorphisms have been related to lens damages like cataract.[55]

In light of this information, the present results verify the existence of oxidative stress in relation to PTZ administration. Diminished GSH and TAC levels and increased AOPP, CAT, SOD, GPx/GR, and GST were observed. These results showed that lens tissue has been strongly affected in PTZ-induced epilepsy models. Besides, Oztay et al[48] had proven that MMSC influenced the activity of nuclear factor-erythroid-2-related factor 2 (Nrf2, a transcription factor), which helps regulate antioxidant levels including GSH. S-methyl methionine sulfonium chloride facilitated the translocation of Nrf2 to nuclei in case of oxidative stress. Interestingly, Nrf2-related protection is lost in lens damages, either due to ROS or because of cataract-dependent conditions.[56] The present results show that MMSC administration reversed altered biochemical parameters probably by the stabilization of proteins/preventing excess oxidation of proteins and decreasing AOPP, and protecting antioxidant system integrity through ameliorating GSH levels, TAC level, and antioxidant enzyme activities via its protective property or Nrf2-regulating effect.

Due to being the primer source of energy, glucose requirement increases owing to seizure-dependent energy demand. Nehlig et al[57] investigated how glucose transporters, GLUT1 and GLUT3, were affected during seizure, and they found elevated expression levels of GLUT mRNAs. Besides, elevated demand for glucose may also cause a hike in AR and SDH activities. This is because glucose must be transformed to avoid osmotic stress into sorbitol and in turn, to fructose by AR and SDH, respectively. The results of the present research are in accordance with these hypotheses indicating elevated levels of polyol enzymes in lenses of PTZ-induced group. Sulfur-containing amino acid derivatives like taurine act as osmolytes in lens, and they help protect this tissue against DNA damage and regulate AR activity.[58,59] According to this information, it may be suggested that MMSC may have acted like taurine and protected the lens against PTZ-induced epilepsy-related injury. Likewise, the results for AR and SDH are in accordance with reports by Tunali et al[24] which revealed the importance of MMSC in decreasing the activities of these enzymes.

Carbonic anhydrase plays many vital roles like carbon dioxide-bicarbonate transportation, regulating pH balance, and excretion of electrolytes from tissues.[60] However, elevated CA activity has been associated with glaucoma. Researchers have been trying to find a way to treat ophthalmologic trouble by targeting/inhibiting CA. The most preferred CA inhibitors have been found to be sulfur-containing/derived compounds like sulfonamides and their isoesters, pyrazole-associated structures, etc.[61] In the present study, increased CA activity was observed in lens tissues of PTZ groups. The administration of MMSC halted the increase in CA activity of the PTZ group. Considering this outcome, it can be suggested that MMSC acted as an inhibitor of CA, thus ameliorating lens injury.

## CONCLUSION

The epilepsy model was designed using PTZ to investigate its possible deleterious effect on lens tissue in relation to other toxicity reports. The administration of MMSC ameliorated the weakened antioxidant system of the lens, regulated the polyol pathway enzymes, and **may have** protected lens tissue from defects like glaucoma by inhibiting carbonic anhydrase.

**Ethics Committee Approval:** The experimental protocols were approved by the Istanbul University Animal Care and Use Committee (Date: November 28, 2014, Decision No: 2014/105).

**Informed Consent:** Written informed consent was obtained from all participants who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept – R.Y., I.B.T., S.B., G.B.K.; Design – R.Y., I.B.T.; Supervision – R.Y.; Funding – R.Y.; Materials – I.B.T., S.B., G.B.K.; Data Collection and/or Processing – I.B.T., S.B.; Analysis and/or Interpretation – R.Y., I.B.T., S.B.; Literature Review – R.Y., I.B.T.; Writing – R.Y., I.B.T.; Critical Review – R.Y., I.B.T.

**Declaration of Interests:** The authors have no conflicts of interest to declare.

**Funding:** This study was supported by Scientific Research Projects Coordination Unit of Istanbul University Cerrahpaşa (project number: 31000).

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