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Effect of captopril on brain oxidative damage in pentylenetetrazole-induced seizures in mice

Fereshteh Asgharzadeh¹, Mahmoud Hosseini¹, Rahimeh Bargi², Mohammad Soukhtanloo³, Farimah Beheshti⁴, Zohreh Mohammady², Akbar Anaeigoudari⁵

¹Division of Neurocognitive Sciences, Psychiatry and Behavioral Sciences Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

²Neurogenic Inflammation Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

³Department of Biochemistry, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

⁴Neuroscience Research Center, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran.

⁵Department of Physiology, School of Medicine, Jiroft University of Medical Sciences, Jiroft , Iran

Corresponding author:

Akbar Anaeigoudari

Address: Department of Physiology, School of Medicine, Pasdaran Steet, Jiroft, Iran

Tel: 98-3443318084, Fax: 98-34318084, E-mail: Anaeiga317@gmail.com

Running title: Protective effects of captopril in seizure

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Abstract:

Background: Frequent seizure is followed by overproduction of free radicals and brain oxidative stress. Renin angiotensin system (RAS) has some effects on central nervous system. We designed this research to challenge the effect of captopril as an angiotensin converting enzyme (ACE) inhibitor against brain oxidative stress in pentylenetetrazole (PTZ) -induced seizures in mice.

Methods: The groups were including (1) Control (saline); (2) PTZ (100 mg/kg, i.p.), (3-5) PTZ-captopril (Capto) that received three doses of Capto 10, 50 and 100 mg/kg 30 min before PTZ injection. Latency time in the onset minimal clonic seizures (MCS) and generalized tonic-clonic seizures (GTCS) were recorded. The level of malondialdehyde (MDA) and total thiol, as well as superoxide dismutase (SOD) and catalase (CAT) activity in the hippocampus and cortex were measured.

Results: All doses of captopril postponed the onset of MCS and GTCS. Accumulation of MDA in the brain tissues of PTZ group was higher than control group, while total thiol content and CAT activity were lower. Pretreatment with captopril (100 mg/kg) diminished MDA concentration compared with PTZ group. Captopril (50 and 100 mg/kg) also increased the level of total thiol groups versus PTZ group. Captopril injection (50 and 100 mg/kg) elevated the activity of SOD and CAT in the brain tissues. In addition captopril administration diminished mortality rate caused by PTZ.

Conclusion: Findings demonstrated that convulsions caused by PTZ were followed by oxidative stress status in the brain tissues. Pretreatment with captopril attenuated the effect of PTZ on brain tissue oxidative damage.

Keywords: Captopril, Pentylenetetrazole, Seizures, Mice, Oxidative Stress

Introduction

Epileptic seizures are considered as a severe abnormal activity of neurons in specific areas of brain such as temporal lobe.¹ This abnormal activity of neurons can be followed by brain oxidative stress and disturbance of cognitive behaviors when they take place repeatedly.² In the epileptic seizure models, antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase have been also shown to protect the brain of rats against oxidative damage.³

In large number of animal studies, pentylenetetrazole (PTZ) has been repeatedly administered for induction an epilepsy model.¹ It has been documented that PTZ-stimulated seizures was accompanied with motor activity impairment, learning and memory dysfunction, anxiety and increased level of hippocampal inflammatory cytokines in rats.⁴ In addition, seizures followed by PTZ administration have been propounded as an important cause of oxidative status imbalance in some brain areas including hippocampus.⁵ For example, PTZ infusion associated with the severe stimulation of N-Methyl-D-aspartate (NMDA) receptors has been shown to elevate the level of free radicals such as nitric oxide (NO) and peroxynitrite in the brain of rats.⁶

On the other hand, the antioxidant properties of angiotensin-converting enzyme (ACE) inhibitors have been accepted.⁷ Captopril as a known ACE inhibitor scavenges free radicals in various tissues.⁸ Captopril has been demonstrated to reinforce enzymatic and non-enzymatic defense in different tissues.⁹ Scientific evidence has indicated that captopril and valsartan (a specific AT1

receptor inhibitor) attenuate oxidative/nitrosative stress in the brain and consequently prevent neuronal damage.¹⁰ Researchers suggested that captopril administration can rescue the neurons against neuro-inflammation caused by accumulation of β -amyloid plaque in the brain.¹¹ Protective effects of captopril on dopamine releasing neurons in nigrostriatal pathway in rat model of Parkinsonism have been also reported.¹² Notwithstanding these data, we did not find any report about effects of captopril on brain oxidative damage followed by PTZ- induced seizure; therefore we decided to design this study to clear the effects of captopril on brain oxidative damage in PTZ-induced seizures in mice.

Materials and Methods

Animals and experimental groups

This work was done on male mice that were prepared from animal house of Mashhad University of Medical Sciences, Iran. The groups were including: Control, PTZ, PTZ + captopril (Capto) 10, PTZ + Capto50 and PTZ + Capto100. The number of animals in each group was 10.

PTZ injection and pharmacological intervention

In order to induction of seizure, PTZ (100mg/kg) (Sigma-Aldrich Chemical Co.) was used. PTZ-induced seizure in mice was monitored for 60 min after injection. The latency to first minimal clonic seizure (MCS), latency to first generalized tonic-clonic seizure (GTCS), and the percentage of mortality were recorded. Captopril (10, 50 and 100 mg/kg)¹³ (Sigma-Aldrich Chemical Co.) was administered for 3 days and also 30 minutes before PTZ. The control group received 0.9% normal saline instead of PTZ. Injections were done intraperitoneally (i.p.). At the end of the tests, the mice were sacrificed and hippocampal and cortical tissues were prepared for studying brain oxidative stress status.

Cortical and hippocampal malondialdehyde

Lipid peroxidation was checked using measurement the level of malondialdehyde (MDA) in the brain tissues. For this purpose a solution containing HCL (hydrochloric acid), TBA (thiobarbituric acid) and TCA (trichloroacetic acid) was mixed with supernatant of the brain tissues. After incubating the mixture in a water bath for 40 minutes, the absorbance was read at 535 nm using a spectrophotometer. The brain concentration of MDA was declared as nanomol/g tissue.¹⁴

Cortical and hippocampal total thiol concentration

To determine the total thiol concentration, the brain supernatant was added to DTNB (5,5'-Dithiobis(2-nitrobenzoic acid)) (20 µL). Finally the absorbance was read at 412 against blank reagent by a spectrophotometer. The level of brain total thiol was announced as µmol/g tissue.¹¹

Cortical and hippocampal superoxide dismutase

The assay of SOD is based on inhibition of MTT [3-(4,5-dimethyl-thiazol-2-yl) 2,5-diphenyl tetrazolium bromide] to its formazan. In this measurement, DMSO (dimethyl sulfoxide) was used to stop the reaction and SOD concentration was estimated at 570 nm. The brain SOD activity was expressed as U/g tissue.¹⁴

Cortical and hippocampal catalase

The brain level of CAT was determined by the breakdown rate of H₂O₂, as substrate of CAT, into H₂O and O₂. In this method, decline in absorption is considered as more amount of breakdown of H₂O₂ by CAT. The brain activity of CAT was declared as U/g tissue.¹⁵

Statistical analysis

The extracted data were exhibited as the Mean ± standard error of mean. Data analysis was carried out by one-way ANOVA followed by LSD post hoc test. P<0.05 was expressed statistically significant.

Results

Effect of captopril on PTZ-induced seizures

In this work MCS and GTCS was induced in all treated groups with PTZ. As shown in Figure 1, pretreatment with captopril (10, 50 and 100 mg/kg) delayed the onset of MCS in mice when it was compared with PTZ group ($P<0.01$ and $P<0.001$). The results of GTCS (Figure 2) also exhibited that the onset of seizures were later in captopril injected groups compared to the PTZ group ($P<0.001$). Additionally, in present study PTZ-caused seizures were accompanied with a mortality rate 100% of mice. The results also showed that all three doses of captopril attenuated mortality rate in mice ($P<0.001$). Based on the results administration of the highest dose of captopril (100 mg/kg) reduced the mortality rate to 0%. (Table1).

Effect of captopril on cortical and hippocampal tissues oxidative stress

Estimation of MDA in hippocampal and cortical tissues of mice indicated that the accumulation of this substance in PTZ group was higher than the control group ($P<0.001$). Based on our results, the cumulation of MDA in brain tissues of the mice had a meaningful reduction in PTZ-Capto100 group than PTZ group ($P<0.001$). Administration of captopril did not result in a significant decrease in concentration of MDA in brain tissues of PTZ-Capto10 and PTZ-Capto50 groups than PTZ group (Figure 2A, 2B).

The hippocampal and cortical tissues of mice also had a low thiol content in PTZ group compared to the control group ($P<0.001$). Administration of captopril led to a significant increase in level of brain tissues thiol in PTZ-Capto50 and PTZ-Capto100 groups when it was compared with the PTZ group ($P<0.01$ and $P<0.001$) (Figure 3A and 3B).

Measurement of SOD and CAT activity in the brain tissues displayed that administered animals with PTZ had a lower activity of these two enzymes compared to control group ($P<0.5$ and

P<0.001) (Figure 4A, 4B, 5A and 5B). The Figure 4B demonstrates that injection of captopril (50 and 100 mg/kg) enhanced the activity of CAT in the cortical tissue with respect to the PTZ group (P< 0.05 and P<0.001). Data did not indicate any significant different in the hippocampal CAT activity in the captopril treated groups than the PTZ group (Figure 4A). As presented in Figure 5A and 5B, SOD had a more activity in the hippocampal and cortical tissues of PTZ-Capto50 and PTZ- Capto100 groups when it was compared with PTZ group (P<0.001).

Discussion

Seizures are abnormal neuronal discharges that can happen after brain strokes and neurodegenerative diseases such as Alzheimer's disease.^{16,17} In animal works, one of the widely used compounds to induce seizure is PTZ.¹⁸ In a similar manner with previous studies,⁶ in our study injection of PTZ was associated with the development of seizure attacks in mice.

On the other hand, Lukawski K and et al (2010) suggested that captopril is able to potentiate the effect of many antiepileptic medicines including carbamazepine and lamotrigine in excited seizures by maximal electroshock in mice.¹⁹ In this study we also administered captopril against PTZ- stimulated seizures in mice. As it has been presented in Figure 1 and 2, captopril significantly delayed the development of seizures in captopril- treated mice with respect to PTZ- injected animals. In addition, uncontrolled seizures have been demonstrated to enhance the chance of mortality.²⁰ In a research, Asadi-Shekaari and et al (2014) reported that PTZ administration promoted the emersion of seizures resulting in mortality rate 100% of rats. These researchers also reported that pretreatment with walnut kernel extract reduced mortality rate to 0%.²¹ In the present research also the convulsions caused by PTZ were associated with a significant increase in mortality rate of mice (100% of mice). Pretreatment with all three doses of

captopril significantly reduced mortality rate of mice. As it was characterized in Table 1 the mortality rate was reached to zero in PTZ-Capto100 group.

It has been well documented that brain oxidative status is imbalanced during epileptic seizures.²² For instance over generation of superoxide anion in the hippocampus of kindled mice by PTZ was reported.⁵ In another example, thiol groups including glutathione (GSH) and glutathione disulfide (GSSG) decreased in the cerebral cortex of mice when they were kindled by PTZ.²³ Researchers also reported that enhanced level of intracellular Ca^{2+} as a result from activation of inotropic glutamate receptors during abnormal neuronal excitations leads to uncontrolled production of reactive oxygen species (ROS).²⁴ Along with these findings in current research, PTZ administration disturbed oxidative status in the brain tissue of mice. This subject was supported by elevated concentration of MDA and diminished level of total thiol group as well as reduction of SOD and CAT activity in cortical and hippocampus of PTZ group compared to control group.

There are numerous documents that confirm the treatment with ACE inhibitors can diminish the oxidative stress.^{25,26} Captopril has been shown to act as a potent free radical scavenger.²⁷ It has been reported that captopril reduces oxidative stress through attenuating of lipid peroxidation in diabetic patients.²⁸ Captopril also has been demonstrated to rescue hepatocytes against oxidative stress caused by paraquat.²⁹ In this work pretreatment with captopril improved malic effects of seizure induced by PTZ on brain oxidative damage. Our findings indicated that accumulation of MDA in the cortical and hippocampal tissues of captopril- treated mice was lower than those of PTZ-injected. In addition the brain tissues in the animals of captopril groups had a higher concentration of total thiol groups and a more activity of SOD and CAT than PTZ group. Considering these results it seems that captopril has some neuro-protective effects. Similar to our

results, it was previously shown that captopril improved learning and memory of rats which was accompanied with an improving effect on oxidative stress criteria.¹³ We also previously showed that ACE inhibitors including captopril had some anti-oxidant effects.^{30,31}

Thus, the ability of captopril to reverse PTZ-induced seizures which was observed in the current study may at least in part be due to enhancement of antioxidant defense system and attenuation of oxidative stress in the brain.³² This action might be resulted from its ability to overcome the pro-oxidant effects of PTZ-induced seizures, through increase in antioxidant defense systems including GSH, SOD and CAT and a decrease in MDA and nitrite levels in the brain.³³ Based on the results obtained from the behavioral and biochemical studies, it may be suggested that captopril may act directly as a free radical scavenger or regulator to ameliorate oxidative stress in nervous system. However, the measurement of ROS needs to be done in the future to better determine more precise mechanism(s) of improving effect of captopril on brain oxidative damage caused by PTZ.

Conclusion

Our results demonstrated that PTZ-caused convulsions disturbed oxidative stress status in cortical and hippocampal tissues of mice. Based on our results captopril as a well-known ACE inhibitor weekend the effect of PTZ in development seizures attacks and also had an improving effect on brain oxidative damage.

Ethical issues

Working with the animals was conducted in agreement with instruction of Ethic Committee of Mashhad University of Medical Sciences. IR.MUMS.fm.REC.1396.327

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Conflict of interest

The authors announce that this article content has no conflict of interest

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Table 1: Mortality rate in treated animals group (n = 10 in each group).

***P<0.001 versus the PTZ group.

Legend of Figures

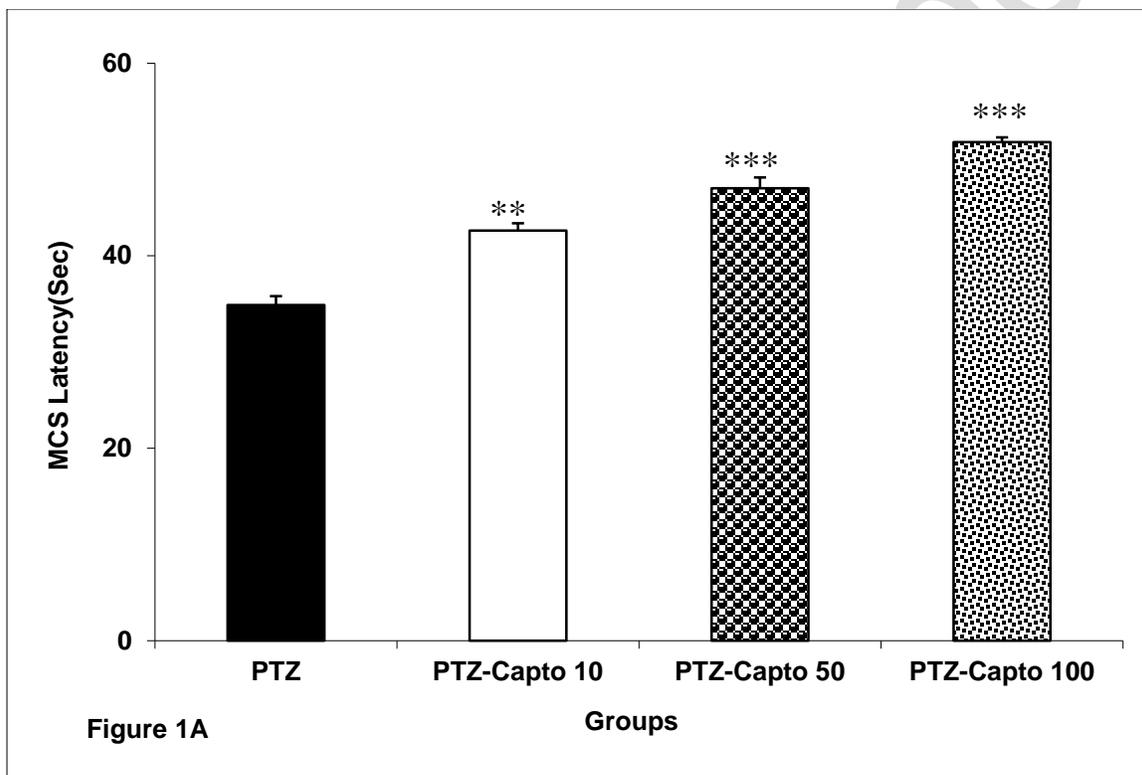
Figure 1: Comparison the effect of injected doses of captopril on latency time in the onset of minimal clonic seizures (MCS) (A) and generalized tonic-clonic seizures (GTCS) (B) in mice. Data were declared as the mean \pm SEM (n= 10). **P<0.01 and ***P<0.001 versus the PTZ group.

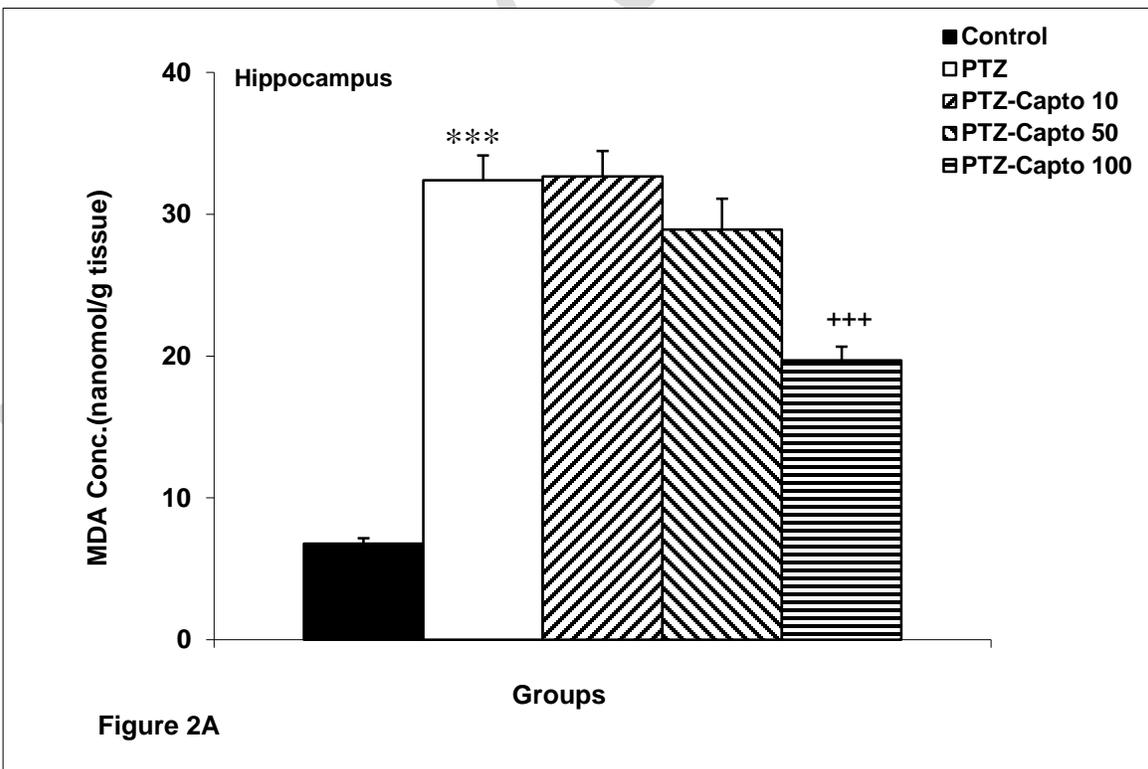
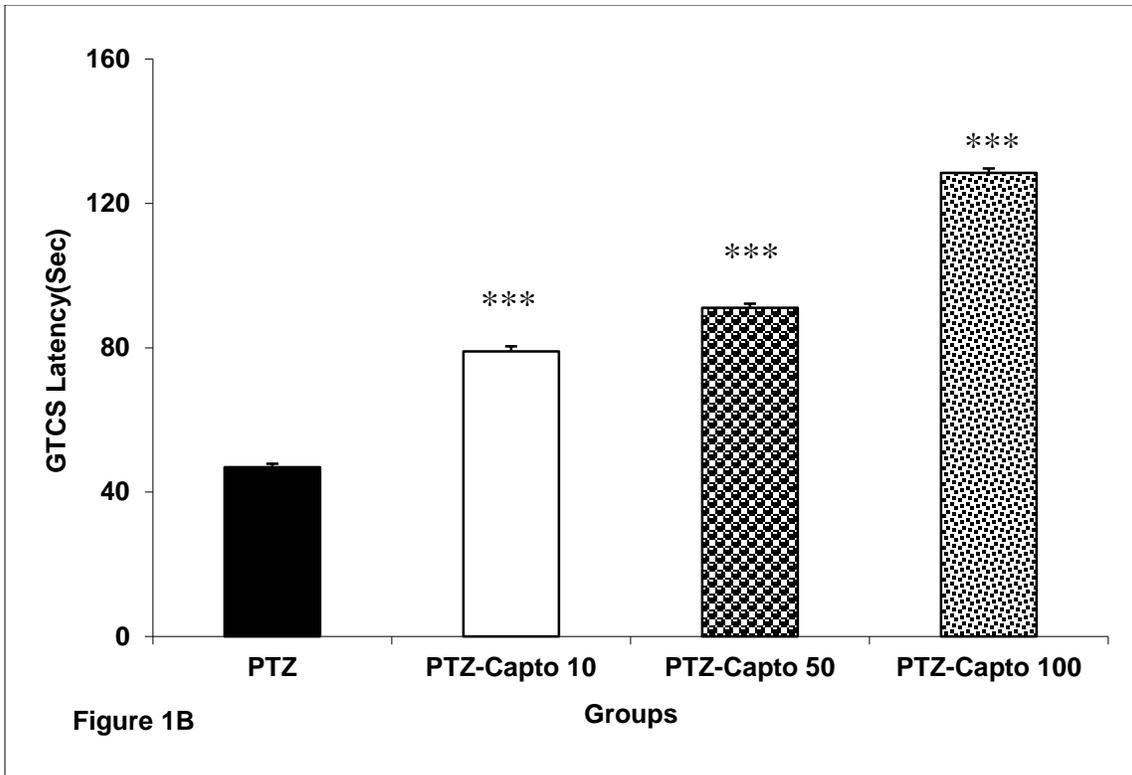
Figure 2: Comparison of MDA concentration in hippocampus (A) and cortical (B) tissue in experimented groups. Data were shown as the mean \pm SEM (n= 10). ***P<0.001 versus control group and ⁺⁺⁺P<0.001 versus PTZ group.

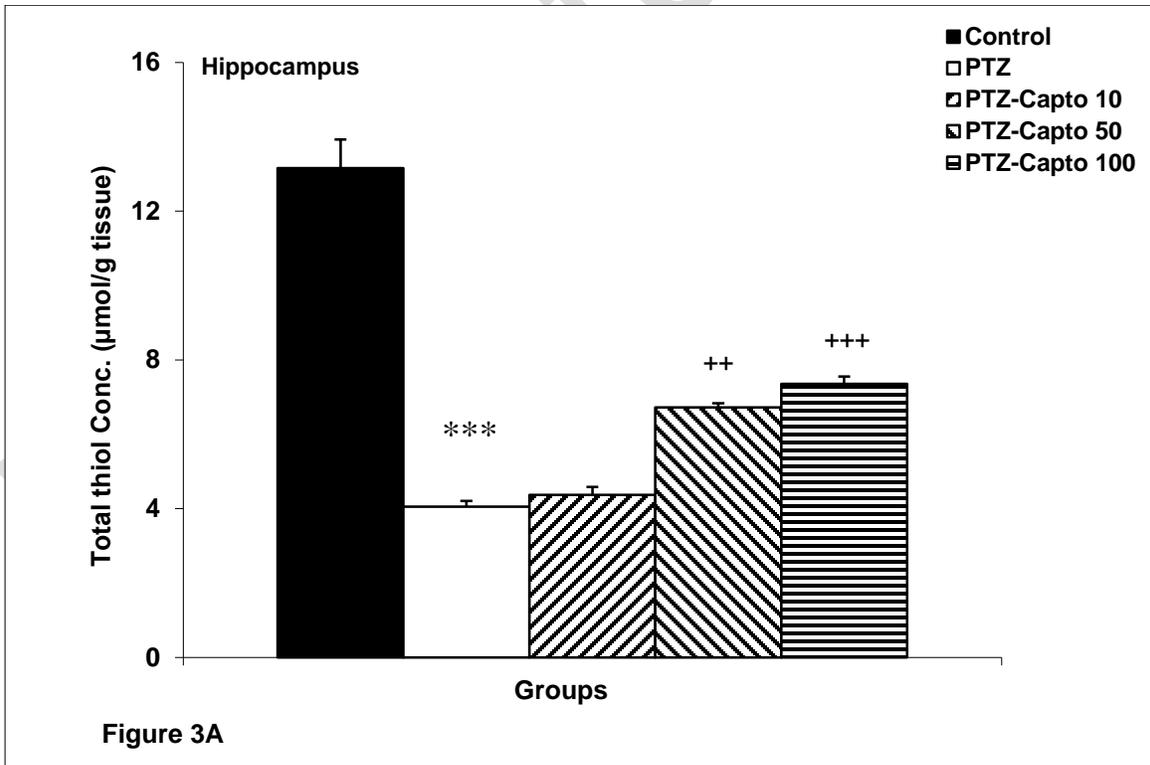
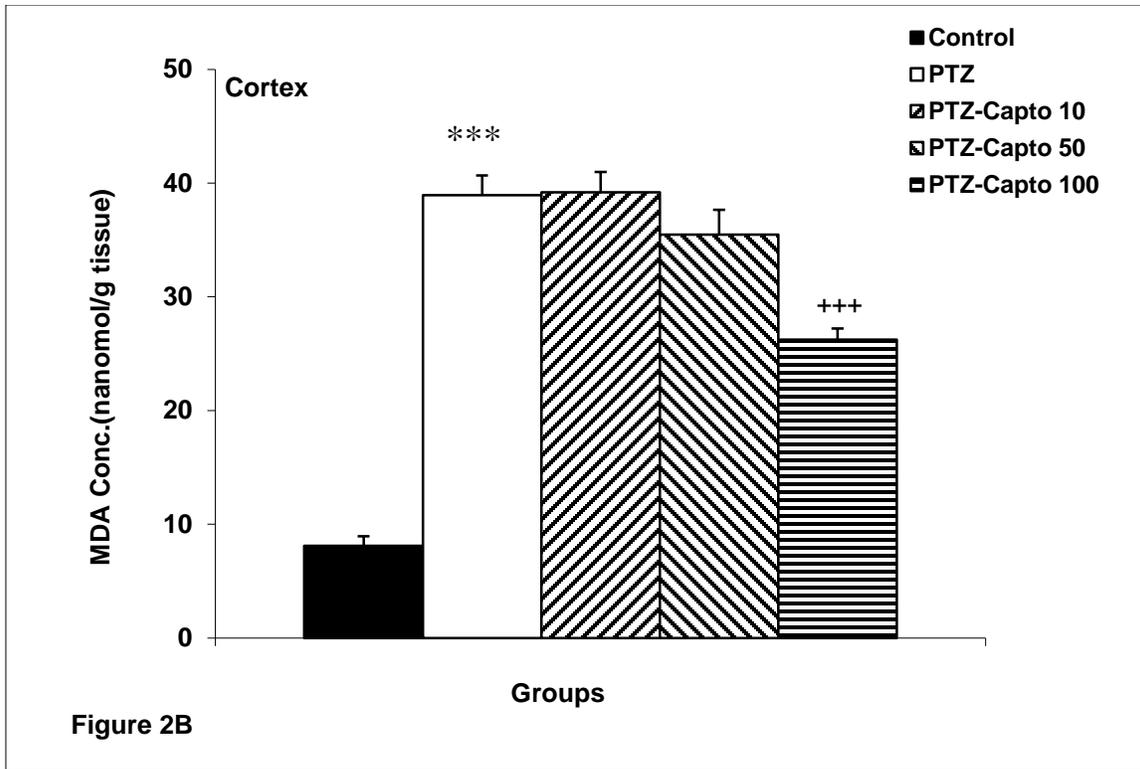
Figure 3: Comparison of total thiol concentration in hippocampus (A) and cortical (B) tissue in experimented groups. Data were shown as the mean \pm SEM (n= 10). *** P<0.001 versus control group. ⁺⁺P<0.01 and ⁺⁺⁺P<0.001 versus PTZ group.

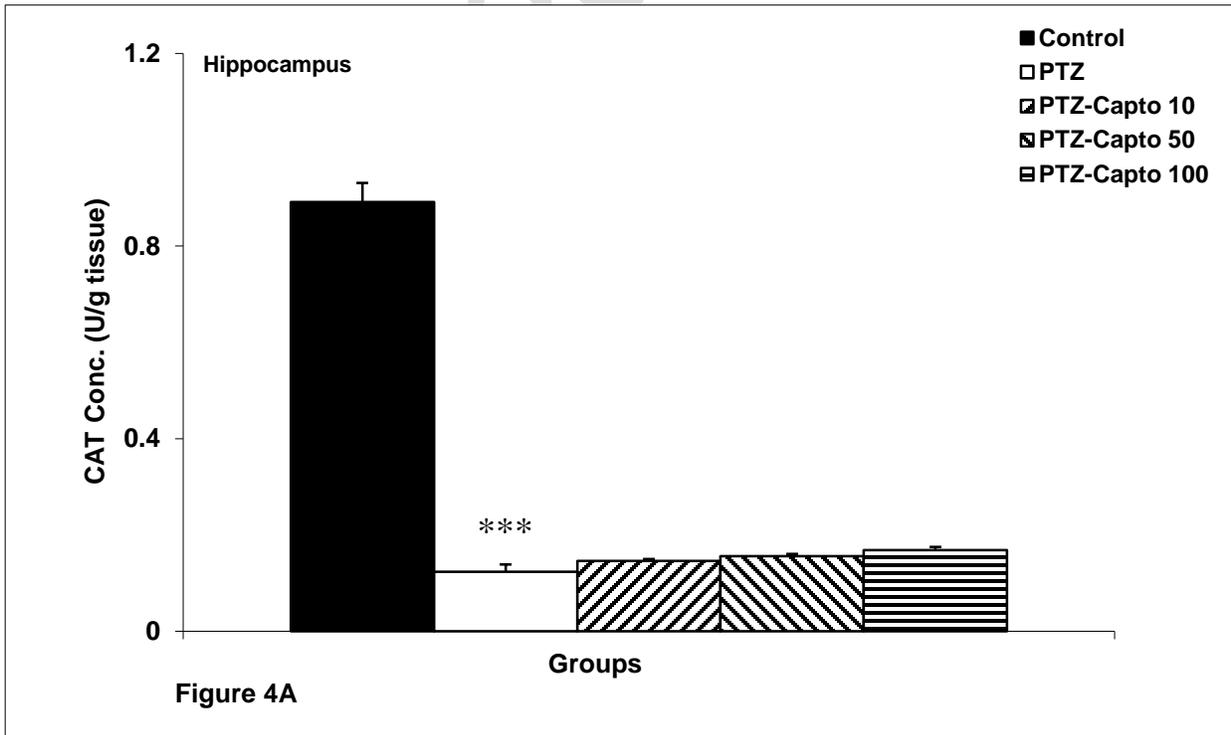
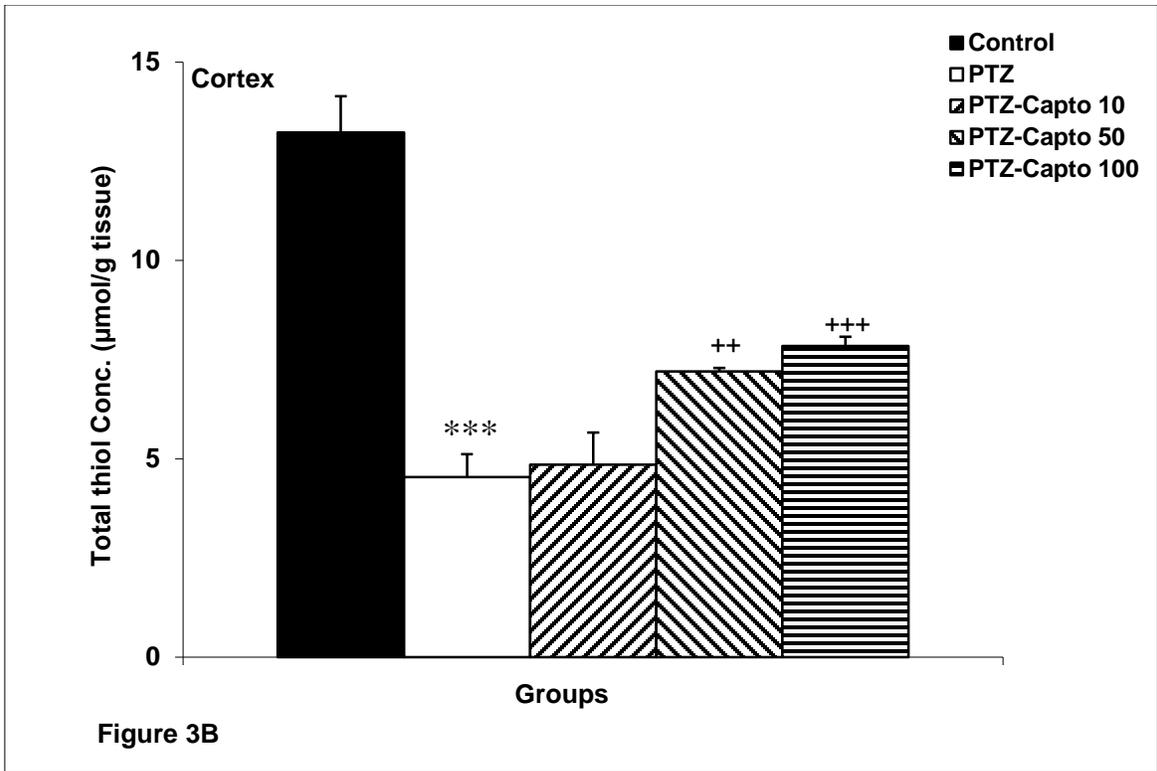
Figure 4: Comparison of CAT activity in hippocampus (A) and cortical (B) tissue in experimented groups. Data were shown as the mean \pm SEM (n= 10). *** P<0.001 versus control group. ⁺P<0.05 and ⁺⁺⁺P<0.001 versus PTZ group.

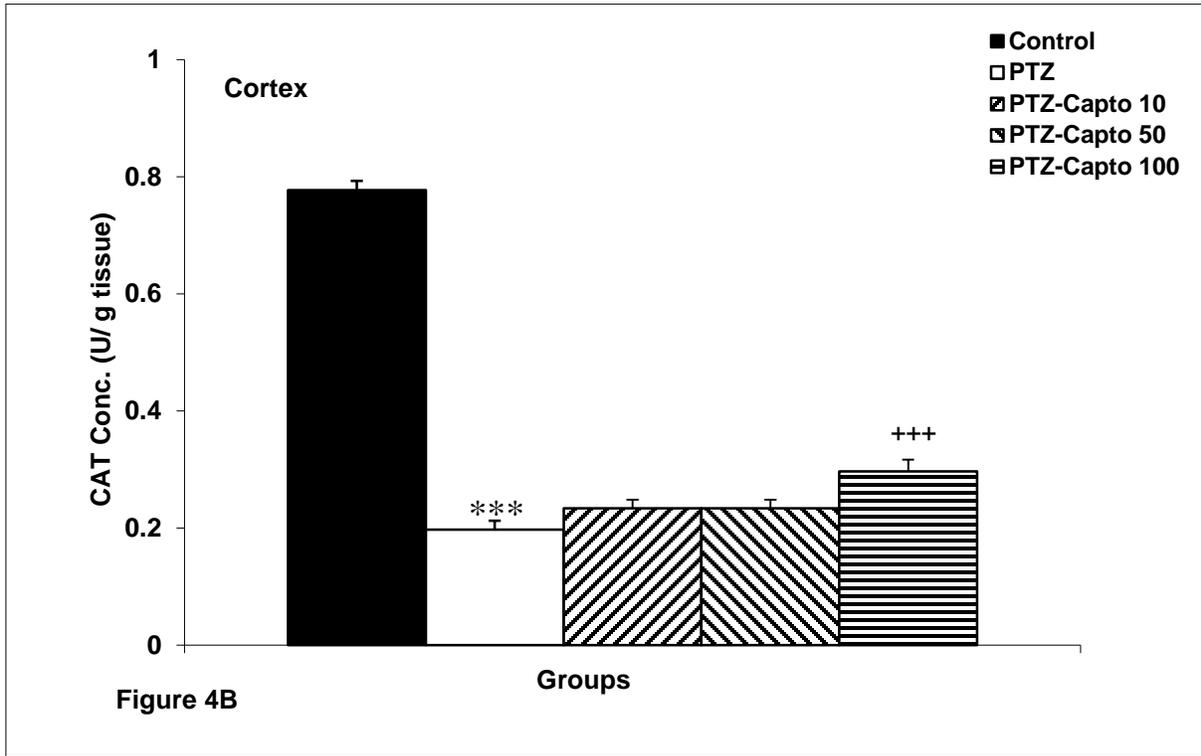
Figure 5: Comparison of SOD activity in hippocampus (A) and cortical (B) tissue in experimented groups. Data were shown as the mean \pm SEM (n= 10). ***P<0.001 versus control group and +++P<0.001 versus PTZ group.











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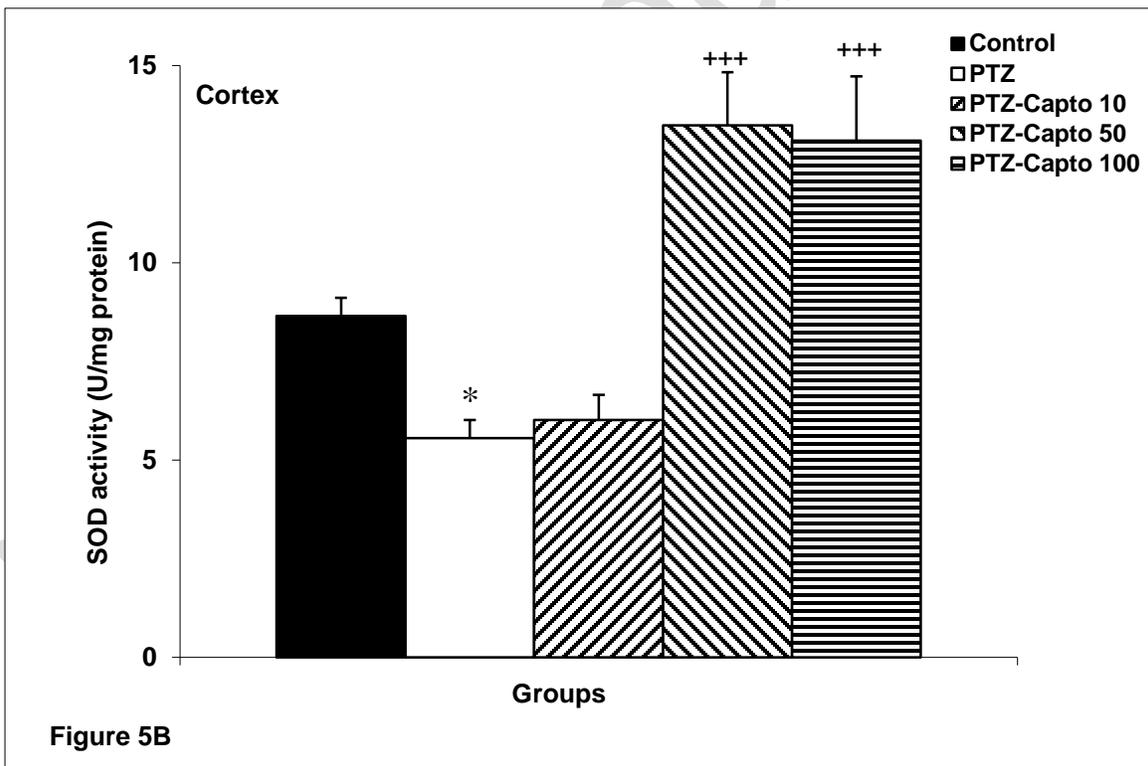
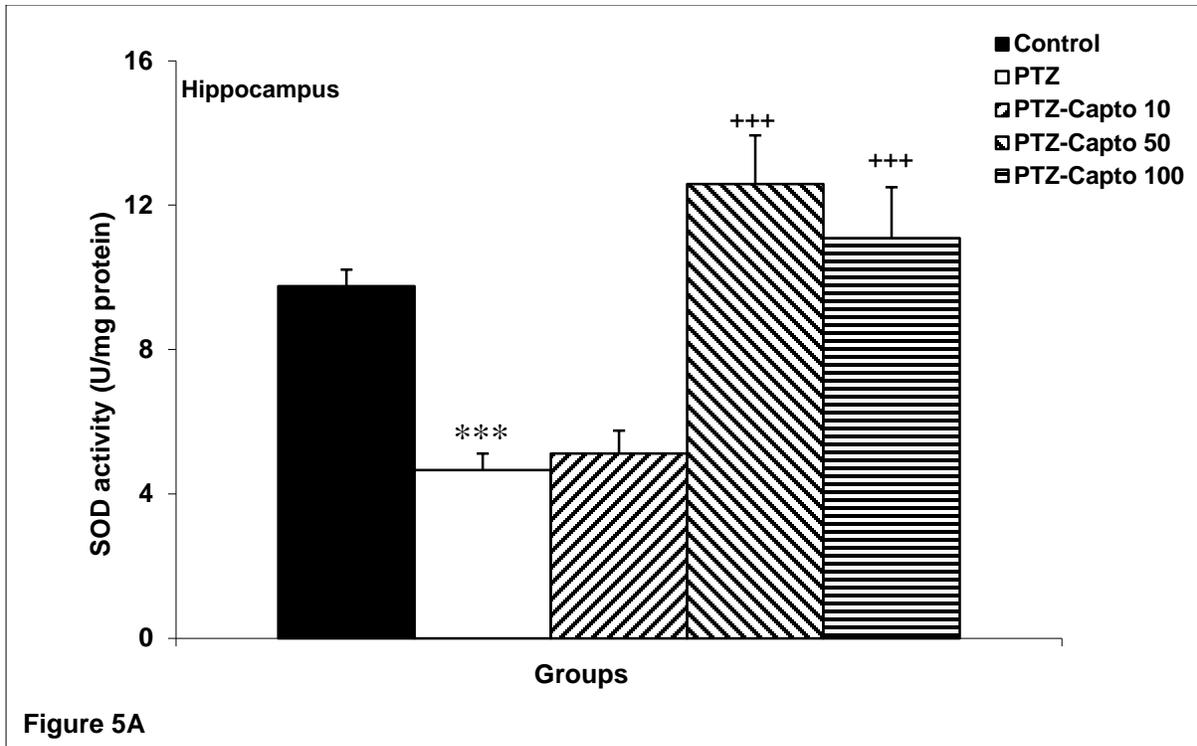


Table 1

Group	PTZ	PTZ- Capto 10	PTZ- Capto 50	PTZ- Capto 100
Mortality (%)	100	30***	20***	0***

Uncorrected Proof