The Effects of Dexmedetomidine on Trauma-Induced Secondary Injury in Rat Brain

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Abstract

Secondary traumatic brain injury is a potentially modifiable and important determinator of the outcome. Sedation and analgesia are common components of the therapy. However current drug therapies have disadvantages like respiratory depression. The objective of this study was to investigate the effect of dexmedetomidine (Dex), a sedative drug with little to no depressive effect on respiratory centers, on secondary injury in rat brain tissue. Eighteen rats were randomized into three groups: Trauma group received anesthesia, followed by head trauma with Mild Traumatic Brain Injury Apparatus, the Trauma+Dex group received additional treatment of 100μg/kg intraperitoneal dexmedetomidine daily for three days, The Control group received anesthesia only. Malondialdehyde (MDA), glutathione (GSH), Na+, K+-ATPase, or sodium/potassium (Na/K-ATPase), and cysteine-aspartic proteases, cysteine aspartates-3 (caspase-3) levels were measured. MDA levels were highest in the Trauma group ($p = 0.002$ vs Control group). Mean levels in the Trauma+Dex group were lower, albeit still significantly high compared to the Control group ($p = 0.002$). Glutathione levels were similar in all groups ($p = 0.99$). Na/K-ATPase levels were lowest in the Trauma group, which is significant compared to the Control group ($p = 0.002$) and the Trauma+Dex group ($p = 0.026$). Histopathologic findings of tissue degeneration like edema, vascular congestion and neuronal injury, and cleaved caspase-3 levels were lower in the Trauma+Dex group compared with the Trauma group. Dexmedetomidine administered during the early stage of traumatic brain injury may inhibit caspase-3 cleavage. However, the mechanism does not seem to be related to the improvement of MDA or GSH levels.

1. Introduction

As traumatic brain injury (TBI) has the highest morbidity and mortality rates among all traumatic injuries in the world, it is generally referred to as a silent epidemic. In the USA, there are 1.4 million cases of TBI and 50000 deaths per annum (Gean and Fischbein 2010; Dewan et al. 2019; Steinmann et al. 2020). Though one has no control over the initial injury, the secondary injury is an important and modifiable determinator of the outcome (Langlois et al. 2006).

The primary injury is due to an external mechanical force causing diffuse neurodegeneration (Gaetz 2004). This initial injury may be limited, or widespread to neurons and vascular endothelium, leading to edema and hemorrhage (Martins et al. 2009). In any case, the ensuing inflammatory response, stimulated by injury to the endothelial cells or ischemia of the neurons, may progress to secondary injury (Sud'ina et al. 1993). Increased intracranial pressure due to cellular damage or patient-related factors like coughing, agitation or hypertension further aggravates ischemia. This vicious cycle will ultimately lead to death (Wallace 2002). Therefore it is vital to follow these patients in the ICU to promptly recognize the signs of secondary injury and provide the appropriate treatment (Wallace 2002).

Sedation and analgesia are commonly utilized to prevent coughing or agitated movements, which may increase intracranial pressure (Procaccio et al.; Xiong et al. 2009). On the other hand, current drug therapy involving the use of barbiturates, benzodiazepines, opioids or propofol may cause respiratory depression

or loss of protective airway reflexes and necessitate endotracheal intubation. Dexmedetomidine is a potent agonist of α2 adrenoreceptors (Schoeler et al. 2012). Presynaptic activation of α2 receptors inhibits norepinephrine release and pain, while postsynaptic activation in the central nervous system decreases sympathetic activity, heart rate and blood pressure (Ertler et al. 1999).

The actors of the secondary injury are still incompletely understood; however malondialdehyde is used to identify destruction of lipid membrane; and glutathione and caspases are commonly used to identify apoptosis of neuronal cells (Nicholson 1999). Malondialdehyde (MDA) is an indicator of membrane lipid peroxidation (Turrens 2018). Glutathione is a non-enzymatic antioxidant; its depletion is the hallmark of progression to apoptosis (Kizhakkayil et al. 2012). Caspase-3 plays an essential role in apoptotic cell death; and is shown to appear within hours and days of traumatic brain injury (Nicholson 1999; Sullivan et al. 2002).

Traumatic brain injury is a vastly varying clinical condition. Therefore several animal models were used to investigate the pathophysiology of the secondary injury. We hypothesized that dexmedetomidine may alleviate the destruction of lipid membrane due to inflammatory peroxidation, and apoptosis of neuronal cells. We designed an experimental study to investigate the effects of dexmedetomidine on the abovementioned pathophysiologic mechanisms in the trauma-induced secondary injury of the brain tissue in rats.

2. Materials And Methods

3-5 months old Spraque Dawley female rats, weighing 250-350 g, were procured from Recep Tayyip Erdogan University Animal Care and Research Unit. All animals received humane care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health. The study was approved by Recep Tayyip Erdogan University Animal Ethical Committee (ID: 2017/1).

2.1 Study Design

Animals' care was performed in Recep Tayyip Erdogan University Animal Care and Research Unit. All animals were maintained and fed in aseptic conditions. Humidity and room temperature were kept at 55-60% and 22±2 °C, respectively. A 12 h light,12 h dark cycle was provided. Rats were allowed access to commercially available standard rat chow (Bayramoglu Feed and Flour Industry Trading Corporation Erzurum, Turkey) and tap water ad libitum along with the experiment.

After sufficient time to adaptto the laboratory conditions, eighteen experimental animals were randomized into three groups. The trauma group (n=6) received head trauma following anesthesia. Trauma+Dex group (n=6) received anesthesia and head trauma the same day; and 100μg/kg intraperitoneal dexmedetomidine daily for three days, beginning in the next day. The dose was determined according to the literature (Garrity et al. 2015). Control group (n=6) received anesthesia only.

Anesthesia was provided with 50 mg/kg intraperitoneal ketamine hydrochloride (Ketalar®, Eczacibası Parke-Davis, Istanbul, Turkey) and 10 mg/kg intraperitoneal xylazine HCl (Alfazyne®, Alfasan International BVWoerden, Holland).

Head trauma was performed with the Mild Traumatic Brain Injury Apparatus developed by Richelle Mychasiuk et al., by dropping a 150 mg weight onto the skull of sedated rats through a 100 cm long tube in free-fall motion (Mychasiuk et al. 2014). Each rat was prepositioned on a 38x27x27 cm3 box-shaped platform which housed a 38x25x15 cm3 sponge. There was a 10 cm gap between the 15 cm tall sponge and the upper edge of the box. An aluminum foil was placed on the upper surface of the box where the rats were laid during trauma. The distal end of the tube and the head of the rat were positioned at a distance of 4 cm.

On the fourth day, all rats were sacrificed by decapitation; the brain was immediately removed and divided into two parts for biochemical and histopathological analysis (Dixon et al. 2016). Biochemical, histopathological, and immunohistochemical analysis procedures are detailed below.

2.2 Tissue homogenization

Homogenization solution was prepared by mixing 20 mM sodium phosphate and 140 mM potassium chloride; the pH was adjusted to 7.4 (Rojas et al. 2012). One mL of this solution was mixed with 100 mg tissue and was centrifuged at 800 G for 10 mins at 4°C. The supernatant was used to measure the concentration of malondialdehyde produced due to degradation of unstable lipid peroxides via Thiobarbituric Acid Reactive Substances (TBARS), and concentration of intracellular glutathione.

2.3 TBARS measurement

TBARS level was measured by the protocol of Ohkawa (Ohkawa et al. 1979). The mixture of 200 µL of tissue supernatant, 50 µL of 8.1% sodium dodecyl sulfate, 375 µL of 20% acetic acid at pH of 3.5, and 375 µL of 0.8% thiobarbituric acid was vortexed. After incubation in boiling water bath for 1 h, it was cooled in ice water for 5 min and centrifuged at 750 G for 10 min. The resulting pink color was read on a spectrophotometer at 532 nm. The results were calculated as nmol/mg prt.

2.4 Total Sulfhydryl Content measurement

The sulfhydryl groups were measured with Ellman's reagent. 1000 µL of 3M Na2HPO4 and 250 µL of DTNB (4 mg DTNB prepared in 10 mL of 1% sodium citrate solution) were added to 250 µL of supernatant and vortexed. The absorbance was measured at 412 nm. The results were determined by the standard curve of reduced glutathione (1000 µM-62.5 µM) and given as nmol/mg prt.

2.5 Na/K ATPase measurement

Na/K ATPase activity was measured by the method of Yoshimura (YOSHIMURA 1973). 100 mg of brain tissue was homogenized after adding 1 mL of 10mM Tris-HCl at a pH of 7.4. Homogenates were

centrifuged at 4000 rpm for 10 minutes at 4°C. One tube was prepared by adding 250 µL of KCl (0.8M), 250 µL of NaCl (4M), 250 MgL of MgCl (0.4) and 200 µL of Tris-HCl (1M) to 50 µL of the supernatant and incubated for 10 minutes. Another tube was prepared by adding 250 µL of ouabain (40mM), 250 µL of NaCl (4M), 250 MgL of MgCl (0.4) and 200 µL of Tris-HCl (1M) to 50 µL of the supernatant and incubated at 37°C for 10 minutes. After 10 min, 60 µL of ATP (25mM) was added to each tube and left at 37°C for 1 h. The reaction was stopped by adding 500 TCL of 10% TCA to each tube. Both tubes were centrifuged at 3000 G for 10 min at 4°C. Inorganic phosphate was measured by the Fiske and Subbarow method and the results were given as nmol/Pi/min/mg prt (Fiske and Subbarow 1925). A commercially available bicinchoninic acid kit was used for protein measurement.

2.6 Histopathological analysis procedure

The brain tissue samples were fixed in 10% neutral formaldehyde. After the fixation, specimens were dehydrated in an ascending series of alcohol, cleared in xylene and embedded in paraffin by routine laboratory methods. Hematoxylin&Eosin staining and evaluation with a light microscope (Leica DM6200, Germany; with Olympus DP20 camera attached) was performed by two histologists blinded to the study groups.

2.7 Immunohistochemical analysis

The following steps were performed for cleaved caspase-3 staining: the sections were deparaffinized and treated with 20 µg/mL proteinase-K solution in Phosphate Buffered Saline (PBS), rinsed in distilled water, immersed in 3% hydrogen peroxide. After several washes with PBS (pH 6.0), the sections were immersed in an equilibration buffer. Sections were incubated with anti-Cleaved Caspase-3 (1:200, ab2302, Rabbit polyclonal to active Caspase-3, Abcam, UK). After several washes, all sections were incubated in anti-digoxigenin-peroxidase. Cleaved caspase activity was revealed with 0.06% 3,3-diaminobenzidine tetrahydrochloride in PBS, and the sections were counterstained with Harris hematoxylin.

2.8 Semi-Quantitative analysis

Brain tissue sections were stained with Hematoxylin&Eosin. For each animal, three slides were selected randomly from eight slides containing the brain tissues. On each slide, the presence of ischemic neurons, edema and vasoconstriction were evaluated. All sections (3-4µm thick) prepared from brain tissue were stained with Caspase-3 staining. The evaluation was performed with a light microscope (Leica DM6200, Germany, x40 magnification). For each animal, six slides were selected randomly from 8 slides random areas. On each slide, the presence of Cleaved caspase-3 positive cells was determined. Two histologists blinded to the study groups evaluated and graded the slides in four categories as neuronal ischemia, brain edema, vascular congestion and cleaved caspase-3 staining positivity. The findings were graded as none (<5%), mild (6-25%), moderate (26-50%) or severe (>50%).

2.9 Statistical analysis

Statistical analysis was performed with SPSS ver. 12 (SPSS Statistical Program, IBM Corporation, USA). The normality of data was tested with the Kolmogorov-Smirnov test and QQ-plots. TBARS and total sulfhydryl values were presented as mean±standard deviation. Groups were compared with a one-way analysis of variance, followed by the Tamhane test. Na/K-ATPase activity, histological injury scores and caspase-3 positivity scores were presented as median (25%-75% interquartile range). Groups were compared with Kruskal-Wallis Test. In case of significant difference, two-group comparisons were performed with one-way analysis of variance, followed by the Tamhane test., a p-value < 0.05 was considered as statistical significance.

3. Results

3.1 Biochemical analysis

Results of biochemical tests are given in Figure 1. Briefly, TBARS levels were higher in the Trauma and Trauma+Dex groups compared with the Control group (p = 0.002 for both comparisons). Glutathione levels were similar among groups (p = 0.99). Na/K-ATPase levels were significantly lower in the Trauma group (p = 0.002 vs Control group, 0.026 vs Trauma+Dex group). The values in the Trauma+Dex group ranged between values similar to both the Control and the Trauma groups (p=0.065 and 0.002, respectively).

3.2 Histopathological analysis

Histological injury scores are given in Table 1. Representative light microscopic images for each group are shown in Figure 2. Briefly, edema, vascular congestion and neuronal injury were milder in the Trauma+Dex group compared to the Trauma group. However neuronal ischemia did not differ significantly between the two study groups and was significantly higher compared to the Control group.

3.3 Immunohistochemical analysis

Progression of the neuronal apoptosis as labeled with cleaved Caspase-3 staining is shown in Figure 3. Results are given in Table 2. Briefly, the Trauma group had the highest number of apoptotic neurons, glial cells and oligodendrocytes. The Trauma+Dex group was similar to the Control group except for the number of apoptotic glial cells.

4. Discussion

This study showed that dexmedetomidine administered in the early post-traumatic period may alleviate some aspects of secondary brain injury in a rat model. Besides, this study found that MDA levels, measured as TBARS, are increased in both groups of rats receiving trauma. Lipid peroxidation is a consequence of the disruption of the cell membrane. MDA levels are higher in trauma patients (Yeler et al. 2005). An experimental study demonstrated that lipid peroxidation and MDA increased approximately one hour after spinal cortex injury (Kaya 1993). However, an increase in MDA levels in rats receiving

dexmedetomidine was limited compared to the Trauma group. This may be explained by dexmedetomidine's inhibitory effect on inflammatory cell response or oxidative stress (Zeng et al. 2016).

The neuronal membranes in the central nervous system are rich in unsaturated fatty acids and the antioxidant system is weak (Öztürk et al. 2001). Therefore it is very susceptible to oxidant activity. Antioxidants are thought to accelerate neurological recovery after trauma by limiting or preventing secondary injury (Kaptanoglu et al. 2002). On the other hand, Ignowski and coworkers observed no difference in brain tissue GSH levels 72 hours after trauma (Ignowski et al. 2018). Similarly, this study, we did not observe any difference in GSH levels between the groups.- This suggests that dexmedetomidine is not involved in thiol metabolism. However, there are other pathways involved in the inhibition of oxidative metabolism and these were not investigated in this study, which is a major limitation (Di Meo et al. 2016). As discussed above, this study was not designed to explore the comprehensive state of oxidative metabolism due to ethical and financial reasons.

Na/K-ATPase activity was significantly reduced in the Trauma group, possibly related to the effect of primary and secondary injury. Na/K-ATPase activity was partly improved in rats that received dexmedetomidine, which suggests that secondary injury was limited in this group. Oxidative damage is a significant component of the secondary injury (Lima et al. 2008). Lower levels of MDA in rats receiving dexmedetomidine may suggest that the wide range of Na/K-ATPase activity in the Trauma+Dex group is a result of dexmedetomidine's antioxidant effect. Since animal studies investigating intraperitoneal TQ reported consistent effects of the drug, a difference in distribution or metabolism is unlikely. The most likely explanation is the low number of rats,

The effects of two molecules with supposedly antioxidant properties, propofol and dexmedetomidine, were compared in rats with spinal cord injury-induced by Allen's weight-drop method (Ger and Konya 2007). They found the lowest MDA levels in the dexmedetomidine group.

Aslan et al. showed similar results in a similar setting with rabbits (Aslan et al. 2009). They showed lower glutathione levels in the dexmedetomidine group. The findings of this study are in agreement with them.

The components of histological injury score obtained in this study suggest that the degree of ischemia is similar in Trauma and Trauma+Dex groups. However cleaved caspase-3 positivity scores suggest that cellular injury is lower in the Trauma+Dex group compared to the Trauma group. In particular, although there is significant neuronal damage in the Trauma+Dex group, neuronal cleaved caspase-3 positivity is similar to the Control group.

Three recent studies may shed light on possible pathways in the rat brain. Shen et al showed that activation of the PI3K/Akt/mTOR signaling pathway by dexmedetomidine may inhibit the apoptosis (Shen et al. 2017). Zhang et al showed that 15 μg/kg intravenous dexmedetomidine increased expression of both Bcl-2 and heat-shock protein 70 (Zhang et al. 2018). Finally, Li et al reported that 25 μg/kg intraperitoneal dexmedetomidine upregulates the nuclear factor erythroid 2-related factor may promote these changes in Bax- and Bcl-protein families (Li et al. 2019). These and similar studies

performed in rats suggest that dexmedetomidine is capable of diverting the neuronal cells to an anabolic path rather than apoptosis. Our study showed similar findings; albeit with a comparatively high dose of 100µg/kg. Additionally, it suggests a possible role of dexmedetomidine as an inhibitor of the inflammatory pathway.

Secondary injury due to post-traumatic neurodegenerative changes is mediated by many metabolites such as monoamines, free oxygen radicals, neuropeptides and extracellular calcium (Ikeda and Long). An anti-inflammatory role of dexmedetomidine was previously suggested by Can et al (Can et al. 2009). They showed that administration of dexmedetomidine to rats with spinal cord injury reduced the production of inflammatory cytokines. Cosar et al. (Cosar et al. 2009) administered dexmedetomidine to rabbits with subarachnoid hemorrhage and found that dexmedetomidine was neuroprotective based on the histopathological and biochemical findings in hippocampal tissues. This study found typical histological appearance and no pathology in the Trauma+Dex group, in contrast with prominently ischemic neurons, perineural vacuolization and severe brain edema in the Trauma group. Also, this study showed that dexmedetomidine limited the degree of neuronal apoptosis.

Hall et al. (Hall et al. 2004) investigated the cardioprotective effects of dexmedetomidine in rats with induced cerebral hypertension. They showed that dexmedetomidine inhibited the increase in plasma catecholamine levels and decreased myocardial MDA levels. Catecholamines are known to increase the production of free radicals and the accumulation of calcium inside the cell. Both of these effects are associated with lipid peroxidation. Finally, Laudenbach et al. showed that dexmedetomidine reduced neuron damage not only in the cortex but also in the white matter of cystic lesions in five-day-old rats with hypoxic-ischemic brain injury induced by injections of NMDA-receptor agonists (Laundenbach et al. 2002). They reported that dexmedetomidine inhibited NMDA receptor activity and intracytoplasmic accumulation of calcium. Therefore dexmedetomidine may directly or indirectly reduce the production of free radicals and limit lipid peroxidation and cellular apoptosis.

This study has several limitations. Different animal models are used to investigate the pathophysiology of secondary injury after traumatic brain injury. Weight-drop models utilize the gravitational forces of free-falling (Flierl et al. 2009). They aim to achieve focal and diffuse brain damage as a result of the external force delivered to the rat's skull (Blaha et al. 2010). There are as many animal models of traumatic brain injury as there are mechanisms of pathogenesis, and this study used only one of these models. Secondly, this study only investigated the biochemical and histopathological end-results of trauma. Therefore, no conclusions can be made about possible interactions between actors of secondary injury. In our study, TBARS, MDA and GSH levels in brain tissue were examined. Our study needs to be supported by studies examining other oxidant / antioxidant molecules that play a role in oxidative stress. Besides, apoptosis should be supported by studies dealing with TUNEL, Ki-67, intracellular and mitochondrial calcium levels.

Another limitation is the lack of hemodynamic and respiratory monitoring. Dexmedetomidine is a sedative drug with unique properties such as vasodilation and bradycardia. The resulting decrease in cardiac output may increase cerebral ischemia. Sedative doses of dexmedetomidine decrease respiratory

rate but do not affect respiratory response to carbon dioxide (Jo et al. 2016). The dose of dexmedetomidine in this study was based on a previous study, which showed that an intraperitoneal dose of 100 µg/kg reliably induced sedation in Sprague-Dawley rats (Garrity et al. 2015). However, this dose is either toxic or does not have a possible human application.

As dexmedetomidine was mostly studied on the hypoxic-ischemic and hyperoxia-induced injury, this pilot study on traumatic brain injury investigated only a single dose of dexmedetomidine due to ethical reasons (Alam et al. 2017; Endesfelder et al. 2017). To limit the sample size, dose-response curves were not generated. Further laboratory studies investigating the specific mechanisms, dose-response relationship, and the impact of time to initiation of treatment are required before clinical translational studies.

5. Conclusion

Dexmedetomidine administered during the early stage of traumatic brain injury may alleviate neuronal injury by inhibiting lipid peroxidation. The mechanism of action and possible alterations to the therapy, such as timing, dosing and duration, should be investigated further.

Declarations

Supplementary Information

The online version contains supplementary material available at http://dx.doi.org/10.17632/k75f5mmgxw.1

Author contributions

AS, BE and LT designed the project. AS, BE, LT, HAU and TM designed the experiments. AS, BE, TM, SB and AO wrote the manuscript. The authors declare that all data were generated in-house and that no paper mill was used. The authors declare that all data were generated in-house and that no paper mill was used.

Availability of data and materials

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Ethical Approval

The study was approved by Recep Tayyip Erdogan University Animal Ethical Committee (ID: 2017/1).

This research was approved by the Animal Research Ethics Committee of the Recep Tayyip Erdogan University (2017–1). Procedures involving animals and their care were carried out in conformity with

international laws and policies (Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 2011)).

Consent to participate

Not applicable

Consent to publish

Not applicable

Competing interests

The authors declare no competing interests.

Availability of data and materials

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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Tables

Table 1. Histological injury scores. Data are given as median (25%-75% interquartile range) and analyzed with the Kruskal-Wallis test.

	Control	Trauma	Trauma+Dex
Atypical neurons	1(1-1)	2(2-3)[a]	1.5(1-2)
Edema	0(0-0	2(2-2.5)[b]	1(0.5-1)[c]
Vascular congestion	0(0-0.5)	2(2-2)[b]	1(0-1)[d]
Histopathological Damage Score (HPDS)	1(1-2)	6.5(5.5-7)[b]	3(2-3.5)[e,f]

[a]p=0.007 versus Control group,

[b]p=0.000 versus Control group,

[c]p=0.028 versus Trauma group,

[d]p=0.005 versus Trauma group,

[e]p=0.037 versus Control group,

[f]p=0.005 versus Trauma group,

Kurkal Wallis/Tamhane T2 test

Table 2. Caspase-3 positivity scores. Data are given as median (25%-75% interquartile range) and analyzed with the Kruskal-Wallis test.

	Control	Trauma	Trauma+Dex
Neuronal Caspase-3 positivity	0(0-0)	1(1-1.5)[a]	0(0-0.5)[b]
Oligodendrocytes Caspase-3 positivity	0(0-0.5)	3(3-3)[c]	1.5(1-2)[d]

[a]p=0.008 versus Control group,

[b]p=0.024 versus Trauma group,

[c]p=0.000 versus Control group,

[d]p=0.000 versus Trauma group,

Figures

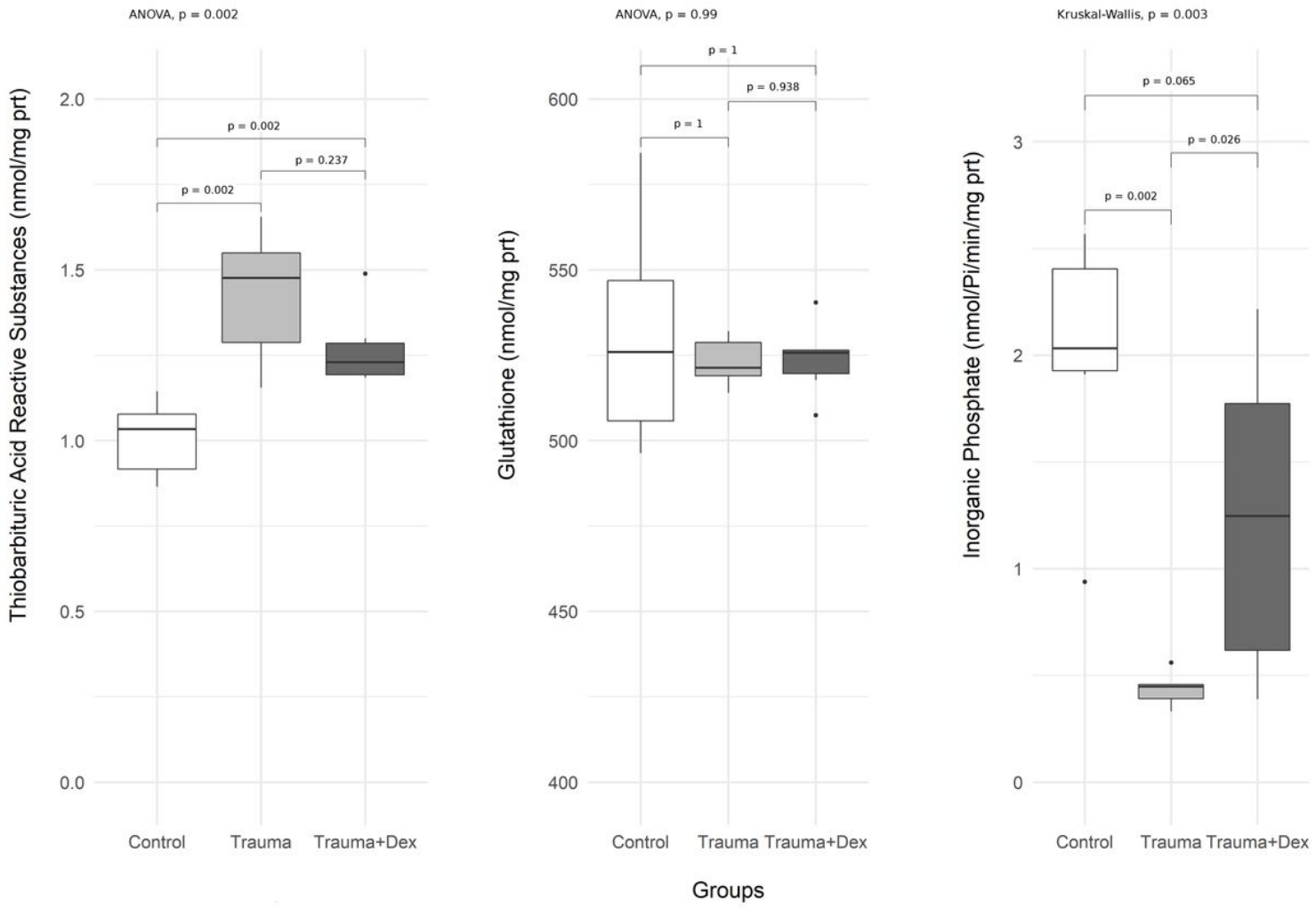

Figure 1

Results of biochemical tests. Each group contained 6 rats.

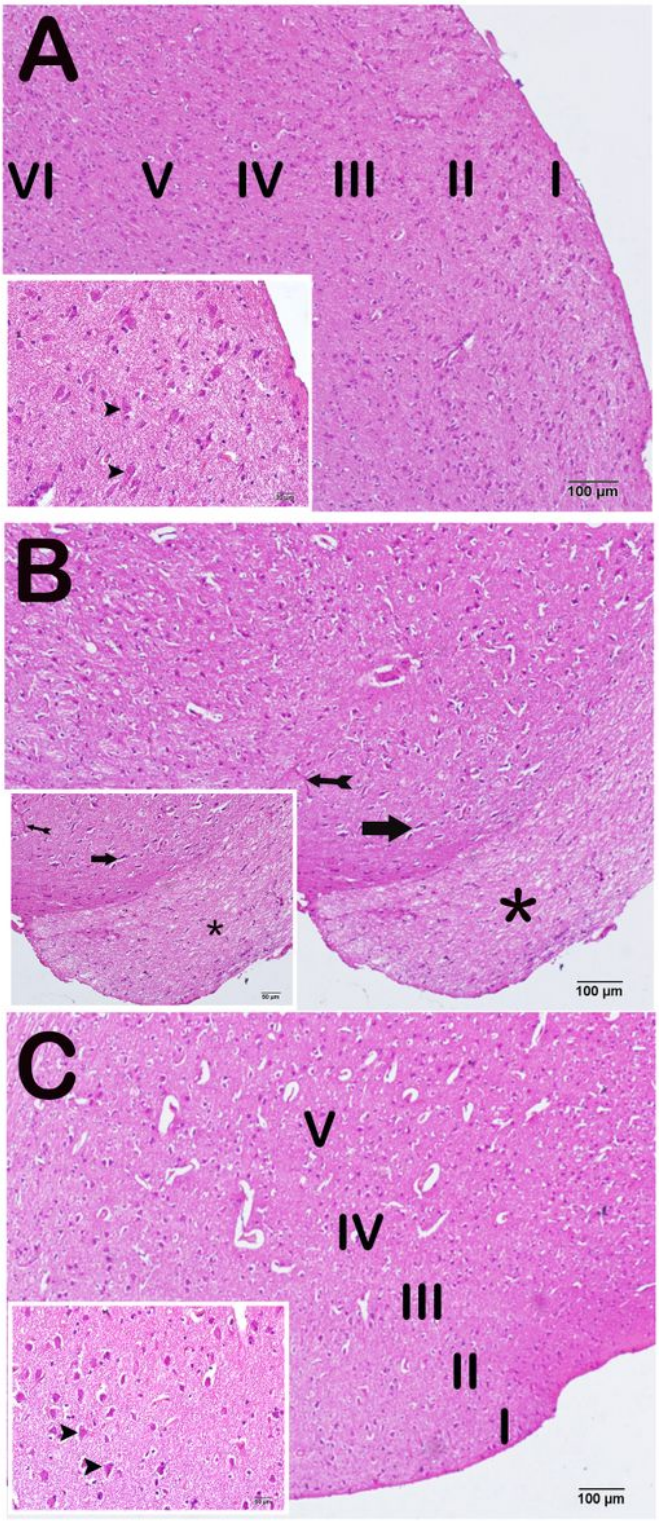

Figure 2

Representative light microscopic photographs from cerebral cortex tissue sections stained with Hemotoxylene&Eosin. Molecular layer (I), External granular layer (II), External pyramidal neuron (III), Internal glanular layer (IV), Internal pyramidal neuron (V), Multıform layer (VI). (A, x100 magnification) Control Group; Healthy morphological structures of the cerebral cortex tissue. (B, x100 magnification) Trauma Group; Cerebral cortex area demonstrates a great number of ischemic neurons with perineural

vacuolizations (arrow). Intense brain edema, prominent in subpia mater region (asterisk). Vascular congestion (tailed arrow). (C, x100 magnification) Trauma+Dex Group; Cerebral cortex area demonstrates typical neurons (arrowhead).

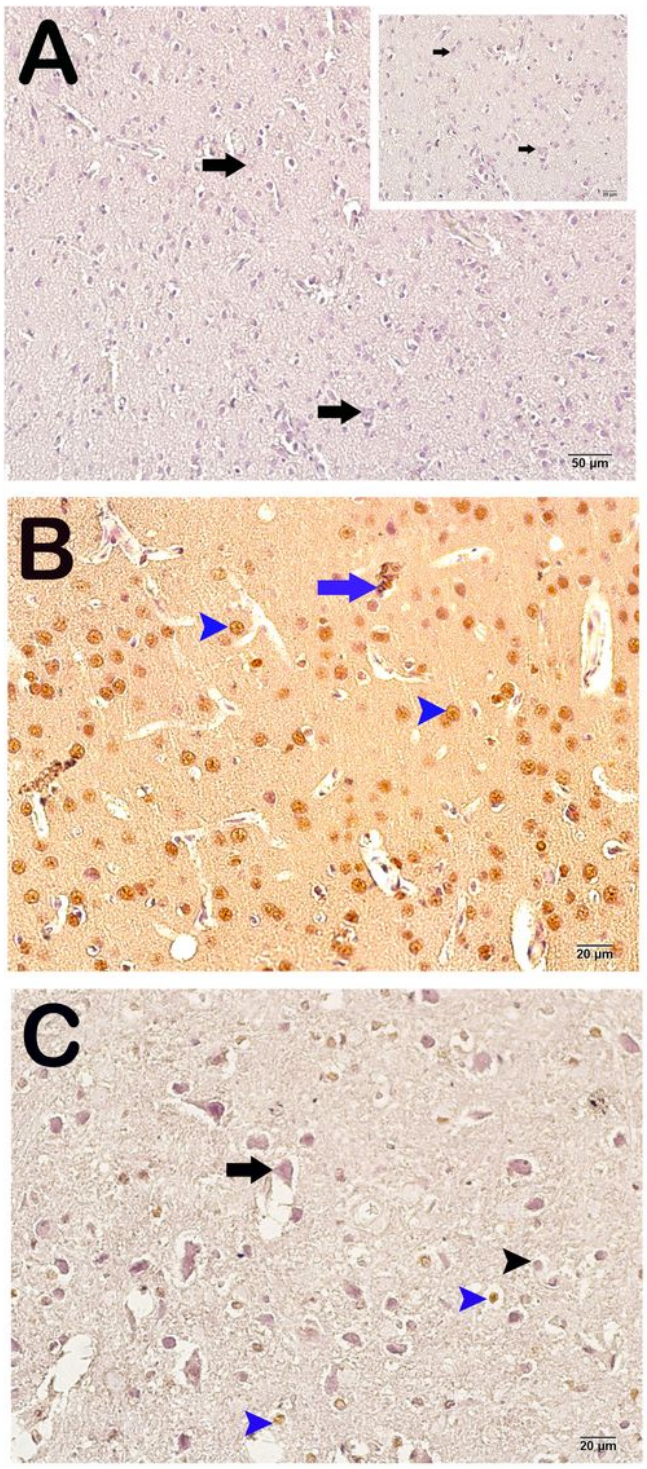

Figure 3

Representative photomicrographs of the Caspase-3 stained sections from the brain tissue by light microscopy. (A, x200 magnification) Control Group; Caspase-3-negative neurons (arrow) and

oligodendrocytes (arrowhead). (B, x400 magnification) Trauma Group; Cerebral cortex area demonstrating a great number of apoptotic neurons (blue arrow) and oligodendrocytes (blue arrow head). (C, x400 magnification) Trauma+Dex Group; Typical neurons (arrow) in addition to a smaller number of apoptotic oligodendrocytes (blue arrow head) were observed.

Supplementary Files

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