UNIVERSIDAD DEL ZULIA
Serbiluz
Sistema de Servicios Bibliotecarios y de Información
Biblioteca Digital
Repositorio Académico

https://doi.org/10.52973/rcfcv-e361799 Revista Científica, FCV-LUZ / Vol. XXXVI

Histopathological and real–time PCR analysis of alterations in the rat brain within the Glycerol–induced Crush Syndrome

Análisis histopatológico y PCR en tiempo real de alteraciones en el cerebro de rata en el síndrome de aplastamiento inducido por Glicerol

***Ayşegül Bulut*[1]*, *Mehmet Tuzcu*[1], *Mehmet Burak Ateş*[1], *Şeyma Hazal Çetin*[2], *Vedat Kaya*[1]**

[1]*Selcuk University, Faculty of Veterinary Medicine, Department of Pathology. Secuklu, Konya, Türkiye.*
[2]*Selçuk University, Faculty of Medicine, Department of Medical Biology. Selcuklu, Konya, Türkiye.*
**Corresponding author: vetaysegulbulut@gmail.com*

ABSTRACT

This study attempted to examine the neuroprotective benefits of vitamin C and tadalafil in an experimental crush syndrome model induced by intramuscular glycerol injection in rats, using histopathological and real–time polymerase chain reaction. 50 females Wistar albino rats were used in the study. The animals were divided into 7 groups: healthy control (n: 6), vitamin C (n: 6), tadalafil (n: 6), glycerol administered (n: 8), glycerol + vitamin C (n: 8), glycerol + tadalafil (n: 8), and glycerol + vitamin C + tadalafil (n: 8). After the 6–day experimental study, the animals were anesthetized and euthanized, and necropsies were performed. For histopathological and real–time polymerase chain reaction analyses brain tissues were fixed in 1% formaldehyde solution. Sections of 5 μm thickness were obtained from tissues processed by routine histological procedures and stained with hematoxylin and eosin. Microscopic examination revealed that glycerol administration caused neuronal necrosis, neuronophagia, gliosis, edema, congestion, endothelial cell damage, and mononuclear cell infiltration. Brain Natriuretic Peptide, Heat Shock Protein 70 and Hypoxia–Inducible Factor 1–alpha expression levels were measured in real–time polymerase chain reaction analysis. Glycerol administration caused increases in Brain Natriuretic Peptide and Heat Shock Protein 70 levels in the brain, while it did not cause any significant changes in Hypoxia–Inducible Factor 1–alpha levels. Decreases in Brain Natriuretic Peptide and Heat Shock Protein 70 expression levels were detected in the glycerol + Vit C, glycerol + tadalafil, and glycerol + Vit C + tadalafil groups. According to the research results, it is thought that the combined application of Vit C, tadalafil and Vit C + tadalafil can provide protection against oxidative and inflammatory stress against brain damage that may occur after glycerol–induced crush syndrome.

Key words: Brain; crush syndrome; glycerol, histopathology, real–time PCR

RESUMEN

Este estudio tuvo como objetivo investigar los efectos neuroprotectores de la vitamina C y el tadalafilo en un modelo experimental de síndrome de aplastamiento inducido por inyección intramuscular de glicerol en ratas, utilizando histopatología y reacción en cadena de la polimerasa en tiempo real. Se utilizaron cincuenta ratas albinas Wistar hembras en el estudio. Los animales fueron divididos en 7 grupos: control (n: 6), vitamina C (n: 6), tadalafilo (n: 6), glicerol (n: 8), glicerol + vitamina C (n: 8), glicerol + tadalafilo (n: 8), y glicerol + vitamina C + tadalafilo (n: 8). Después del estudio experimental de 6 días, los animales fueron anestesiados y sacrificados, y se realizaron necropsias. Para los análisis histopatológicos y de reacción en cadena de la polimerasa en tiempo real y la puntuación, los tejidos cerebrales se fijaron en una solución al 1% de formaldehído. Se tomaron secciones de 5 μm de grosor de los tejidos que fueron sometidos a procedimientos rutinarios de seguimiento de tejidos, y se realizó una tinción con hematoxilina–eosina. El examen microscópico reveló que la administración de glicerol causó necrosis neuronal, neuronofagia, gliosis, edema, congestión, daño a las células endoteliales e infiltración de células mononucleares. Los niveles de expresión de Péptido Natriurético Cerebral, Proteína de Choque Térmico 70 y Factor Inducible por Hipoxia–1 alfa se midieron en un análisis de reacción en cadena de la polimerasa en tiempo real. La administración de glicerol causó aumentos en los niveles de Péptido Natriurético Cerebral y Proteína de Choque Térmico 70 en el cerebro, mientras que no causó cambios significativos en los niveles de Factor Inducible por Hipoxia–1 alfa. Se detectaron disminuciones en los niveles de expresión de Péptido Natriurético Cerebral y Proteína de Choque Térmico 70 en los grupos glicerol + Vit C, glicerol + tadalafilo y glicerol + Vit C + tadalafilo. Según los resultados de la investigación, se piensa que la aplicación combinada de Vit C, tadalafilo y Vit C + tadalafilo puede proporcionar protección contra el estrés oxidativo e inflamatorio contra el daño cerebral que puede ocurrir después del síndrome de aplastamiento inducida por glicerol.

Palabras clave: Cerebro; síndrome de aplastamiento; glicerol, histopatología, PCR en tiempo real

Received: 16/09/2025 Accepted: 23/12/2025 Published: 07/01/2026

CC BY NC SA

INTRODUCTION

Crush syndrome (CS) is a systemic condition that arises when toxic chemicals infiltrate the bloodstream due to severe rhabdomyolysis, potentially leading to fatality. [1]. Traumatic rhabdomyolysis is a syndrome characterized by the release of myoglobin, calcium, potassium, creatine kinase, lactate dehydrogenase, and various proinflammatory mediators into the systemic circulation due to damage to striated muscle cells, usually due to natural disasters, traffic accidents, and wars, and the emergence of various clinical and laboratory findings [2, 3, 4].

In muscle groups under pressure, myocytes rupture, their sodium, calcium, potassium, lactic acid, purines, organic acids, myoglobin, thromboplastin, creatinine, and creatine phosphokinase are released into the cell, causing damage to many organs, especially the kidney [4, 5]. It has been stated that these changes can lead to the development of hypovolemic shock, acidosis, heart failure, acute toxaemia, cardiac arrhythmias, respiratory failure, hyperphosphatemia, infections and acute renal failure [5, 6, 7, 8, 9].

Systemic complications that occur after CS can cause damage to the brain. Increased nitric oxide due to muscle injury causes vasodilation and hypovolemia, leading to reduced blood flow to the brain and subsequent hypoxic damage. In CS, impaired enzyme activities and increased Na^+, K^+–ATPase activities have been reported. After a decrease in blood flow to the brain, the brain's energy metabolism is disrupted, and an energy crisis occurs. [1, 2, 3, 4].

Tadalafil is a phosphodiesterase isoenzyme type 5 (PDE–5) inhibitor that was first approved by the US Food and Drug Administration (FDA) in 2003 for the treatment of erectile dysfunction [5, 6]. It inhibits the PDE–5 enzyme, causing vasodilation in vascular smooth muscle [7]. It is absorbed more slowly after oral administration compared to other PDE–5 inhibitors and is unaffected by food and alcohol intake. Its half–life is 17.5 hours (h) [8, 9, 10]. In the brain, it protects by reducing neuroinflammation, neutralizing reactive oxygen species, suppressing neuronal necrosis and apoptosis, and promoting angiogenesis [11, 12].

Vitamin C is a six–carbon lactone essential for life that cannot be synthesized by humans, primates, and guinea pigs [13]. It is a powerful antioxidant that exerts its primary effect by neutralizing free radicals and reactive oxygen species in the body [14]. This antioxidant activity reduces lipid peroxidation of cell membranes and the risk of cell death [15]. Vitamin C has a protective effect against neuronal damage by inhibiting microglial activation and proinflammatory cytokine release in the brain [16, 17].

Brain Natriuretic Peptide (BNP) is a 32–amino acid hormone that was first isolated from the brain (pig) but is mostly secreted from the ventricles of the heart [18, 19]. Plasma or serum concentrations of BNP and its inactive form, N–terminal pro–B–type natriuretic peptide (NT–proBNP), are used clinically as biomarkers for the diagnosis of cardiac function and heart diseases [20, 21]. BNP release from astrocytes in the brain is increased, especially in hypoxia and stress states [22].

Hypoxia–inducible factor–1 alpha (HIF–1α) is a transcription factor that is a key regulator of hypoxia–induced gene expression [23, 24]. It is expressed at high levels in response to decreased oxygen levels in organs [25]. Genes regulated by HIF–1α generally include genes involved in oxygen homeostasis and glucose–energy metabolism [26]. HIF–1α activation provides protection against ischemic injury [27]. It plays a key role in many aspects, such as vascular structure, production of blood elements, proliferation and migration of epithelial cells, erythropoietin synthesis in the kidney, glucose metabolism, cellular immunity, inflammation, cancer, autophagy, apoptosis, and epigenetic regulation [25, 28, 29].

Heat shock proteins (HSPs) are molecular chaperones with a molecular weight of less than 100 kDa that are involved in the proper folding of proteins. The production of HSPs increases in cells exposed to temperatures of 42–44°C [30, 31]. In addition to heat, HSP increases are caused by factors such as hypoxia, infection, inflammation, toxins such as ethanol, heavy metals, trace metals, and UV light, as well as starvation. For this reason, HSPs are also called "stress proteins." They exhibit cytoprotective properties and play a role in neurodegenerative disorders (multiple sclerosis, Alzheimer's disease, etc.) and cancer. Furthermore, HSPs have been reported to exhibit neuroprotective effects in a wide variety of brain injuries, including ischemia and haemorrhage [32].

Glycerol is a safe agent frequently used to induce rhabdomyolysis via intramuscular injection [33]. In the glycerol–induced CS model, both macroscopic and microscopic local changes such as necrosis, edema, inflammatory reactions, and fibrosis in muscle fibers have been reported, as have systemic cytokine release and increased oxidative stress markers due to the release of many substances into the circulation [34, 35, 36]. The extent to which systemic changes in CS affect the brain remains largely unclear. This study aimed to investigate the neuroprotective effects of vitamin C and tadalafil using histopathological and real–time polymerase chain reaction (PCR) in an experimental CS model induced by intramuscular glycerol injection in rats. It is expected that the findings of the research will provide evidence of the potential central effects of CS. In this way, by contributing to a better understanding of this clinical condition.

MATERIALS AND METHODS

Animals and experimental design

The study was approved by the Selçuk University Faculty of Veterinary Medicine, Experimental Animal Production and Research Center Ethics Committee (SÜVDAMEK) with decision number 181 dated 29.07.2024. In the study, 50 female healthy adult Wistar Albino rats (*Rattus norvegicus*) weighing 250–300 g and 10 weeks old, obtained from Selçuk University Experimental Medicine Research and Application Center, were used. The experiment was terminated after 6 d. Experimental protocols were conducted in accordance with the European Economic Community's animal welfare directives (86/609/EEC and 2010/63/EU).

During the experiment, the rats were housed in cages with a 12-h day (d) and 12–h nite light cycle, a room temperature of 22 ± 2°C, and a humidity of 50 ± 1%. Animals were divided into 7 groups, designated as: healthy control (n: 6, 10 mg·kg^{-1} physiological saline, first d, a single dose, intramuscular (i.m.)), Vitamin C (Vit C, n: 6, 20 mg·kg^{-1} of Vit C at 24, 72 and 120th h, gavage), Tadalafil (T, n: 6, 10 mg·kg^{-1} of Tadalafil at 24, 72 and 120th h, gavage), Glycerol administered group (G, n:8, 10 mg·kg^{-1}·first^{-1} d, a single dose of 5% hypertonic glycerol, equal amounts to both hind legs, i.m.), G

+ Vit C (n: 8, 10 mg·kg^{-1}·first^{-1} d, a single dose of 5% hypertonic glycerol, equal amounts to both hind legs, i.m. + 20 mg·kg^{-1} Vit C at 24, 72 and 120th h, gavage), G + T (n: 8, 10 mg·kg^{-1}·first^{-1} d, a single dose of 5% hypertonic glycerol, equal amounts to both hind legs, i.m. + 10 mg·kg^{-1} Tadalafil at 24, 72 and 120th h, gavage) and G + Vit C+ T (n: 8, 10 mg·kg^{-1}·first^{-1} d, a single dose of 5% hypertonic glycerol, equal amounts to both hind legs, i.m. + 20 mg·kg^{-1} Vit C and 10 mg·kg^{-1} Tadalafil at 24, 72 and 120th h, gavage). Animals were deprived of water 24 h before the start of the experiment. Throughout the trial, rats were fed regular food and water to drink. Euthanasia was carried out under general anesthesia at the conclusion of the experiment, and the brain tissues that had been dissected using the necropsy procedure.

Histopathological analysis

Brain tissues obtained from necropsied animals were preserved in 1% formaldehyde solution for 24 h. They were then sliced to proper sizes, placed in tissue processing cassettes. The formaldehyde solution was then removed by washing in running water for 12 h. Then, using a routine tissue processing procedure (Leica, TP1020, Germany), the tissues were embedded in paraffin blocks, and 5 μm thick sections were cut from the blocks using a microtome (Leica, RM2125, Germany) and stained with Hematoxylin and eosin (H&E). Brain sections were examined under a light microscope (Olympus BX51, Tokyo, Japan) and scored for findings of neuronal necrosis, neuronophagia, gliosis, edema, congestion, endothelial cell damage, and mononuclear cell infiltration (MCI) (0: none, 1: mild, 2: moderate, 3: severe).

Real Time PCR analysis

Formalin–fixed brain tissues were washed in running tap water for 72 h to remove the fixation solution. Total RNA isolation was performed using the SanPrep Column microRNA Miniprep Kit (Cat: SK8811, BIO BASIC) according to the manufacturer's guidelines. cDNA synthesis was performed with the High–Capacity cDNA Reverse Transcription Kit (Cat: 4368813, Thermo Fisher Scientific). mRNA levels of BNP (F:5'–CACCTCTCAAGTGATCCTGTTT–3', R:5'–GCAAGTTTGTGCTGGAAGATAAG–3'), HIF–1α (F:5'–GATGGAATGGAGCAGAAGACA–3', R:5'–TAACTGGTCAGCTGTGGTAATC–3'), HSP70 (F:5'–GGTCTCAAGGGCAAGATCAG–3', R:5'–TTTCTCAGCCAGCGTGTTAG–3'), genes were analyzed by Real–Time PCR device (LightCycler® 96 System, Roche, Switzerland) using the LightCycler 480 SYBR Green Master Mix Kit (Cat: 04707516001, Roche). The PCR protocol was as follows: Following an initial denaturation of 10 min at 95°C, the procedure comprised 40 cycles, each consisting of 10 s of denaturation at 95°C, 10 s of annealing at 58°C, and 10 s of extension at 72°C. The Ct values acquired were standardized to the Ct values established for GAPDH in the same tissue and quantified using 2^–ΔΔCt.method.

Statistical analysis

IBM SPSS Statistics 22 and GraphPad Prism were used to compare the outcomes of histopathological and Real–Time PCR analyses. The Shapiro–Wilk test was applied to determine the normal distribution, whereas the Levene test was utilized to evaluate homogeneity. One–Way ANOVA and post–hoc Duncan testing were performed on data identified to be normally distributed [37]. Results were expressed as mean ± standard error of the mean, with a *P* value less than 0.05 being statistically significant.

RESULTS AND DISCUSSION

Histopathological results

For each case, neuronal necrosis, neuronophagia, gliosis, edema, congestion, endothelial cell damage, and MCI were evaluated, and lesion scores were determined (TABLE I). Mild neuronal necrosis, neuronophagia, gliosis, edema, congestion, endothelial cell damage and MCI were observed in the healthy control group. No substantial differences were identified in other findings except edema in the Vit C and T groups compared to the healthy control group (FIG. 1B, 1C). With glycerol administered group, neuronal necrosis, neuronophagia, gliosis, edema, congestion, endothelial cell swelling, and MCI findings were markedly elevated in comparison to the healthy control group (P<0.05) (FIG. 1D, 1E). All findings were noted to be milder in the G + Vit C and G + T groups compared to the G group. Compared to the Vit C and T treatment groups, the combined treatment group (G + Vit C + T) showed statistically closer results to the healthy control group (FIG. 1F, 1G, 1H).

TABLE I
Statistical analysis of histopathological scores of Vitamin C, Tadalafil, and combined administration of both in a glycerol–induced brain injury model

Groups	Neuronal necrosis/ apoptosis	Neurono–phagia	Gliosis	Edema	Congestion	Endothelial cell swelling	MCI
Control	0.58 ± 0.20^{a}	0.67 ± 0.11^{a}	0.66 ± 0.17^{ab}	0.16 ± 0.10^{a}	0.41 ± 0.20^{a}	0.08 ± 0.08^{a}	0.41 ± 0.15^{a}
Vit C	0.66 ± 0.10^{a}	0.58 ± 0.15^{a}	0.41 ± 0.15^{a}	0.91 ± 0.15^{b}	0.92 ± 0.15^{ab}	0.16 ± 0.10^{a}	0.41 ± 0.15^{a}
T	0.75 ± 0.11^{a}	0.75 ± 0.11^{a}	0.58 ± 0.15^{ab}	0.91 ± 0.15^{b}	0.91 ± 0.30^{ab}	0.33 ± 0.11^{a}	0.50 ± 0.13^{a}
G	3.00 ± 0.31^{c}	2.66 ± 0.17^{c}	2.92 ± 0.42^{d}	1.33 ± 0.11^{b}	2.33 ± 0.16^{d}	2.00 ± 0.22^{c}	1.91 ± 0.24^{c}
G + Vit C	1.91 ± 0.30^{b}	2.00 ± 0.18^{b}	2.16 ± 0.21^{c}	1.25 ± 0.21^{b}	1.75 ± 0.17^{c}	1.25 ± 0.21^{b}	1.41 ± 0.15^{b}
G + T	1.83 ± 0.25^{b}	2.16 ± 0.24^{b}	2.08 ± 0.30^{c}	1.41 ± 0.15^{b}	1.67 ± 0.11^{c}	1.00 ± 0.18^{b}	1.33 ± 0.11^{b}
G + Vit C + T	1.41 ± 0.20^{b}	1.08 ± 0.15^{a}	1.33 ± 0.25^{b}	1.00 ± 0.18^{b}	1.00 ± 0.13^{b}	0.83 ± 0.11^{b}	0.83 ± 0.17^{a}

* ^{a,b,c,d} Different superscript letters in the same column indicate significant statistically differences between groups according to one–way ANOVA followed by Duncan's post hoc test (P<0.05). Values are shown as mean ± standard error of the mean (SEM). Vit C: Vitamin C, G: Glycerol administered group, T: Tadalafil, MCI: Mononuclear cell infiltration

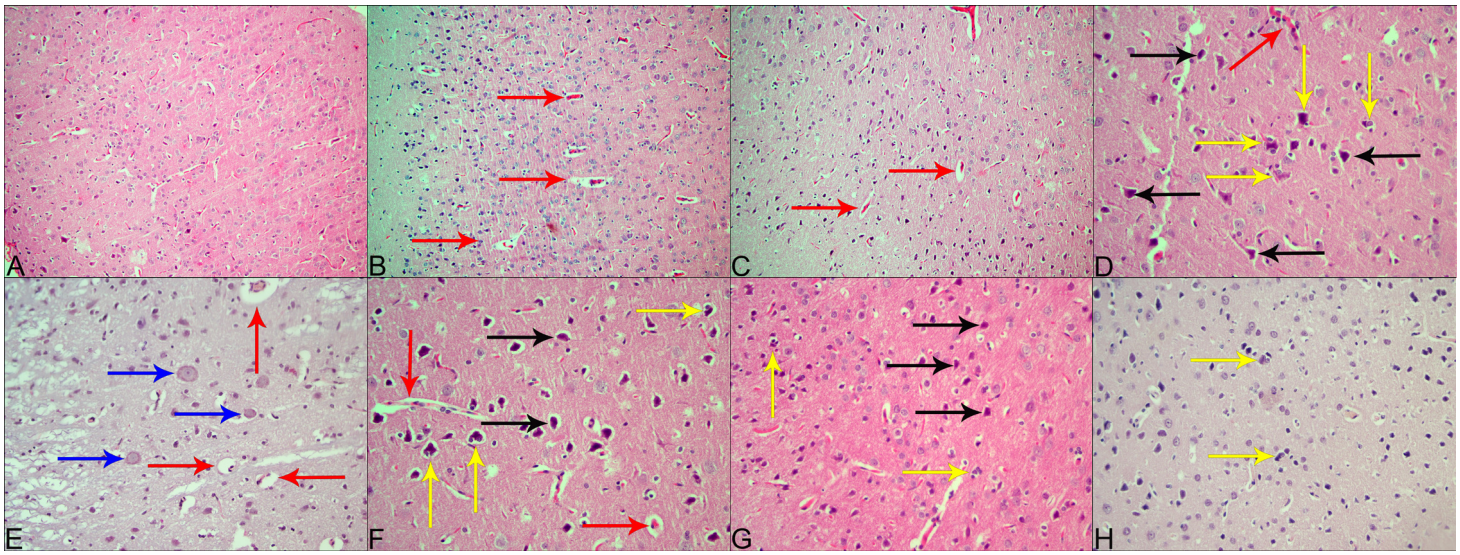

FIGURE 1. Histopathological images of sections from the cerebral cortex of rats, H&E. A: Healthy control group, general view of cerebral cortex, 20x. B: Vit C group, 20x. C: Tadalafil group, 20×. D–E: Glycerol administered group, 40×. F: Glycerol + Vit C group, 40×. G: Glycerol + Tadalafil group, 40×. H: Glycerol + Vit C + Tadalafil group, 40×. Congestion in vessels and perivascular edema (red arrows), necrotic/apoptotic neurons (black arrows), neuronophagia (yellow arrows) and central chromatolysis in neurons (blue arrows)

In the glycerol–induced CS model, there are studies in the literature on acute kidney failure [38, 39, 40]. While there are studies examining the physiological and biochemical changes that occur in brain tissue in glycerol–induced CS, there is limited data in the literature on how they are affected at the histopathological and molecular levels.

Myoglobin is thought to be the most toxic substance released into the bloodstream after rhabdomyolysis. When myoglobin breaks down in the renal tubules, the iron ion within it is released. Free iron undergoes the Fenton reaction, leading to the formation of reactive oxygen species (ROS). Increased ROS are not limited to renal tissue; they can also affect other organs via the bloodstream, resulting in lipid peroxidation, DNA damage, and cell death [41, 42]. The neuronal necrosis/apoptosis observed in the glycerol group in our study was thought to be due to increased ROS resulting from rhabdomyolysis. Myoglobin and other toxic substances act as danger–associated molecular patterns and activate immune cells. Following this activation, various proinflammatory cytokines and chemokines are released, activating microglia cells, resulting in sterile inflammation. Necrotic/apoptotic neurons are phagocytosed by activated microglial cells [43, 44, 45]. In the current study, mononuclear cell infiltration, gliosis, and neuronophagia in necrotic/apoptotic neurons were detected in the glycerol–treated group. Sodium imbalances (hyponatremia) resulting from rhabdomyolysis may trigger brain edema. Inflammation caused by substances entering the systemic circulation causes endothelial cell damage [46, 47, 48].

This study demonstrates that substances entering the systemic circulation because of rhabdomyolysis, either directly or through microglial activation, increase capillary permeability, leading to edema and endothelial cell swelling.

Information currently available indicates that Vit. C is a crucial neuroprotective agent and a part of the brain's intracellular antioxidant system [49]. Vit. C reduces oxidative stress and prevents ROS–induced cell damage. Thanks to its anti–inflammatory activity, it maintains energy balance in neurones, preventing apoptosis and neuronal necrosis by reducing pro–inflammatory cytokine levels and microglial activation [16, 17, 50, 51].

Tadalafil exerts its protective activity by increasing intracellular cyclic GMP (cGMP) levels and activating nitric oxide signaling pathways. Increased cGMP levels cause vasodilation, improving blood flow and protecting endothelial cell functions. Tadalafil enhances antioxidant capacity by increasing the activity of antioxidant enzymes and helps reduce oxidative stress. It reduces proinflammatory cytokine release, suppresses microglial activation, and prevents apoptosis [11, 12, 52].

In the current study, Vit. C and tadalafil are thought to have a neuroprotective effect by reducing ROS accumulation and increasing the release of antioxidant genes and enzymes, resulting in reductions in neuronal necrosis/apoptosis, neuronophagia, gliois, edema, congestion, endothelial cell swelling, and MCI rates.

Real–Time Polymerase Chain Reaction results

The effects of gene expressions in brain tissues are shown in FIG. 2. These findings indicate that glycerol administration increased BNP and HSP70 mRNA transcript levels in the cerebral cortex compared to the control group ($P<0.05$), while no significant change was observed in HIF–1α levels ($P>0.05$). Glycerol + Vit C administration slightly decreased BNP and HSP70 expression levels compared to the glycerol group. G + T administration significantly decreased BNP expression and slightly decreased HSP70 expression compared to the G group. Both Glycerol + Vit C and G + T administrations increased HIF–1α expression compared to the G group, but not to a statistically significant extent.

The combined administration group (G + Vit C+T) showed decreased expression levels of all genes compared to the G group, and the expression levels were found to be similar to those in the healthy control group.

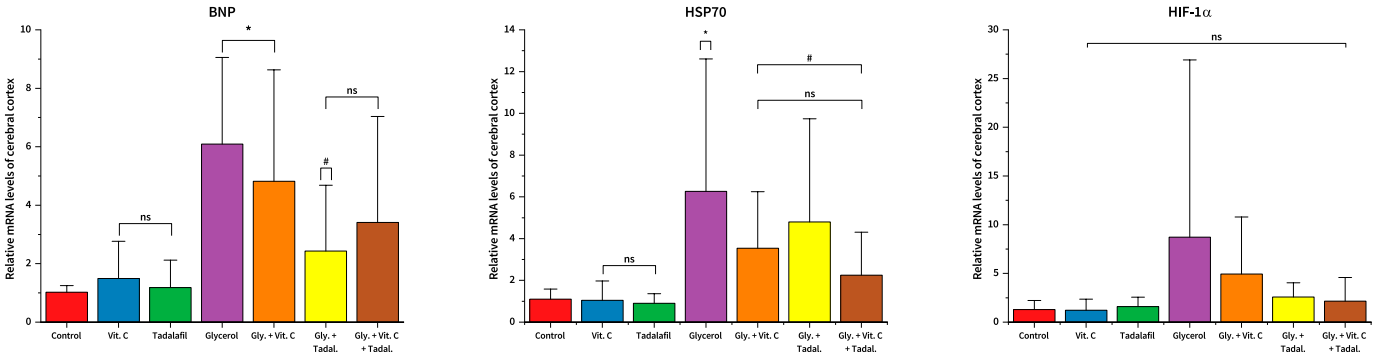

FIGURE 2. Cerebral cortex BNP, HSP70 and HIF–1α alpha levels. Vit. C: Vitamin C, Tadal: Tadalafil, Gly: Glycerol. '*' indicates statistically significant difference compared to the control group (*P*<0.05), '#' indicates statistically significant difference compared to the Glycerol group (*P*<0.05), 'ns' indicates no statistically significant difference compared to the control group (*P*>0.05)

Furthermore, according to the study results, glycerol administration caused a significant increase in BNP and HSP70 mRNA transcript levels in brain tissue. BNP is a hormone synthesized primarily by cardiac ventricular cells with potent vasodilator effects, natriuretic, and diuretic effects [20]. BNP levels increase in parallel with increased oxidative stress in heart diseases. This increase in BNP levels is a protective mechanism aimed at minimizing the harmful effects of mitochondrial oxidative stress in the early stages of heart diseases [53, 54]. In this study, the increase in BNP expression was thought to be an adaptive response to prevent the harmful effects of oxidative stress and mitochondrial dysfunction induced by G administration.

HSP70 has been reported to suppress oxidative stress–induced lipid peroxidation and apoptosis, synthesize antioxidant enzymes, and protect cells from tumor necrosis factor–induced death by inhibiting interleukin–6 and nitric oxide production [55, 56]. In this study, the increase in HSP70 expression was interpreted as protecting brain tissue against oxidative stress. After rhabdomyolysis was induced with glycerol, decreased expression levels of BNP and HSP70 were observed following the combined use of vitamin C, T, and both. This was attributed to the neuroprotective effects of both due to their antioxidant and anti–inflammatory properties.

HIF–1α plays a role in cellular response, angiogenesis, inflammation, and target gene expression in hypoxic environments [25, 28, 29]. This finding showed no significant difference in HIF–1α expression levels between the groups (*P*>0.05). This was interpreted as indicating that intramuscular G administration caused local hypoxia and did not have a systemic effect, and therefore, there was no significant difference in HIF–1α expression levels in the brain.

CONCLUSION

A crucial approach to decreasing mortality rates among patients extricated from debris during disasters, including earthquakes, mining incidents, and natural calamities, is to be informed about CS treatment and protective strategies, as well as to undertake research that will aid in the advancement of novel therapeutic techniques. This research is the inaugural examination of the neuroprotective properties of Vit. C and Tadalafil in an experimental CS model caused by intramuscular G injection in rats, employing histopathological and Real–Time PCR techniques. According to the study results, tissue damage in the experimental CS model was reduced with Vitamin C, Tadalafil, and their combined application, and consequently, histopathological changes were also reduced. Additionally, Vitamin C and Tadalafil treatment reduced BNP and HSP70 expression, suggesting that Tadalafil may protect against oxidative and inflammatory stress like Vitamin C.

Funding

This study was supported by Selcuk University Scientific Research Coordination (BAP) with project number 25401021.

Conflict of interest

The authors declared that they have no conflict of interest.

BIBLIOGRAPHIC REFERENCES

[1] Desai SN, Desai PV. Aspartate aminotransferase and alanine aminotransferase activities of rat brain during crush syndrome. Neurosci. Lett. [Internet]. 2008; 447(1):58–61. doi: https://doi.org/ckfpdq

[2] Cohen BH, Gaspar MP, Daniels AH, Akelman E, Kane PM. Multifocal neuropathy: expanding the scope of double crush syndrome. J. Hand Surg. [Internet]. 2016; 41(12):1171–1175. doi: https://doi.org/f9dmc3

[3] Desai Shanti N, Desai PV. The study of Na^+, K^+–ATPase activity of rat brain during Crush syndrome. Neurochem. Res. [Internet]. 2007; 32(11):1843–1848. doi: https://doi.org/fhbjvs

[4] Martinez T, Laemmel E, Bergis B, Rustin M, Huertas A, Harrois A, Libert N. Beyond acute kidney injury, traumatic rhabdomyolysis associated to hemorrhagic shock is responsible for multiple–organ failure in a rat model. Shock 2025; 2025:10.1097. doi: https://doi.org/qk5v

[5] Özkıdık M, Gökce Mİ, Yaman Ö. Efficacy of tadalafil treatment on erectile dysfunction in patients under dutasteride treatment: A prospective non–randomized comparative study. Turk. J. Urol. [Internet]. 2018; 44(4):294–297. doi: https://doi.org/qk5w

[6] Coward RM, Carson CC. Tadalafil in the treatment of erectile dysfunction. Ther. Clin. Risk Manag. [Internet]. 2008; 4(6):1315–1329. doi: https://doi.org/g78kfq

[7] Nilsson D, Chess–Williams R, Sellers D. Phosphodiesterase-5 inhibitors tadalafil and sildenafil potentiate nitrergic–nerve mediated relaxations in the bladder vasculature. Eur. J. Pharmacol. [Internet]. 2023; 960:176152. doi: https://doi.org/qk5z

[8] Şahin Ç, Yıldırım N, Hortu İ, Akdemir A, Özşener S, Yiğittürk G, Erbaş O. Tadalafil attenuates ischemic damage as well as reperfusion injury in the rat ovary. J. Turk. Ger. Gynecol. Assoc. [Internet]. 2020; 21(1):35–40. doi: https://doi.org/gm9nrv

[9] Seftel AD. Phosphodiesterase type 5 inhibitor differentiation based on selectivity, pharmacokinetic, and efficacy profiles. Clin. Cardiol. [Internet]. 2004; 27(S1):14–19. doi: https://doi.org/fqmkvr

[10] Porst H, Padma–Nathan H, Giuliano F, Anglin G, Varanese L, Rosen R. Efficacy of tadalafil for the treatment of erectile dysfunction at 24 and 36 hours after dosing: a randomized controlled trial. Urology [Internet]. 2003; 62(1):121–125. doi: https://doi.org/c6x22p

[11] Said R, Mahmoud O, Mohammed A, Abd El Maksoud H. Biochemical role of Tadalafil in experimental brain impairment activities in mice. Benha Vet. Med. J. [Internet]. 2024; 46(1):50–53. doi: https://doi.org/qk52

[12] Ko IG, Shin MS, Kim BK, Kim SE, Sung YH, Kim TS, Shin MC, Cho HJ, Kim SC, Kim SH, Kim KH, Shin DH, Kim CJ. Tadalafil improves short–term memory by suppressing ischemia–induced apoptosis of hippocampal neuronal cells in gerbils. Pharmacol. Biochem. Behav. [Internet]. 2009; 91(4):629–635. doi: https://doi.org/cwkn22

[13] Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee JH, Chen S, Corpe C, Dutta A, Dutta SK, Levine M. Vitamin C as an antioxidant: evaluation of its role in disease prevention. J. Am. Coll. Nutr. [Internet]. 2003; 22(1):18–35. doi: https://doi.org/ggrks6

[14] Kaźmierczak–Barańska J, Boguszewska K, Adamus–Grabicka A, Karwowski BT. Two faces of vitamin C–antioxidative and pro–oxidative agent. Nutrients [Internet]. 2020; 12(5):1501. doi: https://doi.org/gmw3bp

[15] Lúcio M, Nunes C, Gaspar D, Ferreira H, Lima JL, Reis S. Antioxidant activity of vitamin E and Trolox: understanding of the factors that govern lipid peroxidation studies in vitro. Food Biophys. [Internet]. 2009; 4(4):312–320. doi: https://doi.org/dzwfms

[16] Kangisser L, Tan E, Bellomo R, Deane AM, Plummer MP. Neuroprotective properties of vitamin C: a scoping review of pre–clinical and clinical studies. J. Neurotrauma [Internet]. 2021; 38(16):2194–2205. doi: https://doi.org/qk53

[17] Ahmad A, Shah SA, Badshah H, Kim M J, Ali T, Yoon GH, H Kim T, B Abid N, Rehman SU, Khan S, O Kim M. Neuroprotection by vitamin C against ethanol–induced neuroinflammation associated neurodegeneration in developing rat brain. CNS. Neurol. Disord. Drug Targets [Internet]. 2016; 15(3):360–370. doi: https://doi.org/f8g2bz

[18] Sudoh T, Kangawa K, Minamino N, Matsuo H. A new natriuretic peptide in porcine brain. Nature [Internet]. 1988; 332:78–81. doi: https://doi.org/b4d9pk

[19] Hosoda K, Nakao K, Mukoyama M, Saito Y, Jougasaki M, Shirakami G, Suga G, Ogawa Y, Yasue H, Imura H. Expression of brain natriuretic peptide gene in human heart. Production in the ventricle. Hypertension [Internet]. 1991; 17(6_pt_2):1152–1155. doi: https://doi.org/qk54

[20] Alcidi G, Goffredo G, Correale M, Brunetti ND, Iacoviello M. Brain natriuretic peptide biomarkers in current clinical and therapeutic scenarios of heart failure. J. Clin. Med. [Internet]. 2022;11(11):3192. doi: https://doi.org/g83qkw

[21] Harpaz D, Seet RC, Marks RS, Tok AI. B–type natriuretic peptide as a significant brain biomarker for stroke triaging using a bedside point–of–care monitoring biosensor. Biosensors [Internet]. 2020; 10(9):107. doi: https://doi.org/qk6h

[22] Katoh C, Osanai T, Tomita H, Okumura K. Brain natriuretic peptide is released from human astrocytoma cell line U373MG under hypoxia: a possible role in anti–apoptosis. J. Endocrinol. [Internet]. 2011; 208(1):51–57. doi: https://doi.org/bz44g9

[23] Semenza GL, Agani F, Booth G, Forsythe J, Iyer N, Jiang BH, Leung S, Roe R, Wiener C, Yu A. Structural and functional analysis of hypoxia–inducible factor 1. Kidney Int. [Internet]. 1997; 51(2):553–555. doi: https://doi.org/bghh74

[24] Cramer T, Johnson RS. A novel role for the hypoxia inducible transcription factor HIF-1alpha: critical regulation of inflammatory cell function. Cell cycle [Internet]. 2003; 2(3):191–192. doi: https://doi.org/btj42h

[25] Adams J, Difazio L, Rolandelli R, Luján j, Haskó Gy, Csóka B, Selmeczy Zs, Németh Z. HIF–1: a key mediator in hypoxia (Review). Acta Physiol. Hung. [Internet]. 2009; 96(1):19–28. doi: https://doi.org/fv5g7f

[26] Schumacker PT. Hypoxia–inducible factor–1 (HIF–1). Crit. Care Med. [Internet]. 2005; 33(12):S423-S425. doi: https://doi.org/ffg57v

[27] Howell NJ, Tennant DA. The role of HIFs in ischemia–reperfusion injury. Hypoxia [Internet]. 2014; 2:107–115. doi: https://doi.org/qk6k

[28] Fulda S, Debatin K–M. HIF-1-regulated glucose metabolism: a key to apoptosis resistance? Cell cycle [Internet]. 2007; 6(7):790–792. doi: https://doi.org/fn3285

[29] Balamurugan K. HIF–1 at the crossroads of hypoxia, inflammation, and cancer. Int. J. Cancer [Internet]. 2016; 138(5):1058–1066. doi: https://doi.org/ggwwk2

[30] Hagymasi AT, Dempsey JP, Srivastava PK. Heat–shock proteins. Curr. Protoc. [Internet]. 2022; 2(11):e592. doi: https://doi.org/qk6m

[31] Janowska MK, Baughman HE, Woods CN, Klevit RE. Mechanisms of small heat shock proteins. Cold Spring Harb. Perspect. Biol. [Internet]. 2019; 11(10):a034025. doi: https://doi.org/qk6n

[32] Stetler RA, Gan Y, Zhang W, Liou AK, Gao Y, Cao G, Chen J. Heat shock proteins: cellular and molecular mechanisms in the central nervous system. Prog. Neurobiol. [Internet]. 2010; 92(2):184–211. doi: https://doi.org/cngkw5

[33] Liu Y, Yu M, Chen L, Liu J, Li X, Zhang C, Xiang X, Li X, Lv Q. Systemic review of animal models used in the study of crush syndrome. Shock [Internet]. 2022; 57(4):469–478. doi: https://doi.org/g5pq9x

[34] Lv Q, Long M, Wang X, Shi J, Wang P, Guo X, Song J, Midgley AC, Fan H, Hou S. The role of alpha–1–acid glycoprotein in the diagnosis and treatment of crush syndrome–induced acute kidney injury. Shock [Internet]. 2021; 56(6):1028–1039. doi: https://doi.org/qk6q

[35] Karamalakova YD, Georgieva ED, Petkova–Parlapanska KV, Slavova VB, Ivanova KV, Ivanov VA, Nikolova GD. *Silybum marianum* reduces acute kidneys injury by modifying biochemical changes and oxidative stress levels in glycerol–induced Crush Syndrome. Bulg. Chem. Commun. [Internet]. 2024; 56(D2):109–115. doi: https://doi.org/qk6r

[36] Koç A. Crush syndrome: a review of current knowledge and treatment strategies. J. Compr. Surg. [Internet]. 2023; 1(1):7–10. doi: https://doi.org/qk6s

[37] Selvi MH. The use of statistics in veterinary sciences and the test methods used. Res. Pract. Vet. Anim. Sci. [Internet]. 2024; 1(1):43–50. doi: https://doi.org/n4v9

[38] Gu H, Yang M, Zhao X, Zhao B, Sun X, Gao X. Pretreatment with hydrogen–rich saline reduces the damage caused by glycerol–induced rhabdomyolysis and acute kidney injury in rats. J. Surg. Res. [Internet]. 2014; 188(1):243–249. doi: https://doi.org/f5w768

[39] Nara A, Yajima D, Nagasawa S, Abe H, Hoshioka Y, Iwase H. Evaluations of lipid peroxidation and inflammation in short–term glycerol–induced acute kidney injury in rats. Clin. Exp. Pharmacol. Physiol. [Internet]. 2016; 43(11):1080–1086. doi: https://doi.org/f9cgbh

[40] Singh AP, Singh AJ, Singh N. Pharmacological investigations of Punica granatum in glycerol–induced acute renal failure in rats. Indian J. Pharmacol. [Internet]. 2011; 43(5):551–556. doi: https://doi.org/dchmpm

[41] Aydogdu N, Atmaca G, Yalcin O, Taskiran R, Tastekin E, Kaymak K. Protective effects of l–carnitine on myoglobinuric acute renal failure in rats. Clin. Exp. Pharmacol. Physiol. [Internet]. 2006; 33(1–2):119–124. doi: https://doi.org/d2znfh

[42] Zager RA. Rhabdomyolysis and myohemoglobinuric acute renal failure. Kidney Int. [Internet]. 1996; 49(2):314–326. doi: https://doi.org/d2hnht

[43] Gong T, Liu L, Jiang W, Zhou R. DAMP–sensing receptors in sterile inflammation and inflammatory diseases. Nat. Rev. Immunol. [Internet]. 2020; 20(2):95–112. doi: https://doi.org/gg264m

[44] Butler CA, Popescu AS, Kitchener EJ, Allendorf DH, Puigdellívol M, Brown GC. Microglial phagocytosis of neurons in neurodegeneration, and its regulation. J. Neurochem. [Internet]. 2021; 158(3):621–639. doi: https://doi.org/gh4h4f

[45] Yu F, Wang Y, Stetler AR, Leak RK, Hu X, Chen J. Phagocytic microglia and macrophages in brain injury and repair. CNS. Neurosci. Ther. [Internet]. 2022; 28(9):1279–1293. doi: https://doi.org/grshnb

[46] Perkins G, Valberg SJ, Madigan JM, Carlson GP, Jones SL. Electrolyte disturbances in foals with severe rhabdomyolysis. J. Vet. Intern. Med. [Internet]. 1998; 12(3):173–177. doi: https://doi.org/btdjbd

[47] Guerrero–Hue M, García–Caballero C, Palomino–Antolín A, Rubio–Navarro A, Vázquez–Carballo C, Herencia C, Martín–Sanchez D, Farré–Alins V, Egea J, Cannata P, Praga M, Ortiz A, Egido J, Sanz AB, Moreno JA. Curcumin reduces renal damage associated with rhabdomyolysis by decreasing ferroptosis–mediated cell death. FASEB J. [Internet]. 2019; 33(8):8961–8975. doi: https://doi.org/ghc46h

[48] Safari S, Aghili SH, Shahlaee MA, Kerachi AJ, Ranjbar MF. Incidence of electrolyte imbalances following traumatic rhabdomyolysis: A systematic review and meta–analysis. Cureus [Internet]. 2024; 16(4):e59333. doi: https://doi.org/g8zrrq

[49] Sánchez–Moreno C, Paniagua M, Madrid A, Martín A. Protective effect of vitamin C against the ethanol mediated toxic effects on human brain glial cells. J. Nutr. Biochem. [Internet]. 2003; 14(10):606–613. doi: https://doi.org/fcjwjs

[50] Kumar RR, Singh L, Thakur A, Singh S, Kumar B. Role of vitamins in neurodegenerative diseases: a review. CNS. Neurol. Disord. Drug Targets [Internet]. 2022; 21(9):766–773. doi: https://doi.org/g9q6st

[51] Yeşilyurt M, Yüksel S. Basınç yaralanmalı hastaların tedavisinde beslenmenin etkisi [The effect of nutrition in the treatment of pressure injury patients]. Genel. Sağlık Bilim. Derg. [J. Gen. Health Sci]. [Internet]. 2020; 2(3):200–207. Turkish. doi: https://doi.org/qk6t

[52] Gulati P, Singh N. Tadalafil enhances the neuroprotective effects of ischemic postconditioning in mice, probably in a nitric oxide associated manner. Canadian J. Physiol. Pharmacol. [Internet]. 2014; 92(5):418–426. https://doi.org/f524nq

[53] Okada A, Kashima Y, Tomita T, Takeuchi T, Aizawa K, Takahashi M, Ikeda U. Characterization of cardiac oxidative stress levels in patients with atrial fibrillation. Heart Vessels [Internet]. 2016; 31(1):80–87. doi: https://doi.org/f75b49

[54] Chang P, Zhang X, Zhang J, Wang J, Wang X, Li M, Wang R, Yu J, Fu F. BNP protects against diabetic cardiomyopathy by promoting Opa1-mediated mitochondrial fusion via activating the PKG–STAT3 pathway. Redox Biol. [Internet]. 2023; 62:102702. doi: https://doi.org/qk6v

[55] Van–Molle W, Wielockx B, Mahieu T, Takada M, Taniguchi T, Sekikawa K, Libert C. HSP70 protects against TNF–induced lethal inflammatory shock. Immunity [Internet]. 2002; 16(5):685–695. doi: https://doi.org/ccq65s

[56] Belenichev IF, Aliyeva OG, Popazova OO, Bukhtiyarova NV. Involvement of heat shock proteins HSP70 in the mechanisms of endogenous neuroprotection: The prospect of using HSP70 modulators. Front. Cell. Neurosci. [Internet]. 2023; 17:1131683. doi: https://doi.org/qk6w