NEAR EAST UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF PHARMACOLOGY

PROTECTIVE EFFECTS OF MESNA ON CAROTID ARTERY ISCHEMIA/REPERFUSION IN RATS

Ph.D. THESIS

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January, 2023

APPROVAL

We certify that we have read the dissertation submitted by **Merve Mercan** titled **'Protective Effects of MESNA on Carotid Artery Ischemia/Reperfusion in Rats'** and that in our combined opinion, it is fully adequate in scope and quality, as a dissertation for the award of the doctorate degree.

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DECLARATION

I hereby declare that this dissertation is my own study, that I have not engaged in any unethical behavior at any stage from the planning of the study to its writing, that I have obtained all information contained in this dissertation according to academic and ethical rules, that I have cited and included in the reference list all information and comments that could not be obtained through this dissertation, and that I have not engaged in any behavior that violates patent rights and copyrights during the study and writing of this dissertation.

MPharm. Merve Mercan

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ABSTRACT

Protective Effects of MESNA Against Carotid Artery Ischemia/Reperfusion in Rats

Mercan, Merve

Ph.D., Department of Pharmacology

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Background

Carotid ischemia/reperfusion is one of the leading causes of adult disability and death worldwide.

Objectives

The purpose of this study was to investigate whether 2-mercaptoethanesulfonate (MESNA) has a protective effect against tissue damage (brain, heart and lungs) in rats exposed to experimental carotid ischemia/reperfusion.

Material and Methods

To induce ischemia in rats, the external and internal carotid arteries were ligated with silk sutures for 10 minutes. A 1 hour reperfusion was then applied. 75 mg/kg and 150 mg/kg of MESNA were administered intraperitoneally half an hour before and immediately after the ischemia. At the end of the experiments, the levels of ALP, AST, ALT, LDH, TNF-α, IL-1β, MDA, MMP (MMP-1, -2, -8) and TIMP-1 were measured in samples of serum in the biochemical analysis. Histopathological scores were measured in the brain, lung and heart tissue collected from the subjects.

Results

The increased biochemical parameters after carotid ischemia/reperfusion were suppressed by the application of MESNA. Specifically, with increasing MESNA dose, TNF-α, ALP, and TIMP-1 parameters were suppressed twice as much with 150 mg/kg treatment as with 75 mg/kg treatment. In the results of examinations of tissues taken from the brain, lungs and heart, MESNA treatment showed significant changes in the degeneration of all tissues.

Conclusion

The results we obtained from this study indicate that MESNA has a protective effect on carotid artery ischemia/reperfusion. These results we have obtained will pave the way for the clinical application of MESNA and should be included in the literature.

Keywords: Ischemia, Reperfusion, MESNA, Oxidative stress

ÖZET

MESNA'nın Sıçanlardaki Karotid Arter İskemi/Reperfüzyonuna Karşı Koruyucu Etkileri

Mercan, Merve

Doktora, Farmakoloji Bölümü

Ocak, 2023

Arka Plan

Karotis iskemisi/reperfüzyonu, dünya çapında erişkin sakatlığının ve ölümün önde gelen nedenlerinden biridir.

Hedefler

Bu çalışmanın amacı, deneysel karotis iskemi/reperfüzyon uygulanan sıçanlarda doku hasarına (beyin, kalp ve akciğer) karşı 2-merkaptoetansülfonatın (MESNA) koruyucu etkisinin olup olmadığını araştırmaktı.

Materyal ve Metodlar

Sıçanlarda iskemiyi indüklemek için, dış ve iç karotid arterler 10 dakika süreyle ipek sütürlerle bağlandı. Daha sonra 1 saatlik reperfüzyon uygulandı. İskemiden yarım saat önce ve hemen sonra 75 mg/kg ve 150 mg/kg MESNA intraperitoneal olarak uygulandı. Deneyler sonunda serum örneklerinde ALP, AST, ALT, LDH, TNF-α, IL-1β, MDA, MMP (MMP-1, -2, -8) ve TIMP-1 seviyeleri ölçüldü. biyokimyasal analizde. Deneklerden toplanan beyin, akciğer ve kalp dokusunda histopatolojik skorlar ölçüldü.

Bulgular

Karotis iskemi/reperfüzyon sonrası artan biyokimyasal parametreler, MESNA uygulamasıyla baskılanmıştır. Spesifik olarak, artan MESNA dozu ile TNF-α, ALP ve TIMP-1 parametreleri 150 mg/kg tedavisi ile 75 mg/kg tedavisine göre iki kat daha fazla baskılanmıştır. Beyin, akciğer ve kalpten alınan dokuların incelenmesi sonucunda MESNA tedavisi tüm dokuların dejenerasyonunda önemli değişiklikler gösterdi.

Sonuç

Bu çalışmadan elde ettiğimiz sonuçlar, MESNA'nın karotis arter iskemisi/reperfüzyonu üzerinde koruyucu etkisi olduğunu göstermektedir. Elde ettiğimiz bu sonuçlar MESNA'nın klinik uygulamasının önünü açacak olup literatüre kazandırılmalıdır.

Anahtar Kelimeler: İskemi, Reperfüzyon, MESNA, Oksidatif Stres

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List of Abbreviations

ACA: Anterior cerebral artery

ALP: Alkaline phosphatase

ALT: Alanine aminotransferase

ANOVA: Analysis of variance

AST: Aspartate aminotransferase

ATP: Adenosine triphosphate

BUN: Blood urea nitrogen

CABG: Coronary artery bypass grafting

CBF: Cerebral blood flow

DNA: Deoxyribonucleic acid

ECM: Extracellular matrix

EDTA: Ethylenediaminetetraacetic acid

ELISA: Enzyme-linked immunosorbent assay

FDA: Food and drug administration

GPX: Glutathione peroxidase

H2O2: Hydrogen peroxide

ICA: Internal carotid

ICAO: Internal carotid artery oclusion

IL: Interleukin

IP: Intraperitoneal

IR: Ischemic reperfusion

IV: Intravenous

LDH: Lactate dehydrogenase

MCA: Middle cerebral arteries

MDA: Malondialdehyde

MESNA: 2-Mercaptoethanesulfonate

MMF: Methoxymethylfurfural

MMP: Matrix metalloproteinase

mRNA: Messenger ribonucleic acid

NaCl: Sodium chloride

O_2: Oxygen

OH: Hydroxyl

PCA: Posterior carotid artery

PO:	Per os
PUFA:	Polyunsaturated fatty acids
RNS:	Reactive Nitrogen Species
ROS:	Reactive Oxygen Species
SC:	Subcutaneous
SOD:	Superoxide dismutase
TBA:	Thiobarbituric acid
TIA:	Transient ischemic attack
TIMP:	Tissue Inhibitor of Metalloproteinase
TNF:	Tumor necrosis factor
VA:	Vertebral artery

1 INTRODUCTION

Carotid ischemia is a major public health problem due to its high mortality and morbidity rates. It first causes damage to brain tissue and then pathological damage to distant organs such as the lungs and heart (Gunata & Parlakpinar, 2021). In particular, reduced blood flow to the brain can lead to death, and if left untreated, diseases such as stroke, Alzheimer's, cardiac arrest, respiratory arrest, cardiac and pulmonary embolism can occur (de la & Jack, 2006). Although reperfusion of ischemic tissue is necessary for tissue survival, it initially increases levels and activation of inflammatory cytokines such as TNF-α (Souza et al., 2002), IL-1β, and proteolytic enzymes such as matrix metalloproteinase (MMP), leading to inflammation and degeneration, resulting in worsening of ischemia (Park et al., 2011). Therefore, the importance of anti-inflammatory agents with immunomodulatory properties is increasing day by day, as inflammatory mediators increasingly play an important role in the pathogenesis of ischemia/reperfusion (Boyle et al., 1996).

2-Mercaptoethanesulfonate (MESNA) is a synthetic sulfur-containing antioxidant and anti-inflammatory drug used to prevent nephrotoxic side effects associated with chemotherapy drugs such as cyclophosphamide and ifosfamide (Saadati et al., 2021). Studies have shown that MESNA inhibits bladder tumors (Merwid et al., 2021), protective effects on spinal cord damage (Yilmaz et al., 2013), intestinal (Ypsilantis et al., 2006), renal (Kabasakal et al., 2004), and hepatic (Sener et al., 2005) ischemia and experimental burns (Sener et al., 2004), ulcerative colitis (ELbatsh et al., 2021), bleomycin-mediated lung injury (El-Medany et al., 2005), drug toxicity (Sener et al., 2005) and many other models of inflammation along with their anti-inflammatory properties (Abd El-Baset et al., 2021). It exerts these effects by regulating the activation of proteolytic enzymes along with the expression of cytokines (Kabasakal et al., 2004).

To date, no successful therapy has proven efficacious in the prevention and treatment of carotid ischemia/reperfusion. There is also no proven effect of MESNA on carotid ischemia/reperfusion. Therefore, our aim in the present study was to investigate whether MESNA induces protection against experimentally induced carotid ischemia/reperfusion in rats by assessing brain, heart and lung injury.

1.1 Background

Cerebrovascular events are one of the most common causes of adult disability and death worldwide (Yano & Kario, 2012). Ischemia is defined as a decrease in cerebral blood flow at a critical threshold that may spread to a selected brain region or the entire brain (Iadecola &

Alexander, 2001). Carotid arteries are blood vessels that carry blood from the neck to the brain. Blockages in the carotid artery reduce blood flow to the brain, causing carotid artery disease. Such interruptions in blood flow to the brain (commonly known as a stroke) can cause permanent damage (Sobieszczyk & Beckman, 2006). Carotid stenosis is caused by plaque (atherosclerosis) buildup in the artery wall that reduces blood flow to the brain. Carotid stenosis is another medical condition that can cause cerebral damage and is responsible for approximately 7% of all ischemic stroke cases (Dharmakidari, Bhattacharya, & Chaturvedi, 2017).

MESNA (2-mercaptoethane sulfonate) is a small, synthetic, water-soluble molecule with the potential to remove reactive oxygen species thanks to its sulfhydryl group. While it is mainly used to reduce hemorrhagic cystitis caused by cyclophosphamide and ifosfamide, it is also widely used as a protective agent against chemotherapy toxicity (Schwerdt et al., 2007). However, to our knowledge, no previous research has been conducted on whether MESNA improves cerebral injury and stroke or affects carotid artery disease/stenosis.

In this context, this study aimed to investigate whether MESNA administered intraperitoneally (i.p.) has a protective effect against tissue (brain, heart, and lung) damage that may develop after cerebral stroke in rats with experimental carotid artery ischemia.

1.2 Research Problem

There is insufficient evidence to confirm the protective effects of MESNA on Carotid artery ischemia/reperfusion.

1.3 Research questions

Does MESNA have a protective effect against carotid artery ischemia/reperfusion?

Does MESNA have a protective effect against stroke?

Does MESNA have a protective effect against cerebral infarction?

1.4 Objectives

This study aimed to examine whether MESNA to be applied intraperitoneally (i.p.) in rats with experimental carotid artery ischemia/reperfusion has a protective effect against tissue (brain, heart, and lung) damage that will develop after spinal cord trauma or stenosis/atherosclerosis.

1.5 Strength of the study

This will be the first study to investigate the protective effects of MESNA in carotid artery ischemia/reperfusion.

1.6 Hypothesis

MESNA has protective effects against carotid artery ischemia/reperfusion (Figure 1).

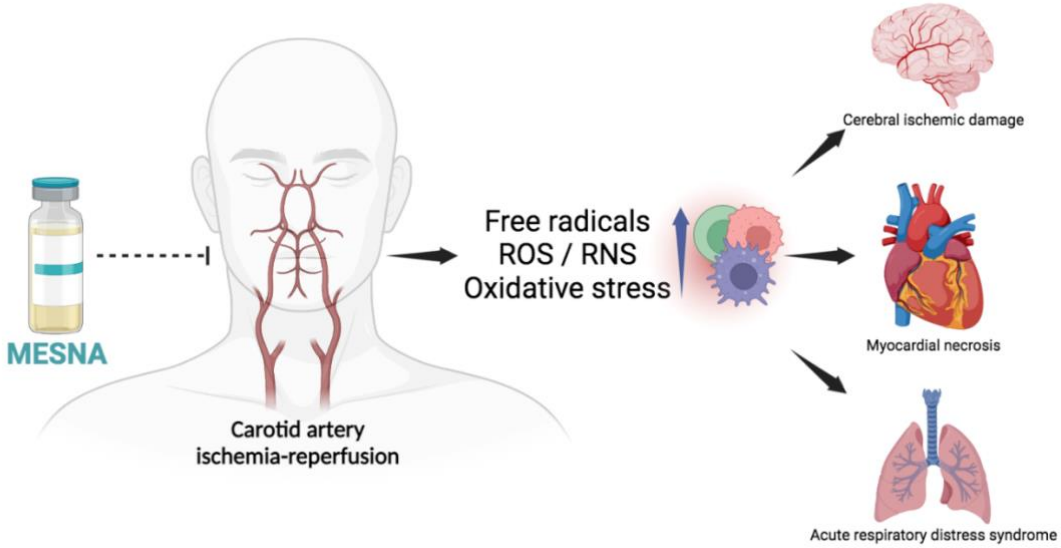


Figure 1: Free radicals, reactive oxygen and nitrogen species and increasing oxidative stress which is occur just after the carotid artery ischemia-reperfusion effects the brain negatively like cerebral ischemic damage. After that ischemia reaches to the remote organs, heart and lung and causes many injuries like myocardial necrosis or acute respiratory distress syndrome. In any inflammation, MESNA inhibits the effects of free radicals, ROS / RNS and oxidative stress and promotes recovery.

1.7 Significance of the study

Finding a new treatment option that will return neurological and physiological functions after cerebral ischemia or stroke with carotid artery ischemia may reduce mortality and morbidity. Today, anticoagulant drugs or surgical intervention (angio/stent) administered within the first 4 hours after detection of carotid artery ischemia or carotid artery disease has been among the most beneficial treatment options results are controversial (Cheng et al., 2018). Although many pharmacological agents have been tried so far in cerebral ischemia and stroke, many side effects have been revealed in clinical studies. While MESNA is mainly used to reduce hemorrhagic cystitis caused by Cyclophosphamide and Ifosfamide, it is also widely used as a protective agent against chemotherapy toxicity (Saadati et al., 2021); however, there is no study about how it will affect carotid artery ischemia. This study will discover whether MESNA, an agent whose effects have not been studied before in carotid artery damage, has protective effects and how it will respond at different doses. In this context, it is possible that MESNA will have a protective effect on carotid artery damage or carotid stenosis (carotid artery occlusion), and any results will make significant contributions to science.

2 LITERATURE REVIEW

2.1 Epidemiology of Carotid Artery Ischemia/Reperfusion

According to a 2020 systematic review published in The Lancet Global Health, carotid plaque prevalence increased by 21.1% (approximately 816 million cases) and carotid stenosis prevalence increased by 1.5 (approximately 58 million cases) (Cachón-Zagalaz et. al., 2020). A difference in genetics, epigenetics, lifestyle, diet, and environment (i.e., lifestyle, diet, and environment) is likely to have contributed to a greater proportion of carotid disease cases in the Western Pacific region, while in the African region, increased intima-media thickness was the least. As men age, they are more likely to develop the carotid disease. The authors found a prevalence of severe asymptomatic carotid stenosis in the general population of 3.1% in a meta-analysis of 23,706 participants from four population-based studies (MalmoDiet and Cancer Study, Tromso, Carotid Atherosclerosis Progression Study, and Cardiovascular Health Study). Carotid stenosis occurs more frequently in white patients than in black patients (Cheng et. al., 2012). Nevertheless, black patients are more likely to have a thick carotid intima-media. An analysis of self-referred individuals who underwent vascular screening tests in the United States found that moderate carotid stenosis (≥50%) varies significantly by race. A prevalence of 3.4% was found in women, while a prevalence of 4.2% was found in men (Columbo et. al., 2020). The highest prevalence was found among Native Americans and Whites. In a multivariate analysis, the chances of African American, Hispanic, and Asian patients developing carotid stenosis were significantly lower than those of White patients, while the chances of Native Americans developing it were higher. In a Southern California hospital, White patients (21.5%) were more likely to have carotid stenosis than other races (10.1% for Hispanics, 8.7% for Blacks, and 10.7% for Asians; P=001) (Yuo et. al, 2016).

2.2 Pathophysiology of Carotid Ischemia/Reperfusion

It is defined as ischemia-reperfusion injury when blood circulation is re-established after a period of decreased perfusion in an organ, resulting in tissue damage (Kalogeris et al., 2012). Ischemia is characterized by disruption of intracellular calcium balance, emptying of the cell's energy stores, increased mitochondrial permeability, and fragility of the cytoskeleton (Kalogeris et al., 2016). Oxidative stress occurs when reactive oxygen derivatives such as superoxide anions, hydroxyl radicals, hydrogen peroxide, and peroxynitrite are produced during ischemia (Afonso et al., 2007). Inflammatory reactions due to ischemia-reperfusion injury lead to multi-organ failure, and multi-organ failure is responsible for 30-40% of mortality in intensive care units (Li et al., 2016).

2.2.1 Micro or Macro Embolic Phenomenon

Atherosclerotic plaques, atheromas, in situ thrombi, or platelet plugs cause micro or macro emboli. A thrombus can occur at the stump of ICAO and embolize to downstream blood vessels. ICAO is frequently characterized by ulcerated atherosclerotic plaques, ruptured atheromas, and distal embolization. The emboli can either be micro, which targets smaller vessels at distal locations, or macro, which targets larger vessels at proximal locations (Malhotra, Goyal & Tsivgoulis, 2017). As a result of recurrent embolization of these atheromas, TIA can occur, accompanied by both ipsilateral cerebral or retinal ischemia and stroke symptoms (Klijn & Kappelle, 2010).

2.2.2 Systemic Hemodynamic Alterations

Patients with reduced cardiac output or varying degrees of hypovolaemia or hypotension often present with systemic haemodynamic changes (Møller & Bendtsen, 2018). Heart procedures such as coronary artery bypass grafting (CABG) can impair hemodynamics, leading to cerebral ischaemia and stroke. Thorough diagnostic work and careful perioperative planning are required when severe ICA or ICAO stenosis is present prior to CABG, as these conditions are associated with worse outcomes. Obstructive lesions in the proximal ICA compromise the vascular territories of the distal boundary zone - the anterior boundary zone between the territories of ACA (Anterior cerebral artery) and MCA and the posterior boundary zone between the territories of MCA and PCA-due to the vulnerability to perfusion. Patients with bilateral carotid occlusive disease with downstream ischaemia may manifest as bilateral proximal weakness in all four limbs, also known as "man in the barrel syndrome" (Silva et al., 2011). However, hemodynamic failure without strong collaterals could involve several vascular regions at risk of extensive cerebral infarction and malignant cerebral edema (Campbell et al., 2019).

2.3 Etiologies of Carotid Ischemia/Reperfusion

The most common etiologies of ICAO are arterithrombosis, cardioembolism and dissection.

2.3.1 Atherothrombosis

Atherothrombosis is present after atherosclerosis with superimposed thrombosis and subsequent progression to a steno-occlusive lesion. A fifth of all stroke subtypes are caused by atherosclerosis of the great vessels, of which carotid atherosclerosis is responsible for about

half (Krasteva et al., 2020). Corresponding to the presence of episodes of ipsilateral ischemia, atherosclerosis includes carotid stenosis ranging from mild (50%), moderate (50-69%), to severe (70-99%). It can be asymptomatic or symptomatic. Acute carotid occlusion is seen in 10% (ie 2% of all stroke types) of patients with extracranial carotid artery disease causing acute stroke. Vascular calcification has often been associated with atherosclerosis and a small number of studies have shown that individuals with cervical ICAO have a 53% frequency of the condition (Paraskevas et al., 2022).

The following are some risk factors for atherothrombosis: hypertension, tobacco use, diabetes and dyslipidemia (Ageno et al., 2008). Carotid atherosclerosis is further affected by additional variables such as hyperhomocysteinemia, past cervical irradiation, and genetic predisposition. A predisposition to the cervical region of the ICA immediately distal to the carotid sinus is demonstrated by atherothrombosis. Atheromas or atherosclerotic plaques develop temporarily due to endothelial damage of the intimal and medial layers (Filis et al., 2017). Although atheromatous plaques can persist for long periods of time, they often change due to disruption of atheroma, platelet activation, and platelet aggregation, resulting in artery-to-artery embolization. Due to the disturbed intermittent flow, severe carotid artery stenosis often causes hemodynamic insufficiency and manifests as stuttering neurological symptoms. Atherosclerotic occlusions usually precede worse clinical outcomes as they are associated with tandem intracranial lesions. Rarely, atherosclerotic blockade of the common carotid artery may result from retrograde expansion of carotid sinus atherothrombosis (Blaser et al., 2002).

Cerebrovascular disease is a broad term that refers to a variety of problems with the brain's vascularization (Vanlandewijck et al., 2018). The current study’s research aim, design, and methodological approach developed from a close understanding and analysis of the existing literature. This part reviewed previous research and published data directly relevant to the research objectives.

2.4 Cerebral Blood Flow

In order to sustain normal function, the human brain gets 15% of total cardiac output and requires 20% of available oxygen; consequently, precise management of blood flow and oxygen delivery is critical for life. Neurodegenerative illnesses are linked to chronic declines in cerebral blood flow and the processes that regulate stable cerebral blood flow, referred to as cerebrovascular function (Willie, Tzeng, Fisher, & Ainslie, 2014).

The internal carotid (ICA) and vertebral arteries (VA) are two of the four main extracranial arteries that provide blood to the brain. The ICAs provide approximately 70% of total cerebral blood flow (CBF), with the two VAs providing the remaining 30%. (Willie et al., 2014). The basilar artery bifurcates to eventually become the posterior cerebral arteries, whereas the ICA continues upwards to the base of the brain to form the anterior (ACA) and middle (MCA) cerebral arteries bilaterally (PCA) (Lassen & Christensen, 1976). On both the left and right hemispheres, the anterior circulation begins distal to the carotid sinus and feeds both the forebrain (frontal and parietal lobes) and midbrain (temporal lobes) (Figure 2).

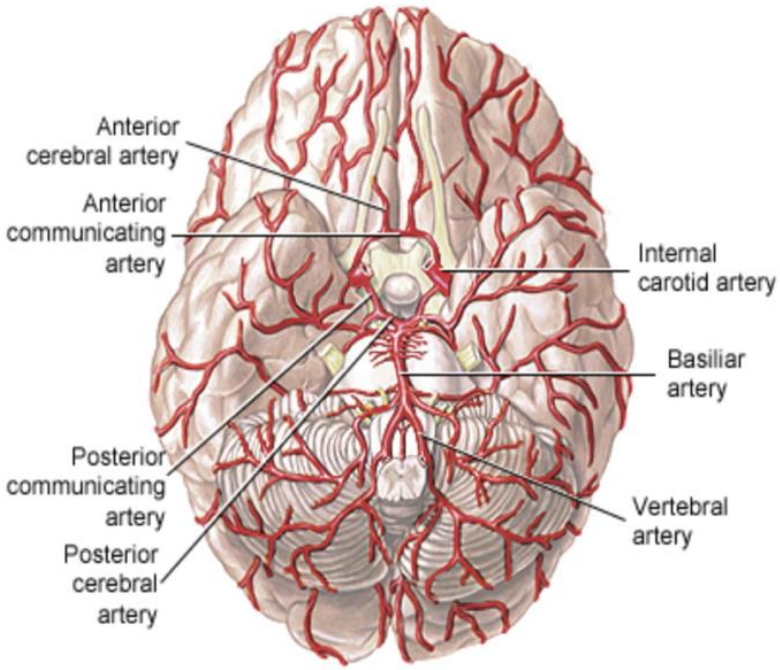


Figure 2: Cerebral blood flow

The Circle of Willis is a vascular structure formed by the joining of the cerebral circulation in a number of sites. The anterior and posterior circulations are linked by the posterior communicating arteries in this anatomical ring near the base of the brain (Figure 3) (Peterson, Wang, & Britz, 2011).

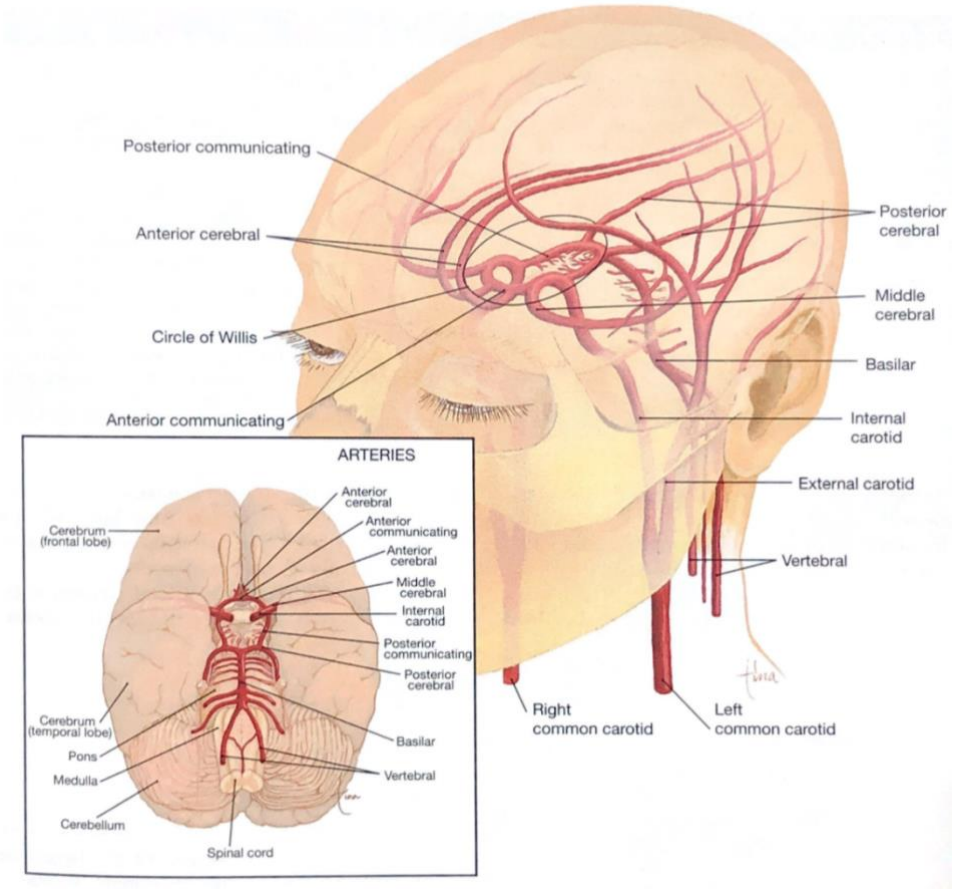


Figure 3: Circle of Willis. This anastomosis is formed by the following arteries: internal carotid, anterior communicating, posterior communicating, and basilar. The cerebral arteries extend from the circle of Willis into the brain. The box shows these vessels in an inferior view of the brain

Because of its high metabolic requirement, the brain requires continual enough food supply to maintain proper functioning. In resting settings, the brain consumes around 25% of total oxygen (O2) and gets 15-20% of total cardiac output.

Cerebrovascular resistance and cerebral perfusion pressure determine cerebral blood flow. The resistive forces operating on blood flow through the brain are referred to as cerebrovascular resistance. Flow resistance is largely found in the cerebral arteries and capillary beds, and as vascular tone rises, so does resistance (Zarrinkoob et al., 2015).

2.5 Cerebral and peripheral vascular function

The internal carotid artery (ICA) and vertebral arteries (VA) are two of the four main extracranial arteries that provide blood to the brain. The ICAs provide approximately 70% of total cerebral blood flow (CBF), with the two VAs providing the remaining 30%. The basilar artery bifurcates to eventually become the posterior cerebral arteries, whereas the ICA continues upwards to the base of the brain to form the anterior (ACA) and middle (MCA)

cerebral arteries bilaterally (PCA). The anterior circulation originates distal to the carotid sinus and serves both the left and right hemispheres' forebrain (frontal and parietal lobes) and midbrain (temporal lobes). Before trifurcating to produce the MCA, ACA, and posterior communicating artery, the ICA contains a branch that feeds the ocular artery. By connecting the two ACAs, the anterior communicating artery permits the anterior circulation to nourish both the left and right hemispheres. The smaller stem and cortical branches of the MCA and ACA branches carry blood throughout the brain, feeding into smaller arterioles before finally feeding into the anterior cerebral capillary beds (Maxwell, 2020; Standl, Steigler, & Janka, 1989).

2.6 Carotid artery disease

The carotid arteries are the blood vessels that carry blood from the heart to the brain through the neck. Each side of the neck has one carotid artery, whose pulsing may be felt with a finger beneath the jaw bone. Carotid artery disease is caused by a blockage in the carotid artery, which reduces blood flow to the brain. Strokes, or interruptions in blood flow to the brain, can result in irreversible damage (Sobieszczyk & Beckman, 2006).

Stroke is the sudden development of localized neurological symptoms that are thought to be caused by a vascular etiology (Hill et al., 2004). These symptoms are the result of brain tissue dying suddenly owing to ischemia as a result of poor blood supply. Occlusion or rupture of intracranial and extracranial arteries might limit cerebral blood flow. Medical treatment is the primary technique of stroke prevention, although in rare cases, surgical treatment is the best option. In large randomized controlled studies, the role of surgical therapy of severe carotid artery stenosis in stroke prevention, both primary and secondary, has been demonstrated (Flumignan, Flumignan, & Navarro, 2017; Wabnitz & Turan, 2017).

Stroke can be caused by atrial fibrillation, an abnormal heart rhythm, can induce stroke by forming minute blood clots when the heart quivers instead of beating regularly (Sorace, Ronai, & Berry, 2019). When a tiny blood clot forms, the heart can send it via the carotid arteries, blocking a blood artery in the brain and depriving that portion of the brain of blood, resulting in a stroke (Razaaq, 2021).

A blockage in the carotid arteries is another prevalent cause of stroke. The carotid arteries deliver blood to the brain, and they, like the blood vessels that feed blood to the heart, can constrict or obstruct (Saw, 2014).

2.6.1 Symptoms of carotid artery disease

Carotid artery blockage frequently has no symptoms, and the patient and doctor are unaware of it until blood flow to the brain is impaired. When a section of the brain is deprived of blood, it ceases to function (Wabnitz & Turan, 2017).

A patient with symptomatic carotid artery stenosis has had a recent central neurological episode with symptoms within the previous 6 months (and typically imaging of cerebral ischemia), matching to the relevant vessel, according to most classifications (Charmoille et al., 2015; Kernan et al., 2014).

Symptoms vary depending on where the blocked brain blood artery is located. A patient may, for example, notice a loss of vision in one eye, begin to slur words, or have trouble locating and expressing himself. Stroke sufferers frequently lose strength or sensation in an arm, leg, or side of the body (Sobieszczyk & Beckman, 2006). Numbness and heaviness in the arm or leg are noticed by the patient. Some patients say their limb or arm feels like it's slipping away from them. A numb and drooping side of the face is possible. These sensations might last anywhere from a few minutes to many hours at a time. Any of these symptoms might be an indication of a mini stroke or transient ischemic attack (Sobieszczyk & Beckman, 2006).

2.6.2 Treatment of carotid artery disease

The optimum therapy for carotid artery stenosis is to stop it from becoming worse. Aggressive medical intervention for all patients with severe symptomatic carotid stenosis should comprise a mix of antiplatelet medication, high-potency statins, blood pressure control, and lifestyle risk factor adjustment, as detailed below. Even in the age of contemporary medical care, less than 20% of patients with carotid stenosis had optimal risk factor control at baseline, demonstrating that the importance of risk factor control is underestimated, even in patients with documented atherosclerotic disease (Meschia et al., 2014).

High blood pressure has been linked to cholesterol deposits in the carotid arteries, which increases the risk of plaque rupture and stroke. The measurement of blood pressure is a standard aspect of a physical examination, and excessive blood pressure is simple to spot. It is suggested that the systolic blood pressure reading be less than 140. This figure should be less than 130 if the patient has diabetes. Many blood pressure-lowering drugs can be used to treat hypertension if your blood pressure is greater than these levels. It's more crucial to drop blood pressure to a certain level than it is to utilize a certain medicine. Weight loss and regular exercise can help to decrease blood pressure and are strongly advised as part of any therapy plan (Haley et al., 2021).

The degree of the blockage in the carotid artery influences the risk of stroke. If the blockage becomes severe, especially if the TIA was brief, a treatment to remove the blockage from the carotid artery and enhance blood flow through it is indicated. There are two procedures for opening clogged carotid arteries that are widely approved.

- Carotid endarterectomy is a procedure that is done under general anesthesia. Through a neck incision, the carotid artery is opened, the cholesterol blockage is removed, and the vessel is sewn back together. The operation is reserved for severe narrowing of the carotid artery and poses a minimal risk of stroke, heart attack, or death. Patients who have had a stroke or a transient ischemic attack (TIA) and have a carotid artery narrowing of 50% or more are candidates for carotid endarterectomy. It is also indicated if the patient has never had a stroke or TIA but has a 70% or greater narrowing of the carotid artery (Sobieszczyk & Beckman, 2006).
- Carotid artery stenting is a treatment that involves inserting a tiny tube into an artery in the groin area of the leg under local anesthetic. A flexible tube-like wire mesh (a stent) is positioned and inflated over the obstruction in the artery under x-ray guidance. The stent pulls the cholesterol deposit out of the path, allowing blood to flow freely again. For individuals who are too unwell to undergo carotid endarterectomy, carotid artery stenting is an alternative to surgery. The chances of having a heart attack, stroke, or dying as a result of this surgery are extremely minimal (Sobieszczyk & Beckman, 2006).

2.7 Carotid Artery Ischemia/Reperfusion and Cytokines

Ischemia results from tissue hypoperfusion (Demeestere et al., 2020). Oxygen support is then supplied to the tissue by infusion. Cellular damage occurs as a result of excessive production of reactive oxygen species in ischemic tissue, with a relative depletion of antioxidants (Douzinas et al., 2019). This is called an ischemia-reperfusion injury. ischemia-reperfusion injury; It is associated with many pathologies, such as myocardial infarction (Cadenas et al., 2018), cerebrovascular disease (Sun et al., 2018), acute kidney injury, injury, sickle cell anemia, cardiac arrest and sleep apnea (Hebbel et al., 2020). May cause high mortality and morbidity.

Cytokines play an important role in I/R lesions by inducing an inflammatory response and regulating the severity of the I/R lesion (Raghay et al., 2020). Tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) are two cytokines commonly found in carotid I / R lesions (Rectenwald et al., 2000). Cytokines are a group of several non-antibody proteins that act as mediators between cells (Sedger et al., 2014). Although they were initially identified as products of immune system cells that act as mediators of immune processes, it is known that

many cytokines are also produced by cells outside the immune system and can affect cells outside the immune system. Cytokines are currently used clinically as modifiers of the biological response to treat a variety of conditions (Rankin et al., 2004). Although the term cytokine is a generic term used to describe many groups of proteins, there are other terms commonly used to describe specific types of cytokines (Haddad et al., 2002). These include:

- Monokines, cytokines produced by mononuclear phagocytic cells
- Lymphokines, Cytokines produced by activated lymphocytes, especially Th cells
- Interleukins, cytokines that act as mediators between leukocytes
- Chemokines, minor cytokines primarily responsible for leukocyte migration

Cytokines function as part of a larger interconnected protein system, known as cytokine and signaling networks (Wojdasiewicz, Poniatowski, & Szukiewicz, 2014). These are complex interactions in which different cells respond differently to the same cytokine, depending on the different signals received by the cell. Cytokine signaling is highly variable and can cause both protective and harmful responses. One cytokine usually affects the synthesis of another. They can initiate a cascade and increase or inhibit the production of other cytokines. In addition, it can often affect the behavior of other cytokines. These effects can be antagonistic, additive or synergistic (Minns et al., 2021).

Cytokines are not usually stored as precursors. Instead, the synthesis begins with gene transcription and the mRNA is short-lived (Wu et al., 2021). They are produced when they are needed for the immune response. Cellular responses to cytokines are usually slow (over time) because new mRNA and protein syntheses are required. During ischemia, hepatocyte oxygen deprivation leads to cessation of mitochondrial energy production, ATP depletion, and changes in homeostasis H^+, Na^+, and Ca^{2+}, which activate hydrolases and reduce cell volume control (Kim, He, & Lemasters, 2003). This condition is associated with an imbalance between nitric oxide and endothelin production, which leads to sinusoidal lumen stenosis and affected lung circulation. Capillary constriction also causes the accumulation of neutrophils in the liver (Enzmann, Kargaran, & Engelhardt, 2018). During I/R, an impressive variety of proinflammatory mediators are released, including ROS, chemokines, cytokines (e.g., TNF and IL-1) and proteases (e.g., MMP). However, Kupffer cells are activated to release reactive oxygen species (ROS) and proinflammatory cytokines, such as TNF-α and IL-1. Chemokines are released during induction. On the other hand, chemokines promote the activation and recruitment of neutrophils, releasing ROS and proteases, helping to accelerate parenchymal lesions (Liberale et al., 2021).

2.7.1 Tumor Necrosis Factor-α (TNF-α)

TNF-α is produced by neutrophils, macrophages and T cells. It is synthesized as a transmembrane precursor protein from activated macrophages and T cells (Baram et al., 2001). TNF-α is an anti-inflammatory cytokine that stimulates an acute phase response (Wang et al., 2018). The release of TNF-α in response to acute lesions is rapid and short-lived. Although it has a half-life of 15-18 minutes, even short-term exposure to TNF-α causes significant metabolic and hemodynamic changes and activation of cytokines later in the cycle (Aulbach et al., 2019. It plays an important role in the pathogenesis of inflammation in many diseases. It causes apoptosis and inflammation, inhibits tumor growth and virus replication (Alharbi et al., 2021).

TNF-α stimulates the release of inflammatory cytokines (IL-1 beta, IL-6, IL-8) and induces the production of ROS in Kupffer cells. This function of TNF-α regulates the initiation and maintenance of events related to the inflammatory response (Kany et al., 2019).

2.7.2 Interleukin-1 (IL-1)

It is the first interleukin identified, affects almost every cell and organ and is an important pathogen of autoinflammatory, autoimmune, infectious and degenerative diseases. The IL-1 family includes seven molecules with agonist activity (IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β and IL-36γ), three receptor antagonists (IL-1Ra, IL -ligand.36γ) 36Ra and IL-38) and proinflammatory cytokines (IL-37). IL-1β signaling initiates a network of self-strengthening cytokines by inducing NF-κB activation and producing other inflammatory mediators, such as IL-1β and TNF-α. It is released in response to TNF-α (Mantovani et al., 2019).

2.7.3 Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS)

Free radicals are divided into two main groups: reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS play key roles in many pathological processes during ischemia reperfusion (Kwon et al., 2021). Currently, the toxicity of free radicals in ischemia-reperfusion lesions is intensively studied.

Oxidative stress is caused by excessive production of ROS (Wu et al., 2020). The main dangerous types of ROS are superoxide anions (O2-), hydroxyl radicals (OH-) and hydrogen peroxide (H2O2). Under physiological conditions, superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase and other antioxidant enzymes can catalytically protect brain tissue from the cytotoxic effects of ROS, while maintaining a neutral balance (Meitha et al., 2020). During the reperfusion process of cerebral ischemia, there is a significant increase in the

production of free radicals leading to the destruction of the antioxidant system, especially in the reperfusion phase.

During the reperfusion of cerebral ischemia, especially with blood regurgitation, mass production of ROS and RNS leads to cell death by DNA (Deoxyribonucleic acid) damage, protein dysfunction and lipid peroxidation. Among all possible pathological mechanisms of ischemic reperfusion injury, free radical damage (mainly oxidative stress / nitrification) has been found to play an important role in this process (Orellana-Urzúa et al., 2020). Free radicals cause protein dysfunction, DNA damage and lipid peroxidation, leading to cell death. In addition, free radical damage is closely linked to the induction of hemorrhagic deformity and cerebral edema, which are major complications of revascularization therapy and mainly affect the neurological outcome resulting from the disruption of the blood-brain barrier (Su et al., 2019. In addition, ROS has physiological roles in regulating immune system function, maintaining redox homeostasis, and participating in multiple pathways as secondary messengers.

2.8 Carotid Artery Ischemia/Reperfusion and Proteolytic Enzymes

The balance between hydrolytic activity and its inhibition regulates tissue homeostasis and extracellular matrix (ECM) (Manou et al., 2019). Disruption of this balance occurs as an integral part of the tissue response to remodeling stimuli, but long-term disorders regulate many biological processes and lead to the development of lesions such as ischemia-pancreatic reperfusion. Elevated levels of MMP characterize acute or chronic disease states and correlate with the severity of the disease, suggesting harmful effects (Cortes-Serra et al., 2020).

2.8.1 Matrix Metalloproteinases (MMPs) and Tissue Inhibitor of Metalloproteinase (TIMP)

- MMP-1 (Interstitial collagenase; collagenase 1),
- MMP-2 (Gelatinase A),
- MMP-8 (Neutrophil collagenase; collagenase 2) and
- TIMP-1

These are the most specific biochemical parameters studied during the pancreatic ischemia-reperfusion lesion. In this paper, we investigated the increase of proteolytic enzymes MMP-1, -2, -8, especially TIMP-1, in ischemia and reperfusion of the carotid artery. Matrix metalloproteinases (MMPs) are a group of over 20 zinc-binding proteases responsible for the breakdown of extracellular matrix proteins and basement membranes. They play a role in various processes such as angiogenesis, tumor invasion, tissue regeneration and remodeling.

Uncontrolled expression of MMP can lead to tissue damage and inflammation (Cabral-Pacheco et al., 2020). MMPs can be divided into four main classes: collagenases, gelatinases, stromelizines, and membranous MMPs (MTs). Serum MMP-2 levels have been shown to be highly elevated during ischemia / pancreatic reperfusion injury. MMP is inhibited by TIME (Chaturvedi, & Kaczmarek, 2014)

The TIMP family consists of four members (TIMP-1, -2, -3 and -4) with significant homology that inhibit specific MMPs. It was initially thought that TIMPs function only as endogenous inhibitors of MMPs that regulate MMP-mediated ECM degradation (Viappiani et al., 2006). However, the accumulated evidence suggests that it is a multifunctional protein that may or may not have inhibitory functions and is involved in a variety of biological activities, from cell growth and differentiation to cell migration, invasion, angiogenesis, survival and apoptosis, organizational context (Samakova et al., 2019). TIMP-1 promotes cell proliferation and regulates apoptosis in different cell types (Allen et al., 2018). Reduced blood circulation associated with various pathological conditions, such as myocardial, cerebral or hepatic ischemia, causes the activation and / or release of various proteolytic enzymes. For example, in a murine model of focal cerebral ischemia, MMP-2 and -9 were found in ischemic brain tissue, which could be suppressed using a neutralizing monoclonal antibody against MMP-9 to reduce the size of the infarction role (Gunata et al., 2021). MMP at the onset of stroke In the heart, one of the organs in which the role of MMF (Methoxymethylfurfural)has been studied a lot, several cytokines such as TNFalpha, IL1-beta and IL-6 are clearly released in the early stages of ischemia, which has an inhibitory effect (Che et al., 2019). MMP expression has been extensively documented in in vitro studies. Also, after myocardial infarction, certain event scenarios (reperfusion) occur when blood circulation is restored to improve circulation and promote recovery. MMP-2 is rapidly activated during reperfusion after ischemia. In the carotid I / R lesion, the target of MMP-2 action was intracellular rather than extracellular (Prado et al., 2021). In fact, MMP-2 has been found to be localized in the sarcomere of a troponin I contractile protein (TnI) regulatory element. Indeed, TnI is very sensitive to MMP-2 degradation. TIMPs are also localized in cardiac sarcomeres and have been shown to be lost due to IR injury, leading to an imbalance between MMP and TIMP in the heart (Cabral-Pacheco et al., 2020). In the heart, MMF activation in the first minutes of reperfusion injury is primarily due to a marked increase in oxidative stress and is responsible for the degradation of key intracellular proteins (eg, TnI and other entities) that mediate contractile dysfunction (Singh et al., 2019). MMPs are involved in tissue remodeling that is harmful (i.e., left ventricular dilation) and compensatory (i.e., wound healing and tissue repair). Because all of this information suggests that MMP

inhibition may be a useful strategy to ameliorate IR-induced carotid lesion, we biochemically measured MMP and TIMP in our study and documented the results.

2.9 2-Mercaptoethane Sulfonate (MESNA)

2.9.1 MESNA Background

MESNA (sodium 2-mercaptoethane sulfonate) is one of the thiolate compounds. MESNA was initially investigated as a possible mucolytic for the treatment of disorders characterized by decreased mucociliary clearance, such as asthma, chronic bronchitis, and cystic fibrosis. It was thought that because MESNA is a low molecular weight chemical with a free sulfhydryl group, it may break down disulfide bonds in glycoproteins that make up mucus, lowering its viscosity and making expectoration easier for patients (Cutler, 2010).

MESNA is made from sodium bromoethanesulfonate. ammonia, and thiourea It comes in 2-ml ampules with 100 mg/ml of the medication and 0.25 mg/ml of EDTA (Ethylenediaminetetraacetic acid), as well as 10-L multidose vials with 10.4 mg of benzylalcohol as a preservative (Reddy & Winston, 2020). It is diluted to a final dosage of 20 mg/ml with different dextrose and NaCl mixes or lactated Ringer's solution prior to intravenous administration. MESNA is oxidized to the equivalent disulfide when exposed to oxygen. Diluted solutions are stable for 24 hours at 25°C, although they should be refrigerated and used within 6 hours of reconstitution. 240 mg/m2 is the standard dose, which is administered at the same time as the initial ifosfamide injection (Burkert, 1983; Shaw & Graham, 1987).

2.9.2 Pharmacodynamics of MESNA

The metabolite acrolein is formed by cyclophosphamide and ifosfamide metabolism, and it has been linked to sterile hemorrhagic cystitis (Matz & Hsieh, 2017).

MESNA prevents acrolein from interacting with host cells in the bladder by attaching to the urotoxic metabolite. MESNA is always given with ifosfamide, although it can also be given with cyclophosphamide, however this is not stated on the label. MESNA can be taken orally or intravenously. The oral dosage is equal to the parenteral dose multiplied by two. There are several dosage regimes that can be employed. Because of its short half-life, MESNA must start concurrently with or before ifosfamide or cyclophosphamide and stop after ifosfamide or cyclophosphamide on any schedule (i.e., MESNA must be present in the bladder when acrolein is present in the bladder) (Cutler, 2010).

Diarrhea, limb discomfort, headache, nausea, exhaustion, and an unpleasant taste in the mouth are all possible side effects. Some patients are hypersensitive to certain things (Shaw & Graham, 1987).

The Pharmacology Department of the German Bielefeld-based Asta-Werke A.G. worked on the development of MESNA for uroprotection (Siu & Moore, 1998) .

In Canada, the United States, Germany, and the United Kingdom, the intravenous solution of mesna can be taken orally to ease outpatient care during short-infusion chemotherapy. The development of both continuous infusion and oral dose regimens would allow MESNA to be delivered as outpatient therapy, allowing patients to spend more time at home and reducing not just patient stress but also the expenditures of protracted patient monitoring in clinics. Since its clearance by the Food and Drug Administration (FDA) in 2002, MESNA tablets have boosted patient convenience by providing the same bioavailability as IV solution while avoiding the IV solution's poor palatability, resulting in higher outpatient compliance (Cutler, 2010).

2.9.3 Pharmacokinetics of MESNA

MESNA is mercaptoethanesulfonic acid's sodium salt (Jeelani et. al., 2017). In the blood, it is oxidized to the equivalent disulfide, but in the kidney, it is reduced back to the free thiol. It interacts with urotoxic ifosfamide metabolites, such as 4-hydroxyifosfamide and aerolein, in this location. Because of this feature, it's been used to prevent hemorrhagic cystitis in Ifosfamide patients.

Ifosfamide-induced hemorrhagic cystitis is treated with MESNA (Vieira et. al., 2003). It is promptly oxidized to the disullide after intravenous injection. It is reduced to free thiol in the kidney, where it interacts with urotoxic ifosfamide metabolites such as acrolein and 4-hydroxyifosfamide. MESNA and its disulfide have half-lives in the blood of 0.36 and 1.17 hours, respectively, and their kinetics are dosage dependent (Verschraagen et. al., 2003).

2.10 Intraperitoneal route of administration

Drugs are administered via various routes in laboratory animals such as intravenous (IV), subcutaneous (SC), intraperitoneal (IP), and oral (PO), each has its own strength and limitation depending on the study being conducted. The route of administration is considered as a significant driver of the ultimate pharmacokinetics, pharmacodynamics, and toxicity of pharmacological drugs. The intraperitoneal route, in which a pharmacological substance is injected into the peritoneal cavity, is one of the most widely employed route of administration in studies conducted in rats (Turner, Brabb, Pekow, & Vasbinder, 2011).

The IV route of drug delivery typically results in the best bioavailability of a medication among all routes of drug administration. However, IV route is often impractical for rodent studies because most investigational pharmacological agents are difficult to fully dissolve in aqueous solutions being hydrophobic in nature, rats have small vessel which requires extra skills, and not suitable for chronic or repetitive doses. To circumvent these issues, researchers frequently administer pharmacological drugs orally, intraperitoneally, or subcutaneously.

While the intraperitoneal route is simple to learn, rapid to administer, ideal for prolonged treatments, and has a modest influence on laboratory mice' stress levels, there is a widespread worry that it may not be an acceptable route for drug delivery in experimental studies. This most likely attributable to a scarcity of data on pharmacokinetics of pharmacological drugs and the methods by which they gain systemic exposure following IP delivery.

A study reviewed the mechanisms of administering IP drugs in comparison with other routes in terms of pharmacokinetic profiles. It also reviewed the anatomy and physiology of peritoneal cavity and linked these processes to the administration of drugs (Al Shoyaib, Archie, & Karamyan, 2020).

The IP route has the fastest rate and extent of drug absorption followed by intramuscular, SC, then oral routes. IP is suitable for both drug solutions and suspensions/emulsions. IP administration provides large surface area which leads to rapid and efficient absorption. The effect produced by a pharmacological drug after IV administration can be similar to IP administration compared to intramuscular or SC administration. The rate of absorption after IP administration is almost as rapid as after IV administration.

On the other hand, a study Ballard (2009) conducted on mice to evaluate how accurate the IP route is, different IP injection methods were examined in their study to see which one was the most accurate. At the start of the investigation, 400 animals (rats and mice) were given an overdose of barbiturate. Altogether, 83 percent of injections given on the left side were successful, whereas 93 percent of injections given on the right side were correct. Failures produced by administration into an organ are commonly acknowledged, but the degree of failure is often unknown. Because the greatest accuracy attained by skilled specialists was only 85%, caution should be exercised while considering this procedure as a viable option. When IP injection is employed, this might be a substantial source of experimental variability. Although there is less of a difference between the two sides in mice, the failure rate discovered in this study implies that caution should be exercised when using this approach in this species (Ballard, 2009).

2.11 Effects of MESNA on neurovascular tissues

The current study is based on the question does MESNA have protective effects carotid artery ischemia/reperfusion, cerebral infarction, or stroke. Hence, it is mandatory to review the literature for studies relating MESNA and neurovascular tissues. There have been few studies establishing this effect, the section below critically reviews them.

A study investigated the protective effect of MESNA against traumatic brain injury in rats. Group 1 (sham), Group 2 (trauma), Group 3 (150 mg/kg MESNA), and Group 4 (30 mg/kg methylprednisolone (Diaz-Ruiz et al., 2000)) were randomized into four groups of eight rats each. The medications were given right after the injury. The animals were slaughtered after 24 hours. The amounts of tissue malondialdehyde, caspase-3, glutathione peroxidase, superoxide dismutase, nitric oxide, nitric oxide synthetase, and xanthine oxidase were measured in brain tissues as they are increased following oxidative stress of brain tissue (Awasthi, Church, Torbati, Carey, & Pryor, 1997). A histological examination of the tissues was also carried out. The findings of the study were interesting, tissue malondialdehyde levels elevated after head trauma, however these levels were considerably reduced by MESNA administration. After trauma, caspase-3 rate had increased, but there was no effect of MESNA on caspase-3 activity. Glutathione peroxidase and superoxide dismutase levels were reduced after trauma, while MESNA enhanced the activity of both antioxidant enzymes. Also, following trauma, the levels of nitric oxide, nitric oxide synthetase, and xanthine oxidase were elevated; however, MESNA treatment dramatically reduced the levels of nitric oxide, nitric oxide synthetase, and xanthine oxidase, indicating that it had antioxidant potential. The brain tissues were well protected from harm by MESNA, according to histopathological examination. MESNA was demonstrated to be at least as beneficial as methylprednisolone in the traumatic brain injury model, while more research with alternative dosing regimens and time intervals is needed (Yilmaz et al., 2013).

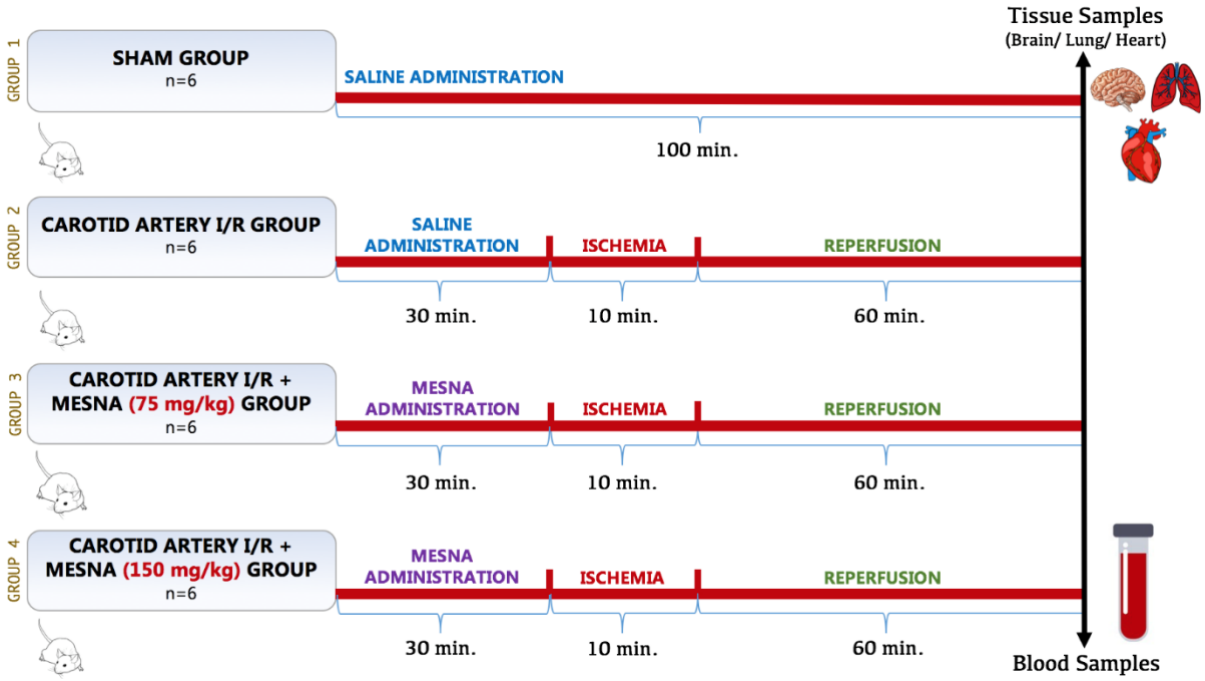
In a recent study, the preventive benefits of MESNA against cisplatin-induced neurotoxicity were examined by Saadti et al. In male Wistar rats, neurotoxicity was caused by administering 2.5 mg/kg cisplatin twice a week for four weeks. Behavioral, electrophysiological, and molecular tests were used to assess the neuroprotective impact of MESNA (150 mg/kg/day). In the cisplatin + MESNA group, the lowered conduction velocity in sensory nerves was regained. MESNA decreased proinflammatory cytokines and partially relieved redox imbalance. MESNA treatment also alleviated morphological abnormalities in cisplatin-treated rats' dorsal root ganglia. Finally, our findings show that MESNA can help to reduce cisplatin-induced central and peripheral nervous system damage (Saadati et al., 2021).

Methylprednisolone is well established to as a protective agent of ischemia following neurovascular injury (Bracken, 2001). A study compared MESNA to methylprednoslone using rabbit models, the researchers wanted to see how MESNA affected caspase-3 activation in a rabbit model. Adult rabbits were given a 20-minute occlusion of the abdominal aorta to cause ischemia damage to their spinal cords. The activity of tissue caspase-3 was evaluated in the spinal cord 24 hours after ischemia. Following ischemia/reperfusion damage, rabbits given a single dosage of 150 mg/kg MESNA showed a reduction in caspase-3 activity in the spinal cord, indicating a protective effect. Caspase-3 activity was lower in methylprednisolone-treated rabbits than in MESNA-treated rabbits (Dolgun et al., 2010).

3 MATERIALS AND METHODS

3.1 Animal Groups

All experimental procedures used were approved by the Near East University Ethics Committee on October 21, 2021 with protocol number 2021/139-139. In our study, 24 male Wistar albino rats, 3 months old and weighing 250-300 g, were selected (such animals were selected based on the extensive available literature, which allowed comparisons with the data obtained). The rats were divided into 4 groups of 6 each (Figure 4).



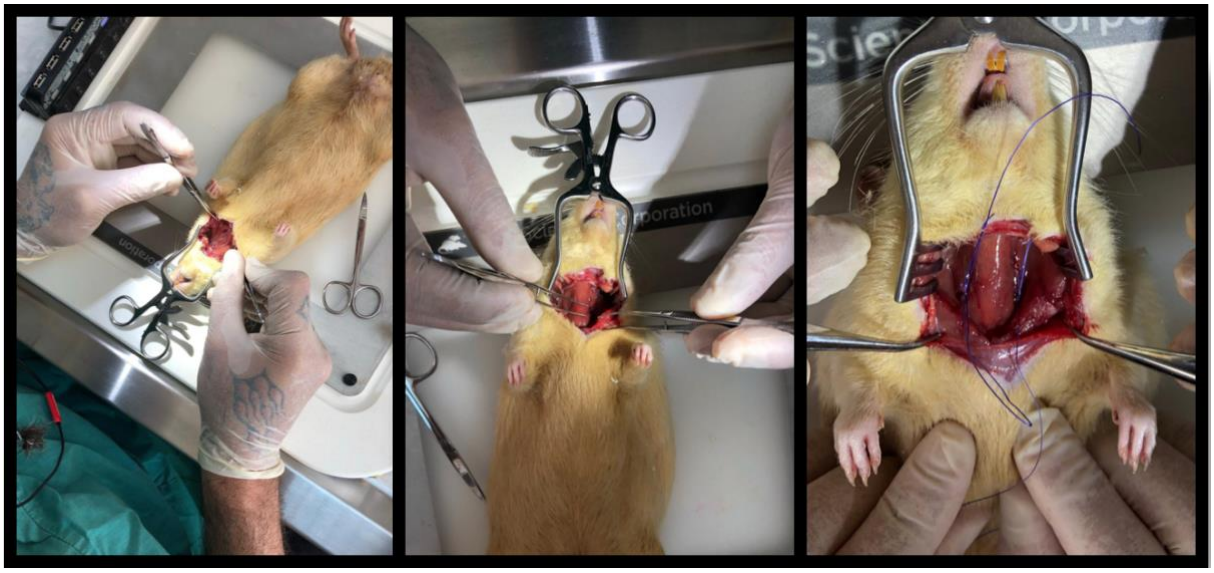
(min.: minute)

Figure 4: Flowchart of experimental study

In the Figure 4, in our study, 24 male Wistar albino rats were divided into 4 groups of 6 animals each. The sham group received only 10 minutes of ischemia followed by 1 hour of reperfusion. Animals in groups 3 and 4 received MESNA (75 mg/kg and 150 mg/kg) half an hour before and immediately after ischemia. In Group 2, subjects were administered only carotid ischemia and reperfusion. At the end of the experiments, tissue samples (brain, lungs, heart) and blood were taken from all subjects.

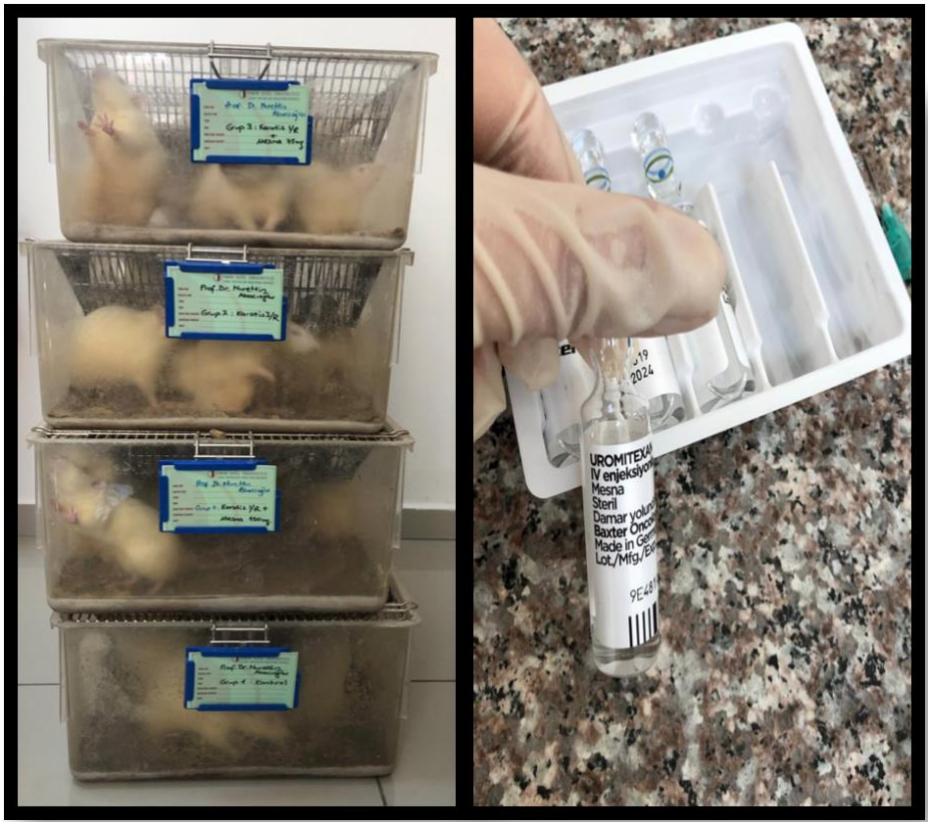
1. **Sham group (n=6):** This group was given normal saline intraperitoneally (i.p.). Sham surgery was performed on the common carotid arteries of subjects in this group.
2. **Carotid artery ischemia/reperfusion group (n=6):** The surgical procedure was performed under general anesthesia induced by intramuscular xylazine (10 mg/kg; Bayer, Istanbul, Türkiye) and ketamine hydrochloride (100 mg/kg; Parke-Davis, Istanbul, Türkiye). It was given normal saline intraperitoneally (i.p.). In the study, the intraluminal

thread method of Handayani et al. was used to provide ischemia and reperfusion of the carotid artery. The rats were placed supine and a skin incision was made 3 cm from the midline of the trachea. The common carotid arteries were exposed by midline blunt dissection of the sternohyoid muscle. The common carotid artery was then ligated bilaterally with 4/0 silk sutures at the level of the 4°-5° tracheal ring (Picture 1). We waited 10 minutes and ischemia was induced. The silk suture was then opened and 1-hour reperfusion achieved. The skin incision was routinely closed (Birol Yanik, Askin Görgülü, Talat Kiris, & Sabahattin Çobanoclu, 2002).



Picture 1: Rat common carotid artery's ligation during the surgery

3. **Carotid artery ischemia/reperfusion + MESNA (75 mg/kg) group (n=6):** 15 minutes before ischemia and just before reperfusion, [MESNA 75 mg/kg; intraperitoneally (i.p)] (Uromitexan, Baxter, Germany) was administered.
4. **Carotid artery ischemia/reperfusion + MESNA (150 mg/kg) group (n=6):** 15 minutes before ischemia and just before reperfusion, [MESNA 150 mg/kg; (i.p)] (Uromitexan, Baxter, Germany) was administered (Cheng et al., 2018) (Picture 2).



Picture 2: Animal groups and MESNA injection from the animal experiments

The animals were decapitated after 1-hour of reperfusion and blood and tissue samples (brain, heart and lungs) were collected (Picture 3). After complete clot formation, the sera were separated by centrifugation at 1500g x 10 min and stored at -80°C (Picture 4).

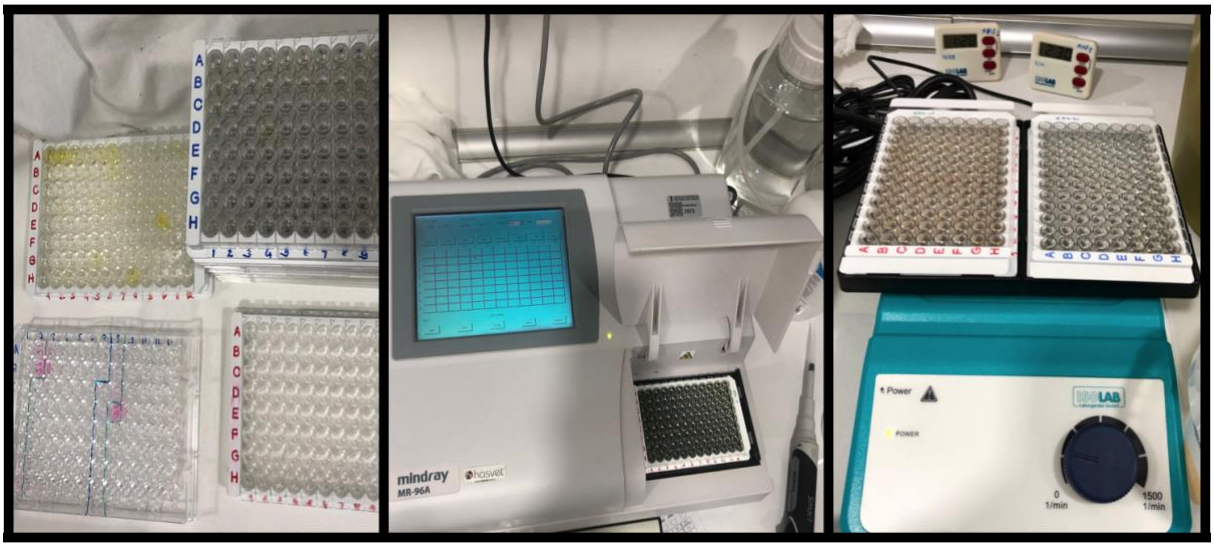
Picture 3: Collecting of tissues (brain, heart and lungs) from the rats

Picture 4: Centrifugation of the sera

3.2 Biochemical Assays

The sera were used to measure the activities of cell-leaking enzymes (alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH)), matrix metalloproteinase activities (MMP-1, -2, -8) and concentrations of tissue inhibitor of metalloprotease-1 (TIMP-1), tumor necrosis factor (TNF-α), interleukin (IL-1β), malondialdehyde (MDA), albumin, total protein, blood urea nitrogen (BUN), creatinin, amilase and lipase. Brain, heart and lung tissue samples obtained from each animal were placed in 10% formaldehyde for histopathological evaluation (Waterborg & Matthews, 1994).

Commercially available diagnostic test kits were run on an automated clinical chemistry analyzer to measure ALT, AST, ALP and LDH activities. Measurement of TNF-α, IL-1β, MMP-1, MMP-2, MMP-8 and TIMP-1 was performed using specific rat ELISA (Enzyme-linked immunosorbent assay) kits and according to the manufacturer's guidelines (Picture 5) (Ma, Ding, Zhang, & Liu, 2015).



Picture 5: ELISA kits that are used according to the manufacturer' guidelines

3.2.1 MDA assay

MDA, the most robust of lipid peroxidation, is also measured. The test is performed according to the spectrophotometric method based on the reaction with thiobarbituric acid (TBA) at 100°C in an acidic environment and measuring the absorbance of the reaction mixture at 530-540 nm (Ito, Sono, & Ito, 2019).

3.3 Histopathological Procedures

Tissues taken from the brain, heart and lungs of the decapitated subjects were washed in tap water for at least 3 hours or 1 night after they were taken into 10% formol, and dehydration with increasing alcohol concentrations (70% alcohol for 15 minutes, 90% alcohol 15 minutes with 96% alcohol, 30 minutes with 100% alcohol, 30 minutes twice with 100% alcohol, 2 times 30 minutes with 100% toluene), then they were kept in paraffin at 60°C for 1 night, the next day. tissue was embedded in paraffin blocks. After blocking, 5-6 mm thick sections were taken from the tissues and a slide was placed and left in toluene for 2 hours for paraffin recovery, then reduced to water with decreasing concentrations of alcohol (2 minutes with 100% alcohol, 2 minutes with 90% alcohol, it was treated with 70% alcohol for 2 minutes) and left in distilled water, after being treated with hematoxylin for 15 minutes, it was left in tap water for 10 minutes for bruising. After applying distilled water with eosin for 5 minutes additionally, dehydration with increasing concentrations of alcohol (70% alcohol for 2 minutes, 90% alcohol for 2 minutes, 96% alcohol for 2 minutes, with 100% alcohol for 10 minutes) and then washed 2 times with toluene (1st bath 5 minutes, 2nd bath 10 minutes) and the tissue was covered with entellan and the tissue was examined at the light microscope level (Otto, Franklin, & Clifford,

2015).

3.4 Statistical Analysis

Statistical analysis was performed using GraphPad Prism 9.3.1 (GraphPad Software, San Diego, CA, USA). Four groups of 6 animals were used. All data were expressed as means ± S.E. Datasets were compared using an one way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Values of $p<0.05$ were considered significant (Shin et al., 2019).

4 RESULTS

4.1 Biochemical Results

In the carotid artery ischemia/reperfusion group, serum levels of ALP, AST, ALT, LDH, TNF-α, IL-1β and MDA were significantly increased compared to the sham group ($p<0.01$-0.0001). After treatment with MESNA (75 mg/kg and 150 mg/kg), the serum biochemical parameters depressed significantly compared with the carotid I/R group ($p< 0.05$-0.0001) (Table 1).

Table 1: Alkaline phosphatase (ALP), aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), tumor necrosis factor (TNF-α), interleukin (IL-1β) and malondialdehyde (MDA) values of all groups in the rat carotid artery ischemia/reperfusion (I/R) model

	Sham	Carotid I/R	I/R + MESNA 75 mg/kg	I/R + MESNA 150 mg/kg
ALP (U/L)	60.67±7.86	109±10.03***	77.75±2.01$^{\#}$	68.37±6$^{\#\#}$
AST (U/L)	129±4.40	161.1±5.97**	129.8±6.69$^{\#\#}$	132.4±5.48$^{\#\#}$
ALT (U/L)	27.18±1.45	114.4±26.03**	35.35±7.40$^{\#\#}$	36.62±7.51$^{\#\#}$
LDH (U/L)	1360±153.7	2833±400.2**	1593±141.2$^{\#\#}$	1848±160.2$^{\#}$
TNF-α (pg/mL)	13.36±3.10	34.17 ±6.17**	19.69±1.58$^{\#}$	8.59±1.61$^{\#\#\#}$
IL-1β (pg/mL)	154.1±17.35	332.2±25.15****	146.3±16.89$^{\#\#\#\#}$	113.4±15.79$^{\#\#\#\#}$
MDA (μmol/L)	6.62±0.90	50.35±11.42***	9.67±1.13$^{\#\#\#}$	7.42±1.12$^{\#\#\#}$

Mean ± standard error

** $p<0.01$, *** $p<0.001$, **** $p<0.0001$ vs. sham

$^{\#}$ $p<0.05$, $^{\#\#}$ $p<0.01$, $^{\#\#\#}$ $p<0.001$, $^{\#\#\#\#}$ $p<0.0001$ vs. carotid I/R

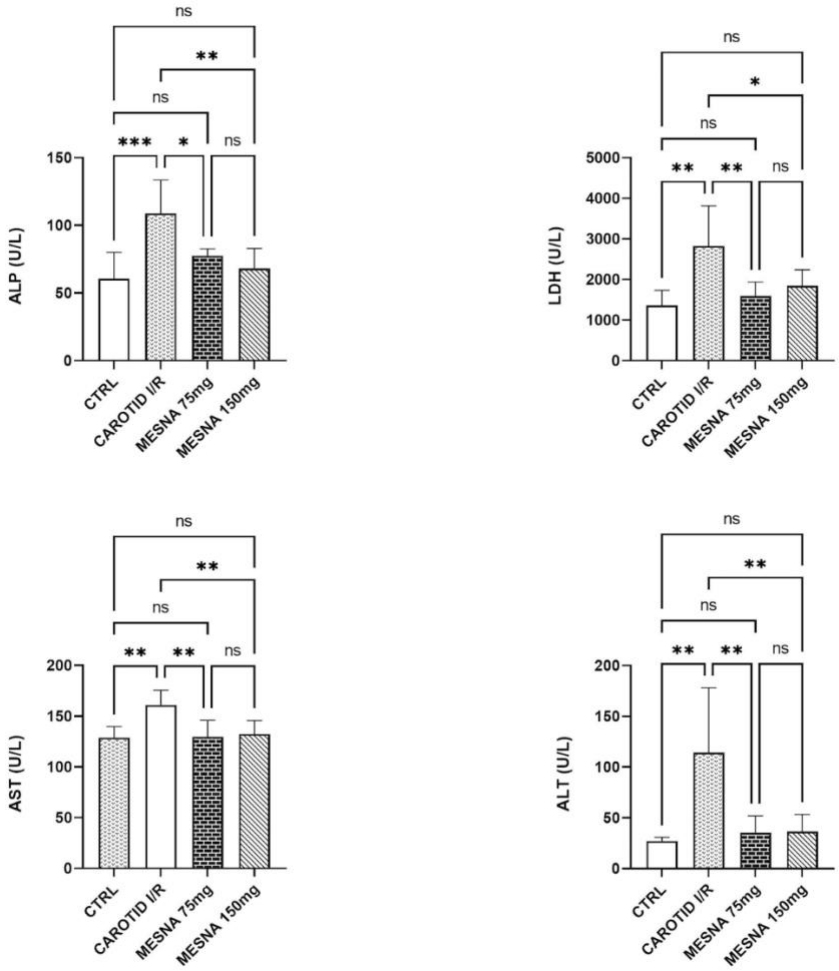


Figure 5: Alkaline phosphatase (ALP), aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) values of all groups in the rat carotid artery ischemia/reperfusion (I/R) model

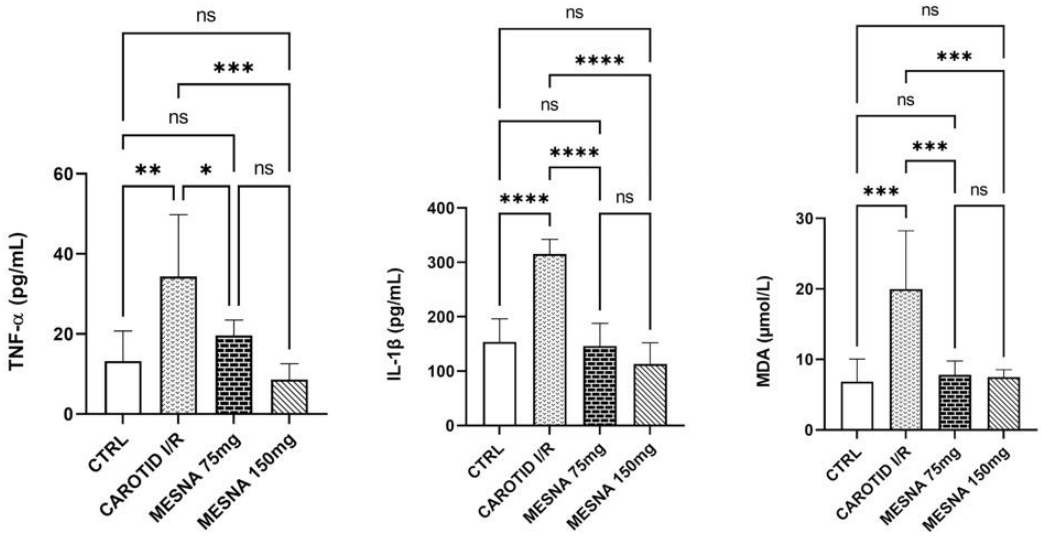


Figure 6: Tumor necrosis factor (TNF-α), interleukin (IL-1β) and malondialdehyde (MDA) values of all groups in the rat carotid artery ischemia/reperfusion (I/R) model

In the carotid artery ischemia/reperfusion group, the serum MMP-1, MMP-2, MMP-8, and TIMP-1 levels increased significantly compared to the sham group ($p<0.01$-0.0001). After treatment with MESNA (75 mg/kg and 150 mg/kg) the serum MMP-1, MMP-2, MMP-8, and TIMP-1 levels reduced significantly compared with the carotid artery I/R group ($p< 0.05$-0.001) (Table 2).

Table 2: Matrix metalloproteinases (MMP-1, -2, -8), tissue inhibitor of metalloprotease-1 (TIMP-1) values of all groups in the rat carotid artery ischemia/reperfusion (I/R) model

	Sham	Carotid I/R	I/R + MESNA 75 mg/kg	I/R + MESNA 150 mg/kg
MMP-1 (pg/mL)	1.51±0.15	3.82±0.19****	2.49±0.28$^{\#\#}$	2.30±0.20$^{\#\#\#}$
MMP-2 (pg/mL)	30.93±5.90	56.32±6.0**	28.69±2.27$^{\#\#}$	22.61±1.57$^{\#\#\#}$
MMP-8 (pg/mL)	72.39±7.71	190.7±43.07***	96.48±4.75$^{\#}$	82.79±4.74$^{\#}$
TIMP-1 (pg/mL)	383.7±45.13	899.2±71.42***	640.2±69.94$^{\#}$	405.6±62.83$^{\#\#\#}$

Mean ± standard error

** $p<0.01$, **** $p<0.0001$ vs. sham

$^{\#}$ $p<0.05$, $^{\#\#}$ $p<0.01$, $^{\#\#\#}$ $p<0.001$ vs. carotid I/R

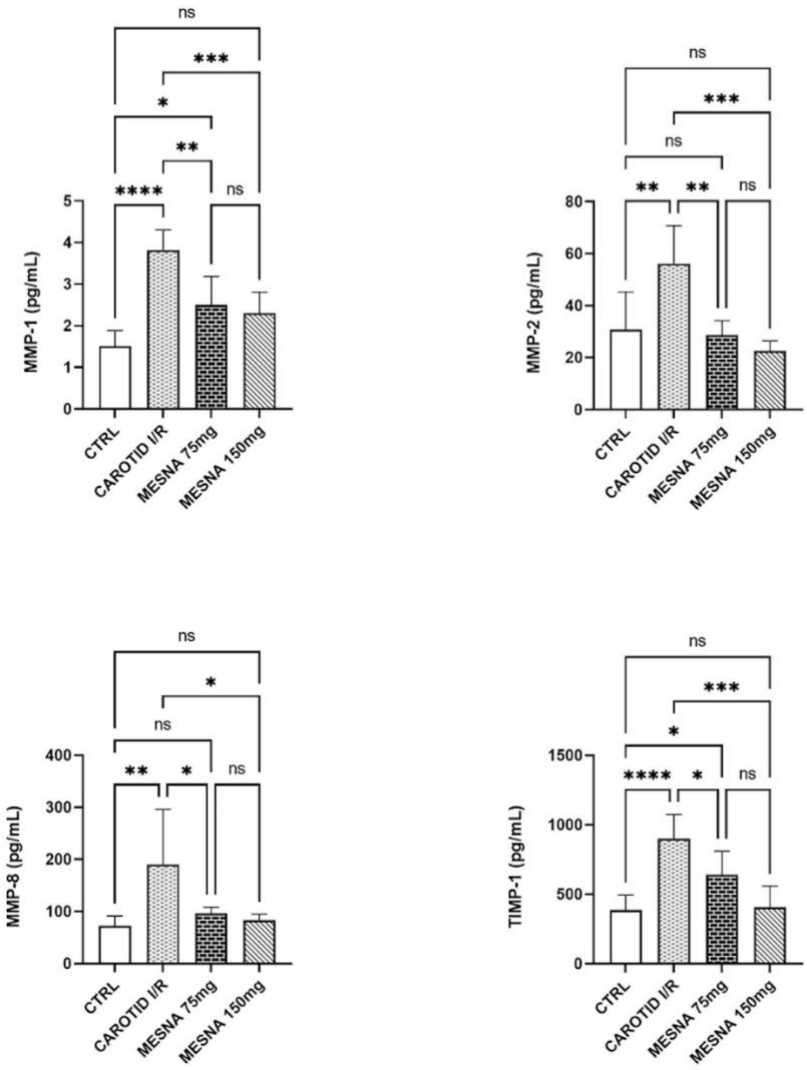


Figure 7: Matrix metalloproteinases (MMP-1, -2, -8), tissue inhibitor of metalloprotease-1 (TIMP-1) values of all groups in the rat carotid artery ischemia/reperfusion (I/R) model

Albumin, total protein and creatinine values did not significantly change in the carotid artery ischemia/reperfusion group. No significant ($p>0.05$) change was observed on these values after the MESNA application. Only BUN value increased in the carotid ischemia/reperfusion group compared to the sham group ($p<0.001$). With the MESNA application, a significant decrease was observed in the BUN value ($p<0.01$).

Table 3: Albumin, total protein, blood urea nitrogen (BUN) and creatinine values of all groups in the rat carotid artery ischemia/reperfusion (I/R) model

	Sham	Carotid I/R	I/R+MESNA 75 mg/kg	I/R+MESNA 150 mg/kg
Albumin (g/dL)	3.12±0.13	3.48±0.06	3.32±0.09	3.22±0.09
Total protein (g/dL)	5.15±0.22	5.53±0.12	5.32±0.09	5.26±0.2
BUN (mg/dL)	23.75±1.34	35.78±2.66***	25.48±1.17##	26.03±1.48##
Creatinine (mg/dL)	0.66±0.08	0.95±0.15	0.77±0.03	0.86±0.05

Mean ± standard error

*** p<0.001 vs. sham

p<0.01 vs. carotid I/R

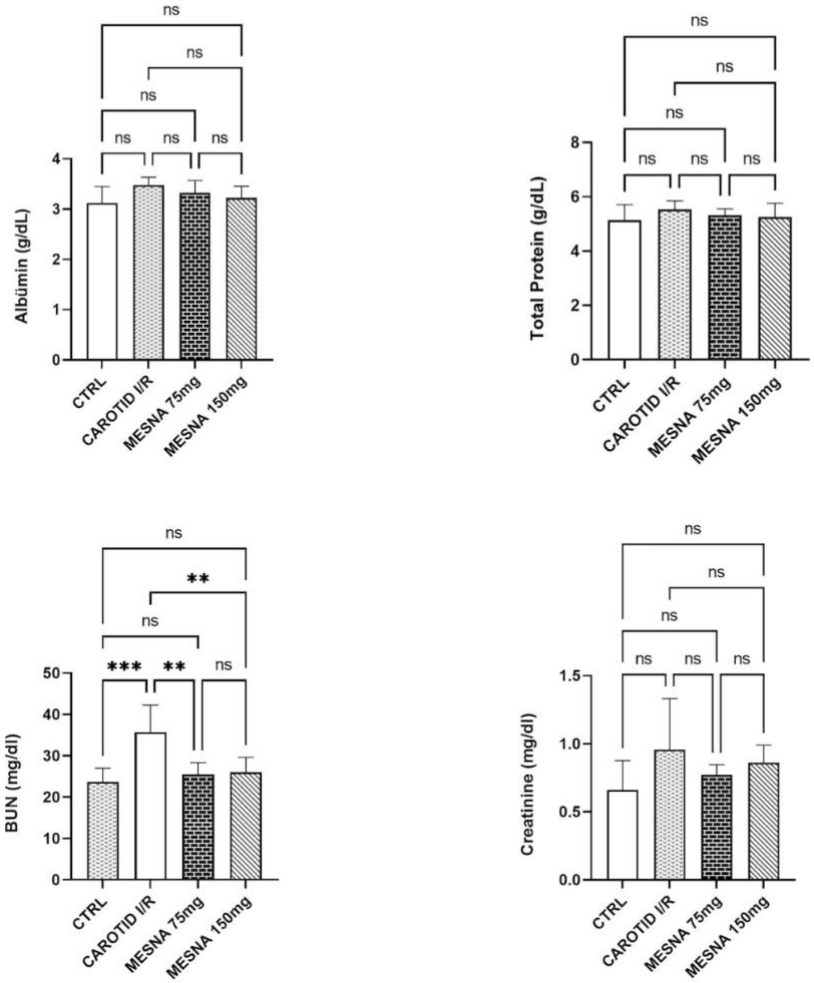


Figure 8: Albumin, total protein, blood urea nitrogen (BUN) and creatinine values of all groups in the rat carotid artery ischemia/reperfusion (I/R) model

When the amylase and lipase values were examined in the study, there was no significant ($p>0.05$) change in the amylase value in any of the groups. Lipase value showed little change in the carotid artery ischemia/reperfusion group compared to the sham group ($p<0.05$). With the MESNA application, it decreased in a non-significant way ($p<0.05$).

Table 4: Amylase and lipase values of all groups in the rat carotid artery ischemia/reperfusion (I/R) model

	Sham	Carotid I/R	I/R+MESNA 75 mg/kg	I/R+MESNA 150 mg/kg
Amylase (U/L)	1856±141.6	2912±584.8	2039±83.75	1857±75.4
Lipase (U/L)	28±2.74	41.17±5.15*	26.33±1.14#	26±2#

Mean ± standard error

* $p<0.05$ vs. sham

# $p<0.05$ vs. carotid I/R

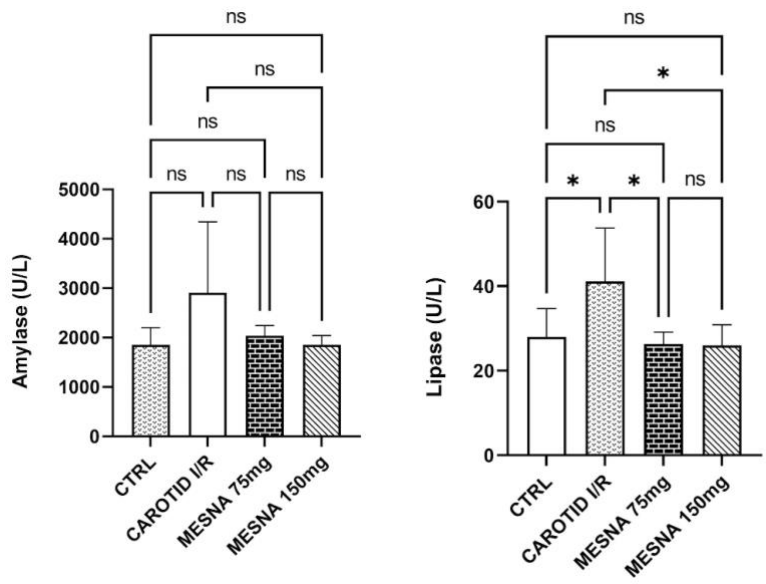


Figure 9: Amylase and lipase values of all groups in the rat carotid artery ischemia/reperfusion (I/R) model

4.2 Histopathological Results

The brain neutrophile layout, brain capillary intensity, and neuronal degeneration scores increased significantly in the carotid I/R group compared to the sham group ($p<0.0001$). After treatment with MESNA (75 mg/kg and 150 mg/kg), the elevated brain neutrophile layout, brain capillary intensity, and neuronal degeneration levels were significantly suppressed ($p< 0.01$-0.0001) (Table 5).

Table 5: In the rat carotid artery ischemia/reperfusion (I/R) model, brain neutrophile layout, brain capillar intensity and neuronal degeneration scores of all groups

	Sham	Carotid I/R	I/R + MESNA 75 mg/kg	I/R + MESNA 150 mg/kg
Brain neutrophile layout	0.28±0.05	2.35±0.19****	1.43±0.05$^{\#\#\#\#}$	1.23±0.04$^{\#\#\#\#}$
Brain capillar intensity	0.25±0.02	2.4±0.07****	1.7±0.05$^{\#\#\#\#}$	1.23±0.04$^{\#\#\#\#}$
Neuronal degeneration	0.33±0.05	2.9±0.07****	2.55±0.04$^{\#\#}$	2.26±0.06$^{\#\#\#\#}$

Mean ± standard error

**** p<0.0001 vs. sham

$^{\#\#}$ p<0.01, $^{\#\#\#\#}$ p<0.0001 vs. carotid I/R

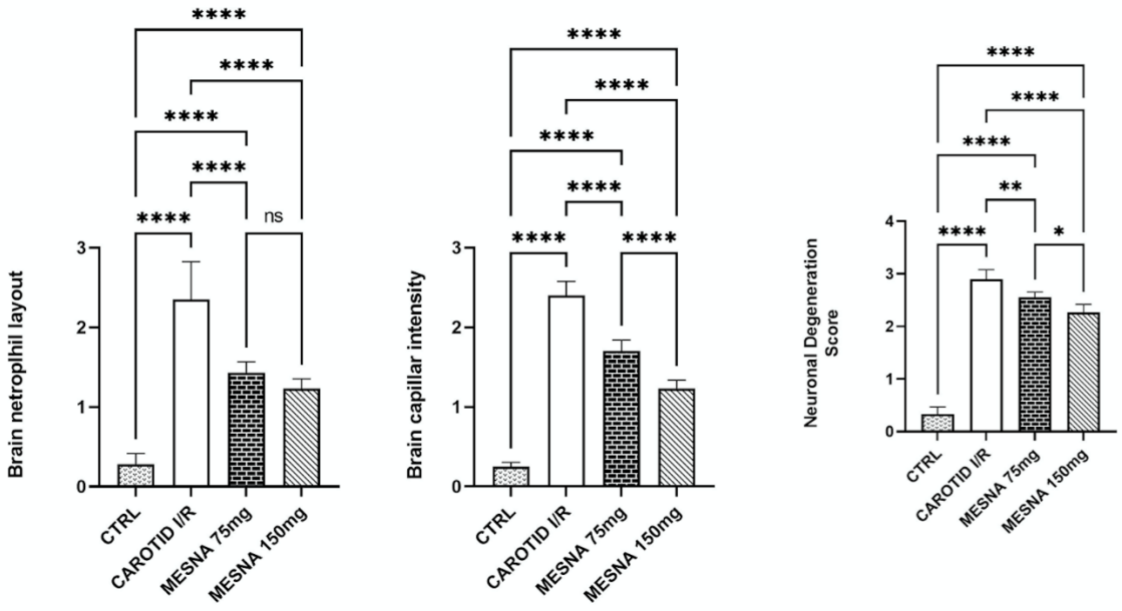


Figure 10: In the rat carotid artery ischemia/reperfusion (I/R) model, brain neutrophile layout, brain capillar intensity and neuronal degeneration scores of all groups

Sham group rats showed regular neuronal and neuropil morphology in the cortex (Fig. 11a). However, rats in the carotid artery I/R group (Fig. 11b) showed degenerated neurons with marked shrinkage of the nucleus and cytoplasm. Rats treated with I/R and 75 mg/kg MESNA revealed decreased neuronal degeneration when compared to rats in the carotid artery I/R group

(Fig. 11c). The carotid artery I/R and 150 mg/kg MESNA group demonstrated regeneration in neuropil morphology (Fig. 11d).

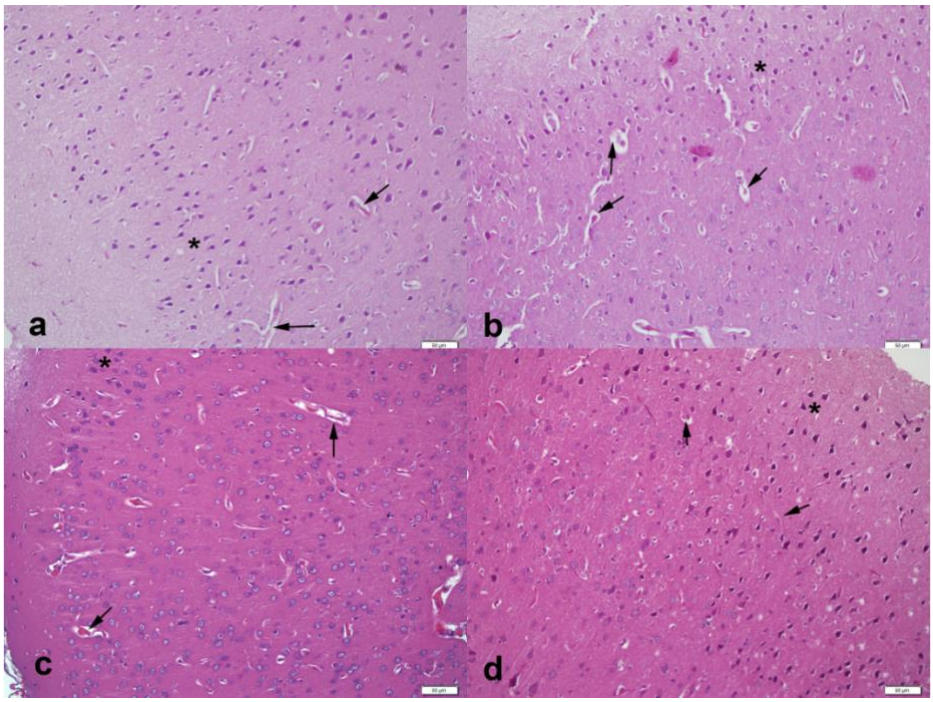


Figure 11: Microscopic examination of brain sections

Fig.11a: Sham group, regular neuropil morphology (*) and capillaries (arrows); Fig.11b: Carotid I/R group, degenerated neurons (*) and capillaries (arrows); Fig.11c: I/ R + MESNA 75 mg/kg group, reduced neuronal degeneration (*) capillaries (arrows); Fig.11d: I/R + MESNA 150 mg/kg group, regenerated neuronal neurons (*) capillaries (arrows).

The lung congestion, alveolar degeneration and interstitial oedema scores in the carotid I/R group were found to be significantly higher than those in the sham group rats ($p<0.0001$). When MESNA (75 mg/kg and 150 mg/kg), was administered after the ischemia and the subsequent reperfusion phase, the elevations in lung congestion, alveolar degeneration and interstitial oedema scores were significantly depressed ($p<0.001$-0.0001) (Table 6).

Table 6: In the rat carotid artery ischemia/reperfusion (I/R) model, lung congestion, alveolar degeneration and interstitial oedema scores of all groups

	Sham	Carotid I/R	I/R + MESNA 75 mg/kg	I/R + MESNA 150 mg/kg
Lung congestion	0.61±0.06	2.95±0.08****	2.11±0.15$^{####}$	1.78±0.08$^{####}$
Alveolar degeneration	0.51±0.06	2.86±0.18****	2.21±0.14$^{###}$	1.68±0.08$^{####}$
Interstitial oedema	0.65±0.07	2.96±0.06****	2.13±0.66$^{####}$	1.61±0.13$^{####}$

Mean ± standard error

**** p<0.0001 vs. sham

p<0.001, #### p<0.0001 vs. carotid I/R

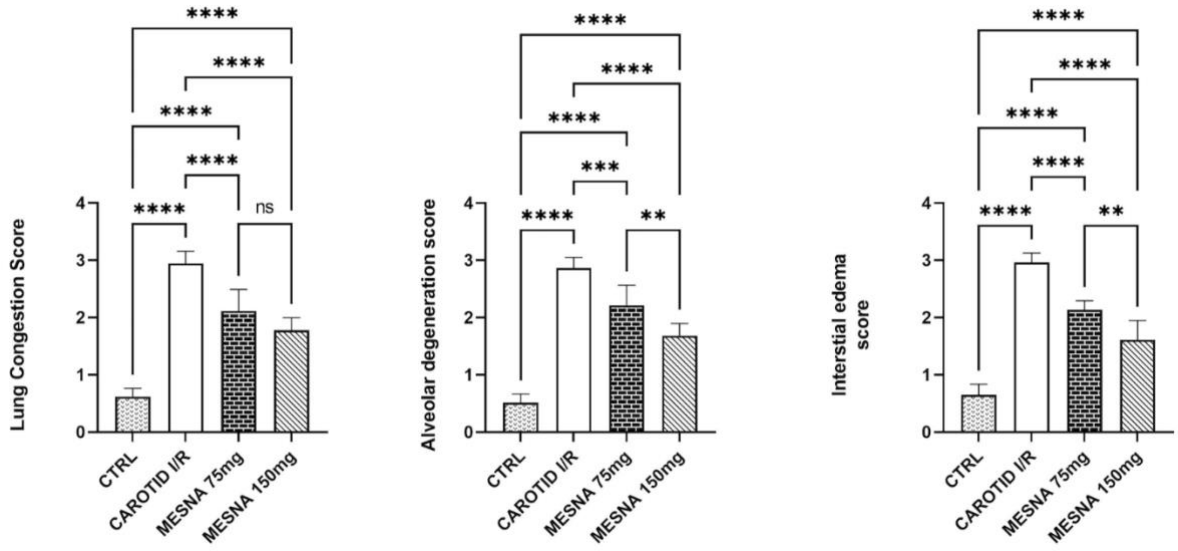


Figure 12: In the rat carotid artery ischemia/reperfusion (I/R) model, lung congestion, alveolar degeneration and interstitial oedema scores of all groups

In light microscopic observation of the lungs in the sham group, alveolar structure and interstitial space showed a uniform appearance (Fig. 13a). In the carotid IR group, diffuse and severe hemorrhage with edema in interstitial spaces was observed. Besides, distended alveolar walls and decreased alveolar space due to edema as well as severe leukocytes accumulation were observed (Fig. 13b). In IR+ 75 mg/kg MESNA group, due to the regression of hemorrhage in the interstitial space, an improvement in alveolar structure and a decrease in leukocyte accumulation were seen (Fig. 13c). In the I/R + 150 mg/kg MESNA group the alveolar structure regenerated besides regression of interstitial edema (Fig. 13d).

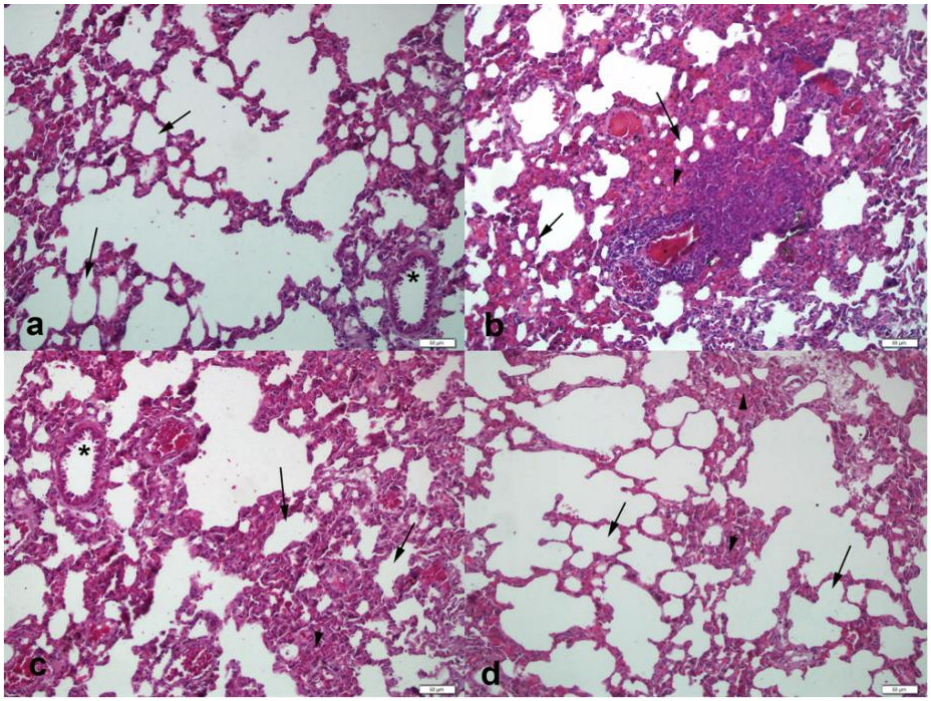


Figure 13: Microscopic examination of lung sections

Fig.13a: Sham group, regular alveolar structure (arrows) and interstitial space, bronchiole (*); Fig.13b: Carotid I/R group, severe and diffuse interstitial edema (arrowhead) and capillary obstruction in hemorrhagic areas and a decrease of the alveolar spaces (arrows); Fig.13c: Group I/R + MESNA 75 mg/kg, structural improvement of the alveolar structures (arrows) by regression of the interstitial edema (arrowhead) and bronchioles (*); Fig.13d: Group I/R+ MESNA 150 mg/kg, recovery highlighted in alveolar structure (arrows) and reduced interstitial edema (arrowheads).

In the carotid I/R group, heart congestion, cardiomyocyte degeneration, and inflammation scores showed a drastic increase ($p<0.0001$) as compared to the sham group. Conversly, the increase in cardiomyocyte degeneration was significantly recovered in both IR+MESNA 75 mg/kg and I/R + MESNA 150 mg/kg groups ($p< 0.05$-0.0001) (Table 7).

Table 7: In the rat carotid artery ischemia/reperfusion (I/R) model, heart congestion, cardiomyocyte degeneration and inflammation scores of all groups

	Sham	Carotid I/R	I/R + MESNA 75 mg/kg	I/R + MESNA 150 mg/kg
Heart congestion	0.5±0.08	2.8±0.03****	2.3±0.07	1.8±0.16$^{\#}$
Cardiomyocyte degeneration	0.3±0.03	2.51±0.04** **	2.08±0.06$^{\#\#\#}$	1.78±0.06$^{\#\#\#\#}$
Inflammation	0.28±0.04	2.3±0.05****	1.733±0.06	1.4±0.07$^{\#}$

Mean ± standard error

**** p<0.0001 vs. sham

# p<0.05, ### p<0.001, #### p<0.0001 vs. carotid I/R

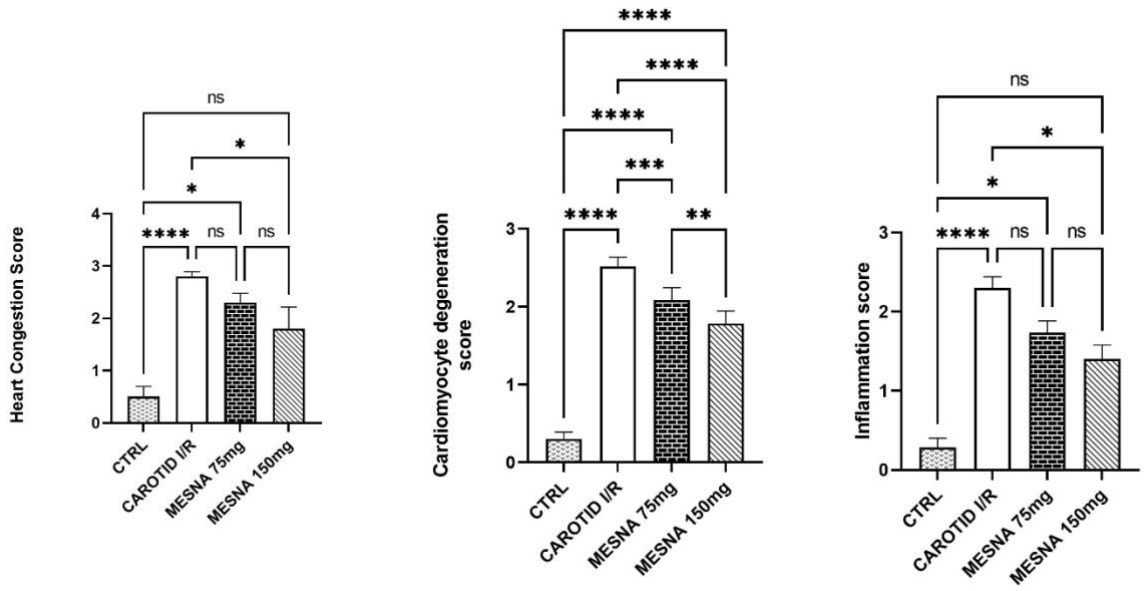


Figure 14: In the rat carotid artery ischemia/reperfusion (I/R) model, heart congestion, cardiomyocyte degeneration and inflammation scores of all groups

In light microscopic observation of the hearts in the sham group, regular alignment of the cardiomyocytes along with numerous capillaries was observed (Fig.14a). In the carotid IR group, remarkable interstitial edema and congestion in capillaries and prominent perinuclear and cytoplasmic disorganization in the cardiomyocytes were observed (Fig.14b). In the IR + 75 mg/kg MESNA group, the general cardiac morphology demonstrated a decrease in interstitial edema and congestion in capillaries in addition to regeneration in the cardiomyocyte (Fig.14c). In the I/R + 150 mg/kg MESNA group the congestion was remarkably reduced and regular cardiomyocytes were prominent (Fig.14d)

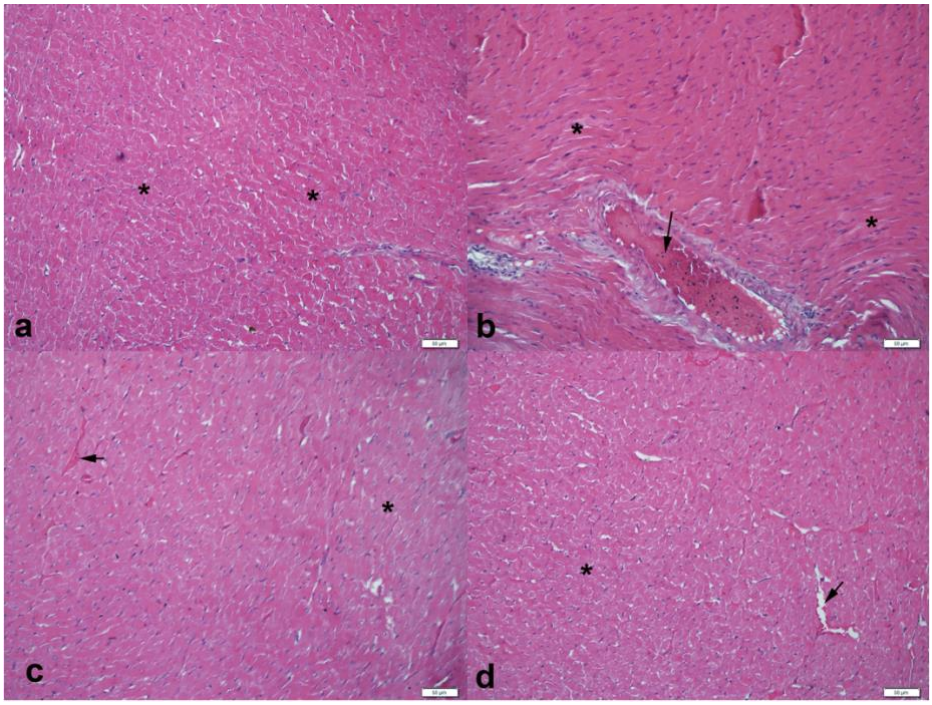


Figure 15: Microscopic examination of heart sections

Fig.15a: Sham group, regular arrangement of cardiomyocytes and capillaries (*); Fig.15b: Carotid I/R group, severe congestion of the capillaries (arrow), cytoplasmic disorganization in cardiomyocytes (*); Fig.15c: I/R+ group MESNA 75 mg/kg, reduction in capillary congestion (arrowhead) and relative organization of cardiomyocytes (*); Fig. 15d: Group I/R+ MESNA 150 mg/kg, marked regression of capillary congestion (arrow) and regular pattern of cardiomyocytes (*).

5 DISCUSSION

In our study, we aimed to investigate local and distant organ damage due to carotid ischemia, investigate the role of proinflammatory cytokines and proteolytic enzymes, and investigate the protective effects of MESNA, which has anti-inflammatory properties. We found that serum levels of proteolytic enzymes along with pro-inflammatory cytokines increased after carotid I/R and this was accompanied by pathological damage to brain, heart and lung tissue. We have obtained the results that the damage seen as a result of carotid artery I/R in all biochemical and histopathological data was reduced by the administration of MESNA at two different doses (75 mg/kg and 150 mg/kg).

Although various agents have been tested against carotid ischemia, it retains its importance as a major health problem. MESNA, which we use for therapeutic purposes, has been shown to have protective effects against cyclophosphamide toxicity and in various models of inflammation with its anti-inflammatory properties. In this study, the protecting consequences of MESNA in opposition to carotid artery I/R damage had been demonstrated. As in all models of inflammation, it has been shown that the rate of biochemical markers indicative of tissue damage is increased in carotid ischemia/reperfusion. In particular, the increase in ALP, LDH, AST and ALT during carotid ischemia are important parameters to show that they destroy tissue. In our study, it was found to be significantly increased in the carotid I/R group, consistent with the literature.

Improving the imbalance of these parameters with MESNA, which we use in the treatment, is considered a positive result. The suppression of ALP, LDH, AST, and ALT activations in previous MESNA studies, particularly in inflammation-related models, is consistent with our results, suggesting that it may have a protective effect against carotid artery I/R injury. It is known that oxidative stress is highly effective during ischemia/reperfusion injury. Lipid molecules are among the biomolecules most affected by oxidative stress. MDA is a product of oxidative stress resulting from the peroxidation of polyunsaturated fatty acids (PUFA). Previous studies have shown increased levels of MDA when I/R injury is induced in tissues. In our study, MDA values increased significantly when the I/R lesion was created. When applying the MESNA treatment, the MDA values were reduced. In the literature, the effect of MESNA on lipid peroxidation products has similar results in I/R injury. The parallelism of this MDA result in our study with the literature indicated that MESNA also prevented damage caused by carotid ischemia/reperfusion in the brain, lungs and heart by lipid peroxidation.

Another parameter we measured were TNF-α and IL-1β, which are cytokine proteins with

diverse biological functions that are particularly effective in inflammatory and immune responses. These are mainly produced by macrophages and T-lymphocytes in response to stressed or damaged tissue and can therefore serve as a systemic marker for tissue injury. Previous studies have shown that carotid I/R increases serum levels of TNF-α and IL-1β. In our study, levels of TNF-α and IL-1β increased significantly in the carotid I/R group, which was consistent with the literature. After MESNA treatment, this increase approached control values and histopathological results indicated that tissue damage was significantly prevented, and our study is consistent with the literature in this regard. In contrast to other studies, in our study TNF-α decreased at the same rate with increasing MESNA dose. Compared to MESNA 75 mg/kg, MESNA 150 mg/kg showed a three-fold improvement in TNF-α, showing how important the MESNA dose was for this biomarker.

Matrix metalloproteinases (MMP-1, -2, -8) regulate the breakdown of the extracellular matrix (ECM). In addition to the degradation of ECM substrates, another biomarker that we also examined in our study, tissue inhibitor of metalloproteinase-1 (TIMP-1), regulates MMP activity. MMPs are believed to be involved in ischemia-induced damage to the brain, lungs and heart, so we gave importance to the results obtained from MMPs in our study based on this literature. MESNA administration suppressed elevated MMP levels in carotid ischemia/reperfusion compared to the sham group. This result indicates that according to the literature it will be effective in treating local and distant organ damage due to ischemia.

Parameters we also measured were Albumin (g/dL), Total protein (g/dL), BUN (mg/dL), and Creatinine (mg/dL). Along with the creatinine test, the BUN test is primarily used to assess kidney function under various conditions, to aid in the diagnosis of renal disease, and to track individuals who have kidney dysfunction or failure. Albumin, which is the most abundant plasma protein in the blood, is a protein synthesized by the liver and together with globulin, they form the total protein. Serum albumin and total protein values are parameters that increase in chronic kidney disease in conjunction with each other. Therefore, in our study, the fact that they did not undergo any change in the group with carotid artery ischemia/reperfusion injury is in line with the literature. Serum albumin and total protein parameters, which did not increase in carotid damage, did not change with the application of MESNA. Since creatine is an increasing parameter in acute renal injury and kidney dysfunction or failure according to the literature, it did not significantly increase in carotid artery ischemia reperfusion injury. In addition, since BUN can increase in ischemic stroke, it increased in the ischemia group in parallel with this in our study. He responded to the treatment at either 75 mg/kg or 150 mg/kg,

regardless of doses, in the MESNA treatment given afterwards. In this context, BUN results decreased after MESNA injection, in line with the literature.Amylase is a type of enzyme used in the digestion of carbohydrates, while Lipase is used in the digestion of fats. They generally increase in pancreatic damage and play an important role in the diagnosis of pancreatitis. In our study, when we checked whether it increased in carotid ischemia/reperfusion injury, although there was no increase in amylase, the lipase value increased in trace amounts in the carotid artery I/R group. The connection of this in the literature is directly proportional to the lipase enzyme, which can increase in some ischemia situations. A very small decrease with the MESNA application shows us that this parameter does not give a meaningful result.

I/R of the carotid artery is a disease that, with its risks, has shown an increasing prevalence in recent years. It is known to cause brain damage and distant tissue damage (lungs and heart). In our study we focused on the damage to these three organs and many values were measured histopathologically. According to the study results, cerebral neutrophilic disposition, cerebral capillary intensity and brain neuronal degeneration were greatly increased in carotid ischemia/reperfusion, and all of these histopathological values decreased after the administration of MESNA. This means that much of the damage caused or caused to the brain by carotid ischemia can be prevented by the use of MESNA. In particular, the rate of recovery from neuronal degeneration paralleled increasing MESNA dose. MESNA 150 mg/kg was 2-fold more effective than MESNA 75 mg/kg administered in relieving neuronal degeneration. Studies have shown that this I/R causes a lot of permanent damage, particularly to the lungs when distant tissue damage is examined. In this study, we also demonstrated histopathologically that carotid artery ischemia and reperfusion cause congestion, alveolar degeneration and interstitial edema in the lungs and obtained results of visible improvement of MESNA treatment. In particular, in Group 4 where MESNA was administered at a dose of 150 mg/kg, the alveolar degeneration was much less than when administered at 75 mg/kg. This information has not been shown in previous studies, and it has been demonstrated that MESNA can inhibit this degeneration much more with a dose increase. Another remote organ injury of carotid artery I/R occurs in the heart. Previous studies have established, that ischemic reperfusion causes a lot of damage to the heart, but there is not enough data for the treatment of these damages, especially in the brain. In this experimental study, we have made an important contribution to the literature by preventing cardiac congestion, cardiomyocyte degeneration and inflammation with MESNA treatment. Cardiomyocyte degeneration improved effectively with increasing doses of MESNA, as we aimed.

6 CONCLUSION

In summary, in our experimental study, we showed that two different doses of MESNA, 75 mg/kg and 150 mg/kg, were effective in carotid ischemia/reperfusion, the prevalence and mortality of which have been increasing in recent years. In addition, according to our results, distant organ injury (brain/lung/heart) caused by carotid ischemia/reperfusion was also treated with MESNA. In this sense, the knowledge that MESNA can prevent this harm in clinical use should be included in the literature. The healing effect of MESNA, which we have gained in particular against degeneration in the brain, lungs and heart, will lead to new treatment methods.

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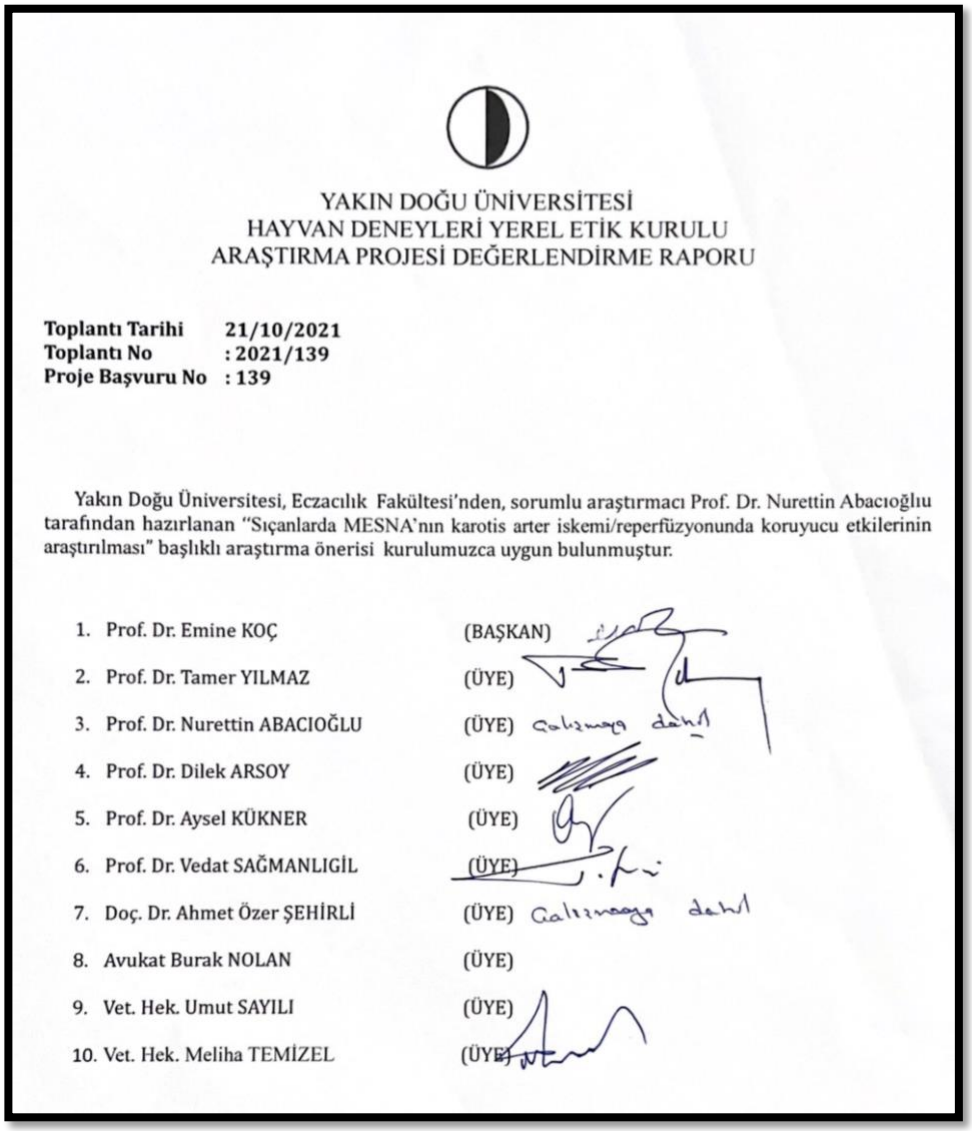
APPENDICES

Appendix A

Ethical Approval Document

The research protocols were approved by the Animal Experiments Ethics Committee of Near East University (Protocol No: 2021/139-139).



YAKIN DOĞU ÜNİVERSİTESİ
HAYVAN DENEYLERİ YEREL ETİK KURULU
ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU

Toplantı Tarihi **21/10/2021**
Toplantı No **: 2021/139**
Proje Başvuru No **: 139**

Yakın Doğu Üniversitesi, Eczacılık Fakültesi'nden, sorumlu araştırmacı Prof. Dr. Nurettin Abacıoğlıu tarafından hazırlanan "Sıçanlarda MESNA'nın karotis arter iskemi/reperfüzyonunda koruyucu etkilerinin araştırılması" başlıklı araştırma önerisi kurulumuzca uygun bulunmuştur.

1. Prof. Dr. Emine KOÇ (BAŞKAN)
2. Prof. Dr. Tamer YILMAZ (ÜYE)
3. Prof. Dr. Nurettin ABACIOĞLU (ÜYE) Çekinmeye dahil
4. Prof. Dr. Dilek ARSOY (ÜYE)
5. Prof. Dr. Aysel KÜKNER (ÜYE)
6. Prof. Dr. Vedat SAĞMANLIGİL (ÜYE)
7. Doç. Dr. Ahmet Özer ŞEHİRLİ (ÜYE) Çekinmeye dahil
8. Avukat Burak NOLAN (ÜYE)
9. Vet. Hek. Umut SAYILI (ÜYE)
10. Vet. Hek. Meliha TEMİZEL (ÜYE)

Appendix B

Curriculum Vitae

Personal Information

Surname, Name: Mercan, Merve

Date of Birth: 21 January 1993

Place of Birth: Şişli, İstanbul

Table B1.

Education.

Degree	Department/Program	University	Year of Graduation
M.Pharm.	Pharmacy	Near East University	2017

Table B2.

Work Experience.

Title	Place	Year
Research Assistant and Lecturer	NEU, Faculty of Pharmacy, Department of Pharmacology	2020-2022

Foreign Languages

Fluent spoken and written English (YÖKDİL: 72.5)

ALES: 74.5

Publications in International Journals

Mercan, M., Şehirli, A. Ö., Chukwunyere, U., & Abacıoğlu, N. (2021). Acute kidney injury due to COVID-19 and the circadian rhythm. *Medical Hypotheses*, *146*, 110463.

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CHUKWUNYERE, U., SEHIRLI, A. Ö., SAYINER, S., CEYLANLI, D., MERCAN, M., ÇETINEL, Ş., ... & ABACIOĞLU, N. (2022). Dose-dependent Effect of Oxytocin on 5-Fluorouracil-induced Intestinal Mucositis in Rats. *Latin American Journal of Pharmacy*, *41*(12), 2476-84.

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hadmek Hayvan Deneyleri Merkezi Etik Kurulu

KOBAY DENEY HAYVANLARI LAB. SAN. VE TİC. A.Ş.

HAYVAN DENEYLERİ YEREL ETİK KURULU

DENEY HAYVANLARI KULLANIM SERTİFİKASI

Sayın MERVE MERCAN

29.11.2021 - 10.12.2021 tarihleri arasında Kobay Deney Hayvanları Lab. San. ve Tic. A.Ş. HADYEK tarafından Ankara İlinde düzenlenen "Deney Hayvanları Kullanım Sertifikası" eğitimini başarı ile tamamlayarak A kategorisi sertifikası almaya hak kazanmıştır.

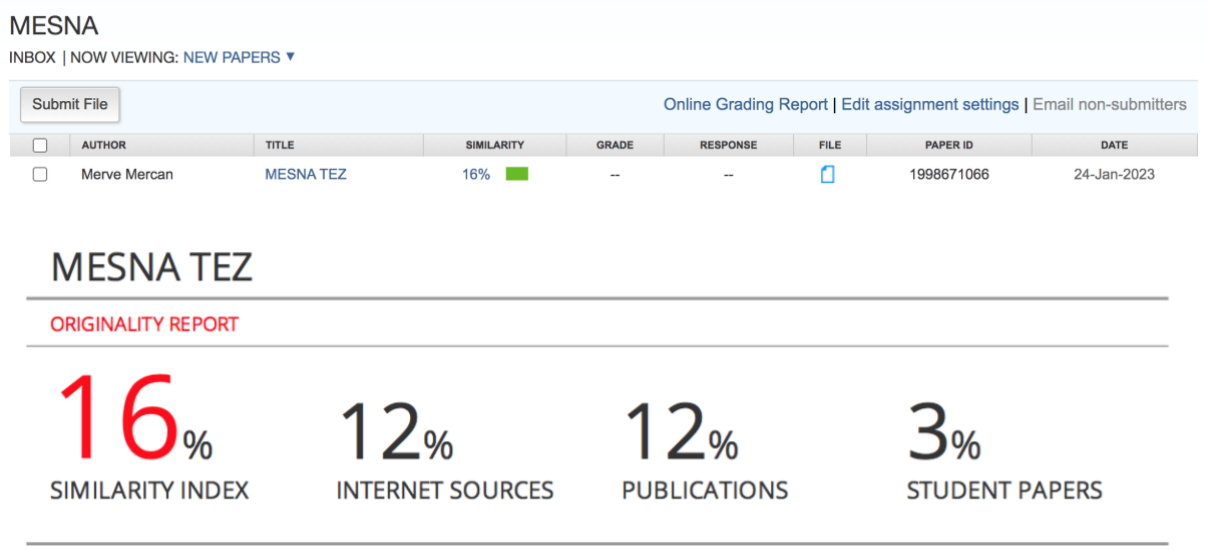
Veteriner Hekim

AYŞE BEGÜM BUĞDAYCI AÇIKKOL

HADYEK Başkanı/Sorumlu Yönetici

Appendix C

Similarity Report



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