T.C.
YEDİTEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF PHARMACEUTICAL TOXICOLOGY

INVESTIGATION OF THE MECHANISMS OF STATIN USE IN PREVENTION OF CONTRAST INDUCED NEPHROPATHY IN RATS

THE DOCTOR OF PHILOSOPHY THESIS

MESUT MOLLAOĞLU, MD

İSTANBUL-2023

İSTANBUL-2023

T.C.
YEDİTEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF PHARMACEUTICAL TOXICOLOGY

INVESTIGATION OF THE MECHANISMS OF STATIN USE IN PREVENTION OF CONTRAST INDUCED NEPHROPATHY IN RATS

THE DOCTOR OF PHILOSOPHY THESIS

MESUT MOLLAOĞLU, MD.

SUPERVISOR
PROF. DR. AHMET AYDIN

İSTANBUL-2023

APPROVAL

Institute : Yeditepe University Institute of Health Sciences

Programme : Doctoral Program in Pharmaceutical Toxicology

Title of the Thesis : Investigation Of The Mechanisms Of Statin Use In Prevention Of Contrast Induced Nephropathy In Rats.

Owner of the Thesis : Mesut Mollaoğlu

Examination Date : 13/10/2023

The Jury judges that the candidate named above has satisfactorily completed his thesis.

Chair of the Jury:	Title, Name-Surname (Institution)
Supervisor:	Prof. Dr. Ahmet AYDIN (Yeditepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology)
Member/Examiner:	Prof. Dr. Semra ŞARDAŞ (İstinye University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology)
Member/Examiner:	Prof. Dr. Turgay ÇELİK (Yeditepe University, Faculty of Pharmacy, Department of Pharmacology)
Member/Examiner:	Assoc. Prof. Dr. Muhammet HAMİTOĞLU (Yeditepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology)
Member/Examiner:	Dr. Öğr. Üyesi Sinem HELVACIOĞLU AKYÜZ (İstinye University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology)

APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated ..... and numbered .....

Prof. Dr. Bayram Yılmaz

Director of Institute of Health Sciences

DECLERATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

13/10/2023

Signature

Mesut MOLLAOĞLU

ACKNOWLEDGEMENT

I would like to thank my thesis advisor and mentor, Prof. Dr. Ahmet Aydın for his support, for giving me the opportunity for this research and for the knowledge and experience that I gained from him. Aside from these properties he also showed me what it means to be a successful researcher, a distinguished scholar and mentor. Without his constant guidance, none of this would be possible.

Additionally I would like to thank Assoc. Prof. Dr. Muhammed Hamitoğlu and Prof. Dr. Turgay Çelik who worked for my thesis committee for their time, insights, audits and guidance during my project. I would like to thank Assoc Prof. Dr. Muhammed Hamitoğlu, Prof. Dr. Gönül Şahin, Prof. Dr. Hande Sipahi for their contributions throughout my training period and to Prof. Dr. Ahmet Aydın for conveying his knowledge and skills with dedication and patience during my training.

I would like to thank Dr. Engin Sümer and his team within Yeditepe University Experimental Research Center for not only sharing their experiences and their support, but also for their friendship. I would also like to thank Dr Enver Yarıkkaya and Dr Tuba Sutaşır, who helped my project with their knowledge and experience.

I would especially like to thank Assoc. Prof. Fatih Doğanay, with whom I work as an Emergency Medicine Doctor, for teaching me a lot about professional practice, colleagues and ethics, as well as for his values and contributions to my thesis project.

And lastly, I would like to thank my wife, Dr Ezgi Mollaoğlu, who is my colleague and advisor in all my work. She always supported me during my residency and doctoral training, and she did not hesitate to offer her help for everything. Without her support, I wouldn't be where I am today.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURE

SYMBOLS/ABBREVIATIONS

ABH	Akut Böbrek Hasarı
ADP	Adenosine diphosphate
AKI	Acute kidney injury
AKI:	Acute kidney injury
AMP	Adenosine Monophosphate
BSA	Bovine Serum Albumin
CAT	Catalase
CHF	Congestive Heart Failure
CI-AKI	Contrast Induced Acute Kidney Injury
CIN	Contrast Induced Nephropathy
CKD	Chronic Kidney Disease
CM	Contrast Media
eGFR	Estimated Glomerular Filtration Rate
ELISA	Enzyme Linked Immunosorbent Assay
eNOS	Endothelial NOS
FRAP	Ferric Reducing Antioxidant Power
GPx	Glutathione Peroxidase
GSH	Glutathione
Hb	Hemoglobin
KM-ABH	Kontrast Madde İlişkili Akut Böbrek Hasarı
LOCM	Low Osmolality Contrast Media
MDA	Malondialdehyde
MMP-9	Matrix Metalloproteinase-9

MPO:	Myeloperoxidase
NAC	N-Acetylcysteine
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NF-κB:	Nuclear transcription factor κB
NGAL	Neutrophil Gelatinase Associated Lipocalin
nNOS	Neuronal NOS
NO	Nitric oxide
NOS	Nitric oxide synthases
NSAIDs	Nonsteroidal Anti-inflammatory Drugs
OS	Oxidative stress
RBC	Red Blood Cell
RCM	Radiocontrast Media
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SCr	Serum Creatinine
SOD	Superoxide Dismutase
TAC	Total antioxidant capacity
TAS	Total antioxidant status
TBH	t-butyl hydroperoxide
TCA	Trichloroacetic Acid Solution
TMP	Tetra-met-oxy-propane

ABSTRACT

MESUT M. (2023). Investigation Of The Mechanisms Of Statin Use In Prevention Of Contrast Induced Nephropathy In Rats. Yeditepe University, Institute of Health Science, Department of Pharmaceutical Toxicology PhD thesis, İstanbul.

AIM: The use of contrast media in diagnostic and other procedural settings has been increasing exponentially, which increases the incidence of a state of iatrogenic kidney function impairment called contrast induced acute kidney injury (CI-AKI). Roughly one third of all hospital acquired kidney injury has been attributed to exposure to radiographic contrast media. The most widely accepted definition of contrast induced nephropathy (CIN) is either a 25% or more increase from the baseline value of serum creatinine or a 0.5 mg/dl or more absolute elevation within 24 to 72 hours of contrast media administration. CI-AKI greatly increases the mortality risk and hospital stay of patients which creates a need for preventative strategies against this condition. Many studies have suggested that alongside their cholesterol lowering properties, statins may exert pleiotrophic effects, including renoprotection, and significantly lower the incidence of CI-AKI. The aim of this study is to observe the efficacy of statins in preventing CI-AKI and demonstrate their mechanism of action.

Material & Method For our animal experiment protocol, 30 adult male Spraque-Dawley rats were used. The subjects were equally divided into 5 groups: Rosuvastatin group (rosuvastatin + iohexol + furosemide + dehydration), Atorvastatin group (atorstatin + iohexol + furosemide + dehydration), CFD group (iohexol + furosemide + dehydration), FD group (furosemide + dehydration) and D group (dehydration). For generation of acute kidney injury, the fluid intake of subjects was restricted. CI-AKI was created by administering iohexol 15 ml/kg to three groups. Two of these were selected and they received 20 mg/kg/day Atorvastatin and 10 mg/kg Rosuvastatin, respectively. The renal tissues of all groups were studied histopathologically. From the blood samples, erythrocytes were used to determine the formation of malondialdehyde (MDA), activities of the enzymes glutathione peroxidase (GPx) and catalase (CAT). These parameters were measured spectrophotometrically. Serum creatinine levels were used for the diagnosis of AKI and serum neutrophil gelatinase associated lipocalin (NGAL) levels were measured with ELISA Kit to evaluate tubular damage.

Results: 3 out of 5 groups that received contrast media, had higher average values of serum creatinine compared to the control groups. There was a statistically significant difference in serum creatinine levels of the CFD group, compared to Atorvastatin, FD and D groups. There was no statistically significant difference between creatinine levels of Rosuvastatin and CFD groups. Concerning the erythrocyte MDA level and erythrocyte GPx and CAT enzyme activities of all groups, no statistically significant difference was observed. In the histopathological examination, medullary congestion and tubular damage scores were evaluated and there was no statistically significant correlation detected.

Conclusion: While atorvastatin is effective in preventing contrast media induced acute kidney injury, the same reno-protective property is not applicable to Rosuvastatin. Although atorvastatin prevented the elevation of serum creatinine in response to contrast media, the same correlation was not evident with prevention of tubular injury or increase in oxidative stress, which were associated with contrast media in priorly published literature.

Keywords: Contrast-induced nephropathy, Statin Reno-protective effect, oxidative stress

ÖZET

MESUT M. (2023). Kontrast ilişkili nefropatiyi önlemede statinlerin etki mekanizmasının sıçanlar üzerinde araştırılması. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü Farmasötik Toksikoloji Anabilim Dalı Doktora tezi, İstanbul.

Amaç: .Iyotlu kontrast maddeler teşhis ve tedavi amaçlı girişimsel prosedürlerde giderek daha fazla kullanılmaktadır. Bu kontrast madde ilişkili akut böbrek hasarı (KM-ABH) olarak adlandırılan iyatrojenik böbrek fonksiyon bozukluğu insidansının artmasına neden olur. Hastane kaynaklı edinilmiş akut böbrek hasarı (ABH) vakalarının yaklaşık üçte birini oluşturmaktadır. KM-ABH'NIN en yaygın tanımı, KM maruz kaldıktan 24-72 saat sonra serum kreatinin değerinde başlangıç değerine göre %25 veya daha fazla artış veya 0,5 mg/dl veya daha fazla mutlak artıştır. KM-ABH nedeniyle hastanede kalış süreleri ve mortalite riski artmaktadır Bu nedenle KM-ABH için önleyeci stratejilere ihtiyaç vardır. Birçok çalışma, renoproteksiyon dahil olmak üzere pleiotropik etkiler sergileyen statinlerin kolesterol düşürücü etkilerinin yanında KM-ABH insidansını önemli ölçüde azalttığını bildirmiştir. Çalışmamızdaki temel amacımız statinlerin KM-ABH' nı önlemedeki etkisini göstermek ve etki mekanizmalarını tespit etmektir.

Materyal & Metot: Çalışmada yetişkin erkek Spraque-Dawley ırkı sıçanlar kullanılmıştır. 30 sıçan eşit olarak Rosuvastatin grubu (rosuvastatin + ioheksol + furosemide + dehidratasyon), Atorvastatin grubu (atorstatin+ioheksol + furosemide + dehidratasyon), CFD grubu (ioheksol + furosemide + dehidratasyon), FD grubu (furosemide + dehidratasyon) ve D grubu (dehidratasyon) olmak üzere 5 gruba ayrılmıştır. Deneyde oluşturulan gruplarda sıvı kısıtlaması yoluyla akut böbrek hasarı oluşturulmuştur. Üç gruba ioheksol 15 ml/kg uygulanarak KM-ABH oluşturulmuştur. Bu üç gruptan ikisi seçilerek bir grup 20 mg/kg/gün Atorvastatin ve diğeri 10 mg/kg Rosuvastatin ile tedavi edilmiştir. Tüm grupların böbrekleri histopatolojik olarak incelenmiştir. Alınan kan örneklerinden malondialdehit (MDA) oluşumu, glutatyon peroksidaz (GPx) ve katalaz (CAT) enzim aktivitelerinin spektrofotometrik yöntemle belirlenmesi için eritrosit kullanılmıştır. ABH tanısı için serum kreatinin seviyeleri kullanılmış ve tubuler hasarın değerlendirilmesi amacıyla ELISA Kit ile serum nötrofil jelatinaz ilişkili lipokalin (NGAL) düzeyleri ölçülmüştür.

Bulgular: Çalışmamızdaki her biri 6 denekten oluşan 5 gruptan kontrast madde verilen 3 grubun da ortalama kreatinin seviyeleri kontrol gruplarına göre yüksek saptanmıştır. CFD grubu kreatinin seviyeleri Atorvastatin, FD ve D gruplarıyla karşılaştırıldığında istatiksel anlamlı fark saptanmıştır. Rosuvastatin ve CFD grubunun kreatinin seviyeleri arasında

istatistiksel anlamlı fark saptanmamıştır. Tüm grupların eritrosit MDA seviyesi ve eritrosit GPx ve CAT enzim seviyeleri karşılaştırıldığında gruplar arasında istatistiksel anlamlı fark saptanmamıştır. Histopatolojik incelemede meduller konjesyon ve tubuler hasar skorları değerlendirilmiştir gruplar arasında istatistiksel anlamlı fark saptanmamıştır.

Sonuç: Atorvastatin, kontrast madde ilişkili akut böbrek hasarı oluşumunu önlemede etki gösterirken rosuvastatinin renoprotektif bir etkisi görülmemiştir. Atorvastatin, kontrast madde nedenli kreatin seviyelerinin yükselmesini önlemesine ragmen, literatürde kontrast maddenin olşturduğu düşünülen tubuler toksisiteyi ve oksidatif stress artışını önlemedeki ilişkisi tespit edilememiştir.

Anahtar kelimeler: Kontrast madde ilişkili nefropati, Statin renoprotektif etki, oksidatif stress

1. INTRODUCTION

Acute kidney injury (AKI) is a serious cause of morbidity and mortality among especially hospitalized patients as well as significant financial loss [1]. Risk factors for AKI in intensity care unit and emergency department include hypovolemia, sepsis, trauma, nephrotoxic drugs, use of radiocontrast media, renal vascular diseases, malignancy and liver disease [2]. The most widely accepted definition of CIN is either a 25% or more increase or a 0.5 mg/dl or more increase in serum creatinine. This increase is in regards to the patients baseline value within 48 - 72 h of exposure to contrast media, with the first 24 h being the determining time period for CIN development [2].

The use of contrast media in diagnostic and other procedural settings has been increasing exponentially. This increased exposure in turn causes significant increase in iatrogenic kidney function impairment. Roughly 10% of all hospital acquired kidney injury has been attributed to exposure to radiographic contrast media which is the third most common cause of renal insufficiency [3].

In experimental studies regarding contrast induced nephropathy, varying responses to ischemic injury have been documented. Following the postischemic reperfusion and subsequent oxidative stress, free radicals emerge and cause acute kidney injury by endothelial damage and apoptotic cell death [4]. In summary, following the renal hypoperfusion and increased oxygen demand triggered by contrast media, renal insufficiency occurs [5].

The preventative effects of statins on contrast induced nephropathy have been demonstrated by numerous studies. In addition to their effect on hypercholesterolemia, statins also have anti-inflammatory, antithrombotic, and antioxidative properties. It is through these additional properties that statins are believed to alleviate renal vascular inflammation and oxidative stress. Lastly, statins decrease reabsorption of contrast media from renal tubules and thus exert a direct preventative effect [6].

Our aim is to investigate the mechanism of action of frequently prescribed statins in preventing contrast media-related acute kidney injury and to compare the effectiveness of statins among themselves.

2. GENERAL DESCRIPTION

2.1. Contrast Nephropathy

Radiographic contrast media have been used for over 70 years, for visualization of structures during interventional procedures such as cardiac catheterization and percutaneous coronary intervention and x-ray dependent scans such as plain radiography and computed tomography. Since these procedures are mostly performed on geriatric population or patients with serious comorbidities, as the use of CM increases, so do incidence of iatrogenic renal function impairment, caused by the exposure to CM, a condition known as contrast induced nephropathy (CIN) [3, 7]. This increase in CIN has been proven with numerous observational studies and nowadays it is widely accepted that CIN is not a condition of the healthy kidney, but rather affects patients that already have impaired kidney function. Radiographic CM are responsible for 11% of cases of hospital-acquired renal insufficiency, the third most common cause of renal failure after impaired renal perfusion and the use of nephrotoxic medications [3, 7]. The most common definition of CIN is an increase of 25% or more, or an absolute increase of 0.5 mg/dl or more in serum creatinine from baseline value, at 48–72 h following the exposure to CM. However, in rats the first 24 h post-exposure appears to be crucial in the development of CIN [7]. Number of studies have shown that the pleiotropic properties of statins exert a beneficial impact in the prevention of contrast nephropathy. Apart from their cholesterol-lowering effects, statins have anti-inflammatory, antithrombotic, and antioxidative properties and may exercise nephron-protective action, thereby improving the endothelial function and reducing vascular inflammation and oxidative stress. Furthermore, statins may reduce tubular reabsorption of contrast media, mitigating their direct toxic effects on the renal tubules [6].

2.1.1. Prevalence

In 2021 guidelines published by Kidney Disease Improving Global Outcomes (KDGOI), contrast media induced acute kidney injury was the third most common cause of AKI in hospitalized patients, after decreased renal perfusion and nephrotoxic medications, with an overall incidence of 11%. The same guideline stated that the incidence of CI-AKI in patients with normal renal function is significantly low (1-2%) even with a prior diagnosis of diabetes, whereas the incidence reached 25% in patients with renal impairment or other comorbidities such as advanced age, congestive heart

failure or administration of nephrotoxic drugs. In a recent retrospective study, the incidence of CIN was shown to be more than 25% in patients with GFR ≤30 ml/min/1.73 m2 and 4.3% of these patients required dialysis. This number diminished significantly in patients with a GFR ≥60 ml/min/1.73 m2 to 5.2% and only 0.07% of these patients required dialysis [8].

Additionally, dialysis requirement in patients with CI-AKI is associated with increased mortality, where in one study the mortality of patients with CI-AKI was 7.1%, whereas this number increased to 35.7% in patients who required dialysis. It is agreed upon that development of CIN is associated with increased duration of hospitalization, morbidity and mortality [9].

2.1.2. Pathophysiology

Although the exact mechanism is yet to be uncovered, recent studies suggest concurrent mechanisms of altered perfusion to the kidney and direct nephrotoxic effects, caused mainly by increased viscosity in the tubules and impaired function of endothelial cells. Changes in renal perfusion have been attributed to increased blood viscosity and osmolarity and alterations in vasoactive agent levels. Following the initial, short-lived vasodilatation seen after the CM administration, there is a period of sustained vasoconstriction and increased renal vascular resistance, led locally by increased endothelin and adenosine and decreased NO and prostoglandins, thus decreasing glomerular blood flow, causing cellular hypoxia. Additionally, increased blood viscosity contributes to impairment of plasticity of erythrocytes, increasing the risk for microvascular thrombosis [10].

The same vasoconstriction is also seen in descending vasa recta (DVR), the long and thin micro-vessels that constitute the entire blood supply of renal medulla. In 2012, Sendeski et al. demonstrated that iodinated CM caused structural vascular endothelial cell damage, rather than just vasoconstriction in rat and human DVR, at physiological concentrations [11]. Renal medulla, specifically thick ascending limb is rich with mitochondria and as the medullary perfusion decreases, interrupted oxidative phosphorylation and decreased ATP causes oxidative stress and enhances the formation of reactive oxygen species (ROS). ROS like superoxide, hydrogen peroxide and free hydroxy radicals are known to cause lipid membrane oxygenation, DNA damage and ER stress, among many others. Particularly, this ER stress causes intracellular Ca^{++} to

increase and induces apoptosis by activating caspase 3 and 9, causing cell death. In 2010, Hsiang-Chun Lee et al revealed that in human embryonic kidney cells and kidney tissue, apoptosis in response to CM is most likely the result of c-Jun-N-terminal kinase (JNK) pathway activation [12].

ROS also interfere with the production of vasodilatory substances such as NO. Superoxide radicals decrease bioavailability of NO by reacting with NO to form oxidative peroxynitrite, which in turn worsen the direct cellular damage CM have on endothelial cells. One of the other ways of ROS induced damage is seen on plasma membranes. In an animal study conducted in 2008, following the injection of CM, levels of ROS metabolites such as malondialdehyde (MDA) and F2 isoprostane, markers of lipid peroxydation were increased [13]. To date, many animal studies have shown increases in urine MDA and/or other lipid peroxidation metabolites in setting of CIN, establishing a clear association between oxidative cell membrane damage and CIN [14].

2.1.3. Risk factors

The major risk factors involved in CIN can be divided into two, namely, patient related and procedure related.

2.1.3.1. Patient-related Factors

The most relevant patient related factors are diabetes mellitus and preexisting renal insufficiency (estimated glomerular filtration rate (eGFR) <60 ml/min). Other less relevant factors include other preexisting conditions such as congestive heart failure (CHF), hypertension, liver cirrhosis, anemia, hypoalbuminemia and advanced age (>75 years) [7, 9, 15, 16].

2.1.3.2. Procedure-related factors

The volume of the CM, osmolality and viscosity all contribute to generation of CIN. Additionally, recurrent exposure to CM within 72 hours and nephrotoxic drug use such as NSAIDs and aminoglycosides are also procedure related factors contributing to CIN [17, 18].

2.1.4. Diagnosis

2.1.4.1. Creatinine and NGAL As Biomarkers

As aforementioned, Contrast-induced nephropathy (CIN) is the state of impaired kidney function, occurring within 48-72 hours following intravenous contrast administration. It is defined as either a 25% increase in serum creatinine (sCr) from baseline or a 0.5 mg/dL (44 μmol/L) increase in absolute sCr value [7]. Other than creatinine, laboratory findings may include metabolic acidosis, hyperkalemia and uremia. Urinalysis may show no significant findings and the urine output can be oliguric, anuric or normal [19].

Generally, serum creatinine increase takes place 24 to 48 hours after exposure, which makes serum creatinine a relatively late indicator of renal function impairment and creates a need for a quicker and more sensitive marker for renal injury. Several potential markers have been researched and proposed for tubular injury [20].

One of these proposed markers is plasma neutrophil gelatinase associated lipocalin (NGAL). NGAL is a mammalian small 25-kDa peptide belonging to the lipocalin superfamily which consists of ~20 small lipoproteins, including lipocalin 2, siderocalin, uterocalin, proteinase-3 and 24p3. NGAL was initially discovered in 1993 from the supernatant of activated human neutrophils as an acute-phase protein [21]. In physiological conditions, the level of NGAL is kept. After an ischemic insult or a nephrotoxic event, NGAL levels increase rapidly in urine and cortical tubules [22]. NGAL as an early predictive marker for AKI is being frequently studied as its levels increase well before creatinine and has higher sensitivity. Studies have shown that in critically ill patients, tubular secretion of NGAL can be a specific marker for early detection of CI - AKI and after two hours, urine NGAL increases significantly in patients with CIN [23]–[27]. This early response to renal injury and the fact that it is readily excreted to serum and urine, makes NGAL a novel marker for AKI [28].

2.1.4.2. Cystatin C

One of the other means of measuring renal function, cystatine C is a protein with low molecular weight that has a constant rate of production by all nucleated cells. Plasma cystatine C is filtered freely through the glomerular basal membrane and since it is not secreted or reabsorbed along the neutron, serum levels perfectly reflect the GFR. In the proximal tubule, cystatine is almost completely broken down and has no renal clearance. In patients with CIN, levels of cystatine were shown to increase after the eight-hour mark. However cystatin levels are highly dependent on other conditions such as systemic

inflammation, neoplasia, age, muscular mass, corticosteroids administration and thyroid dysfunction [29].

2.1.5. Treatment and Prevention

To date, there is no proven definitive treatment of CIN. With the current increase in use of diagnostic radiocontrast media, the risk of CIN should always be kept in mind when any diagnostic imaging using contrast is ordered and if possible, repeated imaging studies should be avoided.

Currently, prevention of CIN in high-risk patients includes pharmacological and non-pharmacological strategies. High risk patient demographics include those with AKI or preexisting kidney disease. Most effective prevention measures include preventing prior volume depletion, avoiding renal vasoconstriction, avoiding any other nephrotoxic drug use and diuretics and when possible, minimizing the amount of radiocontrast media [30].

2.1.5.1. Hydration

One of the more low risk and low cost prevention strategies, hydration with intravenous saline prior to RCM administration has been reported the most effective in preventing CIN [31]. Increased intravenous volume dilutes radiocontrast media introduced while simultaneously inducing diuresis [32]. Additionally, increasing intravascular volume inhibits Renin - Angiotensin System, reducing production of vasoconstrictive substances that are known to play a crucial role in pathogenesis of renal hypoxia and CIN, such as endothelin [33]. In a 2002 randomized study comparing two hydration regimens in patients receiving coronary angioplasty, the patients that benefited most from intravenous saline were patients with diabetes mellitus, patients receiving greater amounts of CM (more than 250 ml) and patients undergoing emergency interventions. The study concluded that patients benefited more from isotonic saline (0.9% saline) than ½ saline (0.45% sodium chloride plus 5% glucose), given in the morning of an elective coronary angioplasty and right before any emergency intervention [34].

In a randomized study on routes of administration, oral and intravenous routes were compared and found no difference in outcome or incidence of CIN [35]. However, another more recent study conducted with a larger cohort reported a reduction of

incidence of CIN in favor of intravenous fluid administration [35]. Another study in 2011, an observational study on oral hydration in patients with normal kidney function undergoing CT angiography showed clear correlation between oral hydration and changes in eGFR [36]. As with any other regimen of hydration, patients with left ventricular dysfunction (EF< 40%) and patients with CKD are at risk of volume overload and therefore volume status in these patients should be carefully evaluated.

In a recent observational study, the effects of oral fluid intake in patients undergoing CT angiography that had normal kidney function was closely correlated with the percentage changes in sCr and the absolute changes in eGFR [37].

2.1.5.2.HMG CO A reductase inhibitors

Statins are drugs belonging to the group of HMG-CoA reductase inhibitors, used for the treatment of hypercholesterolemia, which are known to possess antioxidative and anti-inflammatory properties [38]. There are studies suggesting long term statin use has a positive influence on the risk of CIN, reducing the need for dialysis and long term mortality [39, 40]. A systematic review and meta-analysis conducted in 2011 revealed that, long term statin intake (≥7 days) effectively reduced the risk of CIN, while short-term high dose treatments did not have the same effect [40]. However, there are also studies concluding, that statins either have no effect on CIN incidence or that statin use may even increase the risk of CIN [41, 42]. Therefore, there is still no consensus on whether the use of statins is effectively preventative of CIN and even so, what the appropriate treatment regimen is. There is still an evident need for a large controlled randomized trial on the efficacy of statins on CIN [43].

Although they are generally prescribed for hypercholesterolemia and treatment of atherosclerotic cardiovascular disease, Statins have been under research for their ability to decrease oxidative stress and inflammation, and increase endothelial function [44]. Studies have shown that myocardial damage during PCI can be avoided by a short-term pretreatment with statins. This success has been attributed to their anti-inflammatory and antioxidant properties [45]–[48]. Also their ability to decrease endothelin secretion [49]. In addition, statins have been suggested to impair tubular reabsorption of CM, which reduces their toxicity [16], [50]. With all these promising positive properties of statins, they stand out as potential candidates for CIN prevention, albeit to date no definitive consensus has been reached.

2.1.5.3. Ascorbic acid

Vitamin C is a water soluble antioxidant that can scavenge oxygen radicals and prevent renal injury with different etiologies, including ischemic and nephrotoxic drugs such as aminoglycosides and cisplatin [51, 52]. In a randomized, double blind study on the ability of ascorbic acid to prevent CIN, it was revealed that prophylactic oral administration of ascorbic acid was effective in reducing development of CIN in patients with prior renal disease. The study, conducted in 2005, included 231 patients and followed the regimen of 3 g at least two hours before the procedure and 2 g in the night and the morning after the procedure, which apparently was effective [53]. However, another randomized, controlled trial, involving 156 consecutive patients with chronic renal insufficiency and following the regimen of 3 g intravenous injection before the procedure and oral 1 g per day for 2 days after the procedure, failed to show such correlation [54].

2.1.5.4. N-Acetylcysteine

N-Acetylcysteine (NAC), a more stable form of the sulfhydryl-containing amino acid cysteine is an antioxidant with protective properties. It is an inexpensive drug that is well tolerated whether it is administered orally or intravenously [55]. NAC was first suggested for CIN in patients with chronic kidney disease receiving contrast-enhanced computed tomography (CT) in 2000. Tepel et al demonstrated in their double blind and randomized study that when patients with a baseline creatinine value of >1.2 mg/dL or creatinine clearance of <50 mL/min were treated with NAC (600 mg orally twice daily) and 0.45% saline intravenously (before and after CM administration) their creatinine values showed significant decrease compared to the placebo group [56]. Afterwards, many studies on the subject matter have been reporting inconsistent results, failing to demonstrate an association [57]–[60]. The suggested role of NAC in reducing the risk of CIN lies in antioxidant and vasodilator properties of NAC. N-acetylcysteine is a direct free radical scavenger and precursor of glutathione. It is also known to cause vasodilation through increased expression of nitric oxide synthase [61]. Animal studies have shown that NAC increases nitric oxide synthesis, thus causing vasodilatation and hinders hypoxic injury [62]. Efrati et al. reported a similar result on their animal model and demonstrated enhanced renal blood flow in rats that were treated with NAC and showed increase in renal prostaglandin E2 and renal cortical NO [63]. Studies on the impact NAC has on renal cells demonstrated that on a dose dependant manner, NAC inhibits apoptosis.

[64]. A meta analysis conducted in 2016 states that according to their findings there was a correlation between NAC and decrease in risk for CIN in patients undergoing coronary angiography and contrast enhanced computed tomography. However the same correlation was not evident for patients receiving peripheral angiography [65]**.** Currently, approved preventative measures for CIN include intravenous hydration and the use of low osmolality contrast media (LOCM) but due to its low cost, availability and minimal adverse effects, NAC stands as the most common pharmacological therapy in CIN.

2.2. Oxidative Stress

After being first introduced as a concept by H. Sies in 1985, oxidative stress and harmful effects of free radicals have been an inspiring topic of continuous research and development. The term oxidative stress is defined as an "An imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage." [66]. Free radicals were defined by Moses Gomberg in the early 20th century and as the century progressed the extensive research over the topic revealed substantial information supporting FR involvement in a variety of pathologic conditions atherosclerosis, diabetes, cancer, chronic inflammatory diseases, cell damage and aging [67]. Free radicals are short-lived, highly reactive oxygen byproducts that contain one or more uncoupled electrons on their atomic orbits. Due to this uncoupled electron, free radicals can easily draw electrons from other molecules, or give up their own, which gives them the ability to act as both oxidants and reductants. Free radicals are created within the biological systems as a result of physiological aerobic metabolism and also pathological events generated by disease and xenobiotics [68]. When this continuous production is not adequately matched by the antioxidant scavenger mechanisms, the end result is oxidative stress.

2.2.1. Reactive Oxygen and Nitrogen Species

2.2.1.1. Superoxide Radical

Superoxide molecule, considered the primary free radical, is generated from the molecular oxygen either by metabolic processes or irradiation. Although it does not interact directly with polypeptides, sugars or nucleic acids, it can cause DNA damage and lipid $_{f2reaction}$ or through enzymatic or metal catalyzed reactions [69].

$O_2^{\cdot -} + H_2O_2 \rightarrow O_2 + HO^{\cdot} + OH^-$ (Haber-Weiss reaction).

Superoxide radicals are scavenged by the enzyme Superoxide Dismutase, which yields hydrogen peroxide. Hydrogen peroxide itself is not a free radical but an oxidizing agent. H_2O_2 is then removed by other enzymes that work together with SOD, such as catalase and glutathione peroxidase [69].

2.2.1.2. Hydrogen Peroxide (H_2O_2)

Although not an active radical by itself, hydrogen peroxide still poses a threat to the biological system. Unlike superoxide anion, H_2O_2 is uncharged, and due to its close molecular structure to water, it can utilize aquaporin channels, passing cell membranes rapidly. In the presence of different redox active transition metals, H_2O_2 gives rise to hydroxyl radical, which is far more active [70].

$$H_2O_2 + Fe^{+2} \rightarrow Fe^{+3} + OH\cdot + OH$$

(Fenton reaction)

Within the phagocytic cells, myeloperoxidases utilize H_2O_2 to yield hypochlorous acid and hydroxyl radical. This respiratory burst constitutes the backbone of their bactericidal activity [70].

$$H_2O_2 + CI^- \rightarrow HOCI + OH$$

2.2.1.3. Hydroxyl radical (OH)

Hydroxyl radical has the highest reactivity and shortest half-life among free radicals. By reacting with H^+ atoms in DNA bases or the deoxyribose sugars, hydroxyl radicals are known to cause significant DNA damage. It can be produced through different mechanisms. Ionizing radiation can cause decomposition of H_2O into hydrogen atoms and OH, photolytic decomposition of alkyl hydroperoxides or with Fenton reactions as explained above [71].

2.2.1.4. Nitric oxide (NO)

First described as one of the reactive nitrogen species, nitric oxide is an abundantly found radical with major roles in physiological processes involving blood pressure regulation, muscle relaxation, neurotransmission and many more. Similar to other radicals, this gas form radical has a short life span and contains uncoupled electrons. Generated by nitric oxide synthases (NOS), which are present in three isoforms [endothelial NOS (eNOS), inducible NOS (iNOS), and neuronal NOS (nNOS)] [72].

In aqueous solutions, $O\bullet^{-2}$ can react with •NO and form peroxynitrite anion, which is broken down into NO_2 and OH^- [72].

$ONOO^- + H^+ \rightarrow ONOOH$ and $ONOOH \rightarrow NO_2 + OH^\cdot$

2.2.1.5. Peroxynitrite

A reactive nitrogen species that interacts with many of cellular pathways, one of the early in vivo detections of peroxynitrate was by Beckman et al., in human aortic atherosclerotic plaques with the protein NO_2Tyr [73]. During respiratory burst, phagocytic cells generate nitric oxide and superoxide anion and these molecules react together to form peroxynitrite anion which causes DNA fragmentation and lipid oxidation [74].

2.2.2. Enzymatic Antioxidants

Reactive oxygen species generated throughout the biochemical systems are converted into their non-toxic end - or byproducts by the highly effective defense mechanism against oxidative cell damage called antioxidant enzymes [75].

These enzymes are proteins that can be further classified into groups regarding different properties they harbor, such as size and enzymatic activity. Antioxidants with smaller molecular size neutralizes and removes ROS by scavenging. Main antioxidants in this category are ascorbic acid (Vitamin C), tocopherol (Vitamin E), carotenoids and glutathione (GSH). Antioxidants with larger molecular size are enzymes (SOD, CAT and GPx) that take up the ROS and hinder them from harming other fundamental proteins, or sacrificial proteins such as Albumin [76].

Another mode of classification is according to their enzymatic activity. Non - enzymatic antioxidants exert their effect by intervening the free radical chain reaction. Few examples in this group are ascorbic acid, tocopherol, plant polyphenols, carotenoids and glutathione. Enzymatic antioxidants remove ROS by taking them up and breaking them down. As mentioned above, these enzymes are namely SOD, CAT and GPx, which are concurrently the main antioxidant mechanisms in RBCs [77]. These enzymes convert the highly toxic oxidative species first into hydrogen peroxide and then water and utilize cofactors such as copper, zinc, manganese and iron in the process [76].

2.2.2.1. Catalase

Due to their role in tissue perfusion RBCs are perpetually exposed to oxygen and thus oxidative stress. Catalase, an enzyme with a molecular weight of 250 kDa and one of the main defenses against this continuous insult, contains four hemoprotein groups. CAT catalyzes conversion of H2O2 to H2O and O2 by utilizing Fe or Mn as cofactor. It is mainly found in erythrocytes, peroxisomes of mammalian renal cells and hepatocytes [78, 79].

2.2.2.2. Glutathione Peroxidase

GPx was initially discovered in 1957 in erythrocytes with its protective properties against hydrogen peroxide. During the following decade, glutathione peroxidase was identified in other cells and tissues with its role in degradation of hydroperoxides, including H_2O_2 [80].

$$H_2O_2+2GSH \rightarrow 2H_2O+GSSG$$

GPx is found both in cytosol and mitochondria and contains Selenium at its active site. In comparison to catalase, GPx has a higher affinity to H_2O_2 and has the ability to reduce lipid peroxidase (LOOH) with a redox reaction using glutathione as an electron donor [81].

$$2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$$

$$2GSH + LOOH \rightarrow GSSG + LOH + H_2O$$

In this reaction, GSH is oxidized into its disulfide (GSSG) form. In this form, it is unable to reduce peroxide unless once more reduced to its GSH form by glutathione reductase, which utilizes nicotinamide adenine dinucleotide phosphate (NADPH) as the electron donor. This reaction oxidizes NADPH to $NADP^+$ and relies on the pentose phosphate pathway to once more reduce $NADP^+$ to NADPH and thus creating a cellular redox cycle as an antioxidant defense mechanism [81].

A study conducted in GPx deficient mice demonstrated endothelial progenitor cell dysfunction which resulted in impaired angiogenesis [43]. Another animal study investigating GPx activity in vivo in rat hepatocytes demonstrated that by supplementation with wheat bran, it was possible to stimulate GPx activity, which contains selenium as an important antioxidant [70]. In alloxan treated and diabetes

induced rats, a diet comprising of feruloyl oligosaccharides was proven beneficial in increasing GPx activity in serum, liver and testes. Conclusively, GPx-maintained redox cycles can propose an effective method of innate protection against oxidative stress [82].

2.2.2.3. Superoxide Dismutase

The enzyme Superoxide Dismutase (SOD) , a crucial enzyme against free radicals, oversees reduction of superoxide anions to H_2O_2. The superoxide anion is also responsible in formation of peroxynitrite radical and SOD competes with Nitric oxide (NO) for the superoxide anion, therefore preventing the formation of peroxynitrite and subsequent endothelial and mitochondrial damage [83,84].

It has been long established that free radicals, superoxide anion in particular, and oxidative stress are major contributors of cardiovascular disease such as hypertension and atherosclerosis. In light of these findings, antioxidant therapies and stimulation of existing endogenous defenses has gained popularity as a subject, even though research thus far yielded only controversial evidence. Primary defense against this highly hazardous anion, the SOD enzyme family consist of three isoforms: cytoplasmic (SOD1), mitochondrial (SOD2) and extracellular (SOD3) all isoforms utilize either Cu, Zn or Mn as their cofactor and recent studies show that all isomers, regardless of the subcellular location, catalyze the same $O2^{\bullet-}$ H_2O_2 reaction, suggesting a role in cell signaling. Function and importance of these isoforms were demonstrated in studies involving genetically altered mice and gene transfer by viral mediators [85, 86].

2.2.3. Free Radical Damages on Cell Structures

Free radicals pose a threat to all organic materials such as lipids, polysaccharides, proteins, DNA and RNA. End result of this noxious interaction depends on the duration and severity of the exposure as well as the type of cellular structure affected [87].

2.2.3.1. Lipid peroxidation

Due to the polyunsaturated fatty acid residues of phospholipids, cell membranes are particularly sensitive to oxidation. Since integrity and continuity of cell and organelle membranes are crucial to the cell, disruption of these structures means cell death. Lipid peroxidation can either be non-enzymatic or enzymatic by Cyclooxygenase and lipoxygenase enzymes [89].

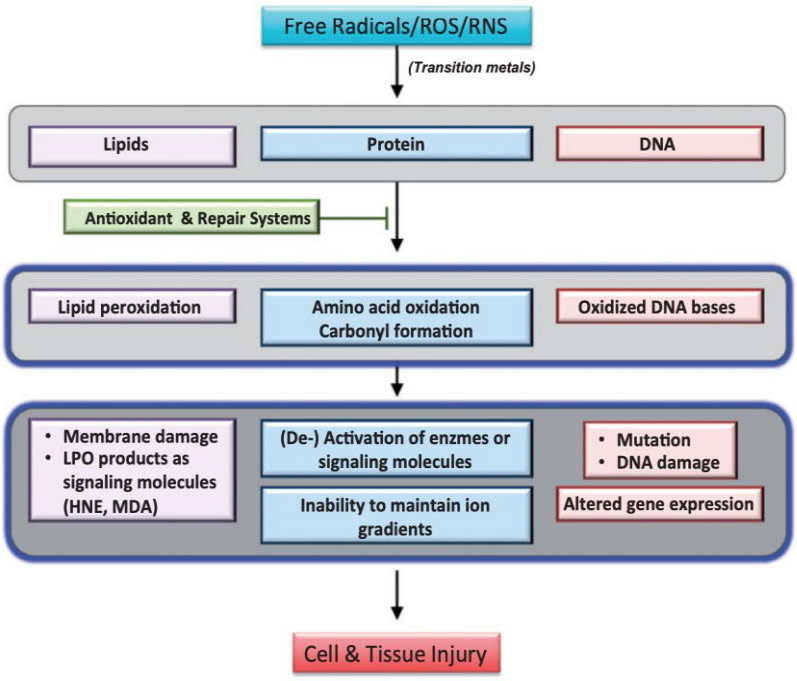

Figure 1: Mechanism by which free radicals damage cells. When free radicals interact with lipids, proteins, and DNA there is the potential for numerous changes that could affect cell function and lea to injury. Antioxidant and repair systems will prevent, or minimize these changes [88].

2.2.3.1.1. Non-Enzymatic Lipid Peroxidation

The main rundown of this process includes abstraction of H atom by radicals, addition of oxygen to carbon radicals, peroxyl radical fragmentation or rearrangement, addition of peroxyl radical to carbon double bonds or cyclization; and peroxyl termination [81]. This overall process occurs in three stages: initiation, propagation and termination.

2.2.3.1.1.1. Initiation

In this stage, a free radical formed within the biological system, interacts with the C-H bond of fatty acid RH chain and breaks down H+ atom. This breakage yields s a carbon-centered lipid radical (R˙) within the fatty acid chain, which quickly rearranges to form a conjugated diene structure[81].

2.2.3.1.1.2. Propagation

Next, molecular oxygen is added to the carbon centered lipid radical and a peroxyl radical is generated[81].

$$L\bullet \underset{-O_2}{\overset{O_2}{\rightleftarrows}} L{-}O{-}O\bullet$$

lipid radical — peroxyl radical

As the newly produced peroxyl is itself also a radical, it affects the neighboring fatty acids by abstracting a H atom and stabilizing itself into a hydroperoxide molecule (LOOH) [81].

2.2.3.1.1.3. Termination

This autocatalytic chain reaction continues until either the recombination of radicals (R• + R• →non-radical product), depletion of the substrate or by an antioxidant, by donation of a H atom, which yields a non-radical molecule [81].

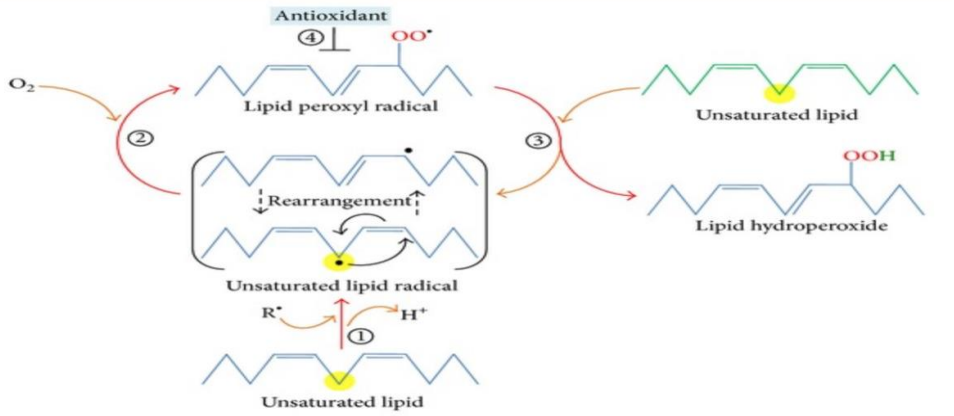

Figure 2: Lipid Peroxidation

2.2.3.1.2. Enzymatic lipid oxidation

Biosynthesis of Thromboxane A2, Prostaglandins and Leukotrienes are carried out by the enzymatic family of cyclooxygenases and lipoxygenases which use arachidonic acid, a polyunsaturated omega 6 fatty acid as a substrate and to a degree achieve lipid oxidation [90].

2.2.3.1.2.1. Malondialdehyde

Enzymatic or non-enzymatic lipid oxidation generates aldehydes such as malondialdehyde, 4-HNE, isoprostanes, and other lipid peroxidation end-products. Among them MDA, a lipid peroxidation derivative of arachidonic acid and larger polyunsaturated fatty acid (PUFA), is being widely used as a marker for lipid peroxidation

and oxidative stress. MDA is normally scarcely found in vivo and is relatively stable. It has the capacity to diffuse across membranes, which gives it ability to covalently modify cytosolic or nucleic proteins located further from its site of production, unlike free radicals that only affect structures immediately close to themselves. MDA is known to alter and cause modifications in many essential structures such as enzymes, carrier proteins, cytoskeletal proteins, and mitochondrial and antioxidant proteins [89 - 92].

3. MATERIALS AND METHODS

3.1. Experimental Design

30 adult female Sprague−Dawley rats with an average weight of 430 g were obtained with the approval of Yeditepe University Animal Experiments Local Ethics Committee (HADYEK; decision number 2022/12-02) and housed at controlled room temperature (21 ± 1°C) with a 12:12 h light-dark cycle in cages.

In order to induce CIN in rats, the methodology that Duan et al described was used [93]. In short, following a 5 day fluid restriction, 10 mg/kg of furosemide was administered [94]. In order to induce CIN, after 20 minutes following the diuretic, iohexol was given with the dose of 15mg/kg. To demonstrate the efficacy of statins in preventing CIN, atorvastatin and rosuvastatin were given to the corresponding groups as detailed below and as seen in the following table 1.

Thirty rats were divided randomly in five groups. As explained below, the groups will receive the corresponding drugs and appropriate sampling will take place. The blood samples were collected under light anesthesia from the jugular vein. All groups except the control group was euthanized at the end of the experiment. Steps of the procedure are explained below and Table 1 & 2 shows experiment procedure and drugs that was administered.

3.1.1. Experimental Groups

3.1.1.1. D Group

Fluid intake of the rats was restricted to 4 ml/100g for six days. No drugs were given to the control group and blood samples was collected at the end of fluid restriction. Normal saline was given by oral gavage to balance out fluid intake with groups given statin solution. Samples was taken in two tubes one with EDTA and one dry biochemistry tube.

3.1.1.2. FD Group

Fluid intake of the rats was restricted to 4 ml/100g for six days. Normal saline was given by oral gavage to balance out fluid intake with groups given statin solution. At the end of day 5, 10 mg/kg of Furosemide was administered intramuscularly. At the 24th hour mark after diuretic, blood samples were collected in two tubes one with EDTA and one dry biochemistry tube. After that, the animals were euthanized, and the kidneys was dissected and stored in formaldehyde for further histopathological examination.

3.1.1.3. CFD Group

Fluid intake of the rats was restricted to 4 ml/100g for six days. Normal saline was given by oral gavage to balance out fluid intake with groups given statin solution. At the end of day 5, 10 mg/kg of Furosemide was administered intramuscularly. 20 minutes after the diuretic, 15 ml/kg iohexol was given intravenously. At the 24th hour mark after contrast media, blood samples were collected in two tubes one with EDTA and one dry biochemistry tube. After that, the animals were euthanized, and the kidneys was dissected and stored in formaldehyde for further histopathological examination.

3.1.1.4. Rosuvastatin Group

Fluid intake of the rats was restricted to 4 ml/100g for six days. At the end of day 5, 10 mg/kg of Furosemide was administered intramuscularly. 20 minutes after the diuretic, 15 ml/kg iohexol was given intravenously. 4 hours after the contrast media, 10 mg/kg of rosuvastatin was given via oral gavage. At the 24th hour mark after contrast media, blood samples were collected in two tubes one with EDTA and one dry biochemistry tube. After that, the animals were euthanized, and the kidneys was dissected and stored in formaldehyde for further histopathological examination.

3.1.1.1. Atorvastatin Group

Fluid intake of the rats was restricted to 4 ml/100g for six days. At the end of day 5, 10 mg/kg of Furosemide was administered intramuscularly. 20 minutes after the diuretic, 15 ml/kg iohexol was given intravenously. 4 hours after the contrast media, 20 mg/kg of atorvastatin was given via oral gavage. At the 24th hour mark after contrast media, blood samples were collected in two tubes one with EDTA and one dry biochemistry tube. After that, the animals were euthanized, and the kidneys was dissected and stored in formaldehyde for further histopathological examination.

Table 1: Groups To Be Applied And Active Substances

	Groups	Active Substances
1.	D Group	Dehydration and no medication was given to this group..
2.	FD Grup	Dehydration + furosemid (10mg/kg) im
3.	CFD Group	Dehydration + furosemid (10mg/kg) im + Iohexol, (350 mg I/mL) 15 ml/kg, iv
4.	Rosuvastatin Group	Dehydration + furosemid (10mg/kg) im + Iohexol, (350 mg I/mL) 15 ml/kg + rosuvastatin 10mg/kg/d
5.	Atorvastatin Group	Dehydration + furosemid (10mg/kg) im + Iohexol, (350 mg I/mL) 15 ml/kg + atorvastatin 20 mg/kg/d

C: Contrast Media, F: furosemide, D: Dehydration

Table 2: Examination Protocol

	1. d	2. d	3. d	4. d	5. d	6. d
D Group	Fluid Restriction 4 ml/ 100 g + 6 ml NS po	Fluid Restriction 4 ml/ 100 g + 6 ml NS po	Fluid Restriction 4 ml/ 100 g + 6 ml NS po	Fluid Restriction 4 ml/ 100 g + 6 ml NS po	Fluid Restriction 4 ml/ 100 g + 6 ml NS po	A blood sample was taken. to be euthanized
FD Group	Fluid Restriction 4 ml/ 100 g + 6 ml NS po	Fluid Restriction 4 ml/ 100 g + 6 ml NS po	Fluid Restriction 4 ml/ 100 g + 6 ml NS po	Fluid Restriction 4 ml/ 100 g + 6 ml NS po	Fluid Restriction 4 ml/ 100 g Furosemid im (10mg/kg) + 6 ml NS po	The kidneys was stored in formaldehyde.
CFD Group	Fluid Restriction 4 ml/ 100 g + 6 ml NS po	Fluid Restriction 4 ml/ 100 g + 6 ml NS po	Fluid Restriction 4 ml/ 100 g + 6 ml NS po	Fluid Restriction 4 ml/ 100 g + 6 ml NS po	Fluid Restriction 4 ml/ 100 g Furosemide im (10mg/kg), 20 minutes after the injection, 15 ml/kg IV Iohexol was injected. + 6 ml NS po	A blood sample was taken. to be euthanized
Rosuvastatin Group	Fluid Restriction 4 ml/ 100 g	Fluid Restriction 4 ml/ 100 g Rosuvastatin 10mg/kg/d oral gavaj	Fluid Restriction 4 ml/ 100 g Rosuvastatin 10mg/kg/d oral gavaj	Fluid Restriction 4 ml/ 100 g Rosuvastatin 10mg/kg/d oral gavaj	Fluid Restriction 4 ml/ 100 g. Furosemide 10 mg/kg and after 20 minutes Iohexol 15 ml/kg. rosuvastatin (10 mg/kg/d oral gavage) 4 hours after KM injection	The kidneys was stored in formaldehyde.
Atorvastatin Group	Fluid Restriction 4 ml/ 100 g	Fluid Restriction 4 ml/ 100 g Atorvastatin 20mg/kg/d oral gavaj	Fluid Restriction 4 ml/ 100 g Atorvastatin 20mg/kg/d oral gavaj	Fluid Restriction 4 ml/ 100 g Atorvastatin 20mg/kg/d oral gavaj	Fluid Restriction 4 ml/ 100 g. Furosemide 10 mg/kg and after 20 minutes Iohexol 15 ml/kg. Atorvastatin (20 mg/kg/d oral gavage) 4 hours after KM injection	The kidneys was stored in formaldehyde.

C: Contrast Media, F: furosemide, D: Dehydration

3.1.2. Samples Planned to Be Examined at the End of the Experiment

Approximately 6 ml of blood sample was obtained from the rats. From the samples, oxidative stress end products such as GPx, CAT ve MDA were assessed via spectrophotometry and serum NGAL levels were assessed with ELISA. In order to demonstrate CIN generation, serum creatinine levels were measured. The kidneys were dissected and stored in formaldehyde for further histopathological examination.

3.1.3. Drug Preparation

3.1.3.1. Atorvastatin

240 mg tablets were crushed in the mortar and triturated until they were powder. 30 mL polyethylene glycol was added on it and levigated. 15 mL of distilled water was added, it was put in the beaker. 15 mL distilled water was put in the mortar in order to take the remaining sample and added in the previous mixture. Then, it was completed to 180 mL with 1% methyl cellulose solution. This suspension was mixed by using a magnetic stirrer at 100 rpm and room temperature for 30 minutes.

3.1.3.2. Rosuvastatin

120 mg tablets were crushed in the mortar and triturated until they were powder. 30 mL polyethylene glycol was added and levigated. 5 mL distilled water was added, it was put in the beaker. 5 mL distilled water was added on the mortar in order to take the remaining sample and added in the previous mixture. Then, it was completed to 120 mL with 1% methyl cellulose solution. This suspension was mixed by using a magnetic stirrer at 100 rpm and room temperature for 30 minutes.

3.2. Biochemical Analysis

3.2.1. Measurement of sCr and serum NGAL

Blood samples were harvested and centrifuged at 1,500 x *g* for 15 min at 4˚C. At the end of centrifuge processes the separated serums of the samples were collected. sCr values was measured by an automatic biochemical analyzer (Roche/Hitachi Cobas 8000 model). The concentration of serum NGAL was evaluated via an enzyme linked immunosorbent assay (ELISA) with an ELISA kit (Shanghai Sunred Bio Tech, China).

3.2.2. Preparation of the Plasma

Blood samples transferred into heparinized tubes were centrifuged at 3000 g at +4°C, and the plasma was transferred to a fresh tube after separation from shaped elements. The separated plasma samples were divided into portions and stored at -20°C for laboratory analysis.

3.2.3. Preparation of Erythrocyte Hemolysate

Blood samples (n=43) kept at room temperature (25ºC) for 2 hours were centrifuged at 4000 *g* for 10 minutes at 4 ºC. After the supernatant was discarded, the remaining part was washed 3 times with 2 layers of 55 isotonic saline solution. With a 1:4 ratio of erythrocytes and cold distilled water, erythrocytes were lysed and incubated at 4ºC for 15 minutes. Then, the sample was centrifuged at 2000 *g* for 10 minutes at 4ºC. Cell remnants were removed, and the obtained erythrocyte hemolysate was stored at -70ºC.

3.2.4. Lowry Protein Assay

Lowry protein assay is a biochemical assay applied to determine the total protein concentration. The presence of proteins can be observed by the concentration-dependent color change in the sample solution, which can be measured by colorimetric techniques. Shortly, 250 mL of 2% 0.1 N Na_2CO_3 (sodium carbonate), 2.5 mL of 0.5% $CuSO_4$ (copper sulfate), and 5 mL of 1% NaKTar (sodium potassium tartarate) are mixed to prepare the Lowry Reagent. Folin solution is diluted by distilled water at a ratio of 1:2 (v/v). Six standard solutions of known protein concentrations were prepared from the 2% stock solution of bovine serum albumin (BSA). Spectrophotometric measurement of the absorbance values of proteins was carried out at 640 nm

3.2.5. MDA Measurement Method In Erythrocyte Hemolysate

3.2.5.1. Preparation of Reagents:

Trichloroacetic acid solution (TCA): 15% concentration of TCA was prepared with distilled water.

EDTA solution was prepared with 0,1 M concentration EDTA disodium dihydrate and distilled water.

TBA solution was prepared with 1% concentration of thiobutyric acid and 0,05 N NaOH.

Phosphate buffer: (pH: 7,5 0,33M): 11.931 g of disodium dihydrogen phosphate was added to 100mls of distilled water (Solution A) 0,907 grams of potassium dihydrogen was added to 20mls of distilled water. (Solution B) Then, 85 ml of solution A and 15 ml of solution B was mixed the pH was adjusted to 7,5 by using phosphoric acid and sodium hydroxide.

3.2.5.2. Procedure

For the aimed result, 0.5 ml of erythrocytes prepared according to the method 1 was mixed with 0.5 ml of phosphate buffer with a pH of 7.5. Then, 0.5 ml of 15% chloroacetic acid solution was added to the mixture and the mixture was vortexed and kept in the refrigerator on ice packs for 2 hours. These tubes were then centrifuged with 4400 rpm at 4C for 10 minutes. 1 ml of the supernatant were isolated into another poly-propylene tube and mixed with 1% TBA solution prepared within 75 ul of 0.1M EDTA and 250 ul 0.05 N NaOH solutions and vortexed for 15 seconds and later held in the boiling water bath for 15 minutes.

Under the same circumstances, 1 ml of phosphate buffer with pH of 7.52 was used blindly and 1 ml of standard was used for the calibration curve.

The absorbances of solutes were measured with spectrophotometry with wavelength of 532nm, against the blind samples. For both samples, two measurements were conducted and their average was taken. As standard, tetrametoxypropane (TMP) solution was used, which was prepared from five standard TMP solutions with concentrations ranging from 0.76 to 3.04 nmol/l. MDA concentrations of samples were calculated directly from calibration curve with the value of nmol/ml. These values were then later converted into nmol/gHb. Each solution was prepared fresh.

Table 3: Preparation of MDA Protocol

Component	Stock Solutions	Volume added
1:5 diluted erythrocyte		0,5
Phosphate Buffer	pH 7.5 0,33 M	0,5
TCA	15 %	0,5
Vortexed, stored in refrigerator on ice packs for 2 hours, then centrifuged at 4400 rpm, at 4C for 10 minutes.		
Supernatant, phosphate buffer or standard		1
EDTA	0,1 M	0,075
TBA	1%	0,25
Total volume		1,325
Incubated in boiling water bath for 15 minutes and then absorbance measurement at 532 nm.		

3.2.6. Erythrocyte GPx Activity Analysis

GPx activities were measured in erythrocyte hemolysate. The reaction mixture was 50 ml, containing 0.303 g of Trizma base, 0.0186 g of Na2EDTA, 0.00834 g of NADPH, 0.0130 g of sodium azide, 0.0307 g of GSH, and 50 U of GR. 10 µl of homogenate and 990 µl of the reaction mixture were mixed, and the reaction was initiated with 10 µl of t-butyl hydroperoxide (TBH). The decrease in absorbance was followed at 340 nm for 3 min (U/g protein).

3.2.7. CAT activity measurement

Catalase activity was expressed in U/gHb.CAT activity in erythrocyte lysate was measured by the method of Aebi [21]. The reaction mixture was 50 mM phosphate buffer pH 7.0, 10 mM H2O2 and erythrocyte lysate. The reduction rate of H2O2 was followed at 240 nm for 30 s at room temperature.

3.3. Histopathologic Examination

Renal tissue was fixated in 10% neutral buffered formalin, later buried in paraffin, cut into 3 μm slices and dyed with Hematoxilen and Eozin (H&E). Histopathological analysis was performed in a blinded manner using a light microscope (Olympus BX53,

Shinjuku, Tokyo, Japan). Histopathological changes were analyzed for tubular necrosis, proteinous deposits and medullary congestion as suggested by Solez et al. At least 10 randomly selected areas of the cortex and outer strip of outer medulla were evaluated and graded at x400 magnification for semi-quantitative analysis at each biopsy. Tubular necrosis and proteinous deposits were graded as follows: no damage (0), mild (+1, patchy isolated damage), moderate (+2, less than 25% damage), severe (+3, 25% to 50% damage)) and very severe (+4, more than 50% damage). The degree of medullary congestion was graded as follows: no congestion (0), mild (+1, vascular congestion where erythrocytes can be seen at x400 magnification), moderate (+2, vascular congestion where erythrocytes can be seen at x200 magnification), severe (+3, vascular congestion where erythrocytes can be seen at x100 magnification) congestion) and very severe (+4, vascular congestion where erythrocytes can be seen at x40 magnification) [95].

3.4. Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics 26.0. Normality test of the continuous variables was performed with Kolmogorov–Smirnov test. It was observed that continuous variables did not fit the normal distribution. Comparisons of the groups in terms of continuous variables were analyzed with Kruskal Wallis H test. Pairwise comparisons of the groups were performed with the Mann Whitney U test for the variables that had a statistically significant relationship as a result of the Kruskal Wallis H test. Comparisons of the groups in terms of categorical variables were analyzed with Chi-Square test. In the crosstabs, Fisher-Freeman-Halton Exact test was used because more than 20% of the entire cells have expected frequencies less than 5. The Fisher-Freeman-Halton Exact test is an analysis method used in the analysis of tables in the RxC dimension, when the assumptions of the Chi-square analysis are not met because the expected frequencies are low [96]. Column ratios were compared with the Z test and Bonferonni correction was performed to identify the cells that caused a statistically significant difference in test results. Continuous data were presented as medians and interquartile ranges, and categorical data were presented as frequencies and percentages. A p value below 0.05 was considered statistically significant difference.

4. RESULTS

Our study consists of 5 groups with 6 subjects each, with a total of 30 animals. Desired amount of blood samples was not obtained due to the fluid restriction and dehydration prior to sampling. Two of the subjects could not be sampled as they became unstable and they only underwent histopathological examinations. Owing to the low volumes of blood, oxidative stress parameters could not be measured in all subjects in Rosuvastatin and CFD groups.

4.1. Study Parameter Results

Renal tissue samples of rats were histopathologically evaluated for tubular injury and medullary congestion and the findings were graded from 0 to 4. Descriptive statistics of categorical variables are presented in Table 10 and 12. Descriptive statistics of continuous variables (NGAL, MDA, Creatinine, GPx, Catalase) are presented below.

4.1.1. Study Parameter Results Of All Groups

In our study, we measured serum NGAL levels of 28 rats. Highest levels of NGAL detected were 6,061 ng/ml and 7,169 ng/ml. Average value of NGAL was 6.809 ng/ml (SD ±0,441).

Serum creatinine values of the rats were also evaluated. Across the groups, the highest and the lowest measured values were 0,320 mg/dl and 4,010 mg/dl, respectively. The average value of serum creatinine was 1,653 mg/dl (SD ±1,501).

Erythrocyte MDA levels of 24 rats were measured. Across all groups, measured highest and lowest values were 1,689 nmol/g protein and 4,366 nmol/g protein. Mean value was 2,805 nmol/g protein (SD ±0,670).

There were 27 rats that Erythrocyte Catalase levels were calculated. Highest and lowest values of these groups were 2,622 kU/mg protein and 0,670 KU/mg protein. Average value of all the groups combined was 1,643 KU/mg protein (SD ±0,493).

Of the same rats, erythrocyte glutathione peroxydase levels were also measured. Highest and lowest values were 0,290 U/mg protein and 0,624 U/mg protein and across all groups the average value was 0,439 U/mg protein (SD ±0,987).

Table 4: Study Parameter Results Of All Groups

	N	Mean	Std. Deviation	Minimum	Maximum	Median (25th-75th)
NGAL ng/ml	28	6,81	0,18	6,06	7,17	6,83 (6,80-,84)
MDA nmol/g protein	24	2,81	0,67	1,69	4,37	2,76 (2,27-,18)
Catalase KU/mg protein	27	1,64	0,49	0,67	2,62	1,72(1,22-2,05)
Glutathione peroxidase U/mg protein	27	0,44	0,10	0,29	0,62	0,43 (0,34–0,52)
Creatinine mg/dl	28	1,71	1,50	0,32	4,01	0,78 (0,39-3,49)

NGAL: Neutrophil Gelatinase-Associated Lipocalin, MDA: Malondialdehyde

4.1.2. Study Parameter Results In Group D

Evaluation of serum NGAL levels of rats that were only dehydrated and received no treatment revealed values ranging from 6,788 ng/ml as the lowest to 7,169 ng/ml as the highest. Average value within the group was 6.932 ng/ml (SD ±0,182).

D group had serum creatinine values ranging from 0,320 mg/dl as the lowest to 0,430 mg/dl as the highest. Average of the group was 0,373 mg/dl (SD ±0,036).

Table 5: Study Parameter Results Of Group D

	N	Mean	Std. Deviation	Minimum	Maximum	Median (25th-75th)
NGAL ng/ml	6	6,93	0,18	6,79	7,17	6,83 (6,82-7,17)
MDA nmol/g protein	6	3,22	0,73	2,22	4,37	3,22 (2,62-3,76)
Catalase KU/mg protein	6	2,00	0,55	1,06	2,62	2,06 (1,62-2,45)
Glutathione U/mg protein	6	0,46	0,09	0,33	0,59	0,46 (0,39-0,53)
Creatinine mg/dl	6	0,37	0,04	0,32	0,43	0,38 (0,34-0,39)

NGAL: Neutrophil Gelatinase-Associated Lipocalin, MDA: Malondialdehyde

Among this group, lowest measured Erythrocyte MDA level was 2,221 nmol/g protein and highest was 4,366 nmol/g protein. Average of the group was 3,223 nmol/g protein (SD ±0,727).

Highest and lowest values of measured erythrocyte catalase in this group were 2,622 KU/mg protein and 1,062 KU/mg protein, respectively. Average of the group was 2,001 KU/mg protein (SD ±0,553).

Erythrocyte glutathione peroxidase levels of the group were 0,325 U/mg protein as the lowest and 0,588 U/mg protein as the highest. Average value of the group was 2,001 U/mg protein (SD±0,553).

4.1.3. Study Parameter Results Of Group FD

Evaluation of serum NGAL levels of rats treated only with furosemide revealed values ranging from 6,735 ng/ml as the lowest to 6,950 ng/ml as the highest. Average value within the group was 6.840 ng/dl (SD ±0,068).

FD group had serum creatinine values ranging from 0,340 mg/dl as the lowest to 0,440 mg/dl as the highest. Average of the group was 0,405 mg/dl with a (SD ±0,037).

Table 6: Study Parameter Results Of Group FD

	N	Mean	Std. Deviation	Minimum	Maximum	Median (25th-75th)
NGAL ng/ml	6	6,84	0,07	6,74	6,95	6,84 (6,81-6,87)
MDA nmol/g protein	6	2,67	0,89	1,69	4,12	2,57 (1,86-3,39)
Catalase KU/mg protein	6	1,51	0,52	0,79	2,09	1,45 (1,09-2,06)
Glutathione U/mg protein	6	0,43	0,13	0,29	0,62	0,42 (0,30-0,56)
Creatinine mg/dl	6	0,41	0,04	0,34	0,44	0,42 (0,37-0,43)

NGAL: Neutrophil Gelatinase-Associated Lipocalin, MDA: Malondialdehyde

Among this group, lowest measured Erythrocyte MDA level was 1,689 nmol/g protein and highest was 4,119 nmol/g protein. Average of the group was 2,667 nmol/g protein with a (SD ±0,894).

Highest and lowest values of measured erythrocyte catalase in this group were 2,089 KU/mg protein and 0,794 KU/mg protein, respectively. Average of the group was 1,505 KU/mg protein (SD ±0,517).

Erythrocyte glutathione peroxidase levels of the group were 0,293 U/mg protein as the lowest and 0,624 U/mg protein as the highest. Average value of the group was 0,431 U/mg protein (SD±0,132).

4.1.4. Study Parameter Results Of Group CFD

Evaluation of serum NGAL levels of 6 rats treated with contrast media revealed values ranging from 6,061 ng/ml as the lowest to 6,840 ng/ml as the highest. Average value within the group was 6.680 ng/dl with a standard deviation of ±0,309.

CFD group had serum creatinine ranging from 2,980 mg/dl as the lowest to 4,010 mg/dl as the highest. Average of the group was 3,488 mg/dl with a standard deviation of ±0,327.

Among this group, lowest measured Erythrocyte MDA level was 2,121 nmol/g protein and highest was 2,729 nmol/g protein. Average of the group was 2,367 nmol/g protein with a standard deviation of ±0,319.

There were 5 rats whose erythrocyte catalase levels were measured in this group. Highest and lowest values were 1,721 KU/mg protein and 0,670 KU/mg protein, respectively. Average of the group was 1,298 KU/mg protein with a standard deviation of ±0,437.

There were also 5 rats whose erythrocyte glutathione peroxidase levels were measured in this group. Highest and lowest values were 0,563 U/mg protein and 0,335 U/mg protein, respectively. Average of the group was 0,422 U/mg protein (SD ±0,085).

Table 7: Study Parameter Results Of Group CFD

	N	Mean	Std. Deviation	Minimum	Maximum	Median (25th-75th)
NGAL ng/ml	6	6,68	0,31	6,06	6,84	6,83 (6,53-6,84)
MDA nmol/g protein	3	2,37	0,32	2,12	2,73	2,25 (2,12-2,73)
Catalase KU/mg protein	5	1,30	0,44	0,67	1,72	1,22 (0,92-1,72)
Glutathione U/mg protein	5	0,42	0,09	0,34	0,56	0,40 (0,36-0,49)
Creatinine mg/dl	6	3,49	0,33	2,98	4,01	3,49 (3,32-3,66)

NGAL: Neutrophil Gelatinase-Associated Lipocalin, MDA: Malondialdehyde

4.1.5. Study Parameter Results Of Rosuvastatin

Among the group that received Rosuvastatin, NGAL levels of 6 rats were measured. Lowest detected NGAL value was 6,626 ng/ml and highest was 6,840 ng/ml. Average value was 6.779 ng/dl (SD±0,088).

Evaluation of serum creatinine levels of 5 rats revealed the highest value to be 3,920 mg/dl and lowest value to be 1,850 mg/dl. The average across the group was 3,328 mg/dl (SD±0,841).

Erythrocyte MDA levels of 3 rats were measured and values ranged from 2,575 nmol/g protein as the lowest, to 3,641 nmol/g protein as the highest. The average value was 2,994 nmol/g protein (SD±0,568).

Erythrocyte catalase (CAT) levels were evaluated in 4 rats. Between the Rosuvastatin group the highest and lowest values were, 2,071 KU/mg protein and 1,189 KU/mg protein, respectively. Average value of the group was 1,578 KU/mg protein (SD±0,438).

Erythrocyte glutathione peroxidase (GPx) levels were evaluated in 4 rats. Between the Rosuvastatin group the highest and lowest values were, 0,521 U/mg protein and 0,290 U/mg protein, respectively. Average value of the group was 0,388 U/mg protein (SD ±0,108).

Table 8: Study Parameter Results Of Group Rosuvastatin

Variables	N	Mean	Std. Deviation	Minimum	Maximum	Median (25th-75th)
NGAL ng/ml	5	6,78	0,09	6,63	6,84	6,80 (6,71-6,84)
MDA nmol/g protein	3	2,99	0,57	2,58	3,64	2,77 (2,58-3,64)
Catalase KU/mg protein	4	1,58	0,44	1,19	2,07	1,53 (1.20-2.01)
Glutathione U/mg protein	4	0,39	0,11	0,29	0,52	0,37 (0,30-0,50)
Creatinine mg/dl	5	3,33	0,84	1,85	3,92	3,61 (2,68-3,84)

NGAL: Neutrophil Gelatinase-Associated Lipocalin, MDA: Malondialdehyde

4.1.6. Study Parameter Results Of Group Atorvastatin

Among the group that received Atorvastatin, NGAL levels of 5 rats were measured. Lowest detected NGAL value was 6,061 ng/ml and highest was 7,169 ng/ml. Average value was 6.809 ng/dl (SD ±0,441).

Evaluation of serum creatinine levels of 28 rats showed the highest value to be 4,010 and lowest value to be 0,320 mg/dl. The average across the group was 1,653 mg/dl (SD±1,501).

Erythrocyte MDA levels of 24 rats were measured and values ranged from 1,689 nmol/g protein as the lowest, to 4,366 nmol/g protein as the highest. The average value was 2,805 nmol/g protein (SD±0,670).

Erythrocyte catalase (CAT) levels were evaluated in 27 rats. Between the Atorvastatin group the highest and lowest values were, 2,622 KU/mg protein and 0,670 kU/mg protein, respectively. Average value of the group was 1,643 KU/mg protein (SD±0,493).

Erythrocyte glutathione peroxidase (GPx) levels were evaluated in 27 rats. Between the Atorvastatin group the highest and lowest values were, 0,624 U/mg protein and 0,290 U/mg protein, respectively. Average value of the group was 0,439 U/mg protein (SD±0,987).

Table 9: Study Parameter Results Of Group Atorvastatin

Variables	N	Mean	Std Deviation.	Minimum	Maximum	Median (25th-75th)
NGAL ng/ml	5	6,81	0,04	6,74	6,84	6,82 (6,78- 6,84)
MDA nmol/g protein	6	2,65	0,42	1,84	2,99	2,76 (2,45-2,94)
Catalase KU/mg protein	6	1,75	0,33	1,28	2,20	1,78 (1,44-2,04)
Glutathione U/mg protein	6	0,48	0,09	0,34	0,58	0,49 (0,41-0,55)
Creatinine mg/dl	5	1,14	0,79	0,41	2,39	1,00 (0,49-1,86)

NGAL: Neutrophil Gelatinase-Associated Lipocalin, MDA: Malondialdehyde

4.2. Histopathologic Statistics Results of All Groups

Renal tissue of all the rats involved in the study were examined histopathologically for tubular necrosis with proteinous cast and medullary congestion. These findings were graded from 0 to 4. The average grade of medullary congestion and tubular injury were 2,333 (SD±0,994) and 2,066 (SD±1,112), respectively.

Microscopic images of renal tissue samples are available as Figures 3-6.

4.2.1. Medullary Congestion

When the pathology results of rats in the Atorvastatin Group were evaluated in terms of medullary congestion, the data showed that 2 (33.3%) rats had mild congestion and 4 (66.7%) rats had Severe congestion. When the pathology results of rats in the Rosuvastatin Group were evaluated in terms of medullary congestion, it was seen that 5 (83.3%) rats had moderate congestion and 1 (16.7) rat had very severe congestion. When

we look at the CFD group, 3 (50%) rats had severe and 3 (50%) rats had very severe medullary congestion. In the FD group, there were 2 (33.3%) rats in mild severity and 4 (66.7%) in moderate severity. And lastly in the D group, which is our control group, there were 3 (50%) rats in mild severity, 1 (16.7%) in moderate severity and 2 (33.3%) rats in severe severity. When the groups were compared in terms of severity of medullary congestion, there was a statistically significant difference between the groups ($p<0.001$). For the detection of the cells that cause this significant difference, the ratio comparison (Z test) was performed. According to the results obtained from the Z test, the ratio of those with moderate medullary congestion severity in the Rosuvastatin group had a statistically significant difference compared to the ratio of the moderate severity of Atorvastatin and CFD groups.

Table 10: Medullary Congestion Score Crosstab Within Every Study Group

			Atorvastatin Group	Rosuvastatin Group	CFD Group	FD Group	D Group	Total
Medullary Congestion Score	Mild	Count	2[a]	0[a]	0[a]	2[a]	3[a]	7
		% within Group	33,3%	0,0%	0,0%	33,3%	50,0%	23,3 %
	Moderate	Count	0[a,b]	5[b]	0[a,b]	4[a]	1[a]	10
		% within Group	0,0%	83,3%	0,0%	66,7%	16,7%	33,3 %
	Severe	Count	4[a]	0[a]	3[a]	0[a]	2[a]	9
		% within Group	66,7%	0,0%	50,0%	0,0%	33,3%	30,0 %
	Very severe	Count	0[a]	1[a]	3[a]	0[a]	0[a]	4
		% within Group	0,0%	16,7%	50,0%	0,0%	0,0%	13,3 %
Total		Count	6	6	6	6	6	30
		% within Group	100,0%	100,0%	100,0%	100,0%	100,0 %	100,0 %

C: Contrast, F: Furosemide, D: Dehydration. a: Each top script letter denotes a subset of group categories whose column proportions do not differ significantly from each other at the 0,05 level. b: Cell with statistically significant difference compared to the moderate scored subjects among Atorvastatin, Rosuvatatin and CFD group. Rosuvastatin group-moderate congestion/atorvastatin group-moderate congestion $p<0,05$. Rosuvastatin group-moderate congestion/CFD group moderate congestion $p<0,05$. No statistically significant difference was detected in comparing the severity of medullary congestion among the other cells. ($p>0.05$)

Table 11: Medullary Congestion Score Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	31,754	12	0,002	,001		
Likelihood Ratio	38,999	12	0,000	,000		
Fisher's Exact Test	25,865			,000		
Linear-by-Linear Association	1,686	1	0,194	,222	,111	,023
N of Valid Cases	30					

4.2.2. Tubular Necrosis and Proteinous Cast

When the pathology results of rats in the Atorvastatin Group were evaluated in terms of tubular damage, 2 (33.3%) rats had mild, 1 (16.7%) had moderate, 2 (33.3%) had severe and 1 (16.7%) had very severe tubular damage. The tubular damage scoring of subjects in the Rosuvastatin Group revealed, 3 (50%) rats in mild severity, 2 in moderate severity (33.3%) and 1 (16.7%) rat in severe severity.

Table 12: Tubular Necrosis and Proteinous Cast Score Crosstab Within Every Study Group

			Groups					Total
			Atorvastatin Group	Rosuvastatin Group	CFD Group	FD Group	D Group	
Tubular Necrosis and Proteinous Cast	Mild	Count	2[a]	3[a]	0[a]	4[a]	3[a]	12
		% within Group	33,3%	50,0%	0,0%	66,7%	50,0%	40,0%
	Moderate	Count	1[a]	2[a]	3[a]	2[a]	1[a]	9
		% within Group	16,7%	33,3%	50,0%	33,3%	16,7%	30,0%
	Severe	Count	2[a]	1[a]	0[a]	0[a]	1[a]	4
		% within Group	33,3%	16,7%	0,0%	0,0%	16,7%	13,3%
	Very Severe	Count	1[a]	0[a]	3[a]	0[a]	1[a]	5
		% within Group	16,7%	0,0%	50,0%	0,0%	16,7%	16,7%
Total		Count	6	6	6	6	6	30
		% within Group	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%

a: Each top script letter denotes a subset of group categories whose column proportions do not differ significantly from each other at the 0,05 level.
No statistically significant difference was detected in comparing the severity of tubular damage among the groups (p>0.05)

When the pathology results of rats in the CFD group were evaluated in terms of tubular damage, there were 3 (50%) rats of moderate severity and 3 (50%) rats of very severe severity. In the FD group, there were 4 (66.7%) rats in mild severity and 2 (33.3%) rats

in moderate severity. When we look at the tubular damage intensities of group D, which is our control group, there were 3 rats in mild intensity (50%), 1 in moderate intensity 16.7%), 1 (16.7%) in severe intensity and 1 rat in very severe intensity. No statistically significant difference was detected in comparing the severity of tubular damage among the groups (p=0.259).

Table 13: Tubular Necrosis and Proteinous Cast Score Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	14,889	12	0,248	0,262		
Likelihood Ratio	18,741	12	0,095	0,244		
Fisher's Exact Test	13,231			0,259		
Linear-by-Linear Association	0,485	1	0,486	0,530	0,265	0,036
N of Valid Cases	30					

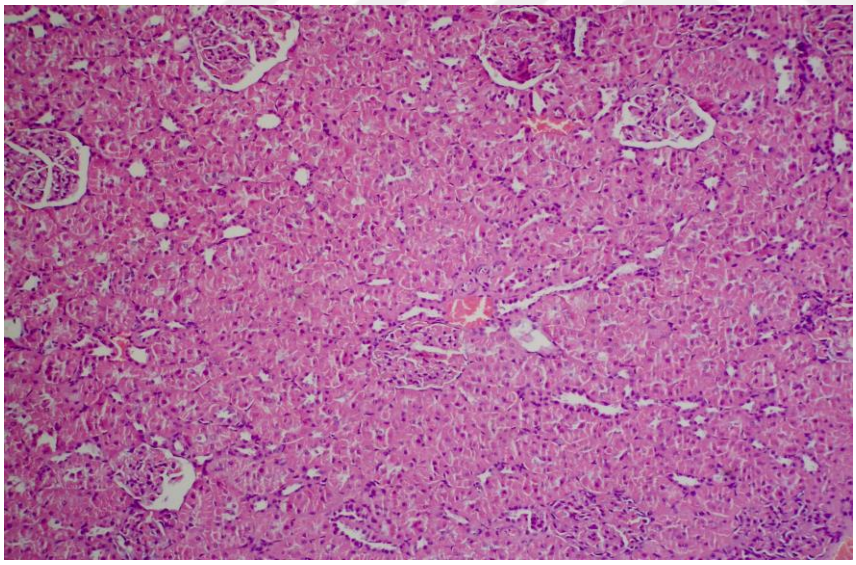

Figure 3: Histopathological examination of kidney tissues by light microscopy. Near-normal-appearing kidney tissue without tubular damage, proteinous deposition, or medullary congestion H&E x100.

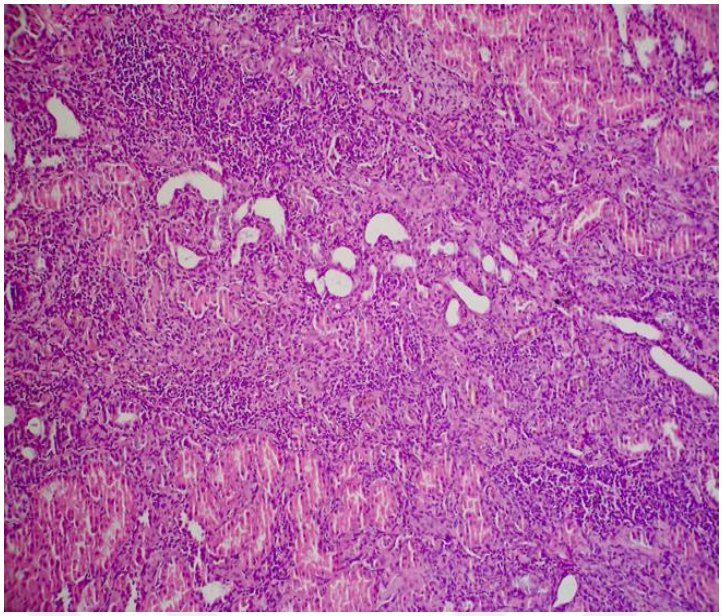

Figure 4: Histopathological examination of kidney tissues by light microscopy. Severe tubular damage and lymphoid cell infiltration H&E x100

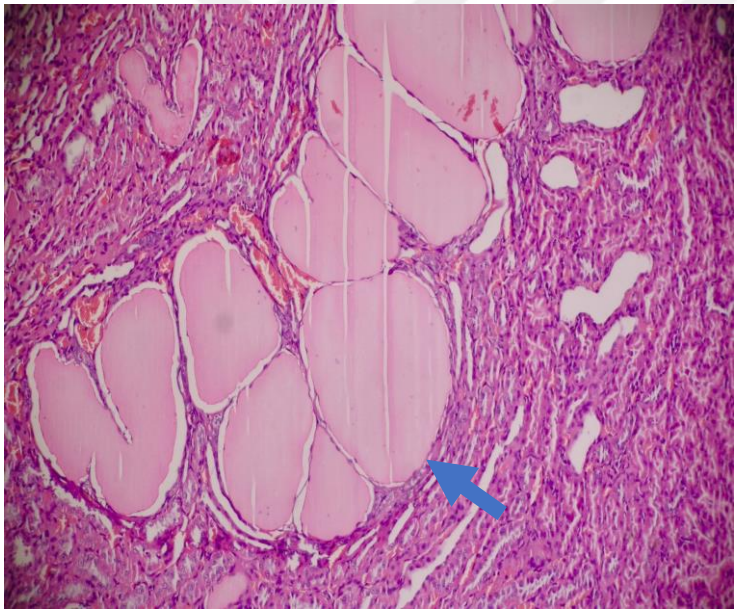

Figure 5: Histopathological examination of kidney tissues by light microscopy. Tubular dilatation and proteinaceous substance deposition (arrow) H&E x200

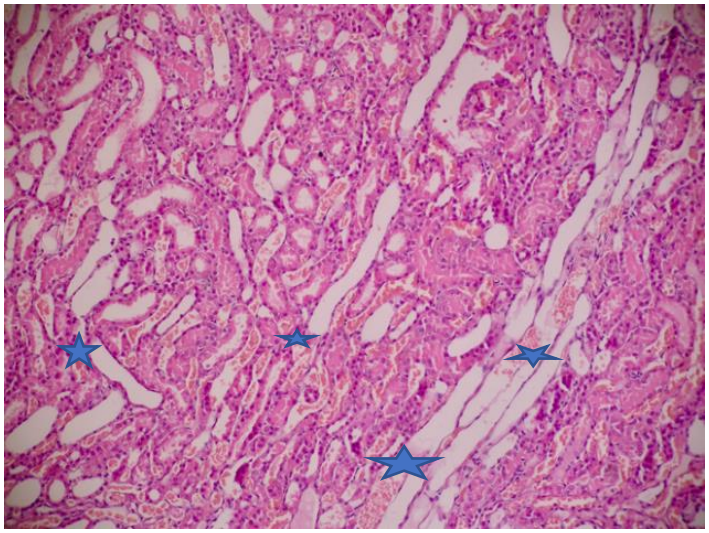

Figure 6:Histopathological examination of kidney tissues by light microscopy. Severe medullary congestion findings (asterix) H&E x100 H&E: Hemotoxylin & Eosin

4.3. Statistical Analysis Of Biochemical Parameters Between Groups

4.3.1. Statistical Analysis Of NGAL, MDA, Cat and GPx

No significant difference was found between the groups in the analyzes performed between serum NGAL levels. Among the oxidative stress parameters, erythrocyte MDA, CAT, GPx levels were measured. In the statistical evaluation of erythrocyte MDA, CAT and GPx levels, no significant difference was found between the groups (p>0,05). Statistical analysis between biochemical parameters of all groups is shown in table 14. Mean values of the aforementioned biochemical parameters are shown in Graphics 1-4.

Table 14: Statistical Analysis Between Oxidative Stress Parameters and Serum NGAL

GROUPS	NGAL Mean ±SD	MDA Mean ±SD	Catalase Mean ±SD	Glutathione Mean ±SD
Group D	6,93 ±0,18	3,22 ±0,73	2,00 ±0,55	0,46 ±0,09
Group FD	6,84 ±0,07	2,67 ±0,89	1,51 ±0,52	0,43 ±0,13
Group CFD	6,68 ±0,31	2,37 ±0,32	1,30 ±0,44	0,42 ±0,09
Group Rosuvastatin	6,78 ±0,09	2,99 ±0,57	1,58 ±0,57	0,39 ±0,11
Group Atorvastatin	6,81 ±0,04	2,65 ±0,42	1,75 ±0,33	0,48 ±0,09
Asymptotic Sig.(2-sided test)	0,500	0,305	0,160	0,629

NGAL (ng/ml): Neutrophil Gelatinase-Associated Lipocalin, Cat (KU/mg protein): Catalase, MDA (nmol/g protein): Malondialdehyde, GPx (U/mg protein): Glutathione Peroxidase. No significant difference was detected between groups in NGAL, Catalase, GPx and MDA levels. (p > 0,05).

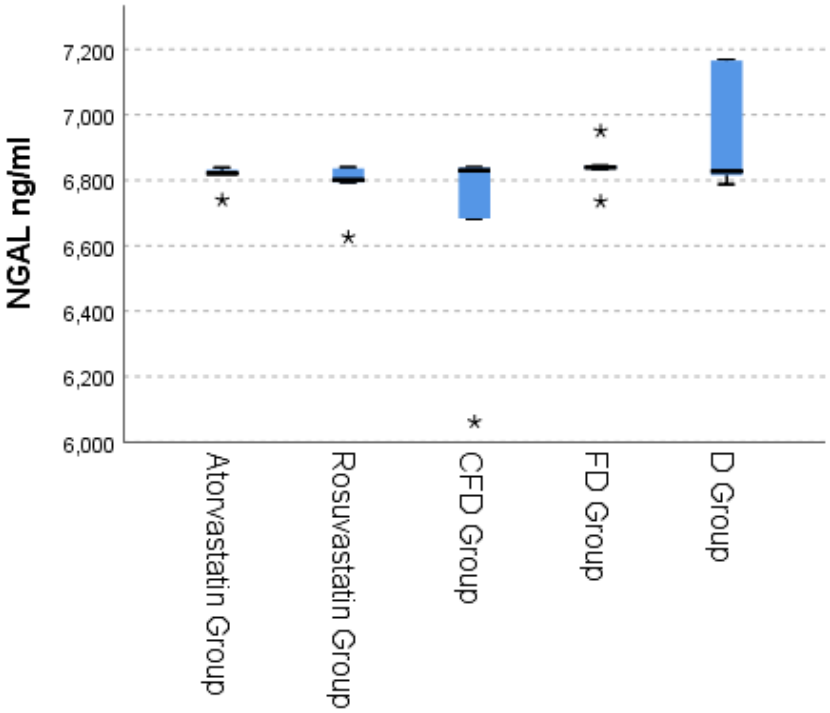

Figure 7:Serum NGAL Level (ng/ml) Statistical Analysis**.** No significant difference was detected between groups (p > 0,05).

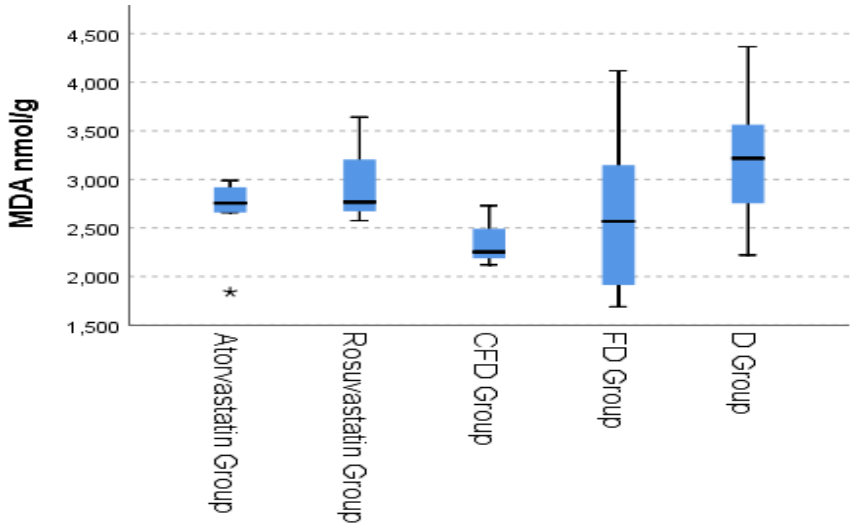

Figure 8: Erythrocyte MDA Level (nmol/g protein) Statistical Analysis. No significant difference was detected between groups (p > 0,05).

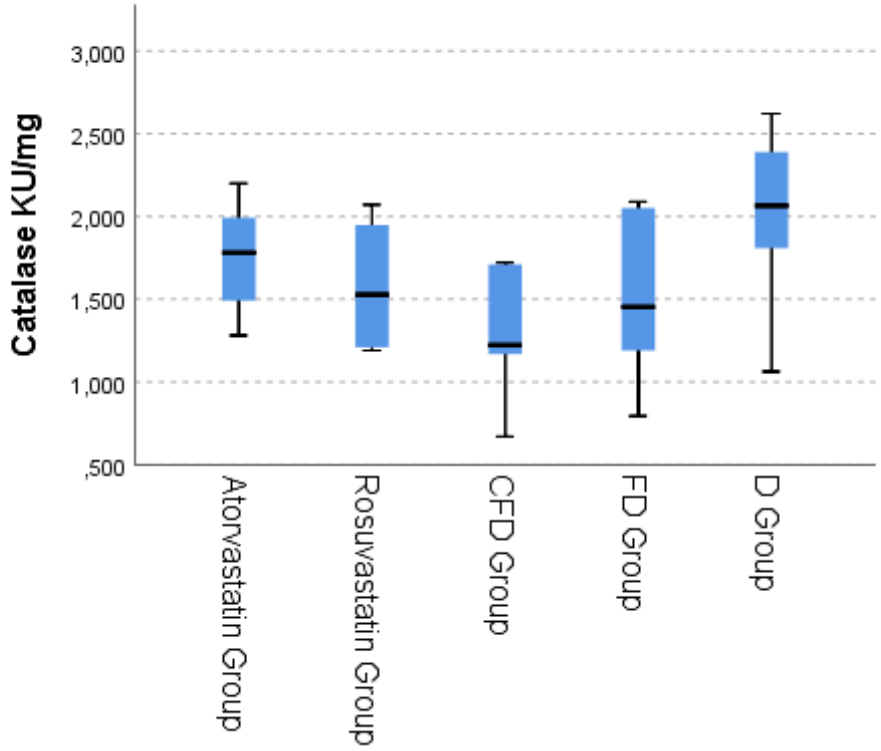

Figure 9: Erythrocyte Catalase (U/mg protein) Statistical Analysis. No significant difference was detected between groups ($p > 0,05$).

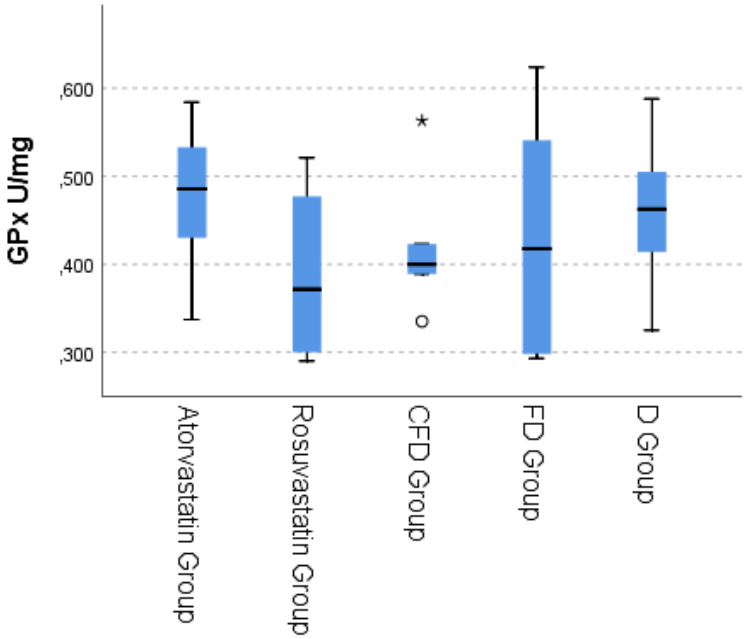

Figure 10: Erythrocyte GPx Level (U/mg protein) Statistical Analysis. No significant difference was detected between groups ($p > 0,05$).

4.3.2. Serum Creatinine Statistical Analysis

Of the 3 groups given contrast material; The median creatinine value of the atorvastatin group was 1,000 mg/dl (IQR 1.375), the rosuvastatin median value was 3.61 mg/dl (IQR 1.165), and the median value of the CFD group was 3.485 mg/dl (IQR 0.340). In the control groups, the creatine median value of the D group was 0.380 mg/dl (IQR 0.050) and th median value of the FD group was 0.420 mg/dl (IQR 0.063). Mean creatinine values of all groups are shown in Graphic 5.

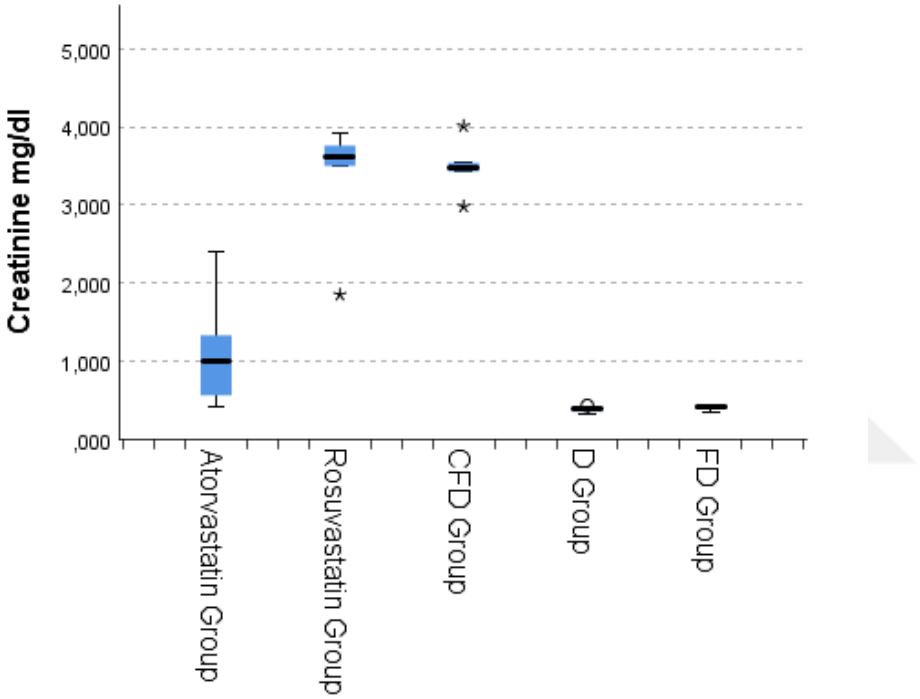

Figure 11: Serum Creatinine (mg/dl) Statistical Analysis. D-FD p>0.05, D-Atorvastatin p<0,05, D-CFD p<0,05, D-Rosuvastatin p<0,05, FD-Rosuvastatin p>0,05, FD-Atorvastatin p>0,05, FD-CFD p<0,05, FD-Rosuvastatin p<0,05, Atorvastatin-CFD p<0,05, Atorvastatin-Rosuvastatin p>0,05, CFD-Rosuvatatin p>0,05

The creatine levels of all groups were compared with each other using the Pairwise comparison method. For Post-hoc, Bonferonni test was applied to calculate statistical significance in pairwise comparisons. Pairwise comparisons of groups showed that there was no statistically significant difference between creatinine values of D and FD groups (p>0.240). However, there were statistically significant differences between Atorvastatin and D group (p<0.010), D group and CFD group (p<0.002) and D group and Rosuvastatin group (p>0.004). There was no statistically significant difference between the two groups

in the comparison of the FD group with the Atorvastatin group (p>0.05). There was a significant difference between creatine levels in the comparison of FD and Rosuvastatin group (p0.004). In comparison of the FD group with the CFD group, creatine levels in the CFD group were higher and a statistically significant difference was found (p<0.002). There was a statistically significant difference between creatine levels between the atorvastatin group and the CFD group (p<0.004). In the comparison of the Atorvastatin and Rosuvastatin groups, the creatine levels of the atorvastatin group were lower and a statistically significant difference was detected (p<0.016). There were no similar levels of creatine levels and no statistically significant differences in the CFD group and Rosuvastatin groups (p>0.662). These findings are summarized below in Table 15.

Table 15: Pairwise Comparisons of Groups in Terms of Creatinine

Groups	Z	Asymp. Sig. (2-tailed)	Exact Sig. [2*(1-tailed Sig.)]
D Group-FD Group	-1,309	0,191	0,240
D Group-Atorvastatin Group	-2,580	0,010	0,009
D Group-CFD Group	-2,903	0,004	0,002
D Group-Rosuvastatin Group	-2,764	0,006	0,004
FD Group-Atorvastatin Group	-2,013	0,044	0,052
FD Group-CFD Group	-2,887	0,004	0,002
FD Group-Rosuvastatin Group	-2,745	0,006	0,004
Atorvastatin Group-CFD Group	-2,739	0,006	0,004
Atorvastatin Group-Rosuvastatin Group	-2,402	0,016	0,016
CFD Group-Rosuvastatin Group	-0,548	0,584	0,662

D: Dehydration FD: Furosemid and Dehydration CFD: Contrast media, Furosemid and Dehydration D-FD p>0.05, D-Atorvastatin p<0,05, D-CFD p<0,05, D-Rosuvastatin p<0,05, FD-Rosuvastatin p>0,05, FD-Atorvastatin p>0,05, FD-CFD p<0,05, FD-Rosuvastatin p<0,05, Atorvastatin-CFD p<0,05, Atorvastatin-Rosuvastatin p>0,05, CFD-Rosuvatatin p>0,05.

5. DISCUSSION

In our study we investigated the efficacy and mechanism of action of statins in preventing contrast induced nephropathy. Our study was conducted with 5 groups of animals, each containing 6 rats. The groups were divided as follows: Group D, which only received fluid restriction, Group FD, which received Furosemide with fluid restriction, Group CFD which received contrast media, furosemide and fluid restriction and two groups which received Atorvastatin and Rosuvastatin in addition to receiving contrast media, furosemide and fluid restriction, Group Atorvastatin and Group Rosuvastatin, respectively. The two drugs, namely Atorvastatin and Rosuvastatin were chosen due to the frequency of their use in current clinical settings. As diagnostic criteria for contrast induced nephropathy, an increase of equal to or more than 25% in serum creatinine was accepted. For determining the mechanism of action of statins, histopathological examination of renal samples and oxidative stress markers such as serum NGAL, erythrocyte MDA, CAT and GPx were utilized.

All groups which received contrast media had higher serum creatinine levels compared to the ones that received only fluid restriction and furosemide. When comparing oxidative stress markers and serum NGAL levels among the groups, there was no significant difference among the ones that received statins with contrast media and ones that received only contrast media. Furthermore, we were not able to demonstrate a statistically significant difference in tubular injury or medullary congestion scores between the same groups in histopathological examinations.

To summarize the currently accepted mechanism behind CI-AKI, it is generally associated with renal parenchymal damage and tissue hypoxia. Parenchymal injury is caused by iodinated contrast media induced hypoxia, which in turn generates ROS that directly insults renal parenchyma. This hypoxia and concurrent ischemia results in occlusion in renal medullary capillaries, which yields to tubular and vascular damage and inflammation. Administration of contrast media substantially decreases medullary oxygenation, without decreasing reuptake of the tubules. Response to this capillary endothelial damage is secretion of endothelin and prostaglandins and subsequent neurohumoral vasoconstriction. Ultimately, an oxygen imbalance occurs and ROS production accelerates. In hypoxic settings, ATP is rapidly hydrolyzed, yielding ADP, AMP and finally adenosine. Adenosine is in turn hydrolyzed by 5’-nucleotidase into Inosine and then into Hypoxanthine by Nucleoside phosphorylase. Hypoxanthine is

substrate of Xanthine Oxidase enzyme, which generates uric acid and hydrogen peroxide (H2O2). Hydrogen peroxide causes further damage to renal medullary microcirculation by decreasing NO. Additionally, superoxide radicals also decrease bioavailability of NO by forming peroxynitrite. Therefore, under physiological circumstances, NO provides protection against ROS induced endothelial damage [97].

In a similar study conducted by Lin Yan et al. In 2020, the preventative effect of atorvastatin on contrast induced nephropathy was demonstrated [98]. In line with our study, atorvastatin was able to reduce serum creatinine. However in contrast to our study, Lin Yan Et al. Were also able to demonstrate statistically significant changes in tubular injury scores, serum NGAL and MDA levels, which can be attributed to the higher volume of contrast media that was used in our study. In our study plan the subjects were tested for nephropathy 24 hours after injection with contrast media. In previously reported articles, CI-AKI is predicted to occur within 24 - 72 hours. Therefore it would be plausible that an histopathological examination conducted within the 48 - 72 hour period would yield increased tubular injury in subjects that received contrast media in contrast to the subjects that were treated with statins.

In their recent study in 2019 Sharif et al. Reported that Atorvastatin was effective in preventing contrast induced nephropathy [99]. This effect was demonstrated by reduction of oxidative stress markers such as MDA and GSH. The study also concluded that the effect Atorvastatin had was dose dependent and as the concentration of Atorvastatin increased, the degree of oxidative stress was reduced. Parallel to their findings, in our study the group that received Atorvastatin had significantly lower levels of serum creatinine compared to Rosuvastatin and control groups. The same difference was not evident when comparing oxidative stress markers such as MDA or GPx. However, the serum creatinine values of Atorvastatin group were higher than of the groups that did not receive any contrast media, which may be due to the fact that our study protocol used higher volumes of contrast media and therefore induced higher nephrotoxic effects compared to the study of Sharif et al. These findings can be interpreted as at higher concentrations, atorvastatin can reduce oxidative stress and consequent contrast induced nephropathy. It is plausible to assume that similar studies conducted with varying drug concentrations would hele further explain the mechanisms involved in CI-AKI.

In their 2015 study Deng et al. Demonstrated the effect of Rosuvastatin on preventing contrast nephropathy in rats with preexisting diabetes by showing significant

decrease in serum creatinine [100]. Our findings showed no effect of Rosuvastatin on either serum creatinine or histopathological examinations of subjects. Regarding the histopathological examination, the difference in findings can be attributed to the difference in kidney injury protocols used in the two studies. Jiang et al. used three groups, each containing 16 rats in their study on efficiency of Rosuvastatin in preventing contrast induced nephropathy [101]. In contrast to our study, Jiang et al. Divided each group into two subgroups and measured serum creatinine levels and conducted histopathological examinations at the 24th hour and 72th hour marks, respectively. At 24th hour, even though there was statistically significant difference between serum creatinine levels of subjects that received both contrast media and Rosuvastatin and the group that received only contrast media, when compared to the control group, serum creatinine values of the Rosuvastatin group were higher. Parallel to the serum creatinine, tubular injury scores of the Rosuvastatin group were higher than of the control group. The outcome was the same for the findings at the 72th hour mark. When histopathological findings from each subgroup at 24 hours and 72 hours are compared, no statistically significant difference was present. However, when we look at serum creatinine values of each subgroup at the 72 hours mark, while the serum creatinine levels of the contrast group continued to increase, the same elevation was not present in control or the Rosuvastatin groups. Ultimately Rosuvastatin was effective in preventing elevation of serum creatinine. Our study protocol included only the evaluation of parameters at 24 hours. At 24 hours, serum creatinine levels in our study resemble that of the Rosuvastatin group of and the contrast group of Jiang et al. We could name the absence of a second measurement of our parameters at 72 hours as a limitation of our study. In contrast to our findings, Jiang et al. Reported that tubular injury scores of contrast and Rosuvastatin groups were higher than the control group. However, the study protocol of this study does not include any fluid restriction or any drug administration that would decrease GFR, prior to contrast media. Therefore any and all tubular injury can only be explained by contrast media exposure. Whereas our experiment protocol includes both fluid restriction and GFR reduction among the groups. Since effects of contrast media and the statins are dependent on dose and duration of use, one could argue that although duration of use was longer for statins, the higher volumes of contrast media administered could have hindered protective activity of Rosuvastatin.

In the study published by Wang et al. In 2017, both Atorvastatin and Rosuvastatin were shown to have renal protective effects against contrast induced nephropathy [102]. The study further demonstrated that although both drugs had favorable effects on oxidative stress, Atorvastatin was considerably more effective in terms of kidney function parameters and histopathological examination findings. Similarly, in our study the Atorvastatin receiving group had significantly lower levels of serum creatinine levels when compared to CFD Group. However, the same effect was not evident in the Rosuvastatin group, there was no statistically significant difference in serum creatinine levels of the Rosuvastatin group and CFD group. There were no significant differences in oxidative stress markers among the groups in our study and contradictory to the study by Wang et al., oxidative stress cannot be associated with development of CI-AKI.

NGAL is constantly produced from numerous different cells and tissues and it is detectable in low concentrations in healthy subjects. In kidneys, NGAL is filtered in glomerulus, followed by the megaline dependent reuptake of luminal NGAL in the proximal tubules [103, 104]. Therefore initial low levels of urine NGAL is to be expected. Following the acute kidney injury, intracellular levels of NGAL in the epithelium of ascending limb of Henle, distal tubule and collecting duct are elevated. This elevation is apparent in plasma and the urine, which can be attributed to the secretion of NGAL from both the apical and basolateral parts of the epithelium. Furthermore, the present tubular injury may intervene with proximal tubular reabsorption and increase urinary levels of NGAL [104]–[106]. These properties of NGAL makes it a serious candidate for replacing serum creatinine in measuring acute tubular injury.

In the absence of acute kidney injury, production of Neutrophil gelatinase associated lipocalin is upregulated in extrarenal tissues during malignancy and sepsis [107]–[110]. On the other hand, in the setting of lower urinary tract infection and subsequent leukocyturia, urine NGAL levels are elevated without apparent kidney injury [111]. Therefore, for interpretation of acute kidney injury, extrarenal sources of NGAL may be misleading. In a study conducted in a large and heterogeneous population, Tomonaga et al. Demonstrated that urinary NGAL levels were also determined by age, sex and liver function and highly correlated with inflammatory parameters [112]. The study also concluded that according to the conventional criteria, 6.5% of subjects who had no kidney disease had elevated levels of urinary NGAL.

In light of these findings, it is clear that serum NGAL levels and tubular injury scoring used in AKI are closely correlated. The absence of a significant difference in serum NGAL among groups in our study can be attributed to the fact that there were no significant differences among histopathological scoring of the groups.

In their 2016 study on CI-AKI and N-acetylcysteine, Li et al. Reported that while serum creatinine levels increase at 48 hours, renal extract MDA and serum NGAL levels begin to rise in 2 hours [27]. In the evaluation of their histopathological examination findings, tubular injury scores of groups that received contrast media were substantially higher than of the control group. The tubular injury scores of groups that received both contrast media and NAC were significantly lower than of the groups which received only contrast media, however when compared to the control group, the statistically significant difference was not evident. In a more recent animal study by Sancho-Martínez et al. on acute tubular injury and renal biomarkers such as serum NGAL and serum creatinine, no significant correlation between the biomarkers and the degree of tubular injury [113]. Similarly, the animal study carried out by Cusomona et al. on the effects of NAC and Atorvastatin on oxidative stress demonstrated that, while Atorvastatin had an impact on MPO and GPx enzymatic activity, there was no significant difference in catalase activity or tubular injury scoring among the groups [114]. A similar study conducted by Boyacıoğlu et al. in 2014 on the effect of L-carnitine in preventing contrast induced nephropathy also included oxidative stress markers and histologic examinations of renal tissue of the subjects [115]. The study concluded that L-carnitine was in fact effective in prevention of CI-AKI. Even though they successfully demonstrated the association between creatine levels and tubular injury, there were no significant correlation between CAT activity and renal parenchymal injury and serum creatinine or any statistically significant difference among the groups in terms of CAT activity.

These studies suggest that renal biomarker levels and oxidative stress is not always correlated with tubular injury. In line with these suggestions, our study concluded that statin use has no statistically significant impact on serum creatinine and serum NGAL levels in the setting of acute kidney injury caused by contrast media. Considering the pleiotropic characteristics that biomarkers share with possible comorbidities and the inter-individual phenotypic heterogeneities, it would be plausible to expect far greater differences in humans than in animal studies.

The main limitation of our study is the sample size of the groups. Given the fact that both fluid restriction and tubular injury reduce the amount of blood that can be drawn from the rats, to evaluate the anti-inflammatory, antioxidant or antithrombotic effects of statins, additional experimentations are necessary. Additionally, dosage and duration dependent responses to these drugs were not included in our study. Therefore, further research is needed to determine the optimal dose and duration of statin therapy to prevent CI-AKI. Lastly, the reasons behind the differences in effects of the two statins in this study are unclear. Having said that, lipophilicity of statins could be a possible explanation. Lipophilic statins such as Atorvastatin, enter cells through passive diffusion and are more easily distributed in the body compared to hydrophilic statins such as Rosuvastatin [116]. In a sense, Atorvastatin could be more "Renophilic" than Rosuvastatin. Therefore further research on chemical properties, bioavailability and pharmacokinetics of statins are required.

Observations of oxidative stress is possible either through measuring end products of oxidative injury or by investigating the potential of an organism, tissue or bodily fluid to withstand excess oxygenation. Unfortunately, there is little to no consensus on the selection of appropriate antioxidants or oxidative stress parameters specific to a disease or a patient. This is not only due to uncertainty as to whether a particular parameter plays a causal role. Moreover, the methods for selecting the appropriate parameters described in the literature represents varying analytical applicability, cost and quality levels. In addition to control materials, generally accepted reference values and interpretations of pathological conditions are lacking [117]. On that account, the inability of this study in demonstrating a difference in levels of oxidative stress parameters can be due to the fact that oxidative stress end products can be affected by many different factors.

6. CONCLUSION

In conclusion, we observed that although both Atorvastatin and Rosuvastatin were unable to prevent contrast induced nephropathy, when compared to the contrast group, Atorvastatin was effective in preventing elevation of serum creatinine. In histopathological examinations, the impact of Rosuvastatin was greater than of Atorvastatin on the severity of medullary congestion and made a statistically significant difference. In terms of tubular injury, both of the drugs were ineffective. However since there were no statistically significant difference in serum NGAL levels, which we associated with severity of tubular injury, and in oxidative stress parameters, which we measured to explain the mechanism of protective properties of statins, between the groups, we were unable to establish a correlation between CI-AKI and statins with these parameters.

The data we obtained may have been affected, due to the fact that fluid restriction utilized in the study may affect GFR and consequently the subjects' responses to the nephrotoxic effects of the contrast media. Additionally, it is important to take into consideration that levels of biochemical parameters such as serum NGAL and oxidative stress markers can be altered by many factors in the metabolism. And most importantly, experiment protocols of the future research that aims to investigate mechanisms of action should be constructed with consideration that contrast media and statins exert their effects in a dosage and duration dependent manner.

7. REFERENCES

[1] E. A. J. Hoste *et al.*, "RIFLE criteria for acute kidney injury are associated with hospital mortality in critically ill patients: A cohort analysis," *Crit. Care*, vol. 10, no. 3, pp. 1–10, 2006, doi: 10.1186/cc4915.

[2] P. Karagöz, A. Kefi, P. Erbay Dündar, S. Canan, and M. Çivi, "Acute Kidney Injury Incidence According to The RIFLE Criteria and Risk Factors in Critically Ill Patients," *Türk Yoğun Bakım Derneği Derg.*, vol. 13, no. 1, pp. 16–25, 2015, doi: 10.4274/tybdd.30502.

[3] K. Nash, A. Hafeez, and S. Hou, "Hospital-acquired renal insufficiency," *Am. J. Kidney Dis.*, vol. 39, no. 5, pp. 930–936, 2002, doi: 10.1053/ajkd.2002.32766.

[4] R. Schräder, "Contrast material-induced renal failure: An overview," in *Journal of Interventional Cardiology*, 2005, vol. 18, no. 6, pp. 417–423, doi: 10.1111/j.1540-8183.2005.00081.x.

[5] T. Yoshioka, A. Fogo, and J. K. Beckman, "Reduced activity of antioxidant enzymes underlies contrast media-induced renal injury in volume depletion," *Kidney Int.*, vol. 41, no. 4, pp. 1008–1015, 1992, doi: 10.1038/ki.1992.153.

[6] J. Zhang, Y. Guo, Q. Jin, L. Bian, and P. Lin, "Meta-analysis of rosuvastatin efficacy in prevention of contrast-induced acute kidney injury," *Drug Des. Devel. Ther.*, vol. 12, pp. 3685–3690, 2018, doi: 10.2147/DDDT.S178020.

[7] R. Mehran and E. Nikolsky, "Contrast-induced nephropathy: Definition, epidemiology, and patients at risk," *Kidney Int.*, vol. 69, no. SUPPL. 100, pp. S11–S15, 2006, doi: 10.1038/sj.ki.5000368.

[8] A. Manuscript and C. Incidence, "Tsai 14 (AKI from PCI 288,000)," vol. 7, no. June 2009, pp. 1–9, 2014, doi: 10.1016/j.jcin.2013.06.016.Contemporary.

[9] P. A. McCullough, R. Wolyn, L. L. Rocher, R. N. Levin, and W. W. O'Neill, "Acute renal failure after coronary intervention: Incidence, risk factors, and relationship to mortality," *Am. J. Med.*, vol. 103, no. 5, pp. 368–375, Nov. 1997, doi: 10.1016/S0002-9343(97)00150-2.

[10] R. Mehran, G. D. Dangas, and S. D. Weisbord, "Contrast-Associated Acute Kidney Injury," *N. Engl. J. Med.*, vol. 380, no. 22, pp. 2146–2155, 2019, doi:

10.1056/nejmra1805256.

[11] M. M. Sendeski *et al.*, “Pathophysiology of Acute Kidney Injury Iodinated contrast media cause endothelial damage leading to vasoconstriction of human and rat vasa recta,” pp. 1592–1599, 2012, doi: 10.1152/ajprenal.00471.2012.

[12] H. C. Lee, S. H. Sheu, H. W. Yen, W. Ter Lai, and J. G. Chang, “JNK/ATF2 pathway is involved in iodinated contrast media-induced apoptosis,” *Am. J. Nephrol.*, vol. 31, no. 2, pp. 125–133, Feb. 2010, doi: 10.1159/000259899.

[13] M. Çetin, “Ionic High-Osmolar Contrast Medium Causes Oxidant Stress in Kidney Tissue : Partial Protective Role of Ascorbic Acid,” pp. 567–572, 2008, doi: 10.1080/08860220802064739.

[14] C. Mamoulakis *et al.*, “Pharmacology & Therapeutics Contrast-induced nephropathy : Basic concepts , pathophysiological implications and prevention strategies ☆,” *Pharmacol. Ther.*, 2017, doi: 10.1016/j.pharmthera.2017.06.009.

[15] P. Parfrey, S. Griffiths, B. Barrett, and et al., “Contrast material-induced renal failure in patients with diabetes mellitus, renal insufficiency, or both. A prospective controlled study,” *NEJM.*, vol. 320, pp. 143–149, 1989.

[16] P. A. McCullough *et al.*, “Risk Prediction of Contrast-Induced Nephropathy,” *Am. J. Cardiol.*, vol. 98, no. 6 SUPPL. 1, pp. 27–36, 2006, doi: 10.1016/j.amjcard.2006.01.022.

[17] S. Hou, D. Bushinsky, J. Wish, and E. Al., “Hospital-acquired renal insufficiency: a prospective study,” *Am J Med.*, vol. 74, pp. 243–248, 1983.

[18] M. Rudnick, S. Goldfarb, L. Wexler, and et al., “Nephrotoxicity of ionic and nonionic contrast media in 1196 patients: a randomized trial. The Iohexol Cooperative Study,” *Kidney Int.*, vol. 47, pp. 254–261, 1995.

[19] N. M. A. Mohammed, A. Mahfouz, K. Achkar, I. M. Rafie, and R. Hajar, “Contrast-induced Nephropathy 1,” vol. 14, no. 3, 1995, doi: 10.4103/1995-705X.125926.

[20] M. Tublin, M. Murphy, and N. Tessler, “Review Current Concepts in Contrast Mediaâ € ” Induced Neph ropathy,” no. October, pp. 933–939, 1998.

[21] A. Patsaoura *et al.*, “Plasma neutrophil gelatinase-associated lipocalin levels are markedly increased in patients with non-transfusion-dependent thalassemia: Lack of association with markers of erythropoiesis, iron metabolism and renal function,” *Clin. Biochem.*, vol. 47, no. 12, pp. 1060–1064, 2014, doi: 10.1016/j.clinbiochem.2014.03.017.

[22] W. F. Finn, “The clinical and renal consequences of contrast-induced nephropathy,” *Nephrol. Dial. Transplant.*, vol. 21, no. SUPPL. 1, pp. 2–10, 2006, doi: 10.1093/ndt/gfl213.

[23] M. Haase *et al.*, “The Outcome of Neutrophil Gelatinase-Associated Lipocalin (NGAL)-positive Subclinical Acute Kidney Injury: A Multicenter Pooled Analysis of Prospective Studies,” *J Am Coll Cardiol*, vol. 57, no. 17, pp. 1752–1761, 2016, doi: 10.1016/j.jacc.2010.11.051.The.

[24] H. Bachorzewska-Gajewska, B. Poniatowski, and S. Dobrzycki, “NGAL (neutrophil gelatinase-associated lipocalin) and L-FABP after percutaneous coronary interventions due to unstable angina in patients with normal serum creatinine,” *Adv. Med. Sci.*, vol. 54, no. 2, pp. 221–224, Dec. 2009, doi: 10.2478/V10039-009-0036-1.

[25] Mishra Jaya *et al.*, “Neutrophil gelatinase-associated lipocalin (NGAL) as abiomarker for acute renal injury after cardiac surgery,” *Lancet*, vol. 365, pp. 1231–1238, 2005, [Online]. Available: https://pubmed.ncbi.nlm.nih.gov/15811456/.

[26] H. Bachorzewska-Gajewska, J. Malyszko, E. Sitniewska, J. S. Malyszko, and S. Dobrzycki, “Neutrophil-gelatinase-associated lipocalin and renal function after percutaneous coronary interventions,” *Am. J. Nephrol.*, vol. 26, no. 3, pp. 287–292, 2006, doi: 10.1159/000093961.

[27] W. H. Li, L. Wang, H. Y. He, J. Chen, and Y. R. Yu, “Expression of neutrophil gelatinase-associated lipocalin in low osmolar contrastinduced nephropathy in rats and the effect of N-acetylcysteine,” *Exp. Ther. Med.*, vol. 12, no. 5, pp. 3175–3180, 2016, doi: 10.3892/etm.2016.3779.

[28] M. Tepel, P. Aspelin, and N. Lameire, “Contrast-induced nephropathy: A clinical and evidence-based approach,” *Circulation*, vol. 113, no. 14, pp. 1799–1806,

2006, doi: 10.1161/CIRCULATIONAHA.105.595090.

[29] K. Soto *et al.*, “Cystatin C as a marker of acute kidney injury in the emergency department,” *Clin. J. Am. Soc. Nephrol.*, vol. 5, no. 10, pp. 1745–1754, 2010, doi: 10.2215/CJN.00690110.

[30] T. Perrin, E. Descombes, and S. Cook, “Contrast-induced nephropathy in invasive cardiology: Incidence, pathophysiology, diagnosis, prevention and prognosis,” *Swiss Medical Weekly*, vol. 142, no. JUNE. pp. 1–11, 2012, doi: 10.4414/smw.2012.13608.

[31] P. Pattharanitima and A. Tasanarong, “Pharmacological strategies to prevent contrast-induced acute kidney injury,” *Biomed Res. Int.*, vol. 2014, 2014, doi: 10.1155/2014/236930.

[32] J. H. Ellis and R. H. Cohan, “Prevention of Contrast-Induced Nephropathy: An Overview,” *Radiologic Clinics of North America*, vol. 47, no. 5. pp. 801–811, 2009, doi: 10.1016/j.rcl.2009.06.003.

[33] K. A. Sterling, T. Tehrani, and M. R. Rudnick, “Clinical significance and preventive strategies for contrast-induced nephropathy,” *Curr. Opin. Nephrol. Hypertens.*, vol. 17, no. 6, pp. 616–623, 2008, doi: 10.1097/MNH.0b013e32830f45a3.

[34] 329. doi:10.1001/archinte.162.3.329 Mueller, C., Buerkle, G., Buettner, H. J., Petersen, J., Perruchoud, A. P., Eriksson, U., … Roskamm, H. (2002). Prevention of Contrast Media–Associated Nephropathy. Archives of Internal Medicine, 162(3), “Prevention of Contrast Nephropathy,” *Jama*, vol. 289, no. 5, pp. 606–608, 2003, doi: 10.1001/jama.289.5.606.

[35] A. J. Taylor, D. Hotchkiss, R. W. Morse, and J. McCabe, “PREPARED: PREParation for Angiography in REnal Dysfunction: A Randomized Trial of Inpatient vs Outpatient Hydration Protocols for Cardiac Catheterization in Mild-to-Moderate Renal Dysfunction,” *Chest*, vol. 114, no. 6, pp. 1570–1574, 1998, doi: 10.1378/chest.114.6.1570.

[36] H. S. Trivedi *et al.*, “A randomized prospective trial to assess the role of saline hydration on the development of contrast nephrotoxicity.,” *Nephron. Clin. Pract.*, vol. 93, no. 1, pp. 29–34, 2003, doi: 10.1159/000066641.

[37] D. Yoshikawa *et al.*, “Importance of oral fluid intake after coronary computed tomography angiography: An observational study,” *Eur. J. Radiol.*, vol. 77, no. 1, pp. 118–122, 2011, doi: 10.1016/j.ejrad.2009.07.011.

[38] S. Bellosta, N. Ferri, F. Bernini, R. Paoletti, and A. Corsini, “Non-lipid-related effects of statins,” *Ann. Med.*, vol. 32, no. 3, pp. 164–176, 2000, doi: 10.3109/07853890008998823.

[39] S. Khanal *et al.*, “Statin therapy reduces contrast-induced nephropathy: An analysis of contemporary percutaneous interventions,” *Am. J. Med.*, vol. 118, no. 8, pp. 843–849, 2005, doi: 10.1016/j.amjmed.2005.03.031.

[40] T. Zhang, L. H. Shen, L. H. Hu, and B. He, “Statins for the prevention of contrast-induced nephropathy: A systematic review and meta-analysis,” *American Journal of Nephrology*, vol. 33, no. 4. pp. 344–351, 2011, doi: 10.1159/000326269.

[41] S. H. Jo *et al.*, “Prevention of radiocontrast medium-induced nephropathy using short-term high-dose simvastatin in patients with renal insufficiency undergoing coronary angiography (PROMISS) trial-a randomized controlled study,” *Am. Heart J.*, vol. 155, no. 3, pp. 499.e1-499.e8, 2008, doi: 10.1016/j.ahj.2007.11.042.

[42] P. Kandula, R. Shah, N. Singh, S. J. Markwell, N. Bhensdadia, and S. D. Navaneethan, “Statins for prevention of contrast-induced nephropathy in patients undergoing non-emergent percutaneous coronary intervention,” *Nephrology*, vol. 15, no. 2, pp. 165–170, 2010, doi: 10.1111/j.1440-1797.2009.01204.x.

[43] G. Patti *et al.*, “Short-term, high-dose atorvastatin pretreatment to prevent contrast-induced nephropathy in patients with acute coronary syndromes undergoing percutaneous coronary intervention (from the ARMYDA-CIN [atorvastatin for reduction of myocardial damage during an,” *Am. J. Cardiol.*, vol. 108, no. 1, pp. 1–7, 2011, doi: 10.1016/j.amjcard.2011.03.001.

[44] *Farmer, J. A. (2000). Pleiotropic effects of statins. Current Atherosclerosis Reports, 2(3), 208–217. doi:10.1007/s11883-000-0022-3.* .

[45] G. Patti *et al.*, “Protection From Procedural Myocardial Injury by Atorvastatin Is Associated With Lower Levels of Adhesion Molecules After Percutaneous

Coronary Intervention. Results From the ARMYDA-CAMs (Atorvastatin for Reduction of MYocardial Damage during Angioplasty-C," *J. Am. Coll. Cardiol.*, vol. 48, no. 8, pp. 1560–1566, 2006, doi: 10.1016/j.jacc.2006.06.061.

[46] V. Pasceri, G. Patti, A. Nusca, C. Pristipino, G. Richichi, and G. Di Sciascio, "Randomized trial of atorvastatin for reduction of myocardial damage during coronary intervention: Results from the ARMYDA (Atorvastatin for Reduction of MYocardial Damage during Angioplasty) study," *Circulation*, vol. 110, no. 6, pp. 674–678, 2004, doi: 10.1161/01.CIR.0000137828.06205.87.

[47] G. Patti *et al.*, "Atorvastatin Pretreatment Improves Outcomes in Patients With Acute Coronary Syndromes Undergoing Early Percutaneous Coronary Intervention. Results of the ARMYDA-ACS Randomized Trial," *J. Am. Coll. Cardiol.*, vol. 49, no. 12, pp. 1272–1278, 2007, doi: 10.1016/j.jacc.2007.02.025.

[48] G. Di Sciascio, G. Patti, V. Pasceri, A. Gaspardone, G. Colonna, and A. Montinaro, "Efficacy of Atorvastatin Reload in Patients on Chronic Statin Therapy Undergoing Percutaneous Coronary Intervention. Results of the ARMYDA-RECAPTURE (Atorvastatin for Reduction of Myocardial Damage During Angioplasty) Randomized Trial," *J. Am. Coll. Cardiol.*, vol. 54, no. 6, pp. 558–565, 2009, doi: 10.1016/j.jacc.2009.05.028.

[49] B. C. Zhang, W. M. Li, and Y. W. Xu, "High-Dose Statin Pretreatment for the Prevention of Contrast-Induced Nephropathy: A Meta-analysis," *Canadian Journal of Cardiology*, vol. 27, no. 6. pp. 851–858, 2011, doi: 10.1016/j.cjca.2011.05.005.

[50] M. Leoncini, A. Toso, M. Maioli, F. Tropeano, and F. Bellandi, "Statin treatment before percutaneous cononary intervention," *J. Thorac. Dis.*, vol. 5, no. 3, pp. 335–342, 2013, doi: 10.3978/j.issn.2072-1439.2013.05.09.

[51] I. Durak, H. Özbek, M. Karaayvaz, and H. S. Öztürk, "Cisplatin induces acute renal failure by impairing antioxidant system in guinea pigs: Effects of antioxidant supplementation on the cisplatin nephrotoxicity," *Drug Chem. Toxicol.*, vol. 25, no. 1, pp. 1–8, 2002, doi: 10.1081/DCT-100108468.

[52] B. H. Ali, "Agents ameliorating or augmenting experimental gentamicin nephrotoxicity: Some recent research," *Food and Chemical Toxicology*, vol. 41,

no. 11. pp. 1447–1452, 2003, doi: 10.1016/S0278-6915(03)00186-8.

[53] K. Spargias *et al.*, “Ascorbic acid prevents contrast-mediated nephropathy in patients with renal dysfunction undergoing coronary angiography or intervention,” *Circulation*, vol. 110, no. 18, pp. 2837–2842, 2004, doi: 10.1161/01.CIR.0000146396.19081.73.

[54] L. Zhou and H. Chen, “Prevention of contrast-induced nephropathy with ascorbic acid,” *Intern. Med.*, vol. 51, no. 6, pp. 531–535, 2012, doi: 10.2169/internalmedicine.51.6260.

[55] S. M. Anderson, Z. H. Park, and R. V. Patel, “Intravenous N-acetylcysteine in the prevention of contrast media-induced nephropathy,” *Ann. Pharmacother.*, vol. 45, no. 1, pp. 101–107, 2011, doi: 10.1345/aph.1P275.

[56] M. Tepel, M. van der Giet, C. Schwarzfeld, U. Laufer, D. Liermann, and W. Zidek, “Prevention of Radiographic-Contrast-Agent–Induced Reductions in Renal Function by Acetylcysteine,” *N. Engl. J. Med.*, vol. 343, no. 3, pp. 180–184, 2000, doi: 10.1056/nejm200007203430304.

[57] D. Castini *et al.*, “Prevention of contrast-induced nephropathy: A single center randomized study,” *Clin. Cardiol.*, vol. 33, no. 3, pp. 63–68, 2010, doi: 10.1002/clc.20576.

[58] L. C. Coyle, A. Rodriguez, R. E. Jeschke, A. Simon-Lee, K. C. Abbott, and A. J. Taylor, “Acetylcysteine In Diabetes (AID): A randomized study of acetylcysteine for the prevention of contrast nephropathy in diabetics,” *Am. Heart J.*, vol. 151, no. 5, pp. 1032.e9-1032.e12, 2006, doi: 10.1016/j.ahj.2006.02.002.

[59] L. J. Diaz-Sandoval, B. D. Kosowsky, and D. W. Losordo, “Acetylcysteine to prevent angiography-related renal tissue injury (the APART trial),” *Am. J. Cardiol.*, vol. 89, no. 3, pp. 356–358, 2002, doi: 10.1016/S0002-9149(01)02243-3.

[60] M. Demir, A. Kutlucan, H. Akin, O. Aydin, and M. T. Sezer, “Comparison of different agents on radiographic contrast agent induced nephropathy,” *Eur. J. Gen. Med.*, vol. 5, no. 4, pp. 222–227, 2008, doi: 10.29333/ejgm/82611.

[61] J. D. Durham *et al.*, “A randomized controlled trial of N-acetylcysteine to prevent

contrast nephropathy in cardiac angiography," *Kidney Int.*, vol. 62, no. 6, pp. 2202–2207, 2002, doi: 10.1046/j.1523-1755.2002.00673.x.

[62] M. Erturk *et al.*, "Does intravenous or oral high-dose N-acetylcysteine in addition to saline prevent contrast-induced nephropathy assessed by cystatin C?," *Coron. Artery Dis.*, vol. 25, no. 2, pp. 111–117, 2014, doi: 10.1097/MCA.0000000000000073.

[63] S. Efrati, S. Berman, I. Ilgiyeav, Y. Siman-Tov, Z. Averbukh, and J. Weissgarten, "Differential effects of N-acetylcysteine, theophylline or bicarbonate on contrast-induced rat renal vasoconstriction," *Am. J. Nephrol.*, vol. 29, no. 3, pp. 181–191, 2009, doi: 10.1159/000154471.

[64] M. Droppa *et al.*, "Impact of N-acetylcysteine on contrast-induced nephropathy defined by cystatin C in patients with ST-elevation myocardial infarction undergoing primary angioplasty," *Clin. Res. Cardiol.*, vol. 100, no. 11, pp. 1037–1043, 2011, doi: 10.1007/s00392-011-0338-8.

[65] R. Xu, A. Tao, Y. Bai, Y. Deng, and G. Chen, "Effectiveness of N-Acetylcysteine for the Prevention of Contrast-Induced Nephropathy: A Systematic Review and Meta-Analysis of Randomized Controlled Trials," *J. Am. Heart Assoc.*, vol. 5, no. 9, 2016, doi: 10.1161/JAHA.116.003968.

[66] H. Sies, "Oxidative stress: A concept in redox biology and medicine," *Redox Biol.*, vol. 4, pp. 180–183, 2015, doi: 10.1016/j.redox.2015.01.002.

[67] J. Luo, X. F. Rong, Y. Y. Ye, W. Z. Li, X. Q. Wang, and W. Wang, "Research Progress on Triarylmethyl Radical-Based High-Efficiency OLED," *Molecules*, vol. 27, no. 5. 2022, doi: 10.3390/molecules27051632.

[68] H. Sies, "Biochemistry of oxidative stress," *Eur. J. Cancer Clin. Oncol.*, vol. 23, no. 11, p. 1798, 1987, doi: 10.1016/0277-5379(87)90716-4.

[69] I. Fridovich, "Biological effects of the superoxide radical," *Arch. Biochem. Biophys.*, vol. 247, no. 1, pp. 1–11, May 1986, doi: 10.1016/0003-9861(86)90526-6.

[70] F. Vieceli Dalla Sega *et al.*, "Specific aquaporins facilitate Nox-produced hydrogen peroxide transport through plasma membrane in leukaemia cells,"

Biochim. Biophys. Acta - Mol. Cell Res., vol. 1843, no. 4, pp. 806–814, 2014, doi: 10.1016/j.bbamcr.2014.01.011.

[71] B. Lipinski, "Hydroxyl radical and its scavengers in health and disease," *Oxidative Medicine and Cellular Longevity*, vol. 2011. 2011, doi: 10.1155/2011/809696.

[72] M. A. Cinelli, H. T. Do, G. P. Miley, and R. B. Silverman, "Inducible nitric oxide synthase: Regulation, structure, and inhibition," *Med. Res. Rev.*, vol. 40, no. 1, pp. 158–189, 2020, doi: 10.1002/med.21599.

[73] J. S. Beckmann *et al.*, "Extensive nitration of protein tyrosines in human atherosclerosis detected by immunohistochemistry.," *Biol. Chem. Hoppe. Seyler.*, vol. 375, no. 2, pp. 81–88, 1994, doi: 10.1515/bchm3.1994.375.2.81.

[74] A. C. Carr, M. R. McCall, and B. Frei, "Oxidation of LDL by myeloperoxidase and reactive nitrogen species: Reaction pathways and antioxidant protection," *Arterioscler. Thromb. Vasc. Biol.*, vol. 20, no. 7, pp. 1716–1723, 2000, doi: 10.1161/01.ATV.20.7.1716.

[75] "Sáez, G. T., & Están-Capell, N. (2014). Antioxidant Enzymes. Encyclopedia of Cancer, 288–294. doi:10.1007/978-3-662-46875-3_7210," .

[76] S. B. Nimse and D. Pal, "Free radicals, natural antioxidants, and their reaction mechanisms," *RSC Adv.*, vol. 5, no. 35, pp. 27986–28006, 2015, doi: 10.1039/c4ra13315c.

[77] D. Melo, S. Coimbra, S. Rocha, and A. Santos-Silva, "Inhibition of erythrocyte's catalase, glutathione peroxidase or peroxiredoxin 2 – Impact on cytosol and membrane," *Arch. Biochem. Biophys.*, vol. 739, p. 109569, May 2023, doi: 10.1016/J.ABB.2023.109569.

[78] O. M. Ighodaro and O. A. Akinloye, "First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid," *Alexandria J. Med.*, vol. 54, no. 4, pp. 287–293, 2018, doi: 10.1016/j.ajme.2017.09.001.

[79] M. H. Hadwan, "Simple spectrophotometric assay for measuring catalase activity in biological tissues," *BMC Biochem.*, vol. 19, no. 1, pp. 1–8, 2018, doi:

10.1186/s12858-018-0097-5.

[80] G. C. Mills, “HEMOGLOBIN CATABOLISM,” *J. Biol. Chem.*, vol. 229, no. 1, pp. 189–197, 1957, doi: 10.1016/s0021-9258(18)70608-x.

[81] H. Yin, L. Xu, and N. A. Porter, “Free radical lipid peroxidation: Mechanisms and analysis,” *Chem. Rev.*, vol. 111, no. 10, pp. 5944–5972, 2011, doi: 10.1021/cr200084z.

[82] M. Higuchi, *Antioxidant Properties of Wheat Bran against Oxidative Stress*. Elsevier, 2014.

[83] J. N. Keller *et al.*, “Mitochondrial manganese superoxide dismutase prevents neural apoptosis and reduces ischemic brain injury: Suppression of peroxynitrite production, lipid peroxidation, and mitochondrial dysfunction,” *J. Neurosci.*, vol. 18, no. 2, pp. 687–697, 1998, doi: 10.1523/jneurosci.18-02-00687.1998.

[84] J. S. Beckman, T. W. Beckman, J. Chen, P. A. Marshall, and B. A. Freeman, “Apparent hydroxyl radical production by peroxynitrite: Implications for endothelial injury from nitric oxide and superoxide,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 87, no. 4, pp. 1620–1624, 1990, doi: 10.1073/pnas.87.4.1620.

[85] T. Fukai, R. J. Folz, U. Landmesser, and D. G. Harrison, “Extracellular superoxide dismutase and cardiovascular disease,” *Cardiovasc. Res.*, vol. 55, no. 2, pp. 239–249, 2002, doi: 10.1016/S0008-6363(02)00328-0.

[86] T. Fukai and M. Ushio-fukai, “Superoxide Dismutases :,” vol. 15, no. 6, 2011, doi: 10.1089/ars.2011.3999.

[87] P.-H. C. Pham-Huy LA, He H, “Free radicals, antioxidants in disease and health.,” *Int J Biomed Sci.*, vol. 4, no. 2, pp. 89–96, 2008.

[88] J. P. Kehrer and L. O. Klotz, “Free radicals and related reactive species as mediators of tissue injury and disease: Implications for Health,” *Crit. Rev. Toxicol.*, vol. 45, no. 9, pp. 765–798, 2015, doi: 10.3109/10408444.2015.1074159.

[89] A. Ayala, M. F. Muñoz, and S. Argüelles, “Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal,” *Oxid. Med. Cell. Longev.*, vol. 2014, 2014, doi: 10.1155/2014/360438.

[90] A. A. Hajeyah, W. J. Griffiths, Y. Wang, A. J. Finch, and V. B. O'Donnell, "The Biosynthesis of Enzymatically Oxidized Lipids," *Front. Endocrinol. (Lausanne).*, vol. 11, no. November, pp. 1–32, 2020, doi: 10.3389/fendo.2020.591819.

[91] N. Zarkovic, A. Cipak, M. Jaganjac, S. Borovic, and K. Zarkovic, "Pathophysiological relevance of aldehydic protein modifications," *J. Proteomics*, vol. 92, pp. 239–247, 2013, doi: 10.1016/j.jprot.2013.02.004.

[92] M. Valko, C. J. Rhodes, J. Moncol, M. Izakovic, and M. Mazur, "Free radicals, metals and antioxidants in oxidative stress-induced cancer," *Chem. Biol. Interact.*, vol. 160, no. 1, pp. 1–40, 2006, doi: 10.1016/j.cbi.2005.12.009.

[93] C. Duan, N. C. do Nascimento, S. Calve, A. Cox, and M. P. Sivasankar, "Restricted Water Intake Adversely Affects Rat Vocal Fold Biology," *Laryngoscope*, vol. 131, no. 4, pp. 839–845, 2021, doi: 10.1002/lary.28881.

[94] K. Liu *et al.*, "A novel rat model of contrast-induced nephropathy based on dehydration," *J. Pharmacol. Sci.*, vol. 141, no. 1, pp. 49–55, 2019, doi: 10.1016/j.jphs.2019.09.003.

[95] K. Solez, E. C. Kramer, J. A. Fox, and R. H. Heptinstall, "Medullary plasma flow and intravascular leukocyte accumulation in acute renal failure," *Kidney Int.*, vol. 6, no. 1, pp. 24–37, 1974, doi: 10.1038/ki.1974.74.

[96] J. H. H. G H FREEMAN, "Note on an exact treatment of contingency, goodness of fit and other problems of significance," *Biometrika*, vol. 38, no. 1–2, pp. 141–149, 1951.

[97] A. Pisani *et al.*, "Role of reactive oxygen species in pathogenesis of radiocontrast-induced nephropathy," *Biomed Res. Int.*, vol. 2013, 2013, doi: 10.1155/2013/868321.

[98] L. Yan *et al.*, "Atorvastatin protects against contrast-induced acute kidney injury via upregulation of endogenous hydrogen sulfide," *Ren. Fail.*, vol. 42, no. 1, pp. 270–281, 2020, doi: 10.1080/0886022X.2020.1740098.

[99] S. J. Sharif, B. A. R. Mshimesh, A. F. Hameed, and S. S. Suzan, "Evaluating the Proposed Mechanism by Which Atorvastatin Can Protect Renal Tissue against Contrast Media: An Animal study," *Al Mustansiriyah J. Pharm. Sci.*, vol. 19, no.

1, pp. 75–84, 2019, doi: 10.32947/ajps.v19i1.546.

[100] B. Qiao, J. Deng, Y. Li, X. Wang, and Y. Han, “Rosuvastatin attenuated contrast-induced nephropathy in diabetes patients with renal dysfunction,” *Int. J. Clin. Exp. Med.*, vol. 8, no. 2, pp. 2342–2349, 2015.

[101] Z. Jiang, J. Zhang, Y. Lu, and Y. Lu, “Protective Effects and Mechanisms of Rosuvastatin on Acute Kidney Injury Induced by Contrast Media in Rats,” *Int. J. Nephrol.*, vol. 2020, 2020, doi: 10.1155/2020/3490641.

[102] X. L. Wang *et al.*, “Comparison of Effects of Different Statins on Contrast-Induced Acute Kidney Injury in Rats: Histopathological and Biochemical Findings,” *Oxid. Med. Cell. Longev.*, vol. 2017, 2017, doi: 10.1155/2017/6282486.

[103] V. Hvidberg, C. Jacobsen, R. K. Strong, J. B. Cowland, S. K. Moestrup, and N. Borregaard, “The endocytic receptor megalin binds the iron transporting neutrophil-gelatinase-associated lipocalin with high affinity and mediates its cellular uptake,” *FEBS Lett.*, vol. 579, no. 3, pp. 773–777, 2005, doi: 10.1016/j.febslet.2004.12.031.

[104] K. Mori *et al.*, “Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury,” *J. Clin. Invest.*, vol. 115, no. 3, pp. 610–621, 2005, doi: 10.1172/JCI23056.

[105] K. M. Schmidt-Ott, “Neutrophil gelatinase-associated lipocalin as a biomarker of acute kidney injury - Where do we stand today?,” *Nephrol. Dial. Transplant.*, vol. 26, no. 3, pp. 762–764, 2011, doi: 10.1093/ndt/gfr006.

[106] K. M. Schmidt-Ott *et al.*, “Neutrophil gelatinase-associated lipocalin-mediated iron traffic in kidney epithelia,” *Curr. Opin. Nephrol. Hypertens.*, vol. 15, no. 4, pp. 442–449, 2006, doi: 10.1097/01.mnh.0000232886.81142.58.

[107] Y. Zhang, Y. Fan, and Z. Mei, “NGAL and NGALR overexpression in human hepatocellular carcinoma toward a molecular prognostic classification,” *Cancer Epidemiol.*, vol. 36, no. 5, pp. e294–e299, 2012, doi: 10.1016/j.canep.2012.05.012.

[108] V. Barresi, L. Reggiani-Bonetti, C. Di Gregorio, E. Vitarelli, M. Ponz De Leon,

and G. Barresi, “Neutrophil gelatinase-associated lipocalin (NGAL) and matrix metalloproteinase-9 (MMP-9) prognostic value in stage I colorectal carcinoma,” *Pathol. Res. Pract.*, vol. 207, no. 8, pp. 479–486, 2011, doi: 10.1016/j.prp.2011.05.012.

[109] D. S. Wheeler *et al.*, “Serum neutrophil gelatinase-associated lipocalin (NGAL) as a marker of acute kidney injury in critically ill children with septic shock,” *Crit. Care Med.*, vol. 36, no. 4, pp. 1297–1303, 2008, doi: 10.1097/CCM.0b013e318169245a.

[110] S. M. Bagshaw *et al.*, “Plasma and urine neutrophil gelatinase-associated lipocalin in septic versus non-septic acute kidney injury in critical illness,” *Intensive Care Med.*, vol. 36, no. 3, pp. 452–461, 2010, doi: 10.1007/s00134-009-1724-9.

[111] A. Yilmaz *et al.*, “Early prediction of urinary tract infection with urinary neutrophil gelatinase associated lipocalin,” *Pediatr. Nephrol.*, vol. 24, no. 12, pp. 2387–2392, 2009, doi: 10.1007/s00467-009-1279-6.

[112] Y. Tomonaga, T. Szucs, P. Ambühl, S. Nock, M. Risch, and L. Risch, “Insights on urinary NGAL obtained in a primary care setting,” *Clin. Chim. Acta*, vol. 413, no. 7–8, pp. 733–739, 2012, doi: 10.1016/j.cca.2012.01.001.

[113] S. M. Sancho-Martínez, M. Herrero, M. Fontecha-Barriuso, J. Mercado-Hernández, and F. J. López-Hernández, “The Urinary Level of Injury Biomarkers Is Not Univocally Reflective of the Extent of Toxic Renal Tubular Injury in Rats,” *Int. J. Mol. Sci.*, vol. 23, no. 7, 2022, doi: 10.3390/ijms23073494.

[114] G. Cusumano *et al.*, “N-Acetylcysteine and High-Dose Atorvastatin Reduce Oxidative Stress in an Ischemia-Reperfusion Model in the Rat Kidney,” in *Transplantation Proceedings*, 2015, vol. 47, no. 9, pp. 2757–2762, doi: 10.1016/j.transproceed.2015.09.035.

[115] M. Boyacioglu, H. Turgut, C. Akgullu, U. Eryilmaz, C. Kum, and O. A. Onbasili, “The effect of L-carnitine on oxidative stress responses of experimental contrast-induced nephropathy in rats,” *J. Vet. Med. Sci.*, vol. 76, no. 1, pp. 1–8, 2014, doi: 10.1292/jvms.13-0202.

[116] M. Schachter, “Chemical, pharmacokinetic and pharmacodynamic properties of

statins: An update," *Fundam. Clin. Pharmacol.*, vol. 19, no. 1, pp. 117–125, 2005, doi: 10.1111/j.1472-8206.2004.00299.x.

[117] S. V. Palmieri B, "Oxidative stress tests: overview on reliability and use.," *Part I. Eur Rev Med Pharmacol Sci.*, vol. 11, no. 5, pp. 309–342, 2007.

8. APPENDIX

8.1. Ethic Committee Approval

T.C.
YEDİTEPE ÜNİVERSİTESİ
Hayvan Deneyleri Yerel Etik Kurulu (HADYEK)

ETİK KURUL KARARI

Protokol No	Toplantı Tarihi	Toplantı Sayısı	Karar No	Proje Yürütücüsü
2022-50	13.12.2022	2022/12	2022/12-02	Prof.Dr.Ahmet Aydın

'Kontrast Madde İlişkili Nefropati Gelişimini Önlemede Statinlerin Etki Mekanizmasının Sıçanlarda Araştırılması'isimli proje oy birliğiyle etik açıdan uygun görülmüştür.

Hayvan Türü / Irkı	Toplam Hayvan Sayısı	Hayvanın Cinsiyeti
Sıçan / Sprague-Dawley	30	Dişi

Görevi	Adı Soyadı	Katılım Durumu
Başkan	Prof. Dr. Bayram YILMAZ	
Başkan Vekili	Prof. Dr. Erdem YEŞILADA	
Üye	Dr. Engin SÜMER	
Üye	Prof. Dr. M. Ece GENÇ	
Üye	Prof. Dr. Rukset ATTAR	
Üye	Prof. Dr. Gamze TORUN KÖSE	
Üye	Prof. Dr. Özlem MALKONDU	
Üye	Doç. Dr. Aylin YABA UÇAR	
Üye	Doç. Dr. Burcu GEMICI BAŞOL	
Üye	Atakan Mücahil YAVUZ	
Üye	Ahmet ŞENKARDEŞLER	

8.2. Certificates

YEDİTEPE ÜNİVERSİTESİ

hadmek Hayvan Deneyleri Merkezi Etik Kurulu

YEDİTEPE ÜNİVERSİTESİ / *YEDITEPE UNIVERSITY*

HAYVAN DENEYLERİ YEREL ETİK KURULU / *ANIMAL RESEARCH LOCAL ETHICS COMMITTEE*

DENEY HAYVANLARI KULLANIM SERTİFİKASI

CERTIFICATE OF ANIMAL USE IN EXPERIMENTAL RESEARCH

Sayın Mesut MOLLAOĞLU

02.11.2020 – 11.11.2020 tarihleri arasında Yeditepe Üniversitesi, Hayvan Deneyleri Yerel Etik Kurulu (HADYEK) tarafından İstanbul İlinde düzenlenen "Deney Hayvanları Kullanım Sertifikası" eğitimini başarı ile tamamlayarak A kategorisi sertifikası almaya hak kazanmıştır.

has successfully completed the "Certificate of Use of Experimental Animals" training organized by Yeditepe University Animal Experiments Local Ethics Committee (HADYEK) in Istanbul between 02.11.2020 - 11.11.2020, and qualified to receive category A certificate.

YEDİTEPE ÜNİVERSİTESİ

hadmek Hayvan Deneyleri Merkezi Etik Kurulu

Ders Adı / *Lecture Name*	Ders Saati *Lecture Hour*	
	Teorik *Lecture*	Uygulama *Practice*
Hayvan deneyleri mevzuatı* / *Legislation and Regulations in Laboratory Animal Research*	1	-
Hayvan deneyleri etiği ve 3R* / *The Ethics of Animal Research and the 3Rs*	2	-
Hayvan davranışı ve refahı* / *The Behaviour and Welfare of Laboratory Animals*	2	-
Deney hayvanlarının karşılaştırmalı anatomisi / *Comparative Anatomy of Laboratory Animals*	2	2
Deney hayvanlarının fizyolojisi / *Physiology of Laboratory Animals*	1	1
Deney hayvanlarının histolojisi / *Histology of Laboratory Animals*	1	1
Deney hayvanlarının biyokimyası / *Biochemistry of Laboratory Animals*	1	-
Kan ve örnek alma teknikleri / *Tecnhiques of Blood and Tissue Sampling*	1	4
Tutuş teknikleri / *Animal Handling Techniques*	1	4
İlaç verme ve enjeksiyon teknikleri / *Drug Administration and Injection Techniques*	1	4
Anestezi teknikleri / *Anesthesia Techniques*	1	2
Ötanazi teknikleri / *Euthanasia techniques*	1	2
Ağrı, sıkıntı ve insani sonlandırma noktalarının belirlenmesi / *Determination of pain, distress and humanitarian experiment termination points*	1	-
Laboratuvar işleyişi (temizlik, güvenlik)* / *Operation of the Laboratory (hygiene, safety)*	2	1
Hayvan tesis/ünitelerinde iş sağlığı ve güvenliği* / Occupational *health and safety in animal facilities/units*	1	-
Deney hayvanlarının beslenmesi / *Feeding of Laboratory Animals*	2	-
Hayvan hastalıkları / *Diseases of Laboratory Animals*	3	2
Biyoistatistik* / *Biostatistics*	4	-
Standardizasyon / *Standardization*	2	-
Hayvan deneyi modeli kavramı ve spesifik deney modelleri / *The concept of animal experiment model and specific experimental models*	2	2
Hayvan deneylerinin tasarlanması/ *Designing Animal Experiments*	2	4
Temel Cerrahi / *Basic Surgery of Laboratory Animals*	2	4
Deney hayvanlarının üretimi, yetiştirilmesi ve barındırılması / *Production, cultivation and Housing of Laboratory Animals*	2	2
Alternatif Yöntemler* / *Alternative Methods*	2	-
TOPLAM / *TOTAL*	40	35

*: Hayvan grubuna özgü olmayan dersler / Lessons not specific to the animal group

Başarı Puanı / *Qualification score*: 78

Sertifikanın geçerli olduğu hayvan türleri / *Animal species for which the certificate is valid*:

1.Grup: Kemirgenler (fare, sıçan, kobay türleri) ve Tavşan / *Group 1: Rodents (Mouse, Rat, Guinea pig species)* and *Rabbit*

Bu Sertifika Orman ve Su İşleri Bakanlığı'nın 02.04.2019 tarih ve 2019/3 sayılı Genelgesine uygun olarak düzenlenmiştir.

8.3. Curriculum Vitae

Personal Informations

Name	Mesut	**Surname**	MOLLAOĞLU

Education

Degree	Department	The name of the Institution Graduated From	Graduation year
Doctorate	Pharmaceutical Toxicology	Institute of Health Sciences Yeditepe University	2023
Residency	Emergency Medicine	Sağlık Bilimleri Üniversitesi Şişli Hamidiye Etfal Eğitim Araştırma Hastanesi	2020
University	Faculty of Medicine	Marmara University	2014
High school		Zonguldak Atatürk Anadolu Lisesi	2006

Languages	**Grades (#))**
English	YÖKDİL 70

Work Experience (Sort from present to past)

Position	Institute	**Duration (Year - Year)**
Practitioner	Zonguldak Kozlu Toplum Sağlığı Merkezi	2014 – 2015
Residency	Sağlık Bilimleri Üniversitesi Şişli Hamidiye Etfal Eğitim Araştırma Hastanesi	2016 - 2021
Attending	Sağlık Bilimleri Üniversitesi Bakırköy Dr. Sadi Konuk Eğitim Araştırma Hastanesi	2021- Present

Computer Skills

Program	**Level**
MS office	Good
SPSS	Good

Proceedings presented in international scientific meetings and published in proceedings book.

E.Yiğit, M.Mollaoğlu, M.Hamitoğlu, 'Cardiac Arreset Presented With Brugada Syndrome Due To Bonzai Abuse; A Case' Report Presented 10th International Congress Of The Turkish Society Of Toxicology Antalya, Turkey 16 - 19th October, 2019
Mesut Mollaoglu, Erkan Gunay, Ertugrul Altinbilek, Hakan Kilci, "Comparison of Neutrophil/Lymphocyte Ratio and Trombocyte/Lymphocyte Ratio With Gensini and Syntax Scores for Non-ST- Elevation Myocardial Infarction" (2022), SAEM22 Abstracts. Acad Emerg Med, 29: S8-S429. https://doi.org/10.1111/acem.14511

Others (Projects / Certificates / Rewards)

Certificate of Animal Use in Experimental Research