Siklofosfamid Nedenli Akut Böbrek Hasarında Escinin Antiapoptotik, Antioksidan ve Renoprotektif Etkileri

ALANNA TEIXEIRA ANDRADE

YÜKSEK LİSANS TEZİ

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ALANNA TEIXEIRA ANDRADE

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by

Alanna Teixeira Andrade

Supervisor: Prof. Dr. Adnan AYHANCİ
Co-supervisor: Prof. Dr. MUSTAFA CENGİZ

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ETHICAL STATEMENT

I hereby declare that this thesis study titled “Antiapoptotic, Antioxidant and Renoprotective Effects of Escin in Cyclophosphamide-induced Acute Kidney Injury” has been prepared in accordance with the thesis writing rules of Eskişehir Osmangazi University Graduate School of Natural and Applied Sciences under academic consultancy of my supervisor (Prof. Dr. Adnan Ayhanci). I hereby declare that the work presented in this thesis is original. I also declare that, I have respected scientific ethical principles and rules in all stages of my thesis study, all information and data presented in this thesis have been obtained within the scope of scientific and academic ethical principles and rules, all materials used in this thesis which are not original to this work have been fully cited and referenced, and all knowledge, documents and results have been presented in accordance with scientific ethical principles and rules.

The present study obtained approval from the Experimental Animals Ethics Committee (Deney Hayvan Etik Kurulu Yönergesi) [2018/670-1] of the Eskişehir Osmangazi University and agreed with the requirements of this Committee and the Universal Declaration of Animal Rights (Hayvan Hakları Evrensel Bildirgesi). 23.05.2022.

Alanna TEIXEIRA ANDRADE

Signature

ÖZET

Akut Böbrek Hasarı (AKI), yüksek morbidite ve mortalite oranları ile ilişkili olduğu için, tüm dünyada sağlık sistemlerine büyük yük ve genel insan iş yükü kaybına neden olan karmaşık bir böbrek yetmezliği durumudur. Genellikle böbrek fonksiyonunda saatler veya günler içinde meydana gelen hızlı düşüş ile karakterizedir. Aynı zamanda, üre ve kreatinin gibi nitrojen metabolizması ürünlerinin birikmesine ve bazen azalmış idrar miktarlarına, metabolik asitlerin birikmesine ve artan potasyum ve fosfat konsantrasyonlarına yol açar/neden olur. AKI genellikle diğer patolojiler için hastanede olan hastalarda görülür ve çoğu kez siklofosfamid (CP), bir nitrojen hardalı ve tümörlerle savaşan ve immünosupresif olarak işlev gören klasik ilaç gibi ilaçların kullanımıyla ilişkilidir. CP hızlı çoğalan hücrelerin hücre döngüsünün kesintiye uğramasına neden olan bir alkilleyici ajandır. Daha spesifik olarak, bu ilacın etkisi, alkil gruplarının DNA bazlarına, özellikle guanine bağlanmasından gelir ve DNA zincirleri sentez veya transkripsiyon için ayrılamadığından hücre döngüsünün daha da ilerlemesini engelleyen çapraz bağlar oluşturur. Onarım mekanizmaları çalışmadığından durum, hücreleri apoptoza götürür. Siklofosfamid uygulandıktan sonra biyolojik olarak dönüştürülmelidir ve metabolizması, alkilleyici ajan olarak hareket eden ve bu ilacın antikanser etkisinden sorumlu olan fosforamid hardalı (FAM) üretir; ve akrolein (ACR)'e antianjiyogenik aktivitenin yanı sıra ürotoksik, nörotoksik, nefrotoksik ve genel sitotoksik etkilere sahip olan, doku antioksidan savunma sistemini baskılayan, reaktif oksijen türleri (ROS) üreten ve diğer maddelerdeki serbest tiyol gruplarının tükenmesi yoluyla hemorajik sistite yol açabilen akrolein. Bu etkiler, böbrek fonksiyonunda AKI'ye yol açan ağır bir bedel üretebilir. AKI, böbrek fonksiyonunda hızlı düşüş ve nitrojen metabolizması ürünleri kreatinin ve üre birikimi ile tanımlanır ve tedavi edilmediğinde, uzun süreli kardiyovasküler olaylar riski ve artan oksidatif stres (OS) reaksiyonları ile ilişkili kronik böbrek hastalığı, inflamasyon ve apoptoz gibi komplikasyonlara yol açar. OS, hücreler ve dokularda reaktif oksijen türlerinin (ROS) ve reaktif nitrojen türlerinin (RNS) birikmesine yol açan ve adı geçen yapılara zarar veren bir redoks dengesizliğidir. Aslında, ROS üretimi, NO tükenmesi, otofaji ve mikrovasküler disfonksiyon gibi OS oluşturma mekanizmaları AKI'de çeşitli zararlı etkilerin oluşumunda rol oynar. OS, bu hastalığın evriminin çok önemli bir parçası olduğundan, cazip bir terapötik yaklaşım, organizmayı bir

redoks dengesine yönlendirmek için antioksidan bileşiklerin kullanılması gerenmektedir. Antioksidanlar, redoks homeostazını korumak için aşırı miktarda serbest radikalleri nötralize edebilen ve bazı endojen olanlar dışında, sindirim yoluyla sağlanan moleküllerdir. Bitki antioksidanları, böbreği korumak için sisplatin gibi nefrotoksisiteye sahip terapötik ajanlarla kombinasyon halinde önerilmektedir. Escin (ES), Avrupa genelinde, özellikle Akdeniz ve Balkan bölgelerinde çeşitli topluluklar tarafından geleneksel tıp olarak kullanılan, at kestanesi olarak da bilinen Aesculus hippocastanum bitkisinde bulunan triterpen saponin glikozitlerin kompleks bir karışımıdır. Bu bitkinin tohumları genellikle yutulur veya halk ilacı olarak kullanılmak üzere çaya dönüştürülür. Bilimsel olarak, Escin, güçlü antioksidan aktivitenin yansıra anti-inflamatuar, anti-ödem, antiviral, anti-tümör ve kronik venöz yetmezlikte vazoprotektif etkiler gibi diğer birçok faydalı etki göstermektedir ve bu da onu AKI'nin tedavisi için harika bir aday haline getirmektedir. ES genellikle α- ve β-escin'e bölünür. β-escin en biyoaktif bileşen olarak kabul edilir ve farmasötik ürünlerde en çok kullanılan türdür. Escin Ia, antidiyabetik, antiinflamatuar ve mide koruyucu bir madde olarak rolü nedeniyle en aktif bileşenlerden bir diğeridir. Bu çalışmanın amacı, Siklofosfamidin neden olduğu Akut Böbrek Hasarında Escin'in olası koruyucu etkisini Sprague-Dawley albino sıçanlarında araştırmaktı. Hayvanlar, her biri 6 adet olmak üzere dört gruba ayrıldı: kontrol, 0,5 mL salin alan sağlıklı hayvanlar; ikinci grup 10 mg/kg ES; üçüncü grup 200 mg/kg intraperitoneal CP; ve dördüncü grup CP+ES alan grup. Hayvanlar uygun şekilde yerleştirildi ve ağırlıkları enjeksiyondan önce ve sakrifiye edilmeden önce ölçüldü. Bir gün sonra sakrifiye edildi ve intrakardiyak yol ile kan örnekleri alındı. Nekroz, inflamasyon, kan üre nitrojeni (BUN) ve kreatinin (Cre) düzeylerinin yanı sıra glomerüler alanlar, apoptotik belirteçler olan Bax, Bcl-2, Kaspaz-3'ü değerlendiren histolojik ve biyokimyasal analizler olmak üzere iki grup analiz yapıldı. Histolojik analizler için tüm hayvanların böbrek dokuları %10'luk formalin solüsyonunda sabitlendi ve parafin bloklara gömüldü. Kesitler hematoksilen-eozin (HE) kullanılarak boyandı ve ışık mikroskobu altında analiz edildi. Standart immünhistokimyasal takip yöntemi ile kaspaz-3, Bax ve Bcl-2 analizleri yapıldı. Biyokimyasal analizlerde ise, hayvanların serumları santrifüj ile elde edildikten sonra BUN ve Cre seviyeleri otomatik biyokimyasal oto-analizör ile belirlenmiştir. Bir klinik kimyasal analiz cihazı ile, OS'nin durumunu araştırmak için serum tiyol-disülfid profil testleri yapıldı ve karşılaştırmalı bir test yoluyla hesaplandı. Disülfid (SDS) (S-S) miktarları, nativ tiyol (NT) konsantrasyonlarının total tiyol (TT)

konsantrasyonlarından çıkarılmasıyla belirlendi. Sonuç olarak SDS/TT, SDS/NT ve NT/TT oranları belirlendi. Sonuçların istatistiksel analizi, ortalamanın standart hatası (±SEM) olarak sunuldu. Bağımsız ölçümlerin ve normal dağılıma sahip sürekli verilerin analizinde One-Way-Anova kullanılmıştır. Anormal dağılım gösteren varyantlar için Kruskal-Wallis testi kullanıldı. P değeri <0,001, <0,01 ve <0,05 ise deney gruplarındaki farklılıkların anlamlı olduğu kabul edildi. Böbreğin histopatolojik analizi, normal glomerüler yapıya ve küçük kapsüler alanlara sahip kontrol ve ES gruplarını gösterdi. CP grubu, glomerülde yapısal anormallik, bowman mesafesinde belirgin daralma, dejenere tübüller ve ayrıca dikkate değer tıkanıklık ve inflamatuar hücre infiltrasyonu sunarken. CP+ES grubunda CP grubuna göre Bowman kapsül mesafesinde daralma, glomerülde hasar, tübüllerde dejenerasyon, konjesyon ve inflamasyonda belirgin azalma görüldü. Böyle bir değişiklik, ES'nin CP'nin neden olduğu böbrek hasarını azaltmada bir rolü olabileceğini düşündürmektedir. Kontrol ve ES gruplarında, sadece birkaç Bax ve Caspase-3 pozitif hücre tespit edildi ve Bcl-2 (antiapoptotik) pozitif hücrelerin istatistiksel anlamlılık elde ettiği gözlemlendi. CP grubuna gelince, boyanmış Caspase-3 ve Bax pozitif hücrelerin sayısal artışı dikkat çekiciydi, ancak Bcl-2 pozitif hücrelerin sayısı oldukça düşüktü ve CP+ES grubunda Es, CP'nin neden olduğu apoptozu önemli ölçüde azalttı. Böbrek fonksiyon bozukluğuna işaret eden serum BUN ve Cre seviyeleri, ES ve kontrol gruplarına kıyasla CP grubunda önemli ölçüde artarken (p <0,001), CP+ES grubunda sadece CP verilen gruba kıyasla bu düzeyler önemli ölçüde azaldı (p<0,05). Kontrol ve ES gruplarında NT ve TT düzeyleri yüksek bulunsa da, CP grubunda belirgin bir düşüş vardı (p<0.05). CP+ES grubunda SDS/NT düzeyleri açısından kontrol ve ES gruplarına göre istatistiksel olarak anlamlı bir fark bulunamadı. Bu sonuçlara dayanarak, ES'nin, CP tarafından indüklenen oksidatif stresi azalttığı görülmektedir. Sonuç olarak, CP+ES grubu ile CP grubu karşılaştırıldığında, glomerüler alan, nekroz, BUN, kreatinin, tiyol-disülfid ve Bcl-2 seviyeleri düşerken nativ tiyol, toplam tiyol, Bax ve Kaspaz-3 yükseldi. Buradan CP'nin Kaspaz-3 ve Bax ekspresyonlarını artırarak apoptoza yol açtığı anlaşılmaktadır. Bu nedenle, bu çalışma, ES'nin böbrek fonksiyonu üzerinde dikkate değer derecede koruyucu etkilerle sonuçlandığını ve AKI için tedavi olarak kullanılma potansiyelini gösterdiğini öne sürüyor. Ayrıca escin'in OS ve böbrek fonksiyonu arasındaki patolojik ilişki üzerindeki faydalı etkisini de gösterir.

Anahtar Kelimeler: Siklofosfamid, Akut Böbrek Hasarı, Escin, oksidatif stress.

SUMMARY

Acute Kidney Injury (AKI) is a complex condition of renal failure responsible for great burden to healthcare systems throughout the world, being related to high morbidity and mortality rates. AKI generally occurs in patients that are already in hospital for other pathologies and is many times related to the use of medication, such as Cyclophosphamide (CP), a classical drug that combats tumors and acts as an immunosuppressive. AKI is delineated by rapid decrease in renal function and accumulation of nitrogen metabolism products creatinine and urea and, when untreated, leads to complications such as long-term risk of cardiovascular events and chronic kidney disease related to increased oxidative stress (OS) reactions, inflammation, and apoptosis. As OS is a crucial part of the evolution of this disease, the objective of this study was to investigate the use of Escin (ES), a complex mixture of triterpene saponin glycosides that has shown antioxidant activity, in Cyclophosphamide-induced AKI in Sprague-Dawley albino rats. Four groups (n = 6) were created: control, Escin, Cyclophosphamide, and CP+ES. These two last groups developed AKI through intraperitoneal injection of CP, while the CP+ES Group was given a single dose of ES shortly after the administration of CP. At the end of the experiment, histological and biochemical analyses of the glomerular areas, necrosis, inflammation, blood urea nitrogen (BUN), and creatinine levels, as well as of the apoptotic markers Bax, Bcl-2, Caspase-3 were done. The glomerular area, necrosis, BUN, creatinine, thiol-disulfide, and Bcl-2 levels decreased while native thiol, total thiol, Bax, and Caspase-3 rose when comparing the CP+ES Group with the CP Group. This work suggests that ES resulted in remarkably protective effects upon kidney function, showing potential to be utilized as therapy for AKI, and demonstrates the impact of ES upon the relationship between OS and kidney dysfunction.

Keywords: Cyclophosphamide, acute kidney injury, Escin, oxidative stress.

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LIST OF ABBREVIATIONS AND SYMBOLS

Symbols	Description
°C	Celsius degree
kg	Kilogram
mg	Milligram
ml	Milliliter
rpm	Revolutions per minute
µm	Micrometer

Abbreviation	Description
ADH	Alcohol dehydrogenase
A1DH	Aldehyde dehydrogenase
AGE	Advanced glycation products
AKI	Acute kidney injury
BUN	Blood urea nitrogen
CKD	Chronic Kidney Disease
COVID-19	Coronavirus disease
CP	Cyclophosphamide
Cre	Creatinine
DAMP	Damage-associated molecular patterns
EDTA	Ethylenediaminetetraacetic acid
eNOS	Nitric oxide synthase
ES	Escin
ETC	Electron chain transport
FDA	United States Food and Drug Administration
GPx	Glutathione peroxidase

LIST OF ABBREVIATIONS AND MEASUREMENTS (continuation)

Abbreviation	Description
HE	Hematoxylin-eosin
HO	Hydroxyl radical
H_2O_2	Hydrogen peroxide
ICU	Intensive Care Unit
iNOS	Inducible nitric oxide synthase
NADP	Nicotinamide adenine dinucleotide phosphate
NF-κB	Nuclear factor kappa B
NO	Nitric Oxide
NO_2-	Nitrite
NO_3-	Nitrate
NT	Native thiol
O_{2-}	Superoxide anion radical
ONOO-	Peroxynitrite
OS	Oxidative Stress
PARP	Poly (ADP-ribose) polymerase
PKC	Protein kinase C
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RRT	Renal replacement therapy
SARS- CoV-2	Severe acute respiratory syndrome coronavirus2
SDS	Disulfide
-SH	Sulfhydryl
SOD	Superoxide dismutase
TGF-β	Transforming growth factor beta
TLR	Toll-like receptor
TT	Total thiol

1. INTRODUCTION AND PURPOSE

The kidney is vulnerable, as a primary target organ, to many toxic xenobiotic substances, such as drugs, metals, and environmental chemicals. Acute kidney injury (AKI) due to drugs and other catalysts often frustrates those working on developing clinically promising drug candidates for some diseases (Shelton, Park, and Copple, 2013; Cengiz et al., 2016).

Drug-based nephrotoxicity is not an uncommon side-effect of many illnesses and is responsible for various pathological effects (Dhodi et al., 2014). For example, cyclophosphamide (CP), a potent anti-cancer agent, which has also proved effective in treating a diverse spectrum of malignancy, such as leukemia and lymphoma, as well as breast, lung, ovarian, and prostate cancers, is a highly restrictive drug due to its detrimental physiological impacts upon such organs as the liver, kidney, heart, and bladder (Khan et al., 2004; Ayhanci et al., 2010; Motawi, Sadik, and Refaat, 2010).

Furthermore, its clinical use is limited due to the noticeable organ toxicity associated with increased oxidative stress (OS), which plays a crucial role in CP-induced AKI, one of the side-effects of this drug (Stankiewicz and Skrzydlewska, 2003; Abraham and Rabi, 2009; Abraham and Rabi, 2011; Nafees et al., 2015). This being the case, it is tempting to think of using various detoxifying and protective antioxidant agents that can scavenge free radicals in treating CP-induced damage so that the toxic effects of CP can be either completely eliminated or significantly reduced (Sabolić, 2006; Yoruk et al., 2010; Neboh and Ufelle, 2015).

Thiols, or mercaptans, are such components. They are organic compounds capable of binding to mercury, which contains the sulfhydryl (-SH) group. The -SH group present in thiols has a protective impact upon not just oxidative stress, but also on apoptosis. Reversible disulfide bonds do form during the reaction between thiols and oxygen radicals. Once oxidative stress is reduced, these disulfide bonds separate from the thiol groups, thus achieving a thiol-disulfide balance. In recent years, thiol-disulfide homeostasis is

acknowledged to be an indicator of oxidative stress (Taysı et al., 2008; Erel and Neselioğlu, 2014).

Escin (ES), *Aesculus hippocastanum* (Hippocastanaceae) is the active substance of the horse chestnut tree that has been used for treating diarrhea, varsity, hemorrhoid, phlebitis, fever, and prostate hyperplasia since ancient times (Hu and Zeng, 2004; Cengiz, Ayhanci, and Kutlu, 2020). However, recent studies have determined its anti-inflammatory, antioxidant, anti-edematous, antiviral, and anti-tumoral effects (Zhang et al., 2010; Cengiz et al., 2020; Gür et al., 2021).

ES, which is especially used in treating peripheral vessels, has two forms; α – and β– escin (Fig. 1.1). ES has had its scope of use expanded, being increasingly more common in cosmetic and skin improvement products. No studies are available in the literature into the protective effects of ES upon CP-induced AKI. The purpose of the present study is to examine the protective impacts of ES against histopathological changes, apoptosis, and oxidative stress due to CP in experimental rats.

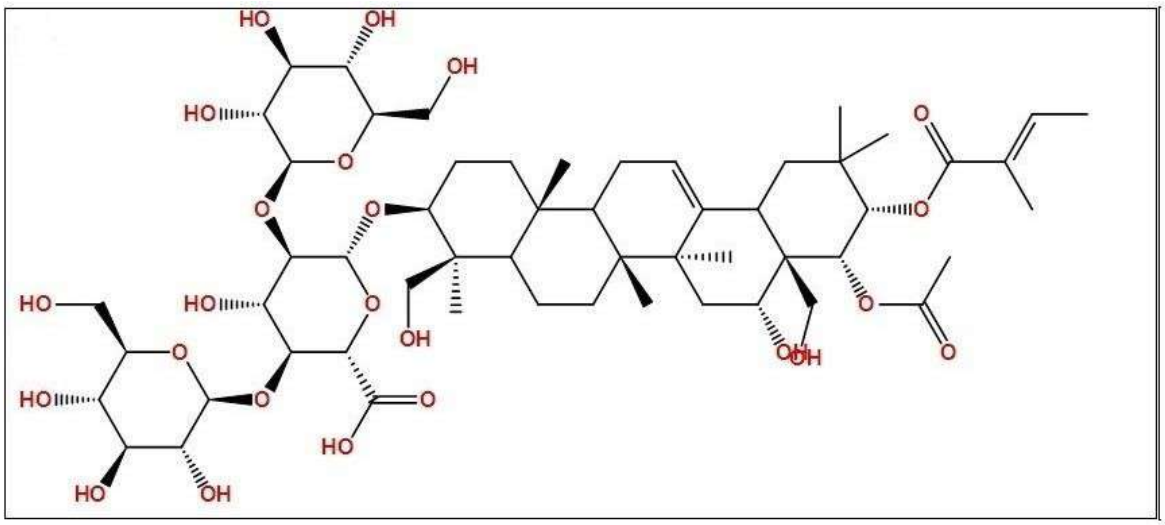

Figure 1.1 The chemical structure of Escin (ES).

2. LITERATURE REVIEW

2.1. Cyclophosphamide

Cyclophosphamide is a classic medication in the treatment of neoplasia, with its first trials and approval by the United States Food and Drug Administration (FDA) more than 60 years ago (Emadi, Jones, and Brodsky, 2009). They came into the public eye when they were firstly developed and used as chemical warfare during World War I (Duchovic and Vilensky, 2007), though by World War II, many different scientists were searching for medical applications for these compounds (Smith, 2017).

CP is qualified as a nitrogen mustard, an organic compound with the functional group chloroethylamine ($Cl(CH_2)_2NR_2$) (IUPAC, 1997). It is an alkylating agent, whose effects cause interruption of the cell cycle of rapid multiplying cells impeding it to reach the S phase (Bruce, Meeker, and Valeriote, 1966; McDonald et al., 2003). Because not only cancer cells are rapidly dividing, but also notably immune cells, cyclophosphamide generates immune suppression, which justifies its use in organ transplantation and autoimmune diseases (Bhat et al., 2018).

Another incredible accomplishment of cyclophosphamide is how it is used as an effective to the treatment of numerous other diseases, such as autoimmune disorders, hematologic malignancies, solid tumors, stem cell mobilization, and as a conditioning regimen for bone marrow transplantation, known as BMT (Emadi, Jones, and Brodsky, 2009; Bhat et al., 2018).

Cyclophosphamide must be bio transformed after administered, which means it needs to be activated inside the body to act (Sládek, 1988). This effectively occurs intermediated by the enzyme cytochrome P450 in the liver, hydroxylated at the fourth carbon of the oxazaphosphorine ring into 4-hydroxycyclophosphamide (4-OH-CP) circulation (Chang et al., 1993), where it is carried by erythrocytes to the tumor cells

(Zhang et al., 2005). 4-hydroxycyclophosphamide is then rapidly converted to aldophosphamide via a ring opening reaction, which then is non-enzymatically split by β-elimination into two cytotoxic compounds: phosphoramide mustard, which acts the alkylating agent and is responsible for the anticancer effect of this drug; and acrolein (Sládek, 1988; Zhang et al., 2005).

More specifically, phosphoramide mustard connects its alkyl groups to DNA bases, in particular guanine, forming crosslinks that prevent the further advancement of the cell cycle as the strands of DNA cannot separate for synthesis or transcription (Colvin et al., 1976; Povirk and Shuker, 1994; Dong et al., 1995; Shulman-Roskes et al., 1998). Without correction from the cellular apparatus, the cell often starts an apoptotic process (O'connor et al., 1991; Banker et al., 1997; Zhang et al., 2005).

Acrolein, besides antiangiogenic activity, has urotoxic, neurotoxic, nephrotoxic, and overall cytotoxic effects, it impairs the tissue antioxidant defense system, produces reactive oxygen species (ROS), and can lead to hemorrhagic cystitis through the depletion of free thiol groups of other substances (Grafström et al., 1988; Sládek, 1988; Günther, Wagner, and Ogris, 2008; Giraud et al., 2010; Aldini, Orioli, and Carini, 2011; Benedikter et al., 2017; Khorwal, Chauhan, and Nagar, 2017).

2.1.1. Deactivation and resistance to cyclophosphamide

Acrolein is detoxified by conjugation with glutathione GSH, but the major mechanism for the detoxification of CP is 4-OH-CP being primarily detoxified by aldehyde dehydrogenase (AlDH) into O-carboxyethylcyclophosphoramide mustard, though alcohol dehydrogenase (ADH) to 4-ketocyclophosphamide can also fulfill this role (Chen et al., 1997; Zhang et al., 2005). Thus, the concentration of AlDH is responsible for the differential cellular responses to CP (Russo, Hilton, and Colvin, 1989).

AlDH is a superfamily of enzymes that, in humans, includes 19 isoenzymes with many splice variants identified until the present date (Jackson et al., 2011; Rodriguez-Torres and Allan, 2016). These enzymes have been recorded in the cytosol, nucleus, mitochondria, and endoplasmic reticulum (Sládek, 2003; Marchitti et al., 2008). These

NADP-dependent enzymes catalyze the conversion of aldehydes to their corresponding carboxylic acids (Jackson et al., 2011).

While AlDH1A1 is the key isoform responsible for the detoxification of cyclophosphamide (Sládek et al., 2002), it also acts in ethanol metabolism and, as another main function, in the biosynthesis of retinoic acid from retinol (Duester, 2001). Retinoic acid is fundamental for cellular growth and differentiation and thus, cells with a high potential to proliferate, such as hematopoietic stem cells, intestinal mucosa and liver cells, express high values of AlDH1A1 and are consequentially relatively resistant to CP (Jones et al., 1995).

Thus, DNA damage that leads to apoptosis is the main susceptibility and determinant in how responsive cancer cells will be to CP (Banker et al., 1997; Zhang et al., 2005; Emadi, Jones, and Brodsky, 2009). Though the key determinant to normal cell sensibility to CP is AlDH1A1 expression, which has interestingly shown association with resistant cancer cell lines (Sládek, 2002).

2.1.2. Cyclophosphamide drawbacks

The use of CP is not without consequences, as earlier mentioned it has toxic effects on the body, importantly on the liver and kidney. Sakr and El-messady (2017) and Bhat et al. (2018) both observed pathological changes in the kidney, such as atrophy of glomerular tuft, dilation, and congestion of renal blood vessels, infiltration of acute inflammatory cells in the cortex, oedema of tubular cells, loss of brush border, vacuolations of epithelial lining renal tubules, and the presence of pyknotic cells, a sign of necrosis or apoptosis. They also observed increased oxidative stress markers, suggesting its relation to this pathological state. Particularly, the use of CP is associated with Acute Kidney Injury (AKI) through a condition of increased oxidative stress (Stankiewicz and Skrzydlewska, 2003; Abraham and Rabi, 2009; Abraham and Rabi, 2011; Nafees et al., 2015).

2.2. Acute Kidney Injury

Acute Kidney Injury (AKI), previously called acute renal failure (Bellomo et al., 2004), is a condition characterized by renal function rapidly decreasing (in hours or days) and the accumulation of nitrogen metabolism products, such as urea and creatinine. Though, there has been debate over the definition of AKI, but it is generally accepted to be an abrupt decline in glomerular function (Dennis and Witting, 2017; Negi et al., 2018). It can also feature decreased urine quantities, accumulation of metabolic acids, and increased potassium and phosphate concentrations (Bellomo, Kellum, and Ronco, 2012). Thus, it is usually clinically evaluated by an increase in serum creatinine (Tögel and Westenfelder, 2014).

As for the triggers of AKI, hospital and ICU inpatients most commonly suffer from AKI derived from sepsis; major surgeries, such as open-heart surgery; cardiogenic shock, multiple organ dysfunction and acute decompensated heart failure, with sepsis accounting for nearly 50% of the generation of AKI (Tögel and Westenfelder, 2014; Le Guen, Tobin, and Reid, 2015). Many of these causes are related to ischemia and acute hypoxia from general or regional decreases in renal blood flow (Dennis and Witting, 2017). Though it is noticeable that the renal artery is usually not occluded in any of these situations. (Hoste and De Corte, 2011; Bellomo, Kellum, and Ronco, 2012). Another note is that AKI is not usually the main cause of patients going into ICU, but a cause for great complications once the patient is already there (Pavlakou et al., 2017).

AKI occurs more frequently in critically ill patients and shows association with high morbidity and mortality rates even in children and young adults, with primary renal diseases still being main causes of AKI in the pediatric sector of developing countries (Kaddourah et al., 2017; Cleto-Yamane et al., 2018; Negi et al., 2018). It is important to mention that children are not usually monitored for complications related to AKI, so its consequences can be especially devastating in this demographic (Lebel, Teoh, and Zappitelli, 2020).

According to Hoste et al. (2015), over 50% of patients in Intensive Care Units (ICUs) are afflicted with AKI during their first week of admission. The incidence of AKI has been increasing in the last years as it is also related to age-related diseases, such as diabetes mellitus and cardiovascular diseases; complications, as hypertension and preexisting chronic kidney diseases; and major medical interventions, namely major surgery, percutaneous coronary intervention, and chemotherapy (Hsu et al., 2013; Pinho, Oliveira, and Pierin, 2015; Kaddourah et al., 2017; Negi et al., 2018).

AKI is also related to an array of short and long-term complications if left untreated, such as hyperkalemia, metabolic acidosis, volume overload, hyponatremia, inflammation, infection, organ crosstalk. All derived of an acid-base disorders and electrolyte abnormalities that impair homeostatic functioning of many processes in the body (Hoste and De Corte, 2011).

As well as long-term risk of cardiovascular events (acute myocardial infarction, congestive heart failure, or stroke after an AKI episode), chronic kidney disease, end-stage renal disease (Negi et al., 2018; Legrand and Rossignol, 2020), and even some respiratory complications, such as inflammatory lung injury (Faubel and Edelstein, 2016). Additionally, AKI is a risk factor for chronic kidney disease (CKD), both occurring secondarily to imbalanced oxidative stress (OS) reactions, inflammation, and apoptosis. Indeed, CKD many times follows AKI even in patients who seem to have fully recovered renal function after AKI episodes (Wald et al., 2009; Lameire et al., 2013; Basile et al., 2016).

Keeping these related consequences in mind, it is not difficult to wonder the costs brought to the healthcare system with such a prevalent impairment. As there are such severe cases that require renal replacement therapy (RRT) in the ICU, need for dialysis, re-hospitalisations, which result in loss of productive years, life expectancy, quality of life, and can lead to a dependency of social services (Collister et al., 2017; Negi et al., 2018; Ostermann and Cerdá, 2018).

Various studies have investigated the expenditures related to this disease in different countries (Ostermann and Cerdá, 2018). In 2014, Kerr et al. estimated the yearly cost of AKI-related inpatient care in England at 1.02 billion British pounds. A cohort study in Canada calculated that the mildest cases of AKI adjusted costs to 1.2–1.3 times greater than those for patients without AKI, while more severe cases could make this figure reach values 1.8–2.5 times greater. It was also assessed that patients with AKIN stage 1 (most moderate stage of the AKI classification from the Acute Kidney Injury Network) had incremental costs of approximately $3800 per patient, with patients that needed dialysis reaching numbers of $18,300 for the period between admission and assessment of kidney recovery or 90 days (Collister et al., 2017).

Aside from financial costs, AKI delivers severe short- and long-term health outcomes and is still responsible for a high mortality and terrible prognosis even with substantial advances in basic research and management of AKI (Tögel and Westenfelder, 2014; Dennis and Witting, 2017; Negi et al., 2018; Ostermann and Cerdá, 2018). Thus, the pursuit of effective treatments for AKI is still relevant and necessary (Dasta and Kane-Gill, 2019).

2.2.1. AKI and coronavirus disease 2019

Another factor that brings more attention to AKI and the search for new treatment options is the recent occurrence of the pandemic of coronavirus disease 2019 (COVID-19). The city of Wuhan, in China, gained abrupt global attention as, during December of 2019, it became the first epicenter of the disease 2019. In January of 2020, Chinese scientists isolated the virus that is now known as severe acute respiratory syndrome coronavirus 2, officially abbreviated as SARS-CoV-2 (Zhou et al., 2020).

Alongside Turkey, many countries tried enforcing “social distancing” as one of the main methods to refrain the rapid contamination of large amounts of people (Bakir, 2020; Kanbur and Akgül, 2020; Naharci, Katipoğlu and Tasci, 2020; Özatay and Sak, 2020). Despite that, on a significant number of them, a percentage of the population did not adhere to the rules and tried to confront the action, gather and follow ordinary patterns of life (Thu, Ngoc and Hai, 2020).

According to the most recent Weekly Epidemiological Update on COVID-19 of 13 of July of 2022 published by the World Health Organization (WHO), the pandemic directly took the lives of more than 6 million people worldwide. Thankfully, vaccines for this ailment are already available in many countries, but a new challenge that poses itself is that of long term COVID-19 side effects and of how to deal with them.

A relation is being established between COVID-19 and AKI. The works of Silver et al. (2020) and Moledina et al. (2021) identify that the percentage of the total prevalence, absolute risk and possibility of death are all increased when comparing patients that had COVID-19 and then AKI with the traditional AKI patients. Those patients also had greater values of C-reactive protein and ferritin, which are used as inflammatory markers and had to make a more significant use of other medications, such as diuretic and vasopressors.

Nadim et al. (2020) from the Acute Disease Quality Initiative (ADQI) Workgroup released a detailed report covering the probable direct and indirect pathological mechanisms of COVID-19-associated AKI. Direct mechanisms include possible tropism of the virus that would directly affect the kidney, as well as activation of the complement system, coagulopathy, and endothelial dysfunction. In short, immune system dysfunction and systemic inflammation. Indirect mechanisms cited are patients baseline characteristics, organ crosstalk and even necessary interventions to treat the infection and other of its effects.

2.2.2. Oxidative stress and AKI

Factors composing the pathological process are apoptosis, necrosis, reactive oxygen species, and micro-vessel damage causing local ischemia, endothelial dysfunction, leaks, and inflammation, and although many stages of the disease have been delineated, they are often overlapping in a clinical situation (Tögel and Westenfelder, 2014).

A considerable part of the complications seen in AKI is related to oxidative stress (OS). In fact, OS mechanisms such as autophagy, DAMP generation and TLR activation, microvascular dysfunction, NO depletion, and ROS generation are involved in the generation of various deleterious effects in AKI (Dennis and Witting, 2017; Pavlakou et

al., 2017). Additionally, OS is related to a series of other pathological conditions, namely ischemia, atherosclerosis, cardiovascular diseases, neurodegenerative diseases, cancer, aging, and many others (Sorg, 2004; Valko et al., 2007; Salmon, Richardson, and Pérez, 2010; Singh et al., 2019), as well as being an important factor in the development of kidney damage (Himmelfarb et al., 2004). OS is linked to hypoxia and ischemia, two elements that are also associated with AKI (Dennis and Witting, 2017).

OS occurs when there is a redox imbalance that leads to accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in cells and tissues, which ends up damaging said structures. Different metabolic pathways can lead to OS while in hyperglycemic conditions, such as the glycolytic, hexosamine, polyol, PKC activation and formation of advanced glycation products (AGE) pathways (Rochette et al., 2014; Darenskaya, Kolesnikova and Kolesnikov, 2021). Unrestrained OS results in cellular damage (Lushchak, 2014), disruption of protein function, and damage to DNA, lipids, and enzymes (Sies, Berndt, and Jones, 2017).

ROS and RNS are common radical species produced as by-products of many metabolic pathways in living systems and contribute to the redox balance, in a homeostatic state. They also act in important intracellular signaling pathways and regulate diverse cellular actions. Prominent examples of this regulator role are in stimulating inflammatory response and participating of the energetic process of aerobic organisms (Lemineur, Deby-Dupont, and Preiser, 2006; Lushchak, 2014; Rochette et al., 2014; Darenskaya, Kolesnikova and Kolesnikov, 2021).

ROS are generated primarily by the reduction of oxygen by cytochrome oxidase in mitochondrial electron chain transport (ETC) which ends in the production of hydrogen peroxide (H_2O_2), superoxide anion radical (O_{2-}), and hydroxyl radical (HO) (Lushchak, 2014).These ROS end up “attacking” lipids, proteins, and amino acids creating unstable molecules that act as radicals and convert into compounds that trigger a multitude of metabolic effects (Lemineur et al., 2006; Dennis and Witting, 2017).

As for RNS, they are by-products of nitric oxide (•NO) and include nitrite (NO_2-), nitrate (NO_3-) and peroxynitrite (ONOO-) and in high levels contribute to many pathologies (Palipoch, 2013). NO is mainly formed by the endothelial isoform of nitric oxide synthase (eNOS), which is vital for normal endothelial function and vascular tone, the prevention of platelet aggregation, and presents anti-inflammatory properties (Lemineur et al., 2006; Aksu, Demirci, and İnce). When eNOS is deprived of its cofactors (i.e., calmodulin and tetrahydrobiopterin), oxygen is oxidated and its superoxide is released, acting as a free radical specie and contributing to the condition of oxidative stress. This happens during inflammatory situations (such as sepsis) where there is an incremental cellular NO release, mediated by the activity of inducible nitric oxide synthase, also known as iNOS (Rabelink and van Zonneveld, 2006).

One theory hypothesizes that the heterogeneous iNOS expression in AKI leads to increase of NO levels and that microcirculatory dysfunction further enhances this, causing the continuation of oxygen deprivation in the region (Gomez et al., 2014), meaning that more than maintained, kidney damage is expanded (Pavlakou et al., 2017). The iNOS-dependent inhibition of eNOS degrades endothelial function, in turn strengthening the connection between NO, oxygen, and its radical species (Rabelink and van Zonneveld, 2006; Gomez et al., 2014) in the pathophysiology of AKI and oxidative stress.

In AKI, the accumulation of these inflammatory products mediates the progression of injury and hemodynamic imbalance, that further stimulates inflammation, promotes dysfunction of the vasculature, and induces cytotoxicity on renal tubule cells, creating a bidirectional relationship between AKI and OS (Glodowski and Wagener, 2015; Ratliff et al., 2016 Pavlakou et al., 2017). Thus, OS is considered an integral aggravator of AKI (Ruiz et al., 2013; Tanaka, Tanaka, and Nagaku, 2014).

2.3. Antioxidants

Seeing that the pathological process of CP in the kidney is strongly related to the mechanism of oxidative stress, it is important to include the administration of

renoprotective drugs, in special antioxidants, before using chemotherapeutic agents (Bhat et al., 2018).

Antioxidants are molecules that can help restore the redox balance by inhibiting oxidation, and thus, the excessive formation of these reactive species (Riley, 1994 Vanlallawmzuali, Devi and Pushpa, 2021). They can neutralize overabundant free radicals to maintain the redox homeostasis and, apart for some endogenous ones, are supplied through ingestion (Flieger et al., 2021).

These compounds can be classified in many ways, for example according to their origin as natural or synthetic/artificial antioxidants (Dontha, 2016) or based on their mechanism of action, as chain breaking or preventive antioxidants (Benzie and Strain, 1999). Preventive antioxidants deactivate metals, quench singlet oxygen and remove hydroperoxides, including catalase, ceruloplasmin, ethylenediaminetetraacetic acid (EDTA), ferritin, glutathione peroxidase (GPx), superoxide dismutase (SOD) and transferrin, thus decreasing chain initiation. While chain breaking antioxidants receive or donate an electron from a reactive species, forming stable byproducts (Halliwell, 1995; Young and Woodside, 2001).

Natural antioxidants can be further divided into biological antioxidants, from endogenous and exogenous sources, and then into enzymatic antioxidants (such as CAT, GPx and SOD) and nonenzymatic antioxidants such as antioxidant enzyme cofactors (Selenium, coenzyme Q10), oxidative enzyme inhibitors, ROS/RNS scavengers (vitamin C and E), and transition metal chelators (Huang and Prior, 2005).

Another important difference among the various antioxidant substances is that they can act at different steps of the oxidative radical process, from the lipid peroxidation in cell membranes, which implies the successive steps of initiation, propagation, and chain termination as targets of antioxidant action (Yin, Xu, and Porter, 2011). Multiple antioxidant responses are acknowledged (Mut-Salud et al., 2016), such as inhibiting the creation of new radicals (superoxide dismutase, catalase, Se, Cu, Zn); catching the free

radicals to evade chain reaction (vitamins E and C, carotenoids); restoring the impairment affected by free radicals (lipases, proteases).

Recently, there has been a wave of interest in replacing the use of artificial antioxidants with natural ones, as some of the most widely used synthetic antioxidants in food have shown some association to toxicity and side effects, such as carcinogenesis (Komes et al., 2011; Carocho, Morales and Ferreira, 2015; Caleja et al., 2017; Sarıkürkçü et al., 2020). Additionally, natural antioxidants prevent oxidants from in cancer cases that use cisplatin, a chemotherapy medication that also has deleterious effects, without altering its efficacy against tumors (Hajian, Rafieian-Kopaei, and Nasri, 2014).

Thus, supplements containing antioxidants may reduce the increased levels of OS that the endogenous sources are unable to inactivate (Poljsak, Šuput, and Milisav, 2013). Therefore, antioxidant intake can help to forage free radicals so that acute and chronic diseases such as Alzheimer, cancer, cardiovascular and liver diseases are prevented (Neha et al., 2019).

2.4. Escin

As reviewed, the consequences of AKI are mainly connected to oxidative stress and the accumulation of cytotoxic free radical species. The combat of such ailments with antioxidants has also been explored and suggested as a logical target in combating AKI (Palipoch, 2013; Pavlakou et al., 2017; Tomsa et al., 2019).

Plant antioxidants are suggested paired with therapeutic agents that possess nephrotoxicity, such as cisplatin, contrast media and others to protect the kidney against the cytotoxic effects of these agents (Neha et al., 2019). The use of escin from the plant *Aesculus hippocastanum*, also known as common horse chestnut and Conker tree, stands out as there are many accounts describing its anti-inflammatory, antioxidant, antiangiogenic, and antitumor activities (Cheong et al., 2018; Shu-Qi et al., 2018; Idris, Mishra, and Khushtar, 2020; Omi et al., 2021).

The genus *Aesculus* L. of the family Hippocastanaceae contains 12 living species, *Aesculus hippocastanum* being one of the two Eurasian species of this group. This deciduous tree is endemic to the Balkans peninsula and extensively used in garden and home decoration (Hardin, 1960), it has also been utilized as a natural medicine by many communities.

The seeds of the plant are used in Europe to treat venous and inflammatory disorders such as varicose veins, hemorrhoids, and arthritis (Sirtori, 2001). Particularly in Turkey, where *A. hippocastanum* is prevalent (Küçükkurt et al., 2010), folk medicine utilizes tea from the crushed seeds of this plant to against stomachache, and a fraction of the seed is swallowed to alleviate hemorrhoids symptoms (Yeşilada, 2002).

Later science has discovered that the seeds of this plant contain a variety of bioactive substances, from which the predominant responsible for many of the effects demonstrated is a complex mixture of triterpene saponin glycosides known as Escin (Zhang, Li, and Lian, 2010; Cheong et al., 2018). ES is also found in small amounts in the leaves, bark, and immature fruit pericarp of this plant, but the seed cotyledons are its main source (Ćalić-Dragosavac et al., 2010).

Escin, a triterpenoid glycoside with three sugar units linked at the third carbon of the aglycone moiety (Vanti et al., 2019), has varied pharmacological actions such as anti-edematous, anti-inflammatory, antioxidant, antiviral, and vasoprotective in chronic venous insufficiency (Matsuda and Yoshikawa; 2000; Zhang et al., 2010; Cengiz et al., 2020; Idris, Mishra, and Khushtar, 2020; Gür et al., 2021).

ES is often divided into α- and β-escin, which are differentiated through melting point, hemolytic index, solubility in water and by specific rotation (Sirtori, 2001). β-escin appears to be the most important constituent and it is the most utilized in pharmaceutical products (Cheong et al., 2018), more specifically, escin Ia is one of the most active components in ES, having antidiabetic, anti-inflammatory and gastroprotective activities (Wu et al., 2014).

Several *in vitro* and preclinical studies (mice and rats) have shown ES activity against a variety of pathologies. The seeds of *A. hippocastanum* have even acted in diabetic neuropathy with decrease in the glomerular area, blood urea nitrogen (BUN), creatinine (Cre), immunoexpression of fibronectin, MDA, transforming growth factor beta (TGF-β), , and urine proteinuria levels (Wang et al., 2008) and Qiao et al. (2022) has shown that escin inhibits the ncreatiuclear factor kappa B (NF-κB) pathway and, in doing this, inhibits oxidative stress, apoptosis, and inflammation induced by H_2O_2 in H9c2 cells.

In tumors, ES inhibited HT29 colon cancer cell proliferation at G1-S phase, decreased oxidative stress and inflammation in mice models of Parkinson′ s disease cells (Patlolla et al., 2006). It also inhibited ALDH1A1, and p-Akt and induced p21 expression in lung tumor cells, alluding to an effective growth arrest of tumor cells (Patlolla et al., 2013).

ES also caused cell cycle arrest on G0/G1 phase and induced apoptosis by caspase-3 activation and expression of the protein Bax on A549 lung adenocarcinoma (Çiftçi Işcan, and Kutlu, 2015). Additionally, ES increased the sub-G1 population by causing G2/M arrest, with activation of caspase-9/-3, annexin V binding, cleavage of poly (ADP-ribose) polymerase (PARP) and Bax protein, as well as decreasing the anti-apoptotic protein levels of Bcl-2, X-linked inhibitor of apoptosis protein and surviving. Thus, diminishing the survival of 786-O and Caki-1, human renal cancer cells (Yuan et al., 2017).

β-escin has ample effects on vascular diseases as it conserves ATP during oxygen shortage (Arnould et al., 1996), decreases histamine response (Frick and Frick, 2000), and cytokine release (Montopoli et al., 2007), attenuates serotonin-induced capillary hyperpermeability (Matsuda et al., 1997), suppresses extravasation and migration of leukocytes (Guillaume and Padioleau, 1994), and preserves the morphology of endothelial cells (Montopoli et al., 2007). We also have studies on the antioxidant potential of β-escin (Küçükkurt et al., 2010; Wang et al., 2014). ES has also inhibited angiogenesis by reducing the secretion of VEGF and IL-8 by suppressing NF-κB activation in pancreatic cancer cell lines (Omi et al., 2021).

As for escin's effects on inflammation, it has shown hepatoprotective action against acetaminophen, a drug commonly used for its antipyretic and analgesic properties, but that can cause oxidative stress, hepatocyte apoptosis and necrosis, and even result in acute liver failure. This result occurred mechanisms such as attenuated inflammatory immune cell infiltration and decreased pro-inflammatory cytokine production, as well as the inhibition of ERK signaling pathway (Lee et al., 2019). Many studies are recently investigating the relation of the anti-inflammatory activity of ES with glucocorticoids or glucocorticoid-like action (Xin et al., 2011; Shu-Qi et al., 2018; Gallelli et al., 2021).

Reiterating the significance of ES, recent research has been done investigating ES-based nanovesicles, escinosomes, that maintain characteristics of the original component, namely inhibition activity of hyaluronidase and the ability to load other molecules such as berberine chloride. This can be useful to strengthen or broaden the activity of the drug that is being carried by it (Vanti et al., 2019).

3. MATERIAL AND METHODS

3.1. Chemicals

Escin and cyclophosphamide were purchased from the Sigma-Aldrich company (CAS Number: 6805-41-0) the Baxter company (1001995501, Halle, Germany), respectively. In preparation for injection, 500 mg of CP was dissolved in saline of 25 mL. The animals were given 200 mg/kg of CP intraperitonially via sterile and disposable injectors. As for the orally prepared solution (via gavage), 10 mg/kg of ES (Li, Li, and Jia, 2015) was prepared by getting dissolved in 0.5 mL saline.

3.2. Treatment

The present study was launched after approval issued by the Experimental Animals Ethics Committee [2018/670-1] of the Eskişehir Osmangazi University. Sprague-Dawley albino rats were obtained from the Centre of Experimental animals and randomly separated into 4 experimental groups (n=6). Then, they were fed with drinking water and diligently prepared standard pellets in a standard environment. They were also placed under standard humidity (% 45–50), heat (22 ± 2 ° C), and light conditions (12 hours of daylight / 12 hours of darkness). The weight of each rat was measured not only during the injection but also before the sacrificing process. While the rats in the Control Group received 0.5 mL of saline, the other study groups were given CP, ES, and CP+ES, respectively. They all were sacrificed the very next day (Cengiz et al., 2019). Meanwhile, their blood samples were obtained through a cardiac puncture.

3.3. Histopathological Analysis

The kidney tissues of all the study animals were fixed in a 10% formalin solution for 48 hours and rinsed with running water for nearly 10 hours. Following routine tissue

follow-up procedures, the kidneys were subjected to alcohol and xylene to be deparaffinized and rehydrated, before being embedded into paraffin blocks.

Incisions of 4μm were done in each block, which were then prepared for analysis. The samples were submerged into a solution of 1% H2O2 in ice-cold methanol for 20 minutes to neutralize endogenous peroxidase. After, they were rinsed in phosphate-buffered saline (PBS) and incubated for 1 hour in blocking solution of 3%BSA, 0.1% Tween-20 in PBS at room temperature.

Then they were incubated with respective anti-Bax, anti-Bcl-2, and anti-Caspase-3 antibodies (goat polyclonal) (1:500) at 4 °C for 12 hours in a blocking solution. The samples were let to return to room temperature and washed with PBS. After that, they went through a second incubation with horse radish peroxidase (HRP) antibody conjugates (1:2500) in a blocking solution that didn't contain Tween-20 for 2 hours. The samples were cleansed utilizing PBS again and incubated with 0.2% solution of 3,3′-diaminobenzidine (DAB) until they reached appropriate stain intensity. After, they were washed with distilled water.

Finally, using hematoxylin-eosin (HE) as a counterstain and and mounted with di-n-butylphthalate-polystyrenexylene (DPX), these incisions were visualized under the light microscope for histopathological and immunohistochemical analyses (Cengiz et al., 2019; Teksoy et al., 2019; Ayhanci et al., 2020).

3.4. Biochemical Analysis

3.4.1. Determination of BUN and Cre levels

The serums of the study animals were obtained by centrifuging their blood samples for 10 minutes at 3000 rpm. Not only serum BUN levels but also those of Cre were determined through an automated biochemical auto-analyzer (HITACHI-917) (Cengiz, 2018a).

To perform this experiment, calibration curves of BUN and Cre were prepared, and the reagent blank absorbance was determined as to diminish any interference from the reagents in the results. The samples were stored at 4 to 8 °C until the analysis as recommended by the manufacturer of the test. Then, they were placed in individual barcoded analyzer tubes and the levels of BUN and Cre were analyzed through an automated biochemical auto-analyzer (HITACHI-917) (Cengiz, 2018a).

The BUN test works by measuring kinetically the consumption of NADH, that occurs after its reaction with ammonia that was in turn generated by a hydrolyzation of urea that wasn't properly filtered by the kidney, signaling this way kidney damage. Creatinine is another classic kidney function test, specifically glomerular filtration. It uses the Jaffé reaction, in which picric acid and creatinine react in an alkaline solution, forming a yellowish red compound, that is then used to photometrically calculate the amount of Cre.

3.4.2. Determination of TT, NT, and SDS levels

To assess OS, the serum thiol-disulfide profile tests were performed. Plasma samples were separated from total blood content by centrifuging at 1500 ×g for 10 minutes. The samples were then set to run at a sophisticated clinical chemical analyzer (Roche, Cobas 501, Mannheim, Germany), according to the instructions of the inventor of the method (Figure 3.1). TT (-SH+S-S) and NT (-SH) quantities were calculated with the aid of a comparative test. The amounts of disulfide (SDS) (S-S) were determined by subtracting the NT concentrations from the TT concentrations. As a result, SDS/TT, SDS/NT, and NT/TT ratios were determined (Erel, 2005).

Reagent 1 volume	225 µL (Reagent 1: xylenol orange 150 µM, NaCl 140 mM and glycerol 1.35 M in 25 mM H_2SO_4 solution, pH 1.75)
Sample volume	35 µL (serum or other fluids, pure or complex oxidant solutions)
Reagent 2 volume	11 µL (Reagent 2: ferrous ion 5 mM and *o*-dianisidine 10 mM in 25 mM H_2SO_4 solution)
Wavelength	Main wavelength 560 nm, secondary wavelength 800 nm (Bichromatic)
Reading point	End-point measurement. The first absorbance is taken before the mixing of R1 and R2 (as sample blank) and the last absorbance is taken when the reaction trace draws a plateau line (about 3–4 min after the mixing)
Calibration type	Linear

Figure 3.1 Setup of the the serum thiol-disulfide profile tests extracted from Erel (2005).

3.5. Statistical Analyses

Determination of the data obtained from the animal experiments was established via the standard error of the mean (±SEM). One-Way-Anova was utilized in analyzing the independent measurements and continuous data with a normal distribution. Besides, for scoring the variants that showed an abnormal distribution, the Kruskal-Wallis test was utilized. The differences in the experimental groups were acknowledged to be of significance if the p-value was <0.001, <0.01, and <0.05.

4. RESULTS AND DISCUSSION

4.1. ES Protects The Kidney From The Harmful Effects Of CP

Histopathological analysis of the kidney showed Control and ES Groups to have a normal structure; namely, the glomerular structure and the small capsular area, as well as the cells in proximal convoluted tubules and epithelial distal convoluted tubules were normal. However, negligible congestion was observed in the Control Group while a small number of inflammatory cell infiltrations were detected in the ES Group. The possibility that the tissues got damaged during the follow-up period seems to account for such an unexpected result. In CP Group, structural abnormality in the glomerulus, noticeable narrowing in the bowman distance, degenerated tubules, as well as remarkable congestion and inflammatory cell infiltration, were observed. As for CP+ES Group, there was narrowing in the Bowman capsule distance, damage in the glomerulus, degeneration in the tubules, as well as a significant reduction in congestion and inflammation when compared to CP Group. Such a change as this seems to suggest that ES may have a part in reducing CP-induced kidney damage (Fig. 4.1 and Table 4.1).

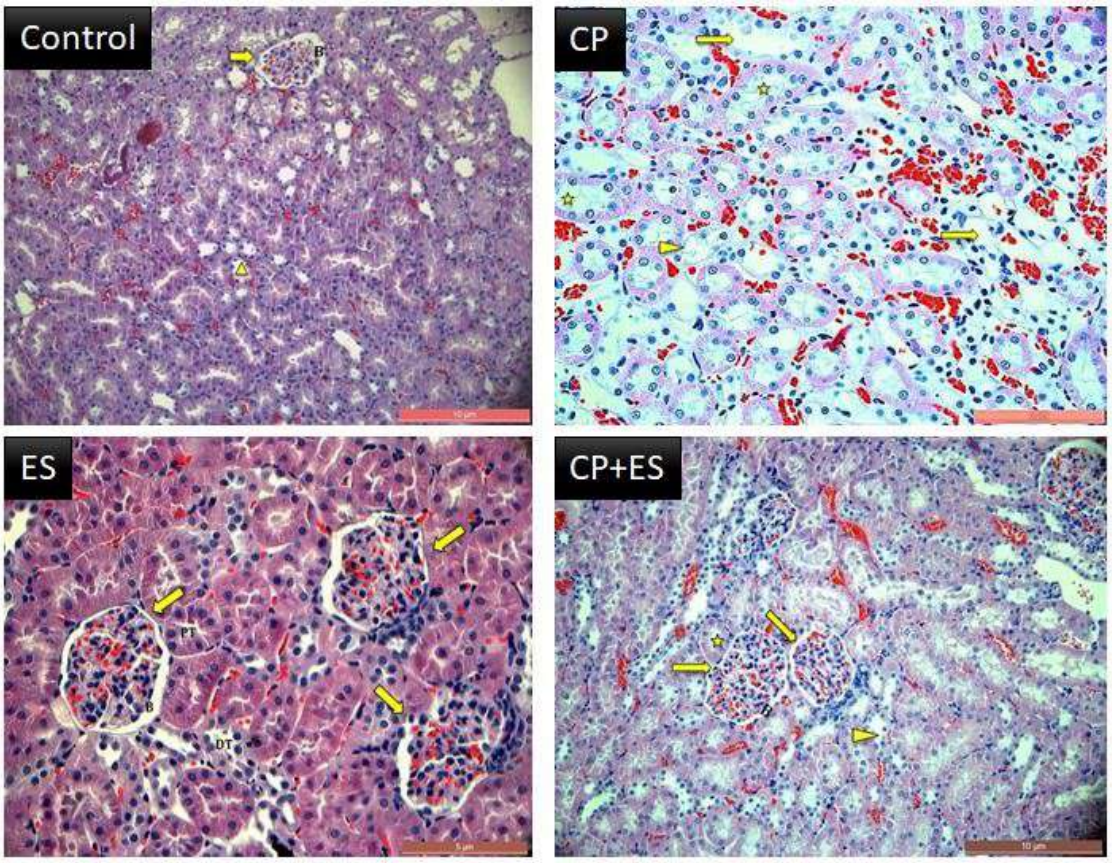

Figure 4.1 Kidney sections stained with Hematoxylin & Eosin. Noticeable Bowman distance capsule, intact glomerulus (arrow), and minimal congestion (arrowhead) (Control Group). Structural abnormality in the glomerulus, noticeable narrowing in the bowman distance, degenerated tubules (arrow), as well as remarkable congestion (star) and inflammatory cell infiltration (arrowhead) (CP Group), c: a small number of inflammatory cell infiltrations (arrow) (ES Group), d: narrowing in the Bowman capsule distance, damage in the glomerulus, degeneration in the tubules (arrow), as well as a significant reduction in congestion (star) and inflammation (arrowhead) (CP+ES Group) when compared to CP Group (X20, X40).

Table 4.1 Histological scoring of the study groups in terms of narrowing in Bowman, tubular damage, inflammation, glomerular damage, and congestion.

Groups	Narrowing in Bowman	Tubular damage	Inflammation	Glomerular damage	Congestion	Total Score
Control	0	0	0	0	1	1
CP	3	3	1	2	3	12***
ES	0	0	1	0	0	1
CP+ES	1	1	1	1	1	5*

*p < 0.01differentcompared to control; ***p < 0.001 significantly different compared to control

4.2. ES Attenuates CP-Induced Apoptosis In The Kidney Of Rats

Apoptotic marker results are presented in Figures 4.2-4.4 and Table 4.2. In both Control and ES Groups, just a few of Bax and Caspase-3 positive cells were spotted in the kidney incisions of the animals. The quantity of Bcl-2 positive cells observed achieved statistical significance. As for the CP Group, stained Caspase-3 and Bax positive cells were remarkably present but that of Bcl-2 positive cells were in very low numbers. In CP+ES Group, ES significantly diminished CP-induced apoptosis. In other words, Bax and Caspase-3 positive cells were observed to have been reduced while Bcl-2 positive stained cells had in turn risen in CP+ES Group (Table 4.2 and Figs. 4.2-4.4).

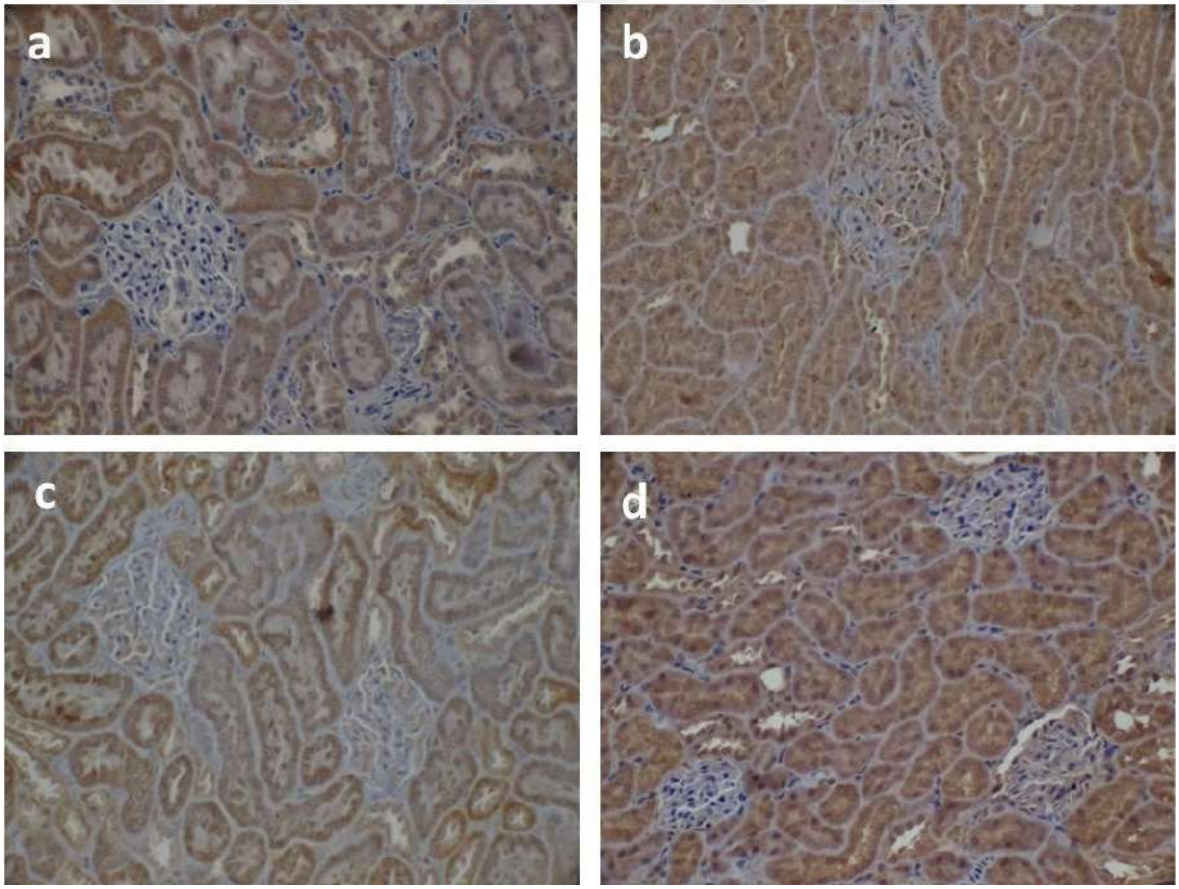

Figure 4.2 Kidney sections stained for Bax expression sections. a: sporadic weak staining in the tubules of Control Group b: noticeable intense staining in the tubules and glomerulus of CP Group c: obscure staining in the glomerulus of ES Group d: weak staining in the tubules of CP+ES Group and focal staining in the local areas (X20).

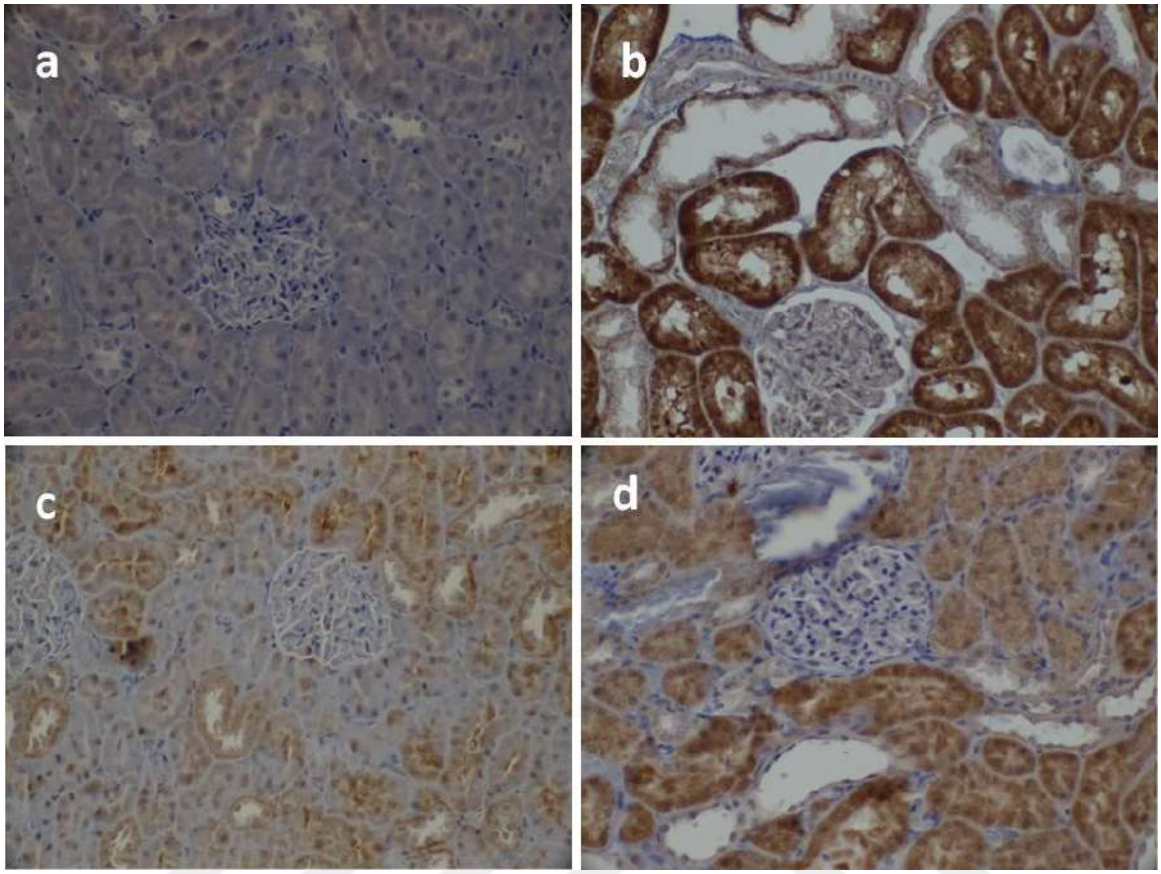

Figure 4.3 Kidney sections of Bcl-2 expression. a: moderate positive immunochemical staining in Control Group b: weak staining in all the tubules but moderate staining in the glomerulus of CP Group c: moderate staining the tubules but obscure staining in the glomerulus of ES Group d: moderate staining in the wide areas of the tubules but obscure staining in the glomerulus of CP+ES Group (X20).

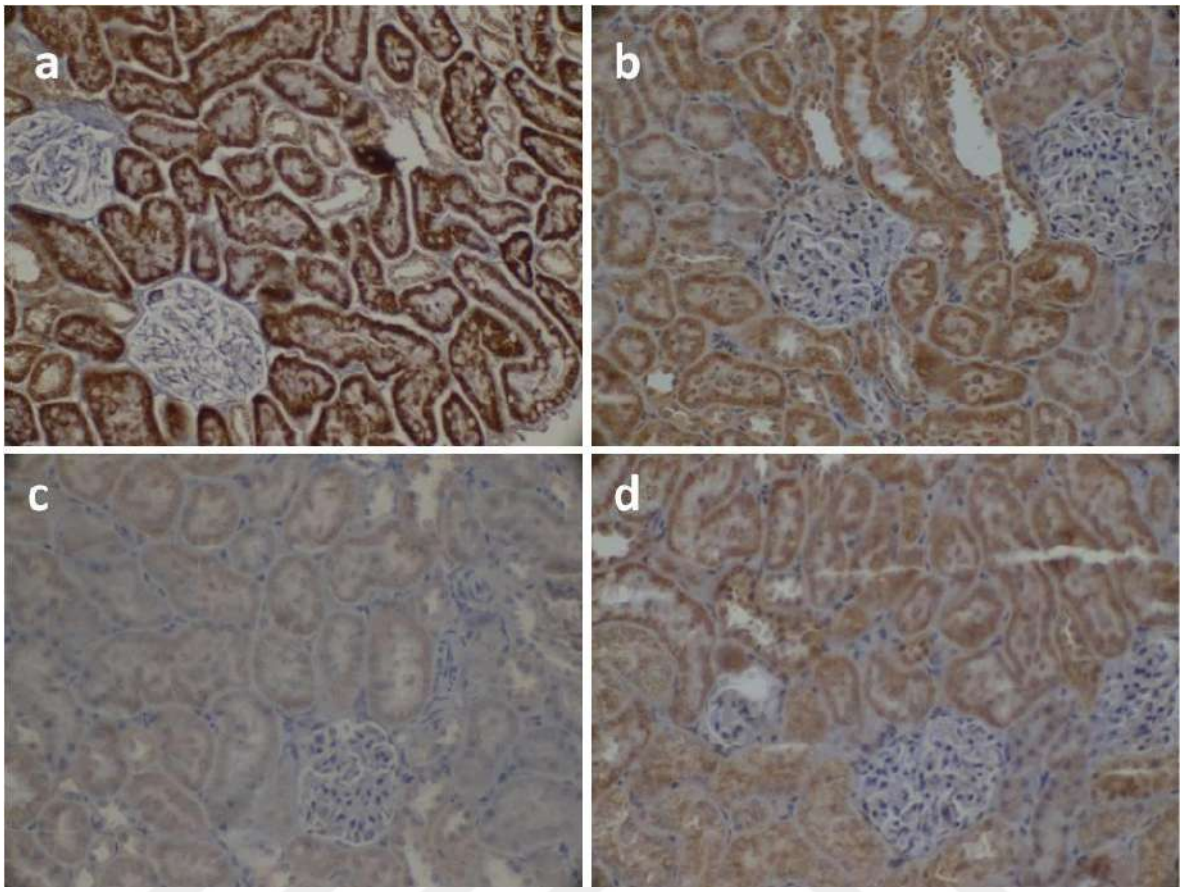

Figure 4.4 Caspase-3 expression sections. a: sporadic weak staining in the tubules of Control Group b: noticeable intense staining in the tubules and glomerulus of CP Group c: obscure staining in the glomerulus of ES Group d: weak staining in the tubules of CP+ES Group and focal staining in the local areas (X20).

Table 4.2 Immunohistochemical findings and their scores in the kidney tissue.

Groups	Caspase-3	Bcl-2	Bax
Control	+	++	+
CP	++++	+	++++
ES	+	++	+
CP+ES	+	++	+

None (-), weak (+), mild (++), moderate (+++) and severe (++++)

4.3. ES Safeguards Kidney Function

BUN and Cre are indicators of a dysfunctional kidney. As seen in Figures 4.5 and 4.6, serum BUN and Cre levels considerably increased in CP Group compared to ES and Control Groups ($p < 0.001$), whereas these levels considerably decreased in CP+ ES Group when compared to CP Alone Group ($p < 0.05$). Assuming from these results, we concluded that ES had improved the renal dysfunction attributable to CP.

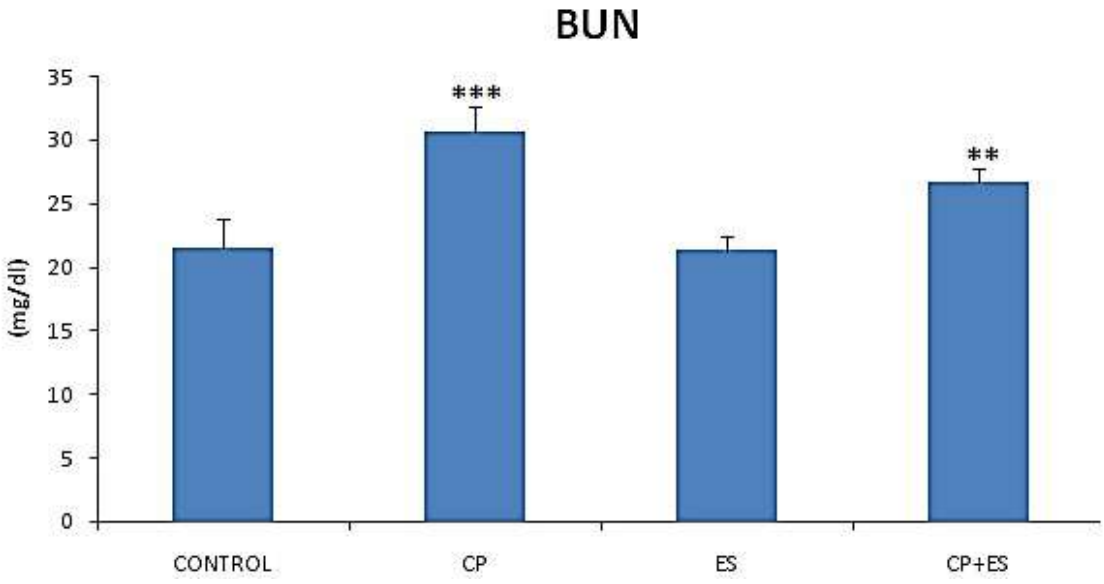

Figure 4.5 Effects of CP and ES on the activities of BUN in the kidney. All the values are expressed as mean ± SD (n = 6). ***p < 0.001 compared to Control Group, **p<0.05 compared to Control Group.

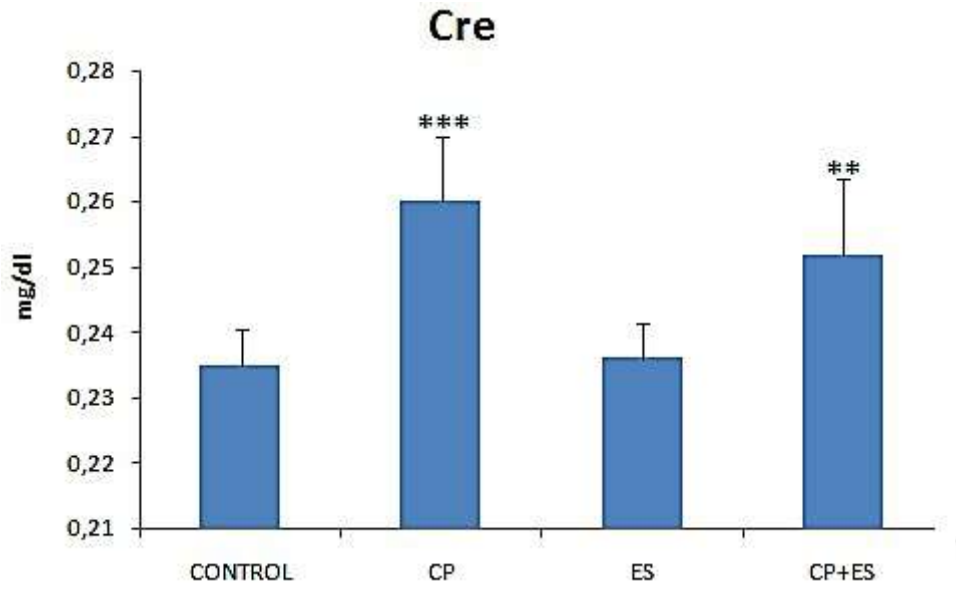

Figure 4.6 Effects of CP and ES on the activities of Cre in the kidney. All the values are expressed as mean ± SD (n = 6). ***p < 0.001 compared to Control Group, **p<0.05 compared to Control Group.

4.4. ES Is Protective Against Oxidative Stress

Oxidative stress analysis results have been shown in Table 4.3. Even though Control and ES Groups had high NT and TT levels, these had decreased remarkably CP Group ($p < 0.05$). No difference of statistical significance could be found in CP+ES Group in terms of SDS/NT levels compared to Control and ES Groups. Based upon these results, ES appears to have reduced oxidative stress induced by CP.

Table 4.3 Serum Thiol Disulfide, Total Thiol and Natural Thiol values and ratios.

Variables	Control	CP	ES	CP+ES
NT (mmol/L)	267,944 ±41.8	205.28±17.73***	269.31±46.33	239.53±31.78*
TT (mmol/L)	380,92±24.9	321.23±23.68***	381.896±73.31	346.71±57.61*
SDS (mmol/L)	50.27±13.5	64.72±8.3**	50.59±8.3	57.51±8.73*
SDS/TT ratio	0.11030.015	0.1932±0.032**	0.1156±0.021	0.1567±0.033*
SDS/NT ratio	0.1736±0.009	0.3025±0.059***	0.1789±0.040	0.2312±0.045*

Native thiol (NT), total thiol (TT), serum disulfide (SDS)

*$p < 0.01$ different compared to Control Group; **$p < 0.05$ significantly different compared to Control Group, ***$p < 0.001$ significantly different compared to Control Group.

4.5. Discussion

The kidney is known as the target of various xenobiotic toxicants, many drugs included. Numerous factors cause sensitivity of the kidney, which is associated with many metabolizing enzymes and xenobiotic transporters, and large blood flow. Furthermore, the physiological, anatomical and biochemical features of the kidney make it very vulnerable to different toxins and drugs.

In line with current events, it is also important to note that severe acute respiratory syndrome coronavirus 2 (SARS- CoV-2) infection raises the prevalence of AKI and tends to lead to a worse prognosis, with a higher number of patients requiring dialysis and lower rates of recover (Fu et al., 2020; Nadim et al., 2020; Moledina et al., 2021). A greater usage of Kidney Replacement Therapy has also been identified in patients with Coronavirus disease (COVID-19)–associated AKI (Silver et al., 2021) generating concerns for the capacity of healthcare systems to provide this service (Goldfarb et al., 2020). Nadim et al. (2020) and Grand et al. (2021) identify possible mechanisms to explain the relationship between AKI and COVID-19. This new surge of patients with AKI just reinforces the need for effective treatments to Acute Kidney Injury.

The present study was conducted to evaluate the possible role of ES in improving the *in vivo* acute kidney injury (AKI) generated by CP, which has two active metabolites,

namely phosphoramide and acrolein. Whereas phosphoramide has antineoplastic activity, acrolein may give rise to nephrotoxicity, lipid peroxidation, and structural change in the tissue apart from altering the antioxidant activity (Abraham and Isaac, 2011; Singh et al., 2014).

Studies have revealed that CP induces oxidative stress and apoptosis after CP administration in various tissues of mice and rats (Mansour et al., 2017; Cengiz, 2018; Cengiz et al., 2019). The status of dynamic thiol/disulfide homeostasis exerts a great impact upon antioxidants, apoptosis, detoxification, and enzymatic activity regulation (Erel and Neselioğlu, 2014). Measuring oxidative stress via thiol/disulfide parameters thanks to a new method developed by Erel et al. has recently gained a lot of attention in the scientific world, given that it reflects accurately the oxidation and TT capacity in the organism (Erel and Neselioğlu, 2014). A previous study of ours reported that 200 mg/kg of CP caused a reduction in serum NT and TT levels while it increased SDS levels (Ayhanci et al., 2019). It should be noted that there are no scientific papers published in the literature on CP-induced thiol/disulfide parameters. In the present study, NT and TT levels of CP Group decreased while SDS levels increased.

In tandem with oxidative stress, CP administration interferes with the balance of apoptosis-associated proteins like Bcl-2, Bax, and caspase-3. Incidentally, apoptosis is strictly controlled by the Bcl-2 protein family (Almeida et al., 2000). Up-regulation of the anti-apoptotic protein Bcl-2 can diminish the amount of damage to the kidney while an increased expression of Bax triggers cellular apoptosis. Added to that, Bcl-2 blocks the mitochondrial Cytochrome C from getting released and thus inhibits apoptosis. Excessive production of reactive oxygen species can also initiate cell death by way of inhibiting Bcl-2 and triggering the pathway of both Bax and mitochondrial apoptosis (Herrera et al., 2001). According to our immunohistochemical data, in the group given CP, Bax, and caspase 3 expressions rose while those of Bcl-2 went down (Figures 4.2-4.4 and Table 4.2). These results seem to be consistent with those of the studies by Liu et al. (2016), Casteels et al. (1998), and AlHaithloul et al. (2019).

The noticeable increase in serum BUN and Cre levels is a marker for kidney damage (Cengiz, 2018b). CP-induced rise in serum BUN and Cre levels observed in the present study are consistent with those published by Rehman et al. (2012), Ayhanci et al. (2010), and Cengiz (2018a). Our histopathological studies also provided reassuring confirmation for the biochemical parameters illustrated by the photomicrographs. Kidney tissues (Fig. 4.1) of CP-given rats show structural abnormality in the glomerulus, noticeable narrowing in the bowman distance, degenerated tubules, as well as remarkable congestion and inflammatory cell infiltration, tubular necrosis, which is consistent with those published by Rehman et al. (2012), Ayhanci et al. (2010), AlHaithloul et al. (2019), Cengiz (2018a), and Sinanoğlu et al. (2012).

ES, which is a chief extracted component of *Aesculus hippocastanum*, supposedly exhibits anti-inflammatory, anti-edematous, and antioxidant properties (Büyükokuroğlu, Taysi, and Özabacıgil, 2007; Daban et al., 2016; Elmas, Erbas, and Yiğittürk, 2016). ES has been reported to possess protective effects upon experimentally-induced liver and lung damage as well as diabetes-related kidney damage (Jiang et al., 2011; Daban et al., 2016; Elmas, Erbas, and Yiğittürk, 2016). Nevertheless, the effects of ES against CP-induced kidney damage have not been investigated in the literature up to the present time. To this end, our study is the first of its kind in that it presents the impacts of ES upon CP-induced AKI.

On one hand, it has been shown *in vitro* that when CP and ES are co-administered, there is a decrease in ES-induced Caspase-3 and Bax expression, thus inhibiting apoptosis (Iqubal et al., 2019). On the other hand, while there was a decrease in the level of Bcl-2 protein expression in the group administered solely with CP, an increase in the level of Bcl-2 protein expression occurred due to ES when it was applied together with CP. It was evaluated that this increase was compatible with the change in Caspase-3 and Bax protein levels.

In conclusion, our biochemical, immunohistochemical, and histopathological results show that ES significantly reduces CP-induced oxidative stress, apoptosis, and tissue damage (Figs. 4.1-4.6 and Tables 4.1-4.3). Based upon our study results, we are of

the opinion that the protective effects of ES upon CP-induced AKI can be accounted for by its antioxidant properties. It is tempting to speculate that ES shows this protective effect by providing cellular membrane integrity and inhibiting ROS-induced apoptotic pathways (Figure 4.7). Therefore, further studies are needed that target molecular pathways involved in the modulatory action of ES upon CP-induced AKI.

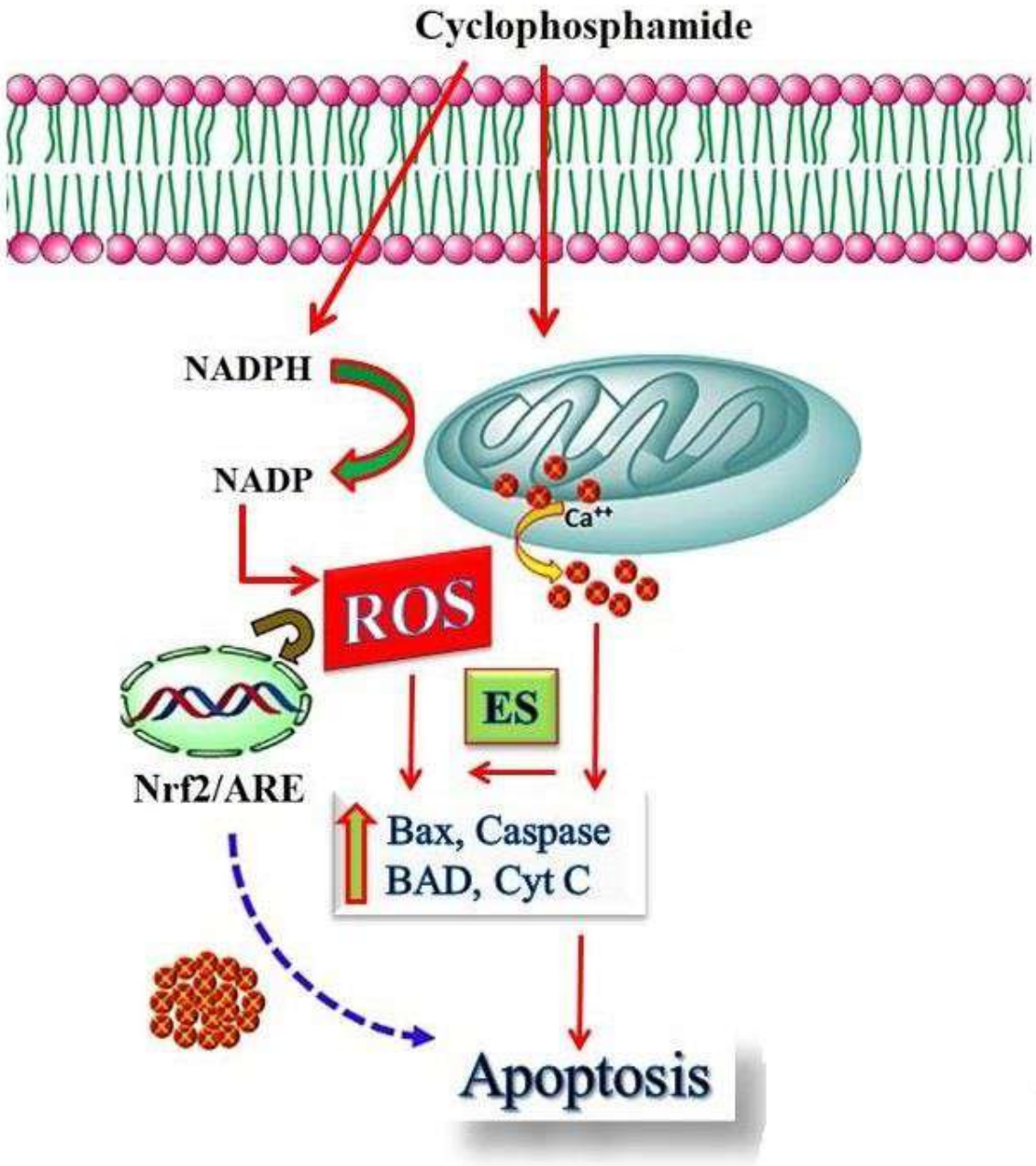

Figure 4.7 The possible protective mechanism of ES upon CP-induced cellular damage (Iqubal et al., 2019).

5. CONCLUSIONS AND RECOMMENDATIONS

Not only does the present study call attention to the therapeutic potential of ES on CP-induced AKI but it also emphasizes the impact of ES upon the relationship between oxidative stress and kidney dysfunction as well. Besides, considering our experimental results, it is understood that CP leads to apoptosis by increasing Caspase-3 and Bax expressions. Finally, more work is needed to elucidate the mechanisms behind ES renal protection against CP toxicity.

REFERENCES

Abraham, P., Rabi, S., 2009, Nitrosative stress, protein tyrosine nitration, PARP activation and NAD depletion in the kidneys of rats after single dose of cyclophosphamide. *Clinical and experimental nephrology*, *13*(4), 281-287.

Abraham, P., Isaac, B., 2011, The effects of oral glutamine on cyclophosphamide-induced nephrotoxicity in rats. *Human & experimental toxicology*, *30*(7), 616-623.

Abraham, P., Rabi, S., 2011, Protective effect of aminoguanidine against cyclophosphamide-induced oxidative stress and renal damage in rats. *Redox report*, *16*(1), 8-14.

Aksu, U., Demirci, C., İnce, C., 2011, The pathogenesis of acute kidney injury and the toxic triangle of oxygen, reactive oxygen species and nitric oxide. *Controversies in Acute Kidney Injury*, *174*, 119-128.

Aldini, G., Orioli, M., Carini, M., 2011, Protein modification by acrolein: relevance to pathological conditions and inhibition by aldehyde sequestering agents. *Molecular nutrition & food research*, *55*(9), 1301-1319.

ALHaithloul, H.A., Alotaibi, M.F., Bin-Jumah, M., Elgebaly, H., Mahmoud, A.M., 2019, Olea europaea leaf extract up-regulates Nrf2/ARE/HO-1 signaling and attenuates cyclophosphamide-induced oxidative stress, inflammation and apoptosis in rat kidney. *Biomedicine & Pharmacotherapy*, *111*, 676-685.

Almeida, O.F.X., Conde, G.L., Crochemore, C., Demeneix, B.A., Fischer, D., Hassan, A. H.S., ... Michaelidis, T.M., 2000, Subtle shifts in the ratio between pro-and antiapoptotic molecules after activation of corticosteroid receptors decide neuronal fate. *The FASEB Journal*, *14*(5), 779-790.

REFERENCES (continuation)

Arnould, T., Janssens, D., Michiels, C., Remacle, J., 1996, Effect of aescine on hypoxia-induced activation of human endothelial cells. *European journal of pharmacology*, *315*(2), 227-233.

Ayhanci, A., Güneş, S., Şahintürk, V., Appak, S., Uyar, R., Cengiz, M., ... Yaman, S., 2010, Seleno L-methionine acts on cyclophosphamide-induced kidney toxicity. *Biological trace element research*, *136*(2), 171-179.

Ayhanci, A., Tanriverdi, D.T., Şahintürk, V., Cengiz, M., Appak-Baskoy, S., Şahin, I.K., 2020, Protective effects of boron on cyclophosphamide-induced bladder damage and oxidative stress in rats. *Biological Trace Element Research*, *197*(1), 184-191.

Bakir, C., 2020. The Turkish state's responses to existential COVID-19 crisis. *Policy and Society*, 39(3), 424-441.

Banker, D.E., Groudine, M., Norwood, T., Appelbaum, F.R., 1997, Measurement of spontaneous and therapeutic agent-induced apoptosis with BCL-2 protein expression in acute myeloid leukemia. *Blood, The Journal of the American Society of Hematology*, *89*(1), 243-255.

Basile, D.P., Bonventre, J.V., Mehta, R., Nangaku, M., Unwin, R., Rosner, M.H., ... Ronco, C., 2016, Progression after AKI: understanding maladaptive repair processes to predict and identify therapeutic treatments. *Journal of the American Society of Nephrology*, *27*(3), 687-697.

Bellomo, R., Ronco, C., Kellum, J.A., Mehta, R.L., Palevsky, P., 2004, Acute renal failure–definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Critical care*, *8*(4), 1-9.

REFERENCES (continuation)

Bellomo, R., Kellum, J.A., Ronco, C., 2012, Acute kidney injury. *The Lancet*, *380*(9843), 756-766.

Benedikter, B.J., Volgers, C., van Eijck, P.H., Wouters, E.F., Savelkoul, P.H., Reynaert, N. L., ... Stassen, F.R., 2017, Cigarette smoke extract induced exosome release is mediated by depletion of exofacial thiols and can be inhibited by thiol-antioxidants. *Free Radical Biology and Medicine*, *108*, 334-344.

Benzie, I.F., Strain, J.J., 1999, [2] Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. In *Methods in enzymology* (Vol. 299, pp. 15-27). Academic press.

Bhat, N., Kalthur, S.G., Padmashali, S., Monappa, V., 2018, Toxic effects of different doses of cyclophosphamide on liver and kidney tissue in Swiss albino mice: a histopathological study. *Ethiopian journal of health sciences*, *28*(6).

Bruce, W.R., Meeker, B.E., Valeriote, F.A., 1966, Comparison of the sensitivity of normal hematopoietic and transplanted lymphoma colony-forming cells to chemotherapeutic agents administered in vivo. *Journal of the National Cancer Institute*, *37*(2), 233-245.

Büyükokuroğlu, M., Taysi, F., Özabacıgil, F., 2007, Dantrolene: in doxorubicin toxicity. *Asian Journal of Chemistry*, *19*(5).

Caleja, C., Barros, L., Antonio, A.L., Oliveira, M.B.P., Ferreira, I.C., 2017, A comparative study between natural and synthetic antioxidants: Evaluation of their performance after incorporation into biscuits. *Food chemistry*, *216*, 342-346.

REFERENCES (continuation)

Ćalić-Dragosavac, D., Zdravković-Korać, S., Šavikin-Fodulović, K., Radojević, L., Vinterhalter, B., 2010, Determination of escin content in androgenic embryos and hairy root culture of Aesculus hippocastanum. Pharmaceutical biology, 48(5), 563-567.

Carocho, M., Morales, P., Ferreira, I.C., 2015, Natural food additives: Quo vadis?. *Trends in Food Science & Technology*, *45*(2), 284-295.

Casteels, K., Waer, M., Bouillon, R., Depovere, J., Valckx, D., Laureys, J., Mathieu, C., 1998, 1,25-Dihydroxyvitamin D3 restores sensitivity to cyclophosphamide-induced apoptosis in non-obese diabetic (NOD) mice and protects against diabetes. *Clinical & Experimental Immunology*, *112*(2), 181-187.

Cengiz, M., Ayhanci, A., Kutlu, H.M., & Musmul, A., 2016, Potential therapeutic effects of silymarin and silymarin-loaded solid lipidnanoparticles on experimental kidney damage in BALB/c mice: biochemical and histopathological evaluation. *Turkish Journal of Biology*, *40*(4), 807-814.

Cengiz, M., 2018a, Boric acid protects against cyclophosphamide-induced oxidative stress and renal damage in rats. *Cellular and Molecular Biology*, *64*(12), 11-14.

Cengiz, M., 2018b, Renoprotective effects of Silybum marianum (L.) Gaertn (Silymarin) on thioacetamide-induced renal injury: Biochemical and histopathological approach. *Pakistan journal of pharmaceutical sciences*.

Cengiz, M., Yıldız, S.C., Demir, C., Şahin, İ.K., Teksöy, Ö., Ayhanci, A., 2019, Hepato-preventive and anti-apoptotic role of boric acid against liver injury induced by cyclophosphamide. *Journal of Trace Elements in Medicine and Biology*, *53*, 1-7.

REFERENCES (continuation)

Cengiz, M., Ayhanci, A., Kutlu, H.M., 2020, Investigation into the protective effects of escin on blood cells and cyclophosphamide-induced bone marrow toxicity in rats. *Bilecik Şeyh Edebali Üniversitesi Fen Bilimleri Dergisi*, *7*(2), 730-738.

Cengiz, M., Kutlu, H. M., Peker, Cengiz, B., Ayhanci, A., 2020, Escin attenuates oxidative damage, apoptosis and lipid peroxidation in a model of cyclophosphamide-induced liver damage. *Drug and Chemical Toxicology*, 1-8.

Chang, T.K., Weber, G.F., Crespi, C.L., Waxman, D.J., 1993, Differential activation of cyclophosphamide and ifosphamide by cytochromes P-450 2B and 3A in human liver microsomes. *Cancer research*, *53*(23), 5629-5637.

Chen, T.L., Kennedy, M.J., Anderson, L.W., Kiraly, S.B., Black, K.C., Colvin, O.M., Grochow, L.B., 1997, Nonlinear pharmacokinetics of cyclophosphamide and 4-hydroxycyclophosphamide/aldophosphamide in patients with metastatic breast cancer receiving high-dose chemotherapy followed by autologous bone marrow transplantation. *Drug metabolism and disposition*, *25*(5), 544-551.

Cheong, D.H., Arfuso, F., Sethi, G., Wang, L., Hui, K.M., Kumar, A.P., Tran, T., 2018, Molecular targets and anti-cancer potential of escin. *Cancer letters*, *422*, 1-8.

Cleto-Yamane, T.L., Gomes, C.L.R., Suassuna, J.H.R., Nogueira, P.K., 2018, Epidemiologia da lesão renal aguda em pediatria. *Brazilian Journal of Nephrology*, *41*, 275-283.

Collister, D., Pannu, N., Ye, F., James, M., Hemmelgarn, B., Chui, B., ... Klarenbach, S., 2017, Health care costs associated with AKI. *Clinical journal of the American Society of Nephrology*, *12*(11), 1733-1743.

REFERENCES (continuation)

Colvin, M., Brundrett, R.B., Kan, M.N.N., Jardine, I., Fenselau, C., 1976, Alkylating properties of phosphoramide mustard. *Cancer research*, *36*(3), 1121-1126.

Çiftçi, G.A., Işcan, A., Kutlu, M., 2015, Escin reduces cell proliferation and induces apoptosis on glioma and lung adenocarcinoma cell lines. *Cytotechnology*, *67*(5), 893-904.

Daban, U., Uğur, M., Oğuzhan, O., Yaldız, M., Akküçük, S., Özbilen, O., Oruç, C., Kılıç, E., and Aydoğan, A., 2016, Protective effects of escin on hepatic ischemia/reperfusion injury in rats. *Acta Medica*, *32*, 973.

Darenskaya, M.A., Kolesnikova, L.I., Kolesnikov, S.I., 2021, Oxidative stress: Pathogenetic role in diabetes mellitus and its complications and therapeutic approaches to correction. *Bulletin of Experimental Biology and Medicine*, *171*(2), 179-189.

Dasta, J.F., Kane-Gill, S., 2019, Review of the literature on the costs associated with acute kidney injury. *Journal of Pharmacy Practice*, 32(3), 292-302.

Dennis, J.M., Witting, P.K., 2017, Protective role for antioxidants in acute kidney disease. *Nutrients*, *9*(7), 718.

Dhodi, D.K., Bhagat, S.B., Pathak, D., Patel, S.B., 2014, Drug-induced nephrotoxicity. *International Journal of Basic & Clinical Pharmacology* no. 3 (2014): 591-597.

Dong, Q., Barsky, D., Colvin, M.E., Melius, C.F., Ludeman, S.M., Moravek, J.F., ... Friedman, H.S., 1995, A structural basis for a phosphoramide mustard-induced DNA interstrand cross-link at 5'-d (GAC). *Proceedings of the National Academy of Sciences*, *92*(26), 12170-12174.

REFERENCES (continuation)

Dontha, S., 2016, A review on antioxidant methods. *Asian J. Pharm. Clin. Res*, *9*(2), 14-32.

Duchovic, R.J., Vilensky, J.A., 2007, Mustard gas: its pre-World War I history. *Journal of chemical education*, *84*(6), 944.

Duester, G., 2001, Genetic dissection of retinoid dehydrogenases. *Chemico-biological interactions*, *130*, 469-480.

Elmas, O., Erbas, O., Yiğittürk, G., 2016, The efficacy of Aesculus hippocastanum seeds on diabetic nephropathy in a streptozotocin-induced diabetic rat model. *Biomedicine & Pharmacotherapy*, *83*, 392-396.

Emadi, A., Jones, R.J., Brodsky, R.A., 2009, Cyclophosphamide and cancer: golden anniversary. *Nature reviews Clinical oncology*, *6*(11), 638-647.

Erel, O., 2005, A new automated colorimetric method for measuring total oxidant status. *Clinical biochemistry*, *38*(12), 1103-1111.

Erel, O., Neselioğlu, S., 2014, A novel and automated assay for thiol/disulphide homeostasis. *Clinical biochemistry*, *47*(18), 326-332.

Faubel, S., Edelstein, C.L., 2016, Mechanisms and mediators of lung injury after acute kidney injury. *Nature Reviews Nephrology*, *12*(1), 48-60.

Flieger, J., Flieger, W., Baj, J., Maciejewski, R., 2021, Antioxidants: Classification, natural sources, activity/capacity measurements, and usefulness for the synthesis of nanoparticles. *Materials*, *14*(15), 4135.

REFERENCES (continuation)

Frick, R.W., Frick, R.W., 2000, Three treatments for chronic venous insufficiency: escin, hydroxyethylrutoside, and Daflon. *Angiology*, *51*(3), 197-205.

Fu, E.L., Janse, RJ., de Jong, Y., van der Endt, V.H., Milders, J., van der Willik, E.M., ... van Diepen, M., 2020, Acute kidney injury and kidney replacement therapy in COVID-19: a systematic review and meta-analysis. *Clinical kidney journal*, *13*(4), 550-563.

Gallelli, L., Cione, E., Wang, T., Zhang, L., 2021, Glucocorticoid-like activity of escin: a new mechanism for an old drug. *Drug Design, Development and Therapy*, *15*, 699.

Giraud, B., Hebert, G., Deroussent, A., Veal, G.J., Vassal, G., Paci, A., 2010, Oxazaphosphorines: new therapeutic strategies for an old class of drugs. *Expert opinion on drug metabolism & toxicology*, *6*(8), 919-938.

Goldfarb, D.S., Benstein, J.A., Zhdanova, O., Hammer, E., Block, C.A., Caplin, N. J., ... Charytan, D.M., 2020, Impending shortages of kidney replacement therapy for COVID-19 patients. *Clinical Journal of the American Society of Nephrology*, *15*(6), 880-882.

Gomez, H., Ince, C., De Backer, D., Pickkers, P., Payen, D., Hotchkiss, J., Kellum, J.A., 2014, A unified theory of sepsis-induced acute kidney injury: inflammation, microcirculatory dysfunction, bioenergetics and the tubular cell adaptation to injury. *Shock (Augusta, Ga.)*, *41*(1), 3.

Grafström, R.C., Dypbukt, J.M., Willey, J.C., Sundqvist, K., Edman, C., Atzori, L., Harris, C.C., 1988, Pathobiological effects of acrolein in cultured human bronchial epithelial cells. *Cancer research*, *48*(7), 1717-1721.

REFERENCES (continuation)

Guillaume, M., Padioleau, F., 1994, Veinotonic effect, vascular protection, antiinflammatory and free radical scavenging properties of horse chestnut extract. *Arzneimittel-forschung*, *44*(1), 25-35.

Günther, M., Wagner, E., Ogris, M., 2008, Acrolein: unwanted side product or contribution to antiangiogenic properties of metronomic cyclophosphamide therapy?. *Journal of Cellular and Molecular Medicine*, *12*(6b), 2704-2716.

Gür, F., Cengiz, M., Kutlu, H. M., Cengiz, B. P., Ayhanci, A., 2021, Molecular docking analyses of Escin as regards cyclophosphamide-induced cardiotoxicity: In vivo and in Silico studies. *Toxicology and Applied Pharmacology*, *411*, 115386.

Hajian, S., Rafieian-Kopaei, M., Nasri, H., 2014, Renoprotective effects of antioxidants against cisplatin nephrotoxicity. *Journal of nephropharmacology*, *3*(2), 39.

Halliwell, B., 1995, How to characterize an antioxidant: an update. In *Biochemical Society Symposium* (Vol. 61, pp. 73-101).

Hardin, J.W., 1960, Studies in the Hippocastanaceae, V. Species of the old world. *Brittonia*, 26-38.

Herrera, B., Fernández, M., Alvarez, A. M., Roncero, C., Benito, M., Gil, J., Fabregat, I., 2001, Activation of caspases occurs downstream from radical oxygen species production, Bcl-xL down-regulation, and early cytochrome C release in apoptosis induced by transforming growth factor β in rat fetal hepatocytes. *Hepatology*, *34*(3), 548-556.

REFERENCES (continuation)

Himmelfarb, J., McMonagle, E., Freedman, S., Klenzak, J., McMenamin, E., Le, P., ... Picard Group, 2004, Oxidative stress is increased in critically ill patients with acute renal failure. Journal of the American Society of Nephrology, 15(9), 2449-2456.

Hoste, E.A., De Corte, W., 2011, Clinical consequences of acute kidney injury. *Controversies in Acute Kidney Injury*, 174, 56-64.

Hoste, E.A., Bagshaw, S.M., Bellomo, R., Cely, C.M., Colman, R., Cruz, D.N., ... Kellum, J.A., 2015, Epidemiology of acute kidney injury in critically ill patients: the multinational AKI-EPI study. *Intensive care medicine*, *41*(8), 1411-1423.

Hsu, R.K., McCulloch, C.E., Dudley, R.A., Lo, L.J., Hsu, C.Y., 2013, Temporal changes in incidence of dialysis-requiring AKI. *Journal of the American Society of Nephrology*, *24*(1), 37-42.

Hu, X.M., Zeng, F.D., 2004, Protective effects of sodium beta-aescin on ischemia-reperfusion injury in rat brain. *Yao xue xue bao= Acta Pharmaceutica Sinica*, *39*(6), 419-423.

Huang, D., Ou, B., Prior, R.L., 2005, The chemistry behind antioxidant capacity assays. *Journal of agricultural and food chemistry*, *53*(6), 1841-1856.

Idris, S., Mishra, A., Khushtar, M., 2020, Phytochemical, ethanomedicinal and pharmacological applications of escin from Aesculus hippocastanum L. towards future medicine. *Journal of Basic and Clinical Physiology and Pharmacology*, *31*(5).

REFERENCES (continuation)

Iqubal, A., Iqubal, M.K., Sharma, S., Ansari, M.A., Najmi, A.K., Ali, S.M., ... Haque, S.E., 2019, Molecular mechanism involved in cyclophosphamide-induced cardiotoxicity: Old drug with a new vision. *Life Sciences*, *218*, 112-131.

IUPAC, 1997, Compendium of Chemical Terminology, 2nd ed. (the "Gold Book"). Compiled by A. D. McNaught and A. Wilkinson. Blackwell Scientific Publications, Oxford. Online version (2019-) created by S. J. Chalk. ISBN 0-9678550-9-8. https://doi.org/10.1351/goldbook.

Jackson, B., Brocker, C., Thompson, D.C., Black, W., Vasiliou, K., Nebert, D.W., Vasiliou, V., 2011, Update on the aldehyde dehydrogenase gene (ALDH) superfamily. *Human genomics*, *5*(4), 1-21.

Jiang, N., Xin, W., Wang, T., Zhang, L., Fan, H., Du, Y., ... Fu, F., 2011, Protective effect of aescin from the seeds of Aesculus hippocastanum on liver injury induced by endotoxin in mice. *Phytomedicine*, *18*(14), 1276-1284.

Jones, R.J., Barber, J.P., Vala, M.S., Collector, M.I., Kaufmann, S.H., Ludeman, S.M., ... Hilton, J., 1995, Assessment of aldehyde dehydrogenase in viable cells.

Kaddourah, A., Basu, R.K., Bagshaw, S.M., Goldstein, S.L., 2017, Epidemiology of acute kidney injury in critically ill children and young adults. *N Engl J Med*, *376*, 11-20.

Kanbay, M., Medetalibeyoğlu, A., Kanbay, A., Cevik, E., Tanriover, C., Baygul, A., ... Tukek, T., 2022. Acute kidney injury in hospitalized COVID-19 patients. *International urology and nephrology*, *54*(5), 1097-1104.

Kanbur, N., Akgül, S., 2020. Quaranteenagers: A single country pandemic curfew targeting adolescents in Turkey. *Journal of Adolescent Health*, *67*(2), 296-297.

REFERENCES (continuation)

Kerr, M., Bedford, M., Matthews, B., O'Donoghue, D., 2014, The economic impact of acute kidney injury in England. *Nephrology Dialysis Transplantation*, *29*(7), 1362-1368.

Khan, T.S., Sundin, A., Juhlin, C., Wilander, E., Öberg, K., Eriksson, B., 2004, Vincristine, cisplatin, teniposide, and cyclophosphamide combination in the treatment of recurrent or metastatic adrenocortical cancer. *Medical Oncology*, *21*(2), 167-177.

Khorwal, G., Chauhan, R., Nagar, M., 2017, Effect of cyclophosphamide on liver in albino rats: A comparative dose dependent histomorphological study. *International Journal of Biomedical and Advance Research*, *8*(3), 102-107.

Komes, D., Belščak-Cvitanović, A., Horžić, D., Rusak, G., Likić, S., Berendika, M., 2011, Phenolic composition and antioxidant properties of some traditionally used medicinal plants affected by the extraction time and hydrolysis. *Phytochemical analysis*, *22*(2), 172-180.

Küçükkurt, I., Ince, S., Keleş, H., Akkol, E. K., Avcı, G., Yeşilada, E., Bacak, E., 2010, Beneficial effects of Aesculus hippocastanum L. seed extract on the body's own antioxidant defense system on subacute administration. *Journal of ethnopharmacology*, *129*(1), 18-22.

Lameire, N.H., Bagga, A., Cruz, D., De Maeseneer, J., Endre, Z., Kellum, J.A., ... Vanholder, R., 2013, Acute kidney injury: an increasing global concern. *The Lancet*, *382*(9887), 170-179.

Lebel, A., Teoh, C.W., Zappitelli, M., 2020, Long-term complications of acute kidney injury in children. *Current Opinion in Pediatrics*, *32*(3), 367-375.

REFERENCES (continuation)

Lee, H.C., Yu, H.P., Liao, C.C., Chou, A.H., Liu, F.C., 2019, Escin protects against acetaminophen-induced liver injury in mice via attenuating inflammatory response and inhibiting ERK signaling pathway. *American Journal of Translational Research*, *11*(8), 5170.

Legrand, M., Rossignol, P., 2020, Cardiovascular consequences of acute kidney injury. *New England Journal of Medicine*, 382(23), 2238-2247.

Legrand, M., Bell, S., Forni, L., Joannidis, M., Koyner, J.L., Liu, K., Cantaluppi, V., 2021, Pathophysiology of COVID-19-associated acute kidney injury. *Nature Reviews Nephrology*, *17*(11), 751-764.

Lemineur, T., Deby-Dupont, G., Preiser, J.C., 2006, Biomarkers of oxidative stress in critically ill patients: what should be measured, when and how?. *Current Opinion in Clinical Nutrition & Metabolic Care*, *9*(6), 704-710.

Li, X., Li, B., Jia, Y., 2015, The hepatoprotective effect of Haoqin Qingdan decoction against liver injury induced by a chemotherapeutic drug cyclophosphamide. *Evidence-Based Complementary and Alternative Medicine*, *2015*.

Liu, Q., Lin, X., Li, H., Yuan, J., Peng, Y., Dong, L., Dai, S., 2016, Paeoniflorin ameliorates renal function in cyclophosphamide-induced mice via AMPK suppressed inflammation and apoptosis. *Biomedicine & Pharmacotherapy*, *84*, 1899-1905.

Lushchak, V.I., 2014, Free radicals, reactive oxygen species, oxidative stress and its classification. *Chemico-biological interactions*, *224*, 164-175.

REFERENCES (continuation)

Mansour, D.F., Salama, A.A., Hegazy, R.R., Omara, E.A. and Nada, S.A., 2017, Whey protein isolate protects against cyclophosphamide-induced acute liver and kidney damage in rats. *Journal of applied pharmaceutical science*, *7*(6), 111-120.

Marchitti, S.A., Brocker, C., Stagos, D., Vasiliou, V., 2008, Non-P450 aldehyde oxidizing enzymes: the aldehyde dehydrogenase superfamily. *Expert opinion on drug metabolism & toxicology*, *4*(6), 697-720.

Matsuda, H., Li, Y., Murakami, T., Ninomiya, K., Yamahara, J., Yoshikawa, M., 1997, Effects of escins Ia, Ib, IIa, and IIb from horse chestnut, the seeds of Aesculus hippocastanum L., on acute inflammation in animals. *Biological and Pharmaceutical Bulletin*, *20*(10), 1092-1095.

Matsuda, H., Li, Y., Yoshikawa, M., 2000, Possible involvement of 5-HT and 5-HT2 receptors in acceleration of gastrointestinal transit by escin Ib in mice. *Life Sciences*, *66*(23), 2233-2238.

McDonald, G.B., Slattery, J.T., Bouvier, M.E., Ren, S., Batchelder, A.L., Kalhorn, T.F., ... Gooley, T., 2003, Cyclophosphamide metabolism, liver toxicity, and mortality following hematopoietic stem cell transplantation. *Blood, The Journal of the American Society of Hematology*, *101*(5), 2043-2048.

Moledina, D.G., Simonov, M., Yamamoto, Y., Alausa, J., Arora, T., Biswas, A., ... Wilson, F.P., 2021, The association of COVID-19 with acute kidney injury independent of severity of illness: A multicenter cohort study. *American Journal of Kidney Diseases*, *77*(4), 490-499.

REFERENCES (continuation)

Montopoli, M., Froldi, G., Comelli, M.C., Prosdocimi, M., Caparrotta, L., 2007, Aescin protection of human vascular endothelial cells exposed to cobalt chloride mimicked hypoxia and inflammatory stimuli. *Planta medica*, *73*(03), 285-288.

Motawi, T.M., Sadik, N.A., Refaat, A., 2010, Cytoprotective effects of DL-alpha-lipoic acid or squalene on cyclophosphamide-induced oxidative injury: An experimental study on rat myocardium, testicles and urinary bladder. *Food and Chemical Toxicology*, *48*(8-9), 2326-2336.

Mut-Salud, N., Álvarez, P.J., Garrido, J.M., Carrasco, E., Aránega, A., Rodríguez-Serrano, F., 2016, Antioxidant intake and antitumor therapy: toward nutritional recommendations for optimal results. *Oxidative Medicine and Cellular Longevity*, *2016*.

Nadim, M.K., Forni, L.G., Mehta, R.L., Connor, M.J., Liu, K.D., Ostermann, M., ... Kellum, J.A., 2020, COVID-19-associated acute kidney injury: consensus report of the 25th Acute Disease Quality Initiative (ADQI) Workgroup. *Nature reviews nephrology*, *16*(12), 747-764.

Nafees, S., Rashid, S., Ali, N., Hasan, S.K., Sultana, S., 2015, Rutin ameliorates cyclophosphamide induced oxidative stress and inflammation in Wistar rats: role of NFκB/MAPK pathway. *Chemico-biological interactions*, *231*, 98-107.

Naharci, M. I., Katipoğlu, B., Tasci, I., 2020. Coronavirus 2019 (COVID-19) outbreak and geropsychiatric care for older adults: a view from Turkey. *International Psychogeriatrics*, *32*(10), 1193-1197.

REFERENCES (continuation)

Neboh, E.E., Ufelle, S.A., 2015, Myeloprotective activity of crude methanolic leaf extract of Cassia occidentalis in cyclophosphamide-induced bone marrow suppression in Wistar rats. *Advanced biomedical research*, *4*.

Negi, S., Koreeda, D., Kobayashi, S., Yano, T., Tatsuta, K., Mima, T., ... Ohya, M., 2018, Acute kidney injury: Epidemiology, outcomes, complications, and therapeutic strategies. In *Seminars in dialysis* (Vol. 31, No. 5, pp. 519-527).

Neha, K., Haider, M.R., Pathak, A., Yar, M.S., 2019, Medicinal prospects of antioxidants: A review. *European journal of medicinal chemistry*, *178*, 687-704.

O'connor, P.M., Wassermann, K., Sarang, M., Magrath, I., Bohr, V.A., Kohn, K.W., 1991, Relationship between DNA cross-links, cell cycle, and apoptosis in Burkitt's lymphoma cell lines differing in sensitivity to nitrogen mustard. *Cancer Research*, *51*(24), 6550-6557.

Omi, K., Matsuo, Y., Ueda, G., Aoyama, Y., Kato, T., Hayashi, Y., ... Takiguchi, S., 2021, Escin inhibits angiogenesis by suppressing interleukin-8 and vascular endothelial growth factor production by blocking nuclear factor-κB activation in pancreatic cancer cell lines. *Oncology reports*, *45*(5), 1-9.

Ostermann, M., Cerdá, J., 2018, The burden of acute kidney injury and related financial issues. *Acute Kidney Injury-Basic Research and Clinical Practice*, *193*, 100-112.

Özatay, F., G., Sak, 2020. "What If Turkey Imposes a Curfew Due To COVID-19." *Economic Policy Research Foundation of Turkey (TEPAV), Policy Note N* 202008.

REFERENCES (continuation)

Palipoch, S., 2013, A review of oxidative stress in acute kidney injury: protective role of medicinal plants-derived antioxidants. *African Journal of Traditional, Complementary and Alternative Medicines*, *10*(4), 88-93.

Patlolla, J.M., Raju, J., Swamy, M.V., Rao, C.V., 2006, β-Escin inhibits colonic aberrant crypt foci formation in rats and regulates the cell cycle growth by inducing p21waf1/cip1 in colon cancer cells. *Molecular Cancer Therapeutics*, *5*(6), 1459-1466.

Patlolla, J.M., Qian, L., Biddick, L., Zhang, Y., Desai, D., Amin, S., ... Rao, C.V., 2013, β-Escin inhibits NNK-induced lung adenocarcinoma and ALDH1A1 and RhoA/Rock expression in A/J mice and growth of H460 human lung cancer cells. *Cancer Prevention Research*, *6*(10), 1140-1149.

Pinho, N.A.D., Oliveira, R.D.C.B.D., Pierin, A.M.G., 2015, Hipertensos com e sem doença renal: avaliação de fatores de risco. *Revista da Escola de Enfermagem da USP*, *49*, 101-108.

Poljsak, B., Šuput, D., Milisav, I., 2013, Achieving the balance between ROS and antioxidants: when to use the synthetic antioxidants. *Oxidative medicine and cellular longevity*, *2013*.

Povirk, L.F., Shuker, D.E., 1994, DNA damage and mutagenesis induced by nitrogen mustards. *Mutation research/reviews in genetic toxicology*, *318*(3), 205-226.

Qiao, P., Zhang, B., Liu, X., Xu, J., Li, X., 2022, Effects of Escin on Oxidative Stress and Apoptosis of H9c2 Cells Induced by H2O2. *Disease Markers*, *2022*.

REFERENCES (continuation)

Rabelink, T.J., van Zonneveld, A.J., 2006, Coupling eNOS uncoupling to the innate immune response. *Arteriosclerosis, thrombosis, and vascular biology*, *26*(12), 2585-2587.

Ratliff, B.B., Abdulmahdi, W., Pawar, R., Wolin, M.S., 2016, Oxidant mechanisms in renal injury and disease. *Antioxidants & redox signaling*, *25*(3), 119-146.

Rehman, M.U., Tahir, M., Ali, F., Qamar, W., Lateef, A., Khan, R., ... Sultana, S., 2012, Cyclophosphamide-induced nephrotoxicity, genotoxicity, and damage in kidney genomic DNA of Swiss albino mice: the protective effect of Ellagic acid. *Molecular and cellular biochemistry*, *365*(1), 119-127.

Riley, P.A., 1994, Free radicals in biology: oxidative stress and the effects of ionizing radiation. *International journal of radiation biology*, *65*(1), 27-33.

Rodriguez-Torres, M., Allan, A.L., 2016, Aldehyde dehydrogenase as a marker and functional mediator of metastasis in solid tumors. *Clinical & experimental metastasis*, *33*(1), 97-113.

Rochette, L., Zeller, M., Cottin, Y., Vergely, C., 2014, Diabetes, oxidative stress and therapeutic strategies. *Biochimica et Biophysica Acta (BBA)-General Subjects*, *1840*(9), 2709-2729.

Ruiz, S., Pergola, P.E., Zager, R.A., Vaziri, N.D., 2013, Targeting the transcription factor Nrf2 to ameliorate oxidative stress and inflammation in chronic kidney disease. *Kidney international*, *83*(6), 1029-1041.

REFERENCES (continuation)

Russo, J.E., Hilton, J., Colvin, O.M., 1989, The role of aldehyde dehydrogenase isozymes in cellular resistance to the alkylating agent cyclophosphamide. *Progress in clinical and biological research*, *290*, 65-79.

Sabolić, I., 2006, Common mechanisms in nephropathy induced by toxic metals. *Nephron Physiology*, *104*(3), p107-p114.

Sakr, S.A., El-messady, F.A., 2017, Cyclophosphamide Induced Histologial and Immunohistochemical Alterations in Kidney of Albino Rats: The Ameliorative Effect of Fennel Oil. *International Journal of Sciences*, *6*(08), 78-87.

Salmon, A.B., Richardson, A., Pérez, V.I., 2010, Update on the oxidative stress theory of aging: does oxidative stress play a role in aging or healthy aging?. *Free Radical Biology and Medicine*, *48*(5), 642-655.

Sarıkürkçü, C., Locatelli, M., Tartaglia, A., Ferrone, V., Juszczak, A.M., Özer, M.S., ... Tomczyk, M., 2020, Enzyme and biological activities of the water extracts from the plants aesculus hippocastanum, olea europaea and Hypericum perforatum that are used as folk remedies in Turkey. *Molecules*, *25*(5), 1202.

Shelton, L.M., Park, B.K., Copple, I.M., 2013, Role of Nrf2 in protection against acute kidney injury. *Kidney International*, *84*(6), 1090-1095.

Shu-Qi, Z.H.A.O., Shi-Qiang, X.U., Cheng, J., Xiao-Lu, C.A.O., Zhang, Y., Wei-Ping, Z.H.O.U., ... Xia-Min, H.U., 2018, Anti-inflammatory effect of external use of escin on cutaneous inflammation: possible involvement of glucocorticoids receptor. *Chinese journal of natural medicines*, *16*(2), 105-112.

REFERENCES (continuation)

Shulman-Roskes, E.M., Noe, D.A., Gamcsik, M.P., Marlow, A.L., Hilton, J., Hausheer, F.H., ... Ludeman, S.M., 1998, The partitioning of phosphoramide mustard and its aziridinium ions among alkylation and P− N bond hydrolysis reactions. *Journal of medicinal chemistry*, *41*(4), 515-529.

Sies, H., Berndt, C., Jones, D.P., 2017, Oxidative stress. *Annual review of biochemistry*, *86*, 715-748.

Silver, S.A., Beaubien-Souligny, W., Shah, P.S., Harel, S., Blum, D., Kishibe, T., ... Harel, Z., 2021, The prevalence of acute kidney injury in patients hospitalized with COVID-19 infection: a systematic review and meta-analysis. *Kidney Medicine*, *3*(1), 83-98.

Sinanoğlu, O., Yener, A.N., Ekici, S., Midi, A., Aksungar, F.B., 2012, The protective effects of spirulina in cyclophosphamide induced nephrotoxicity and urotoxicity in rats. *Urology*, *80*(6), 1392-e1.

Singh, M., Kumar, N., Shuaib, M., Garg, V.K., Sharma, A., 2014, A review on renal protective agents for cyclophosphamide induced nephrotoxicity. *World J Pharm Pharmaceut Sci*, *3*, 737-747.

Singh, A., Kukreti, R., Saso, L., Kukreti, S., 2019, Oxidative stress: a key modulator in neurodegenerative diseases. *Molecules*, *24*(8), 1583.

Sirtori, C.R., 2001, Aescin: pharmacology, pharmacokinetics and therapeutic profile. *Pharmacological Research*, *44*(3), 183-193.

Sládek, N.E., 1988, Metabolism of oxazaphosphorines. *Pharmacology & therapeutics*, *37*(3), 301-355.

REFERENCES (continuation)

Sládek, N.E., 2002, Leukemic cell insensitivity to cyclophosphamide and other oxazaphosphorines mediated by aldehyde dehydrogenase (s). *Clinically Relevant Resistance in Cancer Chemotherapy*, 161-175.

Sládek, N.E., Kollander, R., Sreerama, L., Kiang, D.T., 2002, Cellular levels of aldehyde dehydrogenases (ALDH1A1 and ALDH3A1) as predictors of therapeutic responses to cyclophosphamide-based chemotherapy of breast cancer: a retrospective study. *Cancer chemotherapy and pharmacology*, *49*(4), 309-321.

Sládek, N.E., 2003, Human aldehyde dehydrogenases: potential pathological, pharmacological, and toxicological impact. *Journal of biochemical and molecular toxicology*, *17*(1), 7-23.

Smith, S.L., 2017, War! What is it good for? Mustard gas medicine. *CMAJ*, *189*(8), E321-E322.

Sorg, O., 2004, Oxidative stress: a theoretical model or a biological reality?. *Comptes rendus biologies*, *327*(7), 649-662.

Stankiewicz, A., Skrzydlewska, E., 2003, Protection against cyclophosphamide-induced renal oxidative stress by amifostine: the role of antioxidative mechanisms. *Toxicology Mechanisms and Methods*, *13*(4), 301-308.

Tanaka, S., Tanaka, T., Nangaku, M., 2014, Hypoxia as a key player in the AKI-to-CKD transition. *American Journal of Physiology-Renal Physiology*, *307*(11), F1187-F1195.

REFERENCES (continuation)

Taysi, S., Memisoğulları, R., Koc, M., Yazıcı, A. T., Aslankurt, M., Gümüştekin, K., ... Tahsin Özder, H., 2008, Melatonin reduces oxidative stress in the rat lens due to radiation-induced oxidative injury. *International Journal of Radiation Biology*, *84*(10), 803-808.

Teksöy, O., Şahıntürk, V., Cengiz, M., İnal, B., Ayhanci, A., 2020, The Protective Effects of Silymarin on Thioacetamide-Induced Liver Damage: Measurement of miR-122, miR-192, and miR-194 Levels. *Applied Biochemistry and Biotechnology*, *191*(2), 528-539.

Thu, T. P. B., Ngoc, P. N. H., Hai, N. M., 2020. Effect of the social distancing measures on the spread of COVID-19 in 10 highly infected countries. *Science of the Total Environment*, *742*, 140430.

Tögel, F., Westenfelder, C., 2014, Recent advances in the understanding of acute kidney injury. *F1000prime reports*, *6*.

Tomsa, A.M., Alexa, A.L., Junie, M.L., Rachisan, A.L., Ciumarnean, L., 2019, Oxidative stress as a potential target in acute kidney injury. *PeerJ*, *7*, e8046.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M., Telser, J., 2007, Free radicals and antioxidants in normal physiological functions and human disease. *The international journal of biochemistry & cell biology*, *39*(1), 44-84.

Vanlallawmzuali, C., Pushpa, N., 2021, Production of Natural Antioxidant Rich Food Products (Bread, Tofu & Curd)–An Alternate to Synthetic Food Products. *Annals of the Romanian Society for Cell Biology*, *25*(6), 5683-5693.

REFERENCES (continuation)

Vanti, G., Bani, D., Salvatici, M.C., Bergonzi, M.C., Bilia, A.R., 2019, Development and percutaneous permeation study of escinosomes, escin-based nanovesicles loaded with berberine chloride. *Pharmaceutics*, *11*(12), 682.

Wang, X.H., Xu, B., Liu, J.T., Cui, J.R., 2008, Effect of β-escin sodium on endothelial cells proliferation, migration and apoptosis. *Vascular pharmacology*, *49*(4-6), 158-165.

Wang, T., Zhao, S., Wang, Y., Yang, Y., Yao, L., Chu, L., ... Fu, F., 2014, Protective effects of escin against indomethacin-induced gastric ulcer in mice. *Toxicology Mechanisms and Methods*, *24*(8), 560-566.

Wald, R., Quinn, R.R., Luo, J., Li, P., Scales, D.C., Mamdani, M.M., ... University of Toronto Acute Kidney Injury Research Group, 2009, Chronic dialysis and death among survivors of acute kidney injury requiring dialysis. *Jama*, *302*(11), 1179-1185.

World Health Organization, 2022. COVID-19 weekly epidemiological update, edition 100, 13 July 2022. World Health Organization.

Wu, X.J., Cui, X.Y., Tian, L.T., Gao, F., Guan, X., Gu, J.K., 2014, Pharmacokinetics of escin Ia in rats after intravenous administration. *Journal of ethnopharmacology*, *156*, 125-129.

Xin, W., Zhang, L., Sun, F., Jiang, N., Fan, H., Wang, T., ... Fu, F., 2011, Escin exerts synergistic anti-inflammatory effects with low doses of glucocorticoids in vivo and in vitro. *Phytomedicine*, *18*(4), 272-277.

REFERENCES (continuation)

Yeşilada, E., 2002, Biodiversity in Turkish Folk Medicine in "Biomolecular Aspects of Biodiversity and Innovative Utilization" Ed. B. Sener.

Yin, H., Xu, L., Porter, N.A., 2011, Free radical lipid peroxidation: mechanisms and analysis. *Chemical reviews*, *111*(10), 5944-5972.

Yoruk, O., Gür, F., Uyanık, H., Yaşar, M., Mutlu, V., Altaş, E., ... Taysı, S., 2010, Antioxidant effects of Nigella sativa in the treatment of experimentally induced rhinosinusitis. *Macedonian J Med Sci*, *3*, 132-137.

Young, I.S., Woodside, J.V., 2001, Antioxidants in health and disease. *Journal of clinical pathology*, *54*(3), 176-186.

Yuan, S.Y., Cheng, C.L., Wang, S.S., Ho, H.C., Chiu, K.Y., Chen, C.S., ... Ou, Y.C., 2017, Escin induces apoptosis in human renal cancer cells through G2/M arrest and reactive oxygen species-modulated mitochondrial pathways. *Oncology reports*, *37*(2), 1002-1010.

Zhang, J., Tian, Q., Chan, S.Y., Duan, W., Zhou, S., 2005, Insights into oxazaphosphorine resistance and possible approaches to its circumvention. *Drug resistance updates*, *8*(5), 271-297.

Zhang, Z., Li, S., Lian, X.Y., 2010, An overview of genus Aesculus L.: ethnobotany, phytochemistry, and pharmacological activities. *Pharmaceutical Crops*, *1*(1).

Zhang, L., Fu, F., Zhang, X., Zhu, M., Wang, T., Fan, H., 2010, Escin attenuates cognitive deficits and hippocampal injury after transient global cerebral ischemia in mice via regulating certain inflammatory genes. *Neurochemistry international*, *57*(2), 119-127.

REFERENCES (continuation)

Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., ... & Cao, B., 2020. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *The lancet*, *395*(10229), 1054-1062.

APPENDIXES

T.C.
ESKİŞEHİR OSMANGAZİ ÜNİVERSİTESİ REKTÖRLÜĞÜ
HAYVAN DENEYLERİ YEREL ETİK KURULU (HADYEK)

HAYVAN DENEYLERİ YEREL ETİK KURULU KARARI

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Siirt Üniversitesi Matematik ve Fen Bilimleri Eğitimi Bölümü Fen Eğitimi Anabilim Dalında görevli **Dr. Öğr. Üyesi Mustafa CENGİZ'**in araştırma yürütücüsü olduğu 670-1/2018 kayıt numaralı ve **"Escin yüklü katı lipid nanopartikülün siklofosfamid nedenli karaciğer hasarı ve oksidatif stres üzerine koruyucu etkileri"** konulu çalışma; Deney Hayvanları Etik Kurulu Yönergesi'ne göre değerlendirilmiş ve gerekçede belirtildiği şekilde yapılması uygun bulunmuştur.

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Adres: Meşelik Yerleşkesi
26480 Eskişehir

Tel : 0 222 239 29 79 (4563)
Fax: 0 222 239 10 74