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**INVESTIGATION OF FERULIC ACID AND CURCUMIN
EFFECTS ON CISPLATIN-INDUCED NEPHROTOXICITY IN
RATS**

ERLIASA BAMI
MASTER OF SCIENCE THESIS

CLINICAL PHARMACY DEPARTMENT

SUPERVISOR
Assist.Prof. Dr. Betul Okuyan

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Danışman (Unvan, Adı, Soyadı)

Yrd.Doç.Dr. Betül Okuyan



Kurumu

M.Ü. Eczacılık Fakültesi Klinik
Eczacılık Abd.

İmza

Sınav Jüri Üyeleri (Unvan, Adı, Soyadı)

Prof.Dr. Fikret Vehbi İzzettin



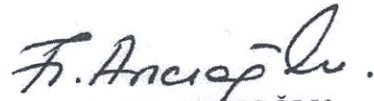
M.Ü. Eczacılık Fakültesi Klinik
Eczacılık Abd.

Yrd.Doç.Dr. Özlem Bingöl Özakpınar



M.Ü. Eczacılık Fakültesi Klinik
Eczacılık Abd.

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IV. ABBREVIATIONS and SYMBOLS LIST

ARF: Acute Renal Failure

BUN: Blood Urea Nitrogen

CIS: Cisplatin

CMN: Curcumin

COX-2: Cyclo Oxygenase Two

ELISA: Enzyme-Linked ImmunoSorbent Assay

FA: Ferulic Acid

GSH: Glutathione

H&E: hematoxylin and eosin

HO-1: Heme Oxygenase-1

I.P: Intra Peritoneal

I.V: Intra Venous

iNOS: Inducible Nitric Oxide Synthase

MDA: Malondialdehyde

MPO: Myeloperoxidase

PtNT: Protein Nitrotyrosine

PBS: Phosphate buffer saline

ROS: Reactive Oxygen Species

RT-PCR: Reverse Transcription Polymerase Chain Reaction

SOD: Superoxide Dismutase

TAS: Total Antioxidant Status

TGF- β 1: Tumor Growing Factor Beta One

TOS: Total Oxidant Status

TUNEL: Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling

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1. ÖZET

Sıçanlarda Ferulik Asit ve Curcuminin Sisplatin Kaynaklı Nefrotoksisite Üzerine Etkilerinin İncelenmesi

Erliasa Bami; Danışman: Yrd. Doç. Dr. Betül Okuyan, Klinik Eczacılık Anabilim Dalı.

Birçok çalışmada, klinik eczacının ilaçla ilgili problemlerin önlenmesi ve çözümlemesinde faydalı görevleri olduğu gösterilmiştir. Çalışmamızda, ferulik asit ve curcuminin sıçanlarda sisplatin kaynaklı akut nefrotoksisiteye karşı protektif etkilerinin incelenmesi amaçlanmıştır. Toplamda 36 sıçan, kontrol, toksisite ve tedavi olmak üzere 6 farklı gruba ayrılmıştır. İlk günden itibaren kontrol ve tedavi gruplarına sırasıyla serum fizyolojik, ferulik asit veya curcumin uygulanırken, toksisite ve tedavi gruplarına 2. gün tek doz sisplatin uygulanmıştır. Sisplatin uygulanmasından 72 saat sonra hayvanlar sakrifiye edilmiştir. Hayvanların kan örnekleri ve böbrekleri ileri inceleme amacıyla alınmıştır. Hayvan ağırlıkları, böbrek fonksiyon parametreleri, oksidatif stres parametreleri ve hayvan serum veya böbrek dokularında antioksidatif durum ölçülmüştür. Sisplatin uygulaması BUN (Blood Urea Nitrogen), kreatinin, MDA (Malondialdehyde), MPO (Myeloperoxidase), TOS (Total Oxidative Status), PtNT (Protein Nitrotyrosine) seviyelerinin yükselmesine sebep olarak böbrek bozukluğunun gerçekleştiği gösterilmiştir ($p<0.05$). Toksisite grubundaki histolojik incelemeler anormal böbrek morfolojisi göstermiştir; ayrıca apoptozu gösteren pozitif TUNEL hücrelerine en çok sisplatin uygulanan hayvanların böbreklerinde rastlanmıştır. RT-PCR (Real Time Quantitative Protein Chain Reaction) yöntemiyle ölçülen HO-1 (Heme Oxygenase-1) seviyeleri toksisite grubunda düşük olarak seyretmiştir, aynı düşüş TAS (Total Antioxidative Status) seviyelerinde de görülmüştür. Bütün bu parametrelerde ferulik asit ve curcumin tedavisinden sonra iyileşme görülmüştür ($p<0.05$). Sonuç olarak ferulik asit ve curcumin tedavisi anlamlı derecede oksidatif stresi önleyerek, antioksidatif durumu yükselterek ve histolojik değişimleri normale dönüştürerek, renoprotektif ve antioksidan etkilere sahip oldukları görülmüştür.

Anahtar Sözcükler: nefrotoksisite, sisplatin, ferulik asit, curcumin, klinik eczacılık

2. SUMMARY

Investigation of Ferulic Acid and Curcumin Effects on Cisplatin-Induced Nephrotoxicity in Rats

Erliasa Bami; Supervisor: Assist. Prof. Dr. Betul Okuyan, Clinical Pharmacy Department.

Clinical Pharmacists' beneficial role on preventing and solving drug induced problems appears in several studies. This study aimed to determine the protective effects of ferulic acid (FA) and curcumin against cisplatin induced nephrotoxicity in rats. A total of 36 animals were divided into 6 groups consisting of control, toxicity and treatment groups. Starting from the first day of the experiment, control and treatment groups have been administered saline, FA and curcumin respectively, while toxicity and treatment groups were injected single cisplatin doses on the second day of the experiment in order to obtain acute nephrotoxicity. Animals were sacrificed 72 hours after cisplatin administration. Blood samples and kidneys were taken for further investigations. Animal weights, renal function parameters, oxidative stress parameters, antioxidant status were assessed in serum samples or kidney tissues. Cisplatin administration resulted in high BUN (Blood Urea Nitrogen), creatinine, MDA (Malondialdehyde), MPO (Myeloperoxidase), TOS (Total Oxidative Status), PtNT (Protein Nitrotyrosine) levels, indicating renal function disorder ($p < 0.05$). Histological observations showed abnormal morphology of the kidney; in addition TUNEL positive cells indicating apoptosis appeared mostly in cisplatin administered animals kidneys. HO-1 (Heme Oxygenase-1) levels measured by RT-PCR (Real Time Quantitative Protein Chain Reaction), revealed antioxidant depletion due to cisplatin administration in toxicity group animals as well as TAS (Total Antioxidative Status). All parameters showed improvement in groups treated with FA and curcumin ($p < 0.05$). FA and curcumin treatments were found significant in preventing oxidative stress, increasing antioxidative status and regaining histological parameters to normal, indicating renoprotective and antioxidant effects for these phenolic compounds.

Key Words: nephrotoxicity, cisplatin, ferulic acid, curcumin, clinical pharmacy

3. INTRODUCTION and AIM

Acute Renal Failure (ARF) is a clinical complication with high morbidity and mortality ratio. It is known that ARF arises as a result of several inflammation reactions deriving from damaged tubular and vascular cells; still its pathophysiology is much more complicated (Qi et al., 2013). Nowadays treatments consist on supportive care and renal transplant in general. In spite of these treatments, mortality percentage in five years of ARF patients is higher than 50% (Waikar et al. 2008). Thus it is very important to intervene with novel treatment choices in order to improve life span in these patients.

ARF may result as a reason of various factors. For example it can result as a side effect of some specific drugs usage. One of these is the chemotherapeutic agent cisplatin. Of patients using cisplatin, 25% develops heavy renal dysfunction (Sanchez-Gonzalez et al. 2011).

Cisplatin (cis-diamindichloroplatin) is a high effective anti-neoplastic agent used against many types of cancers such as: cervical, ovarian, testicular cancer (Delord et al. 2009). Even cisplatin high dosages are more effective, they are not preferred due to its toxic side effects such as: nephrotoxicity, neurotoxicity, bone marrow toxicity, gastrointestinal toxicity, etc. leading thus in lower therapeutic profile (Sohn et al. 2011). One of the main reasons for reduction of cisplatin dose during treatment is its nephrotoxic potential (Arany et al. 2003).

The first target spots that cisplatin effects within kidney are: proximal and distal tubule cells. Gathering in these areas, cisplatin represents harmful effects by causing DNA damage, oxidative stress and apoptosis (Sohn et al. 2011).

The damage that cisplatin causes in the renal tubules may lead to reduced glutathione levels (GSH), rising in blood urea nitrogen (BUN) level, high serum creatinine and malondialdehyde (MDA) levels. Moreover it is known that tissue is injured by the interaction of reactive oxygen species (ROS) with various macromolecules such as unsaturated fatty acids.

ROS are endogenously produced in the kidney cells and are cleared off by endogenous antioxidants such as glutathione (GSH) and superoxide dismutase

(SOD). Elevation in ROS level has been associated with lowered glomerular filtration (Sohn et al. 2011).

Phenolic acids are widely found plant metabolites. They are produced by plants in order to fight pathogens. Thus they play an important role in plants growing and generation phases. Phenolic compounds are known for their roles on neurodegenerative diseases, cancer, diabetes, coronary heart diseases, inflammation states and idleness as potential therapeutic agents (Ferguson et al. 2001).

FA, is a plant phenolic acid that comes out from the metabolism of phenylalanine and tyrosine. It turns out to be a potential antioxidant phenoxy radical due to the conjugated chain at one side and its stable resonance (Maistro et al. 2011). FA is found in many plants seeds and leaves.

According to some studies, FA with its antioxidant properties is able to neutralize nitric oxide and hidroxy-radical groups that lead to DNA damage (Kanski et al. 2002; Roy et al. 2014).

In a study astragalozide IV and FAFA combination has shown reno-protective effects by reducing serum creatinine level and tubulointerstitial fibrosis in obstructive nephropathy (Meng et al. 2011).

In previous studies FA has been able to reduce the urine protein excretion and inhibit tumor growing factor beta 1 (TGF- β 1), reducing thus kidney damage (Fujita et al. 2008).

FA has been supportive in the regeneration of a peripheral nerve during a sciatic nerve damage rat model study (Lee et al. 2013). In addition, FA has played important role in the temporary focal cerebral ischemia by showing antioxidant and anti-apoptotic effects (Rukkumani et al. 2012).

During a study made on diabetic rats, FA has appeared to be able to significantly reduce high malonedialdehyde levels, inhibit thickening of the glomerular membranes due to diabetes, and reduce the increased ROS production due to high glucose level (Choi et al. 2011).

Various antioxidant compounds, including FA, have appeared effective in inhibiting cytokine production responsible of COX-2, iNOS protein expression and inflammation (Ronchetti et al. 2009).

In a study consisting of a hypertensive rat model, FA has been able to improve the antioxidant status and to widen the endothelium linked thorax aorta rings by increasing SOD and catalase enzyme activities in heart and kidneys. Moreover FA has reduced plasma liver enzymes and creatinine concentrations. As a conclusion FA is found to be effective in improving heart, vascular, liver and kidney functions in hypertensive rats (Alam et al. 2013).

Another study has showed that FA could inhibit the damage caused by nicotine on tissues, by exerting anti-hyperlipidemic and antioxidant effects (Adluri et al. 2008).

In another study, rats stimulated with glycerol have appeared to experience increase in urine nitric oxide levels, and reduction in serum and kidney lipid peroxidation, SOD, catalase, glutathione peroxidase, glutathione S-transferase activities and reduction in glutathione levels. All these effects caused by glycerol have been reduced after the treatment with FA in a dose dependent manner. It is concluded that FA protects the cells from the negative effects caused by glycerol by increasing the antioxidant status (Manikandan et al. 2014).

At the other hand, curcumin is another polyphenolic compound that exerts antioxidant, anti-inflammatory and anti-cancer effects (Sahin et al. 2014). In a study made on rats, it is seen that curcumin possesses reno-protective effects based on its ability to scavenge free radicals and increase the antioxidant mechanisms in kidney mitochondria (Waseem et al. 2013).

In another study, curcumin treatment has resulted in an improvement in the tubular necrosis score induced by cisplatin. Here curcumin has shown to reduce cisplatin-induced nephrotoxicity by its anti-inflammatory properties (Ueki et al. 2013).

In a study including laboratory animals, the reduction of antioxidant activities noticed after cisplatin administration has turned to normal after treatment with curcumin (Waly et al. 2011). As a result, it has been pointed out that curcumin exhibits protective effects on cisplatin-induced nephrotoxicity, and this effect is based on its antioxidant and anti-inflammatory properties (Kuhad et al. 2007).

During the literature research we have observed that FA reno-protective effects on cisplatin-induced nephrotoxicity have not been studied. Our study aims to

examine the effects of ferulic acid on cisplatin induced ARF, and compare it to the other poly-phenolic compound curcumin which reno-protective effects have been concluded in several studies.

4. GENERAL INFORMATION

4.1. Acute Renal Failure (ARF)

ARF is defined as the reduction of the glomerular filtration through a couple of hours or weeks period characterized by the accumulation of waste materials such as urea and creatinine (Dagger et al., 2008). Still, it is a complex disorder for which no definition has been accepted (Mehta et al., 2007).

4.1.1. Pathophysiology

ARF can be pre-renal, intrinsic and post-renal. Pre-renal ARF may be a result non-efficient blood circulation within the kidney (decrease in renal perfusion). Intrinsic kidney failure has its origin in a possible injury within the kidney structure. Post-renal failure may result from an obstruction through the way from renal tubules to urethra. Functional renal failure not being linked to a reduced perfusion or any structure injury of kidney, is known to be caused by the hemodynamic changes within the glomerulus (Hoste et al., 2006). Abnormalities causing renal failure categories can be ranked as follows (Dagger et al., 2008; Mehta et al., 2004).

Pre-Renal

- Dehydration
- Diabetes inspidus
- Hemorrhage
- Hypo-albuminemia
- Anaphylactic shock
- Heart Failure
- Renal artery stenosis
- Emboli
- Medications
- Radiocontrast materials
- Hepatorenal syndrome

Intrinsic

- Vasculitis
- Emboli
- Accelerated hypertension
- Systemic lupus erythematosus
- Poststreptococcal glomerulonephritis
- Antiglomerular basement membrane disease
- Exogenous toxins
- Endogenous toxins
- Drugs
- Infections

Post-Renal

- Prostatic hypertrophy, infection, cancer
- Nephrolithiasis

4.1.2. Clinical Properties

ARF may develop and its state of appearing may change according to the cause. Outpatients may experience changes in urination habit, increase in body weight and flank pain. The symptoms of inpatients are usually noticed by the doctor before the patient himself. These symptoms include edema, colored or foamy urine, and orthostatic hypertension in low volume patients (Palevsky, 2006; Dagger et al., 2008).

4.1.3. Diagnosis

In order to diagnose ARF patients overall medical and drugs history, measurement of laboratory data and scanning implementations if needed should be present.

Serum creatinine and blood urea nitrogen levels can not be used by themselves for diagnosis as they are not sensitive to sudden glomerular filtration changes, meaning that they may not be showing the proper current kidney function.

Volume changes in urine can be helpful for diagnosis. Acute anuria (less than 50 mL urine/day) may be following a urinary obstruction or a shock state. Oliguria (400-500 mL urine/day) may be the forerunner for pre-renal azotemia. Non-Oliguric renal failure (more than 400-500 mL urine/day) means the intrinsic renal failure (Dagger et al., 2008).

4.1.4. Treatment Aim

The primary aim in renal failure treatment is to prevent further renal damage. In case renal failure has already developed, the aim is to support kidney function by minimizing the substances that would cause further kidney damage or would slow down the improvement.

4.1.5. Prevention of ARF

The risks for developing ARF include aging, acute infections, previous respiratory or cardiovascular diseases, dehydration, chronic kidney disease, perfusion reduction as a result of heart by-pass or abdominal operations, acute blood loss in a trauma or uric acid nephropathy (Dagger et al., 2008).

Nephrotoxic ingredients should be eliminated at any opportunity (ex. radiocontrast dyes). In case the patient experiences nephropathy as a result of the radiocontrast dye, renal perfusion should be increased to maximum. Hydration should be regulated by saline and sodium bicarbonate solutions, and four total oral doses of acetylcysteine administrated at an interval of 12 hours between each dose. In addition, it has been noticed that an intense of glycemic control with insulin in diabetic patients reduces kidney failure development rate, so it is very important to choose the right treatment for diabetes in these patients (Bellomo et al., 2004).

4.1.6. Control of Current Renal Failure

There is no special drug generated to improve renal failure yet. Thus ARF patients have to be supported pharmacologically and non-pharmacologically during the disease period (Bellomo et al., 2004).

Non-pharmacological Approach

One of the main supportive care intentions is to regulate tissue perfusion and heart output at its best in order to turn the kidney function at its previous state.

Drugs that reduce kidney blood circulation must be stopped. Sufficient liquid replacement must be started and it is very important to stay away from nephrotoxic ingredients.

Renal replacement treatments (ex.: hemodialysis and peritoneal dialysis) helps in maintaining the electrolyte and liquid balance while cleansing the blood from waste materials. Continuous or intermittent types have advantages and disadvantages but they show similar results after the disease is under control.

Intermittent hemodialysis is wide spread and lasts for only 3-4 hours. Disadvantages are the difficulty in reaching the veins of hypotensive patients, and the hypotension risk following the removing of high amount of liquid in a short period.

Continuous treatments are well-tolerated form the patients as it helps by removing the waste materials slowly form the blood. The need for nurse care, not being wide spread, its occurrence for 24 hours, being expensive and the non-presence of all drug types administration guidelines during these therapies are of disadvantages of this method (Dagger et al., 2008; Mehta et al., 2002).

Pharmacological Approach

Loop diuretics are effective in maintaining water retention though they do not appear to increase the improvement rate of renal failure. The most effective diuretic types are mannitol and loop diuretics (Mehta et al., 2002).

Manittol 20% i.v administration is started from 12.5 - 25 g / 3-5 minutes. I.V administration is a disadvantage because of the hyper osmolality risk resulting in worsening of renal failure state. Loop diuretic type equivalents show similar effects, except that patients with sulphur allergy is usually administered ethacrynic acid. Loop diuretics administered continuously have appeared to be more effective and to have fewer side effects than intermittent bolus dosages. IV infusion starting dose consists of 40-80 mg furosemide or equivalent and continues with 10-20 mg/h (Dagger et al., 2008).

Several strategies are used in order to reduce the diuretic tolerance development in renal failure patients. Diuretics that affect the distal tubule such as thiazides, or gathering tubules such as amiloride, triamteren, spironolactone are added to loop diuretics resulting in higher effect by the synergy that comes out. Metholasone is also used in renal failure patients as it is effective even when glomerular filtration is less than 20 ml/min (Dagger et al., 2002; Bellomo et al., 2004).

4.1.7. Electrolyte Balance Regulation and Nutrition

Hyperkalemia is one of the most present electrolyte disorders in ARF patients. Potassium intake should not exceed 3 g/day and serum potassium levels should be monitored daily. Hyponatremia and water retention are other often-occurring problems, thus daily sodium intake should also not exceed 3 g. While calculating sodium intake everything should be counted including sodium found within drugs such as antibiotics, etc. Phosphor and Magnesium levels should be monitored as well as none of them is removed properly by the means of dialysis.

Regarding nutrition, it has been noticed that instead of parenteral nutrition, enteral nutrition has been more effective (Dagger et al., 2008; Bellomo et al, 2004).

4.1.8. Drug Dosing During ARF

Drug treatment optimization is kind of difficult in ARF patients. Using of dialysis types, differences in drug residual clearance and water retention makes the situation even more complicated.

In case of edema presence drugs dissolved in water are distributed more than normal. (Dagger et al., 2008; Mehta et al., 2002).

Drug dosing guidelines for ARF patients receiving renal replacement therapies may need to get individualized and doses may be determined by estimating the clearance of medications associated with a high risk of toxicity by the patient and the renal replacement therapy. (Dagger et al., 2008)

Frequent drug level assessments should be performed on these patients in addition to thorough monitoring procedures, leading to a more secure dosing.

4.1.9. Therapeutic Outcome Evaluation

It is very important that patient situation is monitored very sensitively. Drug concentration in ARF patients may change due to several factors such as volume changes, renal replacement treatments, abnormal renal function, etc. thus drug levels should be monitored continuously (Dagger et al., 2008).

4.2. Drug Induced Renal Failure

The complex nature of various disorders usually leads us to use multiple therapeutic agents, a part of which may have the potential to cause renal injury when used alone or in combination (Neesh et al. 2008). Of the important complications resulting from the use of such agents include ARF, acute interstitial nephritis, and pre-renal failure, which could be reversed in case of early diagnosis (Garella, 1993).

Specific drugs are able to cause renal failure through several different ways. The mechanisms of toxicity caused by medications differ a lot according to the pharmacologic actions, metabolism, and excretion ways of the agent. One of the most common forms of nephrotoxicity is the change in kidney hemodynamics. This type of renal failure is caused by medications that are able to reduce renal prostaglandins leading thus in reduction of renal blood flow and glomerular filtration rate.

Rarely renal failure may be commenced as a result of hyperosmolar substances administrations such as intravenous immunoglobulin or the plasma expander hydroxyethyl starch. They may cause swollen and partially emptied cells in proximal tubules (Garella, 1993).

The principle renal injury types mostly affect the tubules, being thus the courier of acute tubular injuries and necrosis, or interstitial nephritis with inflammatory tubular injury. Renal damage may also occur as a result of vascular damage or glomerular injury (Pazhayattil et al., 2014).

Nephrotoxins in general are the reason for mostly acute tubular necrosis or other types of renal failure (Pannu et al., 2008). They may cause harm to the kidney by evading glomerular and tubular epithelial cells by mechanisms that destroy normal function of renal cells. They may obstruct tubules by crystal depositions or may

support swelling of the cells and the obstruction of tubules by specific osmotic effects. Crystal deposition in kidney is one of the main reasons for development of kidney failure (Garella, 1993). Various drugs derive to urine insoluble substances that appear to precipitate inside the tubules leading thus to acute renal insufficiency. In such situations appropriate drug dosing, volume expansion with high urinary flow and urine alkalinization may play important roles for the prevention of acute renal injury (Perazella, 1999).

4.2.1. Prevention of Drug Induced Renal Failure

The predisposition of a patient to nephrotoxicity from medications or toxic compounds counts on various factors. These include factors related with the drug itself, with the patient and the kidney situation.

Patient factors: Being of female sex; being older than 60 years; possession of underlying renal failure; hypertension, diabetes, heart failure, acid-base disorders, hypoalbuminemia, sepsis, etc (Perazella, 2010).

Drug factors: High dosage or long period of usage; drugs or metabolites that are not dissolved within the intratubular lumen; direct contact with nephrotoxic agents or combinations.

Kidney factors: Kidney perfusion; high drug concentration within renal medulla and interstitium; high metabolic rate of Henle loop cells; re-uptake of toxins from the proximal tubule (Perazella, 2010).

The primer principle of the prevention of drug-induced nephrotoxicity is avoiding the usage of nephrotoxic agents especially in high-risk patients. In case avoiding such agents is not possible, some other interventions may be performed to help lower the damage. Raising the tubular flow rate by providing sufficient hydration could be helpful. Other precaution measures can be taken according to the specific properties of the drug (Okuyan, 2010).

4.2.2. Pharmacist's Role on Drug Induced Renal Failure

Taking responsibility in determining drug problems in renal failure patients is one of the roles a clinical pharmacist should take in order to provide good pharmaceutical care service. Drug-induced problems keep increasing especially with age, current underlying kidney disease and specific drugs usage.

Pharmacists are expected to be effective by controlling renal failure patients drugs, symptoms, side effects, interactions, treatment period for specific drugs, helping thus with the avoidance of nephrotoxic compounds and drug metabolites that may turn to be dangerous to improper functioning kidneys. Pharmacists' review of renal patient treatments has shown that pharmacists are beneficial in identifying and resolving drug related problems.

Renal failure patients' treatments are medically complex, due to various complications and morbidities. These patients require multiple medications through the same period, thus drug related problems are highly encountered. Problems of this type may result to morbidity and mortality in addition to healthcare cost.

It has been observed that pharmaceutical care applied to renal patients' treatment by pharmacists has leaded to better improvement (Manley et al., 2002).

In addition, it has been suggested that healthcare systems might be able to cut approximately \$3.98 from the healthcare expenses as an equivalent to \$1 payoff to pharmaceutical care (Manley et al., 2002).

Patient compliance is another problem where pharmacist intervention may produce beneficial results. Pharmacists can make patient counselling sessions for renal failure patients, especially the ones that have to obey a complex treatment chart for a long period (Okuyan, 2010). Pharmacists' roles on renal failure patients may be briefly lined up as below:

- Reaching the aimed blood pressure values.
- Reaching the needed glycemic levels in diabetes patients
- Early diagnosis, assessment and treatment of proteinuria, hypoalbuminemia and hyperparathyroidism
- Reaching of aimed lipid levels

- Management of suitable drug dosages
- Lowering of drug-induced nephrotoxicity incidence
- Patient counselling regarding drug usage
- Choosing suitable and tolerable drugs.

Clinical pharmacy services have shown to be able to reduce drug related problems, increasing thus patients' quality of life (Joy et al., 2005).

4.2.3. Cisplatin

Cisplatin is a widely used antitumor agent that exerts potential efficiency against various tumor types (Takahara et al., 2002). It is a platinum based alkylating-like agent. The platinum atom binds to bases in the DNA, especially to guanine.

Cisplatin changes the structure of cancer cells by crosslinking DNA, damaging them (Eastman, 1989; Takahara et al., 2002).

Cisplatin is used in the treatment of testicular, ovarian, breast, cervix, endometrium, vulva, prostate, brain, lung, digestive tract cancers, etc. Both Hodgkin's and non-Hodgkin's lymphomas respond to cisplatin.

DNA crosslink occurs in various manners. After cisplatin interacts with DNA, different signals are activated by the means of intrastrand crosslink adducts, which then transverse their ways resulting in cytotoxic effect (Takahara et al., 2002).

The damaged DNA will elicit DNA repair mechanisms but they will be unable to repair the DNA. This causes apoptosis to happen, the natural death of the cell. By decreasing the number of new cells the organism tries to stop the growth of cancer cells. DNA toxic signals can be reduced though, leading to cisplatin resistance. Mechanisms responsible for this resistance may rank as: drug uptake reduction, inactivated drug amount increase, repairing of DNA adducts. These mechanisms are of molecular basis (Siddik, 2003).

Unfortunately, chemotherapy drugs cannot tell the difference between normal and cancer cells so normal cells are also affected. However, they are usually dividing slower but still there are plenty of side effects due to which dosages may

need to be changed and the patient should be monitored carefully (Chang et al., 2014).

Cisplatin is an irritant, thus it may cause inflammation of the vein through which it enters the body. Nephrotoxicity (kidney damage) is a major concern. Changes in kidney function are dose-limiting side effect. Adequate hydration and diuresis are used to help prevent renal damage. Moreover cisplatin may increase levels of uric acid within the body. In addition development of nausea and vomiting is a high incidence side effect. Vomiting symptoms may be controlled by the means of antiemetic drugs in combination with steroids. Unfortunately ototoxicity (hearing loss) may also occur. The administration of other ototoxic medications is usually avoided while the patient is on cisplatin treatment. Also electrolyte disturbances can occur including changes in magnesium, potassium, and calcium levels (Roila et al., 2010).

Besides, cisplatin can affect blood cells in a couple of different ways. It can suppress bone marrow production of blood cells. Blood counts of red or white cells may start decreasing about 10 days after therapy. It can also cause hemolytic anemia because of the red cells damage. Another serious side effect is peripheral neuropathy, which occurs in less than 30% of patients. It manifests by decreased sensation, numbness and tingling of the hands, feet, arms and legs. Neurotoxicity (nerve damage) can also occur. In such cases patients can experience seizures or loss of reflexes, blurred vision or loss of color vision, agitation or confusion. Loss of appetite and changes in the ability to taste things correctly might be noticed characterized by a metallic taste in the mouth without any reason. Liver function can be affected and increase in liver enzymes in the blood may be present. These changes return to normal once treatment is discontinued. Fertility can also be affected by cisplatin. Most patients do not experience all of the side effects. Some side effects will occur only at specific time and are of limited duration (Sun et al., 2005; Roila et al., 2010; Chang et al., 2014)

Patients should not attempt pregnancy if they are on cisplatin as it can cause birth defects. They must be sure to use reliable birth control to prevent pregnancy.

This is important for men as well. Women should not breast feed while on cisplatin treatment.

Since cisplatin treatment patients are more likely to pick up infections, it is an advantage to stay away from people with flu or cold any other symptoms. Hands should be washed very often. Any immunization or vaccination should be avoided during cisplatin treatment. Patients should try to drink 2 to 3 liters of fluid every day unless otherwise instructed by the health care provider and should avoid caffeinated or alcoholic beverages as they act as diuretics and can be hard on kidney function.

Chewing on ice chips 10 to 15 minutes right before and after infusion as well as intermittently throughout the day may reduce the incidence and severity of mouth sores. Chilled drinks may also feel good and help. Good nutrition measures should be cared for while on cisplatin treatment. Patients should get plenty of rest and should try to reduce stress.

4.2.4. Ferulic Acid

FA is a plant chemical found in many fruit and vegetable types, corn and rice bran. This phytochemical comes out as a result of the metabolism of phenylalanine and tyrosine in plants. As FA has appeared to be a potential antioxidant compounds it exerts a wide range of activities against diabetes mellitus, cardiovascular and neurodegenerative disorders, even cancer (Srinivasan et al., 2007).

The chemical structure of FA is responsible for its antioxidant properties. Free radicals that contact FA molecules can easily turn to stabilized compounds by loosing a hydrogen atom, forming thus phenoxy radicals. Moreover these radicals possess an additional unsaturated side chain, which helps in higher stabilization of the entire molecule (Srinivasan et al., 2007).

FA is taken out with urine in humans as 3-hydroxyphenyl and 3-methoxy-4-hydroxy phenyl derivatives of phenyl propyonic acid, hydracrylic acid and glycine conjugates (Deeds et al., 1957).

FA has also shown have potential as cancer chemopreventive agent. As a conclusion FA has various therapeutic properties many of which are attributed to its antioxidant properties (Huang et al., 1988).

4.2.5. Curcumin

Turmeric is a spice derived from the plant *Curcuma longa*. It is yellow colored. Curcumin has been included in several studies where it has exhibited antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal, and anticancer activities (Aggarwal et al., 2007).

Curcumin has demonstrated effectiveness against reactive oxygen and nitrogen species (Sreejayan et al., 1997).

Curcumin antioxidant activity is enhanced by increasing glutathione synthesis and by inhibiting inflammatory enzymes.

Turmeric is the dried root of *Curcuma longa* (Joe et al., 2004).

It is used as a spice especially in India, Southeast Asia, and Middle Eastern countries. Curcumin is the most found curcuminoid in turmeric, counting of approximately 75% of curcuminoids. It is also found in curry powder as turmeric combined with other spice types. In addition curcumin is widely used as a food-coloring agent.

Turmeric has been recognized safe by the FDA as a food additive. Curcumin oral high doses have not resulted in serious adverse effects in humans. A dose trial study performed on 24 adults demonstrated that single oral dosages of 12g curcumin appeared to be safe in general and side effects were not related to the administered dosage (Lao et al., 2006).

In another study performed on various patients, was observed that supplements containing up to 8g curcumin ingested daily for three months were well-tolerated (Cheng et al., 2001).

The safety of curcumin as a dietary supplement during pregnancy/lactation has not been established so it is safest to not use curcumin supplements during pregnancy and lactation.

Curcumin has appeared to possess antiplatelet effect by inhibiting aggregation of platelets. This way it is suggested that it may enhance the risk of bleeding. Thus, patients who have a high risk of bleeding or that are under any anticoagulant/antiplatelet treatment should consult their doctor before starting to use curcumin supplements (Shah et al., 1999).

5. MATERIALS and METHODS

Animal experiments for this thesis study were supplied from "Marmara University Experimental Animal Center (DEHAMER)" being previously approved by Marmara University Animal Experiments Ethical Committee referring to the protocol number 37.2014 mar. (Attachment 1).

Animals: The study was performed on male *Rattus norvegicus* Wistar Albino rats weighing approximately 250-350 g. All the animals were fed with standard laboratory pellets and tap water *ad libitum*.

Experimental animals were randomly divided into six main groups consisting of three control, one toxicity and two treatment groups. Each group contained six animals(n=6). (Table 5.1.)

Table 5.1. Experiment Protocol

Classification of Experimental Animals		
Groups	Compounds	Dosage and Administration
Control (n=6)	Saline	1 mL/100 g i.p on 2 nd day; 1mL distilled water by oral gavage from day 1 st to day 5 th
Control (n=6)	Ferulic Acid (FA)	50 mg/kg oral gavage from 1 st day to 5 th day
Control (n=6)	Curcumin (CMN)	100 mg/kg oral gavage from 1 st day to 5 th day
Toxicity (n=6)	Cisplatin (CIS)	10 mg/kg i.p on 2 nd day
Treatment(n=6)	Ferulic Acid + Cisplatin	FA- 50 mg/kg oral gavage from 1 st day to 5 th day + CIS- 10 mg/kg i.p only on 2 nd day
Treatment(n=6)	Curcumin + Cisplatin	CMN- 100 mg/kg oral gavage from 1 st day to 5 th day + CIS- 10 mg/kg i.p only on 2 nd day

Materials: Compounds such as FA, curcumin and cisplatin were purchased from Sigma-Aldrich Chemie GmbH, Riedstr. 2D-89555 Steinheim. FA and curcumin were suspended in distilled water and administered by oral gavage. Cisplatin was dissolved in saline and was administered intraperitoneally.

Study Design: Animals were housed within Marmara University Experimental Animals Laboratory for five days under 12 hours light and 12 hours dark cycle. Each group was kept in metal cages, each one containing 3 animals, in an environment with suitable humidity and temperature.

Animals in group I served as control and were treated with 1 mL distilled water given by oral gavage for five consecutive days and one single dose of 1mL i.p saline

only on the second day of the experiment. Animals in group II received 50 mg/kg FA oral gavage suspension for five consecutive days. FA dosage was selected according to previous studies estimating no toxicity or negative effects even in higher dosages (Gim et al., 2013). Group III animals received 100 mg/kg curcumin oral gavage suspension for 5 days consecutively. Curcumin dosage was as well decided according to previous studies (Palipoch et al., 2014). Group IV served as toxicity group and received one single dose of 10 mg/kg i.p cisplatin only on the second day of the experiment. Cisplatin dose was selected according to previous studies that demonstrated significant nephrotoxicity in rats even in lower dosages (Okuyan, 2010; Sahu et al., 2013). Group V served as FA treatment group and received 50 mg/kg FA oral gavage suspension for five consecutive days in addition to 10 mg/kg single dose i.p cisplatin administered on the second day of the experiment. Animals in group VI represented curcumin treatment group and received 100 mg/kg curcumin oral gavage suspension in addition to 10 mg/kg single dose i.p cisplatin on the second day of the experiment. Seventy-two hours after cisplatin injection, blood samples were obtained from animals under ether anesthesia by cardiac puncture for further biochemical analysis. After this procedure animals were sacrificed by cervical dislocation. Kidneys were removed and deposited under available conditions (-80°C) in order to be used for further biochemical and histological observations.

Parameter Evaluation Methods

Weights: Animal weights were registered at the beginning and at the end of the experiment and the difference between were calculated.

Blood Urea Nitrogen (BUN) and Creatinine: Animals blood samples were provided from anesthetized animals hearts before sacrifice. Blood samples were transferred to available serum separator tubes (Vacutainer) and were centrifuged immediately. Obtained serums were transferred to tubes and stored under -80 °C for further biochemical measurements. Serum samples were processed with a special autoanalyser (Beckman Coulter AU 5800) for BUN and creatinine level estimations.

Malondialdehyde (MDA) Analysis

MDA levels were assessed in kidney tissues in order to evaluate lipid peroxidation levels in this organ. The measure of lipid peroxidation was analyzed through thiobarbituric acid-reactive substances ([Ohkawa et al., 1979](#)). Supernatants were prepared by adding potassium chloride 150 mM to kidney tissues and homogenizing with a tissue homogenizer (Janke & Kunkel, IKA-WERK), providing thus 10% tissue homogenates. The reactive mixture was made of: 0.2 mL of 8.1% sodium dodecyl sulphate, 1.5 mL of 20% acetic acid (pH=3.5, adjusted with sodium hydroxide), 1.5 mL aqueous solution of thiobarbituric acid (pH=3.5). This mixture was added to 0.1 mL of kidney tissue supernatant. The sample was completed to 4 mL with distilled water and heated at 95 °C for 30 minutes. After the mixtures were cooled they were mixed with 1.0 mL distilled water and 5.0 mL n-butanol-pyridine (15:1 v/v) mixture. The new mixtures were vortexed in order to mix thoroughly. Then each one was centrifuged under 4000 rpm for 10 minutes. The upper organic liquid layer was separated and was measured in a spectrophotometer (Thermo) at 532 nm. Results were represented as nmol/mL (nanomoles of MDA per milligram of protein).

Myeloperoxidase (MPO) Analysis

MPO activity was determined within kidney tissues according to the o-dianisidine method (Bradley et al., 1982). Kidney tissues were diluted with a 10 times bigger volume of cold 20 mM potassium phosphate buffer (K_2HPO_4 ; pH=7,4) and were processed with a tissue homogenizer (Janke & Kunkel, IKA-WERK). Tissue homogenates were centrifuged (Universal 32R, Hettich) under 4 °C at 12.000 rpm for 10 minutes in order to pellet insoluble cellular debris. Formed pellets were re-diluted in a cooled 50 mM potassium phosphate (K_2HPO_4 ; pH=6) buffer, 0.5% of which was made of hexa-1,6-bis-decyltrimethylamonium bromide (HETAB), and were homogenized again. Samples were sonificated (Bandelin Sonopuls UW 3100) for 30 sec. Finally; MPO activity was defined by spectrophotometric (Thermo) measure of o-dianisidine 2HCL, linked to H_2O_2 oxidation. One unit of enzyme activity has been defined as the MPO amount that caused absorbance changes under 460 nm for 3

minutes. One unit MPO activity has been defined as the reduction of 1 μmol H_2O_2 under 25 °C during 1 minute and was represented as Unit/gram.

Total Oxidant Status (TOS)

TOS was evaluated in rat kidney tissues supernatants, with an available commercial fully automated TOS Assay Kit (Rel Assay Diagnostics®). The principle of measurement was related to the oxidation of ferrous ion-chelator complex to ferric ion due to the presence of oxidants within the sample. The ferric ion creates a colored complex with chromogen in an acidic environment. The color intensity provided by the reaction is measured spectrophotometrically at 530 nm, indicating the number of the oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Equiv./L).

Total Antioxidant Status (TAS)

TAS was evaluated in rat kidney tissues supernatants, with the means of an available commercial fully automated 3rd generation TAS Assay Kit (Rel Assay Diagnostics®). Antioxidants in the sample reduce dark blue-green colored radical to colorless form. Absorbance of the color was measured spectrophotometrically at 660 nm indicating the total antioxidant level found in the sample. The assay is calibrated with a stable antioxidant standard solution, which is traditionally named as Trolox Equivalent that is a vitamin E analog.

Protein Nitrotyrosine (PtNT)

Nitrotyrosine levels were measured on rat kidneys with an available competitive ELISA nitrotyrosine quantitation kit (Oxiselect™ Nitrotyrosine ELISA Kit). Samples were first added to nitrated BSA (Bovine Serum Albumin) pre-absorbed EIA plate. After a brief incubation, an anti-nitrotyrosine antibody was added followed by a second antibody conjugated with HRP (horse radish peroxidase). The protein nitrotyrosine content in the sample was determined spectrophotometrically using 450 nm as the primary wave length.

Heme Oxygenase - 1 (HO-1)

Real - Time Quantitative Chain Reaction (RT-PCR) Analysis for the assessment of HO-1 levels in rat kidney tissues were carried out on an Applied Biosystems 7500/7500 Fast Real-Time PCR System. Total RNA was isolated from renal tissues by using the commercially available kit (PureLink® RNA Mini Kit-ambion® by life technologies™). Complementary DNA (cDNA) was reverse-transcribed from total RNA samples using a High-Capacity cDNA Reverse Transcription Kit (RT Step). PCR products were quantitatively synthesized from cDNA samples using the TaqMan® Gene Expression Master Mix (PCR Step). Primer sets used: HO-1 (forward:AGATCACATTACGGTGCTG; reverse:AGCTCAATGTTGAGCAGG). The resulting DNA amount was normalized to the beta actin signal (forward: 5' TGGCGCTTTTGACTCAGGAT -3'; reverse: 5' GGGATGTTTGCTCCAACCAA 3') amplified in a reaction.

Histological Evaluation

For light microscopical evaluation, kidney samples were fixed in 10% buffered formalin for 48 hours and processed for routine paraffin embedding. For general morphological evaluation, approximately 4-µm thick sections were stained with hemathoxylin and eosin (H&E).

Determination of Apoptosis - TUNEL Method

The TUNEL (terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling) method was used in accordance with the user's manual of the manufacturer (Apoptag Plus Peroxidase in situ Apoptosis Kit, Chemicon International, S7101, Temecula, CA, USA). The procedure was as follows: Approximately 4-µm thick sections were incubated with proteinase K for 5 min, washed with distilled water and incubated with 3% hydrogen peroxide in PBS for 5 min. The sections were then washed with PBS, put in the equilibrium buffer for 30 min and incubated in TdT enzyme at 37°C for 1 h. The sections were agitated in washing buffer for 15 sec, washed in PBS, put into anti-digoxigenin conjugate for 30 min and then washed with PBS. After incubation with peroxidase for 6 min, they were washed with distilled water, stained with Mayer's hemathoxylin and covered with Entellan (Merck,

Darmstadt, Germany). In each section, TUNEL positive cells as brown color were evaluated at x400 magnification.

In both of the staining technique at least 5 similar microscopical area were observed. All of the stained sections were observed and photographed with a digital camera (Olympus C-5060, Tokyo, Japan) attached to a photomicroscope (Olympus BX51, Tokyo, Japan). The histological examination was performed by a histologist unaware of the treatment groups.

Statistical Analysis Method

Statistical analysis was carried out using GraphPad Prism 5.0 (GraphPad Software, Inc. La Jolla, CA, USA). All data are expressed as means $\pm$ SEM. Relationship within and between groups is measured with Mann Whitney U and ANOVA tests by using Tukey test as a post-hoc test. Group differences of $p < 0.05$ were considered statistically significant.

6. RESULTS

6.1. Pre- and Post-Experimental Weights in Rats

Animals' pre-experiment and post-experiment mean weight results are shown on Table 6.1.1. Considering weight changes before and after the experiment it is noticed that animals belonging to control groups have experienced significant weight elevation ($p<0.001$). In other all groups rather than control saline group have shown significant weight reduction when compared with pre and post experiment weights ($p<0.05$) (Table 6.1.1).

FA control and curcumin control groups had slight, similar to each other and non-significant weight decrease when compared to control saline group animals ($p>0.05$).

Meanwhile, weights of animals that belong to toxicity group have decreased significantly after cisplatin administration in accordance with control saline ($p<0.01$).

Treatment FA group animals have shown significant weight improvement in accordance with alone cisplatin-administered animals ($p<0.05$).

At the other hand, curcumin treated animals weight percentage changes have shown a little improvement in weights, yet these values have not appeared significant in accordance with the toxicity group ($p>0.05$).

Animals' weight percentages changes during experiment are shown on Figure 6.1.1.

Table.6.1.1. Weight assessment of experimental rats.

	Mean±SEM	Mean±SEM	<i>p</i>*
	Pre	Post	
<i>Control- Serum saline</i>	383.33±12.11	389.83±13.64	<0.001
<i>Control- FA</i>	377.83±28.07	362.50±30.28	<0.10
<i>Control-Curcumin</i>	410.00±48.99	389.83±42.18	<0.05
<i>Toxicity- Cisplatin</i>	347.60±15.77	301.60±21.56	<0.10
<i>Treatment- FA</i>	327.80±35.06	299.00±37.60	<0.10
<i>Treatment- Curcumin</i>	344.00±5.48	304.40±7.70	<0.001

* Difference between pre-experiment and post-experiment values.

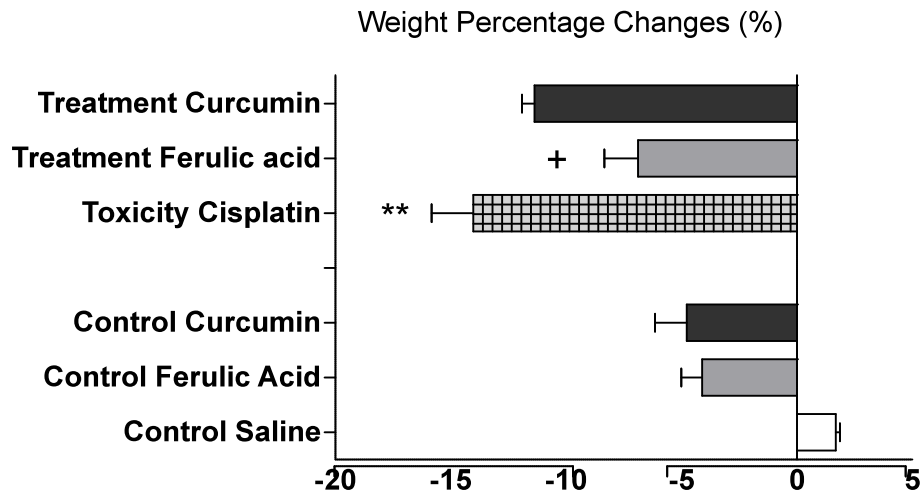


Figure 6.1.1. Rats' weight percentage changes before and after the experiment.

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$, in comparison with saline control group

+: $p < 0.05$; ++: $p < 0.01$; +++: $p < 0.001$, in comparison with cisplatin toxicity group

6.2. Renal Function Tests in Rats

Blood samples were collected from rats' hearts just before sacrifice. BUN levels that were measured in these serum samples are ranked in Figure 6.2.1.

Control groups have shown to possess similar to each other and normal BUN levels. FA and curcumin alone have not shown any changes in BUN levels according to saline control group ($p>0.05$).

Cisplatin toxicity group BUN has had a significant increase in accordance with saline control group ($p<0.01$).

Treatment FA and treatment curcumin groups have shown increased BUN levels when compared with saline control group, however this increase has not appeared significant ($p>0.05$). In addition, curcumin treatment group has recorded higher BUN levels in comparison with FA treatment group; but the difference between these groups hasn't been significant when compared to each other. Although FA + cisplatin combination administered group has shown statistically improvement in comparison with the toxicity group ($p<0.01$), while curcumin + cisplatin combination has not been appeared significant ($p>0.05$).

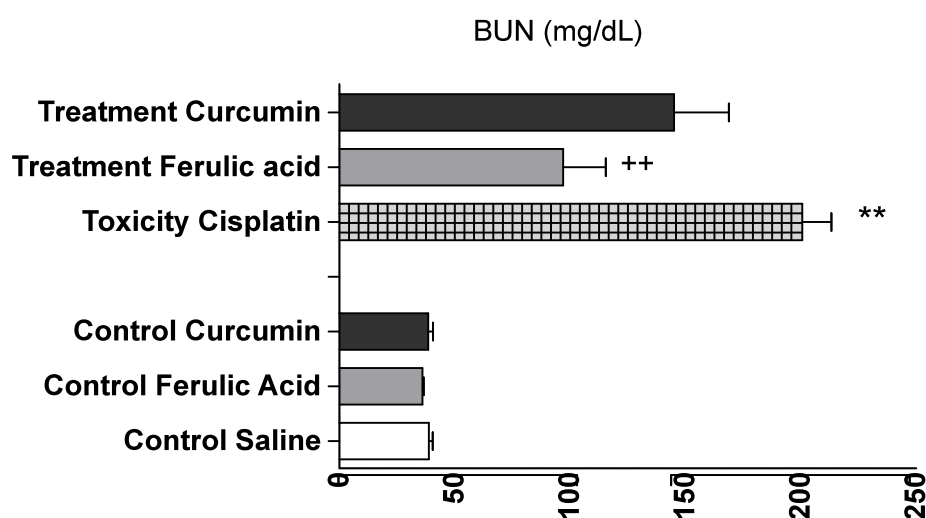


Figure 6.2.1. Serum samples BUN levels in rat kidney tissues.

*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$, in comparison with saline control group
+: $p<0.05$; ++: $p<0.01$; +++: $p<0.001$, in comparison with cisplatin toxicity group

Creatinine levels were estimated in serum samples of animals of which heart blood samples were collected just before sacrifice. Creatinine levels measured in these samples take place in Figure 6.2.2.

In control saline, FA and curcumin groups, serum creatinine has been similar to each other and within the normal range.

In cisplatin toxicity group, creatinine levels have appeared obviously higher when compared to saline control group creatinine levels ($p < 0.01$).

These two treatment groups have also been almost similar to each other with the exception that FA treatment group creatinine levels have appeared lower than that of curcumin, even not significantly ($p > 0.05$).

According to cisplatin toxicity group, FA + cisplatin combination administered treatment group has recorded significant lower creatinine levels, indicating that FA has been able to decrease creatinine levels significantly ($p < 0.05$), while curcumin + cisplatin administered treatment group has shown a slight but not statistically significant decrease on creatinine levels of curcumin treatment group ($p > 0.05$).

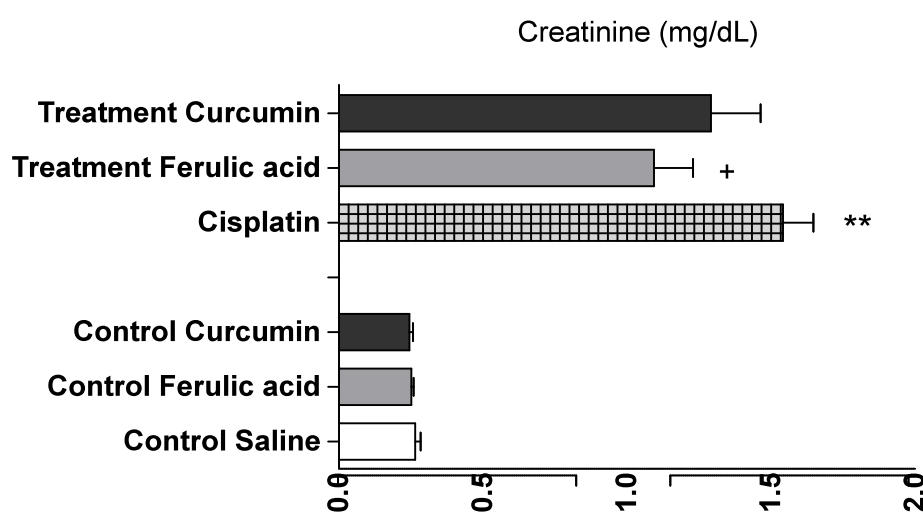


Figure 6.2.2. Creatinine levels in rat serum samples.

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$, in comparison with saline control group

+: $p < 0.05$; ++: $p < 0.01$; +++: $p < 0.001$, in comparison with cisplatin toxicity group

6.3. MDA Levels

MDA levels have been assessed on animals' kidney tissues, and the results take place on Figure 6.3.1.

As noticed control saline, FA and curcumin groups have demonstrated similar, slightly increased but non-significant MDA levels ($p>0.05$).

On the contrary to control group, cisplatin toxicity group has experienced high increase in MDA levels, indicating thus significant oxidative stress in rats kidneys due to cisplatin administration ($p<0.01$).

Both combinations, cisplatin + FA administration as well as cisplatin + curcumin administered treatment groups have been able to significantly decrease MDA levels in rats' kidneys after cisplatin administration ($p<0.01$).

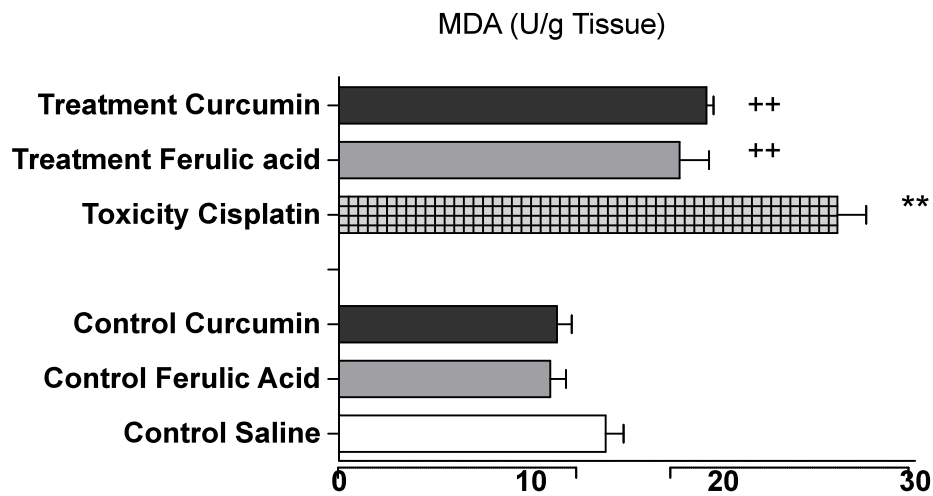


Figure 6.3.1. Malondialdehyde (MDA) levels in rat kidney tissues.

*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$, in comparison with saline control group
+: $p<0.05$; ++: $p<0.01$; +++: $p<0.001$, in comparison with cisplatin toxicity group

6.4. TOS Levels

TOS has been assessed in rat kidney tissues and results obtained are shown on Figure 6.4.1.

In agreement with previously displayed MDA levels, TOS has not been different in control FA and control curcumin groups when compared with the cisplatin control group ($p>0.05$).

On the contrary to control TOS, cisplatin toxicity group TOS has revealed to be significantly high, indicating thus higher oxidant status due to cisplatin administration ($p<0.05$).

FA and curcumin treatment groups have also registered high TOS in comparison with the saline control group and no significant changes in accordance with each other ($p>0.05$).

In addition, FA and curcumin combinations with cisplatin has revealed significant TOS decrease on the basis of cisplatin toxicity group, indicating ability of reducing oxidative status induced by cisplatin administration ($p<0.05$).

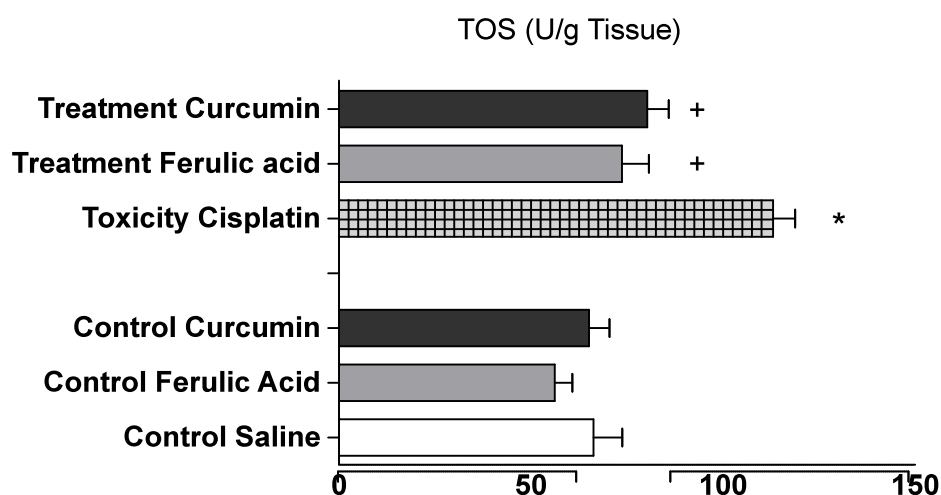


Figure 6.4.1. Total Antioxidant Status (TOS) in rat kidney tissues.

+: $p<0.05$; **: $p<0.01$; ***: $p<0.001$, in comparison with saline control group
 +: $p<0.05$; ++: $p<0.01$; +++: $p<0.001$, in comparison with cisplatin toxicity group

6.5. MPO Levels

MPO levels have been assessed on rat kidney tissues and are displayed on Figure 6.5.1.

Control FA group MPO levels have been similar to control saline group's, while control curcumin group has shown slightly higher MPO levels in comparison with saline control, however this increase hasn't been recorded as statistically significant ($p>0.05$).

Cisplatin administered toxicity group has experienced obvious increase in MPO levels in accordance with control saline group levels ($p<0.01$).

FA + cisplatin combination administered group MPO levels have not increased when compared with control groups ($p>0.05$). Thus, FA has revealed the ability of significantly reducing MPO levels increased due to cisplatin administration ($p<0.01$).

Same way, curcumin + cisplatin administration has resulted in significantly lower MPO levels, indicating the ability of curcumin to decrease neutrophil infiltration boosted by cisplatin administration ($p<0.05$).

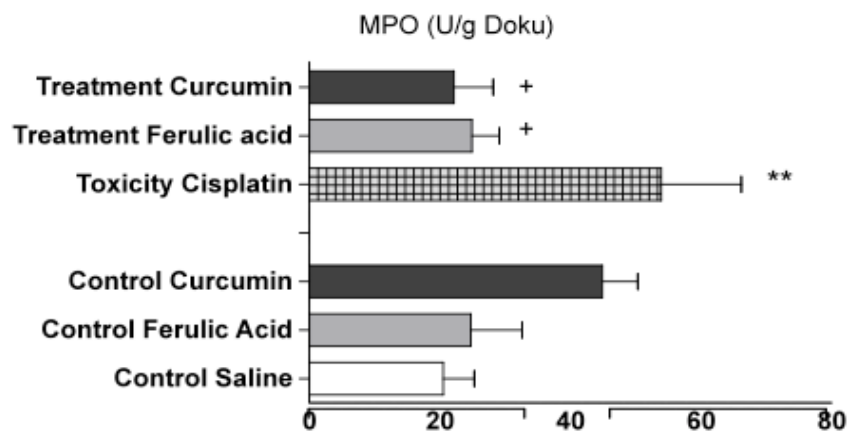


Figure 6.5.1 Myeloperoxidase (MPO) levels in rat kidney tissues.

*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$, in comparison with saline control group
+: $p<0.05$; ++: $p<0.01$; +++: $p<0.001$, in comparison with cisplatin toxicity group

6.6. TAS Levels

TAS has been assessed on kidney tissues of experimental animals and is displayed on Figure 6.6.1.

Control saline group TAS have appeared high. Control FA and control curcumin groups have not shown any significant difference in accordance with the control saline group ($p>0.05$).

On the contrary, cisplatin administered group TAS has shown to be significantly lower in accordance with the control saline group TAS indicating depletion of antioxidative proteins due to cisplatin toxicity ($p<0.05$).

FA treatment group TAS has appeared significantly higher in comparison with cisplatin toxicity group, indicating antioxidant effect of FA ($p<0.05$).

Similarly, curcumin + cisplatin combination has revealed significant TAS increase in accordance with cisplatin administered group, indicating ability of preventing and regaining TAS in curcumin presence even after cisplatin administration ($p<0.05$).

FA and curcumin treatment groups have not revealed significant differenced between each other regarding TAS in rat kidney tissues ($p>0.05$).

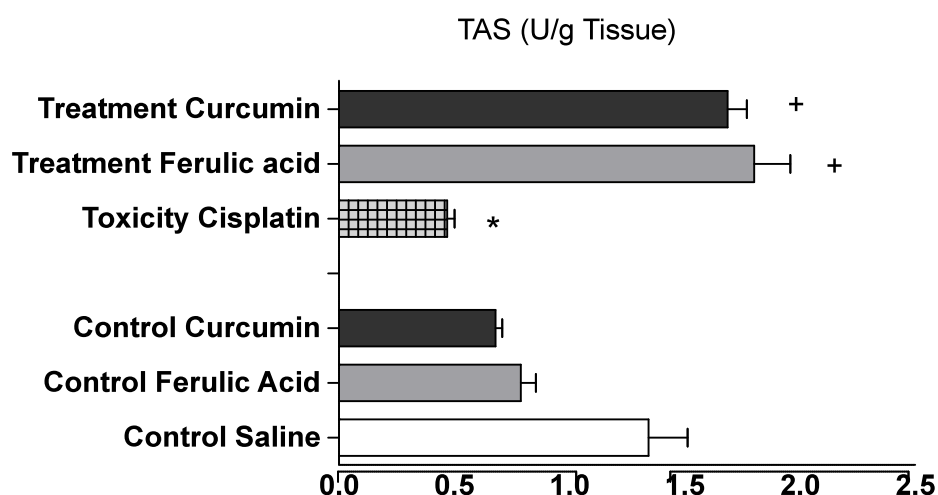


Figure 6.6.1 Total Antioxidant Status (TAS) in rat kidney tissues.

+: $p<0.05$; **: $p<0.01$; ***: $p<0.001$, in comparison with saline control group
 +: $p<0.05$; ++: $p<0.01$; +++: $p<0.001$, in comparison with cisplatin toxicity group

6.7. PtNT Levels

PtNT levels were assessed on kidney tissues of rats and the results are displayed on Figure 6.7.1.

As noticed control group nitrotyrosine levels were similar to each other representing no nitrosative stress presence in control saline, FA and curcumin group rats ($p>0.05$).

On contrary, cisplatin administered toxicity group exhibited significantly high nitrotyrosine levels, indicating high nitrotyrosine production due to cisplatin toxicity when compared with the control saline group ($p<0.01$).

Treatment group, treated with cisplatin in combination with FA or curcumin represented insignificantly high nitrotyrosine levels due to cisplatin presence in comparison with control saline group ($p>0.05$).

However treatment group nitrotyrosine levels were significantly decreased due to antioxidants such as FA and curcumin when compared with alone cisplatin administered toxicity group ($p<0.01$). FA and curcumin treatment abilities to reduce nitrosative status didn't differ versus each other, indicating similar effects on protein nitrotyrosine levels ($p>0.05$).

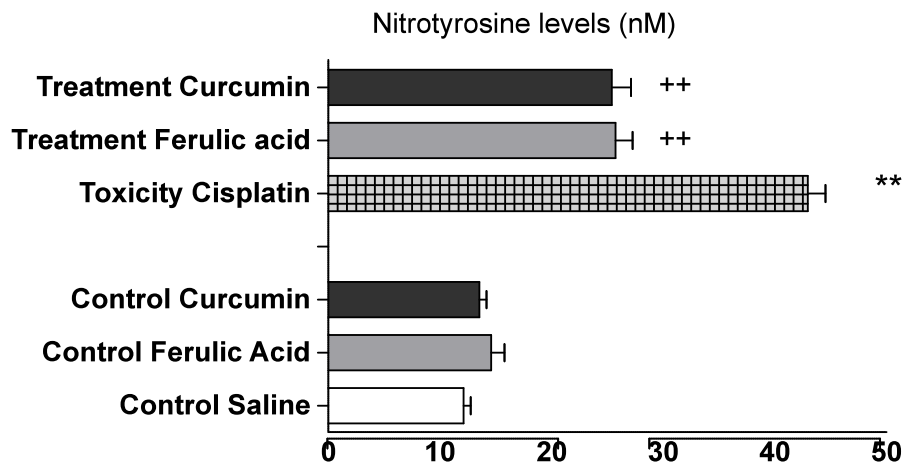


Figure 6.7.1. Protein Nitrotyrosine (PtNT) levels in rat kidney tissues.

*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$, in comparison with saline control group
+: $p<0.05$; ++: $p<0.01$; +++: $p<0.001$, in comparison with cisplatin toxicity group

6.8. Heme Oxygenase - 1 (HO-1) Assessment

HO-1 levels were assessed in rat kidney tissues and are displayed in figure 6.8.1. Results were evaluated on a basis of control saline group and are expressed as % of control. Control FA and control curcumin groups didn't show any significant increase in HO-1 levels in comparison with the control saline group.

Cisplatin administration resulted in significantly high levels of HO-1 versus control FA and control curcumin groups ($p < 0.001$).

FA + cisplatin treated rats kidney HO-1 levels were insignificantly higher when compared to control FA and control curcumin groups ($p > 0.05$), however a significant decrease in HO-1 levels was obtained due to FA presence in this treatment group compared to alone cisplatin toxicity group ($p < 0.01$).

Similarly, curcumin + cisplatin administered rats exhibited insignificantly high levels of HO-1 according to control curcumin and control FA groups, while a significant decrease was also noticed due to curcumin presence in this treatment group when compared to alone cisplatin toxicity HO-1 levels ($p < 0.05$).

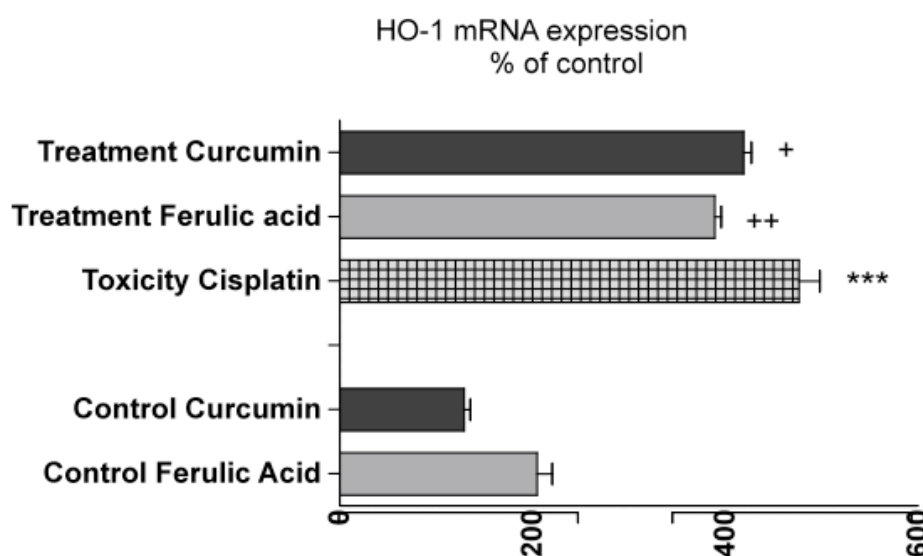


Figure 6.8.1. Heme Oxygenase - 1 (HO-1) mRNA expression % of control

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$, in comparison with saline control group

+: $p < 0.05$; ++: $p < 0.01$; +++: $p < 0.001$, in comparison with cisplatin toxicity group

6.9. Histological Evaluations

Histological analysis was performed on rat kidneys of each group and results obtained are displayed on Figure 6.9.1.

In the saline (Fig. 6.9.1A), curcumin (Fig. 6.9.1B) and FA (Fig. 6.9.1C) applied control groups, regular morphology of kidney with renal corpuscles and tubuli were observed.

In the cisplatin applied toxicity group severe degeneration of the tubular epithelium, dilatation of Bowman's space and glomerular congestion and inflammatory cell infiltration in kidney were observed (Fig. 6.9.1D).

Curcumin + cisplatin treatment group showed mild degeneration in renal corpuscles and tubules in kidney (Fig. 6.9.1E).

FA + cisplatin applied treatment group showed quite regular renal corpuscles and tubules, with mild vascular congestion in kidney (Fig. 6.9.1F).

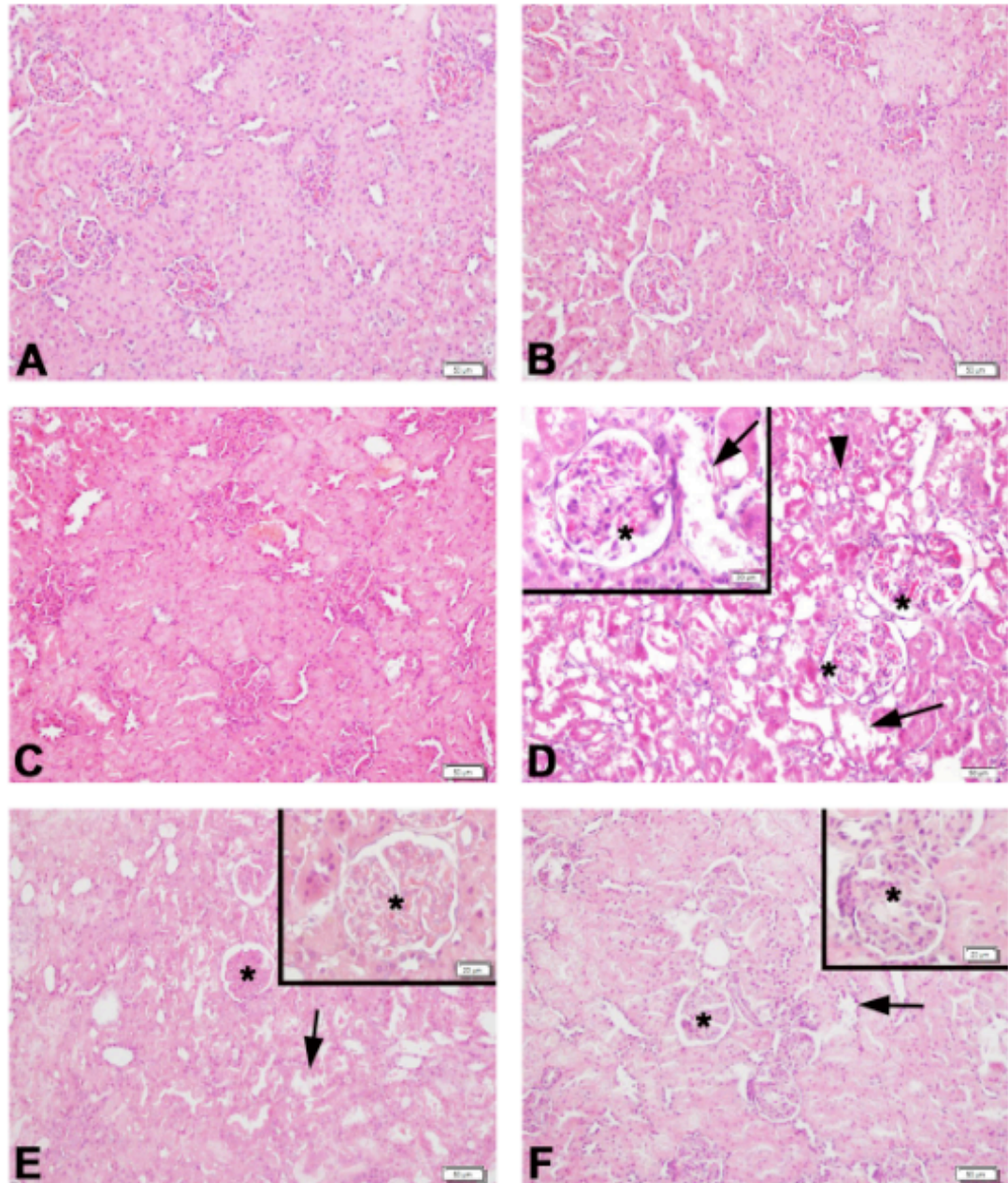


Figure 6.9.1: Representative photomicrographs of kidney in experimental rat groups. Regular parenchyma morphology of kidney in the saline (A), curcumin (B) and FA (C) applied control groups; glomerular congestion (*), degenerated tubules (arrow) and vascular congestion (arrowhead) in cisplatin applied toxicity group (D); quite regular renal corpuscles with decreased glomerular congestion (*), a few degenerated tubules (arrow) in curcumin + cisplatin applied (E) and FA + cisplatin applied (F) treatment groups.

H&E staining, scale bars: 50µm, and inset: 20 µm

Apoptosis

Brown- colored TUNEL- positive cells were observed in all groups. In the saline (Fig. 6.9.2A), curcumin (Fig. 6.9.2B) and FA (Fig. 6.9.2C) applied control groups observations were noticed either no TUNEL positive cells or 1-2 TUNEL positive cells in each microscopical area.

In the cisplatin applied toxicity group (Fig. 6.9.2D) showed more than 6 TUNEL positive cells in each microscopical area.

Both curcumin + cisplatin applied (Fig. 6.9.2E) and FA + cisplatin applied (Fig. 6.9.2F) treatment groups showed less than 5 TUNEL positive cells in each microscopical area.

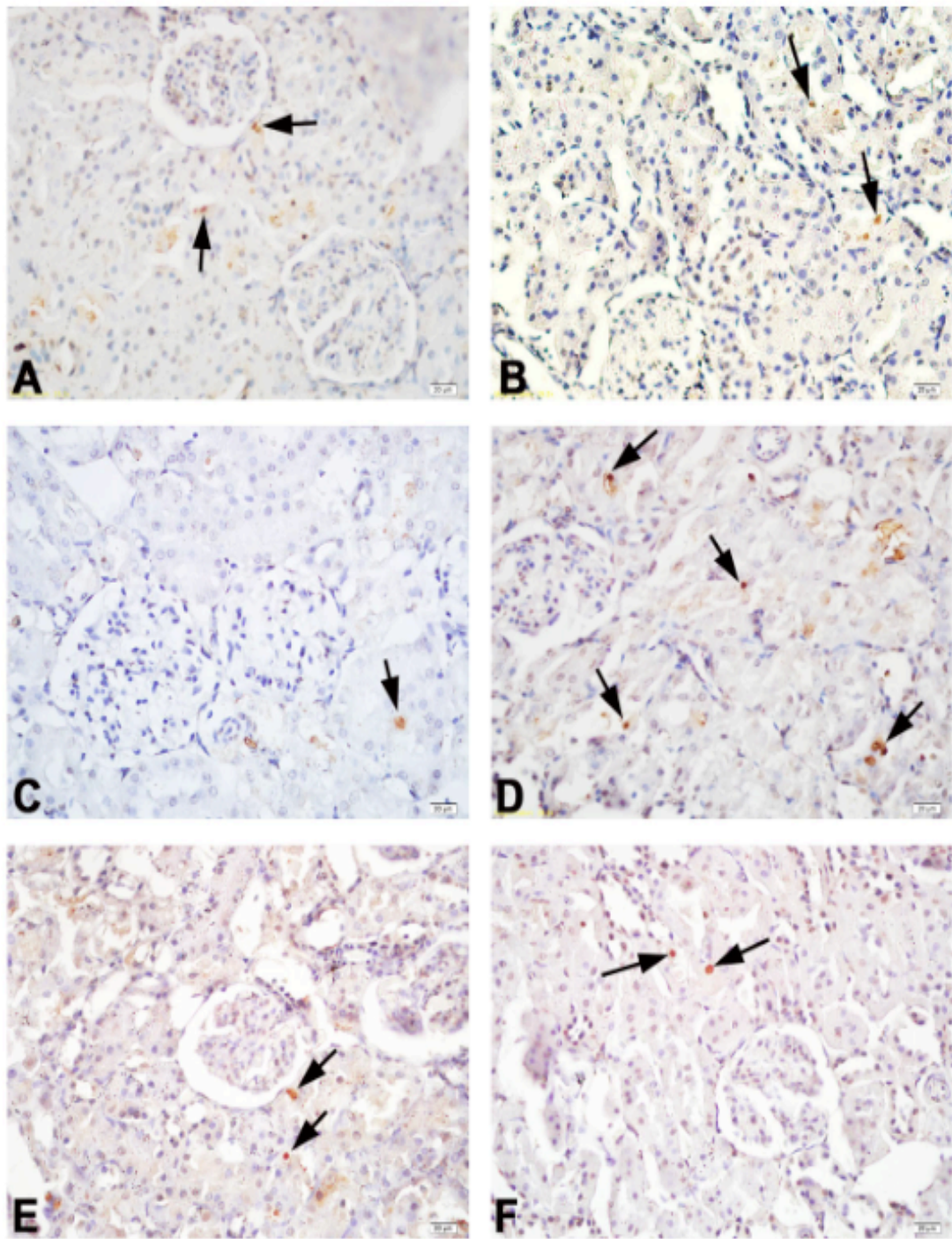


Figure 6.9.2. Representative photomicrographs of TUNEL stained sections of kidney in experimental groups. Brown colored TUNEL positive cells (arrow) in the saline (A), curcumin (B) and FA (C) applied control groups, cisplatin (D) applied toxicity group, curcumin + cisplatin (E) and FA+ cisplatin (F) applied treatment groups.

7. DISCUSSION and CONCLUSION

Cisplatin is a powerful anti-neoplastic agent widely used within chemotherapeutic protocols against various cancer types. Cisplatin and its metabolites are majorly eliminated from kidneys (Goldstein et al., 1983). The kidney is directly exposed to cisplatin metabolites and cisplatin itself, increasing thus the risk for nephrotoxicity (Jonker et al., 2003). In one animal model study it was found that the occurrence of nephrotoxicity was the first side effect during cisplatin treatment, therefore special attention must be paid to kidneys during chemotherapy with cisplatin (Nematbakhsh et al., 2012). Cisplatin induced renal failure is generally determined by responses such as metabolic reactions, cell generation events and inflammatory status. In order to clinically reduce cisplatin toxicity, these outcomes should be manipulated (Arany et al., 2003).

Nephrotoxicity related to cisplatin is noted to especially be aiming the signaling pathways that lead to tubular cell death and inflammation (Pabla et al., 2008).

Cisplatin has appeared to harm renal function as well as the kidney itself by oxidative stress in long-term treatments, especially by inducing lipid peroxidation and free radical production, and by changing renal tubular structure (Hassan et al., 2012; An et al., 2011).

In this study we examined FA and curcumin which have exhibited renoprotective effects on injured kidneys by inhibiting injury development and improving renal function in rats (An et al., 2011; Ueki et al., 2013).

The phenolic compound FA has served as high potential antioxidant in many studies (Barone et al., 2009). It has positive effects against oxidative stress that is implicated in various disease types (Kanski et al., 2002). FA has demonstrated abilities of inhibiting and reducing lipid peroxidation by interacting with the inflammation pathways and by scavenging free radicals (Trombino et al., 2013; Alam et al., 2013).

Yet, no study that has directly examined FA effects on cisplatin-induced nephrotoxicity in rats was encountered.

The other phenolic compound included in our study, curcumin, has often demonstrated dual antioxidant functions by scavenging ROS due to its phenolic structure, and by inducing the upregulation of several endogenous cytoprotective and antioxidant proteins (Dinkova-Kostova et al., 2008). According to previous studies curcumin has shown renoprotective properties on rats affected by diabetic nephropathy (Pan et al., 2013). In a study it was demonstrated that curcumin was effective in the prevention of renal damage as well as in the progression of renal disease. The renoprotective effect of curcumin has been related to the down-regulation of pro-inflammatory mechanisms. Lowered glomerular damage, responsible for the kidney structure changes, was evidenced in rat kidneys after curcumin treatment (Soetikno et al., 2011).

As demonstrated in many cisplatin-induced nephrotoxicity studies, cisplatin causes weight reduction on rats (Ademiluyi et al., 2013). In this study cisplatin exhibited significant decreasing ability on rat weights in comparison with control groups which weights didn't alter significantly at the end of the experiment ($p < 0.001$). While on treatment groups significant weight preserve was noticed as a result of FA treatment ($p < 0.05$). Curcumin treatment also appeared to preserve rats' weights, though the improvement was not statistically significant ($p > 0.05$).

Several studies have shown that cisplatin administration has caused increase in serum BUN and creatinine levels in rats due to glomerular and tubular damage (Domitrovic et al., 2014; Helmy et al., 2014). The current study showed obvious increased serum BUN and creatinine levels in cisplatin administered toxicity group. Cisplatin combined with FA/curcumin administered treatment groups also experienced high BUN and creatinine levels in accordance with the control group, however FA has been able to significantly reduce BUN and creatinine levels in comparison with the toxicity group ($p < 0.01$), while curcumin could only slightly and non-significantly reduce BUN and creatinine levels ($p > 0.05$).

Lipid peroxidation in this study has been determined by measurement of MDA levels in rat kidney tissues. Previous studies, in agreement with this one, have demonstrated increased MDA levels due to cisplatin induced nephrotoxicity (An et al., 2011; Kahtani et al., 2014; Amirshahrokhi et al., 2014).

In an animal model study high serum MDA levels related to cisplatin administration have been distinguished (Motamedi et al., 2014; Helmy et al., 2014).

As noticed in several previous studies, the present study also showed that MDA levels of rat kidney tissues were significantly increased after cisplatin administration in the toxicity group and treatment groups ($p < 0.001$), indicating lipid peroxidation occurrence due to cisplatin administration, while control groups MDA levels were lower. However, cisplatin administration in combination with FA or curcumin revealed significantly lowered MDA levels, according to toxicity group ($p < 0.001$).

In addition, MPO measurements in rat kidney tissues were significantly higher in the toxicity group comparing with the MPO levels of the control groups ($p < 0.01$). In agreement with other studies (Amirshahrokhi et al., 2014), toxicity group of the current study exhibited the highest levels of MPO due to cisplatin-induced toxicity. FA and curcumin control groups exhibited similar MPO levels as saline control group, indicating no oxidative stress presence due to both antioxidant administrations. Cisplatin combined FA and curcumin treatment administered group measurements of MPO levels were high, still FA and curcumin have been able to significantly reduce MPO levels in these treatment groups ($p < 0.05$), indicating neutrophil infiltration reduction ability. FA and curcumin treatment were found significant to reduce MPO levels induced by cisplatin ($p < 0.05$).

Literature screening has showed that cisplatin-induced nephrotoxicity leads to changes in oxidative status by increasing oxidative stress (Pabla et al., 2008). TOS and TAS were evaluated in previous studies, and cisplatin appeared to increase TOS while decreasing TAS in kidney tissues, indicating changes in oxidative proteins and antioxidant deposits (Toplu et al., 2014).

In previous studies it was observed that TOS is increased by cisplatin administration in rats (Toplu et al., 2014). Results of this study were in agreement with statements of cisplatin increasing TOS in kidney tissues due to oxidative stress presence. It appeared that oxidant status was significantly higher in cisplatin administered rat kidneys than in groups that didn't receive cisplatin ($p < 0.05$). FA and curcumin control groups did not register any significant increase in TOS, showing that these compounds did not play any role on oxidative stress increasing. FA +

cisplatin and curcumin + cisplatin combination treatments resulted in lower TOS, indicating significant oxidative stress reducing ability of these two compounds ($p < 0.05$). Activity exerted by FA and curcumin on TOS was similar to each other ($p > 0.05$).

In previous studies TAS levels have been reduced due to depletion of antioxidant protein deposits (Chandran et al., 2014). Analysis in the present study revealed high TAS in control saline, FA and curcumin groups. Cisplatin administered toxicity resulted in significant lower TAS than normal ($p < 0.05$). On contrary, cisplatin combined with FA and curcumin administrations led to significantly higher TAS measurements ($p < 0.05$). The current study resulted in altered TAS due to cisplatin administration, and improvements of these measurements were obtained due to curcumin and FA treatments. FA and curcumin were able to increase TAS in rats, informing thus about the ability of restoring TAS in rat kidney tissues.

Nitrotyrosine formation is based on the modification of tyrosine residues to 3-nitrotyrosine by potential nitrating agents (ex: peroxynitrite), and has been reported in many human, animal or cellular models of disease (Gow et al., 2004). Previous studies have shown increased nitrotyrosine levels in rat kidneys after cisplatin administration. (Domitrovic et al., 2013). Kidney nitrotyrosine levels in the current study resulted high in cisplatin administered toxicity group, indicating thus significant oxidative and nitrosative status presence due to cisplatin toxicity ($p < 0.01$). FA and curcumin administered alone did not exhibit nitrotyrosine-increasing effects, indicating reliance in the nitrosative status point of view. Groups administered cisplatin combined with FA or curcumin resulted in higher than normal nitrotyrosine levels due to cisplatin administration. Still, protein nitrotyrosine levels in these treatment groups were significantly lower than only cisplatin administered toxicity group, indicating protective effect of FA and curcumin against nitrotyrosine production due to cisplatin induced toxicity ($p < 0.01$).

Heme oxygenase-1 is a prescriptive parameter of oxidation. It is hypothesized that HO-1 protects the kidney from further injury due to its antioxidant and anti-inflammatory properties (Elmarakby et al., 2012). HO-1 is a protein inducible by stress presence, upregulated as a response to current chemical and physical stress

(Gozzelino et al., 2010). It is also noticed to increase due to oxidative stress (Vile et al., 1994). HO-1 level modifications due to various compound administrations such as berberine, curcumin, metalloporphyrines, melatonin, has been targeted in several previous studies, resulting in high HO-1 levels of the toxicity group and decreased ones in treatment groups (Kilic et al., 2013; Domitrovic et al., 2013; Fetoni et al., 2014; Pan et al., 2014). In agreement with previous studies, the current study observed high levels of HO-1 in cisplatin treated toxicity group rats, while saline, FA and curcumin administered rats experienced lower kidney tissue HO-1 levels. Rats treated with cisplatin combined to FA or curcumin respectively, showed significant decreases in HO-1 levels ($p < 0.05$). Thus, the current study indicated that HO-1 significantly increased due to cisplatin induced oxidative stress in order to scavenge free radicals and protect renal tissue from toxicity ($p < 0.001$). Meanwhile antioxidants such as FA and curcumin were able to decrease HO-1 levels in rat kidney tissues, suggesting lower needs for free radical scavenging and protection by HO-1 as a result of oxidative stress and free radical reduction due to antioxidants presence.

Histological findings of previous studies demonstrated changes in kidney structure due to cisplatin (Sahu et al., 2013). Glomerular and tubular modifications as a result of cisplatin induced nephrotoxicity have been noticed in several studies (McDuffie et al., 2013; Sahu et al., 2013; Sahu et al., 2013). Present histological analysis resulted in severe denegeration of the tubules, congestion and inflammatory cell infiltration in kidneys of cisplatin toxicity group. Meanwhile, saline, curcumin and FA control groups represented regular kidney morphology. FA + cisplatin combination administered treatment group showed regular renal corpuscles and tubules with mild vascular congestions in kidneys, indicating renoprotective ability of FA. Curcumin and cisplatin applied treatment group showed mild degeneration in renal corpuscles and tubules, showing that curcumin also had positive effects against cisplatin induced nephrotoxicity.

Apoptosis evaluations in this study in agreement with other studies demonstrated cisplatin ability to enhance apoptosis in kidney tissues (Yang et al., 2014; Choi et al., 2014). Oxidation and inflammation, which mainly results in apoptosis of tubular epithelial cells, plays a main role on renal function and tubular

structure damage (Jose et al., 2011). Studies have showed cisplatin induced ARF resulting in increased tubular apoptosis (Terada et al., 2013). The present study revealed that cisplatin toxicity group possessed the greatest number of apoptotic cells when compared with saline, curcumin and FA control groups which contained very few or no apoptotic cells per microscopical area. These results indicate that FA was able to significantly prevent apoptosis in kidney tissues as well as curcumin, which was also able to reveal anti-apoptotic activity.

Based on our findings, cisplatin induced lipid peroxidation by enhancing oxidative stress and free radicals. It also led to glomerular and tubular alterations by enhancing cell death in kidney tissues. On the other hand, FA could significantly prevent or slow down oxidative stress by scavenging free radicals and by increasing antioxidative status. It was also able to decrease morphological changes of kidney structure by preventing apoptosis. Curcumin was also effective in regaining antioxidant status and reducing oxidative stress in kidney tissues, though its preventing or improving abilities were generally registered as significant ($p < 0.05$).

In the present study it has been concluded that cisplatin administration led to renal damage by inducing oxidative stress and inflammatory reactions, depleting natural antioxidative status, damaging renal morphology and enhancing apoptosis. On contrary, treatment with antioxidants such as FA and curcumin resulted in renal damage prevention and improved renal morphology, indicating renoprotective effects for FA, and for curcumin.

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9. ATTACHMENTS

Attachment 1: Marmara University Institute of Health Sciences Animal Experiments Ethical Comity study approval document.

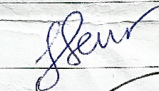
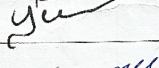
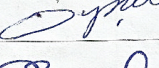
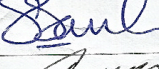
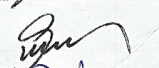
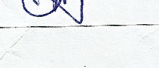


MARMARA ÜNİVERSİTESİ HAYVAN DENEYLERİ YEREL ETİK KURULU
PROJE ONAY FORMU

BAŞVURU BİLGİLERİ	PROTOKOL KODU	37.2014.mar	Çalışma: Yüksek lisans tezi
	PROJE ADI	Sıçanlarda Ferulik Asid ve Curcuminin Sisplatin Kaynaklı Nefrotoksisite Üzerine Etkilerinin İncelenmesi	
	SORUMLU ARAŞTIRICI ÜNVANI/ADI	Yrd.Doç.Dr. Betül OKUYAN	
	ARAŞTIRMA MERKEZİ	Marmara Üniversitesi Eczacılık Fakültesi - Klinik Eczacılık Anabilim Dalı Laboratuvarı-Marmara Üniversitesi Deneysel Araştırma ve Hayvan Laboratuvarı	
	DESTEKLEYİCİ	BAPKO	

KARAR BİLGİLERİ	Tarih Yukarıda başvuru bilgileri verilen araştırma başvuru dosyası ve ilgili belgeler araştırmanın gerekçe, amaç, yaklaşım ve yöntemleri dikkate alınarak incelenmiş ve gerçekleştirilmesinde sakınca bulunmadığı için Kurulumuzca onaylanmasına oy birliği ile karar verilmiştir. Onay sonrasında yapılacak her türlü proje değişiklikleri (katılımcılar, başlık vb.) veya protokol değişikliklerinin Etik Kurula bildirilerek proje onayının yenilenmesi gerekmektedir.
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ETİK KURUL BİLGİLERİ	
ÇALIŞMA ESASI	Deney hayvanları ile yapılacak olan bilimsel araştırma, test, sağlık hizmetleri uygulamaları ve eğitim-öğretim gibi temel etkinliklerde kullanılan yöntem ve materyaller ile ilgili etik standartları gözetmek, etik ilkeler doğrultusunda görüş bildirmek, araştırma önerilerini incelemek ve sertifikası olmayanların deney hayvanı kullanmalarını engellemektir.

ÜYELER						
Ünvanı / Adı / Soyadı	Uzmanlık Dalı	Kurumu / EK Üyeligi	Onaylanan Proje ile ilişkisi		Toplantıya katılım	İmza
Prof. Dr. Gökse ŞENER	Farmakoloji	M.Ü Tıp Fakültesi ve Deney Hayvanları Uygulama ve Araştırma Merkezi Müdürü	Var	Yok	Evet Hayır	
Prof. Dr. İnci ALICAN	Fizyoloji	Yürütücü Sekreteri	Var	Yok	Evet Hayır	
Prof. Dr. Ayşen YARAT	Biyokimya	M.Ü Diş Hekimliği Fakültesi	Var	Yok	Evet Hayır	
Doç. Dr. Serap ŞIRVanci	Histoloji Embriyoloji ABD	M.Ü Tıp Fakültesi	Var	Yok	Evet Hayır	
Doç. Dr. Hasan Raci YANANLI	Farmakoloji	M.Ü Tıp Fakültesi	Var	Yok	Evet Hayır	
Doç. Dr. Gürkan SERT	Tıp Tarihi ve Etik	M.Ü Tıp Fakültesi	Var	Yok	Evet Hayır	
Vet. Hek. Dilek ÖZBEYLİ	Veteriner Hekim	M.Ü Tıp Fakültesi ve Deney Hayvanları Uygulama ve Araştırma Merkezi Sorumlu Veterineri	Var	Yok	Evet Hayır	
Bio. Arif GÜMÜŞ	Biyoloji	İstanbul Hıfzıssıhha Enstitüsü Müdürü, Kuruma ilişkisi olmayan TC vatandaşı üye	Var	Yok	Evet Hayır	
Billur AYGÖR	Emekli Memur	Kuruma ilişkisi olmayan TC vatandaşı üye	Var	Yok	Evet Hayır	

Attachment 2: Congress Report Obtained from this Study.

Erlisa Bami, Betul Okuyan, Ozlem Bingol Ozakpinar, Zarife Ozdemir Kumral, Kutay Koroglu, Feriha Ercan, Zeynep Cirakli, Turgut Sekerler, Fikret Vehbi Izzettin, Mesut Sancar. Protective Effects of Ferulic Acid and Curcumin against Cisplatin-Induced Nephrotoxicity in Rats. Fourth International Meeting on Pharmacy and Pharmaceutical Sciences. 18-21 September 2014, p86.

PP 33 EVALUATION OF DRUG BURDEN INDEX IN ELDERLY PATIENTS UTILIZED INHALER

BETÜL OKUYAN, ZEHRA AYDINLI, IPEK CELIK, EFE DOĞUKAN DİNCEL, TUGCE SAY, DILAY YAVUZ, AYSU SELÇUK, SEONKYEONG YANG, MESUT SANCAR

CLINICAL PHARMACY DEPARTMENT, MARMARA UNIVERSITY- FACULTY OF PHARMACY, ISTANBUL, TURKEY

The aim of the present study was to evaluate patients' inhalation skills and calculate drug burden index (DBI) among elderly patients utilized metered-dose inhalers, diskuses, turbuhalers and aerolizers at community pharmacy. This cross sectional descriptive study was conducted between February and May 2014. Patients were eligible for the present study if they were 65 years old or older, used at least one inhaler device, did not take any support for medication utilization and provided their consent. Patient demographic, clinical and medication data were recorded with face-to-face interview. Cognitive function has been evaluated by using revised Turkish version of Mini Mental State Examination (rMMSE-T) was scored between 0-30 (1). Patients' inhaler use attitudes were evaluated essential steps during inhaler use for each inhaler device. Drug burden index has been also calculated. A total of thirty-seven elderly patients (mean of age: 71.65±5.47 years old; male/female: 20/17) utilized aerolizer (70.27%), metered dose inhaler (54.05%), diskus (21.62%), and turbuhaler (10.81%) were included in this study. The number of inhaler utilized by elderly patients was one (48.60%), two (45.90%) and three (5.40%). The mean of rMMSE-T score was calculated as 23.62±4.08. Twenty seven elderly patients utilized five or more medications. Among twenty five elderly patients who utilized anticholinergic and sedative medications; DBI index was calculated equal and less than one in twenty two and more than one in three patients. When evaluating patients' steps while using their inhaler; only five patients could correctly follow all steps during inhaler utilization. Most of patients were failure to hold their breath for ten second after inspiration and wait a few seconds before next dose. Although present study had a little sample size; our results presented the importance of evaluation drug burden index and cognitive function in elderly patients, who used inhalers which required special skills and had polypharmacy.

1. Keskinoglu P, Ucku R, Yener G, Yaka E, Kurt P, Tunca Z. Reliability and validity of revised Turkish version of Mini Mental State Examination (rMMSE-T) in community-dwelling educated and uneducated elderly. *Int J Geriatr Psychiatry*. 2009;24(11):1242-50.

PP 34 PROTECTIVE EFFECTS OF FERULIC ACID AND CURCUMIN AGAINST CISPLATIN-INDUCED NEPHROTOXICITY IN RATS

ERLIASA BAMI¹, BETÜL OKUYAN¹, OZLEM BINGOL OZAKPINAR², ZARIFE N. OZDEMIR KUMRAL³, KUTAY KOROGU⁴, FERİHA ERCAN⁴, ZEYNEP CIRAKLI⁵, TURGUT SEKERLER², FIKRET VEHBI IZZETTİN¹, MESUT SANCAR¹

¹ DEPARTMENT OF CLINICAL PHARMACY, MARMARA UNIVERSITY FACULTY OF PHARMACY, ISTANBUL, TURKEY

² DEPARTMENT OF BIOCHEMISTRY, MARMARA UNIVERSITY FACULTY OF PHARMACY, ISTANBUL, TURKEY

³ DEPARTMENT OF PHYSIOLOGY, MARMARA UNIVERSITY FACULTY OF MEDICINE, ISTANBUL, TURKEY

⁴ DEPARTMENT OF HISTOLOGY AND EMBRYOLOGY, MARMARA UNIVERSITY FACULTY OF MEDICINE, ISTANBUL, TURKEY

⁵ DEPARTMENT OF BIOCHEMISTRY, BAKIRKOY DR. SADI KONUK TRAINING AND RESEARCH HOSPITAL, ISTANBUL, TURKEY

This study aims to evaluate protective effects of phenolic compound ferulic acid and curcumin against cisplatin induced nephrotoxicity in rats. Rats were divided into six groups consisting of six animals: three control groups, one toxicity group, and two treatment groups. Serum saline (1mL/kg), ferulic acid (50 mg/kg), and curcumin (100 mg/kg) were administered by oral gavage throughout five days. Single dose (10mg/kg) i.p. cisplatin was administered on the second day of experiment. Kidneys were removed after 72 hours of cisplatin administration within each group. Malondialdehyde (MDA) levels, myeloperoxidase (MPO) levels, and total antioxidant status (TAS) were measured in rat kidney tissues. Ferulic acid and curcumin significantly decreased kidney MPO levels, which had increased as a result of cisplatin administration ($p < 0.05$). Cisplatin caused an obvious decrease in kidney TAS levels, while ferulic acid (50 mg/kg) and curcumin (100 mg/kg) administration significantly increased TAS levels in treatment groups ($p < 0.05$). Although a slight decrease was noticed in kidney MDA levels after treatment with ferulic acid and curcumin when compared to toxicity group, no statistically significant differences were observed ($p > 0.05$). Based on this study we suggest that ferulic acid and curcumin supplementation expelled beneficial effects against cisplatin toxicity on rat kidneys, therefore it appears that both ferulic acid and curcumin may be effective in preventing cisplatin induced nephrotoxicity.

10. CURRICULUM VITAE

Name	Erliasa	Surname	Bami
Birth Place	Albania	Birth Date	11.08.1984
Nationality	Albanian	E-mail	eli.bami@hotmail.com

Education

	Name of Graduation Foundation	Graduation Year
Master Degree	Marmara University Clinical Pharmacy Department	2014
University	Istanbul University Faculty of Pharmacy	2008
High School	Turgut Ozal Private College	2003

Working Experience

Job	Company	Duration (Year - Year)
Pharmacist	Various Pharmacies	2008-2011

Foreign Languages	Reading*	Speaking*	Writing*
English	Very Good	Very Good	Very Good
Albanian	Very Good	Very Good	Very Good
Italian	Medium	Medium	Medium
Turkish	Very Good	Very Good	Very Good

Foreign Language Exam Evaluation*								
YDS	ÜDS	IELTS	TOEFL IBT	TOEFL PBT	TOEFL CBT	FCE	CAE	CPE
Eng.89								

	Quantitative	Equally Weighted	Verbal
ALES Points	59.983	51.801	43.551

Computer Knowledge

Program	Usage Skill
Microsoft office	Very Good

* Please estimate as very good, good, medium, weak.