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ARAŞTIRMA / RESEARCH

Investigation of uroprotective effects of seed methanol extracts of Hypericum triquetrifolium Turra. on cyclophosphamide-induced bladder hemorrhagic cystitis and nephrotoxicity in Wistar albino rats

Wistar albino sıçanlarında Hypericum triquetrifolium Turra. tohum metanol ekstraktlarının siklofosfamid-nedenli mesane hemorajik sistiti ve nefrotoksisitesi üzerine üroprotektif etkilerinin incelenmesi

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Abstract

Purpose: This study investigated the possible uroprotective effects of Hypericum triquetrifolium Turra. (HT) seed methanol extracts (25,50,100 mg/kg, i.p., for 6 days) against cyclophosphamide (CYP)-induced (150 mg/kg, single dose, i.p.) acute bladder hemorrhagic cystitis (HC) and nephrotoxicity in rats.

Materials and Methods: Wistar albino rats used in this study were divided into nine groups, each including seven rats. Group 1 (control) was treated with 0.5ml saline (SF) and Group 2 was treated with CYP (150 mg/kg). Groups 3, 4, 5 were treated with 25, 50, 100 mg/kg HT, respectively while groups 6, 7, 8 were treated with 25, 50, 100 mg/kg CYP + HT, respectively. Finally, Group 9 (control-2) was treated with 0.5ml-%0.2 dimethyl sulfoxide (DMSO). The serum creatinine, blood urea nitrogen (BUN), superoxide dismutase (SOD) and catalase (CAT) levels were measured in blood serum.

Results: The CYP-treated rats histopathologically had mild-moderate bladder and renal injuries. The serum creatinine and BUN levels, which are the biochemical markers of renal injury, significantly increased compared to the control group.

Conclusion: HT showed a protective effect on CYP-related bladder HC and nephrotoxicity in rats by inhibiting inflammation and apoptosis.

Keywords:. Cyclophosphamide, Hypericum triquetrifolium, nephrotoxicity, hemorrhagic cystitis

Öz

Amaç: Bu çalışmada, Hypericum triquetrifolium Turra (HT) tohum metanol ekstraktlarının (25,50,100 mg/kg, i.p., 6 gün boyunca) siklofosfamid (CYP) nedenli (150 mg/kg, tek doz, i.p.) akut mesane hemorajik sistit (HC) ve nefrotoksisiteye karşı olası üroprotektif etkileri araştırıldı.

Gereç ve Yöntem: Bu çalışmada kullanılan Wistar albino sıçanlar, her biri yedi sıçan olmak üzere dokuz gruba ayrıldı. Grup 1 (kontrol) 0.5ml salin (SF) ile muamele edildi ve Grup 2, CYP (150 mg/kg) ile muamele edildi. Grup 3, 4, 5 sırasıyla 25, 50, 100 mg/kg HT ile tedavi edilirken, grup 6, 7, 8 sırasıyla 25, 50, 100 mg/kg HT + CYP ile tedavi edildi. Son olarak, Grup 9 (kontrol-2) 0.5ml-% 0.2 dimetil sülfoksit (DMSO) ile muamele edildi. Kan serumunda serum kreatinin, kan üre azotu (BUN), süperoksit dismutaz (SOD) ve katalaz (CAT) seviyeleri ölçüldü.

Bulgular: CYP ile tedavi edilen sıçanların histopatolojik olarak hafif-orta derecede mesane ve böbrek yaralanmaları vardı. Böbrek hasarının biyokimyasal belirteçleri olan serum kreatinin ve BUN düzeyleri, kontrol grubuna göre önemli ölçüde artmıştır.

Sonuç: HT, enflamasyonu ve apoptozu inhibe ederek sıçanlarda CYP ile ilişkili mesane HC'si ve nefrotoksisite üzerinde koruyucu bir etki göstermiştir.

Anahtar kelimeler: Siklofosfamid, Hypericum triquetrifolium; nefrotoksisite, hemorajik sistit

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INTRODUCTION

Cyclophosphamide (CYP), an alkylating antineoplastic agent, which is used to kill cancerous cells in vivo, also damages normal cells and has a wide usage area[1]. However, its clinical usage is generally limited by its severe toxicities such as nephrotoxicity[2,3] and bladder dysfunction[4]. CYP also causes hemorrhagic cystitis (HC)[4,5] and nephrotoxicity thereby limiting its clinical utility[3]. Acrolein and phosphoramide are two active metabolites of CYP and considered to be the reason for its toxic and antineoplastic effects such as apoptosis, oncosis and necrosis[6]. The responsible mechanism of CYP-related HC is the direct effect of uroepithelium induced by acrolein that is the CYP'urotoxic metabolite[7]. Acrolein is a highly reactive aldehyde and can cause epithelial inflammatory process which permeates to the entire bladder wall. Then, it leads to an increase in the production of reactive oxygen species (ROS) in the bladder and the epithelium causes to increase transepithelial and transendothelial permeabilities[5]. CYP-related HC is the reason for both acrolein contact with urinary bladder mucosa and oxidative stress[5,8].

Oxidative stress caused by acrolein is important for the pathogenesis of CYP-related acute urotoxicity[9,10]. The most common symptoms of HC are dysuria, nocturia, intense suprapubic pain and gross hematuria[11,12]. In addition, oxidative stress induces various renal failures[13,14]. Nephrotoxicity is a prevalent case and the reason for various pathological impacts on renal tissues[15]. Drugs used in cancer treatment can cause damage and dysfunction in three main parts of nephron (proximal tubules, distal tubules and glomeruli) in the kidney[16,17]. The CYP-induced renal damage and nephrotoxicity are characterized by the remarkable rise in the serum creatinine and uric acid levels[2].

In recent years, alternative medicine has been used to treat many diseases by utilizing antioxidants from herbal agents[18]. In this regard, a number of researches have demonstrated that extracts or isolated compounds of medicinal plants can reduce cystitis and treat CYP-related HC[7,19,20], as well as nephrotoxicity[20-22]. The aerial parts of HT with methanolic extract possesses high antioxidant activities[23] which might be useful in protecting against or decelerating the development of diverse oxidative stress-induced disorders[24]. Thus, HT is a strong candidate to protect the healthy cells from the toxic side effects of CYP[25].

MATERIALS AND METHODS

Drug and chemicals

Cyclophosphamide Monohydrate, C0768 (CYP), serum creatinine, BUN, SOD and CAT assay kits were commercially purchased from Sigma-Aldrich, Taufkirchen, Germany. A solution was prepared with 500 mg CYP and 25 mL bidistilled water at its proper concentration and saved at 4 ˚C before usage. A single dose of 150 mg/kg b.w - i.p CYP was administered to the rats.

Herbal material (*Hypericum triquetrifolium* Turra.)

The HT samples were collected at seeding stages from August to September 2015. Voucher specimens were stocked at Mardin Artuklu University Herbarium (C.Keskin 2015-14) located in Mardin, Turkey. The taxonomic identification of the plant samples was approved by Dr Cumali Keskin. 20 g of seeds were ground into powder. The seed powder was extracted three times with 200 ml absolute methanol under magnetic stirrer. After the extraction process, approximately 4 g of the crude methanol extracts were obtained and stored at -20^{o}C before the experiments. The obtained crude methanol extract was dissolved with 0.2% dimethyl sulfoxide (DMSO) into the ultrasonic bath to obtain different concentrations (25, 50 and 100 mg/kg).

Experimental design

The Wistar Albino rats used in the present study were obtained from Kobay Experimental Animals Lab. San. Tic. A.S.S. and this study were carried out in accordance with the approval of the ESOGU Experimental Animals Ethics Committee (Date-Protocol no: 04.03.2015-444-1/2015) obtained prior to the experiment.

Healthy 220±20 g and 3-4 months old male rats were kept in transparent polypropylene cages under standard environmental conditions and at 25±2°C room temperature, 60-70% humidity. The rats were supplied 12h light-dark cycle under the standard environmental condition and were allowed to freely access standard pellet food with regular tap water. The rats were housed in rat cages for 2 weeks so that they could adapt to the environment.

The rats were separated into nine groups, each group including seven rats (63 rats in total). All injections were made intraperitoneally (i.p.). Group 1 (control) was treated with 0.5 ml serum physiologic (SF) so-called normal saline for 6 days. Group 2 was treated with SF for 5 days and with a single dose of CYP (150 mg/kg) on the 6th day. Groups 3, 4 and 5 were treated with 25, 50 and 100 mg/kg HT for 6 days, respectively. Groups 6, 7 and 8 were treated with 25, 50 and 100 mg/kg HT, respectively, and a single dose of 150 mg/kg CYP on the 6th day. Group 9 was treated with 0.2% dimethyl sulfoxide (DMSO) for 6 days. Before the rats were sacrificed on the 7th day, blood samples were taken with cardiac puncture via the ketamine / xylazine anaesthesia.

Histological measurement

Cardiac tissues were stained in a 10% formaldehyde solution. During the routine histologic preparation, the tissue specimens were embedded in paraffin and then 5.0 micron thick serial sections were done and kept with Hematoxylin-Eosin. Then, the histopathological properties were measured. The consequences were assayed with One Way Analysis of Variance and Kruskal-Wallis test to score the variants with an abnormal distribution. Differences between the groups were considered significant if $p<0.05$.

Immunohistochemistry

The renal and bladder tissue samples were deparaffinized and rehydrated routinely. Antigen retrieval by citrate buffer (pH 6.0) was conducted by heating the samples in a microwave for 10 min at 700 W. Then by blocking with 3 mL/L H_2O_2 and swine serum, the samples were incubated with the primary antibodies, managed against Caspase-3 (Thermo), Bax (Abcam) and Bcl-2 (Abcam), and at dilutions of ultravisionquanto detection system (Thermo Scientific).

Statistical analysis

The result of the test was signified as means±SD. Statistical analyses were performed by One Way Analysis of Variance and Kruskal-Wallis One Way Analysis of Variance on Ranks Test. $p<0.05$ was considered as statistically significant. Each experiment was repeated at least three times.

RESULTS

The biochemical levels of lipid peroxidation marker indicated a remarkable increase while the antioxidant defence enzyme indicated a remarkable increase in the CYP-treated rats and nephrotoxicity. In this study, the SOD and CAT levels were found to be low in Group 2 which shows that CYP causes kidney damage. CYP + HT treatment increased SOD and CAT levels. As can be seen from the results of this study, while SOD and CAT levels decreased in the CYP treated group 2, it increased in the CYP + HT treated groups as HT dose increased (Table 1).

The serum creatinine and blood urea nitrogen (BUN) levels were found to be high in Group 2, whereas these levels were lower in the CYP + HT groups (Table 1). The effects of HT on BUN and serum creatinine and renal function in CYP-treated rats were also determined in this study. Moreover, HT doses showed an important increase in the serum creatinine levels (Table 1).

The bladder tissue had normal histology in the control and 25, 50, 100 mg/kg HT treated groups and 0.2% DMSO treated group. In the CYP treated group (Group 2), bladder tissue was inflamed and hemorrhage was observed. In this study, symptoms such as hematuria, dysuria and polyuria were also observed in rats after the CYP administration (Figures 1-3). Inflammation and haemorrhage could not be prevented completely in the bladder tissues of groups 6, 7 and 8 that were given CYP + HT, while damage and changes in the bladder tissue decreased further as HT dose increased (Figures 1-3). Furthermore, in Group 2 treated with CYP, inactivity, fatigue and bloody urine were observed. There was no bleeding in urine observed in the CYP + HT groups.

In the bladder sections to which Bcl-2 immunohistochemistry was applied, there were no significant differences between the control, 25, 50, 100 mg/kg HT treated groups and 0.2% DMSO treated group. It was found that Bcl-2 positivity was low in Group 2 treated with CYP, and in the groups 6, 7 and 8 treated with CYP + HT, positive staining increased gradually as HT dose increased. In the bladder sections to which Caspase-3 and Bax immunohistochemistry were applied, the positivity was higher in Group 2. The positivity increased in the groups 6, 7 and 8 treated with CYP + HT as the HT dose increased (Figures 1-3).

The renal tissue had normal histology in the control, 25, 50, 100 mg/kg HT treated groups and 0.2% DMSO treated group. In the kidneys of the rats in Group 2 treated with CYP, the dilatation of distal tubules, compacting of glomeruli and narrowing of bowman capsule were observed (Figures 4-6). Serum creatinine level increased in group 2 due to glomerular dysfunction. In the present study, it was found that the tubular and glomerular contents of the renal tissues of groups 6, 7 and 8 treated with CYP + HT decreased and these changes improved greatly as HT dose increased (Figures 4-6).

Table 1. The effects of 150 mg/kg cyclophosphamide (CYP) and 25, 50 and 100 mg/kg doses of *Hypericum triquetrifolium* (HT) on the serum levels of hepatic enzymes (Serum Creatinine, Blood Urea Nitrogen, Superoxide Dismutase, and Catalase).

Groups	Serum Creatinine	Blood Urea Nitrogen (BUN)	Superoxide Dismutase (SOD)	Catalase (CAT)
1-Control (saline)	*0.19 ±0.00a**	11.65±1.44a	21.16±1.34a	31.40±1.16a
2- CYP (150 mg/kg)	0.20±0.00b	23.13±1.47b	12.36±1.43b	19.86±0.90b
3- HT (25 mg/kg)	0.19±0.00a	11.39±1.13a	21.78±0.90a	31.61±1.04a
4- HT (50 mg/kg)	0.19±0.1 c	11.51±1.17a	21.87±1.10a	31.58±0.96a
5- HT (100 mg/kg)	0.19±0.00a	7.16±0.91c	22.78±0.62a	32.04±0.82a
6- CYP+HT (25 mg/kg)	0.19±0.00a	21.66±2.51b	13.74±0.51b	22.04±1.18c
7- CYP+HT (50 mg/kg)	0.19±0.00a	20.36±1.25d	15.53±0.84c	23.62±1.03d
8- CYP+HT (100 mg/kg)	0.19±0.00a	15.13±1.34e	17.02±0.95d	25.50±1.28e
9-Control (DMSO)	0.19±0.00a	12.72±0.72a	21.35±0.82a	31.50±0.80a

*Means are the averages of 3 replicates. **Values reported are means±standard deviation; means followed by different letters in the same columns are significantly different (p<0.001: statistically significant differences).

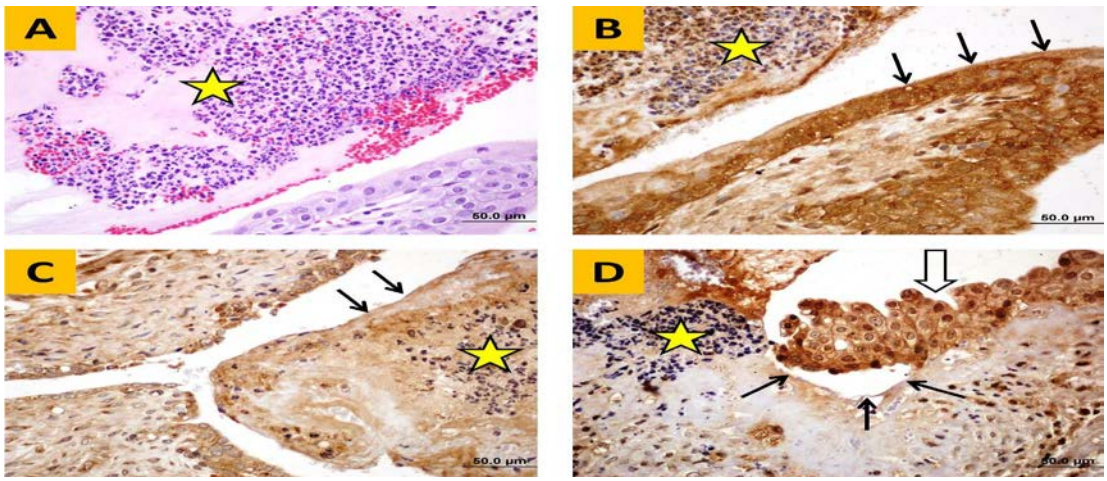

Figure 1. Bladder sections of CYP (150 mg/kg)-treated rats. A: Bladder lumen is full with purulent material *(star)* and haemorrhage, hematoxylin-eosin. B: Bladder tissue is inflammed *(star)* and epithelial cells immunostained positively *(thin arrows)* with apoptotic protein caspase 3. C: Bladder tissue is inflammed *(star)*, epithelium is became thin *(thin arrows)* and epithelial cells immunostained negatively with anti-apoptotic protein Bcl-2. D: Bladder tissue is inflammed *(star)*, epithelium is separated from adjacent tissue *(thin arrows)* and epithelial cells immunostained positively *(thick arrow)* with apoptotic protein Bax. Bars are 50 µm.

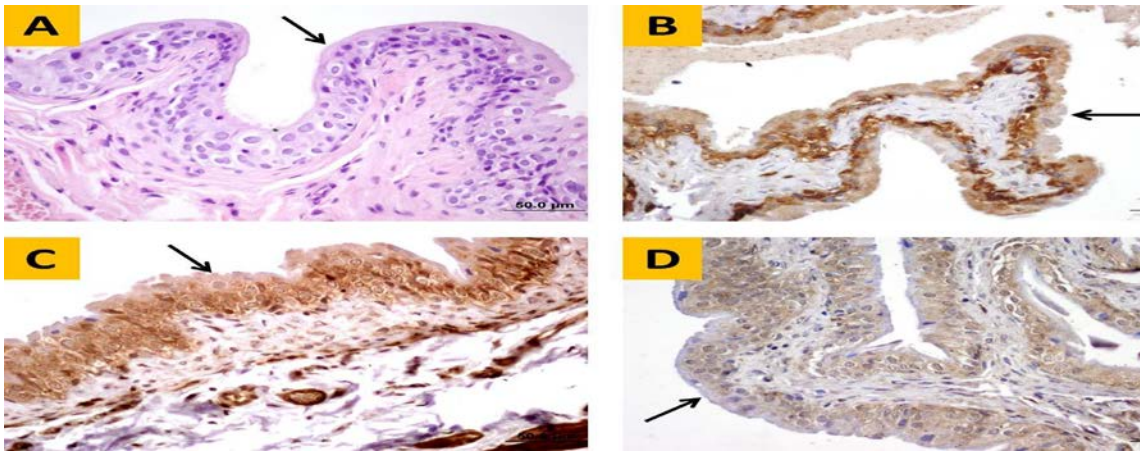

Figure 2. Bladder sections of HT (100 mg/kg)-treated rats. A: Bladder lumen is covered by typical transitional epithelium *(arrow)*, hematoxylin-eosin. B: Epithelial cells immunostained negatively *(arrow)* with apoptotic protein caspase 3. C: Epithelial cells immunostained positively *(arrow)* with anti-apoptotic protein Bcl-2. D: Epithelial cells immunostained negatively *(arrow)* with apoptotic protein Bax. Bars are 50 µm.

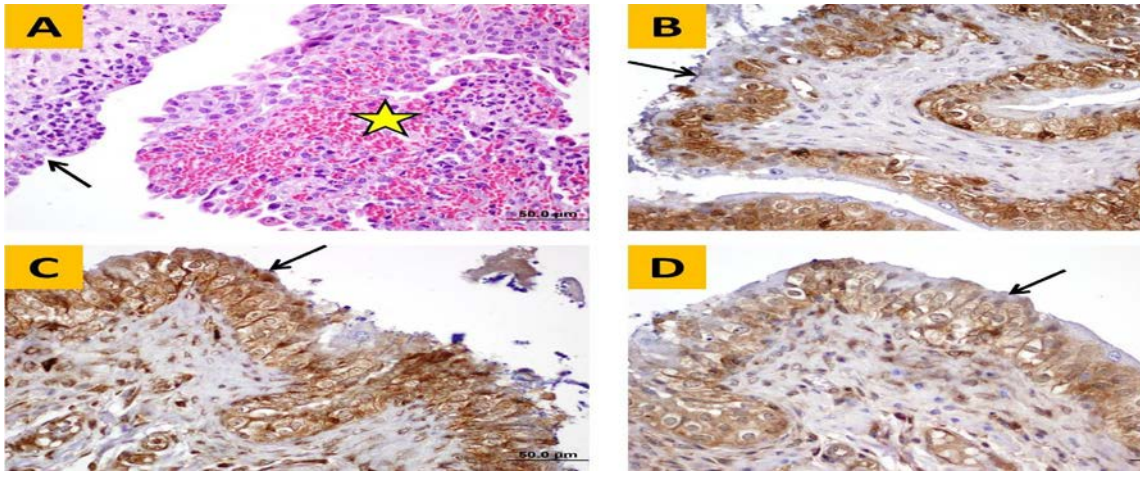

Figure 3. Bladder sections of CYP (150 mg/kg) + HT (100 mg/kg)-treated rats. A: Bladder lumen is full with purulent material *(star)* and haemorrhage. Inflammatory cells also is seen in the epithelium *(arrow)*, hematoxylin-eosin. B: Some of epithelial cells immunostained negatively *(arrow)* while others immunostained positively with apoptotic protein caspase 3. C: Epithelial cells substantially immunostained positively *(arrow)* with anti-apoptotic protein Bcl-2. D: Some of epithelial cells immunostained negatively *(arrow)* while others immunostained positively with apoptotic protein Bax. Bars are 50 µm.

In the renal sections to which Bcl-2 immunohistochemistry was applied, there were no significant differences between the control, 25, 50, 100 mg/kg HT treated groups and 0.2% DMSO treated group. Bcl-2 positivity was found to be low in Group 2 treated with CYP. The positive staining increased in the CYP + HT treated groups (groups 6,7 and 8) as the HT dose increased. In the renal

sections to which Caspase-3 and Bax immunohistochemistry were applied, the positivity was found to be high in Group 2 treated with CYP. The positivity was lower in the CYP + HT treated groups as the HT dose increased (Figures 4-6).

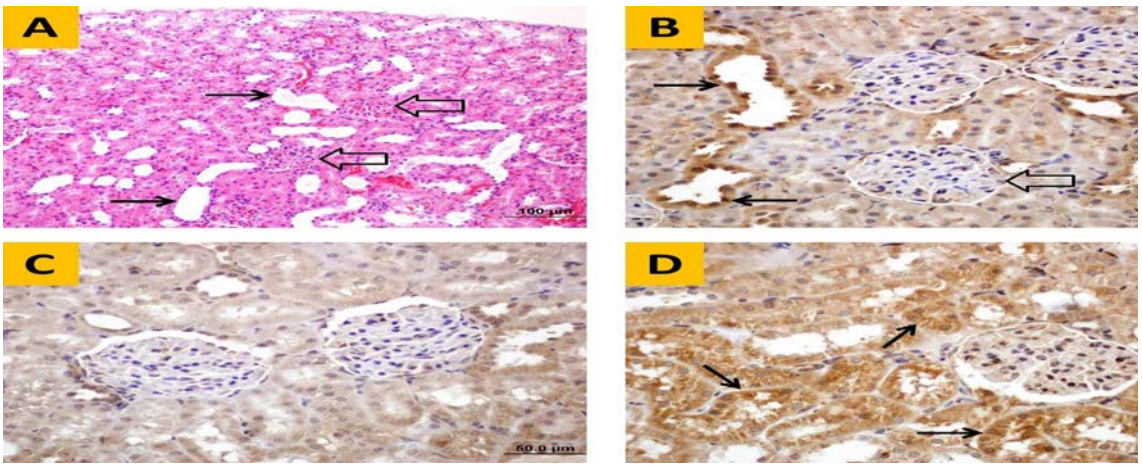

Figure 4. Kidney sections of CYP (150 mg/kg)-treated rats. A: Distal tubules are dilated *(thin arrows)* and glomerules are atrophic with narrowed Bowman's spaces *(thick arrows)*, hematoxylin-eosin. B: Distal tubular cells *(thin arrows)* immunostained with apoptotic protein caspase 3 and atrophic glomerules *(thick arrow)*. C: Tubular cells generally immunostained negatively with anti-apoptotic protein Bcl-2. D: Tubular cells *(thin arrows)* immunostained with apoptotic protein Bax. Bars 100 µm in A and 50 µm in B, C and D.

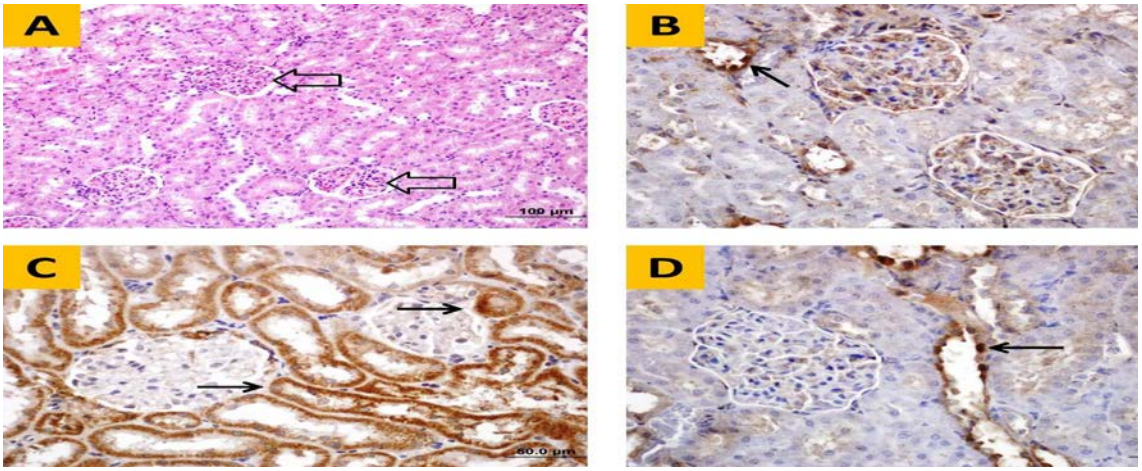

Figure 5. Kidney sections of HT (100 mg/kg)-treated rats. A: Tubules and glomerules *(thick arrows)* show typical histological features, hematoxylin-eosin. B: There is only a few distal tubular cells *(thin arrow)* immunostained with apoptotic protein caspase 3. C: Tubular cells immunostained positively *(thin arrows)* with anti-apoptotic protein Bcl-2. D: There is only a few distal tubular cells *(thin arrow)* immunostained with apoptotic protein Bax. Bars 100 µm in A and 50 µm in B, C and D.

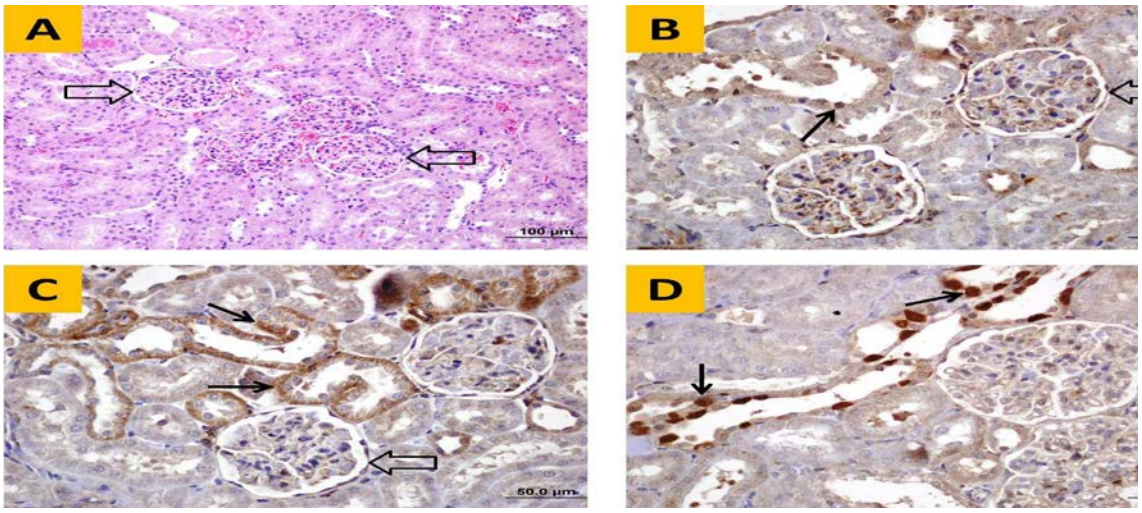

Figure 6. Kidney sections of CYP (150 mg/kg) + HT (100 mg/kg)-treated rats. A: Tubules and glomerules *(thick arrows)* show typical histological features, hematoxylin-eosin. B: There is only a few distal tubular cells *(thin arrow)* immunostained with caspase 3 and typical glomerules *(thick arrow)*. C: Tubular cells of some tubules immunostained positively *(thin arrows)* with anti-apoptotic protein Bcl-2 which are protected tubules and glomerules *(thick arrow)* are typical. D: There is only a few distal tubular cells *(thin arrows)* immunostained with apoptotic protein Bax. Bars 100 µm in A and 50 µm in B, C and D.

DISCUSSION

The aim of a commonly used antineoplastic drug such as CYP is to inhibit the growth and proliferation of the cancer cell without damaging the normal cells of the patient or, if possible, to eliminate them completely. However, while they destroy cancer cells that are proliferating pathologically in the body, they may also destroy normal cells that are rapidly proliferating as well. Yildiz et al.[27] reported that CYP therapy has an anti-angiogenic effect on tumour cells[26]. However, in order for the therapy to be effective, a high dose of CYP needs to be used while eliminating its side effects at the same time (150 mg/kg i.p.). CYP-induced (150 mg/kg) bladder inflammation is a well-established model of interstitial cystitis/painful bladder syndrome. It has been indicated in studies in the literature that rat bladders can be damaged by CYP (200 mg/kg i.p.), which shows its toxic effects when activated in urine[4]. Various studies have also shown that 40% of patients develop HC after a long-term or high-dose CYP therapy[28,29]. In addition, CYP therapy has been shown to cause nephrotoxicity[4].

Oxidative stress is known to have a significant role in CYP-related HC. In the present study, the biochemical levels of lipid peroxidation marker indicated a remarkable increase while the antioxidant defence enzyme indicated a remarkable increase in the CYP-treated rats[3,18] and nephrotoxicity. Superoxide dismutase (SOD) is the most prominent antioxidant enzyme to cope with oxyradicals. Catalase (CAT) is a peroxisomal hem protein that catalyzes the elimination of H_2O_2 created during the reaction catalyzed by SOD. Therefore, SOD and CAT behave as mutually helpful antioxidative enzymes that provide defence against ROS[30]. In this study, the SOD and CAT levels were found to be low in Group 2 which shows that CYP causes kidney damage (Table 1). In parallel to the present study, researches in the literature have showed that there was a significant decrease in the SOD level in the kidneys of CYP-treated rats[31,32]. However, CYP + HT treatment in the present study showed increased SOD and CAT levels. As can be seen from the results of this study, while SOD and CAT levels decreased in the CYP treated group 2, it increased in the CYP + HT treated groups as HT dose increased (Table 1).

Increase in serum creatinine levels is a significant signal for nephrotoxicity and renal injury. Kidney injury is one of the dose-limiting adverse effects of CYP[33]. In this study, the serum creatinine and blood

urea nitrogen (BUN) levels were found to be high in Group 2, whereas these levels were lower in the CYP + HT groups (Table 1). The effects of HT on BUN and serum creatinine and renal function in CYP-treated rats were also determined in this study (Table 1). Moreover, HT doses showed an important increase in the serum creatinine levels. This study showed that HT could effectively reduce CYP-related renal dysfunction. In addition, Liu *et al.*[27] reported a remarkable CYP-related decrease in the serum BUN and creatinine levels. In another study, it was reported that acrolein causes damage to the urinary epithelium and increases creatinine level[34,35] showed that the serum creatinine and BUN levels increased in rats given 75 mg/kg CYP. The results of the present study showed a remarkable increase in the serum creatinine and urea levels after the CYP treatment[36].

Creatinine evaluation is extremely useful in diagnosing renal failure and diseases where a directly proportional relationship exists between renal function and creatinine[37]. After the CYP treatment, the increase in the serum creatinine and uric acid levels might be due to the leakage of the substance into the circulatory system[38]. It was seen that the serum creatinine level significantly increased after the CYP treatment compared to the control group[32]. Nephrotoxicity is an unpleasant complication that develops in chemotherapy patients on CYP therapy[7,39]. Nephrotoxicity and renal injury are characterized by the remarkable increase in the serum creatinine and uric acid levels[2].

As can be seen, there is a broad range of animal model presentations related to CYP cystitis. Researches have demonstrated that CYP can lead to comprehensive bladder inflammation and mucosa damage[40-42]. In the CYP-treated rats, bladder demonstrated severe epithelial ulceration, haemorrhage, inflammatory cells infiltration, submucosal oedema and desquamated granular uroepithelium cells[43]. CYP treatment may significantly increase neuronal activity in the bladder[44]. Inflammation and haemorrhage could not be prevented completely in the bladder tissues of groups 6, 7 and 8 that were given CYP + HT, while damage and changes in the bladder tissue decreased further as HT dose increased (Figures 1-3). Furthermore, in Group 2 treated with CYP, inactivity, fatigue and bloody urine were observed. There was no bleeding in urine observed in the CYP + HT groups.

In the bladder sections to which Bcl-2 immunohistochemistry was applied, there were no significant differences between the control, 25, 50, 100 mg/kg HT treated groups and 0.2% DMSO treated group. It was found that Bcl-2 positivity was low in Group 2 treated with CYP, and in the groups 6, 7 and 8 treated with CYP + HT, positive staining increased gradually as HT dose increased. In the bladder sections to which Caspase-3 and Bax immunohistochemistry were applied, the positivity was higher in Group 2. The positivity increased in the groups 6, 7 and 8 treated with CYP + HT as the HT dose increased (Figures 1-3).

Nephrotoxicity that occurs due to cytotoxic drugs is one of the most common side effects of chemotherapy. The physiological, anatomical and biochemical properties of the kidney make it especially susceptible to many drugs and toxins. In this study, the renal tissue had normal histology in the control, 25, 50, 100 mg/kg HT treated groups and 0.2% DMSO treated group. In the kidneys of the rats in Group 2 treated with CYP, the dilatation of distal tubules, compacting of glomeruli and narrowing of bowman capsule were observed (Figures 4-6). Serum creatinine level increased in group 2 due to glomerular dysfunction. Amien *et al.*[32] reported that CYP (150 mg/kg) caused dilation and congestion of renal blood vessels, vacuolation of epithelial lining renal tubules and atrophy of glomerular tuft. In another study, it was observed that CYP treatment in rats resulted in medium kidney injury on histological examination[45]. In the CYP administration, it is aimed to show that complementary antioxidant agents are able to prevent kidney damages. In the present study, it was found that the tubular and glomerular contents of the renal tissues of groups 6, 7 and 8 treated with CYP + HT decreased and these changes improved greatly as HT dose increased (Figures 4-6).

In the renal sections to which Bcl-2 immunohistochemistry was applied, there were no significant differences between the control, 25, 50, 100 mg/kg HT treated groups and 0.2% DMSO treated group. Bcl-2 positivity was found to be low in Group 2 treated with CYP. Following the CYP treatment, apoptosis was one of the most important mechanisms that play a role in renal function. CPY exposure caused an imbalance in apoptosis-related proteins such as pro-apoptotic protein Bax and anti-apoptotic protein Bcl-2. The overexpression Bcl-2 can reduce renal damage in animal models, while the extreme production of Bax induces cell-apoptosis[46].

The positive staining increased in the CYP + HT treated groups (groups 6,7 and 8) as the HT dose increased. In the renal sections to which Caspase-3 and Bax immunohistochemistry were applied, the positivity was found to be high in Group 2 treated with CYP. The positivity was lower in the CYP + HT treated groups as the HT dose increased (Figures 4-6).

In conclusion: CYP is commonly used in combination with various protective and detoxifying agents in order to decrease or remove its toxic side effects. CYP-related urotoxicity remains a prevalent problem in the setting of treatment of a multitude of oncologic conditions. Antioxidants such as HT possess protective effects against CYP-related acute urotoxicity. Accordingly, CYP in combination with a potent antioxidant may be suitable to lessen the urotoxic side effects of the drugs. Based on the biochemical, histopathological and immunohistological results of CYP on rats' urinary bladder and kidney in the present study, it is possible to say that HT could lessen urotoxicity by means of its antioxidant features. Thus, a therapy carried out using natural agents can be regarded as a remarkable promising future alternative to refrain from toxic side effects of CYP. In conclusion, the present study demonstrated that HT showed a protective effect on CYP-related bladder HC and nephrotoxicity in rats by inhibiting inflammation and apoptosis.

Yazar Katkıları: Çalışma konsepti/Tasarımı: SÇY; Veri toplama: SÇY; Veri analizi ve yorumlama: SÇY, CK, VS, AA; Yazı taslağı: SÇY; İçeriğin eleştirel incelenmesi: SÇY, CK; Son onay ve sorumluluk: SÇY, CK, VŞ, AA; Teknik ve malzeme desteği: -; Süpervizyon: SÇY, CK, AA; Fon sağlama (mevcut ise): yok.
Etik Onay: Bu çalışma, Eskişehir Osmangazi Üniversitesi Rektörlüğü Yerel Etik Deneyleri tarafından 444-1 / 2015 sayı ile onaylanmıştır.
Hakem Değerlendirmesi: Dış bağımsız.
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