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Protective effects of ursolic acid on cyclophosphamide induced hemorrhagic cystitis in rats

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Cyclophosphamide (CP) is a commonly used antineoplastic agent despite its adverse effects. Hemorrhagic cystitis (HC) is one of the most common side effects of CP. Although Mesna is commonly used to prevent HC, it suffers from few side effects. Therefore, search for better alternate substance still continues. In this study, we studied the effects of ursolic acid (UA), one of the active ingredients of *Plantago major*, commonly called plantain or white man's foot, in CP-induced hemorrhagic cystitis in rats. Forty-nine Sprague–Dawley rats in seven equal groups viz. control (C); cyclophosphamide (CP); cyclophosphamide+Mesna (CPMesna); cyclophosphamide+ursolic acid [100 (CPUA100) and 200 mg (CPUA200)], and cyclophosphamide+ mesna+ ursolic acid (100, (CPMesnaUA100) and (200 mg (CPMesnaUA200) were investigated for inducible nitric oxide synthetase (iNOs), total antioxidant status (TAS), total antioxidant status (TOS), TNF–α, interleukin 10 (IL–10) and interleukin 6 (IL–6) levels from blood samples. The data obtained were statistically analyzed using SPSS and both Kruskall Wallis and Dunn tests were performed. Value of P <0.05 was considered as statistically significant. Our results have revealed that the UA applied groups better preserve compared to CP group. However, it was observed that this protection is comparatively weaker than the Mesna and UA-Mesna combinations. Hence, it is suggested that the urosilic acid could prevent cyclophosphamide induced hemorrhagic cystitis, and also extends support to Mesna applications. Overall, it has been observed that ursolic acid has limited protective effects against CP-induced hemorrhagic cystitis, and can serve as a supportive agent in inhibiting hemorrhagic cystitis.

Keywords: Antineoplastic, Cancer chemotherapy, Mesna, Plantain, White man's foot

Cancer is one of the leading causes of adult mortality worldwide, particularly in the economically developed nations. It is reported to be the second most common cause of death in United States[1]. The chemotherapy regimens used to treat different types of cancer are insufficient since they frequently lead to allergies and nephritis and tend to harm DNA in healthy cells[1,2].

The DNA-alkylating chemotherapy drugs cyclophosphamide (CP) and ifosfamide (IF) (oxazaphosphorins), frequently used to treat different leukemias, myelomas, and lymphomas, have cytotoxic and antiproliferative effects[2,3]. Side effects include hemorrhagic cystitis (HC), delayed wound healing and nephropathy, which can develop due to infection, malignancy, chemical exposure and radiotherapy[4,5]. Hemorrhagic cystitis is prevalent in patients receiving intravenous high-dose cyclophosphamide[2,6].

Hemorrhagic cystitis is a symptomatic case with potential mortality that develops as a complication in some systemic situations with exposure to a drug, environmental toxins, pathogens (bacteria or viruses), ionizing radiation, and infectious disease factors. HC is a hemorrhagic and inflammatory condition of the bladder[3,7]. Activation of inflammatory molecular pathways known as pyroptosis, results in a broad cell death reaction whcih causes inflammation in HC[3]. Hematuria, dysuria, pollakiuria, suprapubic discomfort, and consequences like bleeding, necrosis, fibrosis, and bladder contracture are all indications of hemorrhagic cystitis[5,6,8].

Chemoteuropathic medicines, viz. cyclophosphamide and its synthetic equivalent, ifosfamide, though do not directly affect the bladder, produce HC through metabolic byproducts[6,9]. Literature reports 12-41% of HC cases to be CP-induced[10]. Acrolein, a byproduct of cyclophosphamide induces HC as well as lung and cardiac damage[10]. Hepatic microsomal hydroxylation converts CP to acrolein, which is then eliminated by

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the kidney into the urine bladder (UB)[5]. When acrolein comes into direct contact with the urethra, it can cause CP-related hemorrhagic cystitis, which is a sign of bladder inflammation and causes bladder dysfunction by damaging the urothelium and necrosizing bladder tissue[2,5,6].

The cyclophosphamide treatment dose has harmful effects such as declined antioxidant activity and increased acrolein-related lipid peroxidation on a number of systems, including the urinary system[11-14]. Further, levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS) rise with increased oxidative stress, and antioxidant defense mechanisms decline[5].

The medicine 'Mesna', which is now licensed for the treatment of CP-induced hemorrhagic cystitis, works by detoxifying the harmful CP metabolite[2]. When mesna is administered, acrolein is directly bound in the urinary bladder lumen and neutralized to an inactive metabolite (thioether) that can be safely eliminated in the urine without causing urothelial damage[5]. Acrolen's contact time with the bladder mucosa during the biotransformation of CP is minimized by hyperhydration, forced diuresis, frequent urination, urethral catheterization, diuresis alkalization, which is one of the common prophylaxis methods in HC, as well as the concurrent use of mesna or other agents[10,15]. Although prophylactic use of mesna is advised for prevention of HC, reports indicate that mesna is ineffective in treating or alleviating specific clinical symptoms[2,3,5]. To lessen the risks, it is crucial to combine mesna with other preventive antioxidants such as ursolic acid (UA).

Ursolic acid is present in the commono plantain or white man's foot *Plantago major* as well as a variety of other fruits, vegetables and medical plants[16]. It possesses biological advantages such as anti-inflammatory, anticarcinogenic, antiulcerogenic, antimicrobial, hepatoprotective, neuroprotective, and neuroregenerative properties, as well as antidiabetic, antiviral, and antibacterial effects[17-19]. One of the most well known sources of triterpene comes from the Lamiaceae family of medicinal plants, but in recent years, UAs have also been found in a number of other families as well[16,20]. An abundant component of plants and foods, UA is a pentacyclic triterpenoid carboxylic acid, produced by plants and mammals in a manner similar to that of steroid hormones[21,22]. Apple peels, rosemary, thyme, ocimum species, and a variety of other herbs and spices are important sources of phytocomponents[17].

Research on ursolic acid and its derivatives have demonstrated that they have a tendency to lower the levels of TNF-α, NF-κB and other inflammatory proteins, as well as inflammation and oxidative stress in tuberculosis-infected cells[16]. However, there are no studies availabe on the protective/curative effects of UA in hemorrhagic cystitis until now[2,3,5,10,15]. Hence, in this study, we investigated the effects of ursolic acid on hemorrhagic cystitis.

Materials and Methods

Materials

Cyclophosphamide (CP) (endoxan®, 150 mg/kg Eczacibasi Istanbul, Türkiye, $C_7H_{15}Cl_2N_2O_2P$ Cas 50-18-0), Mesna (uromitexan®, 400 mg, Eczacıbaşı Baxter, Türkiye, $C_2H_5NaO_3S_2$, Cas 19767-45-4), and ursolic acid (UA) (Sigma, Cas 77-52-1) were purchased commercially. This study was carried out at Experimental Animal Research and Application Center, Afyon Kocatepe University with necessary approval (AKUHADYEK-493-15, 01.09.2015). Forty-nine male Sprague Dawley rats weighing 220-250 g were made into 7 experimental groups each comprising 7 rats as given in Table 1, and were given *ad libitum*

Table 1 — Experimental and control groups

Experimental and control groups	No. of animals/ group	No. of iterations	Total No. Of animals used/group
Gr. I (Control): Sham (–) control only i.p. isotonic will be applied	7	1	7
Gr. II (CP): CP 150 mg/kg i.p.	7	1	7
Gr. III (CPMesna): CP 150 mg/kg i.p.+ mesna 30 mg/kg (20 min before CP) 30 mg/kg mesna at the 4th h and 30 mg/kg mesna additional dose i.p. at 8 h.	7	1	7
Gr. IV (CPUA100): CP 150 mg/kg i.p.+100 mg UrsolicAsiti.p	7	1	7
Gr. V (CPUA200): CP 150 mg/kg i.p.+200 mg UrsolicAsiti.p	7	1	7
Gr. VI (CPMesnaUA100): CP 150 mg/kg i.p. + mesna 30 mg/kg (20 min before CP) 100 mg/kg Ursolic Acid at the 4th and 8th h	7	1	7
Gr. VII (CPMesnaUA200): CP 150 mg/kg i.p. + mesna 30 mg/kg (20 min before CP) 200 mg/kg Ursolic Acid at the 4th and 8th h	7	1	7

standard rodent feed and tap water during the experiment. They were housed in polycarbonate cages, maintained on 12 h light/dark cycle at room temperature (22±0.5°C) and in an appropriate humid environment and the experiment started after a week of adaptation.

Sample collection and biochemical analysis

At the end of the experiment, the animals were anesthetized with 5 mg/kg of xylazine HCl and 50 mg/kg of ketamine HCl. Anesthetic depth was kept under control by observing pinching, muscle tonus, and palpepral reflexes. Blood samples taken from all animals under anesthesia with the help of an injector with intra-cardiac puncture were taken into gel glass serum and hemogram tubes. Blood samples taken from rats were centrifuged at 2500 rpm for 10 min with NF 1000 R centrifuge device and their serum and plasma were separated. They were kept in a deep freezer until the biochemical measurements were carried out (–80°C). total antioxidant statue (TAS), total oxidant statue (TOS), ınducible nitric oxide synthesis (iNOS), ınterleukin 10 (IL-10), ınterleukin 6 (IL-6), tumour necrosis factor-alpha (TNF-α) levels determined by plasma samples.

Plasma total antioxidant statue (TAS), total oxidant statue (TOS) and ınducible nitric oxide synthetase (iNOS) levels

TAS measurements were carried out through spectrophotometric (Biotek, ELx800) reading 5 min after the samples and reagents were mixed following the method developed by Erel[23]. The measured TAS levels were divided into total protein levels and expressed as mmolTrolox Equivalent/g-protein. TOS measurements were again carried out through spectrophotometric reading at 560 nm 3-4 min after the samples and reagents were mixed by the method developed by Erel[24], and the results were expressed as hydrogen peroxide equivalent liter/g-protein (µmol H_2O_2 Equiv./g-protein).The iNOS level was measured on an ELISA device as per the steps defined in the commercial kit.

Analysis of cytokine levels

Levels of inflammatory cytokine as TNF–α, IL-10 and IL–6 were analyzed on an ELISA device (Biotek ELx800) following rat-specific kit (e-Bioscience, Vienna, Austria) protocols in the present study.

Statistical analysis

The data obtained from the study were evaluated using SPSS 20.0 statistics software. The averages of the data were expressed with mean ± SE. The normality tests of the obtained data were performed and Kruskall-Wallis test was used to detect the statistical differences among the groups and the Dunn test from the multiple comparison tests was utilized in the groups where differences were detected. The statistical significance was accepted as P <0,05[25].

Results

Plasma TAS, TOS and iNOS levels

Total antioxidant statue (TAS), Total oxidant statue (TOS) and Inducible nitric oxide synthesis (iNOS) levels were determined in the plasma samples taken from the seven experimental groups established in our study. Statistical values and comparisons of the findings of the plasma levels of the respective groups are indicated in Table 2. There was no statistically significant difference observed in plasma TAS levels among the groups. However, we noticed a decrease in CPUA100 and CPMESNAUA200 groups, and an increase in other groups as compared to the control group (Gr. I). However, TOS levels showed a significant increase in TOS level in CPMESNA and CPMESNAUA200 groups compared to CP and CPUA100 groups (P <0.05). However, in other groups, it was observed the plasma TOS level to be at par with the control group.

The iNOS levels also showed a significant increase in CPMESNA and CPUA100 groups compared to CPMESNAUA200 group (P <0.05). However, the iNOS levels of other groups, similar to the TOS levels were the same as that of the control group.

Table 2 — Plasma TAS (µmol Trolox Eqv. /L), TOS (µmol H2O2 Eqv. /L) and iNOS levels of groups

Groups	N			Medıan			Q1			Q3			χ^2			P		
	TAS	TOS	iNOS	TAS	TOS	iNOS	TAS	TOS	iNOS	TAS	TOS	iNOS	TAS	TOS	iNOS	TAS	TOS	iNOS
I	7	7	7	0,93	5^{ab}	$20,94^{ab}$	0,85	4,53	19,20	1,32	5,07	22,63						
II	7	7	7	1,43	$4,30^{b}$	$20,64^{ab}$	1,38	3,84	19,01	1,69	4,30	23,46						
III CPMESNA	7	7	7	1,68	$5,92^{a}$	$23,64^{a}$	0,94	5,76	22,05	1,95	6,23	25,09						
IV (CPUA100)	7	7	7	0,84	$4,30^{b}$	$22,46^{a}$	0,66	3,84	20,61	1,49	5,00	26,75	9.045	21.919	21.093	0.171	0.001	0.002
V (CPUA200)	7	7	7	1,55	$4,53^{ab}$	$22,72^{ab}$	0,83	4,00	15,98	1,89	4,76	23,50						
VI (CPMESNAUA100)	7	7	7	1,57	$5,19^{ab}$	$18,09^{ab}$	0,83	3,69	16,63	2,07	6,44	19,36						
VII (CPMESNAUA200)	7	7	7	0,86	$6,00^{a}$	$16,27^{b}$	0,67	5,61	14,64	1,31	8,15	17,79						

Table 3 — Plasma IL-6, IL-10 and levels of groups

Groups	N			Medıan			Q1			Q3			χ^2			P		
	IL-6	IL-10	TNF-α	IL-6	IL-10	TNF-α	IL-6	IL-10	TNF-α	IL-6	IL-10	TNF-α	IL-6	IL-10	TNF-α	IL-6	IL-10	TNF-α
I	7	7	7	91.50^{ab}	37.61	70.64	26.50	15.69	60.75	186.50	46.46	128.28						
II	7	7	7	159.00^{ab}	17.61	60.75	116.50	14.53	60.75	189.00	29.15	75.96						
III CPMESNA	7	7	7	136.50^{ab}	18.76	70.76	114.00	7.81	63.22	144.00	28.76	106.97						
IV (CPUA100)	7	7	7	149.00^{ab}	14.53	70.64	79.00	7.23	51.82	174.00	25.69	128.28	16.44	5.276	4.513	0.012	0.509	0.608
V (CPUA200)	7	7	7	79.00^{b}	26.07	60.87	51.50	2.23	55.08	104.00	26.07	87.78						
VI (CPMESNAUA100)	7	7	7	79.00^{b}	13.76	70.64	46.50	11.76	56.17	116.50	37.61	136.13						
VII (CPMESNAUA200)	7	7	7	214.0^{a}	22.23	106.53	114.00	14.15	60.87	236.50	46.46	399.41						

Plasma IL-6, IL-10 and TNF-α Levels

The IL-6, IL-10 and TNF-α levels were determined in the plasma samples taken from the seven experimental groups as detailed in the Methods. Statistical values and comparisons of the findings of the plasma levels of the respective indicators are given in Table 3. There was a significant increase in IL-6 levels in CPMESNAUA200 group compared to CPUAS200 and CPMESNAUA100 groups (P <0.05). However, no statistically significant difference was observed in either plasma IL-10 or plasma TNF-α levels among the groups. While there was no change in the IL-6 level in other groups, compared to the control group, IL-10 level showed a decrease in all groups compared to the control group. The CPMESNAUA100 group had the lowest level. For TNF-α, the CPUA100 and CPMESNAUA100 groups had the same levels, and the CPMESNAUA200 group had the highest level. The CP group showed the lowest level.

Discussion

In the past, various classical options were tried for prevention and treatment of cyclophosphamide (CP) induced hemorrhagic cystitis (HC). However, the same effect and success could not be achieved for each patient. The purpose of current studies was to keep both the oxidant-antioxidant balance and remove or minimize the symptoms related to HC; therefore, reinforcing the physical and mental adaptations of the patients to daily life.

Mesna (2-mercaptoethan sodium sulfonate) is routinely used in the treatment of hemorrhagic cystitis and it has been shown that this well-tolerated agent cannot reduce the chemotherapeutic side effects[26]. Few studies have shown that mesna could not remove some clinical, macroscopic, and microscopic symptoms including side effects viz. allergic reactions, dermatosis and hypersensitivity[27,28].

The clinical application dose of CP, 150 mg/kg of dose, was adopted in this study and again intraperitoneal administration way was preferred as the clinical application. To see the effect of UA against bladder damage caused by cyclophosphamide, a working mechanism with three controls and the experimental groups were made as per the experimental desgine as given in Table 1. The most effective protocol was obtained in HC related to cyclophosphamide when mesna is given before 20 min through IP and before 60 min orally from the chemotherapeutic agent[29]. Active ingredients were applied alone and also in combination with mesna to protect from the leading acrolein damage in some alternative studies and it was expressed that successful results were obtained from these applications[30]. From this point of view, CPUA100 (Gr. IV), CPUA200 (Gr. V), CPMesnaUA100 (Gr. VI) and CPMesnaUA200 (Gr. VII) groups were established.

Studies on different active substances related to hemorrhagic cystitis were carried out. Kılıç *et al*.[31] in their CP-related HC model administered ankaferd and epinephrine and presented in the light of their results that both two agents had effects in inhibiting congestion and edema. Xu & Malavé[32], in their CP-related HC model with Berberin, have mentioned that the active substance completely blocks the hemorrhage and edema in the bladder in the increasing doses. Santos *et al*.[33] presented that the active contents of *Mandevilla velutina* inhibited the CP-related bladder hemorrhage and the increase in weight and MV8608, one of the contents of *Mandevilla velutina* inhibited polymorphic cell accumulation in the bladder. Keles *et al*.[12] in their study with different doses of resveratrol indicated that the active substance inhibited congestion, edema, and hemorrhage. However, this effect was weaker than that of mesna.

Hamsa & Kuttan[34] stated that *Ipomoea obscura* had an anti-inflammatory effect over proinflammatory cytokines. Boeira *et al*.[35] mentioned the anti-inflammatory effects of *Phyllanthus niruri* in in their CP-induced HC model. In the cyclophosphamide-

related HC model, it was presented that ankaferd and epinephrine had an effect to inhibit necrosis and ulceration; however, ankaferd was found more successful in epithelium regeneration[31]. Curcumin from *Curcuma longa*, was found to have a ureteral protective feature in CP-related HC[36]. It was also determined that *Alpinia oppicinarrum* (Malsyda), *Vitis vinifera* (Baghi) and *Timus vulgaris* included in ankaferd, which are used against hemorrhagic cystitis, had an antioxidant effect and inhibited the lipid peroxidation[37]. Curcumin showed antioxidant features as well in CP-related HC along with its ureteral protective effect[36]. Boeira *et al*.[35] in their CP-induced HC model obtained high antioxidative and anti-inflammatory results from the hydroalcoholic extract of *P. niruri* and suggested this extract against the side effect of mesna besides the toxic effects of CP. TAS and TOS values in blood samples were analyzed in our study, and the results are presented in Table 2. No statistically significant difference was observed in TAS values among the groups. However, there was a decrease in CPUA100 and CPMESNAUA200 groups compared to the control group, but an increase in other groups compared to the control group. In plasma TOS level, a significant increase was observed in CPMESNA and CPMESNAUA200 groups compared to CP and CPUA100 groups (P <0.05). However, in other groups, plasma TOS level is observed as in the control group. These findings are different from the studies indicating the oxidative stress table caused by CP. Kankaya[38] has shown that the CP increased TOS level and mesna repaired the oxidative damage caused by CP. However, in our study, CP decreased oxidative status, but increased antioxidant status contradictory to the above observation. Mesna given with CP again increased the oxidant status decreased by CP. It was observed that ursolic acid (UA) decreased the oxidant status increased by mesna when it was used with CP and adding mesna to UA and CP combination increased the oxidant status. This indicates that UA is probably more effective in reducing oxidant status, not in combination with mesna, but in its alone use. In addition, it was observed that the most effective dose of ursolic acid in reducing total oxidant status was the dose of 100 mg/kg when the UA-given groups were analyzed and on contrary, the dose of 200 mg/kg combined with mesna increased the total oxidant status and the dose of 200 mg/kg alone and 100 mg/kg combined with the mesna increased the total antioxidant status above the control group level although it was not statistically significant. The fact that the dose of UA200 is the dose that both increases the oxidant status most and lowers the antioxidant status most makes us think that it is an ineffective dose for ursolic acid.

Nitric oxide (NO) is a colourless, small molecule, oil-soluble neurotransmitter substance with a toxic effect and a high reaction capability that has a short half-life and can easily pass through cell membranes[39]. As a result of stimuli created by cytokines in the immune system (IL-1α, IL-1β, TNF-α, IFN-γ) and endotoxins, NO synthesis with nanomolar quantity which starts in just a few hours and lasts for days occurs[40]. Tumor necrosis factor-alpha (TNF-α) is a cytokine that plays an important role in inflammation, the progression of cell life, and the process of cell death. This molecule makes the macrophages different and increases NO generation by activating inducible nitric oxide synthesis (iNOS) like IL-1β[41]. The iNOS is not synthesized inside the cell structurally, its synthesis is initiated via an appropriate immune stimulus. Unlike other NO synthetase isoforms, it synthesizes NO as it is in the setting and the amount of NO synthesized by iNOS against cytotoxic and harmful effects is high. It was reported that NO, which was overproduced by macrophages, especially with bacteria, high lipopolysaccharide stimuli, generated cytostatic or cytotoxic effects in foreign cells such as bacteria, parasites, and tumors[40].

Acrolein, which comes out with the transformation of cyclophosphamide in the body used alone or in combination with other antineoplastic drugs in the treatment of various malignancies, leads to hemorrhagic cystitis increasing the release of inflammatory mediators. Acrolein quickly enters the uroepithelium due to its chemical nature and leads to the overproduction of NO in the bladder epithelium and causes both direct and indirect iNOS induction and an increase of reactive oxygen species (ROS). Acrolein enters the uroepithelium and causes ROS production, iNOS induction, and activation of transcription factors (e.g. NF-κB and AP-1). Activated NF-κB and AP-1 cause cytokine (TNF-α, IL-1β) gene expression, iNOS induction and again ROS production.Therefore, harmful molecules such as ROS, No, and cytokines dramatically increase. ROS lipid attacks cellular macromolecules such as DNA and protein and leads to damage[13]. It is known that NO is the ultimate agent for hemorrhage and urothelial damage in the formation of cystitis. The

existence of iNOS in the inflamed bladder necessitates the platelet-activating factor (PAF) and the efficiency of TNF-α. Moraise *et al*.[42], in their study, stated that corticoids which is an inhibitors in the synthesis of cytokines such as TNF-α ve IL-1, PAF, iNOS, and an immune suppressor reduced the incidence of CP-induced cystitis.

Topal *et al*.[43], found the iNOS level high only in the CP-given group in their study they investigated the curative effect of melatonin in CP-induced bladder damage. Again Al-Malki[44] has reported that CP increased MDA and NO levels, but decreased SOD, CAT and GPx levels as compared to the control group. Oter *et al*.[45] analyzed the inhibition of iNOS in CP-induced HC and presented that S-methyliso-thiourea (SMT; 20 mg/kg/day), a picky iNOS inhibitor, inhibited severe cystitis caused by CP. Again Sadir *et al*.[46] in their study found that CP increased the iNOS activity in the bladder, but decreased in the melatonin-applied group. Malley *et al*.[47] designed an experimental animal model that assessed the cytokines at three different times as 4th, 48th and 10th day following the CP administration and identified a significant increase in IL-1β, IL-2 ve IL-6 levels in the 4th h. Sehirli *et al*.[48] identified significant increases in serum levels of proinflammatory cytokines such as TNF-α, IL-1β and IL-6 in the cases treated with iphosphamide and observed that these increases decreased in the groups treated with resveratrol.

Cytokines have important roles in the regulation of the inflammation process in several organs including the bladder. In a clinical study on patients with interstitial cystitis, a significant increase was observed in IL-2, IL-6 and IL-8 levels compared to the control group[49]. Keleş *et al*.[13] in their study found the TNF-α level to be the lowest in the control group compared to the CP group. Further, in the mesna and resveratrol groups, they observed that the TNF-α level decreased compared to the CP group.

Here, we studied iNOS, TNF-α and IL-6 from an interleukin family which had an important role in hemorrhagic cystitis pathogenesis. According to our study results, it was found that the CPMESNA and CPUA100 groups had a significant increase in iNOS level as compared to the CPMESNAUA200 group, however, plasma iNOS level was at the control group level in other groups. Although iNOS level increased in the groups treated with CP in literature findings, this remained at the control group level in our study. iNOS level was not statistically significant in the CPMESNA and CPUA100 groups; however, it slightly increased as compared to the control group and it deviated from the control group level in CPUA100 and CPMESNAUA100 groups. It was concluded that the most effective combination for decreasing the increase in iNOS level was the CPMESNAUA200 group.

We found no statistically significant difference in TNF-α levels among the related groups. In addition, while the control group, CPUA100 (Gr., and CPMESNAUA100 groups were at the same level, the CPMESNAUA200 group had the highest and the CP group had the lowest level in TNF-α level. In this sense, our study is different from the overall literature findings.

According to the obtained data, it is seen that there is a significant increase in IL-6 level of Gr. VII (CPMESNAUA200) as compared to Gr. V (CPUA200) and Gr. VI (CPMESNAUA100) (P <0.05). In other groups plasm IL-6 level was observed to be at par with that of the control group level.

IL-10 is a strong anti-inflammatory cytokine and cytoprotective agent inhibiting the production of other cytokines[50] and pro-inflammatory cytokines suppress the production of ROS and reduce the efficiency of macrophages[51]. Keleş *et al*.[13] in their study found the IL-10 level to be the highest in the control group and the lowest in CP applied group. Further, they determined the existence of a protective effect against the decrease in IL-10 level in the groups treated with mesna and resveratrol. Although no statistically significant difference was observed among the groups in our present study, the IL-10 level was found as the highest in the control group as expected. However, a decrease is observed in other groups as compared to the control group and it is seen that the CPMESNAUA100 group has the lowest level. The most effective dose to protect IL-10 level is ursolic acid (UA) 200 mg combined with CP. This even partially makes us think that organizing anti-inflammatory responses would be important for presenting the useful effects of UA against the negative effects of CP.

Conclusıon

Ursolic acid (UA) has limited protective effects against cyclophosphamide (CP) induced hemorrhagic cystitis (HC). In light of these findings, it was

concluded that UA could be a supportive agent in preventing HC. This protective effect can be attributed to the antioxidant and anti-inflammatory features of UA. The observation that UA reduces the side effects of CP treatment and increases the efficiency of mesna when used together, needs to be further supported with immunohistochemical studies.

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Conflicts of Interest

Authors declare no competing interests.

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