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Effects of histidine and N-acetylcysteine on acute kidney injury induced by doxorubicin in rats: roles of anti-oxidative, anti-inflammatory and anti-apoptotic mechanisms

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Abstract

Doxorubicin (DOX), as a potent anti-cancer agent, exerts side effects in vital organs. Various chemical compounds with tissue protective properties are used to prevent the side effects of DOX. This study was planned to investigate the effects of histidine (HIS) and N-acetylcysteine (NAC) on DOX-induced acute kidney injury. The possible mechanisms were followed by determining the histopathological changes of the kidney along with the biochemical alterations of the blood and kidney tissue. Forty-eight rats were divided into eight groups of six animals each to receive normal saline and DOX after alone and combined treatments with HIS and NAC. The DOX at a single dose of 15.00 mg kg^{-1} was intraperitoneally injected on day one. The separate and combined intraperitoneally injections of HIS and NAC at a similar dose of 100 mg kg^{-1} were began 30 min after DOX administration and continued for seven consecutive days. The DOX increased kidney weight and caused congestion, hemorrhages and degeneration in kidney tissue. It also increased serum urea and creatinine concentrations and kidney tissue levels of malondialdehyde, tumor necrosis factor-alpha and caspase-3, and decreased superoxide dismutase activity in this tissue. Separate and combined treatments with HIS and NAC improved all the above-mentioned effects of DOX. The restoring effects of the combined treatment were more prominent than the effect of amino acids alone. It was concluded that anti-oxidative, anti-inflammatory and anti-apoptotic mechanisms might be related to the tissue protective effects of HIS and NAC against DOX-induced acute renal injury.

Introduction

Acute kidney injury (AKI) is a common clinical syndrome that is characterized by abnormal renal function and structure.[1] The pathophysiology of AKI is very complex, but its main causes are sepsis, ischemia and nephrotoxicity.[2] Conventional chemotherapeutic drugs are first-line agents to treat several malignancies but cause nephrotoxicity, which occurs when kidney excretion properly due to the action of harmful chemicals.[3] Doxorubicin (DOX), has been demonstrated to have significant therapeutic potential for the treatment of various cancers such as breast cancer, carcinomas, sarcomas and hematological malignancies.[4]

Despite widespread use, DOX produces side effects and causes toxicity of the vital organs of the body such as the brain, heart, lungs and kidneys.[5] Many initiatives such as antioxidative, anti-inflammatory, and anti-apoptotic agents, prodrugs and DOX analogues are being used to reduce the side effects of DOX.[6]

Histidine (HIS) is an essential amino acid with important roles in proton buffering, metal ion chelation, scavenging of reactive oxygen and nitrogen species, erythropoiesis, and the histaminergic system.[7] Dietary supplementation of amino acid HIS has demonstrated benefits in various preclinical and clinical conditions such as anorexia, anxiety, stress, sleep disturbances and cancer therapy.[8,9] It has been reported that dietary supplementation of the amino acid HIS in leukemia-bearing mice treated with the commonly used chemotherapy methotrexate significantly improves treatment outcome.[10]

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N-acetylcysteine (NAC) is a synthetic derivative of the endogenous amino acid cysteine and modulates oxidative stress and involves in pathophysiologic processes implicated in disease such as mitochondrial dysfunction, apoptosis, and inflammation.[11] Treatment with NAC, has been demonstrated to exert beneficial effects in dermatological, neurological, renal and pulmonary disorders.[12] The NAC is also a nonantibiotic compound possessing antimicrobial property and exerts anticarcinogenic and antimutagenic effects against certain types of cancer.[13] It has been reported that NAC treatment effectively reduces the reactive oxygen species production and reactive oxygen species-mediated signaling that contribute to cell survival, metastasis, and drug resistance in triple-negative breast cancer.[14]

There is no report describing the effect of HIS on nephropathy caused by DOX, however, separate and combined treatments with HIS and NAC improved toxic effects of DOX on the heart and sciatic nerve.[15,16] On the other hand, NAC has been reported to produce beneficial effects on DOX-induced nephropathy.[17] In the present study the separate and combined treatment effects of HIS and NAC were investigated on the nephropathy induced by DOX. To clarify the possible mechanism of action, kidney functional biomarkers (Urea and creatinine) in the blood and histopathology outcomes and oxidative stress superoxide dismutase (SOD) and malondialdehyde (MDA), inflammatory (tumor necrosis factor-alpha (TNF-α)) and apoptotic (caspase-3) pathways in the kidney tissue were determined.

Materials and Methods

Animals. This study was conducted on male Wistar rats with initial body weights 200 - 220 g. The animals were housed in a ventilated room at a temperature of 22.00 ± 1.00 ˚C with a 12 hr light-dark cycle (lights on: 7:00 AM). Food and water were available *ad libitum*. All research and animal care procedures were approved by Veterinary Ethics Committee of Urmia University Faculty of Veterinary Medicine (Ethical code: IR-UU-AEC-3/32).

Drugs and kits. The HIS and NAC were purchased from Sigma-Aldrich Chemical Co. (St. Louis, USA). Doxorubicin was purchased from Ebedoxo, EBEWE Pharma GmbH, Unterach, Austria. All the analytical chemicals including sodium dodecyl sulphate, acetic acid, thiobarbituric acid (TBA), n-butanol, pyridine, 2,4,6-tripyridyl-S-triazine and $FeCl_3$. $6H_2O$ were purchased from Merck Co. (Darmstadt, Germany). Navand Salamat supplied MDA and SOD assay kits (Urmia, Iran). Moreover, ELISA kits of TNF-α and caspase-3 were purchased form Diaclone SAS, Besancon Cedex, France and Elabscience, Houston, USA, respectively.

Study protocol. After a 2-week adaptation period, on the third week, intraperitoneally (IP) injection of DOX was performed. Separate and combined treatments with HIS and NAC were begun 30 min after DOX treatment on the first day and thereafter continued for six consecutive days. At the end of the experiment (day 7), after weighing the body and kidneys, the animals were anesthetized by intraperitoneal injection of ketamine (100 mg kg^{-1}; Alfasan, Woerden, The Netherlands) and xylazine (10 mg kg^{-1}; Alfasan) and blood and tissue samples were taken for histopathological and biochemical evaluations. Thereafter, the animals were euthanized by intracardiac injection of 20.00 mg xylazine. Initial body weight (day 1), final body weight (day 7) and kidney weight (day 7) were obtained and (final – initial) body weight and kidney-somatic index (KSI) were calculated as below*:*

$$KSI = (kidney\ weight\ /body\ weight) \times 100$$

Animal grouping. In the present study, **48** rats were divided into eight groups of six as follows: Group 1 (normal saline (NS) + NS): In this group, animals received NS twice with an interval of 30 min on the first day and continued once from the second to the 7th day. Group 2 (NS + HIS): In this group, animals received HIS after NS with an interval of 30 min on the first day and continued once from the second to the 7th day. Group 3 (NS + NAC): In this group, animals received NAC after NS with an interval of 30 min on the first day and continued once from the second to the 7th day. Group 4 (NS + HIS + NAC): In this group, animals received HIS plus NAC after NS with an interval of 30 min on the first day and continued once from the second to the 7th day. Group 5 (DOX + NS): In this group, animals received NS after DOX with an interval of 30 min on the first day and continued once (NS) from the second to the 7th day. Group 6 (HIS + DOX): In this group, animals received HIS after DOX with an interval of 30 min on the first day and continued once (HIS) from the second to the 7th day. Group 7 (NAC + DOX): In this group, animals received NAC after DOX with an interval of 30 min on the first day and continued once (HIS) from the second to the 7th day. Group 8 (HIS + NAC + DOX): In this group, animals received HIS plus NAC after DOX with an interval of 30 min on the first day and continued once (HIS plus NAC) from the 2nd to the 7th day. The groups were named based on the prescription made on the first day of the experiment. Groups 2 - 4 were designed to clarify the effects of HIS, NAC and HIS plus NAC in the absence of DOX.

Drug doses and injections. All drugs were dissolved in NS. On the first day, DOX was IP injected at a single dose of 15.00 mg kg^{-1}. The HIS and NAC at a similar dose of 100 mg kg^{-1} were IP injected in separate and combined treatments 30 min after DOX injection on the first day and thereafter continued daily for six days. The IP injections of test drugs were done using a 25-gauge needle in a volume of 1.00 mL kg^{-1}. Drug doses used here were in accordance with previous studies,[18,19] and our preliminary experiments.

Serum and tissue sampling. At the end of the study (day 7), the animals were weighted and deeply anesthetized with IP injection of the previously mentioned mixture of ketamine and xylazine A 23-gauge injection needle was inserted into the heart through seventh and eighth intercostal muscles. Blood samples were collected from the heart into non-heparin containing tubes. These tubes were centrifuged at 3,500 rpm for 10 min; serum samples were separated and transferred to Eppendorf tubes for biochemical determination of urea and creatinine levels. Immediately after blood sampling, the abdomen was opened and the kidneys was removed, washed with NS, blotted dry on filter papers and weighed. One kidney was fixed in 10.00% buffered formalin and used for histopathological studies, and the other was segmented and stored at – 80.00 ˚C for biochemical analysis.

Histopathological evaluations. The fixed kidney tissues were routinely processed for paraffin embedding. For each sample, 4.00 - 5.00 µm-thick sections were cut and stained by Hematoxylin and Eosin and examined under a light microscope. The evaluation of the kidney sections was based on the severity of the pathological changes, including congestion, hemorrhages and degeneration. The following scores were given to lesions observed: 0 - none, 1 - mild, 2 - moderate and 3 - severe.[20]

Biochemical assays. Freshly prepared serum levels of urea and creatinine were measured spectrophotometrically (Ultraspec II; Pharmacia LKB Biochrom Ltd., Cambridge, UK) using urea and creatinine test kits from Navand Salamat. The levels are expressed as mg dL^{-1}. The stored kidney segments placed on ice-cold saline, dissected into pieces and were homogenized in phosphate buffer solution (0.05 M, pH 7.40) as extraction buffer. Then, each homogenized sample was centrifuged for 15 min at 10,000 *g* at 4.00˚C. Total protein concentrations of kidney samples were estimated using Bradford protein assay, as previously described.[21] The kidney SOD activity was determined by SOD Assay Kit based on the manufacture protocol. Briefly, the principle of SOD enzyme assay is inhibition of pyrogallol oxidation. Pyrogallol is a compound that is rapidly autoxidized in the presence of molecular oxygen in an alkaline environment. As a result of autoxidation of pyrogallol, an intermediate compound called semi quinone radical was produced, and then pyrogallol-quinone was produced, and the last compound was measured at 420 nm. The higher the activity of the enzyme, the less pyrogallol-quinone compound was produced. Finally, the activity of SOD was expressed as U mg^{-1} protein. To evaluate kidney tissue MDA level, a commercial MDA assay kit was used. Briefly, estimation of MDA was based on reaction with TBA and generation of the MDA-TBA adduct. The MDA-TBA adduct was simply quantified colorimetrically at the wavelength of 532 nm by a spectrophotometer. The findings were expressed as nmol mg^{-1} protein. Kidney tissue TNF-α content was evaluated by ELISA kit according to the manufacture's instruction. Briefly, rat TNF-α standards and samples were bounded to the coated antibodies followed by binding of the biotinylated anti-rat TNF-α secondary antibody. Next, horseradish peroxidase conjugate solution added to the wells. Then, for color visualization chromogen substrate was added to the wells and after blue color development it was stopped by the addition of acid. The strength of the produced color was directly proportional to the amount of TNF-α present in the samples. Finally, the optical density (OD) of the color of each well was measured and plotted against concentration. The concentration of rat TNF-α in samples estimated using standard curve and presented as pg mg^{-1} protein. Caspase-3 levels in kidney were determined using ELISA assay kit according to the manufacture instruction. This ELISA kit was based on sandwich-ELISA as the method. Briefly, samples were added to the microplate wells which pre-coated with specific caspase 3 antibody. Then, a biotinylated detection antibody specific for both caspase-3 and avidin-horseradish peroxidase conjugate was added up to each microplate well and incubated. The substrate solution was added to each well. Only those wells that contained the complex of biotinylated detection antibody, caspase-3 and avidin-horseradish peroxidase conjugate developed blue color. For termination of enzyme-substrate reaction sulfuric acid solution was added. The OD was measured spectrophotometrically at the wavelength of 450 nm. The OD value was directly proportional to the concentration of caspase-3. The concentration of caspase-3 in samples was estimated by comparing OD of the samples to the standard curve. The results were presented as ng mg^{-1} protein.

Statistical analysis. Statistical comparisons were performed using the GraphPad Prism (version 8.2; GraphPad Software Inc., San Diego, USA). Values of blood and kidney tissue biochemical markers were analyzed using one-way ANOVA followed by Tukey's test. Because of the semi-quantitative nature of data obtained from histopathological changes, Kruskal-Wallis and post hoc Mann-Whitney tests were performed. Values of histopathological changes were presented as quartiles minimum value, first quartile, median, third quartiles, and maximum value, and the other data were expressed as the mean ± SEM. Significance of level was set at $p < 0.05$.

Results

No significant differences were observed in initial ($F[7,40] = 0.0377$, $p > 0.05$) final ($F[7,40] = 0.2843$, $p > 0.05$) and (final – initial) ($F[7,40] = 1.874$, $p > 0.05$) body weights, kidney weight ($F[7,40] = 0.5587$, $p > 0.05$) and body/kidney weight ratio ($F[7,40] = 1.272$, $p > 0.05$) among treated groups (Table 1). Subsequent Tukey's test analysis confirmed no significant differences among treated groups (Table 1).

Table 1. Effects of separate and combined treatments with histidine (HIS) and N-acetylcysteine (NAC) on body and kidney weight changes in normal saline (NS) and doxorubicin (DOX) treated groups

Groups	Initial body weight (g)	Final body weight (g)	Body weight change (g)	Kidney weight (g)	Kidney-Somatic Index
NS + NS	221.33 ± 5.21	226.17 ± 4.62	4.83 ± 1.25	1.12 ± 0.05	0.49 ± 0.02
NS + HIS	222.83 ± 4.28	225.51 ± 3.24	3.11 ± 1.13	1.13 ± 0.06	0.50 ± 0.03
NS + NAC	221.67 ± 3.08	224.83 ± 3.62	3.17 ± 1.11	1.11 ± 0.05	0.48 ± 0.02
NS + HIS + NAC	221.13 ± 3.57	225.11 ± 3.89	3.83 ± 1.19	1.13 ± 0.07	0.51 ± 0.02
DOX + NS	220.17 ± 4.53	220.67 ± 4.94	0.50 ± 1.50	1.25 ± 0.08	0.56 ± 0.03
DOX + HIS	220.67 ± 3.94	221.08 ± 3.29	0.33 ± 1.42	1.21 ± 0.07	0.57 ± 0.04
DOX + NAC	221.67 ± 3.25	222.51 ± 2.98	0.83 ± 0.71	1.17 ± 0.05	0.52 ± 0.02
DOX + HIS + NAC	221.52 ± 3.88	223.48 ± 4.17	2.00 ± 1.24	1.19 ± 0.06	0.53 ± 0.03

Doxorubicin was used at a single dose of 15.00 mg kg^{-1} on day one. Histidine and NAC were used at a similar dose of 100 mg kg^{-1} for 7 consecutive days after DOX administration.
No significant differences were observed among treated groups in each column.

Figures 1 and 2 show the effects of separate and combined treatments with HIS and NAC on DOX-induced kidney tissue histopathological alterations and their corresponding scores, respectively. No histopathological changes were observed in NS + NS, NS + HIS, NS + NAC and NS + HIS + NAC groups with score of 0. The DOX caused congestion, hemorrhages and degeneration in the kidney tissue, and the corresponding scores were 2.79 ± 0.07, 2.19 ± 0.05 and 2.83 ± 0.06. Histopathological alterations and the increased scores were decreased by HIS and NAC used alone. The HIS plus NAC treatment also reduced histopathological and score changes and reached them to approximately normal values.

Table 2 shows the effects of separate and combined treatments with HIS and NAC on serum urea and creatinine concentration changes and kidney tissue SOD activity and MDA, TNF-α and caspase-3 level alterations in NS and DOX treated rats. One-way ANOVA revealed significant differences in serum urea ($F[7,40] = 84.07$, $p < 0.0001$) and creatinine ($F[7,40] = 87.20$, $p < 0.0001$) and kidney tissue MDA ($F[7,40] = 206.9$, $p < 0.0001$), TNF-α ($F[7,40] = 70.96$, $p < 0.0001$) and caspase-3 ($F[7,40] = 58.23$, $p < 0.0001$) levels and SOD activity ($F[7,40] = 34.85$, $p < 0.0001$) among treated groups. The Tukey's test expressed no significant differences among NS + NS, HIS + NS, NAC + NS and HIS + NAC + NS treated groups.

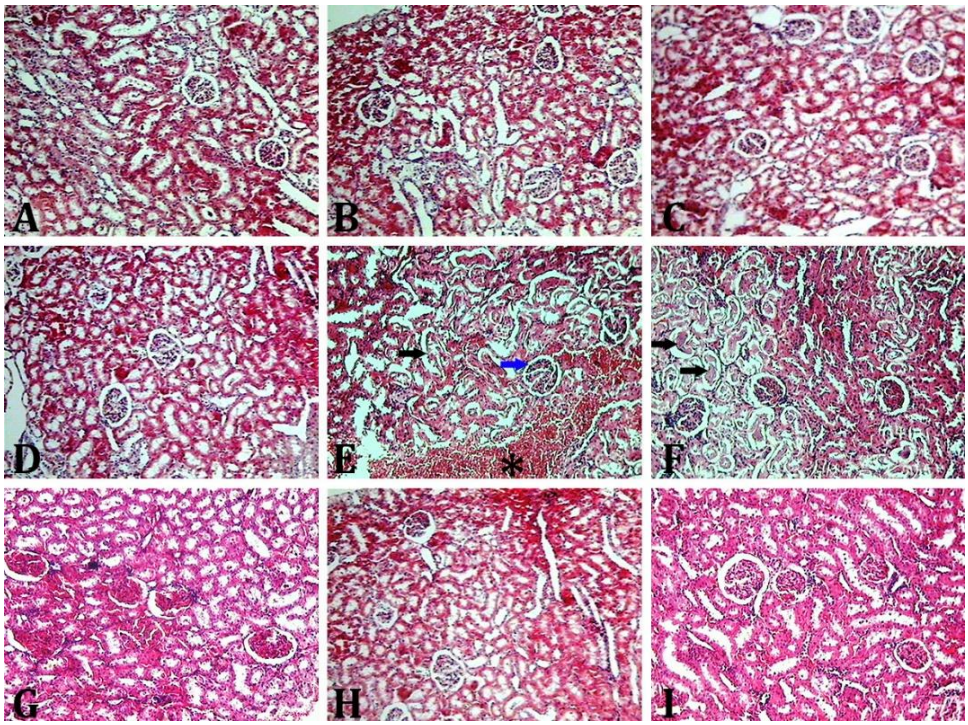

Fig. 1. Micrographs of the kidney tissue (Hematoxylin and Eosin, 400×). Normal histoarchitecture of kidney tissues are seen in **A)** normal saline (NS) + NS, **B)** NS + histidine (HIS), **C)** NS + N-acetylcysteine (NAC), **D)** NS + HIS + NAC groups, **E** and **F)** DOX-treated rats show congestion (blue arrow), hemorrhage (black star), and degeneration (black arrows). The DOX-induced kidney tissue histopathological alterations were attenuated by IP injections of **G)** HIS at a dose of 100 mg kg^{-1}, **H)** NAC at a dose of 100 mg kg^{-1} and **I)** HIS plus NAC at the similar dose of 100 mg kg^{-1}.

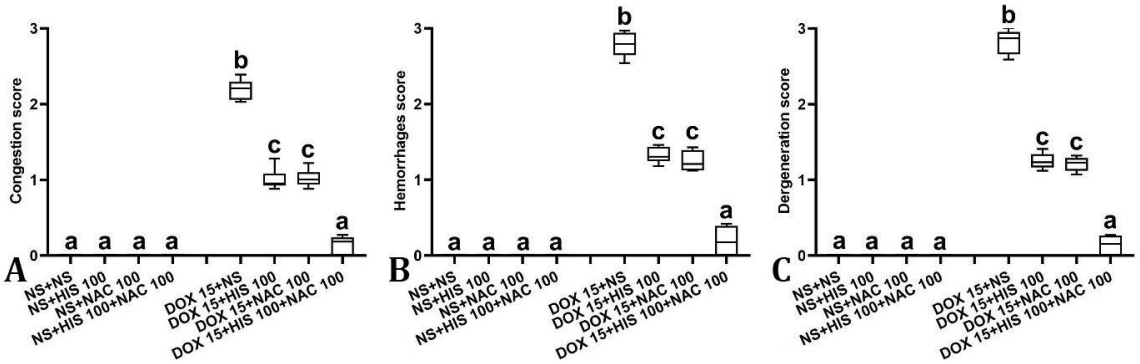

Fig. 2. Histopathological scores of **A)** congestion, **B)** hemorrhage, and **C)** degeneration in different experimental groups. Different letters indicate significant differences among treated groups (a *vs* b: $p < 0.01$, a *vs* c: $p < 0.05$, b *vs* c: $p < 0.05$).

Table 2. Effects of separate and combined treatments with histidine (HIS) and N-acetylcysteine (NAC) on serum urea and creatinine concentration, kidney tissue superoxide dismutase (SOD) activity and malondialdehyde (MDA), tumor necrosis factor-alpha (TNF-α) and caspase-3 level alterations in kidney tissue induced by doxorubicin (DOX).

Groups	Urea (mg dL^{-1})	Creatinine (mg dL^{-1})	SOD (nmol mg^{-1} pr)	MDA (nmol mg^{-1} pr)	TNF-α (pg mg^{-1} pr)	Caspase-3 (ng mg^{-1} pr)
NS + NS	13.78 ± 1.79^{a}	0.61 ± 0.04^{a}	38.91 ± 1.86^{a}	0.51 ± 0.05^{a}	25.07 ± 2.36^{a}	0.98 ± 0.09^{a}
NS + HIS	13.37 ± 1.29^{a}	0.59 ± 0.03^{a}	37.53 ± 1.61^{a}	0.49 ± 0.04^{a}	24.23 ± 1.94^{a}	0.95 ± 0.07^{a}
NS + NAC	14.17 ± 1.15^{a}	0.57 ± 0.05^{a}	36.32 ± 1.83^{a}	0.52 ± 0.04^{a}	25.17 ± 1.91^{a}	0.98 ± 0.06^{a}
NS + HIS + NAC	14.23 ± 1.38^{a}	0.58 ± 0.04^{a}	39.33 ± 1.91^{a}	0.48 ± 0.05^{a}	24.12 ± 1.48^{a}	0.96 ± 0.05^{a}
DOX + NS	53.95 ± 2.29^{b}	2.17 ± 0.09^{b}	9.28 ± 1.22^{b}	5.68 ± 0.26^{b}	78.65 ± 3.46^{b}	2.93 ± 1.17^{b}
DOX + HIS	32.67 ± 1.63^{c}	1.35 ± 0.08^{c}	24.71 ± 1.64^{c}	2.79 ± 0.13^{c}	50.87 ± 2.11^{c}	1.89 ± 0.08^{c}
DOX + NAC	31.53 ± 1.71^{c}	1.31 ± 0.07^{c}	25.92 ± 1.19^{c}	2.86 ± 0.17^{c}	45.73 ± 2.66^{c}	1.79 ± 0.09^{c}
DOX + HIS + NAC	15.62 ± 1.25^{a}	0.69 ± 0.05^{a}	36.32 ± 1.33^{a}	0.64 ± 0.08^{a}	29.42 ± 2.09^{a}	1.03 ± 0.06^{a}

NS: Normal saline; pr: protein. Doxorubicin was used at a single dose of 15.00 mg kg^{-1} on day one. Histidine and NAC were used at a similar dose of 100 mg kg^{-1} for seven consecutive days after DOX administration. Different letters indicate significant differences among groups in each column (a *vs* b: $p < 0.001$, a *vs* c: $p < 0.01$, b *vs* c: $p < 0.05$).

The DOX significantly ($p < 0.001$) increased serum urea and creatinine and kidney tissue MDA, TNF-α and caspase-3 levels and decreased SOD activity in this organ. All the above-mentioned alteration induced by DOX, significantly ($p < 0.01$) reduced by 100 mg kg^{-1} HIS and 100 mg kg^{-1} NAC used alone. In the combined treatment with HIS and NAC, the serum and kidney tissue biomarker contents reached to the normal level.

Discussion

The results of the present study showed that the final body weight, kidney weight and KSI in NS treated groups were not affected by HIS and NAC treatments. The lack of effect of HIS and NAC on body weight observed in the present study might be associated with their used doses. It has been reported that IP injection of HIS at doses of 0.75 - 2.00 g kg^{-1} reduces food intake in rats.[22] In addition, it was found that providing food containing 2.50 and 5.00 % HIS for eight days reduced food intake, fat accumulation and body weight.[23] It has been reported that IP injection of NAC at a dose of 90.00 mg kg^{-1} for 14 days did not cause significant changes in standard chow food consumption and body weight.[24] In the current study, DOX caused a non-significant decrease in final body weight and non-significant elevations in kidney and KSI. Moreover, these non-significant alterations were not affected by HIS and NAC treatments. The reason for the lack of effect of DOX on body and kidney weights observed in the present study might be due to the period of the experiment (7 days). It has been reported that DOX (a single dose of 15.00 mg kg^{-1}) caused food intake and body weight reductions and kidney weight and KSI elevations on day 14 after DOX administration.[25]

In the current study, DOX caused histopathological changes such as congestion, hemorrhages, and degeneration in the kidney tissue. These histopathology outcomes were completely consistent with the findings of other researchers, in which glomeruli with congested capillaries, renal tubules with vacuolar degeneration, tubular lumen filed with shed cells and casts and extensive hemorrhage in interstitial tissue have been reported.[25,26] In the present study DOX-induced histopathological alterations in kidney tissue were improved with HIS and NAC treatments. As far as we know, there have been no reports showing the ameliorative effect of HIS on DOX-induced renal injury. However, the sciatic nerve tissue edema and the heart tissue hemorrhage induced by DOX were found to mitigate with HIS and NAC treatments.[15,16] In addition, proximal tubule necrosis, tubular casts and

infiltration of inflammatory cells into the interstitial tissue induced by DOX (10.00 mg kg^{-1}) have been reported to improve by IP injection of NAC.[27]

In the present study, DOX increased serum levels of urea and creatinine, and restoration was observed with treatments of HIS and NAC, while these treatments did not cause significant changes in NS receiving groups. It is well known that AKI is typically diagnosed by the accumulation of end products of nitrogen metabolism such as urea and creatinine in the serum.[28] It has been reported that intravenous injection of DOX (a single dose of 11.50 mg kg^{-1}) increases kidney dysfunction biomarkers, i.e. urea and creatinine in the serum of rats.[29] There are no reports describing the effects of HIS and NAC on DOX-induced serum urea and creatinine alterations. Although the effects of HIS and NAC on DOX-induced renal dysfunction biomarkers have not been reported, HIS and NAC treatments have been recommended in the cases of AKI.[30,31] In this regard, in cisplatin-induced nephrotoxicity, IP injection of NAC (50.00 mg kg^{-1}, three times a week for 4 weeks) exerted protective effects against serum alterations of urea, creatinine and uric acid.[25]

The results of the present study showed that there were no significant changes in the of tissue biomarker contents in the groups receiving HIS and NAC without DOX. On the other hand, DOX decreased the activity of SOD and increased the levels of MDA, TNF and caspase-3 in the kidney tissue, and these alterations were improved by HIS and NAC treatments. These results stated that HIS and NAC counteracted the harmful effects of DOX by suppressing oxidative stress and stimulating anti-oxidative, anti-inflammatory and anti-apoptotic mechanisms. The SOD is one of the major parts of antioxidant defense enzymatic system in all cells exposed to O_2.[32] The MDA is a molecule which is generated as a result of membrane polyunsaturated fatty acid lipid peroxidation and hence its amount is considered as a primary indicator of lipid peroxidation and oxidative damage in tissue.[33] The TNF-α is a cytokine that has been identified as a major regulator of inflammatory responses.[34] Caspases are crucial mediators of programmed cell death (apoptosis), and among them, caspase-3, a cysteine-aspartic acid protease, is a frequently activated death protease, catalyzing the specific cleavage of many key cellular proteins.[35] In almost all organs of the body, DOX causes extensive side effects such as neuropathy, cardiomyopathy and nephropathy through mechanisms such as DNA damage, reactive oxygen species production, immunomodulation, autophagy, apoptosis, ferroptosis, and pyroptosis while fighting cancer.[4] It has been reported that treatments with HIS and NAC reduce the plasma level of MDA in DOX induced cardiotoxicity and neurotoxicity, and increase the plasma level of the total antioxidant capacity in the lattr.[15,16] In streptozotocin/nicotinamide-induced type 2 diabetic rats, HIS administration normalized TFN-α level in the kidney tissue.[36] In addition, HIS treatment was found to normalize down-regulated caspase-3 and TNF-α in D-galactose-treated SH-Sy5y cell line.[37] On the other hand, NAC was found to exert ameliorative effects on adriamycin (DOX) induced cardiotoxicity and nephrotoxicity by reducing tissue levels of TNF-α and interleukin-1beta.[17] Moreover, NAC has been found to exert a protective effect against vancomycin-induced nephrotoxicity by reducing the tissue level of caspase-3.[38]

The results of the current study showed the synergistic effects of combined treatment of HIS and NAC compared to their separate use against DOX-induced nephrotoxicity. Our previous studies demonstrated these synergistic effects between HIS and NAC in DOX-induced cardiotoxicity and neurotoxicity.[15,16] In this regard, synergistic effects against cyclophosphamide-induced hemorrhagic cystitis have been reported in the anti-oxidative enhancing and oxidative suppressing of combined use of HIS and vitamin C in relation to alone use.[39] In addition, in a combined treatment with NAC and taurine, a synergistic effect against cisplatin-induced nephrotoxicity has been found.[25] The biological redundancies in molecular networks of complex diseases limit the efficacy of monotherapy. Combination therapeutics, as a common therapeutic method, involve pharmacological intervention using several drugs that interact with multiple targets in the molecular networks of diseases and may achieve better efficacy and/or less toxicity than monotherapy in practice.[40]

The results of the present study showed that DOX caused nephrotoxicity and impaired kidney function. Separate and combined treatments with HIS and NAC ameliorated DOX-induced nephrotoxicity by suppressing oxidative stress and stimulating anti-oxidative, anti-inflammatory and anti-apoptotic mechanisms and restored kidney function to normal levels. A synergistic effect was observed in the combined treatment of HIS and NAC compared to the use alone.

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Conflict of interest

The authors declared no conflicts of interest.

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