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Original article

Investigation of the protective effect of chitosan against arsenic-induced nephrotoxicity and oxidative damage in rat kidney tissue

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Abstract

Arsenic is an important metalloid that can cause poisoning in humans and domestic animals. Exposure to arsenic causes cell damage, increasing the production of reactive oxygen species. Chitosan is a biopolymer obtained by deacetylation of chitin with antioxidant and metal ion chelating properties. In this study, the protective effect of chitosan on arsenic-induced nephrotoxicity and oxidative damage was investigated. 32 male Wistar-albino rats were divided into 4 groups of 8 rats each as control group (C), chitosan group (CS group), arsenic group (AS group), and arsenic+chitosan group (AS+CS group). The C group was given distilled water by oral gavage, the AS group was given 100 ppm/day Na-arsenite ad libitum with drinking water, the CS group was given 200 mg/kg/day chitosan dissolved in saline by oral gavage, the AS+CS group was given 100 ppm/day Na-arsenite ad libitum with drinking water and 200 mg/kg/day chitosan dissolved in saline by oral gavage for 30 days. At the end of the 30-day experimental period, 90 mg/kg ketamine was administered intraperitoneally to all rats, and blood samples and kidney tissues were collected. Urea, uric acid, creatinine, P, Mg, K, Ca, Na, Cystatin C (CYS-C), Neutrophil Gelatinase Associated Lipocalin (NGAL) and Kidney Injury Molecule 1 (KIM-1) levels were measured in serum samples. Malondialdehyde (MDA), Glutathione (GSH), Catalase (CAT) and Superoxide dismutase (SOD) levels in the supernatant obtained from kidney tissue were analyzed by ELISA method. Compared with AS group, uric acid and creatinine levels of the AS+CS group were significantly decreased ($p<0.001$), urea, KIM-1, CYS-C, NGAL, and MDA levels were numerically decreased and CAT, GSH, and SOD levels were numerically increased ($p>0.05$). In conclusion, based on both biochemical and histopathological-immunohistochemical-immunofluorescence findings, it can be concluded that chitosan attenuates kidney injury and protects the kidney.

Keywords: arsenic, chitosan, nephrotoxicity, oxidative stress, rat

Introduction

Arsenic is one of the most important metalloids that can cause poisoning in humans and domestic animals, and its main route of distribution and transport in the environment is natural waters (Selby et al. 1977, Hughes 2002). Chronic exposure to groundwater containing unsafe levels of arsenic can cause many human conditions, including dermal, cardiovascular, neurological, pulmonary, renal and metabolic conditions. The toxicity of arsenic compounds can vary greatly depending on their chemical form, solubility and concentration. (Muzaffar et al. 2023).

Arsenic participates in metabolic processes, usually in the structure of mitochondria. Arsenic (5+) (As^{+5}) binds to high-energy phosphate substituents during ATP formation, causing ATP synthesis to stop. As^{+3} inhibits lipoic acid, which is required for pyruvate dehydrogenase, preventing the progression of the citric acid cycle (Bakan 2019).

Increased reactive oxygen species as a result of arsenic exposure cause cell damage. Arsenic (e.g. As^{+3}) has a harmful effect by interacting with the sulfhydryl groups of proteins and enzymes, denaturing proteins and enzymes in the cell and increasing reactive oxygen species in the cell, thereby causing cell damage (Kaya and Eraslan 2013). Oxidative stress induced by trivalent methylated arsenic leads to the impairment of protective mechanisms against oxidation in the cell by inhibiting glutathione (GSH) reductase and thioredoxin reductase. The decrease in GSH in the cell sensitizes the cells to arsenic. In addition, arsenic weakens DNA repair processes, which increases susceptibility to cancer (e.g. skin cancer) and other arsenic-related diseases (Duker et al. 2005).

Oxidative stress and inflammation are believed to play a critical role in the pathogenesis of arsenic--induced nephrotoxicity. Exposure to arsenic can cause kidney damage characterized by tubulointerstitial nephritis and acute tubular necrosis (Aleksunes et al. 2008). Consumption of arsenic-contaminated drinking water increases the risk of kidney damage and hypertension (Zalups 1997, Robles-Osorio et al. 2015).

Found in the exoskeleton of shellfish, insects, and in the cell walls of fungi, chitin is the second most common polysaccharide on Earth after cellulose. Chitosan polymer is a polysaccharide obtained by partial deacetylation of chitin. Chitosan, which can be obtained in abundant amounts from natural sources, is non-toxic to living things, and biodegradability, biocompatibility, and chemical and physical properties make it superior to other biopolymers.

(Elieh-Ali-Komi and Hamblin 2016). In the studies conducted, it has been reported that chitosan reduces oxidative damage caused by various effects on cells with the demonstration of its antioxidant properties (Smith et al. 2002, Wu et al. 2006, Wang et al. 2016, Aboulthana and Ibrahim 2018, Özdek et al. 2019). Chitosan, which is non-toxic, has no side effects since it is completely harmless to the environment and living things. Today, chitosan can be used in many fields such as medicine, pharmacy, cosmetics, agriculture, food, and textiles (Demir and Seventekin 2009, Toz and Değer 2018).

The molecular weight and water solubility of orally ingested chitosan play an important role in the level of intestinal absorption. In addition, the absorption and distribution of chitosan in the body affects its physiological activity. Chitosan absorbed in the body is distributed to organs such as liver, kidney, blood and spleen. The kidney was a main excretion manner (Zeng at al. 2008).

In some recent studies, it has been shown that chitosan shows an antioxidant effect in vitro and strengthens the antioxidant defense mechanism. It has been reported that chitosan shows an antioxidant effect by forming chelates with metals that catalyze oxidative reactions with active hydroxyl and amino groups in polymer chains. The free radical scavenging ability of chitosan is related to the degree of deacetylation and molecular weight. Chitosan has been found to strengthen the body's antioxidant defense mechanism and prevent oxidative damage and lipid peroxidation. In addition, it is reported that chitosan has a significant antioxidant effect and delays lipid oxidation in foodstuffs in which chitosan is used as an additive. This effect of chitosan is closely related to its chelation of metal ions. (Smith et al. 2002, Wu et al. 2006).

The aim of this study was to investigate the capacity of chitosan to prevent arsenic-induced nephrotoxicity and oxidative damage with its antioxidant properties.

Materials and Methods

Chemicals and reagents

Sodium arsenite (NaAsO2, Merck Company), Chitosan (Deacetylated chitin; Poly(D-glucosamine; Molecular Formula:$(C_7H_{15}NO_4)_n$; Molecular Weight: 60-120 kDa; DAC degree:90%+; Solubility: Soluble in 1M Acetic Acid (10 mg/ml); Insoluble in Water; Density: 1.35-1.40 g/cm^3; CAS number: 9012-76-4, Sigma-Aldrich), KIM-1 (BT, Cat. No:E0549Ra), CYS-C (BT, Cat. No: E0145Ra), NGAL (BT, Cat. No: E0762Ra), CAT (BT, Cat. No: E0869Ra), GSH (BT, Cat. No: E1101Ra), MDA (Rel Assay, Cat. No: RL0057), SOD (Rel Assay, Cat. RLD0123) were obtained from commercial companies.

Animal material and experimental protocol

Ethical approval for this study was obtained from the Saki Yenilli Experimental Animals Local Ethics Committee (Approval Number: 2021/06/04/25). In the study, 32 male Wistar albino rats of 10-12 weeks of age, weighing 200-300 g, obtained from Saki Yenilli Experimental Animal Laboratory were used. Prior to the experiment, the rats were adapted to the environment for 7 days.

Rats were randomly selected and divided into 4 groups of 8 rats each:

1 - Control group (C): Rats in this group were given distilled water by oral gavage for 30 days.
2 - Chitosan group (CS group): Rats 200 mg/kg/day chitosan was dissolved in saline and given by oral gavage for 30 days (Toz and Değer 2018).
3 - Arsenic group (AS group): Rats were given 100 ppm Na-arsenite ad libitum with drinking water for 30 days (Kaya and Eraslan 2013).
4 - Arsenic+Chitosan group (AS+CS group): Drinking water and 100 ppm Na-arsenite were given ad libitum for 30 days and 200 mg/kg/day chitosan was dissolved in saline and given by oral gavage for 30 days.

The rats were housed in rooms with a temperature of 22±2°C and 12 h light/dark conditions during the 30-day experiment with standard pellet feed and water ad libitum.

Collection of blood samples and kidney tissues, preparation of homogenates

At the end of the experimental period were anesthetized by intraperitoneal injection of ketamine (90 mg/kg). Blood samples were collected from the left ventricle of the rats' hearts with a syringe and collected in tubes without anticoagulant. In the serum samples obtained, urea, uric acid, creatinine, P, Mg, K, Ca, and Na levels were measured by autoanalyzer (ADVIA 1800 Chemistry System), Cystatine C (CYS-C), Neutrophil Gelatinase-Associated Lipocalin (NGAL), and Kidney Injury Molecule 1 (KIM-1) were determined by ELISA method using commercial kits. One of the kidneys obtained from rats was homogenized with 1/10 of phosphate buffer, pH 7.4, 0.1 M, on ice and centrifuged at 1800xg. Malondialdehyde (MDA), Glutathione (GSH), Catalase (CAT), and Superoxide dismutase (SOD) levels in the supernatant obtained were analyzed by ELISA using commercial kits.

Histopathological analysis

The other kidney obtained at the end of the study was fixed in 10% formaldehyde solution for 48 hours and embedded in paraffin blocks after routine tissue follow-up procedures. Sections were taken from each block and preparations prepared for histopathologic examination were stained with hematoxylin-eosin (HE) and examined by light microscopy (Olympus BX 51, JAPAN). The sections were evaluated as absent (-), mild (+), moderate (++), and severe (+++) according to histopathologic features (degeneration of tubules, necrosis of tubules, hyperemia of the veins) (Ibaokurgil 2023).

Immunohistochemical examination

For immunohistochemical analysis, all tissue sections were deparaffinized and dehydrated. Then, endogenous peroxidase was inactivated by keeping the tissues in 3% H_2O_2 for 10 min. The sections were then boiled in 1% antigen retrieval solution (100X Citrate Buffer pH 6.0) and allowed to cool at room temperature. All sections were incubated with protein block for 5 min to prevent nonspecific background staining in the tissues. Then, primary antibody (Caspase 3 Cat. No: sc-65497, dilution ratio: 1/100, US) was added to the tissues and incubated according to the instructions for use. 3-3' Diaminobenzidine (DAB) chromogen was used as chromogen in the tissues. Stained sections were examined by light microscopy (Zeiss AXIO GERMANY) (Danışman et al 2023).

Double ımmunofluorescence examination

All sections taken for immunofluorescent staining were deparaffinized and dehydrated. The endogenous peroxidase activities were inactivated by keeping the tissues in 3% H_2O_2. The tissues were then boiled in 1% antigen retrieval solution solution (100X Citrate Buffer pH 6.0) and allowed to cool at room temperature. All sections were incubated with protein block for 5 min against nonspecific binding. Then, primary antibody (8-OHdG; Dilution ratio: 1/100; cat. no. sc-66036, Santa Cruz Biotechnology) was added to the tissues and incubated according to the instructions for use. Immunofluorescence marker secondary antibody (FITC; Dilution ratio: 1/1000, Cat No: ab6785, Abcam) was used as secondary marker and kept in the dark for 45 minutes. Then, another primary antibody (JNK; Dilution: 1/100, Cat No: sc-514539, Santa Cruz Biotechnology) was added to the tissues and incubated according to the instructions for use. Immunofluorescence secondary antibody (Texas Red; Dilution ratio: 1/1000, Cat No: ab6719, Abcam) was used as secondary marker and kept in the dark for 45 minutes. Then, DAPI with mounting medium (Dilution ratio: 1/200, Cat no: D1306, Thermo Fisher Scientific) was added to the sections and kept in the dark for 5 min and the sections were covered with

Table 1. Serum urea, uric acid, creatinine, Mg, K, Na, P, and Ca levels in all rat groups (Mean± SE).

Parameters	C	CS	AS	AS+CS	P
Urea (mg/dl)	44.00 ± 1.430^{b}	45.10 ± 1.060^{b}	52.60 ± 1.270^{a}	49.70 ± 0.680^{a}	0.001
Uric Acid (mg/dl)	1.47 ± 0.085^{c}	1.30 ± 0.092^{c}	3.02 ± 0.093^{a}	2.09 ± 0.219^{b}	0.001
Creatinine (mg/dl)	0.33 ± 0.005^{b}	0.33 ± 0.007^{b}	0.36 ± 0.008^{a}	0.34 ± 0.005^{b}	0.001
Mg (mg/dl)	2.42 ± 0.067^{c}	2.29 ± 0.044^{c}	2.97 ± 0.085^{a}	2.76 ± 0.048^{b}	0.001
K (mmol/L)	5.30 ± 0.210^{ab}	4.87 ± 0.093^{b}	5.85 ± 0.276^{a}	5.54 ± 0.179^{a}	0.013
Na (mmol/L)	146.00 ± 0.680	146.90 ± 1.590	149.10 ± 0.880	148.10 ± 0.480	0.152
P (mg/dl)	8.71 ± 0.238^{b}	7.05 ± 0.094^{b}	10.26 ± 0.276^{a}	10.28 ± 0.171^{a}	0.001
Ca (mg/dl)	10.25 ± 0.082^{b}	9.5 ± 0.117^{c}	11.00 ± 0.134^{a}	11.02 ± 0.147^{a}	0.001

^{a,b,c}: Different letters in the same row mark statistical difference between values. ($p<0.05$).
C: Control Group; CS: Chitosan Group; AS: Arsenic Group; AS+CS: Arsenic+Chitosan Group
Mg: Magnesium; K: Potassium; Na: Sodium; P: Phosphorus; Ca: Calcium

Table 2. Serum KIM-1, CYS-C, NGAL, and kidney tissue CAT, GSH, SOD, and MDA levels in all rat groups (Mean± SE).

Parametreler	C	CS	AS	AS+CS	P
KIM-1 (ng/ml)	0.99 ± 0.039	1.03 ± 0.037	1.15 ± 0.062	1.11 ± 0.047	0.095
CYS-C (ng/ml)	6.80 ± 0.553^{b}	5.34 ± 0.658^{b}	10.30 ± 1.090^{a}	9.85 ± 1.112^{a}	0.001
NGAL (ng/ml)	5.15 ± 0.370^{c}	5.85 ± 0.559^{bc}	8.58 ± 0.758^{a}	7.23 ± 0.433^{ab}	0.001
MDA (nmol/L)	9.88 ± 1.030^{c}	12.88 ± 1.523^{bc}	22.68 ± 3.201^{a}	17.56 ± 1.720^{ab}	0.001
CAT (ng/ml)	24.39 ± 1.175^{c}	33.69 ± 2.254^{a}	27.39 ± 1.499^{bc}	30.83 ± 2.578^{ab}	0.013
GSH (mg/L)	229.20 ± 17.440	266.80± 23.200	211.70 ± 13.590	247.20 ± 24.430	0.271
SOD (U/ml)	175.00 ± 6.871	217.50 ± 15.203	175.25 ± 10.481	192.25 ± 16.078	0.081

^{a,b,c}: Different letters in the same row mark statistical difference between values. ($p<0.05$).
C: Control Group; CS: Chitosan Group; AS: Arsenic Group; AS+CS: Arsenic+Chitosan Group
KIM-1: Kidney Injury Molecule-1; CYS-C: Cystatine C; NGAL: Neutrophil Gelatinase-Associated Lipocalin; MDA: Malondialdehyde; CAT: Catalase; GSH: Glutathione; SOD: Superoxide dismutase

coverslips. The stained sections were examined under a fluorescence attachment microscope (Zeiss AXIO GERMANY) (Danışman et al 2023).

Statistics analysis

IBM SPSS Statistics 28 Trial Version package program was used for data analysis. "One-Way ANOVA Analysis" was used to compare group means and "Duncan Multiple Comparison Test" was used to determine the difference between groups. $p<0.05$ was accepted as statistical significance. The Kruskal-Wallis test was used to analyze the differences between groups in terms of histopathologic data, and the Mann-Whitney U test was used to compare paired groups. To determine the intensity of positive staining in immunohistochemical and immunofluorescence images, 5 randomly selected areas were evaluated in Zen Imaging Software (ZEISS). One-way ANOVA followed by the Tukey test was performed to combine positive immunoreactive cells and immunopositive cells with healthy controls. A p value <0.05 was considered significant and data are presented as mean ± SD.

Results

Biochemical findings

Serum urea, uric acid, creatinine, Mg, K, Na, P and Ca levels of all groups are given in Table 1. Urea, K, P and Ca levels were higher in AS and AS+CS groups compared to the other groups, while uric acid, creatinine and Mg levels were higher in AS group compared to the other groups ($p<0.05$). There was no statistical significance when Na levels of the groups were compared ($p>0.05$).

Serum KIM-1, CYS-C, NGAL and kidney tissue MDA, CAT, GSH and SOD levels of all groups are given in Table 2. There was no statistical difference between the groups in terms of serum KIM-1, renal tissue GSH, SOD levels. Statistical significance was found between the groups in terms of CYS-C, NGAL and MDA levels ($p<0.001$) and it was determined that the highest levels for these parameters belonged to the rats in the AS group. Statistical significance was found between the groups in terms of CAT levels ($p<0.05$) and it was determined that the rats in the CS group had the highest CAT level.

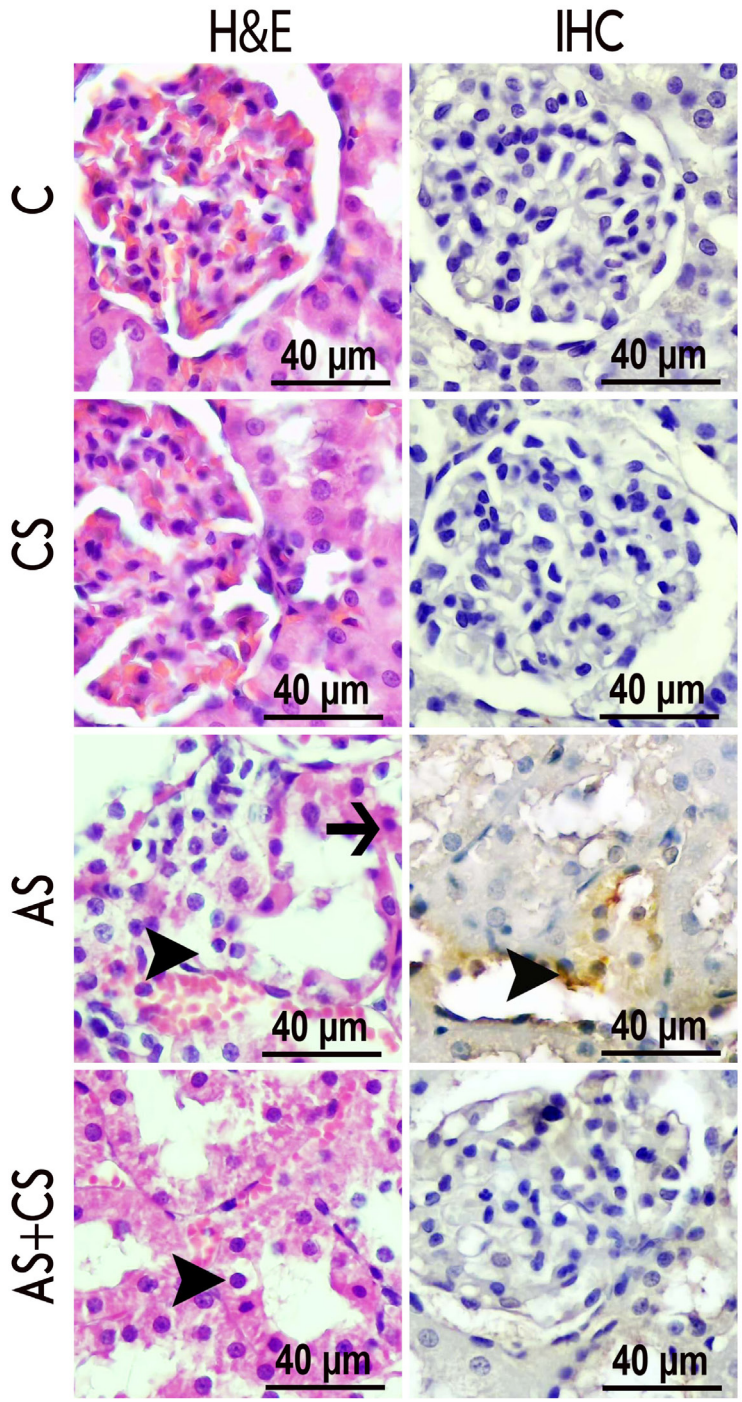

Fig. 1. Kidney tissues of rats; histopathologic, immunohistochemical staining. H&E: Tubular epithelial degeneration (arrowheads), tubular epithelial necrosis (arrows) in kidney tissues; IHC-P: Caspase 3 expression in tubular epithelium of kidney tissues (arrowheads), Bar: 40 µm.

Histopathologic, immunohistochemical, and immunofluorescence findings

When the kidney tissues were examined histopathologically; the C group had a normal histologic appearance, the CS group had a normal histologic appearance in serosa, medulla, and cortex layers, AS group had severe degeneration and necrosis in the tubular epithelium, dilatation in tubules and Bowman capsule, hyperemia in glomerular and parenchymal vessels. Mild degeneration in the tubular epithelium, mild dilatation in tubules, and hyperemia in vessels were observed in the AS+CS group (Fig. 1), with a statistically significant difference when compared to the AS group ($p<0.05$). The scoring of the histopathological findings observed in the kidney tissues is summarized in Table 3.

When the kidney tissues were examined immunohistochemically, Caspase 3 expression was negative

Table 3. Scoring of histopathologic findings observed in rat kidney tissues.

	Degeneration of tubules	Necrosis of tubules	Hyperemia of the veins
C	-	-	-
CS	-	-	-
AS	+++	+++	+++
AS+CS	+	-	++

C: Control Group; CS: Chitosan Group; AS: Arsenic Group; AS+CS: Arsenic+Chitosan Group

Table 4. Caspase-3, 8-OHdG and JNK levels in rat kidney tissues (Mean± SE).

	Caspase 3	8 OHdG	JNK
C	23.42±2.23[a]	24.60±1.08[a]	19.82±0.97[a]
CS	25.18±1.06[a]	26.58±2.23[a]	20.50±0.55[a]
AS	80.26±1.20[c]	83.44±0.84[c]	62.19±1.18[c]
AS+CS	40.22±0.98[b]	44.70±0.60[b]	30.29±1.37[b]

[a,b,c]: Different letters in the same row mark statistical difference between values. ($p<0.05$).
C: Control Group; CS: Chitosan Group; AS: Arsenic Group; AS+CS: Arsenic+Chitosan Group
8 OHdG: 8-hydroxy-2'-deoxyguanosine; JNK: c-Jun N-terminal kinase

in the tubular epithelium of rats in the C and CS groups. The tubular epithelium of the rats in the AS group showed a severe level of cytoplasmic Caspase 3 expressions. In the AS+CS group, the tubular epithelium of the rats observed mild levels of cytoplasmic Caspase 3 expressions (Fig. 1). A statistically significant difference ($p<0.05$) was found when compared to the AS group. In the immunofluorescence method; while 8-hydroxy-2'-deoxyguanosine (8 OHdG) and c-Jun N-terminal kinase (JNK) expressions were negative in the tubular epithelium of rats in the C and CS groups, increased cytoplasmic 8 OHdG and JNK expressions were detected in the tubular epithelium of rats in the AS group. In the AS+CS group, the tubular epithelium of the rats showed a mild level of 8 OHdG and JNK expressions (Fig. 2). A statistically significant difference ($p<0.05$) was found when compared to the AS group. Immunohistochemical and immunofluorescence findings are summarized in Table 4.

Discussion

Arsenic causes toxic effects at the molecular level by altering gene expression, stimulating cell proliferation, disrupting the intracellular respiratory pathway, and activating oxidative stress (Galanis et al. 2009). By activating oxidation-sensitive metabolic pathways, this metaloid produces free radicals, leading to cell damage and even death (Kitchin and Conolly 2010, Flora 2011, Jomova and Valko 2011).

The liver, kidney, and spleen are thought to be the organs most affected by the toxic effects of arsenic exposure (Liu and Waalkes 2008, Singh et al. 2014, Noman et al. 2015). Exposure to high doses of arsenic results in kidney damage. Kidney damage due to arsenic poisoning can be assessed by measuring serum and urine markers (Zheng et al. 2014). Overproduction of free oxygen radicals by arsenic induces tubular necrosis, which results in increased tubular permeability and ultimately increased serum urea levels due to decreased renal excretion (Narayana et al. 2001). Arsenic metabolites may differentially influence renal function, which may then influence the excretion of creatinine (Peters et al, 2014). In this study, SA administration alone caused significant increases ($p<0.001$) in serum urea, uric acid and creatinine concentrations compared to the control group which confirms SA-induced nephrotoxicity reported in previous studies (Aras et al. 2015, Ewere et al. 2019). This indicates that the kidney is unable to excrete these waste products as a result of impaired renal function. Progressive loss of renal function leads to disturbances in blood electrolyte levels and acid-base balance (Dhondup and Qian 2017). Determination of serum electrolyte levels is important in assessing the tubular integrity of the nephron (Bagga et al. 2005) It has been reported that mineral levels are affected due to nephrotoxicity caused by some heavy metals (Tilako et al. 2020). It was reported that sodium arsenite administration in rats caused significant increases in serum K^+ (Ewere et al. 2019, Sharma et al. 2022), and Cl^- and Na^+ (Ewere et al. 2019) levels. Consistent with the studies reported in this study, it was determined that the rats in the arsenic-treated group had the highest K ($p<0.05$) and Na (numerically, $p>0.05$) levels and that sodium arsenite could cause imbalances in electrolyte levels due to impaired kidney function. Roy and Roy (2011) found that serum phos-

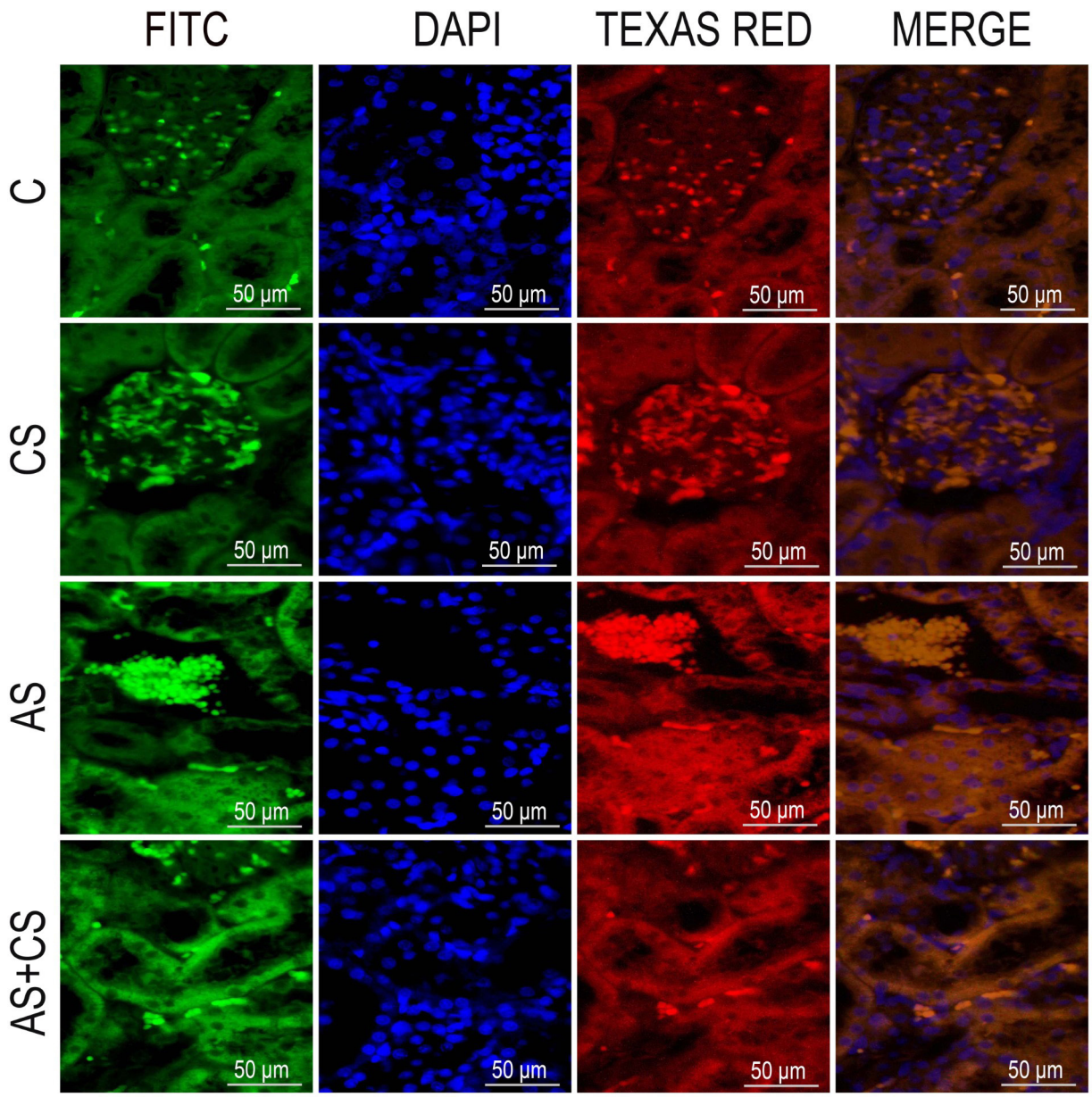

Fig. 2. Kidney tissues of rats immunofluorescent staining. IF: 8OHdG expression (FITC), JNK expression (TEXAS RED), Bar: 50 µm.

phorus levels increased and serum calcium levels decreased in animals exposed to arsenic compared to the control group. Similarly, the phosphorus values of the AS group were higher than the C group in this study. Shen et al. (2000) and Shen et al. (2002) showed that the induction of increased Ca^{2+} and NO levels by As_2O_3 initiates the mitochondria-dependent apoptotic pathway of apoptotic signaling messengers. Liu and Huang (1997) demonstrated the relationship between increased intracellular calcium, DNA damage, and calcium homeostasis disorders after arsenite treatment and concluded that calcium ions play a crucial role in arsenite-induced genotoxicity. Arsenic trioxide-induced apoptosis is associated with changes in intracellular calcium concentration (Akao et al. 2000, Cai et al. 2003). In this study, contrary to Roy and Roy (2011), Ca levels were higher in the arsenic group compared to the control group. In addition, the changes in calcium concentration as a result of arsenic exposure in this study are consistent with the reported studies (Liu and Huang 1997, Akao et al. 2000, Cai et al. 2003). Differences in mineral levels between studies may be related to the dose of toxicant administered, the type and duration of toxicant exposure.

KIM-1, CYS-C, and NGAL are among the biological markers recently identified for renal pathologies. It has been reported that serum CYS-C, KIM-1, IL-18, and NGAL levels are useful in the diagnosis of acute kidney injury in patients hospitalized in the intensive care unit and that NGAL and CYS-C levels are twice as high in patients with acute renal failure compared to controls (Büget et al. 2014). In a study investigating

the effect of chronic arsenic exposure on rat kidneys, it was reported that NGAL levels in urine were higher in all arsenic-treated groups compared to controls, and pathological changes were observed in proximal tubules and glomeruli (Wan Muhamad Salahudin et al. 2021). It is hypothesized that urinary excretion of NGAL is due to proximal tubule damage (Devarajan 2010).

Changes in serum and urine CYS-C levels have been reported in models of kidney injury (Dieterle et al. 2010, Zhang et al. 2011, Ghys et al. 2014). In a study examining the relationship between occupational exposure to heavy metals and CYS-C concentrations, the highest serum CYS-C concentration was found in the group exposed to arsenic and lead simultaneously (Poręba et al. 2011). Very small decreases in glomerular filtration rate (GFR) increase serum CYS-C (Mussap and Plebani 2004). In this study, serum NGAL and CYS-C levels of rats in the arsenic group increased compared to the control group ($p<0.001$). There was a numerical decrease in serum NGAL and CYS-C levels in the AS+CS group compared to the AS group, but this decrease was not statistically significant. This decrease may be related to the partially favorable change in GFR caused by chitosan.

Increased malondialdehyde (MDA) level is an important marker reflecting the extent of arsenic-induced tissue and cell damage in cells (Kamran et al. 2020). In previous studies, it was determined that MDA contents increased significantly in kidney and liver tissue homogenates of Arsenic-poisoned rats (Mehrzadi et al. 2018, Nozohour and Jalilzadeh-Amin 2019, Ishaq et al. 2021). Similarly, in this study, the highest MDA level was detected in rats in the arsenic group, indicating that acute exposure to arsenic causes nephrotoxicity and increases reactive oxygen species. Moreover, the MDA level of the AS+CS group (17.56±1.720 nmol/L) was numerically decreased ($p>0.05$) compared to the AS group (22.68±3.201 nmol/L).

This finding is consistent with the conclusion that CS reduces ROS production in conditions such as liver toxicity (Jeon et al. 2003), diabetes (Yuan et al. 2009), and lead toxicity (Wang et al. 2016, Özdek et al. 2019).

Enzymes such as CAT, SOD, and GP_X play an important role in cellular defense against ROS-induced oxidative damage. In a study investigating the preventive role of arjunolic acid against arsenic-induced nephrotoxicity, a significant decrease in CAT, SOD, and GSH activities was observed in the kidneys of arsenic group animals; this was associated with oxidative damage caused by disruption of pro-oxidant/antioxidant balance (Sinha et al. 2008). It was found that lithium caused significant changes in the antioxidant system in kidney tissue by decreasing the levels of enzymatic and non-enzymatic antioxidants, and SOD, CAT, and GSH values were significantly increased in chitosan-treated kidney tissues compared to the lithium-treated group (Aboulthana and Ibrahim 2018). In this study, SOD, CAT and GSH levels in the kidney tissues of rats in the AS+CS group were numerically higher than those in the AS group, but statistical significance was not detected.

There are studies reporting that CS reduces oxidative stress and strengthens the antioxidant defense system (Smith et al. 2002, Aboulthana and Ibrahim 2018, Özdek et al. 2019). In this study, the reason why there was no statistical difference between the groups in terms of antioxidant parameters and some other markers may be due to the different degree of deacetylation, molecular weight and viscosity of chitosan used in similar studies.

Arsenic has been reported to cause thickening of the parietal layer of Bowman's capsule, dilatation of the urinary cavity, acute necrosis of tubular cells, glomerular atrophy, and interstitial hemorrhages (Nozohour and Jalilzadeh-Amin 2019). Sinha et al. (2008) reported that sodium arsenite administration caused extensive tubular debris and as well as tubular dilatations. In the histopathological examination of the kidney tissues of the rats in the arsenic group in this study, severe degeneration and necrosis in the tubular epithelium, dilatation in tubules and Bowman's capsule, hyperemia in glomerular and parenchymal vessels were observed. It was determined that these symptoms were alleviated in the AS+CS treated group and chitosan showed a protective effect against renal lesions by reducing free radicals.

Arsenic, a metalloid, mediates many cellular mechanisms such as increased oxidative stress, expression of growth factors, suppression of cell cycle checkpoint proteins, promotion of apoptosis, inhibition of DNA repair, changes in DNA methylation by disrupting the pro/antioxidant balance (Flora 2011).

Namgung and Xia (2001) concluded that in rats exposed to sodium arsenite and dimethyl arsenic acid (DMA), arsenite neurotoxicity may result from the activation of c-Jun N-terminal kinase 3 (JNK3) and p38 involved in the apoptotic process. The role of metallothionein in altering DMA genotoxicity in mice was examined and an increase in DNA strand breaks was detected along with an increase in 8-hydroxy-2′-deoxyguanosine formation (Jia et al. 2004). The mechanisms by which trivalent forms of arsenic induce apoptosis include the release of calcium stores, activation of caspases, and changes in intracellular glutathione levels (Florea et al. 2005). In this study, severe levels of cytoplasmic Caspase 3, 8 OHdG, and JNK expressions were determined in the tubular epithelium of rats

treated with sodium arsenite, and it was determined that chitosan administration together with sodium arsenite partially alleviated these findings ($p<0.05$).

In a study investigating the in vivo renoprotective effect of chitosan nanoparticles in rats, it was determined that chitosan nanoparticles provided significant protection and improvement against CCl_4-induced nephrotoxicity. The potential mechanisms underlying the amelioration of CCl_4-induced nephrotoxicity by chitosan NPs were reported to be suppression of oxidative stress, inhibition of inflammatory cytokines and deactivation of caspase-3 enzyme resulting in antioxidant, anti-inflammatory and antiapoptotic effects (Nomier et al. 2022). Chou et al. (2015) investigated the renal protective effects of low molecular weight chitosan administration in dialysis patients and reported that renal tissue morphology improved and renal methylglyoxal accumulation, a damage factor associated with carbonyl stress, was reversed after low molecular weight chitosan treatment. In the present study, changes in some biochemical parameters and histopathologic/immunochemical findings suggest that chitosan attenuates kidney injury by similar mechanisms, in agreement with the findings of the investigators.

Conclusion

This study is one of the pioneering studies investigating the capacity of chitosan to prevent arsenic-induced nephrotoxicity and oxidative damage with its antioxidant properties. Compared to the AS group, uric acid and creatinine levels of the AS+CS group were significantly decreased ($p<0.001$), urea, KIM-1, CYS-C, NGAL, and MDA levels were numerically decreased and CAT, GSH, and SOD levels were numerically increased ($p>0.05$).In conclusion, based on both biochemical and histopathologic-immunohistochemical-immunofluorescence findings, it can be concluded that chitosan attenuates renal damage and protects the kidney.

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