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SODIUM THIOSULPHATE SHOWS PROMISING ANTI-INFLAMMATORY ROLE AGAINST DOXORUBICIN-INDUCED RENAL INJURY DEPENDING ON TLR4 PATHWAY INHIBITION

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Running Title: TLR4 impact on doxorubicin-induced nephrotoxicity .

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Abstract

Doxorubic in nephrotoxicity is always a major cause of death for cancer patients. *objectives:* our study aimed at proving the potential curative mechanisms of sodium thiosulphate, on experimentally-induced nephrotoxicity in rats by doxorubicin explaining the mechanisms of the serious inflammation pathway TLR4/MAPK P38/NF-κB/TNF-α. *Methods:* nephrotoxicity was induced by parenteral administration of doxorubicin (5.2mg/kg/ weekly for 4 weeks). And the treatment depends on giving sodium thiosulphate (400 mg/kg, *p.o.*) One hour before doxorubicin injection for4 weeks.

*D*oxorubicin injection caused severerenal dysfunction evident from a significant increase in the kidney biomarkers; urea,creatinine, KIM.1, and serum cystatin C,together with decreasing serum albumin and total protein.Besides,increased MDA and MPOassociated with a significant decrease inGSH, Nrf-2,SOD and catalase activities, heightened inflammatory markers TLR4, MAPK P38,NF-κB,IL-1β, and TNF- alpha also, induced apoptotic markers expression in renal tissues of doxorubicin group.However, treatment with sodium thiosulphate normalized oxidative markers, inflammatory markers,MDA,MPO, GSH, SOD, Nrf-2and catalase. Also, prevented apoptotic changesthroughsuppressing BAX and increasing Bcl-2.

Conclusion: Our study provides a promising protective use of sodium thiosulphate against doxorubicin nephrotoxicity.

***Keywords*:** Doxorubicin, Nephrotoxicity, oxidative stress, Inflammation, Apoptosis, TLR4

Introduction

Doxorubicin (DOX) is a natural anthracycline anti-tumor agent used in several cancer treatment protocols for several types of cancer including, blood malignancy leukemia, lymphoma and solid cancers such as breast, cervical, uterine, ovarian, pulmonary and liver cancers Shi *et al*. (2018); Zidan *et al*., 2018). Unfortunately, despite being a very important anti-cancer drug, doxorubicin use can cause damage to vital organs including heart and kidney (Zhao *et al*., 2014; Ren *et al*., 2016; Nagai *et al*., 2018).

Nephrotoxicity is an important dose-limiting adverse effect in the doxorubicin treatment protocol. Different pathways are included in doxorubicin-induced renal injury including oxidative stress, inflammation (Benzer *et al*., 2018), fibrotic kidney changes (Cardoso *et al*., 2018), and apoptosis that may trigger renal injury through tubular degeneration (Shaker *et al*., 2018). Furthermore, hyperuricemia can be another causative factor in doxorubicin-induced renal injury (Khames *et al*., 2017). Danmaigoro *et al*. (2018), reported that up till now there is no protective drug that could completely reverse doxorubicin-induced nephrotoxicity. Also, most of the previous work that considers doxorubicin nephrotoxicity was limited only to estimating oxidative stress and hyperuricemia markers (Khan *et al*., 2018). However, in this study, we will try to cast light on the effect of TLR4 activation in doxorubicin-induced nephrotoxicity.

TLR4 has been reported to play a great role in renal diseases and tubular damage, however, its mechanism isn't completely understood (Molteni *et al*., 2016). It is considered to be a leading receptor in the inflammatory cascade induced by doxorubicin, besides being a part of the innate immunity activated by several ligands such as bacterial lipopolysaccharides and drugs (XIAO *et al*., 2013, Molteni *et al*., 2016, Kuzmich *et al*., 2017). Binding of a ligand to TLR4 results in its activation and then activating mitogen-activated protein kinases (MAPK), Nuclear factor-kappa B (NF-κB) signaling pathways and TNF-α inducing inflammation (Zhu *et al*., 2015; Molteni *et al*., 2016; Kuzmich *et al*., 2017).

Sodium thiosulphate is a sulfur salt that has been generally used for decades in human medicine in conjunction with sodium nitrite for the treatment of cyanide intoxications (McGeer and McGeer, 2016; Corona *et al*., 2018). Sodium thiosulphate has been previously reported to protect against oxidative stress and inflammation (Bijarnia *et al*., 2015; Ravindran *et al*., 2017). Furthermore, sodium thiosulphate has many clinical applications including inhibiting calciphylaxis (Cicone *et al*., 2004; Hayden and Goldsmith, 2010), ameliorating chondrocyte mineralization, reducing the severity of murine osteoarthritis (Nasi *et al*., 2016) and protecting cardiac cells against ischemia-reperfusion (Ravindran *et al*., 2017). Importantly, it has been previously reported to protect against nephrotoxicity (Ishikawa *et al*., 2015; Freyer *et al*., 2017)

However, there is no study on sodium thiosulphate role in the doxorubicin-induced nephrotoxicity. This study aimed to investigate the effect of sodium thiosulphate on the doxorubicin-induced renal injury and is considered to be a trial to explain its protective effects and the mechanisms of this protection especially the role of TLR4 pathway.

Materials and Methods

Animals

Adult male Sprague-Dawley rats weighing 200g ± 25 were obtained from the breeding colony of the animal house of the National Organization for Drug Control and Research (NODCAR, Giza, Egypt). Animals had free access to food and water *ad libitum*. They were maintained at 22–24˚C, 40–60% relative humidity and diurnal light cycles in animal holding rooms. Animals were adapted for two weeks in their place before the start of the experiments. Experimental procedures were conducted by the ethical guidelines for investigations in laboratory animals and were approved by the Research Ethical Committee of Faculty of Pharmacy, Beni-Suef University (Beni-Suef, Egypt) to comply with the Guide for the Care and Use of Laboratory Animals (ILAR, 1996). Careful handling of animals in such a manner that they do not suffer from unnecessary pain. Animals were treated in a friendly manner without squeezing or pressure, providing good care towards the health and well-being of animals.

Drugs and Chemicals

Doxorubicin was purchased as Adriablastina vial (10 mg/5 ml doxorubicin hydrochloride), from Pharmacia Italia S.P.A. Italy. Sodium thiosulphate was provided by Sigma-Aldrich, USA. All the other chemicals were of the highest purity and analytical grade.

Induction of nephrotoxicity:

By injecting rats with Doxorubicin cummulative dose: 2.6 mg/kg i.p. every 3 days for 28 days (Elsherbiny and El-Sherbiny, 2014)

Experimental design

Rats were divided and distributed in four groups (n=10-12 rat), and were arranged as follow, the first group is considered as a normal control group receiving saline. The second group was given sodium thiosulphate (400 mg/kg/day; p.o; for four weeks (Bijarnia *et al*., 2015). Thethird group was injected with doxorubicin (21 mg/kg, i.p., for four weeks). The fourth group received a combination of doxorubicin and sodium thiosulphate for four weeks, where sodium thiosulphate was injectedand then doxorubicin injectionwas administeredafter one hour.

Sample preparation

Blood samples were withdrawnfrom the retro-orbital sinus plexusafter light anesthesiathen the serum was separatedby centrifugation at 1000g for 10 min, stored at −80°C for biological measurements of the renaland cardiacfunction biomarkers. Animals were then euthanized by decapitation underanesthesia, the two kidneysof each animal were dissected out, and divided into two separate parts, the first part was fixed in 10% formalin solution for histopathological examination but the other part was homogenized in 50 mM phosphate buffer solution (pH 7.4) and kept at -80°C till the determination of biochemical parameters and western blot examination.

Biochemical analysis

Assessment of renal functions

Colorimetric assay kits were used for the assay of kidney function tests including the levels of, blood urea nitrogen (BUN), serum creatinine, serum albumin and total proteins using (Biomed diagnostics, Cairo, Egypt). Rat Cystatin-C (Cys-C) ELISA kit was used for measurement of serum cystatin and the Rat Kidney injury molecule (KIM-1) ELISA Kit for measurement of serum KIM-1 obtained from MyBioSource (San Diego, CA, USA). All procedures were performed according to the kit manufacturers' instructions.

Assessment of inflammatory markers in renal tissues

Protein levels of TLR-4, NF-κB, Nrf-2, and p38-MAPK were measured using the Western blot technique by TGX Stain-Free™ Fast Cast™ Acrylamide Kit (SDS-PAGE) which was provided by (Bio-Rad Laboratories, TNC, USA Catalog. NO. 161-0181).

The Western Blot analysis procedure was done using the V3 Western Workflow Complete System, Bio-Rad R-Hercules, CA. where, proteins were extracted from tissue homogenates by ice-cold radio-immunoprecipitation assay buffer supplemented with phosphatase and protease inhibitors (50 mmol/L sodium vanadate, 0.5 mMphenylmethylsulphonyl fluoride, 2 mg/mL aprotinin, and 0.5 mg/ml leupeptin) and at 12,000 rpm centrifugation for 20 min. Finally, the protein concentration for the sample was measured using the Bradford method. All procedures were performed according to the manufacturers' instructions

Assessment of IL-1 βand TNF-α in renal tissues

Rat TNF-α and IL-1β were measured by the ELISA kits obtained from Ray Biotech Inc. (Parkway, LaneSuite Norcross, GA).

Estimationof renal oxidative stress

In the kidney homogenate, the assessment of thiobarbituric acid reactive substances (TBARS), glutathione (GSH), myeloperoxidase (MPO), catalase (CAT) and superoxide dismutase (SOD) was done using ELISA kits (My Biosource, San Diego, CA, USA) and the manufacturer's instructions were followed.

Measurement of protein expression of Bax/Bcl2

Protein levels of B-cell lymphoma 2 (Bcl-2) proteins and Bcl-2-Associated-X (Bax)-protein apoptotic markers were examined and assessed using western blot technique, proteins were extracted by triazole reagent, and protein concentrations were estimated by Bradford assay, all procedures followed the manufacturer's instructions.

Renal histopathological examination

Autopsy samples were taken from the kidney of rats in different groups and were fixed in 10% formalin saline for twenty-four hours. Washing was done with tap water, then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and were embedded in paraffin at 56 degrees in a hot air oven for twenty-four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slidge microtome. The obtained tissue sections were collected on

glass slides, deparaffinized, stained by hematoxylin & eosin stain for routine examination through the suitable light electric microscope (Suvarna *et al*., 2018).

Statistical analysis

Results were expressed as mean ± SE. Statistical analysis was donebySPSS version 16 (Chicago, IL, USA), but the graphs were drawn and constructed by (Graph Pad Software Inc. V5, San Diego, CA, USA). Statistical analysis was done by one-way analysis of variance (ANOVA) with the Tukey Multiple Comparison Test as a post hoc test. Probability values that less than 0.05 were considered statistically significant.

Results

Nephrotoxicity biomarkers

Normal control values for the serum levels of urea, creatinine, total protein and albumin were 33.17 ± 1.9 mg/dl, 0.18 ± 0.01 mg/dl, 5.59 ± 0.14 mg/dl and 3.5 ± 0.25 mg/dl, respectively. Normal control values for the serum levels of KIM-1 and cystatin C were 2.20 ± 0.09 pg/ml and 0.6617 ± 0.04 pg/ml. Doxorubicin caused a significant increase in serum levels of urea nitrogen and creatinine associated with significant suppression of total protein and albumin levels as compared to the normal control group. Doxorubicin significantly increased serum levels of KIM-1 and cystatin C.

However, co-treatment with sodium thiosulphate significantly abolished the increase of urea nitrogen, creatinine, KIM-1, and cystatin C levels and restored the ratio of total protein and albumin, respectively, as compared to doxorubicin group (Table 1).

Oxidative stress parameters.

Normal control values for MDA and GSH, SOD, CAT, Nrf-2 and MPO were 5.350 ± 0.17 nmol/g. tissue, 71.15±2.95 mg/g tissue, 5.89± 0.11 mg/g tissue, 120.0 ± 1.09 mg/g tissue,1.03± 0.02 and 39.25 ± 2.39 mg/g tissue, respectively.

Doxorubicin treatment caused a significant increase in the renal MDA content and MPO activity accompanied by a marked decrease in renal content of GSH as well as a significant decrease in Nrf-2, SOD and CAT activities, respectively, as compared to the normal control group.

Treatment with sodium thiosulphate significantly suppressed the renal MDA content and MPO activity and restored the renal deficiencyof Nrf-2, GSH, SOD, and CAT to normal control values as compared to doxorubicin group (Fig. 1A-F).

Inflammatory mediators.

Normal control values for TLR4, P38 MAPK, NF-κB,IL-1 β and TNF-α were 1.00 ±0.01, 1.005 ± 0.001 , 1.01 ± 0.01, 14.93±1.30 and 24.88 ± 0.86 pg/g tissue.

Doxorubicin treatment resulted in a significant increase in renal content of the inflammation pathway TLR4, P38 MAPK, NF-κB, and TNF-α as compared to the normal control group. Co-treatment with sodium thiosulphate significantly suppressed renal content of TLR4, P38 MAPK, NF-κB, IL-1 β, and TNF-α as compared to doxorubicin group (Fig. 2A-D).

Apoptotic markers

We assessed the levels of pro-apoptotic protein BCL2 Associated X protein (Bax), and anti-apoptotic protein B-cell lymphoma 2 (Bcl-2) as apoptotic markers

As shown in Fig.3A-Cdoxorubicin caused a severe increase of Bax while the Bcl-2 level decreased in the renal cells of the doxorubicin group. Sodium thiosulphate inhibitedapoptosis in the renal cells of Sodium thiosulphate_treated groups through decreasing the apoptotic marker Bax and increasing the anti-apoptotic Bcl-2 compared with doxorubicin group.

Renal histopathology(Fig .4). (Table 2)

As shown in Figure 4, renal sections obtained from control and sodium thiosulphate- treated groups showed no histological alterations of kidney architecture. However, the group treated with doxorubicin showed focal inflammatory cell infiltration with few fibroblastic cells proliferation in the tubules and glomeruli at the cortex. Also, vacuolization was shown in the endothelial cells lining the tufts of the glomeruli, Congestion was shown in the cortical stromal blood vessels and edema. Focal fibrosis was shown in the corticomedullary portion and focal hemorrhages in the tubules. There were swelling in the lining epithelium of tubules and obliteration in the tubular lumen but in the other tubules at the corticomedullary portion, there are degenerative changes in the epithelium lining. The sodium thiosulphate co-treated group showed mild fibrosis in between the tubules.

Table 1: Effect of sodium thiosulphate on serum levels of urea, creatinine, total protein, albumin, Kidney injury molecule and cystatin in doxorubicin-induced nephrotoxicity in rats.

Group	Serum urea (mg/dl)	Serum creatinine (mg/dl)	Serum total protein g/dl	Serum albumin g/dl	Kidney injury molecule Pg/ml	Serum cystatin Pg/ml
Control saline	33.17 ± 1.9	0.18 ± 0.017	5.59 ± 0.14	3.48 ± 0.25	2.20 ± 0.09	0.66 ± 0.04
sodium thiosulphate	32.33 ± 2.7^{b}	0.17 ± 0.02^{b}	5.9 ± 0.05^{b}	3.50 ± 0.14 ^{b}	2.40 ± 0.12^{b}	0.76 ± 0.02^{b}
Doxorubicin	± 83.83 2.7^{a}	1.64 ± 0.12^{a}	3.77 ± 0.06^{a}	2.48 ±0.12^{a}	12.17 ± 0.36^{a}	2.86 ± 0.37^{a}
sodium thiosulphate + Doxorubicin	41 ± 3.25^{b}	0.52 ± 0.03^{ab}	5.1 ± 0.07^{ab}	3.33 ±0.13^{b}	4.96 ± 0.32^{ab}	1.12 ± 0.05^{b}

Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer Multiple Comparison Test.

a Significantly different from normal control group value at $p < 0.05$.

b Significantly different from doxorubicin group value at $p < 0.05$.

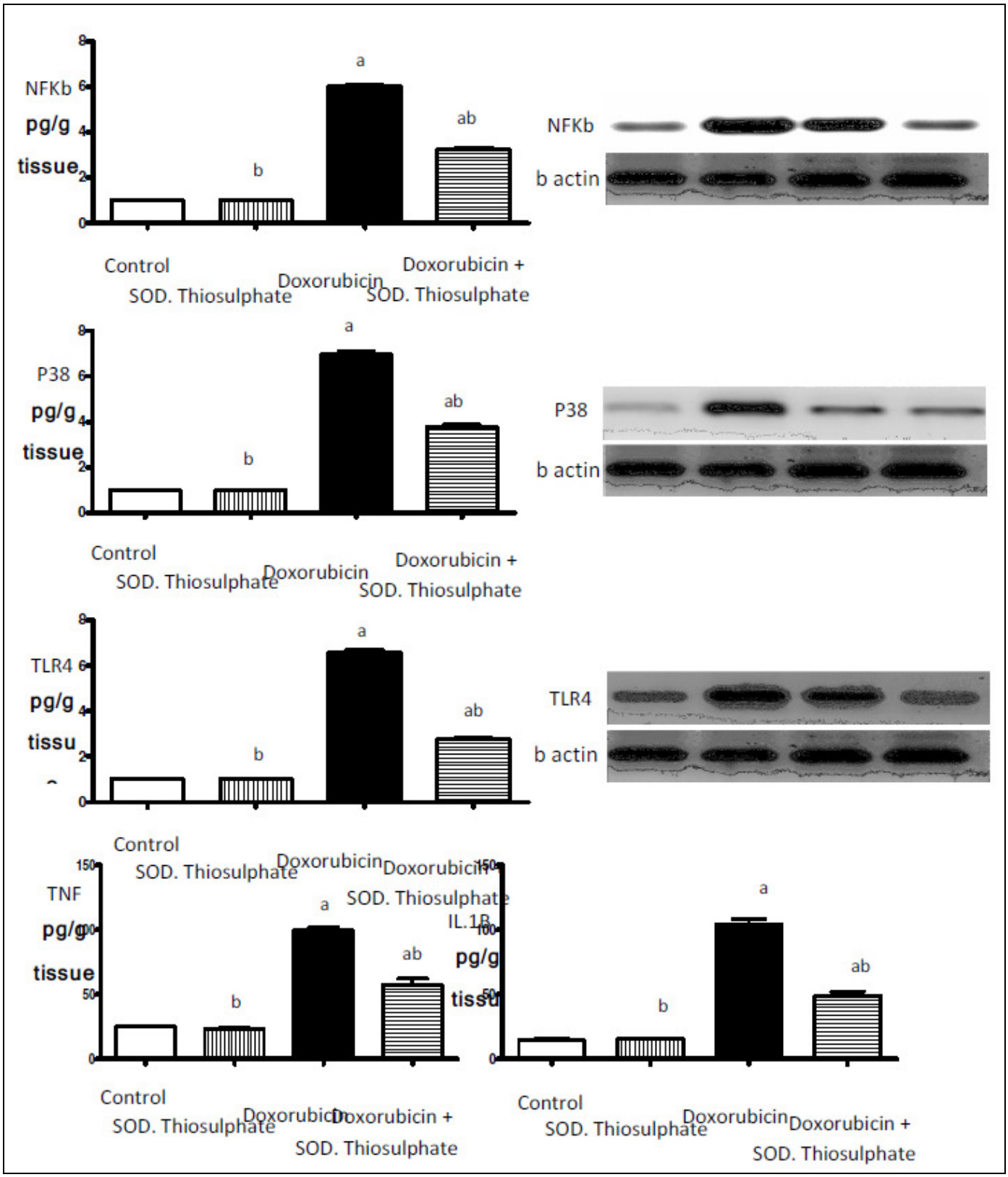

Fig. 1 : Effect of sodium thiosulphate on renal content of NFKb, p38, TLR4, TNF-α and IL-1β in doxorubicin-induced nephrotoxicity in rats.

Each value represents the mean of 8-10 rats ± standard deviation of the mean (SE.).
Statistical analysis was carried out by one way ANOVA followed by Tukey- Kramer Multiple Comparison Test.
[a] Significantly different from normal control group value at $p < 0.05$.
[b] Significantly different from doxorubicin group value at $p < 0.05$.

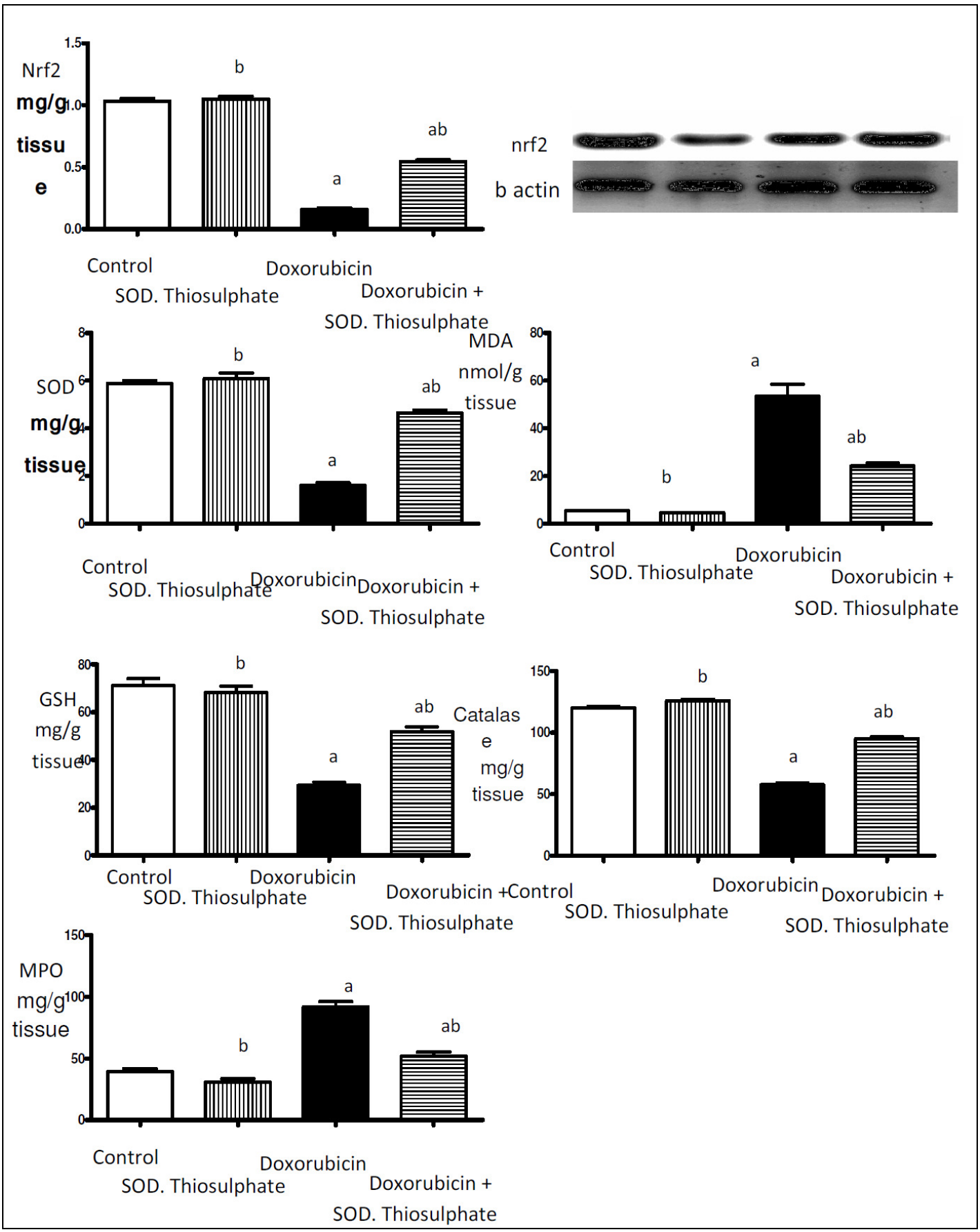

Fig. 2: Effect of sodium thiosulphate on renal content of Nrf2, SOD MDA, GSH, catalase and MPO in doxorubicin-induced nephrotoxicity in rats

Each value represents the mean of 8-10 rats ± standard deviation of the mean (SE.).

Statistical analysis was carried out by one way ANOVA followed by Tukey- Kramer Multiple Comparison Test.

[a] Significantly different from normal control group value at $p < 0.05$.

[b] Significantly different from doxorubicin group value at $p < 0.05$.

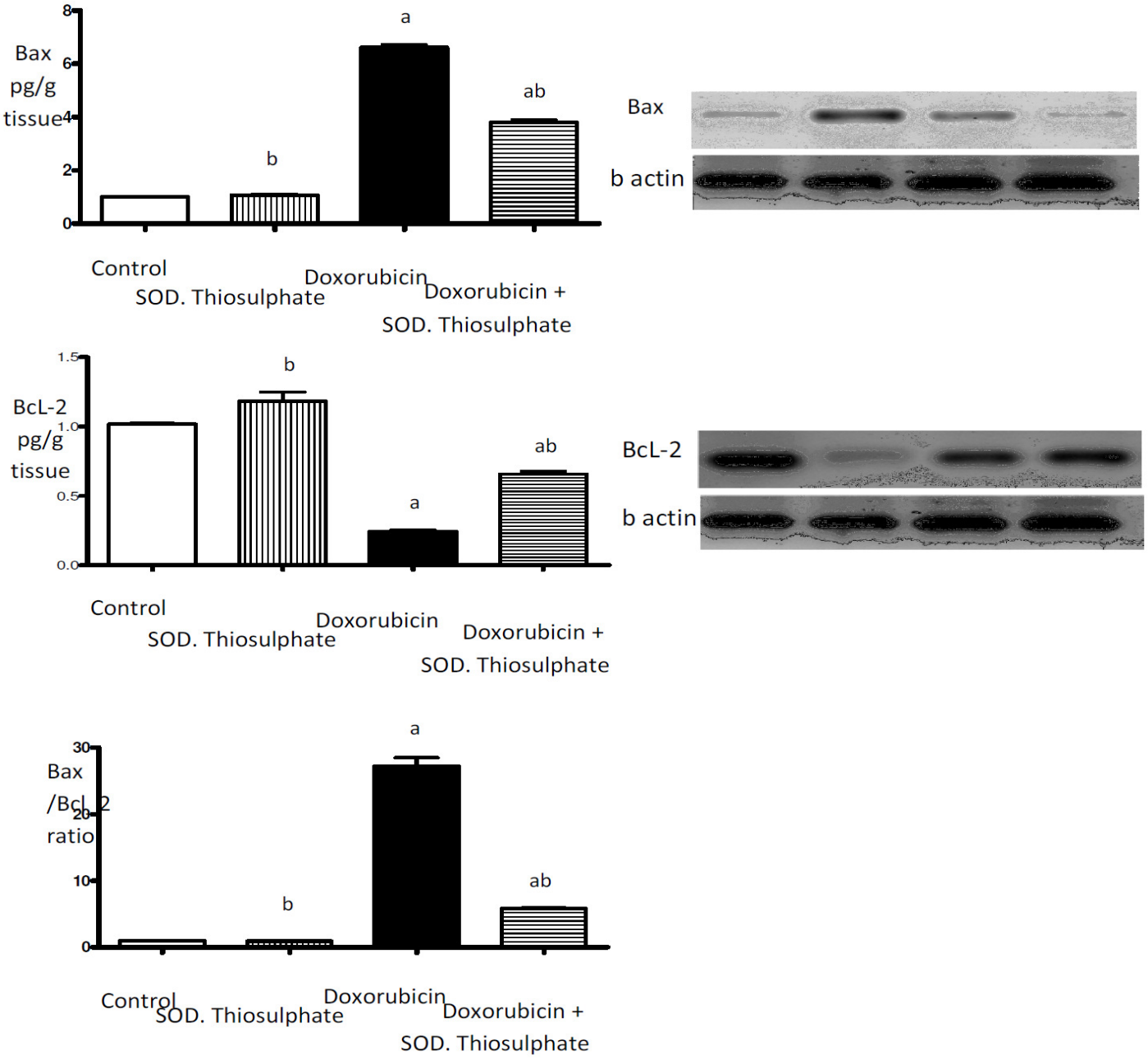

Fig. 3: Effect of sodium thiosulphate on renal content of Bax and Bcl in doxorubicin-induced nephrotoxicity in rats.
Each value repesents the mean of 8-10 rats ± standard deviation of the mean (SE.).
Statistical analysis was carried out by one way ANOVA followed by Tukey- Kramer Multiple Comparison Test.
[a] Significantly different from normal control group value at $p< 0.05$.
[b] Significantly different from doxorubicin group value at $p < 0.05$

Table 2 : Effect of sodium thiosulphate on histopathological alterations in doxorubicin-induced nephrotoxicity in rats.

Group Histopathological alteration	Control saline	Doxorubicin	Doxorubicin + sodium thiosulphate	sodium thiosulphate
Tubular degeneration	–	++	–	–
Focal inflammatory cell infiltration	–	++	–	–
Focal fibrosis	–	++	–	–
Vaculisation of glomerular endothelium	–	+++	–	–
Congestion in blood vessels	–	++	–	–
Perivascular edema	–	++	–	–
Focal fibrosis	–	++	+	–
Focal haemorrhage	–	++	–	–
Degeneration in the tubules	–	++	–	–

Statistical analysis was carried out by one-way ANOVA followed by Tukey- Kramer Multiple Comparison Test.
a Significantly different from normal control group value at $p < 0.05$.
b Significantly different from doxorubicin group value at $p < 0.05$.

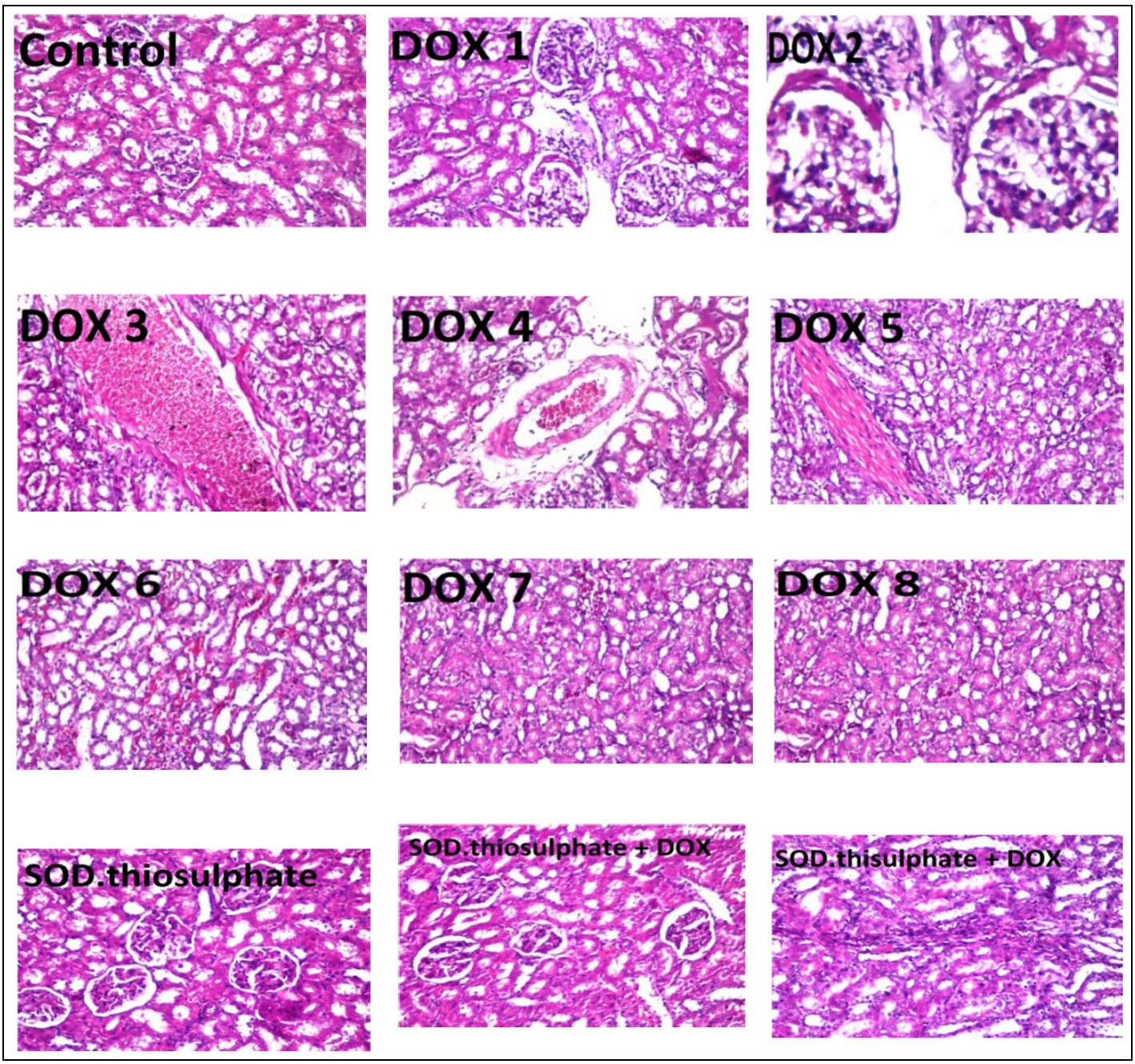

Fig. 4: Effect of sodium thiosulphate on kidney sections in doxorubicin-induced nephrotoxicity in rats stained with hematoxylin/eosin (H/E) and examined under the light microscope

Doxorubicin treated rats showed degenerative changes; focal inflammatory cells infiltration (DOX 1), The endothelial cells lining the tufts of the glomeruli showed vacuolization (DOX 2), The cortical stromal blood vessels showed congestion (DOX 3), as well as perivascular edema (DOX 4), The corticomedullary portion showed focal fibrosis (DOX 5), and focal hemorrhages in between the tubules (DOX 6), There were swelling in the lining tubular epithelium with obliteration in the tubular lumen (DOX 7), while other tubules at the corticomedullary portion had vacuolar degeneration in the lining epithelium (DOX 8),There was no histopathological alteration as recorded in (SOD.thiosulphate only),There was no histopathological alteration in the tubules and glomeruli at the cortical portion, The corticomedullary portion showed focal few fibrosis in between the tubules (DOX+SOD. Thiosulphate)

Discussion

Chemotherapy negatively affects all the body physiological functions through inducing injury to vital organs and systems in the body and increasing mortality. Doxorubicin is a leading anti-cancer that rescued the life of many patients suffering from lymphoma, leukemia, breast and hepatic cancers (Yazd *et al*., 2018). Unfortunately, it causes fatal adverse effects including nephrotoxicity (Bulucu *et al*., 2008, Akindele and Oludadepo, 2018; Benzer *et al*., 2018). Besides, cardiac, pulmonary, testicular and hematological toxicities (Ananthanarayanan Ajith *et al*., 2016; Jaćević *et al*., 2018). The mechanisms by which doxorubicin induces nephrotoxicity depend on several factors including the production of free radicals generated by doxorubicin metabolites, inflammation, apoptosis (Khan *et al*., 2018; Kramer *et al*., 2009) and hyperuricemia(Khames *et al*., 2017b).

In the present study, the administration of doxorubicin increased significantly serum levels of BUNand creatinine, while it significantly deteriorated serum levels of albumin and total protein. These results are in agreement with those of (Qiao *et al*., 2018); (Mansouri *et al*., 2018; Yazd *et al*.,

2018). This is attributed to the direct damaging effect of doxorubicin on renal tissues also, lack of renal blood supply due to cardiac side effects of doxorubicin impair renal filtration and decrease renal function, filtering protein and albumin. Also, this study showed a significant increase in the serum level of Kim-1 and cystatin C in the doxorubicin group as compared to the normal control group. These results agreed with the results of (Lateef *et al*., 2014; Wang *et al*., 2015).

Tubular kidney injury molecule-1 is a transmembrane protein induced in the case of acute kidney injury and chronic renal damage (Huo *et al*., 2010). Doxorubicin-induced KIM-1 in case of nephropathy by an unknown mechanism. Cystatin C is a substance that is filtered by the glomeruli and is reabsorbed again through proximal tubules. Defect in tubules by any chemical will increase cystatin C in serum (Dharnidharka *et al*., 2002). These results could prove that doxorubicin and its metabolites cause renal tubular injury.

The generation of oxidative free radicals and ROS by doxorubicin resulted in oxidative stress inside the kidney tissues leading to structural and functional changes of the kidney, and this is considered to be the main mechanism responsible for DOX-induced nephrotoxicity (Khames *et al*., 2017a). Doxorubicin is metabolized to the semi-quinone form that reacts with molecular oxygen forming hydrogen peroxide (H_2O_2), hydroxyl radicals (OH·) and other free radicals that react with and damage the renal tissue proteins (Liu *et al*., 2009; Mansouri *et al*., 2017). Moreover, the free radicals produced attack DNA and interact with it inducing apoptosis in both normal and cancer cells (Al-Dalaen and Al-Qtaitat, 2014; Benzer *et al*., 2018; Akindele and Oludadepo, 2018). Another mechanism for doxorubicin oxidative stress is dependent on the presence of iron where doxorubicin–iron complex oxidizes molecular oxygen giving hydrogen peroxide and several ROS leading to an increase in MDA and MPO, and depletion of antioxidant enzymes (Tury *et al*., 2018).

Results of the present study showed that doxorubicin administration significantly reduced the activities of the natural antioxidant enzymes superoxide dismutase and catalase besides decreasing the renal contents of GSH and Nrf-2. Doxorubicin not only decreased the antioxidant protective enzymes but also increased oxidative agents as proved by a significant increase observed in the renal MDA content and MPO activity. These findings are in agreement with (Fouad *et al*., 2018). It could be suggested that the GSH and antioxidant enzymes are exploited in neutralizing the ROS and free radical metabolites produced by doxorubicin in addition to counteracting lipid peroxidation (Ashour *et al*., 2011).

Another amazing finding in this study was that DOX significantly increased the renal content of the inflammatory mediators; TLR4, P38 MAPK, NF-κB, IL-1 beta, and TNF-α. These results are in agreement with those of (Kobayashi *et al*., 2016; Ma *et al*., 2012) and Kobayashi *et al*. (2016). This increase in the inflammatory mediators is thought to be due to the excessive oxidative stress caused by ROS generated from the semi-quinone form of doxorubicin after depletion of antioxidant mechanisms predisposing the tissues to injury and inflammation, and this explains why cancer patients receiving doxorubicin are susceptible to an inflammatory response (Wang *et al*., 2016).

(Kumar *et al*., 2004) and (Vyas *et al*., 2014) reported that lipopolysaccharide (LPS), free radicals and anticancer drugs can activate TLR4, inducing an inflammatory response through activating MAPK then NFκB transcription factor that stimulates the synthesis of the inflammatory mediators IL-1β and TNF-α. In the present study, doxorubicin stimulated the TLR4 receptor and started the sequential activation of MAPK and then NF-κB and this will induce the production and release of pro-inflammatory cytokines IL-1β, TNF-α and IL-6. TLR4 signaling is the heart of our study especially when we talk about cancer therapy where TLR4 is included in the regulation of carcinogenesis through increased proliferation, apoptosis inhibition and metastasis (He *et al*., 2007; Lu *et al*., 2013). Also, (Szajnik *et al*., 2009) reported that TLR4 signaling may contribute to resistance to chemotherapy. In the present study, we evaluated the stimulant effect of doxorubicin on TLR4 and its participation in renal injury besides the inhibitory effect of sodium thiosulphate on TLR4 as a mechanism of protection against doxorubicin-induced renal injury.

The results of this study revealed that apoptosis is an important mechanism of doxorubicin-induced nephrotoxicity through increasing BAX and decreasing Bcl-2 contents in the kidney. This is in harmony with the results of (Sun *et al*., 2018; Hoshi *et al*., 2017). (Imam *et al*., 2018) found that oxidative stress and inflammation activate pro-apoptotic factors and induce apoptosis. Furthermore, (Park *et al*., 2014) reported that apoptotic changes occur in the cells are caused by a defect in the mitochondrial membrane due to lipid peroxidation and p53 activation. This augmented the findings of the present study that, the apoptotic response in kidney tissue is due to ROS mediated NF-κB activation. NF-κB was found to regulate DOX-induced apoptosis. Also, the activation of the TLR4 pathway has been observed to be involved in the development of doxorubicin-induced nephropathy.

The doxorubicin-treated group showed histopathological alterations where fibroblastic cell proliferation and focal inflammatory cell infiltrations detected in between the tubules and glomeruli at the cortex. The endothelial cells lining the tufts of the glomeruli appeared with vacuolization. Also, congested cortical stromal blood vessels showed as well as oedematous perivascular area. The corticomedullary portion showed focal fibrosis and hemorrhage between the tubules. There was swelling in the lining tubular epithelium with obliteration in the tubular lumen while other tubules at the corticomedullary portion had vacuolar degeneration in the lining epithelium (Fig.4), (Table 2)

The present study proved that sodium thiosulphate pretreatment prevented doxorubicin-induced nephrotoxicity as proved by decreasing serum levels of urea, creatinine, KIM, and cystatin and increased serum albumin and total protein. Previous results also hypothesized that sodium thiosulphate ameliorates nephrotoxicity in the rat (Shimizu *et al*., 2011; Ozturk *et al*., 2017). This could be explained by the chelating power of sodium thiosulphate, where it can bind oxidative metabolites of doxorubicin and prevent its intercalation with renal protein resulting in improving renal function.

Additionally, sodium thiosulphate suppressed MDA content and myeloperoxidase activity and restored the

antioxidant enzyme activities of Nrf-2, GSH, SOD, and catalase. This is in the agreement with the results of (Bijarnia *et al*., 2015; Nasi *et al*., 2016). This could be attributed to the free radical scavenger activity of sodium thiosulphate and its ability to reduce ROS due to the presence of a sulfur atom.

Regarding the anti-inflammatory effect of sodium thiosulphate, the results of our study declared that sodium thiosulphate has an anti-inflammatory effect proved by decreasing the renal content of TLR 4, p38 MAPK, NF-κB, IL-1β, and TNF–α. This is in agreement with the results of (Nasi *et al*., 2016; Ravindran *et al*., 2017) who explained this by the antioxidant effect of sodium thiosulphate that could neutralize free radicals produced by doxorubicin preventing oxidative stress and thereby avoiding inflammatory response.

In this study, sodium thiosulphate abolished the apoptotic effect of doxorubicin as evidenced by the significant decrease observed in the renal content of BAX and the significant increase in the renal content of Bcl-2 as compared to doxorubicin group. This finding is compatible with those of Nagata *et al*. (2003)and (Ravindran *et al*., 2017). (Nagata *et al*., 2003) proving that sodium thiosulphate counteracts doxorubicin-induced inflammation and thereby preventing the activation of pro-apoptotic proteins and inhibiting apoptosis.

Concerning the histopathological study, it was obvious that sodium thiosulphate can prevent structural damage caused by doxorubicin where it didn't show any histopathological alteration than the normal control group.

As mentioned and proved above doxorubicin caused both structural and functional renal abnormalities. From our results and findings, it could be deduced that the mechanisms of doxorubicin-induced nephrotoxicity are oxidative stress, inflammation, and apoptosis. Sodium thiosulphate appears to be a promising agent to be used concomitantly with many anticancer agents to reduce their adverse effects through several mechanisms including, strong antioxidant ability, anti-inflammatory, and anti-apoptotic mechanisms through suppression of oxidative stress-mediated activation of TLR4/ MAPKp38/NF-κB signaling pathways.

Contributions

KA and designed the study. AK, K.M, and G.A.M performed experiments, analyzed data and wrote the manuscript. M.KA.M supervised the study. KA, G.A.M, K.M, and M.O edited the manuscript.

Declaration of interests

The authors declare that they have no known competing for financial interests or personal relationships that could have appeared to influence the work reported in this paper.animal handling and all procedures were done according to ethical standards and guidelines.

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Disclosure of Conflict of interest

The authors have read the journal's policy on disclosure of potential conflicts of interest and they all declare no personal or financial conflict of interest.

Authorship Statement

All authors have read the journal's authorship statement and agree to it.

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