Research Article

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Clinical Pharmacology and Toxicology Journal
ISSN 2171-6625

2022
Vol.6 No.4:001

Toll like Receptor 4 Pathway Inhibition by Sodium Thiosulphate Ameliorated Doxorubicin: By Induced Kidney Injury

Ali Khames Abd El Twab[1*], Marwa M Khalaf[2], Amany M Gad[3], Ola M Abd El Raouf[3] and Mohamed Ahmed Kandeil[4]

[1]Department of Pharmacology and Toxicology, Deraya University, Minya, Egypt
[2]Department of Pharmacology and Toxicology, Beni-Suef University, Beni-Suef, Egypt
[3]Department of Pharmacology, National Organization for Drug Control and Research (NODCAR), Cairo, Egypt
[4]Department of Biochemistry, Beni-Suef University, Beni-Suef, Egypt

Corresponding author: Ali Khames Abd El Twab, Department of Pharmacology and Toxicology, Deraya University, Minya, Egypt, E-mail: ali_khamies@yahoo.com

Received date: January 15, 2020, Manuscript No. IPRRT-20-3207; **Editor assigned date:** January 21, 2020, PreQC No. IPRRT-20-3207; **Reviewed date:** February 04, 2020, QC No. IPRRT-20-3207 ; **Revised date:** August 22, 2022, QI No. IPRRT-20-3207; Manuscript No. IPRRT-20-3207; **Published date:** September 19, 2022

Citation: Twab AKAE, Khalaf MM, Gad AM, El Raouf OMAE, Kandeil MA (2022) Toll like Receptor 4 Pathway Inhibition by Sodium Thiosulphate Ameliorated Doxorubicin: By Induced Kidney Injury. Clin Pharmacol Toxicol J Vol.6 No.4: 001

Abstract

Aim: Doxorubicin nephrotoxicity is always a major cause of death for cancer patients. Therefore, 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 (2.6 mg/kg/twice weekly for 4 weeks). And the treatment depends on giving sodium thiosulphate (400 mg/kg, p.o.) One hour before doxorubicin injection for4 weeks.

Key findings: Doxorubicin injection caused severe renal dysfunction evident from significant increase in the renal biochemical parameters, urea, creatinine, KIM.1 and serum cystatin C, together with decreasing serum albumin and total protein. In addition, increased MDA and decreased GSH, SOD, Nrf-2 and catalase inflammatory markers TLR4, MAPK P38, nuclear factor kappa-B NF-κB,interlukin-1 and TNF- alpha also, increased apoptosis in renal tissues of doxorubicin group., sodium thiosulphate normalized oxidative markers, inflammatory markers MDA,GSH,SOD,Nrf-2 and catalase doxorubicin prevented apoptotic changes through suppressing BAX and increasing Bcl-2,and finally proved by histopathology study.

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

Keywords: Doxorubicin; Nephrotoxicity; Oxidative stress; In lammation; 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. Unfortunately, despite being a very important anti-cancer drug, doxorubicin use can cause damage to vital organs including heart and kidney [1,2].

Nephrotoxicity is an important dose-limiting adverse effect in doxorubicin treatment protocol. Different pathways are included in doxorubicin-induced renal injury including oxidative stress, inflammation, fibrotic kidney changes, and apoptosis that may trigger renal injury through tubular degeneration. Furthermore, hyperuricemia can be another causative factor in doxorubicin-induced renal injury. It is reported that up till now there is no protective drug that could completely reverse doxorubicin-induced nephrotoxicity. In addition, most of the previous work that considers doxorubicin nephrotoxicity was limited only to estimating oxidative stress and hyperuricemia markers. However, in this study we will try to cast light on the effect of TLR4 activation in doxorubicin-induced nephrotoxicity [3].

TLR4 has been reported to play a great role in renal diseases and tubular damage, however, its mechanism isn't completely understood. 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. 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 [4].

Sodium thiosulphate, is a sulfur salt which has been generally used since decades in human medicine in conjunction with sodium nitrite for the treatment of cyanide intoxications. Sodium thiosulphate has been previously reported to protect against oxidative stress and inflammation. Furthermore, sodium

thiosulphate has many clinical applications including inhibiting calciphylaxis, ameliorating chondrocyte mineralization, reducing the severity of murine osteoarthritis and protecting cardiac cells against ischaemia reperfusion. Importantly, it has been previously reported to protect against nephrotoxicity [5-7].

However, there is no study on sodium thiosulphate role in doxorubicin-induced nephrotoxicity. This study aimed to investigate the effect of sodium thiosulphate on 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 [8-12].

Materials and Methods

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 [13].

Induction of nephrotoxicity

A cummulative dose of doxorubicin 21 mg/kg was divided on four weeks where adult rat was given 2.6 mg/kg I.P. twice per week.

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 normal control group receiving saline. The second group was given sodium thiosulphate (400 mg/kg/day; p.o; for four weeks. The third group was injected with doxorubicin (13 mg/kg, i.p., for four weeks). The fourth group received combination of doxorubicin and sodium thiosulphate for four weeks, where sodium thiosulphate was injected and then doxorubicin injection was administered after one hour [14-18].

Sample preparation

Blood samples were withdrawn from the retro-orbital sinus plexus then the serum was separated by centrifugation at 1000 g for 10 min, stored at -80°C for biological measurements of the renal function tests. Animals were then sacrificed through decapitation under the effect of anesthesia, the two kidneys of 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 determination of biochemical parameters and western blot examination [19].

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 my biosource (San Diego, CA, USA). All procedures were performed according to the kit manufacturers' instructions [20,21].

Assessment of inflammatory markers in renal tissues: Protein levels of TLR-4, NF-κB, Nrf-2, and p38-MAPK were measured using 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 [22,23].

The Western Blot analysis procedure was done using V3 Western Workflow Complete System, Bio-Rad R_Hercules, CA. where, proteins were extracted from tissue homogenates by ice-cold radio-immuno precipitation assay buffer supplemented with phosphatase and protease inhibitors (50 mmol/L sodium vanadate, 0.5 mM phenyl methyl sulphonyl fluoride, 2 mg/mL aprotinin, and 0.5 mg/mLleupeptin) and at 12,000 rpm centrifugation for 20 min. Finally, the protein concentration for the sample was measured using Bradford method. All procedures were performed according to the manufacturers' instructions [24-28].

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, Lane Suite Norcross, GA).

Estimation of renal oxidative stress: ELISA kits purchased from (San Diego, CA, USA) were used for the measurement of Thiobarbituric Acid Reactive Substances (TBARS), Glutathione (GSH), Myeloperoxidase (MPO), Catalase (CAT) and Superoxide Dismutase (SOD) on the basis of the manufacturer's instructions [29].

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 trizol reagent, and protein concentrations were estimated by Bradford assay, all procedures followed the manufacturer's instructions [30,31].

Renal histopathological examination: Autopsy samples were taken from the kidney of rats in different groups and were fixed in 10% formaline 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 degree in 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 and eosin stain for routine examination through the suitable light electric microscope [32,33].

Statistical analysis: Results were expressed as mean ± SE. Statistical analysis was done by the SPSS 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 Tukey-

Kramer Multiple Comparison Test as a post hoc test. Probability values that less than 0.05 were considered statistically significant [34].

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 2.202 ± 0.09 pg/ml and 0.6617 ± 0.04 pg/ml. Doxorubicin caused significant increase in serum levels of urea nitrogen and creatinine associated with a significant suppression of total protein and albumin levels as compared to normal control group. Doxorubicin significantly increased serum levels of KIM-1 and cystatin C [35,36].

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)**.

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.

Serum cystatin Pg/ml	Kidney injury molecule Pg/ml	Serum albumin g/dl	Serum total protein g/dl	Serum creatinine (mg/dl)	Serum urea (mg/dl)	Group
0.66 ± 0.04	0.09 ± 2.20	0.25 ± 3.48	5.59 ± 0.14	0.18 ± 0.017	33.17 ± 1.9	Control saline
0.02 ± 76 0[b]	0.12 ± 2.40[b]	0.14 ± 3.50[b]	5.9 ± 0.05[b]	0.17 ± 0.02[b]	32.33 ± 2.7[b]	Sodium thiosulphate
0.37 ± 2.86[a]	12.17 ± 0.36[a]	± 2.48 0.12[a]	3.77 ± 0.06	0.12 ± 1.64[a]	83.83 ± 2.7	Doxorubicin
1.12 ± 0.05[b]	4.96 ± 0.32[ab]	± 3.33 0.13[b]	0.07 ± 5.1[ab]	0.03 ± 0.52[ab]	± 41 3.25[b]	Sodium thiosulphate +Doxorubicin

Note: 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 $p<0.05$.

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 [37].

Doxorubicin treatment causeda significant increase in the renal MDA content and MPO activity accompanied with 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 supressed the renal MDA content and MPO activity and restored the renal deficiency of Nrf-2, GSH, SOD, and CAT to normal control values as compared to doxorubicin group (**Figure 1**).

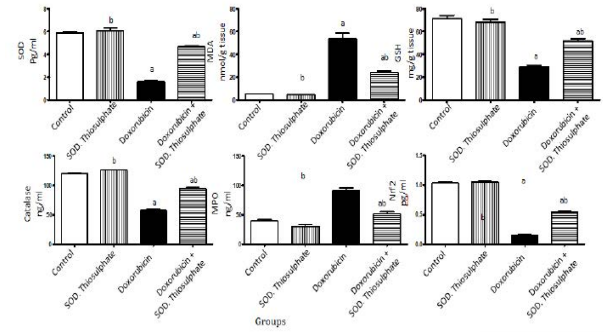

Figure 1: Effect of Sodium thiosulphate on renal content of SOD, MDA, GSH, catalase, MPO, and Nrf2 in doxorubicin-induced nephrotoxicity in rats. Each value represents the mean of 8-10 rats ± standard deviation of the mean (SE.) a) Significantly different from normal control group value at $p<0.05$; b) Significantly different from doxorubicin group value at $p<0.0$.

Statistical analysis was carried out by one way ANOVA followed by Tukey-Kramer multiple comparison test.

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 [38].

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 (**Figure 2**).

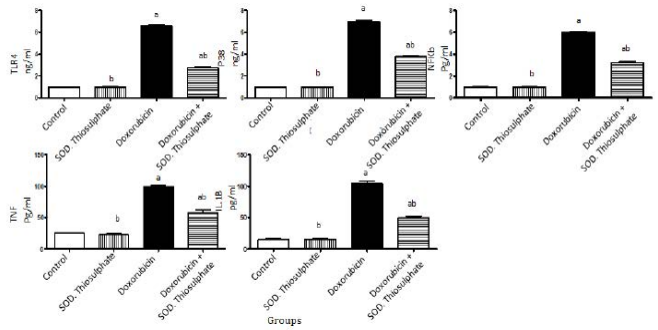

Figure 2: Effect of sodium thiosulphate on renal content of TLR4, P38, NFKb, TNF and IL.1B in doxorubicin-induced nephrotoxicity in rats. a) Significantly different from normal control group value at p<0.05; b) Significantly different from doxorubicin group value at p<0.05.

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.

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 doxorubicin caused severe increase of Bax while Bcl-2 level decreased in the renal cells of doxorubicin group. Sodium thiosulphate inhibited apoptosis in the renal cells of Sodium thiosulphate treated groups through decreasing the apoptic marker Bax and increasing the anti apoptic Bcl-2 compared with doxorubicin group **(Figure 3)**.

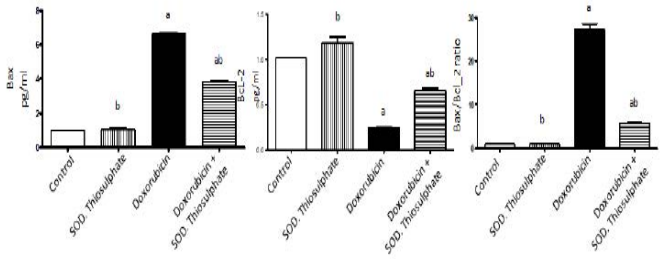

Figure 3: Effect of sodium thiosulphate on renal content of Bax and Bcl-2 in doxorubicin-induced nephrotoxicity in rats; a) Significantly different from normal control group value at p<0. 05; b) Significantly different from doxorubicin group value at p<0.05.

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.

Renal histopathology

As shown in 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 cells infiltration with few fibroblastic cells proliferation in the tubules and glomeruli at the cortex. In addition, vacuolization was shown in the endothelial cells lining the tufts of the glomeruli, Congestion was shown in the cortical stromal blood vessels and oedema. Focal fibrosis was shown in the corticomedullary portion and focal haemorrhages 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 is degenerative changes in the epithelium lining. The sodium thiosulphate co-treated group showed mild fibrosis in between the tubules (**Figure 4 and Table 2**).

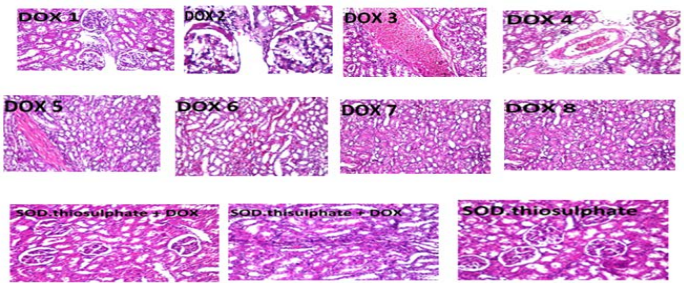

Figure 4: Effect of Nicorandil on kidney sections in doxorubicin-induced nephrotoxicity in rats stained with Hematoxylin/Eosin (H/E) and examined under a microscope.

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

Sodium thiosulphate	Doxorubicin+sodium thiosulphate	Doxorubicin	Control saline	Group Histopathlgical alteration
_	_	++	_	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

Note: Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer multiple comparison test. ++ Significantly different from normal control group value at $p<0.05$; - Significantly different from doxorubicin group value at $p<0.05$.

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 oedema (DOX 4), The corticomedullary portion showed focal fibrosis (DOX 5), and focal haemorrhages 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) [39].

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. Unfortunately, it causes fatal adverse effects including nephrotoxicity. Besides, cardiac, pulmonary, testicular and hematological toxicities. The mechanisms by which doxorubicin induces nephrotoxicity depend on several factors including production of free radicals generated by doxorubicin metabolites, inflammation, apoptosis and hyperuricemia.

In the present study, administration of doxorubicin increased significantly serum levels of BUN and creatinine, while it significantly deteriorated serum levels of albumin and total protein. This is attributed to 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. In addition, this study showed a significant increase in 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 [40].

Tubular kidney injury molecule-1 is a trans-membrane protein induced in case of acute kidney injury and in chronic renal damage. Doxorubicin induced KIM-1 in case of nephropathy by 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. 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 kidney, and this is considered to be the main mechanism responsible for DOX-induced nephrotoxicity. 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. Moreover, the free radicals produced attack DNA and interact with it inducing apoptosis in both normal and cancer cells. Another mechanism for doxorubicin oxidative stress is dependent on presence of iron where doxorubicin–iron complex oxidizes molecular oxygen giving hydrogen peroxide and several

ROS leading to increase in MDA and MPO, and depletion of antioxidant enzymes [41].

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. Nuclear factor erythroid-2 related factor 2 is a transcription factor that regulates the inducible expression of antioxidant genes and other metabolizing enzymes involved in phases I and II metabolic reactions. 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. 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.

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. 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 inflammatory response.

It is 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. Toll-Like Receptor 4 is a member of TLR family that is identified and stimulated by LPS and toxic chemicals. Stimulation of the TLR4 receptor system is followed by production of pro-inflammatory cytokines and interferons precipitating an inflammatory response.

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 specially when we talk about cancer therapy where TLR4 is included in regulation of carcinogenesis through increased proliferation, apoptosis inhibition and metastasis. In addition, it is 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.

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. Furthermore, it is reported that, apoptotic changes occur in the cells are caused by defect in mitochondrial membrane due to lipid peroxidation and p53 activation. This augmented the findings of the present study that, the apoptotic response in kidney tissue are due to ROS mediated NF-κB activation. NF-κBwas found to regulate DOX-induced apoptosis. In addition, the activation of TLR4 pathway has been observed to be involved in the development of doxorubicin-induced nephropathy. Doxorubicin-treated group showed histopathological alterations where, fibroblastic cells proliferation and focal inflammatory cells 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 haemorrhage in 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.

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 rat. 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 [42].

Additionally, sodium thiosulphate suppressed MDA content and myeloperoxidase activity and restored the antioxidant enzyme activities of Nrf-2, GSH, SOD and catalase. This could be attributed to the free radical scavenger activity of sodium thiosuphate and its ability to reduce ROS due to the presence of sulphur atom.

Regarding the anti-inflammatory effect of sodium thiosulphate, the results of our study declared that sodium thiosulphate has 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 results of experts who explained this by the antioxidant effect of sodium thiosuphate that could neutralize free radicals produced by doxorubicin preventing oxidative stress and thereby avoiding inflammatory response.

Conclusion

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. 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 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.

Acknowledgments

The authors would like to thank Prof. Dr. A. Bakear (Pathology Department, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt) for assistance in the histopathological examinations.

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.

References

1. Akindele AJ, Oludadepo GO, Amagon KI, Singh D, Osiagwu DD (2018) Protective effect of carvedilol alone and coadministered with diltiazem and prednisolone on doxorubicin and 5-fluorouracil-induced hepatotoxicity and nephrotoxicity in rats. Pharmacol Res Perspect 6: e00381.
2. Al-Dalaen SM, Al-Qtaitat AI (2014) Review article: Oxidative stress versus antioxidants. Am J Biosci Bioeng 2: 60-71.
3. Ananthanarayanan Ajith T, Hema U, Aswathi S (2016) *Zingiber officinale Roscoe* ameliorates anticancer antibiotic doxorubicin-induced acute cardiotoxicity in rat. J Exp Ther Oncol 11.
4. Ashour OM, Elberry AA, Alahdal AM, Al Mohamadi AM, Nagy AA, et al. (2011) Protective effect of bilberry (*Vaccinium myr illus)* against doxorubicin-induced oxidative cardiotoxicity in rats. Med Sci Monit 17: BR110.
5. Benzer F, Kandemir FM, Kucukler S, Comaklı S, Caglayan C (2018) Chemoprotective effects of curcumin on doxorubicin-induced nephrotoxicity in wistar rats: By modulating inflammatory cytokines, apoptosis, oxidative stress and oxidative DNA damage. Arch Physiol Biochem 124: 448-457.
6. Bijarnia RK, Bachtler M, Chandak PG, van Goor H, Pasch A (2015) Sodium thiosulfate ameliorates oxidative stress and preserves renal function in hyperoxaluric rats. PLoS One 10: e0124881.
7. Bulucu F, Oktenli C, Kenar L, Ocal R, Koc B, et al. (2008) Efficacy of deferoxamine, N-acetylcysteine and selenium treatments in rats with Adriamycin-induced nephrotic syndrome. J Nephrol 21: 576-583.
8. Elsherbiny NM, El-Sherbiny M (2014) Thymoquinone attenuates Doxorubicin-induced nephrotoxicity in rats: Role of Nrf2 and NOX4. Chem Biol Interact 223: 102-108.
9. Fouad AEA, Refaie SM, Dawood AFA (2018) Carnosine mitigates the adverse effect of doxorubicin on rat kidneys.
10. He W, Liu Q, Wang L, Chen W, Li N, Cao X (2007) TLR4 signaling promotes immune escape of human lung cancer cells by inducing immunosuppressive cytokines and apoptosis resistance. Mol Immunol 44: 2850-2859.
11. Hoshi R, Watanabe Y, Ishizuka Y, Hirano T, Nagasaki-Maeoka E, et al. (2017) Depletion of TFAP2E attenuates adriamycin-mediated apoptosis in human neuroblastoma cells. Oncol Rep 37: 2459-2464.
12. Imam F, Al-Harbi NO, Al-Harbi MM, Ansari MA, Al-Asmari AF, et al. (2018) Apremilast prevent doxorubicin-induced apoptosis and inflammation in heart through inhibition of oxidative stress mediated activation of NF-κB signaling pathways. Pharmacol Rep 70: 993-1000.
13. Jaćević V, Dragojević-Simić V, Tatomirović Ž, Dobrić S, Bokonjić D, et al. (2018) The efficacy of amifostine against multiple-dose doxorubicin-induced toxicity in rats. Int J Mol Sci 19: 2370.
14. Khames A, Gad AM, Abd El-Raouf OM (2017) Ameliorative effects of sildenafil and/or febuxostat on doxorubicin-induced nephrotoxicity in rats. Eur J Pharmacol 805: 118-124.
15. Khames A, Khalaf MM, Gad AM, Abd EL-Raouf OM (2017) Ameliorative effects of sildenafil and/or febuxostat on doxorubicin-induced nephrotoxicity in rats. Eur J Pharmacol 805: 118-124.
16. Khan TH, Ganaie MA, Alharthy KM, Madkhali H, Jan BL, et al. (2020) Naringenin prevents doxorubicin-induced toxicity in kidney tissues by regulating the oxidative and inflammatory insult in Wistar rats. Arch Physiol Biochem 126: 300-307.
17. Kobayashi M, Usui F, Karasawa T, Kawashima A, Kimura H, et al. (2016) NLRP3 deficiency reduces macrophage interleukin-10 production and enhances the susceptibility to doxorubicin-induced cardiotoxicity. Sci Rep 6: 1-11.
18. Kramer AB, van Timmeren MM, Schuurs TA, Vaidya VS, Bonventre JV, et al. (2009) Reduction of proteinuria in adriamycin-induced nephropathy is associated with reduction of renal kidney injury molecule (Kim-1) over time. Am J Physiol Renal Physiol 296: F1136-F1145.
19. Kumar A, Takada Y, Boriek AM, Aggarwal BB (2004) Nuclear factor-κB: its role in health and disease. J Mol Med 82: 434-448.
20. Lateef A, Hassan SK, Rashid S, Ali N, Zeeshan M, et al. (2014) D-limonene suppresses doxorubicin-induced oxidative stress and inflammation *via* repression of COX-2, iNOS, and NFiB in kidneys of Wistar rats. Exp Biol Med 239, 465-76.
21. Li J, Ichikawa T, Janicki JS, Cui T (2009) Targeting the Nrf2 pathway against cardiovascular disease. Expert Opin Ther Targets 13: 785-794.
22. Liu J, Qu W, Kadiiska MB (2009) Role of oxidative stress in cadmium toxicity and carcinogenesis. Toxicol Appl Pharmacol 238: 209-214.
23. Lu Q, Ding H, Li W (2013) Role of Toll-like receptors in microbiota-associated gastrointestinal cancer metastasis. J Cancer Res Ther 9: 142.
24. Ma Y, Zhang X, Bao H, Mi S, Cai W, et al. (2012) Toll-Like Receptor (TLR) 2 and TLR4 differentially regulate doxorubicin induced cardiomyopathy in mice. PloS one 7: e40763.
25. Mansouri E, Assarehzadegan MA, Nejad-Dehbashi F, Kooti W (2018) Effects of pravastatin in adriamycin-induced nephropathy in rats. Iran J Pharm Sci 17: 1413.
26. Mansouri E, Jangaran A, Ashtari A (2017) Protective effect of pravastatin on doxorubicin-induced hepatotoxicity. Bratisl Lek Listy 118: 273-277.
27. Khames A, Gad AM, Abd El-raouf OM, Kandeil MA, Khalaf MM (2020) Sodium thiosulphate shows promising anti-inflammatory

role against doxorubicin-induced renal injury depending on Tlr4 pathway inhibition. Plant Arch 20: 2948-2958.

28. Nasi S, Ea HK, Lioté F, So A, Busso N (2016) Sodium thiosulfate prevents chondrocyte mineralization and reduces the severity of murine osteoarthritis. PLoS One 11: e0158196.

29. Ozturk H, Firat T, Tekce BK, Yilmaz F, Ozturk H (2017) Effects of sodium thiosulphate on renal function and histopathology in rats with partial unilateral ureteral obstruction. Kaohsiung J Med Sci 33: 236-245.

30. Park S, Yoon J, Bae S, Park M, Kang C, et al. (2014) Therapeutic use of H_2O_2-responsive anti-oxidant polymer nanoparticles for doxorubicin-induced cardiomyopathy. Biomaterials 35: 5944-5953.

31. Qiao J, Liu Y, Jiang Z, Yang Y, Liu W, et al. (2018) Preparation and renoprotective effects of carboxymethyl chitosan oligosaccharide on adriamycin nephropathy. Carbohydr Polym 201: 347-356.

32. Ravindran S, Hussain SJ, Boovarahan SR, Kurian GA (2017) Sodium thiosulfate post-conditioning protects rat hearts against ischemia reperfusion injury *via* reduction of apoptosis and oxidative stress. Chem Biol Interact 274: 24-34.

33. Shi W, Deng H, Zhang J, Zhang Y, Zhang X, et al. (2018) Mitochondria-targeting small molecules effectively prevent cardiotoxicity induced by doxorubicin. Molecules 23: 1486.

34. Shimizu S, Saito M, Kinoshita Y, Ohmasa F, Dimitriadis F (2011) Sodium thiosulphate ameliorates ischaemia-reperfusion injury in the rat kidney. Br J Pharmacol 163: 272-282.

35. Sun Y, Wang C, Meng Q, Liu Z, Huo X, et al. (2018) Targeting P-glycoprotein and SORCIN: Dihydromyricetin strengthens anti-proliferative efficiency of adriamycin via MAPK/ERK and Ca2+-mediated apoptosis pathways in MCF-7/ADR and K562/ADR. J Cell Physiol 233: 3066-3079.

36. Suvarna KS, Layton C, Bancroft JD (2018) Bancroft's Theory and Practice of Histological Techniques E-Book, Elsevier Health Sciences.

37. Szajnik M, Szczepanski MJ, Czystowska M, Elishaev E, Mandapathil M, et al. (2009) TLR4 signaling induced by lipopolysaccharide or paclitaxel regulates tumor survival and chemoresistance in ovarian cancer. Oncogene 28: 4353-4363.

38. Tury S, Assayag F, Bonin F, Chateau Joubert S, Servely JL, et al. (2018) The iron chelator deferasirox synergises with chemotherapy to treat triple-negative breast cancers. J Pathol 246: 103-114.

39. Vyas D, Laput G, Vyas AK (2014) Chemotherapy-enhanced inflammation may lead to the failure of therapy and metastasis. Onco Targets Ther 7: 1015.

40. Wang L, Chen Q, Qi H, Wang C, Wang C, et al. (2016) Doxorubicin-induced systemic inflammation is driven by upregulation of toll-like receptor TLR4 and endotoxin leakage. Cancer Res 76: 6631-6642.

41. Wang N, Zhang DX, Zhang SC, Du M, Li JL, et al (2015) Biomarkers for kidney injury induced by gentamicin, doxorubicin and adenine in rats.

42. Yazd ZNE, Hosseinian S, Shafei MN, Bideskan AE, Heravi NE, et al. (2018) Protection against doxorubicin-induced nephropathy by plantago major in rat. Iran J Kidney Dis 12: 99.