Doxorubicin-induced nephrotoxicity model in Wistar rats: Characterization of biochemical parameters, histological and immunohistochemical assessment

Article *in* Ceylon Journal of Science · December 2022

DOI: 10.4038/cjs.v51i4.8064

CITATIONS
0

READS
75

4 authors:

Sachinthi S Amarasiri
University of Ruhuna
40 PUBLICATIONS **92** CITATIONS
SEE PROFILE

Anoja Priyadarshani Attanayake
University of Ruhuna
185 PUBLICATIONS **376** CITATIONS
SEE PROFILE

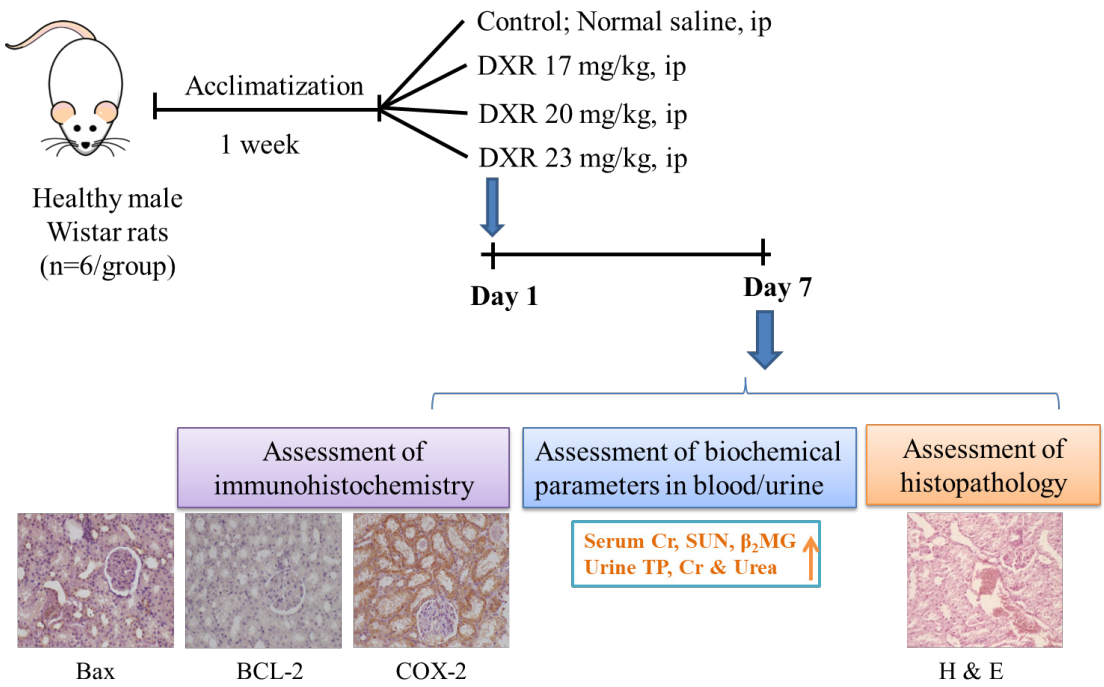
Lakmini Mudduwa
Faculty of Medicine, University of Ruhuna
138 PUBLICATIONS **637** CITATIONS
SEE PROFILE

K.A.P.W. Jayatilaka
University of Ruhuna
109 PUBLICATIONS **657** CITATIONS
SEE PROFILE

RESEARCH ARTICLE

Doxorubicin-induced nephrotoxicity model in Wistar rats: Characterization of biochemical parameters, histological and immunohistochemical assessment

Sachinthi S. Amarasiri, Anoja P. Attanayake, Lakmini K.B. Mudduwa and Kamani A.P.W. Jayatilaka



Highlights

- Experimental models are crucial in assessing therapeutic targets.
- Doxorubicin at a dose of 20 mg/kg was selected for the establishment of the nephrotoxicity model.
- Apoptosis and inflammation were the principal mechanisms of doxorubicin nephrotoxicity.
- This model provides a methodological reference for the exploration of nephroprotective therapeutics.

Ceylon Journal of Science 51(4) 2022: 471-479
DOI: http://doi.org/10.4038/cjs.v51i4.8064

RESEARCH ARTICLE

Doxorubicin-induced nephrotoxicity model in Wistar rats: Characterization of biochemical parameters, histological and immunohistochemical assessment

Sachinthi S. Amarasiri[1], Anoja P. Attanayake[2,*], Lakmini K.B. Mudduwa[3] and Kamani A.P.W. Jayatilaka[2]

[1]Department of Medical Laboratory Science, Faculty of Allied Health Sciences, University of Ruhuna, Galle, Sri Lanka
[2]Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka.
[3]Department of Pathology, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka

Received: 15/08/2022; Accepted:01/11/2022

Abstract: This study characterizes the doxorubicin (DXR)-induced nephrotoxicity model in Wistar rats in terms of biochemical, histological, and immunohistochemical assessments, to provide a methodological reference model to investigate potential nephroprotective therapeutics. The experiments were carried out using four groups of healthy male Wistar rats (six rats per group) administered with a single intraperitoneal dose of normal saline (vehicle group) and DXR at 17, 20, and 23 mg/kg doses, respectively. The rats were sacrificed under anaesthesia on the seventh day after DXR administration and blood, urine, and kidney tissues were collected for investigations. The DXR dose of 17 mg/kg showed mild changes in biochemical parameters, whereas 23 mg/kg resulted in increased mortality (33%). Serum concentrations of creatinine, urea nitrogen, β_2-microglobulin, urine concentrations of total protein, creatinine, and urea increased with the increased dose of DXR while serum concentrations of total protein and albumin decreased ($p<0.05$). Assessment of histopathology revealed the features of acute tubular injury and immunohistochemical studies revealed increased apoptosis and inflammatory changes. Based on the findings, the DXR dose of 20 mg/kg was selected as the optimal dose for the establishment of an acute nephrotoxicity model in Wistar rats to explore potential nephroprotective drug leads.

Keywords: Doxorubicin-induced acute tubular injury, apoptosis, histopathology, immunohistochemistry, inflammation.

INTRODUCTION

Doxorubicin (DXR) is an antineoplastic drug of the anthracycline family, with a wide variety of applications in the treatment of human cancers (Lee and Harris, 2011). Despite the pronounced anticancer efficacy, the organ toxicity associated with the drug has become a major limitation to its effective use in cancer chemotherapy (Horie *et al.*, 2018). Therefore, potential strategies to attenuate the toxic effects of the drug without affecting its anticancer efficacy are a major research field of interest in the present era.

The potential involvement of the kidneys in xenobiotic metabolism as a major elimination pathway for DXR and its metabolites causes kidneys more prone to develop functional impairments during DXR chemotherapy. A series of redox reactions that generate some free radical species and toxic reactive intermediates leading to oxidative damage, apoptosis, and inflammation have been identified as major pathways of causing and amplifying nephrotoxicity by DXR (Mohammadi and Ahmadizadeh, 2018; Ibrahim *et al.,* 2020; Li *et al.,* 2020). Therefore, among the various approaches pursued to ensure safe and effective DXR chemotherapy, approaches made through a combination of drug delivery together with potential therapeutics that attenuate DXR-induced oxidative stress, inflammation, and apoptosis offered the most promising results (Quiles *et al*., 2002). Accordingly, numerous studies have been conducted using various drug therapeutics at different doses in different experimental models of DXR nephrotoxicity (Heravi *et al*., 2018; Khan *et al.,* 2020; Molehin, 2020).

The DXR-induced experimental nephrotoxicity model in rodents is a classical pharmacological animal model that simulates the early pathophysiological characteristics of kidney damage (Li *et al.,* 2019; He *et al*., 2020). Clinical manifestations are similar to human kidney disease, including elevated serum creatinine and serum urea nitrogen concentrations, reduced creatinine clearance, hypoproteinemia, hypoalbuminemia, proteinuria, and morphological changes in kidney damage (Lee and Harris, 2011; Khajavi-Rad *et al.,* 2017; Li *et al.,* 2019). Histopathological changes of nephrotoxicity appear as early as one to two weeks after DXR administration and progress to a severe stage by four weeks (Wang *et al*., 2000; Lee and Harris, 2011). Although the features of acute tubular necrosis could be observed with many nephrotoxic drugs, DXR also results in chronic effects on the kidney, based on published reports (Okuda *et al*., 1986).

Unravelling the mechanistic pathways of DXR nephrotoxicity remains quite challenging due to different clinical presentations of the model, such as acute kidney injury, acute tubular necrosis, nephrotic syndrome, chronic kidney injury, and other types of nephropathies (Okuda *et al*., 1986; Li *et al*., 2019; Molehin, 2020). The rationale behind the variability in clinical response might be the narrow therapeutic index of the drug leading to a significant variation in disease severity with a small difference in the

**Corresponding Author's Email:* anojaattanayake@med.ruh.ac.lk
https://orcid.org/0000-0002-4365-7956

dose administered. The possibility of individual variability in response, even within the same strain of rodents, the potential differences in study duration, and the differences in biochemical and morphological parameters evaluated remain other concerns in the establishment of a consistent experimental model of DXR nephrotoxicity (Lee and Harris, 2011; Heravi *et al*., 2018; Khan *et al.,* 2020; Molehin, 2020). Therefore, the establishment of an experimental model with high fidelity, that suits a particular research setting, is worth ascertaining a fertile animal model for the assessment of therapeutic targets. In this context, proper characterization of the DXR-induced nephrotoxicity model in terms of the pathophysiological features of kidney impairment and the potential mechanisms of nephrotoxicity are crucial. Therefore, the present work was carried out as the first study conducted in a Sri Lankan research setting, on the characterization of the experimental nephrotoxicity model induced by DXR in terms of biochemical, histological, and immunohistochemical assessments to provide a methodological reference to study potential drug therapeutics with nephroprotective effects against DXR nephrotoxicity.

MATERIAL AND METHODS

Experimental animals and ethical aspects

Male Wistar rats weighing 200±25 g and 10-12 weeks old were obtained from the Animal Care Facility of the Medical Research Institute (Colombo, Sri Lanka). The animals were kept under pathogen-free conditions with alternating light and dark cycles. The animals were fed with a standard pelleted rodent diet and provided water ad libitum. The experiments were carried out following the International Ethical Guidelines on the Care and Use of Laboratory Animals (Council NR, 2010). The experimental protocols were approved by the Faculty of Medicine, University of Ruhuna, Institutional Ethical Review Committee, Sri Lanka (No. 14.12.2015:3.1).

Experimental design

Healthy male Wistar rats fasted for 8 h and were divided into four groups with six rats per group. Doxorubicin hydrochloride (DXR, United Biotech Pvt Ltd, India) was injected once intraperitoneally into each experimental rat at three selected doses; 17, 20, and 23 mg/kg body weight. Six male Wistar rats of the same age and weight were injected with an equivalent volume of isotonic saline (vehicle). All rats in the vehicle and experimental groups were housed individually in metabolic cages and a 24-hour collection of spontaneously voided urine was performed on the sixth day after DXR administration (Figure 1).

Rats were sacrificed under CO_2 anaesthesia (EUTH 2A CO_2 euthanasia chamber, Orchid Scientific, India) on the seventh day of the study. Blood samples were collected by cardiac puncture using a 21 G needle mounted on a 3 cc syringe. The samples were left at room temperature (27 °C) for one hour for coagulation before centrifugation at 3000 rpm for 15 min for serum separation. Serum and urine samples were stored at -70 °C to be used in biochemical assays. Kidney tissues were excised and fixed in 10% formalin for the preparation of Hematoxylin and eosin (H and E) sections for the assessment of histopathology and immunohistochemistry.

Measurement of body weight, consumption of food and intake of water

The body weight of rats in all groups was measured using an animal scale (Shinano, Japan), before and daily, after DXR administration. The average percentage change in weights per group was calculated. Consumption of food and intake of water were measured daily during the period of intervention.

Biochemical measurements

Serum concentrations of creatinine, urea nitrogen, and urine concentrations of total protein, creatinine, and urea were measured with commercially available test kits by spectrophotometric methods. The concentration of β_2-microglobulin in serum was estimated using the enzyme-linked immunosorbent assay (ELISA) method.

Histology

Stained kidney sections of the experimental animals were prepared according to the standard procedures. Tissue sections were examined under the light microscope for the presence of indicators of cellular damage by two independent study investigators, including a consultant histopathologist. The investigators were blind to the respective groups of experimental rats during the histopathological assessment.

Immunohistochemistry studies

Immunohistochemical staining was performed on paraffin-embedded kidney sections on microscopic slides coated with 0.01% (w/v) of poly-l-lysine (Sigma- Aldrich, USA). The primary markers used were anti-Bax (ab216494,

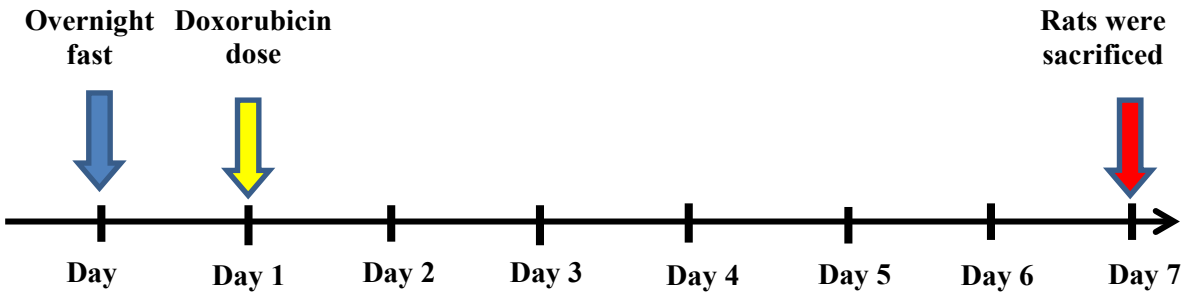


Figure 1: Experimental design for the development of doxorubicin-induced nephrotoxicity model in Wistar rats

Abcam, Cambridge, UK), anti- BCL-2 (M0887, Dako, Denmark), and anti- COX-2 (M3617, Dako, Denmark). The secondary antibody was the HRP- conjugated REAL EnVision detection system from Dako, Denmark (K4061). Brown staining of cells was considered positive immunoreactivity.

Statistical analysis

Biochemical data were evaluated by one-way analysis of variance (ANOVA) and least significant difference (LSD) post hoc test. Quantitative data were expressed as mean±SEM and the differences were considered statistically significant at values of $p<0.05$.

RESULTS AND DISCUSSION

Drug-induced nephrotoxicity is a serious health issue that requires attention due to the increased risk of acute kidney injury and subsequent progression to chronic kidney disease, end-stage renal disease, and mortality (Chinnappan *et al*., 2019; Li *et al,* 2020). Nephrotoxicity induced by various chemical agents in xenobiotics has become a frequent entity in clinical medicine due to the involvement of the kidneys in the metabolism and elimination of xenobiotics and their active metabolites (Iahtisham-Ul-Haq *et al.,* 2019). Despite the numerous efforts made to discover potential therapeutic candidates, the management of nephrotoxicity remains a key area of research. Therefore, the present study was a foundation for exploring potential nephroprotective therapeutics for better management of nephrotoxicity, using the DXR-induced experimental model of nephrotoxicity.

The present study was conducted using three selected doses of DXR, including 17, 20, and 23 mg/kg. During the experiments, Wistar rats from all the experimental groups were sacrificed seven days after DXR administration due to the moribund status of the animals administered with higher doses of DXR. All the rats administered with 17, and 20 mg/kg doses survived until day seven of the intervention whereas, the 23 mg/kg dose increased mortality of rats (33%).

Effect of DXR on body weight, consumption of food and intake of water

Administration of a single intraperitoneal dose of DXR at the three selected doses inhibited weight gain in Wistar rats. Animals treated with DXR showed a significant decrease in body weight compared to the control rats, as shown in Table 1 ($p<0.05$). However, the percentage change in body weight did not deviate in a dose-dependent manner and the lowest dose of DXR (17 mg/kg) caused the highest change in body weight (21%) in experimental rats. The DXR doses at 20 and 23 mg/kg resulted in a 9% and 15% reduction in body weight respectively.

Similarly, a significant reduction in the percentage change in average consumption of food and intake of water was associated with the induction of nephrotoxicity with DXR. The average consumption of food decreased (97%, 100%, and 100% change from the initial consumption). In contrast, the average intake of water increased (85%, 79%, and 73% change from the initial intake) with the increased dose of DXR in test groups ($p<0.05$). However, the percentage change in the average consumption of food and water intake in the vehicle group was 5% and 7%, respectively.

Effect of DXR on biochemical measurements

The diagnosis of nephrotoxicity in rodent models was mainly based on the determination of serum concentration of creatinine and urea in previous studies. Those are the key factors that determine the glomerular filtration rate (Abouzeinab, 2015). Both are nitrogenous end products of metabolism that are cleared from the bloodstream by the kidney (Safhi, 2018). The groups of rats administered with the 17, 20, and 23 mg/kg doses of DXR showed a significant increase in serum creatinine concentration (117%, 158%, and 259%) compared to the rats administered with saline ($p<0.05$). Similarly, a significant increase in the level of serum urea nitrogen was observed in nephrotoxic rats administered with a single dose of DXR at 20 mg/kg (26%), and 23 mg/kg (41%), when compared to the experimental rats of vehicle group ($p<0.05$). These elevated concentrations of serum urea nitrogen and creatinine, especially at 20 and 23 mg/kg doses of DXR substantiate the fact that more than 50% of the nephrons are functionally damaged, which denotes severe nephrotoxicity (Perše and Veceric-Haler, 2018; Aygun and Gul, 2019). The findings are consistent with those of the previous reports on DXR-induced nephrotoxicity model (Yilmaz *et al.*, 2006). Yet, there are few reports on the same animal model which show deviated results in selected biomarkers (Heeba and Mahmoud, 2016;

Table 1: Change in body weight, consumption of food and intake of water in DXR-induced nephrotoxicity in Wistar rats.

Group	Body weight (g)		Average consumption of food (g)	Average intake of water (mL)
	Initial weight	Weight on 7th day		
Vehicle	183±8	215±8	129.40±3.26	269±8.43
DXR (17 mg/kg)	210±6	165±8	14.10±6.71[c]	79.00±30.92[b]
DXR (20 mg/kg)	158±8	140±8	4.60±3.88 [c]	128.00±43.52[a]
DXR (23 mg/kg)	264±4	225±5	5.00±2.24 [c]	153.00±53.47[a]

Data were presented as mean±SEM and [a] $p<0.05$, [b] $p<0.01$ and [c] $p<0.001$ were considered to be statistically significant against the vehicle. DXR; Doxorubicin

Khan *et al.*, 2020) further corroborating the requirement of their protocols for each laboratory to develop experimental models of DXR-induced nephrotoxicity for the evaluation of protective therapeutics.

The latest research on biomarkers has revealed that traditional markers of nephrotoxicity, including serum creatinine and serum urea nitrogen, are specific with low sensitivity in the detection of early kidney damage (Al-Naimi *et al.,* 2019). Therefore, the use of novel biomarkers with sensitivity and high specificity is encouraged for the discovery of initial kidney injury with information on the site of the underlying kidney damage. Urine protein is a potential biomarker of acute and chronic kidney damage induced by nephrotoxic drugs. Normally, glomeruli restrict the transport and migration of high-molecular-weight proteins from the bloodstream to the lumen of the nephron. However, under pathological conditions, high molecular weight proteins can be detected in urine due to nephron dysfunction (Al-Naimi *et al.,* 2019). Therefore, high-molecular-weight proteins such as albumin are regarded as more sensitive proteins in the early detection of glomerular filtration dysfunction. Hence, several studies have focused on the improvement of proteinuria under several therapies (Qi *et al.,* 2012; Zhao *et al.,* 2015). The loss of urinary protein in 24-hour urine samples was significantly increased in rats treated with 20 (110%), and 23 mg/kg (138%) of DXR in the present study ($p<0.05$). These findings were further supported by the results of the urine concentration of creatinine and urea. Although both parameters showed a dose-dependent increase with DXR administration, a statistically significant increase was observed only with creatinine concentration in urine in rats induced with the highest dose of DXR (195%). These discrepancies could be due to the associated limitations in the collection of urine samples and the management of water intake. Since animals with severe kidney damage may develop oliguria, the collection of urine samples is difficult. Furthermore, urinary biomarker concentrations such as urea are altered by fluid intake that cannot be easily controlled in animal experiments (Mohamed *et al*., 2015).

Generally, low-molecular-weight proteins are reabsorbed in the proximal renal tubules. However, the presence of an excessive amount of low-molecular-weight proteins leads to nephron overload exceeding the reabsorbing capacity of proximal renal tubules. Therefore, damage to the proximal renal tubules may result in low-molecular-weight proteinuria (Al-Naimi *et al.,* 2019). Low-molecular weight proteins such as β_2-microglobulin reflect the underlying renal glomerular and/or tubular damage during nephrotoxicity. The concentrations of these low-molecular-weight proteins in serum would be directly proportional to the degree of nephrotoxicity (Izzedine and Perazella, 2017; Al-Naimi *et al.,* 2019). Although the concentration of β_2-microglobulin was increased in a dose-dependent manner according to the dose of DXR (13%, 14%, and 29%), a significant increase was observed only in nephrotoxic rats administered the highest dose of DXR compared to the control group in the present study ($p<0.05$). The findings on biomarkers in serum and urine are shown in Table 2.

These observed results on biochemical parameters and biomarkers of kidney function in the present study substantiate that DXR causes significant nephrotoxicity effects in Wistar rats. These findings corroborate the use of DXR as an inducer of kidney damage in rodents, as recognized in previous studies (Lee and Harris, 2011; Qi *et al.,* 2012). However, in the present study, dose-dependent changes with biochemical parameters such as serum concentration of total protein and albumin were not observed with the administration of DXR. These deviations in results could be attributed to some non-genetic factors which might affect the susceptibility to DXR nephrotoxicity. Such factors include dietary depletion and reduced absorption of magnesium, decreased dietary level of selenium, hydration status of animals, etc. (Perše and Veceric-Haler, 2018). Variation of these factors contributes to the deviations in results even within the same group due to uneven responses of experimental animals even though DXR induced nephrotoxicity model is reported as reproducible.

Effect of DXR on kidney histology

The evaluation of histopathology has become the most reliable method of determining the degree of nephrotoxicity accompanied by mild to moderate kidney damage in animal studies due to the drawbacks in routine biochemical parameters as mentioned above (Perše and Veceric-Haler,

Table 2: The effect of DXR on serum and urine biomarkers of kidney function in experimental rats

Group	Biochemical parameters in serum			Biochemical parameters in urine		
	Creatinine (µmol/L)	SUN (mmol/L)	β_2-microglobulin (µg/ ml)	Total protein (mg/L)	Creatinine (mmol/L)	Urea (mmol/L)
Vehicle	30.15±2.79	6.86±0.55	4.50±0.18	452.27±13.24	0.93±0.20	5.80±1.14
DXR (17 mg/kg)	65.52±2.75^{c}	7.84±0.34	5.06±0.11	811.43±140.14	1.80±0.29	6.06±0.49
DXR (20 mg/kg)	77.93±2.93^{c}	8.63±0.46^{a}	5.15±0.21	948.37±151.26^{a}	2.13±0.34	6.37±0.77
DXR (23 mg/kg)	108.18±10.95^{c}	9.65±1.31^{a}	5.82±0.65^{a}	1075.75±166.65^{a}	2.74±0.75^{a}	6.84±0.33

Data were presented as mean±SEM and ^{a} $p<0.05$, ^{b} $p<0.01$ and ^{c} $p<0.001$ were considered to be statistically significant against the vehicle. DXR; Doxorubicin, SUN; serum urea nitrogen

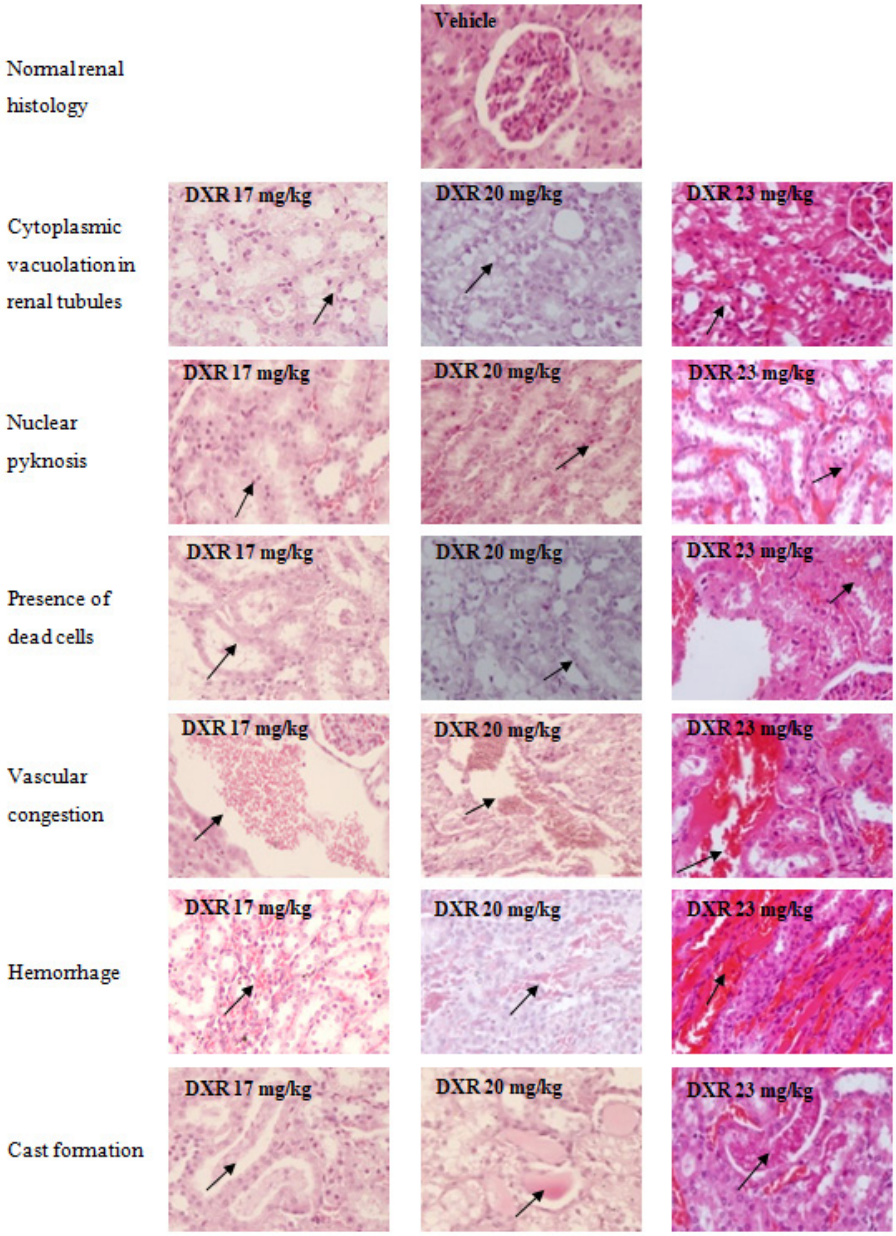


Figure 2: Photomicrographs of normal renal histology (Vehicle) and the features of acute tubular injury including cytoplasmic vacuolation, nuclear pyknosis, presence of dead cells, vascular congestion, hemorrhage and cast formation in DXR induced nephrotoxicity in Wistar rats (x400) seven days following intraperitoneal administration of the 17, 20, 23 mg/kg doses of DXR. DXR; Doxorubicin

2018). The findings of histopathological assessment corroborated the results of biochemical parameters in the present study. Figure 2 shows the photomicrographs of the H and E stained kidney sections of rats from different experimental groups. Histological examination revealed the presence of early features of acute tubular necrosis in experimental animals administered with DXR. The nephrotoxic rats suffered significant cortical damage with considerable glomerular and tubular damage following the administration of DXR. The severity of acute tubular injury increased with the increased dose of DXR as evident by the dose-dependent increase of tubular cell vacuolization, pyknosis of tubular epithelial cells, and the presence of dead cells. A similar increment was noticed with vascular congestion and hemorrhage as well. Tubular casts derived from sloughed cells and proteins could be observed with the highest dose of DXR. However, complete destruction of kidney morphology was not observed in either group of rats administered DXR. Furthermore, no features of chronic kidney injury were observed, including glomerulosclerosis, interstitial fibrosis, and tubulointerstitial inflammation. These findings are in line with the previous reports (Park *et al.,* 2003; Yagmurca *et al*., 2015).

Effect of DXR on apoptotic and anti-inflammatory markers

Several mechanisms have been suggested for the nephrotoxicity induced by DXR. The oxidative stress caused by the increased generation of reactive oxygen species which in turn, causes diverse oxidative damage to biological macromolecules, membrane lipid peroxidation, and oxidation of proteins is the mainstay in explaining

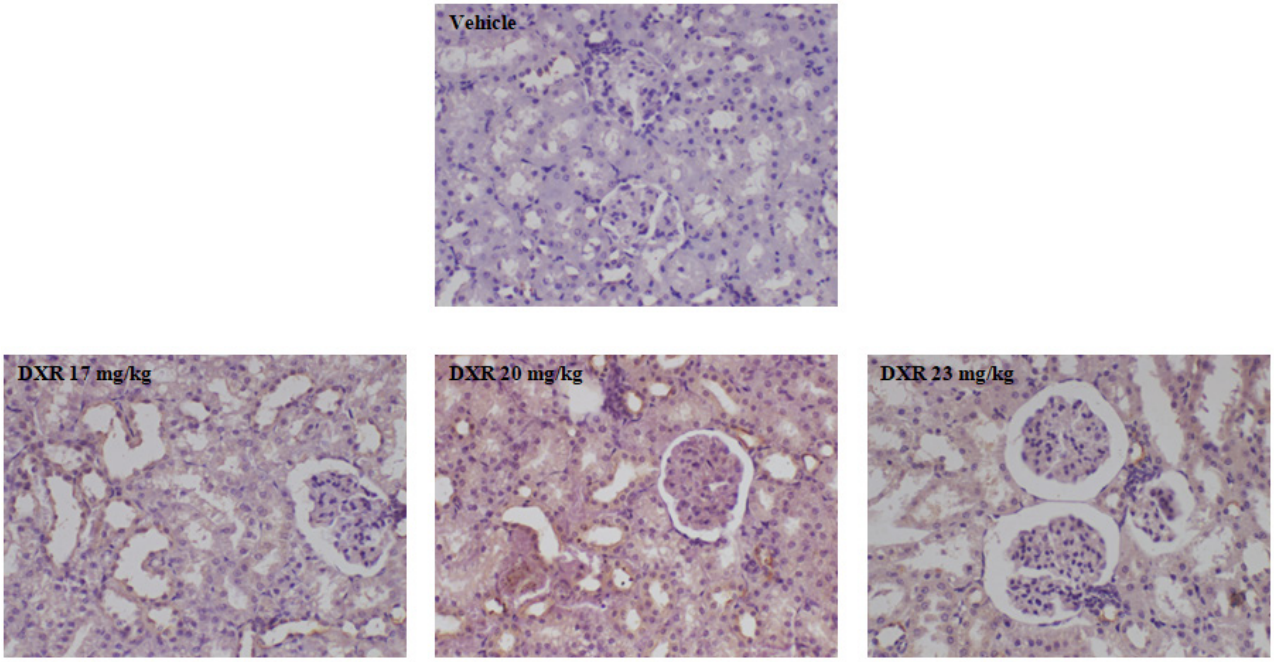


Figure 3(A): Photomicrographs of kidney tissues immune stained with anti-Bax (x400) seven days following intraperitoneal administration of the vehicle or DXR (17, 20, 23 mg/kg). A dose-dependent increase in the intensity of immunostaining (brown color) for anti-Bax was noted in experimental rats administered with 17, 20 and 23 mg/kg doses of DXR, respectively. In contrast, the experimental rats of vehicle group showed less staining for anti-Bax compared to the experimental rats administered with DXR. DXR; Doxorubicin.

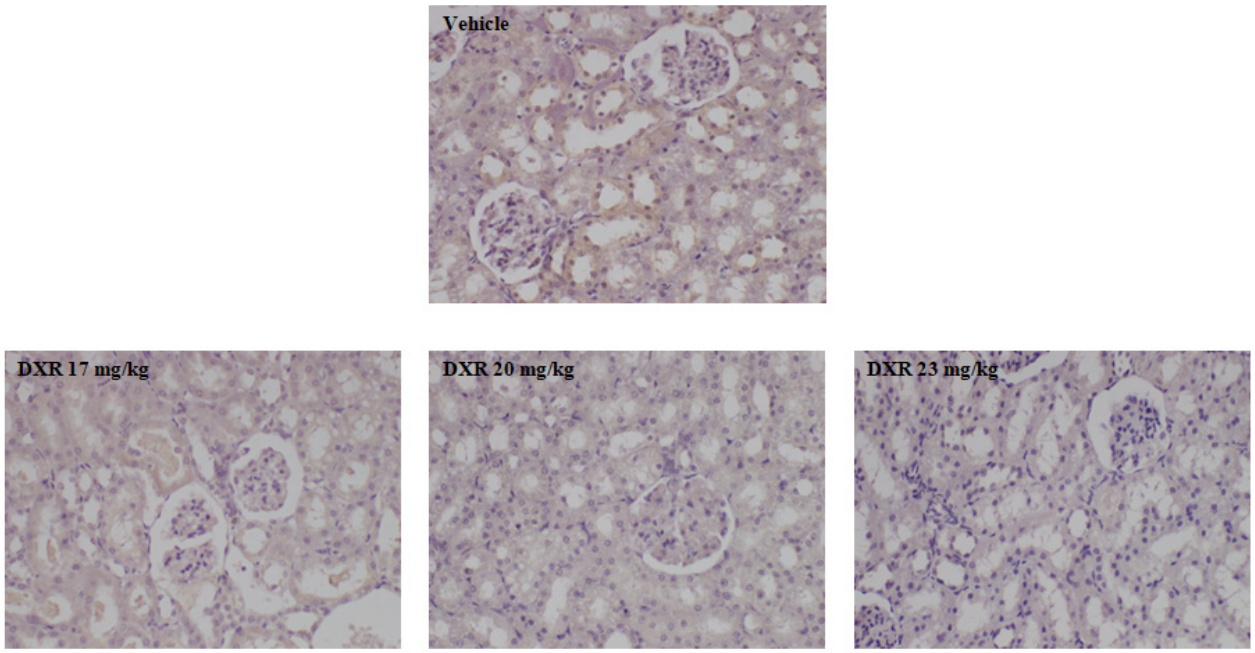


Figure 3(B): Photomicrographs of kidney tissues immune stained with anti BCL-2 (x400) seven days following intraperitoneal administration of the vehicle or DXR (17, 20, and 23 mg/kg). A dose-dependent decrease in the intensity of immunostaining (brown color) for anti BCL-2 was noted in experimental rats administered with 17, 20, and 23 mg/kg doses of DXR respectively. In contrast the experimental rats of vehicle group showed better staining for anti BCL-2 compared to the experimental rats administered with DXR. DXR; Doxorubicin.

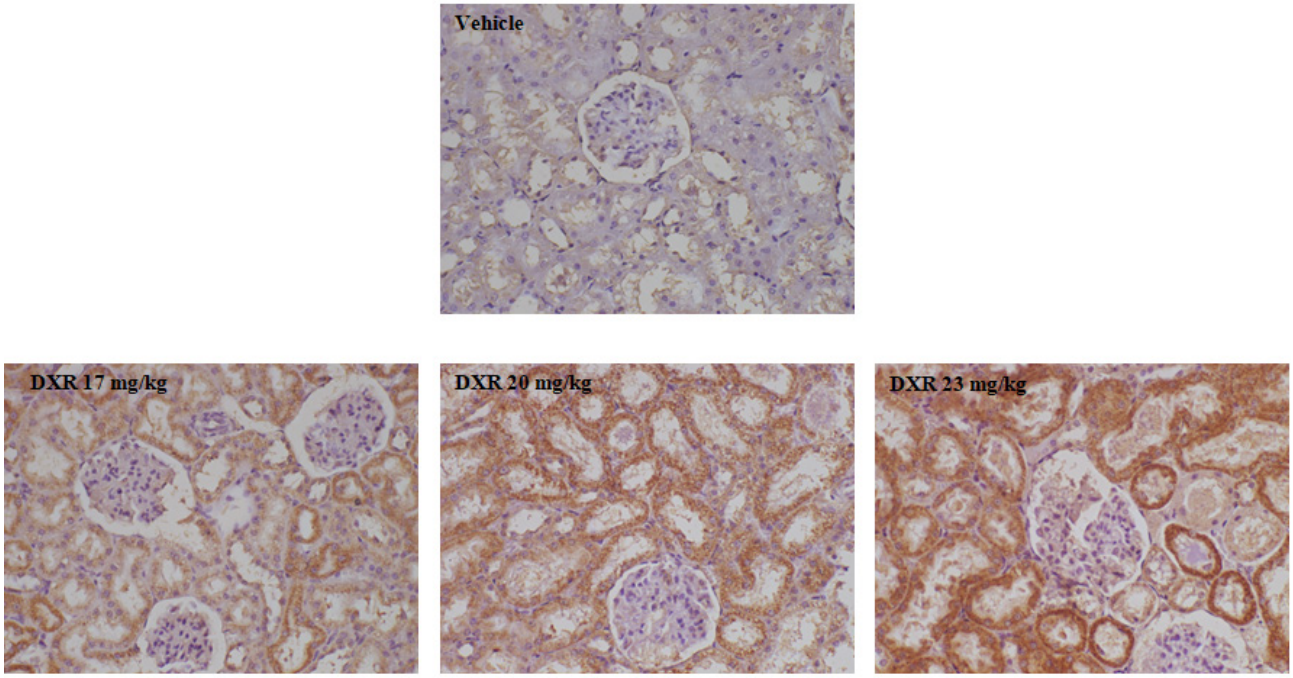


Figure 3(C): Photomicrographs of kidney tissues immune stained with anti COX-2 (x400) seven days following intraperitoneal administration of the vehicle or DXR (17, 20, and 23 mg/kg). A dose-dependent increase in the intensity of immunostaining (brown color) for anti COX-2 was noted in experimental rats administered 17, 20, and 23 mg/kg doses of DXR respectively. In contrast the experimental rats of vehicle group showed less staining for anti COX-2 compared to the experimental rats administered with DXR. DXR; Doxorubicin.

the DXR nephrotoxicity (Ayla *et al*., 2011; El-Sheikh *et al*., 2012). Furthermore, the activation of apoptotic pathways mediated by DXR-induced oxidative stress and the generation of various pro-inflammatory mediators that stimulate inflammatory pathways play an important role in DXR-induced nephrotoxicity. DXR alone or in association with its biologically reactive intermediates induces mitochondrial lesion, switching the intrinsic apoptotic pathway, which is regulated mainly through a series of regulatory proteins of the B-cell lymphoma-2 family, leading to caspase-dependent cell death (Lahoti *et al.,* 2012; Ibrahim *et al.,* 2020). In the present study, immunohistochemical studies revealed a dose-dependent increase in immunostaining for anti-Bax in experimental rats administered DXR. On the contrary, a dose-dependent reduction in the immunostaining was observed with reference to anti- BCL-2. Photomicrographs are shown in Figure 3. This increased expression of Bax and decreased expression of BCL-2 with the increased dose of DXR further substantiate the up-regulation of apoptosis pathways in the course of DXR administration. Furthermore, DXR stimulates the production and release of various pro-inflammatory cytokines causing subsequent up-regulation of the inducible enzyme; cyclooxygenase-2 (COX-2), which triggers inflammatory pathways leading to nephrotoxicity (Yu *et al*., 2017). This was further evidenced by the increased expression of COX-2 with an increase in DXR dose in the present study (Figure 3). Following up-regulation of COX-2, the subsequent release of the potent inflammatory modulator prostaglandin E-2, which is actively involved in the change of glomerular hemodynamics and glomerular filtration might result in increased serum concentrations of urea and creatinine in the nephrotoxicity of DXR in the present study (Ibrahim *et al.,* 2018; Safhi, 2018).

However, it is difficult to develop a perfect animal model to comply with human disease. Animal models are used in hundreds of experiments to develop effective therapeutics for kidney diseases, but most have failed in clinical studies (EL-Arabey and Salama, 2015). Differences in species, low incidence of subjects, and artificial induction of the disease are considered predisposing factors for misleading results in animal studies (Van der Worp *et al.,* 2010). No animal model can exactly simulate or predict the response to kidney disease in humans. Therefore, developing animal models to represent human kidney disease is a challenge.

An ideal animal model of nephrotoxicity is dose-dependent and reflects the effect of the nephrotoxic agents morphologically. Volume depletion is the major predisposing factor for nephrotoxicity since; it may result in intense tubular uptake as well as retention of the toxin (Heyman *et al.,* 2009). However, the selection of an appropriate dose of a nephrotoxic agent is important in scientific studies. Lower doses than recommended may be insufficient to cause kidney injury, while higher doses may become lethal, resulting in extensive tubular damage. According to the present findings, 20 and 23 mg/kg doses showed significant nephrotoxicity, particularly the features of acute kidney injury, in experimental animals. The dose of 17 mg/kg in rats showed mild changes in biochemical parameters which is not sufficient to demonstrate significant changes with a nephroprotective agent. However, a dose of 23 mg/kg was found to be a lethal dose, as the administration of the particular dose resulted in mortality in the study. Since animal experiments should

be conducted following procedures that minimize potential pain, suffering, or distress to the experimental animals, DXR at the dose of 23 mg/kg may not be suitable for the induction of nephrotoxicity (Festing and Wilkinson, 2007; Perše and Veceric-Haler, 2018). Further, a positive control group was not used in the establishment of the model which could be a drawback in the present study.

CONCLUSIONS

The findings revealed a dose-dependent increase in nephrotoxicity with the increased dose of DXR, in which inflammatory and apoptotic pathways play a substantial role. The DXR dose of 23 mg/kg was found to be lethal whereas, the dose of 17 mg/kg resulted in mild changes in biochemical parameters. The Wistar rats administered with a single intraperitoneal dose of DXR at 20 mg/kg produced a reliable animal model of acute kidney injury, which simulates the biochemical changes and morphological characteristics of the respective human disease. These findings would open new vistas to explore new nephroprotective drug leads/ therapeutics for to be used in the management of acute kidney injury, being the first report on characterization of dose-dependent changes of DXR nephrotoxicity.

ACKNOWLEDGEMENT OF SOURCES OF FUNDING

This work was financially supported by the UGC block grant, the University of Ruhuna (RU/PG- R/16/14) and the research grant of the National Science Foundation, Sri Lanka (RG/2016/HS -03).

STATEMENT OF CONFLICT OF INTEREST

The authors declare no competing interests.

REFERENCES

Abouzeinab, N.S. (2015). Antioxidant effect of silymarin on cisplatin-induced renal oxidative stress in rats. *Journal of Pharmacology and Toxicology* **10**(1): 1-19.

Al-Naimi, M.S., Rasheed, H.A., Hussien, N.R., Al-Kuraishy, H.M. and Al-Gareeb, A.I. (2019). Nephrotoxicity: Role and significance of renal biomarkers in the early detection of acute renal injury. *Journal of Advanced Pharmaceutical Technology and Research* **10**(3): 95-99.

Aygun, H. and Gul, S.S. (2019). Protective effect of melatonin and agomelatine on adriamycin induced nephrotoxicity in rat model: a renal scintigraphy and biochemical study. *Bratislava Medical Journal* **120**(2): 113-118.

Ayla, S., Seckin, I., Tanriverdi, G., Cengiz, M., Eser, M., Soner, B.C. and Oktem, G. (2011). Doxorubicin induced nephrotoxicity: protective effect of nicotinamide. *International Journal of Cell Biology*, DOI: 10.1155/2011/390238.

Chinnappan, S.M., George, A., Thaggikuppe, P., Choudhary, Y., Choudhary, V.K., Ramani, Y. and Dewangan, R. (2019). Nephroprotective effect of herbal extract *Eurycoma longifolia* on paracetamol-induced nephrotoxicity in rats. *Evidence-Based Complementary and Alternative Medicine*, doi: 10.1155/2019/4916519.

Council NR. (2010). *Textbook of Guide for the Care and Use of Laboratory Animals*. National Academies Press, Washington.

EL-Arabey, A.A. and Salama, A.S. (2015). Are mice and rats good experimental models to explore novel compounds against cisplatin-induced nephrotoxicity?. *International Journal of Nephrology and Kidney Failure* **1**(2), DOI: 10.16966/2380-5498.107.

El-Sheikh, A.A., Morsy, M.A.,Mahmoud, M.M., Rifaai, R.A. and Abdelrahman, A.M. (2012). Effect of coenzyme-Q10 on doxorubicin-Induced nephrotoxicity in rats. *Advances in Pharmacological Sciences*, DOI: 10.1155/2012/981461.

Festing, S. and Wilkinson, R. (2007). The ethics of animal research: talking point on the use of animals in scientific research. *EMBO Reports* **8:** 526-530.

Heeba, G.H. and Mahmoud, M.E. (2016). Dual effects of quercetin in doxorubicin-induced nephrotoxicity in rats and its modulation of the cytotoxic activity of doxorubicin on human carcinoma cells. *Environmental Toxicology* **31**(5): 624-636.

Heravi, N.E., Hosseinian, S., Yazd, Z.N.E., Shafei, M.N., Bideskan, A.E., Shahraki, S., Noshahr, Z.S., Motejadded, F., Beheshti, F., Mohebbati, R. and Parhizgar, S., Noshahr, Z.S., Motejadded, F., Beheshti, F., Mohebbati, R. and Parhizgar, S. (2018). Doxorubicin-induced renal inflammation in rats: Protective role of Plantago major. *Avicenna Journal of Phytomedicine* **8**(2): 179-187.

He, S., Li, A., Zhang, W., Zhang, L., Liu, Y., Li, K. and Qin, X., (2020). An integrated transcriptomics and network pharmacology approach to exploring the mechanism of adriamycin-induced kidney injury. *Chemico-Biological Interactions* **325**:109096.

Heyman, S.N., Rosen, S. and Rosenberger, C. (2009). Animal models of renal dysfunction: acute kidney injury. *Expert Opinion on Drug Discovery* **4**(6): 629-641.

Horie, S., Oya, M., Nangaku, M., Yasuda, Y., Komatsu, Y., Yanagita, M., Kitagawa, Y., Kuwano, H., Nishiyama, H., Ishioka, C. and Takaishi, H., (2018). Guidelines for treatment of renal injury during cancer chemotherapy 2016. *Clinical and Experimental Nephrology* **22**(1): 210-244.

Ibrahim, A., Al-Hizab, F.A., Abushouk, A.I. and Abdel-Daim, M.M. (2018). Nephroprotective effects of benzyl isothiocyanate and resveratrol against cisplatin-induced oxidative stress and inflammation. *Frontiers in Pharmacology* **9**, DOI: 10.3389/fphar.2018.01268.

Ibrahim, K.M., Mantawy, E.M., Elanany, M.M., Abdelgawad, H.S., Khalifa, N.M., Hussien, R.H., El-Agroudy, N.N. and El-Demerdash, E. (2020). Protection from doxorubicin-induced nephrotoxicity by clindamycin: novel antioxidant, anti-inflammatory and anti-apoptotic roles. *Naunyn Schmiedeberg's Archives of Pharmacology* **393**(4): 739-748.

Iahtisham-Ul-Haq, Butt, M.S., Randhawa, M.A. and Shahid, M. (2019). Nephroprotective effects of red beetroot-based beverages against gentamicin-induced renal stress. *Journal of Food Biochemistry* **43**(7. DOI: 10.1111/jfbc.12873.

Izzedine, H. and Perazella, M.A. (2017). Anticancer drug-induced acute kidney injury. *Kidney International Reports* **2**(4): 504-514.

Khajavi-Rad, A., Mohebbati, R. and Hosseinian, S. (2017). Drug-induced nephrotoxicity and medicinal plants. *Iranian Journal of Kidney Diseases* **11**(3): 169-179.

Khan, T.H., Ganaie, M.A., Alharthy, K.M., Madkhali, H., Jan, B.L. and Sheikh, I.A. (2020). Naringenin prevents doxorubicin-induced toxicity in kidney tissues by regulating the 328 oxidative and inflammatory insult in Wistar rats. *Archives of Physiology and Biochemistry* **126**(4): 300-307.

Lahoti, T.S., Patel, D., Thekkemadom, V., Beckett, R. and Ray, S.D. (2012). Doxorubicin induced in vivo nephrotoxicity involves oxidative stress- mediated multiple pro- and anti-apoptotic signaling pathways. *Current Neurovascular Research* **9**(4):282-295.

Lee, V.W. and Harris, D.C. (2011). Adriamycin nephropathy: A model of focal segmental glomerulosclerosis. *Nephrology* **16**(1): 30-38.

Li, A.P., Yang, L., Zhang, L.C., He, S.S., Jia, J.P. and Qin, X.M. (2019). Evaluation of injury degree of adriamycin-induced nephropathy in rats based on serum metabolomics combined with proline marker. *Journal of Proteome Rsearch* **19**(7): 2575-2584.

Li, X.Y., Chen, H.R., Zha, X.Q., Chen, S., Pan, L.H., Li, Q.M. and Luo, J.P. (2020). Prevention and possible mechanism of a purified Laminaria japonica polysaccharide on adriamycin-induced acute kidney injury in mice. *International Journal of Biological Macromolecules* **148**: 591-600.

Mohammadi, A. and Ahmadizadeh, M. (2018). Effects of antioxidants on xenobiotics-induced nephrotoxicity. *Journal of Renal Injury Prevention* **7**(2): 56-57.

Mohamed, F., Endre, Z.H. and Buckley, N.A. (2015). Role of biomarkers of nephrotoxic acute kidney injury in deliberate poisoning and envenomation in less developed countries. *British Journal of Clinical Pharmacology* **80**(1):3-19.

Molehin, O.R. (2020). Alleviation of doxorubicin-induced nephrotoxicity by Clerodendrum volubile leaf extract in Wistar rats: A preliminary study. *Journal of Herbmed Pharmacology* **9**(2): 138-144.

Okuda, S., Oh, Y., Tsuruda, H., Onoyama, K., Fujimi, S. and Fujishima, M. (1986). Adriamycin-induced nephropathy as a model of chronic progressive glomerular disease. *Kidney International* **29**(2): 502-510.

Park, E.S., Kim, S.D., Lee, M.H., Lee, H.S.S., Lee, I.S., Sung, J.K. and Yoon, Y.S. (2003). Protective effects of N-acetylcysteine and selenium against doxorubicin toxicity in rats. *Journal of Veterinary Science* **4**(2):129-136.

Perše, M. and Veceric-Haler, Z. (2018). Cisplatin-induced rodent model of kidney injury: characteristics and challenges. *BioMed Research International*: 1-29, DOI: 10.1155/2018/1462802.

Qi, Y., Xiao, H., Xu, C., Tian, X., Wu, H., Shen, W. (2012). Cyprinuscarpio decoction improves nutritionand immunity and reduces proteinuria through nephrin and CD2AP expressions in rats with adriamycin-induced nephropathy. *Evidance Based Complementary and Alternative Medicine*. DOI: 10.1155/2012/237482.

Quiles, J.L., Huertas, J.R., Battino, M., Mataix, J. and Ramírez-Tortosa, M.C. 2002. Antioxidant nutrients and adriamycin toxicity. *Toxicology* **180**(1): 79-95.

Safhi, M.M. (2018). Nephroprotective effect of Zingerone against CCl4-induced renal toxicity in Swiss albino mice: molecular mechanism. *Oxidative Medicine and Cellular Longevity*, DOI: 10.1155/2018/2474831.

Van der Worp, H.B., Howells, D.W., Sena, E.S., Porritt, M.J., Rewell, S., O'Collins, V. and Macleod, M.R. (2010). Can animal models of disease reliably inform human studies?. *PLos Medicine* **7**(3), DOI: 10.1371/journal.pmed.1000245.

Wang, Y., Wang, Y.P., Tay, Y.C. and Harris, D.C. (2000). Progressive adriamycin nephropathy in mice: Sequence of histologic and immunohistochemical events. *Kidney International* **58**(4): 1797-1804.

Yagmurca, M., Yasar, Z. and Bas, O. (2015). Effects of quercetin on kidney injury induced by doxorubicin. *Bratislava Lekarske Listy* **116**(8): 486-489.

Yilmaz, S., Atessahin, A., Sahna, E., Karahan, I. and Ozer, S. (2006). Protective effect of lycopene on Adriamycin induced cardiotoxicity and nephrotoxicity. *Toxicology* **218**(2-3): 164-171.

Yu, X., Yang, Y., Yuan, H., Wu, M., Li, S., Gong, W., Yu, J., Xia, W., Zhang, Y., Ding, G. and Huang, S. (2017). Inhibition of COX-2/PGE2 cascade ameliorates cisplatin-induced mesangial cell apoptosis. *American Journal of Translational Research* **9**(3):1222-1229

Zhao, J., Lin, X., Xiao, X., Yang, J., Liu, H., Yi, W., Zhang, Z. and Zhang, X. (2015). Inhibition of Adriamycin-induced nephropathy in rats by herbs based kangshenoral solution. *International Journal of Clinical and Experimental Medicine* **8**(12): 22469-22476.