

## Research Article



## Effect of Pentoxifylline against Doxorubicin-Induced Nephrotoxicity in Rabbits

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### ABSTRACT

This study was designed to evaluate the possible protective effect of pentoxifylline against doxorubicin-induced nephrotoxicity in rabbits. The animals groups were divided into a control group received 5 ml of normal saline intraperitoneally for 5 days, doxorubicin group that received (25mg/kg) intraperitoneally at day 3 and then the animals were sacrificed at day 5 and pentoxifylline + doxorubicin group (80mg/kg/day) intraperitoneally administered for 3 days before and two days after doxorubicin (25mg/kg) intraperitoneally administered at day 3 and then the animals were sacrificed at day 5. All animal were anesthetized by ether and sacrificed, after 48 hour after doxorubicin administered, at day 5. The spectrophotometry measuring of kidney function parameters such as serum urea and creatinine level. In addition, the oxidative stress parameters that included malondialdehyde and reduced glutathione in the renal tissue homogenate. The results obtained by analysis of data, by using independent t-test, showed a significant improvement of urea and creatinine levels in pentoxifylline + doxorubicin group as compared to doxorubicin group at  $p \leq 0.05$ . Also, reduction effect of pentoxifylline on malondialdehyde and a significant elevation of GSH. The results of this study led to conclude the beneficial effect pentoxifylline against doxorubicin-induced kidney toxicity in rabbit's models.

**Keywords:** Doxorubicin, Nephrotoxicity, Pentoxifylline, Rabbits

### INTRODUCTION

Doxorubicin (DOX) is an anthracycline antineoplastic drug<sup>1</sup>. Doxorubicin which is essential in treating breast and oesophageal carcinomas, solid tumors in childhood, osteosarcomas, Kaposi's sarcoma, soft tissue sarcomas, and Hodgkin and non-Hodgkin lymphomas<sup>2</sup>. DOX acts by stabilizing an intermediate reaction in which DNA strands are cut and covalently linked to tyrosine residues of topoisomerase II which blocks subsequent DNA resealing. Failure to relax the supercoiled DNA blocks DNA replication and transcription; furthermore, DNA strand breaks may trigger apoptosis of cancer cells<sup>3-5</sup>. Nephrotoxicity is a well-known DOX side effect, nevertheless, its exact mechanism(s) is not yet known. Dox accumulates mainly in the kidney although is also found in liver, heart and small intestine<sup>6,7</sup>. DOX is metabolized by oxido-reductase cycle leading to production of free radicals such as hydrogen peroxide and hydroxyl free radicals. In addition, there is a non-enzymatic mediated complex formation between DOX and iron ions resulting in formation of hydroxyl radicals<sup>8</sup>. Doxorubicin activation of stress-related signaling pathways and cytokines receptors causes an imbalance between the pro-apoptotic and anti-apoptotic factors; this imbalance will increase the permeability of mitochondrial membrane, enhance the release of cytochrome C oxidase, prolong the opening time of calcium channels in the sarcoplasmic reticulum, and end with cell death<sup>9</sup>. Pentoxifylline (PTX), a methyl xanthine derivative, is an inhibitor of phosphodiesterase enzyme (PDE). PTX had been reported to suppress the production of tumor necrosis factor-alpha (TNF- $\alpha$ ) and

modulate the production of other inflammatory cytokines<sup>10</sup>. Besides, PTX decreases total systemic vascular resistance by its ability to reduce the viscosity of whole blood<sup>11,12</sup>. Also it has the potential to decrease intraglomerular pressure and thus, there was an early interest in pentoxifylline as a therapeutic agent in patients with kidney disease<sup>13</sup>.

PTX administration was found to attenuate different drugs-induced oxidative stress in kidneys tissue<sup>14-17</sup>.

### MATERIALS AND METHODS

#### Experimental design

Eighteen domestic rabbits of either sex weighing between (1-1.5) kg were included in the present study. Animals were housed under standard conditions of temperature and relative humidity. All procedures involving animals were carried out under the institute ethics committee approval.

Animals divided into three groups, each group of six animals as following:

#### Group 1 (Normal group)

Each rabbit was received 5 ml /intraperitoneally of isotonic normal saline / at day 3; then animals were sacrificed at the day 5.

#### Group 2 (DOX group)

Each rabbit was treated with a single dose of DOX (Ebewe Pharma, Austria) (25mg/kg/i.p.) at the day 3; then, these animals were sacrificed at the day 5 (i.e., after 48 hours of DOX administration).



### Group3 (PTX+DOX treated group)

Each rabbit was treated with PTX (Sanofi-Aventis, Turkey) at a dose 80mg i.p./kg/day for 5 days. A single dose of DOX (Ebewe Pharma, Austria) (25mg/kg/i.p.) was administered at the day 3; then animals were sacrificed at the day 5.

#### Preparation of serum samples

At the day 5 of experiment (i.e., after 48 hours of DOX administration) the blood was aspirated via intracardiac puncture, and immediately transferred into plastic test tubes which were not containing anticoagulant. The clot was dispersed with glass rod and then centrifuged at 3000 rpm for 15-20 minutes; then, the supernatant was used for the estimation of serum urea and creatinine.

#### Determination of Serum Urea

Serum urea levels were determined using urease-modified Barthelot reaction<sup>18</sup> by a ready-made auto analyzer kit for this purpose, which can be measured of serum. The measured levels of serum urea were expressed in mg/dl.

#### Determination of Serum Creatinine

Serum creatinine concentrations were determined according to Jaffe reaction<sup>19</sup> using a ready-made auto analyzer kit for this purpose, which can be measured of serum. The measured levels of serum creatinine were expressed in mg/dl.

#### Preparation of Kidney Tissue Homogenate

After the animals being euthanized by anesthetic ether, kidneys were quickly excised, placed in chilled phosphate buffer solution (pH= 7.4) at 4 °C, blotted with filter paper and weighed. One gram of organ was then taken to prepare 10% tissue homogenate by using the same buffer solution and utilizing tissue homogenizer<sup>20</sup> at set 3 for 1 minute at 4 °C.

All preparations were freshly prepared and kept frozen (-70 °C) unless worked immediately.

#### Measurement of Lipid Peroxidation

Malondialdehyde (MDA), the end product of lipid peroxidation, was analyzed according to the method of Buege and Aust<sup>21</sup>.

Light absorbency of clear supernatant solution determined at 535 nm against a blank using Specord-40 spectrophotometer. The results were expressed as nmol MDA/g tissue.

#### Determination of Reduced Glutathione Level

Total thiol groups contents, which could be used as an indicator of reduced glutathione (GSH), was determined according to the method of Elman's<sup>22</sup>.

The light absorbency of the solution at 412 nm was measured after 2 minutes. The results were expressed as nmol reduced GSH / g tissue.

### Histopathological Examination

According to the method of Bauer<sup>23</sup> tissue sections of rabbits' kidneys were prepared for histopathological examination by using paraffin sections technique. They were fixed in 10% neutral buffered formalin solution, and then dehydrated using increasing strength of ethanol (80%, 95%, and absolute alcohol). Clearing of tissue was performed using xylene, then impregnated with paraffin wax, heated to 56-58 °C and blocked in embedding molds. Blocks were cut by microtome into 5 microns thick sections, stained with hematoxylin and eosin, then examined under light microscope.

#### Assessment of Histopathological Changes in Kidney Sections

The slides were coded and semi-quantitative analysis of the kidney sections was performed without knowledge of the treatment protocol<sup>24,25</sup>. The changes seen were limited to the tubulointerstitial areas and graded as follows (0) normal, (1) areas of tubular epithelial cell swelling, vascular degeneration, necrosis and desquamation involving (25%) of cortical tubules, (2) similar changes involving (25%), but less than (50%) of cortical tubules, (3) similar changes involving (50%), but less than (75%) of cortical tubules and lastly (4) similar changes involving (75%) of cortical tubules.

#### Statistical analysis

Mean values  $\pm$  S. E. M. were calculated for each parameter. For the determination of significant differences, each parameter was analyzed separately and independent Student's t-test for comparison between two groups was carried out.  $p < 0.05$  was considered significant<sup>26</sup>.

### RESULTS

Compared to those of control group, rabbits of DOX group showed significant ( $P < 0.05$ ) increments in mean levels of both serum urea and mean serum creatinine [Table 1]. Besides, in kidney tissue homogenate samples of DOX group, a significant ( $P < 0.05$ ) increment in mean MDA level and a significant ( $P < 0.05$ ) increment in mean GSH level could be determined when being compared to those of control group [Table 1]. Pentoxifylline (PTX) could induce a reduction ( $P < 0.05$ ) in mean levels of both serum urea and mean serum creatinine [Table 1; Figures (1 & 2)]; this decrement was found to be significant ( $P < 0.05$ ) when obtained values being compared to those of DOX group and non-significant ( $P > 0.05$ ) (i.e., comparable) when being compared to those of control group [Table 1]. Treatment with PTX significantly ( $P < 0.05$ ) minimized the magnitude of DOX - induced elevation in mean level of MDA and reduction in mean GSH in kidney tissue homogenate samples; nevertheless, such protective effect of PTX could not normalize the mean levels, i.e., they were still significantly ( $P < 0.05$ ) higher (regarding MDA) or less (regarding GSH) than those of control group [Table 1; Figures (3& 4)]. The



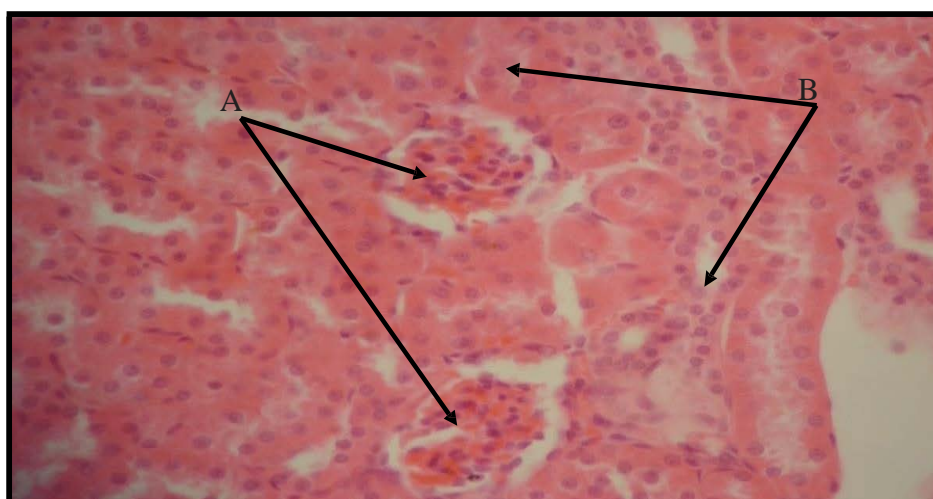
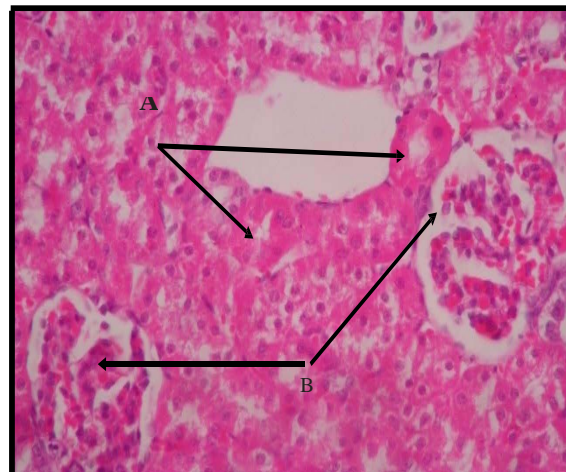
effect of single dose of DOX (25mg /kg i.p.) on kidney tissue evaluated as moderate level by measuring the score mean of histopathological features changes that found to be significantly higher score levels  $P < 0.05$  in comparison to control group score level [Table 1 ; Figures

(1& 2)]. In other hand, the mean of score level of PTX group showed a mild score level with less tubular swelling and vacuolation and significantly less as compared to DOX treated group ( $p=0.008$ ) and significantly different as compared to control group ( $p=0.002$ ), [Table 1 ; Figure 3].

**Table 1:** Effect of pentoxifylline on means of serum creatinine, serum urea, tissue malondialdehyde, tissue glutathione and histopathological scores levels of rabbits with doxorubicin-induced nephrotoxicity.

Groups (6 rabbit/Group)	Serum Level (Mean ± S.E.M.)(mg/dl)		Tissue Level (Mean ± S.E.M.) μmol/g		Histopathological Changes scores (Mean ± S.E.M.)
	Creatinine	Urea	MDA	GSH	Score means
Control	0.60 ± 0.02	28.1 ± 1.9	20.96 ± 1.7	9.05 ± 0.4	0.0 ± 0.0
Doxorubicin	0.94 ± 0.06*	71.8 ± 3.5*	56.29 ± 1.4*	2.7 ± 0.26 <sup>s</sup>	2.33 ± 0.21 <sup>*</sup>
Pentoxifylline	0.64 ± 0.08 <sup>s</sup>	31.5 ± 0.92 <sup>s</sup>	34.67 ± 0.82 <sup>s</sup>	6.5 ± 0.39 <sup>s</sup>	1.5 ± 0.22 <sup>*,s</sup>

<sup>\*</sup> = 80 mg/kg/day i.p. for 5 days, <sup>\*</sup> = significant change ( $p < 0.05$ ) when being compared to that of control group, <sup>s</sup> = significant change ( $p < 0.05$ ) when being compared to that of doxorubicin (single i.p. dose - 25mg/kg) group.



**Figure (1):** Section of kidney of control group shows A) Single glomerular tuft, B) Proximal tubules appear with normal epithelial cells. H & E (40X).

**Figure (2):** Section of kidney of (single dose 25mg/kg i.p.) DOX group reveals A) Moderate tubular epithelial cell swelling and vacuolation B) Glomerular congestion. H & E (40X).



## DISCUSSION

The nephrotoxic effect of DOX characterized by decreasing glomerular filtration rate that leading to a rise in serum urea and creatinine. Toxic effects obtained in the present study are compatible with previous studies were showing a deterioration in renal functions parameters including serum urea and creatinine<sup>23,24</sup>. DOX nephrotoxicity may be attributed to formation of active metabolite and enhancement of free radicals production that had been associated with damaging to tubular epithelial cells membranes and increasing of end products of lipid peroxidation (i.e. MDA as a lipid peroxidation marker). Excessive lipid peroxidation had been reported in DOX-treated rabbits and showed significant increment in the levels of MDA in kidney tissues<sup>23</sup>. DOX found to cause a declined GSH level leading to aggravate potential toxic effect and prevent the important roles of GSH in detoxification of reactive free radicals, conjugation and excretion of toxic compounds<sup>25</sup>. Whereas, administration of PTX prior and after doxorubicin toxic dose associated with improvement of renal function parameters by ameliorating the serum urea and serum creatinine level. PTX administration attenuated renal tissue damage and number of apoptic cells<sup>27</sup>. The nephro-protective effect of PTX mediated by vascular decongestion that may increase blood flow and reduce hypoxia with improvement of the renal microcirculation through the stimulation of renal vasodilator prostaglandins<sup>28</sup>. On the other hand, it had been shown that PTX affects calcium homeostasis and may inhibit calcium accumulation in the kidney, this effect explain the protective effect of calcium channel blockers in the prevention of nephrotoxicity<sup>29</sup>. The nephro-protective effect of PTX involves its ability to down-regulate the production of proinflammatory cytokines and mediated the reduction of cell death due to over production of TNF- $\alpha$ <sup>34</sup>. PTX has proven nephroprotective efficacy through modulating the release of inflammatory cytokines and nitric oxide and restoring the oxidant/antioxidant balance<sup>30</sup>.

PTX had an antioxidant effect which is playing a role in reducing lipid peroxidation (i.e. reduction in MDA level) and elevation of GSH in kidney tissue and render the damage extent of DOX on kidney tissues toward control group.

## CONCLUSION

Results of the present study pointed out that PTX has a protective and antioxidant effect against DOX-induced nephrotoxicity.

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