Protective Effect of Simvastatin Against Adriamycin-Induced Nepherotoxicity in Rats; Biochemical and Histological Study

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Abstract

Introduction: The usefulness of adriamycin (ADR), a potent anti-tumor antibiotic, is limited by the development of life-threatening cardiomyopathy and nephropathy. The cellular changes leading to these toxicities are suggested to be mediated by increased free radicals and lipid peroxidation

Aim of the study: The current study was aimed to investigate the protective role of simvastatin (SIM) on adriamycin-induced nephrotoxicity in rat using biochemical, and histological approaches.

Material and methods: Twenty eight healthy male Swiss albino rats were used and divided into four groups : CONT (control), ADR (adriamycin treated), SIM (simvastatin treated), and SIM+ADR (simvastatin plus adriamycin treated). Blood samples were collected and used to determine the serum urea, creatinine, albumin, and total protein levels. Both kidneys were removed ,one of them was prepared for histological examinations and the other was stored at −70 °C for subsequent measurement of malondialdehyde (MDA), glutathione (GSH) contents and phase II antioxidants enzymes activities.

Results: Glutathione (GSH) level, glutathione-s-transferase (GST) and DT-diphorase activities were decreased, while the lipid peroxidation was increased in kidney tissue. Administration of SIM (cumulative dose, 60 mg/kg body wt) in 12 equal injections (PO), before and concurrent with ADR, more or less prevented these nephropathic changes, normalized kidney function, and eliminated ascitis. Treatment with SIM was also accompanied by an increase in kidney GSH level as well as DT-diphorase activities with a concomitant decrease in lipid peroxidation. Histological examination revealed extensive and marked tubular necrosis in the ADR-treated kidney. Administration of Simvastatin reversed kidney damage with a marked reduction in tubular damage induced by ADR

Conclusion: These data show that SIM can provide coma protection against ADR nephropathy. This protective effect of SIM may be related to the antioxidant status on the kidney

Key wards: Adriamycin, Nephrotoxicity, Simvastatin, antioxidant enzymes, DT diaphorase.

Introduction

Since 1969, adriamycin (ADR) an anthracycline antibiotic is widely used as anticancer agent. In spite of its high anti-tumor efficacy, the use of ADR in therapeutic doses is limited due to its diverse toxicities, including cardiac, renal, hematological and testicular toxicity (Gillick, *et al*., 2002; Kang et al., 2002; Yagmurca *et al.,* 2004). Intravenous administration of ADR was noticed to induce kidney functional changes in rats, nephrotic syndrome like effect, characterized by proteinuria, albuminuria, hypoalbuminemia and hyperlipidemia (Desassis *et al*., 1997). This experimental nephropathy resembles histologically and clinically minimal change nephropathy, or focal and segmental glomerulosclerosis (Zima *et al*., 1997). ADR also increases glomerular capillary permeability and cause glomerular atrophy (Saad *et al*., 2001). ADR-induced toxicity has been believed to be mediated through different mechanisms including free radical

formation; and iron-dependent oxidative damage to the biological macromolecules and membrane lipid peroxidation (Oslon and Mushlin, 1990; Deman et al., 2001 and Fadillioglu *et al.*, 2004). Two different pathways of free radical formation by ADR have been described. **First;** formation of a semiquinone free radical by the action of several NADPH-dependent reductases that produce one-electron reduction of ADR to the corresponding ADR semiquinone. In the presence of oxygen, redox cycling of ADR-derived quinine–semiquinone yields superoxide radicals ($O_{2\bullet}^-$) (Venditti *et al.*, 1998). **Second;** ADR free radicals are produced by a non-enzymatic mechanism that involves reactions with iron. Iron–DXR complex can reduce oxygen to H_2O_2 and other active oxygen species, which cause oxidative damage of a variety of tissues including the kidneys (DeBeer *et al.,* 2001).

However, the dose and the duration of ADR for inducing renal diseases were variable. Wapstra *et al.* (1999) has demonstrated that a dose of 3 mg/kg ADR induced renal damage after 6 weeks. On the other hand, it was shown that nephrotoxicity was induced by a dose of 25 mg/kg ADR after 2 days (Saad *et al.*, 2001). Pharmacological methods of preserving renal function and the investigation of the effects of nephro-protective intervention therapy have been studied by OKasora *et al.* (1992).

Statins are widely used clinically for lowering hypercholesterolemia because of their inhibitory effect on 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the enzyme that catalyzes the rate-limiting step of the cholesterol synthesis in the liver and other tissues (Corsini *et al.*, 1995). Many investigators suggest that statins exhibit" pleiotropic effect" such as anti-inflammatory, anti-thrombotic, anti-angiogenic, anti-hypertropic, and plaque modifying effects that are not directed to their cholesterol-lowering activity (Weitz-Schmidt, 2002; Bonetti *et al.*, 2003). Since inflammatory processes have an important role in the pathogenesis of coronary heart disease, stroke and transplant rejection. Statin

therapy is beneficial to decrease the incidence of rejection and to improve survival in heart-transplant patients (Wenke *et al.*, 1997; Plutzky and Ridker, 2001). Nephrotic syndrome in experi-mental adriamycin nephrotoxicity in rats may be prevented based on suggestion that the possible role of hyperlipidemia in the pathogenesis of glomerular damage and glomerulosclerosis using effective hypolip-idemic therapy (Wahio *et al.*, 1993; Tesar *et al.,* 1995; Montilla *et al.*, 1997). There is increasing evidence for an ameliorative effect of statin in renal disease (Oda and Keane, 1999; Buemi, *et al.*, 2002).

The aim of the present study was to investigate the protective activity of commonly used statin, simvastatin, in particular, against ADR-induced nephroto-xicity and the changes in oxidant-antioxidant status.

Material and Methods :

Chemicals:

Simvastatin (simvastat) was produced by FARCO and Alexandria Pharmac-euticals Co., Egypt. Adriamycin (doxor-ubicin hydrochloride) was used in the form of an injectable commercial product (Adriablastina, Farmitalia Carlo Erba, Milan, Italy). Concentrated, diethylether, Ellman's reagent (5,5′-dithio-bis-(2-nitro-benzoic acid); DTNB, thiobarbituric acid (TBA), trichloroacet-icacid (TCA), glutath-ione (GSH), 2,6-dichloropenol indophenol, 1-chloro-2, 4-dinitrobenzene (CDNB), and Malondi-aldehyde (MDA);1,1,3,3 tetra-methoxyp-ropane used in this study were analyt-ically pure product of Sigma-Aldrich Chemical Co., St. Louis, MO, USA. n-Butanol, orthophosphoric acid, produced by Merck Co., Darmstadt, Germany. Potassium dihydrogen phosph-ate, sodium dihydrogen phosphate, dipota-ssium hydrogen phosphate anhydrous and sodium hydroxide pellets produced by El-Nasr Chemical Co., Cairo, Egypt.

Animals:

Twenty eight healthy male Swiss albino rats, weighing 160±10 g, were used and housed in an animal facility that was maintained with conditioned atmosphere at 25 ± 2°C and kept on standard diet pellets (El-Nasr, Cairo, Egypt), and tap water.

Experimental protocol:

Rats were divided into four groups, seven rats each: CONT (control), ADR (adriamycin treated), SIM (simvastatin treated), and SIM+ADR (simvastatin plus adriamycin treated). Adriamycin (doxorubicin hydrochloride) was injected intra-peritoneally in six equal doses of (2.5 mg/kg ADR) to animals in ADR and SIM+ADR groups over a period of 2 weeks for a total cumulative dose of 15 mg/kg body weight. Simvastatin (cumulative dose 60 mg/kg body wt) was also administered orally to SIM and SIM+ADR groups in 12 equal doses (each treatment containing 5mg/kg) over a period of 4 weeks, 2 weeks before adriamycin administration and 2 weeks alternating with adriamycin injections. CONT animals were injected with saline on the same regimen as ADR group.

Treated as well as control animals were observed during injections for their body weight changes and mortality. Rats were sacrificed 24 hours after the last dose of adriamycin. Blood samples were collected and used for determination of serum urea, creatinine, albumin, and total protein levels. Kidneys were removed and either fixed in 10% formaldehyde for histological examinations or stored at −70 °C for subsequent measurement of malondialdehyde (MDA), glutathione (GSH) contents and phase II antioxidants enzymes activities.

Biochemical analysis:
1. Estimation of lipid peroxidation:

Lipid peroxidation products of kidney homogenate were determined as thiobarbituric acid- reactive substances. Kidneys from control and treated rats were homogenized in ice-cold 0.9% saline to get 10% homogenate. 0.5ml of the supernatant after differential centrifugation was allowed to react with 3 ml of 1% orthophophoric acid and 1 ml of 0.6% thiobarbituric acid. The tubes were heated in a boiling water bath for 45 min, cooled, and then 4 ml of n-butanol was added to each test tube, mixed vigorously and centrifuged for 5 min at 1000 rpm. The supernatant was used for thiobarbituric acid-reactive substance deter-mination at 535 nm against a reagent blank. Commercially available 1,1,3,3-tetraetho-xypropane was used as a standard for MDA. TBARS are expressed as nmol/gm wet wt (Mihara and Uchiyama ,1978).

2. Estimation of reduced glutathione (GSH):

The level of GSH was determined as non-protein sulfhydryl contents (NPSH) of kidney tissue and from the standard curve with commercially available GSH according to Ellman (1959). Add 0.5 ml of tissue homogenate to each tube containing 0.5 ml TCA (10%). The tubes were gently shaken intermittently for 10 min, followed by centrifugation at 2000 rpm for 5 min at room temperature. Accurately, 0.1 ml of the resulting clear supernatant was mixed with 1.8 ml of 0.1 M phosphate buffer (pH 8) in separate test tubes. At least, a duplicate was made for each sample. 0.01 ml Ellman's reagent (0.39%) was added to each tube, and then, after 5 min, the optical density was measured at 412 against a reagent blank. The data were expressed as μmol/g tissue. NPSH content is expressed as μmole per gm wet weight.

3. Phase II antioxidant enzymes:
Determination of DT-diaphorase ctivity:

The activity DT-diaphorase was assayed as described by Benson et al. (1980) which involve measurement of reduction of NADH at 600 nm as the electron donor and 2,6-dichloropenol indophenol as the electron acceptor. The enzyme activity was calculated using the extinction coefficient 21 $mM^{-1} cm^{-1}$.

Determination of glutathione-S-transferase (GST) activity:

The GST activity was determined using spectrophotometry according to Habig et al. (1974). The reaction mixture (3 ml) contained 1.0 ml of 0.3 mM phosphate buffer (pH 6.5), 0.1 ml of 30 mM 1-chloro-

2, 4-dinitrobenzene (CDNB) and 1.7 ml of double distilled water. After pre-incubating the reaction mixture at 37°C for 5 min, the reaction was started by the addition of 0.1 ml of tissue homogenate and 0.1 ml of glutathione as substrate. The absorbance was followed for 3min at 340 nm. Reaction mixture without the enzyme was used as blank. The activity of GST is expressed as µmoles of GSH-CDNB con-jugate formed/min/mg protein using an extinction coefficient of 9.6 mM^{-1} cm^{-1}.

4. Estimation of tissue protein content:
Protein was determined according to Lowry et al. (1951) using Bovine Serum Albumin (BSA) as standard, at 660 nm.

5. Kidney function tests:
Blood urea nitrogen levels were determined according to Patton and Crouch (1977), while creatinine was estimated by the alkaline picrate according to Bonsnes and Taussky (1945). Total protein conc. was measured according to Henry (1974), and serum albumin was determined according to Doumas *et al.* (1971).

Histological technique:
The animals were anaesthetized & scarified, both kidneys from each animal were collected and part of them bisected sagitally the specimens were fixed in 10% neutral buffered formalin then processed to obtain 6 µ/micvare thick paraffin sections and stained with Hx&E for detection of general morphology, PAS to demonstrate mucopolysacchrides and Masson's Trichrome stains to demonstrate conne-ctive tissues (Drury & Wallington, 1980)

Statistical Analysis:
The experimental data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni test for multiple comparisons. Data were expressed as mean ± SEM. Using GRAPHADA INSTAT (ISI Software) computer program (1993).
Optical density for PAS reactivity in renal tissue was done using Optimas Image Analyzer, version 6.2. Then, mean,

standard error were calculated, and using the Student T-test to get the variant significance among all groups.

Results

A- Biochemical results
1- General Observations and Hemodynamics:
Within 24 hours of the last injection, no mortality was seen in any of the control, ADR, SIM, and SIM+ADR group. However, during the treatment period, the mortality rate was approximately 28.5% in the ADR group, with no deaths in the CONT, SIM, nor SIM+ADR groups **(table 1).** After the completion of treatment with adriamycin, animals in the ADR-treated group produced the characteristic signs of a nephrotic syndrome including enlarged abdomen, enlarged kidneys and liver, fluid accumulation in the peritoneal cavity, and animals looked weaker and lethargic. All ADR group animals had a significant amount of peritoneal fluid, while in the SIM+ADR animals showed minimal amount of peritoneal fluid (data is not shown). Animals in the ADR and ADR+SIM groups showed a significant loss in body weights (fig. 1). This reduction in body weight is attributed to reduced food intake and inhibition of protein synthesis due to adriamycin.
2- Effect of Simvastatin on ADR-induced kidney dysfunction:
ADR caused a marked reduction in renal functions as characterized by significant increased serum BUN (71.1 %), creatinine levels (99.65 %), and decreased serum albumin (24.8 %) and total protein levels (42.1 %) compared with control levels (at P ≤ 0.05).
These data indicated that ADR impair kidney function. Treatment with simvast-atin markedly reversed ADR-induced incre-ase in serum creatinine (11.6 %) and BUN levels (11.9 %). Simvastatin was also effec-tive to reverse ADR-induced decrease in serum albumin and total protein (table 2& fig. 2).

3- Effects of Simvastatin on kidney lipid peroxidation as an Index of Oxidative Damage:

Data in (fig. 3) showed that MDA level increased in kidney tissues in the

ADR group in comparison with the control group (27 %). While treatment with simvastatin alone or concurrently with ADR provided marked reduction in MDA concentration (12.65 % & 31.22 %). Compared to control or ADR treated group respectively.

4- Effects of simvastatin on kidney antioxidant status:

The activities of enzymatic antiox-idants GST, DT-diphorase and non-enzym-atic antioxidant GSH were measured to study the effect of SIM on antioxidant status. Administration of SIM to normal rats significantly increased the antioxidants levels (34 % in NPSH content & 88 % in DT diphorase activity) compared to normal control group. In addition, SIM administ-ration to nephropathic rats significantly decreased ADR-induced alterations in NPSH content and DT-diphorase activity in renal tissues, but without significant effect on GST activity. SIM treatment signifi-cantly increased renal NPSH content ~ 2 times and DT-diphorase activity 7 times compared to that produced by ADR alone (table. 3 & fig. 3).

B- Histological results:

Sections stained with Hx & E of control group (fig. 5), showed the normal pattern of renal tissue in the form of renal corpuscles surrounded by cross and oblique sections of proximal and distal convoluted tubules, sections of loops of Henle and collecting tubules were also seen in the medulla. In ADR treated animals, there were distorted and dilated renal tubules

with extensive cellular necrosis, with shrinked renal corpuscles (fig. 6). Whereas, the animals treated with Simvastatin and ADR (fig. 7) showed slightly distorted tubules and corpuscles; but those treated with Simvastatin alone (fig. 8) showed relatively normal renal picture.

PAS stained sections of the normal control group showed a bright red PAS positive reactivity in basement membrane of distal tubules with intense reaction in tubular brush border, and the glomerular basement membrane of the capillary loops (fig. 9). On the other hand, there were a significant reduction of the PAS activity in renal tissues in ADR-treated animals (fig. 10). A moderate PAS positive reactivity, significantly increased from those of ADR treated animals and decreased from those of the control group, were noticed in the basement membrane and brush border in some distal tubules concerning Simvastatin + ADR treated animals (fig. 11). Additionally, PAS reactivity was improved in animals treated with Simvastatin alone, but without statistical significant when compared to the control group (fig. 12). Table 4, and figure 4 show the distribution of PAS activity with its statistical evaluation among all treated groups in comparison to the control group.

In sections stained with Masson's trichrome stain, the capsular and minimal cortical and medullary connective tissue were noticed in normal control group (fig. 13). ADR-treated group showed slightly increased in connective tissue in the glomeruli, and in between the tubules (fig. 14). On the other hand, animals treated with Simvastatin and ADR, or those with Simvastatin alone showed a relatively normal connective tissue distribution of the renal tissues (fig. 15 & fig. 16).

Table (1): Effects of simvastatin on adriamycin-induced changes in body weight and mortality % in rats.

Group	No. of animals	Body weight (g)	Mortality %
CONT	7	213.9±2.05	0
SIM	7	216.0±3.06[b]	0
SIM+ADR	7	169.5±5.21[abc]	0
ADR	7	140.1±3.53[a]	28.5

Values are means of 7 experiments ± SEM.

* Statistical analysis was done using one way ANOVA followed by Bonferroni test for multiple comparisons.

a   significantly different from normal control group at $P \leq 0.05$.
b   significantly different from adriamycin-treated group at $P \leq 0.05$.
c   significantly different from simvastatin-treated group at $P \leq 0.05$.

Fig (1): Effects of simvastatin on adriamycin-induced body weight changes in rats.
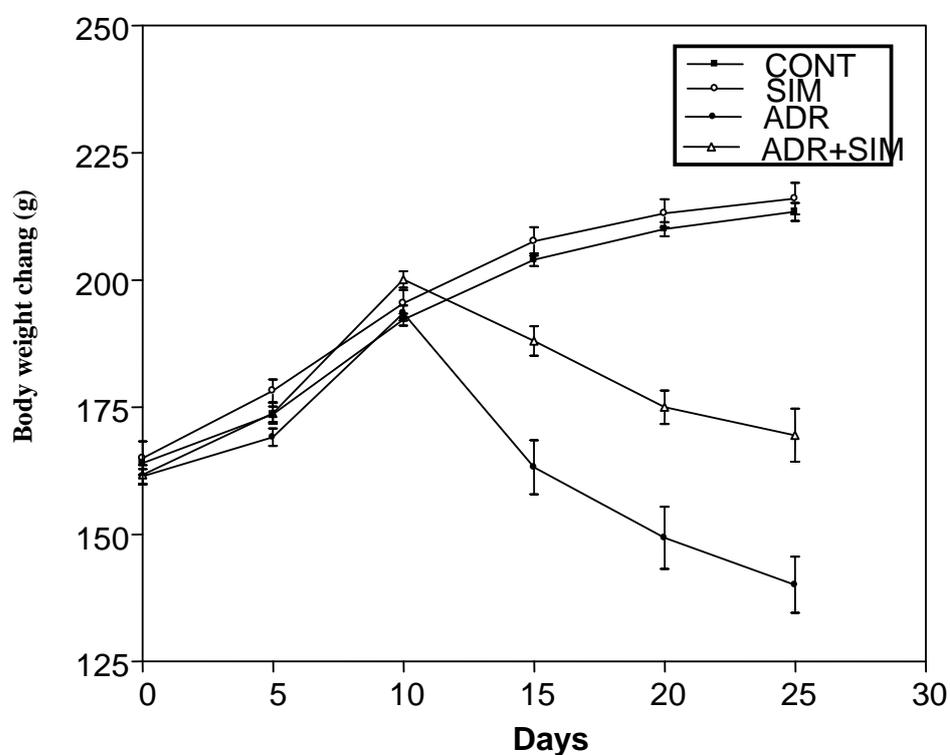
Table (2): Effect of Simvastatin on ADR- induced kidney dysfunction:

Group	Serum BUN mg/dl	Serum creatinine mg/dl	Serum albumin♀ g/dl	Serum total protein g/dl
CONT	32.22± 4.503	0.871± 0.061	4.599± 0.029	6.233± 0.319
SIM	42.11± 1.475[ab]	1.061± 0.0758[b]	4.063± 0.126 [b]	5.429± 0.049 [b]
SIM+ADR	48.57± 1.556 [abc]	1.53± 0.026 [abc]	3.711± 0.207 [abc]	4.978±0.083[ab]
ADR	55.13± 0.541[a]	1.739± 0.331 [a]	3.458± 0.033 [a]	3.61± 0.738 [a]
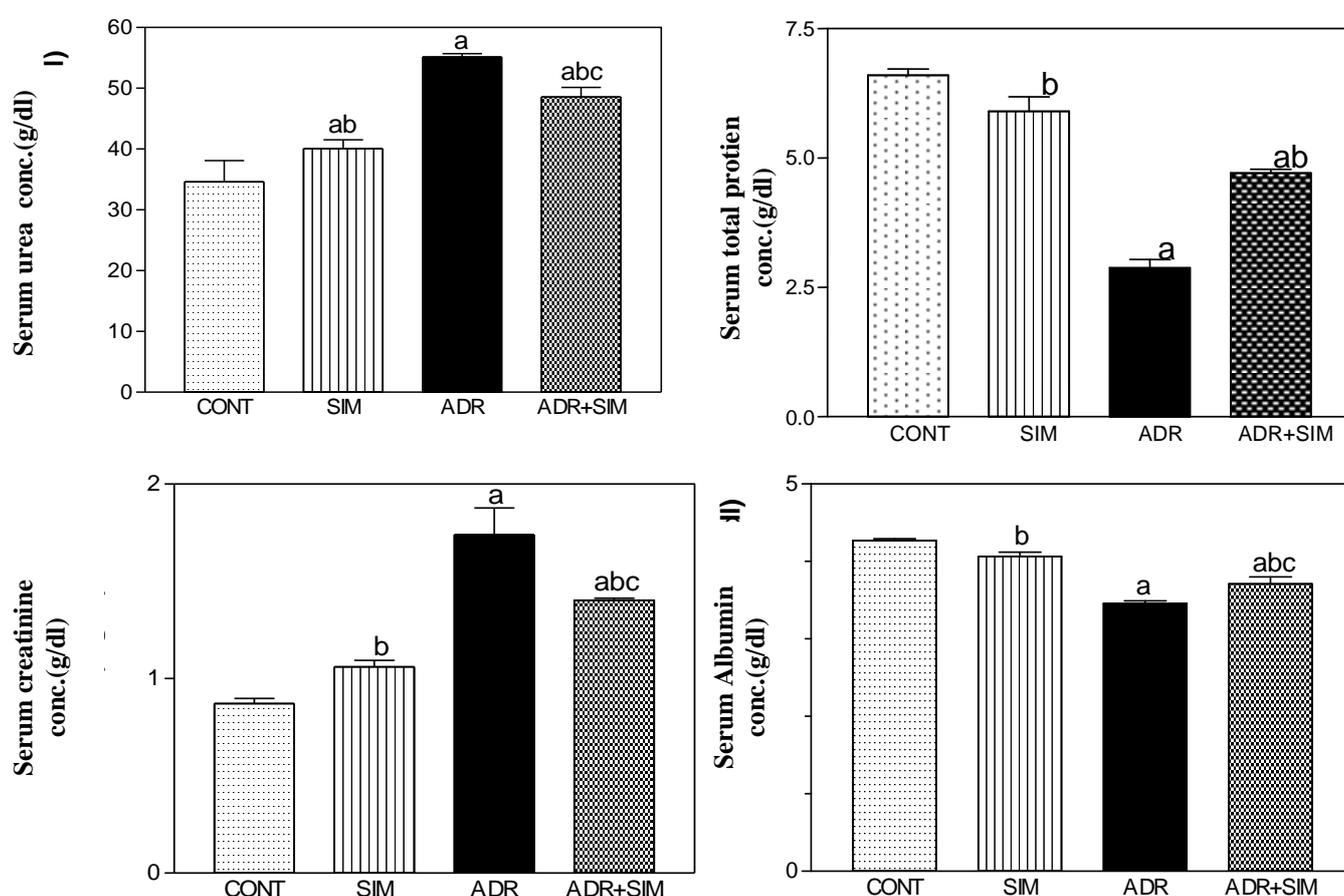
Fig. (2) : Effect of simvastatin (SIM) on serum urea, creatinine, albumin and total protein level of normal and doxorubicin (ADR)-induced nephrotic rats. Rats were treated with SIM (cumulative dose, 60 mg/kg body wt ,o.p. in 12 equal injections) before and concurrent with ADR(cumulative dose, 15 mg/kg body wt, i.p. in six equal injections), and 24 hours after last ADR injection, serum urea, creatinine, albumin and total protein concenteration were determined in renal tissues.

Bars are means ± S.D. of seven observations. a, b or c indicates a significant difference from control, ADR, or SIM groups, respectively, at *P*<0.05 using one-way analysis of variance (ANOVA) followed by Bonferroni test for multiple comparison

Table (3): Effect of Simvastatin on ADR- induced kidney lipid peroxidation as an Index of Oxidative Damage

Group	TBRAS (nmol/g tissue)	NPSH (μmol/g tissue)	GST (μmol/min/g tissue)	DT-diphorase (μmol/min/gtissue)
CONT	123.3± 1.31	4.4± 0.07	3.49± 0.08	1.61± 0.11
SIM	51.8± 0.0758[ab]	5.9± .21[ab]	3.86± 0.15 [b]	3.03± 0.19 [ab]
SIM+ADR	107.7± 4.6[abc]	3.9± 0.01[bc]	2.86± 0.11 [ac]	2.34± 0.03 [abc]
ADR	156.6± .97 [a]	1.8± 0.16 [a]	2.45± 0.10 [a]	0.28± 0.03[a]
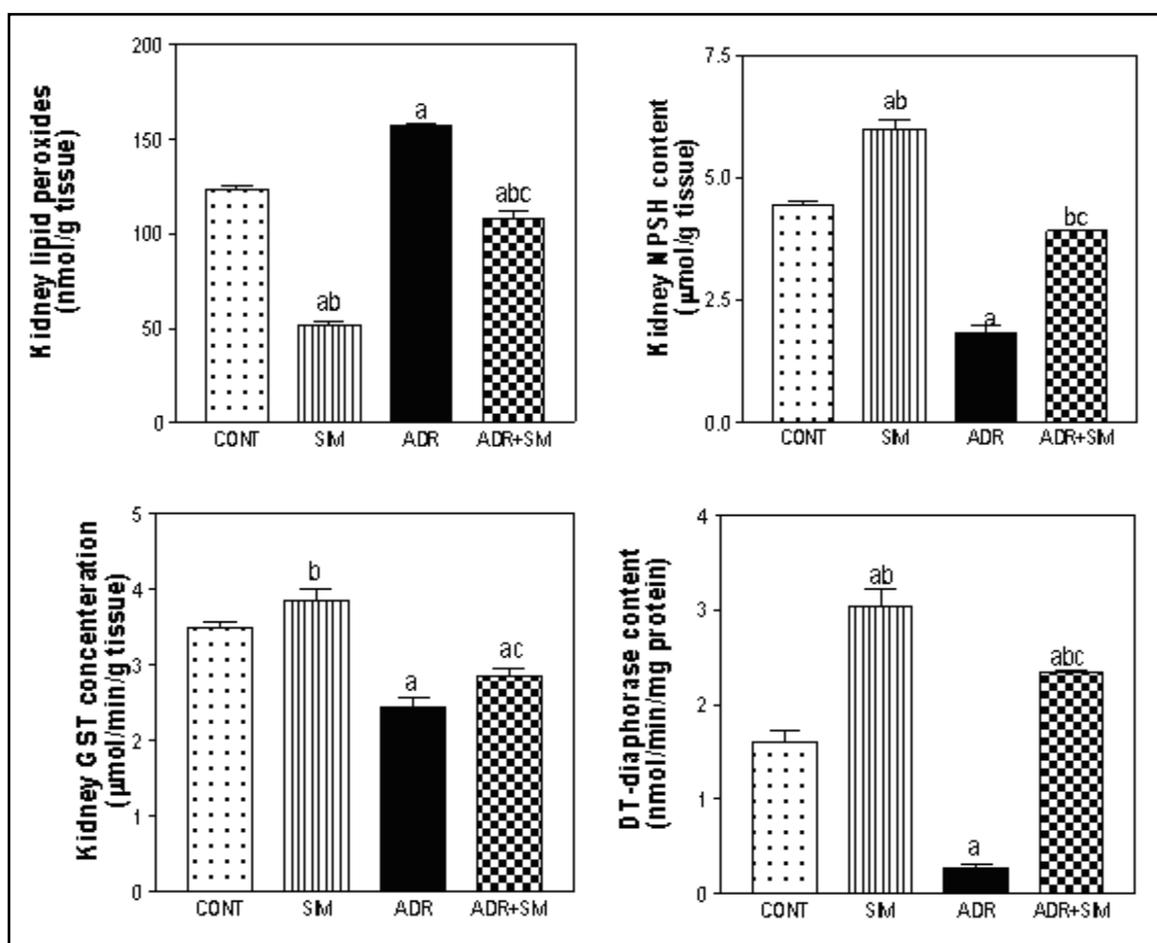
Fig (3) : Effect of SIM on lipid peroxide (thiobarbituric acid – reactive substances, TBARS) level, non-protein sulfhydyl (NPSH) content, glutathione-s- transferees (GST) activity and DT- diphorase activity in the kidney of normal and doxorubicin (ADR)- induce nephritic rats. Rats were treated with SIM (cumulative dose, 60 mg/kg body wt., o.p. in 12 equal doses) before and concurrent with ADR (cumulative dose, 15 mg/kg body wt., i.p. in sis equal doses), and 24 hours after last ADR injection, NPSH, TBARS and GST and DT-diphorase activity were determined in renal tissues.

Bars are means ± S.D. of seven observations. a, b or c indicates a significant difference from control, ADR, or SIM groups, respectively, at *P*<0.05 using one-way analysis of variance (ANOVA) followed by Bonferroni test for multiple comparisons

(Table 4): Optical density of PAS reactivity in the glomerular basement membrane, and in renal tubules in different groups

Group	Optical density of PAS reactivity in brush border of renal tubules	Optical density of PAS reactivity in the glomerular basement membrane
CONT	0.76723± 0.0073	0,1775± 0,0018
SIM	0,7115± 0,0213[b]	0,171± 0,0022[b]
SIM+ADR	0,5455± 0,0062 [abc]	0,1549± 0,0009[abc]
ADR	0.15283± 0.015[a]	0,1151± 0,0022 [a]
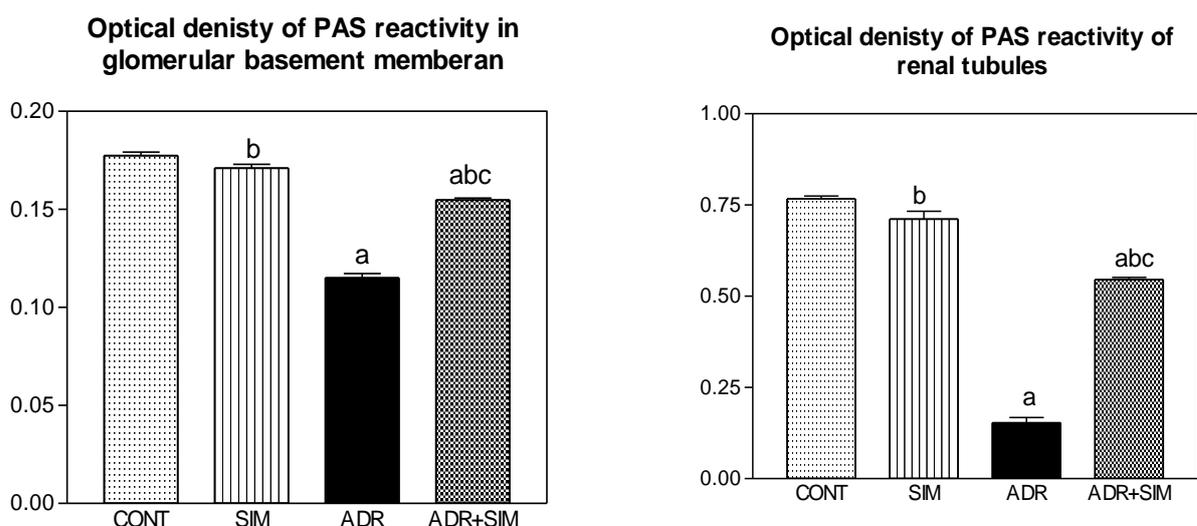
Fig (4): The optical density of the PAS reactivity in the glomerular basement membrane, and renal tubules of different groups.

Bars are means ± S.D. of ten observations. a, and c indicates a significant difference from control, ADR, or SIM&ADR groups, respectively, at $P<0.05$ using one-way analysis of variance (ANOVA) followed by Bonferroni test for multiple comparison
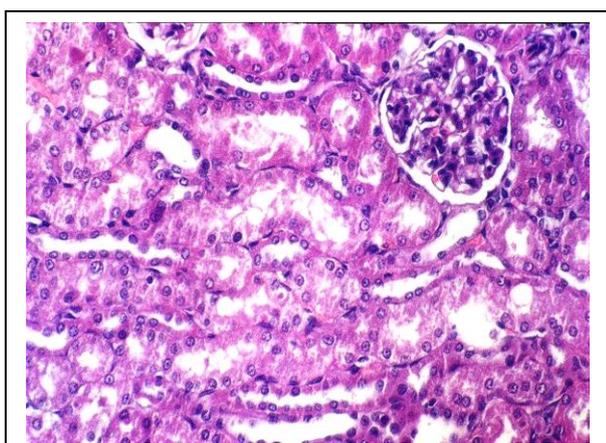

Fig (5): A photomicrograph of a kidney section of the control group showing normal renal tubules, and glumeruli. (Hx&E X400)
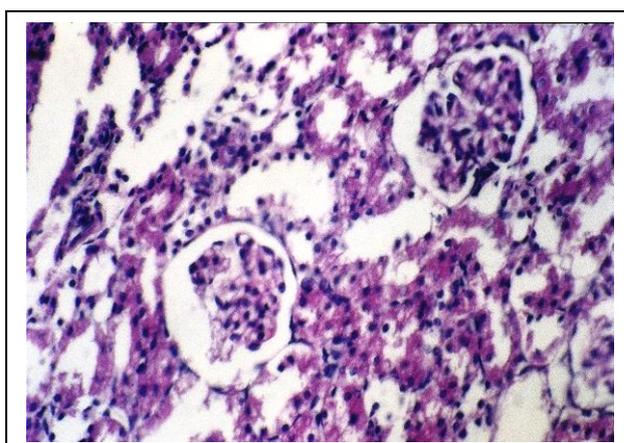
Fig (6) : A photomicrograph of a kidney section treated with ADR showing distorted renal tubules, and collapsed glumeruli.
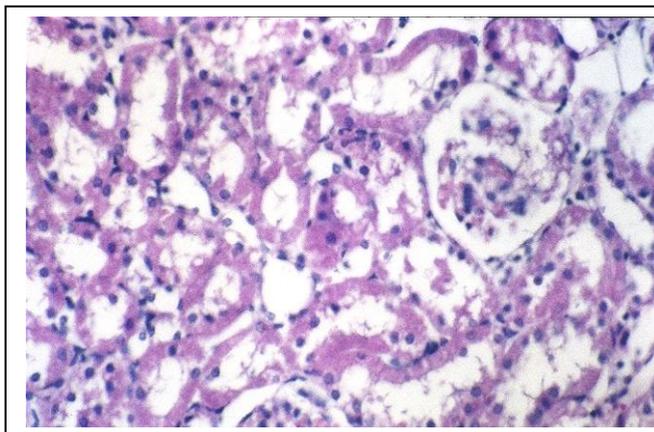
(Hx&E X400)

Fig (7) : A photomicrograph of a kidney section treated with ADR and SIM showing renal tubules, and more or less normal glumeruls slightlydilated. (Hx&E X400)
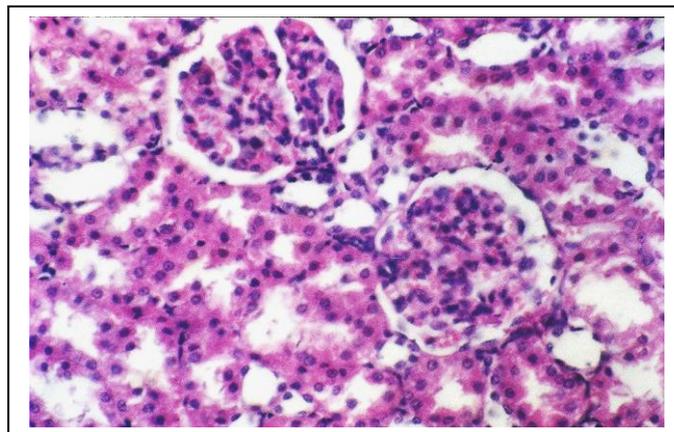

Fig. 8) A photomicrograph of a kidney section treated with SIM showing more or less normal renal tubules, and glumeruli. (Hx&E X400)
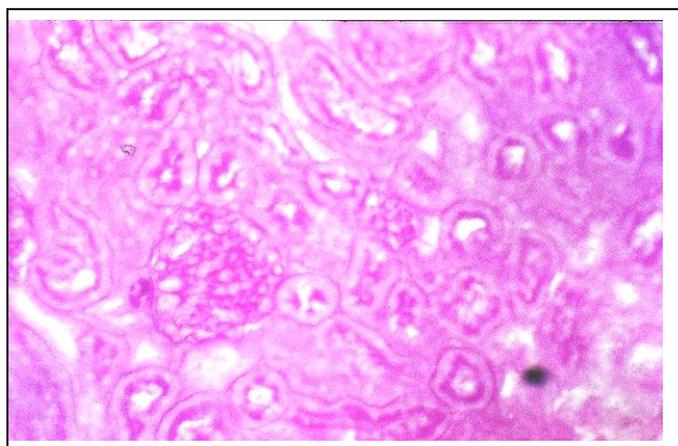

(Fig. 9) A photomicrograph of a kidney section of the control group showing normal PAS reactivity in renal tubules, and glumeruli. (PAS X400)
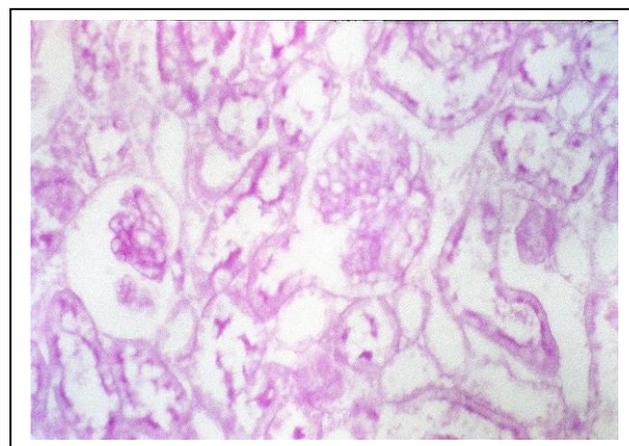
(Fig. 10) A photomicrograph of a kidney section treated with ADR showing distorted PAS reactivity in the renal tubules, and glumeruli. (PAS X400)
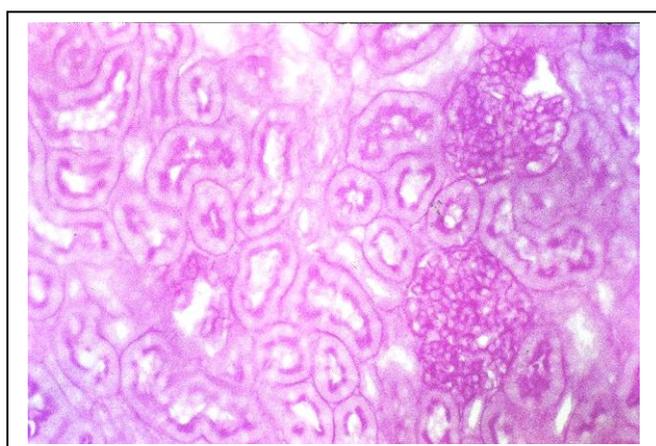

(Fig. 11) A photomicrograph of a kidney section treated with ADR and SIM showing improved PAS reactivity in the renal tubules, and glumeruli. (PAS X400)
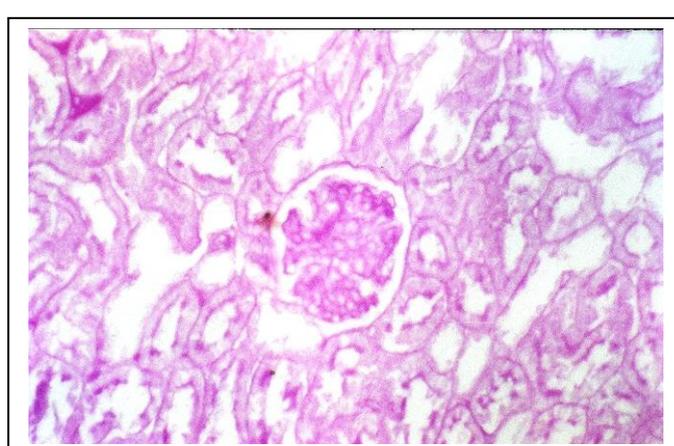

(Fig. 12) A photomicrograph of a kidney section treated with SIM showing more or less normal PAS reactivity in the renal tubules, and glumeruli. (PAS X400)
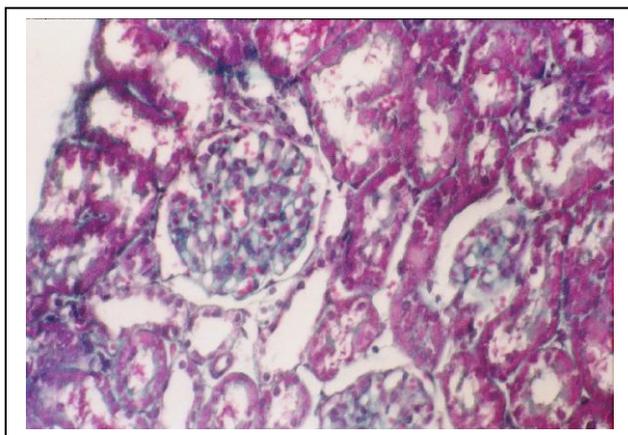
(Fig. 13) A photomicrograph of a kidney section of a control group showing normal distributed connective tissue in the renal tissue.   (Masson`s tricrome X400)
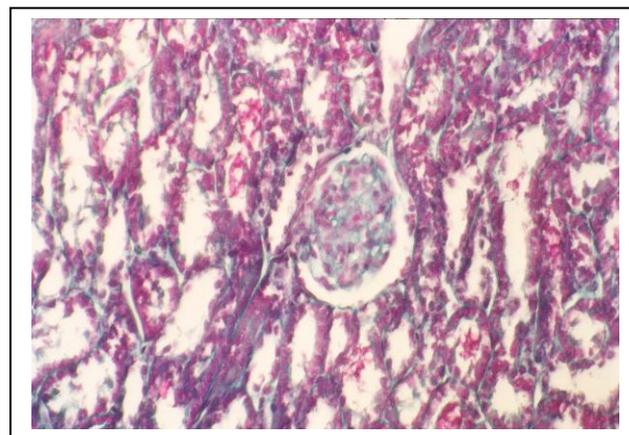
(Fig. 14) A photomicrograph of a kidney section treated with ADR showing connective tissue distribution in the renal tissue.   (Masson`s tricrome X400)
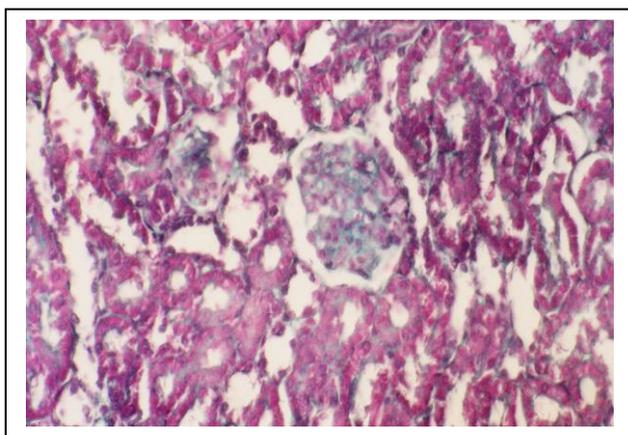
(Fig. 15) A photomicrograph of a kidney section treated with ADR and SIM showing more or less normal distributed connective tissue in the renal tissue.
(Masson's tricrome   X400)
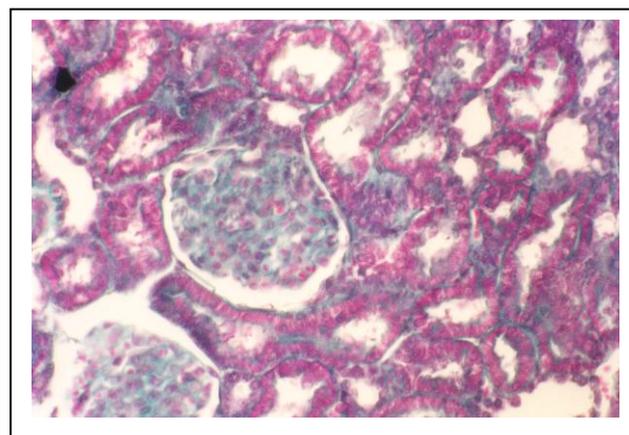
(Fig. 16) A photomicrograph of a kidney section treated with SIM showing more or less normal distributed connective tissue in the renal tissue. (Masson's tricrome   X400)

Discussion

Our results demonstrated that administration of simvastatin improved ADR-induced kidney dysfunction and organ damage as confirmed by microscopic examination and biochemical assays. Functional nephrotoxicity indices such as BUN, serum creatinine, were markedly elevated, whereas serum albumin was reduced in ADR treated group compared to control group. Several mechanisms seem to account for the effect of anthracycline, both in term of anticancer action and of cardiac and other organ toxicity (Quiles *et al*., 2002).

It is well known that oxidative stress and free radicals production are involved in ADR action, in relation to its anticancer and toxic effect. Thus, it has been reported that ADR leads to direct oxidative injury to DNA and generates lipid peroxidation (Mataix *et al*., 1997). Plasma oxidant/ antioxidant studies may reflect the

extracellular response to the external agents or to the tissue status regarding these oxidant and antioxidant systems. The biochemical and physiological changes in the renal tissues could affect extracellular components because of the close relationship between cellular environment and extracellular space. Fadillioglu and Erdogan (2003). have demonstrated that ADR elevated lipid peroxidation in plasma and myocardial tissue. Two different mechanisms of free radical formation by ADR have been described. The first implicates the formation of semiquinone free radical by the action of several NADPH-dependent reductases that produce one-electron reduction of the ADR to the corresponding ADR semiquinone. In the presence of oxygen, redox cycling of ADR-derived quinine-semiquinone yields superoxide radicals. In the second, ADR free radicals are produced by a non-enzymatic mechanism that involves reaction with iron. Iron-ADR complex can reduce oxygen to H_2O_2 and other active species (Nakano and Gemba, 1989; Singal *et al*., 2000; De Beer *et al*., 2001).

The findings of the present study showed that ADR administration increased MDA in rat kidney. The impaired renal function was accompanied by increasing MDA concentrations in kidney tissue. Our results are similar to those obtained by Deman *et al*. (2001) and Yagmurca *et al*. (2004). Where, they demonstrated that treatment with ADR significantly reduces antioxidant capacity in kidney, which accounts for increasing suitability to oxidative stress of the cellular structures.

One of the most important intracellular antioxidant systems is glutathione redox cycle. GSH is one of the essential compounds for maintaining cell integrity because of its reducing properties and participation in the cell metabolism. The increased concentration of GSH in the renal tissue supports the idea of ROS involvement in our experimental conditions. GSH synthesis has been shown to be induced in cells exposed to oxidative stress as an adaptive process (Salvemini *et al*., 1999). Reduced glutathione was reported to protect the cells from cytotoxic

damage induced by many compounds and it is generally known as a potent factor in the control of lipid peroxidation (Ketterer *et al*., 1983). The findings of the present study indicated that ADR administration decreased the glutathione content in rat kidney and increased lipid peroxidation , which are consistent with the previous report done by Babu *et al*. (1995), and Mansour *et al*. (1999), which explained that ADR-induced nephrotoxicity due to depletion of glutathione content in kidney tissues. It seems that lipid peroxidation is the main cause of ADR-induced nephrotoxicity and that ADR-induced lipid peroxidation is probably due to depletion of non-protein sulfhydral containing compounds.

The above observations correlated well with the renal histological finding which revealed extensive and marked tubular necrosis in the ADR-treated kidney. Similar changes were also reported by Wang *et al*. (2000) and Yagmurca *et al*. (2004). They have demonstrated structural changes in renal tissue of ADR-treated animals and the protective effect of various agents. Administration of Simvastatin improved kidney damage with especially a marked reduction in tubular changes induced by ADR.

In summary, the present results demonstrated that simvastatin has a protective effect on the kidney against the toxic effects of ADR both at the biochemical and histological level. Our findings support the idea suggested that the simultaneous use of simvastatin could be effective clinically by its antioxidant properties. However, further investigations should be done to elucidate the exact mechanism of protection and potential usefulness of simvastatin as a protective agent against drugs or xenobiotics toxicity in clinical trials.

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التأثير الوقائي لعقار السيمفستاتين ضد التأثير السام لعقار الأدرياميسين على الكلي للفئران ؛ دراسة كيميائيه حيوية و هستولوجيه

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استخدام عقار الأدرياميسين (مضـاد حيوي و مضـاد للأورام) يعتبر محدودا نظراً لتأثيره السام على عضلة القلب و الكلى. و يعتبر هذا التأثير السلبي نتيجـة لزيادة الشقاق الحرة و الاكسده الفوقية للدهون.

الغرض من البحث: دراسة التأثير الوقائي لعقار السيمفستاتين ضد التأثير السام لعقار الأدرياميسين على الكلي للفئران ؛باستخدام الوسائل الكيميائية الحيوية و الهستولوجيه.

وقد استخدم لهذا البحث ثمان و عشرون من ذكور الفئران السويسرية البيضاء السليمة حيث قسمت إلى أربع مجموعات؛ المجموعة الأولى ضابطة؛ و المجموعة الثانية تم حقنها بعقار السيمفستاتين؛ و المجموعة الثالثة تم حقنها بعقار الأدرياميسين؛ المجموعـة الرابعة تم حقنها بعقار السيمفستاتين و الأدرياميسين. و قد أخذت عينات من الدم لقياس نسبة اليوريـا والكريـاتينين و الـزلال و البـروتين الكلى. كمـا أخـذت عينـات مـن الكلـى لدراسـة التغيـرات الهسـتولوجية و الهسـتوكيميائية و كـذلك بعـض الإنزيمـات مثل الجلوتـاثيون و غيرها فى الدم . وقد لوحظ انخفاض نسبة الجلوتـاثيون و بعض الإنزيمـات الأخرى و زيادة الاكسده الفوقية للدهون في نسيج الكلى؛ كما لوحظ تشوهات و ضمور في النسيج الكلوي و خاصـةً الأنابيب الكلويـة نتيجـة لتأثير الأدرياميسين. و بعد الحقن بعقار السيمفستاتين مـع الأدرياميسين لوحظ تحسن ملحوظ في أنزيمـات و تركيب نسيج الكلى. هذه الدراسـة قد أثبتت التأثير الوقائي لعقار السيمفستاتي على النسيج الكلوي ضد التأثير الضار و السام عقار الأدرياميسين. و هذا التأثير الوقائي لعقار السيمفستاتي قد يرجع لتأثيره كمضاد لأكسدة على النسيج الكلوي.