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Research Article
Losartan and/or Naringenin Ameliorates Doxorubicin Induced Cardiac, Hepatic and Renal Toxicities in Rats

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Abstract

Background and Objective: Doxorubicin (DOX) is conventional chemotherapy for several types of malignancies although it induces considerable oxidative damage in different physiological systems. The present study aimed to examine whether the co-administration of naringenin (NG) and losartan (LOS) can produce additional protective effects against DOX-induced cardiac, hepatic and renal toxicities in rats. **Materials and Methods:** The LT and/or NG 2 week's pre-treated animals were challenged with intraperitoneal DOX (15 mg kg^{-1}). One week later, cardiac, hepatic and renal tissues along with serum were collected for biochemical and histopathological evaluations. **Results:** The altered histological architecture of the cardiac, hepatic and renal by DOX were restored by LT and NG treatments. Combining both compounds showed augmented protective effects. The LT and NG combination significantly ameliorated DOX associated elevation in serum inflammatory cytokines. In addition, DOX challenge induced cardiac, hepatic and renal toxicities along with disturbances in the biological oxidative defense capabilities manifested by elevated lipid peroxidation products, lowered glutathione levels and inhibited antioxidant enzymes activities. Co-administration of LT and NG markedly improved oxidative stress biomarkers and shifted the serum aminotransferases, creatinine, urea, lactate dehydrogenase and creatine kinases to their normal levels. **Conclusion:** Merging LT and NG therapies revealed significant advantages over single therapy with regards to their antioxidant and anti-inflammatory actions, which suggested a synergistic value of their combination.

Key words: Losartan, naringenin, doxorubicin, inflammatory, antioxidant, cytokines, enzymatic activities

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Anthracycline antibiotics are a group of chemotherapeutic agents, which are extensively used in management of different types of malignancies. Doxorubicin (DOX) is considered an important member of anthracycline antibiotics. In clinical practice, DOX exhibits acute and chronic physiological toxicities, which might limit its regular use. These adverse effects may include pericarditis, transient arrhythmias, hepatotoxicity and renal damage, along with progressive left ventricular dysfunction[1]. Several mechanisms of actions were investigated to explain pathways behind these effects. Oxidative damage induced by the excessive production of reactive oxygen species (ROS) and that consider one of the major contributing factors. It is noteworthy that oxidative stress may stimulate pro-inflammatory cytokines production via activation of cellular transcription factors including nuclear factor kappa B (NF-κB)[2,3].

The association of renin angiotensin system (RAS) in DOX mediated physiological toxicities was reported in several studies, particularly cardiotoxicity. The octapeptide angiotensin II (Ang II) is a major pathophysiological factor in DOX-induced cardiomyopathy as well as several other cardiovascular diseases such as; hypertensive and ischemic heart diseases[4]. Furthermore, Ang II was reported to trigger the production of inflammatory biomarkers. Therefore, medications that are known to inhibit RAS cascades were evaluated to ameliorate DOX-induced toxicities. Losartan is an Ang II receptor blocker that was proven to have alleviative effects against oxidative damage induced by DOX[5]. Moreover, LT was found to enhance the therapeutic effectiveness of DOX in other studies[6].

A fast growing number of *in vitro* and *in vivo* studies reported the effectiveness of medicinal plants isolated compounds, particularly flavonoid molecules, in the treatment of metabolic diseases and in ameliorating the cytotoxic effects of chemotherapy. Naringenin is a well-documented member of flavonoids isolated from citrus fruits[7]. Studies proved that NG has a considerable antioxidant and anti-inflammatory properties making it a potential therapeutic natural compound[8]. Notably, NG was found to prevent DOX-induced renal and gonadal toxicities via regulation of inflammatory and oxidative pathways[9,10]. The DOX associated cardiotoxic effects were also attenuated by NG in several studies[5,11]. In addition, NG markedly improved the anti-tumor efficiency of DOX in Zhang *et al.*[12].

There is an accumulative interest towards the combination of therapies or poly-therapy in several studies. In this context, the parallel administration of numerous compounds to cure one or more health disorders in explored. For instance, DOX-induced cardiotoxicity was found to be ameliorated by quercetin and LT combination or p-coumaric acid and NG co-therapy in previous studies[5,13]. Hence, this experiment was designed to explore the impact of combining LT and NG therapies on the oxidative and inflammatory disturbances and toxicities in rodents challenged with DOX.

MATERIALS AND METHODS

Present experimental study was carried out in Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh, in between August, 2018-February, 2019. Experimental protocol of present study was followed with few modifications of an earlier similar study[5].

Animals: Approximately 240-300 g body weights of male albino Wistar rats were attained from Pharmacy College Animal Care Center at King Saud University. All received animals were acclimatized for 10 days prior to start the experiments. The rats were sustained in standard conditions such as; 22±1°C temperature, 50-55% humidity and equal 12 h day/night cycles. All the experimental protocol such as; euthanasia procedure, blood sampling and final sacrifice were followed by National Institute of Health guide care policy (NIH, 1996) and this animal study was approved (EAEA-256-2018) by the Ethical Committee of Pharmacy College, Animal Care Center, King Saud University.

Chemicals and kits: Losartan was obtained from Canada (Toronto Research Chemicals Inc, Toronto). Naringenin obtained from ALDRICH Chemicals, UK. Doxorubicin received from EBEWE Pharma, Austria. The diagnostic kits of CK-MB, LDH, ALT, AST, creatinine and urea were purchased from Human Diagnostics (Wiesbaden, Germany). Pro-inflammatory cytokines such as; TNF-α, IL-1β and IL-6 and oxidative enzymes including SOD, CAT, GPx and GST kits of ELISA system for rats were bought from USA (R and D Systems Minneapolis, MN). The TBARS and GSH ELISA kits were obtained from Cayman Chemicals (Ann Arbor, MI, USA).

Study design: After the acclimatization week rats were randomly separated into 5 groups as follows: (1) Control group (vehicle), (2) DOX group (vehicle), (3) DOX+LT group (LT, 7 mg kg^{-1}/day), (4) DOX+NG group (NG, 100 mg kg^{-1}/day) and (5) DOX + LT + NG group (LT 7 mg kg^{-1}/day and NG 100 mg kg^{-1}/day). After two weeks of LT and NG treatments, DOX single dose (15 mg kg^{-1}) was intraperitoneally injected to 2, 3, 4 and 5 groups of rats

and the treatment continued for one extra week. Rats were weighed then anesthetized with ketamine/xylazine (94/14 mg kg^{-1}) ratio. Blood was collected from heart puncture in a plain tube, which centrifuged at 4,000 rpm for 10 min to get the serum. Organs including liver, kidneys and heart were dissected and stored as follows; a part was preserved in aluminum foil and then kept at deep freezer (-80°C) for the biochemical analysis and the another part stored in 10% formalin for histopathological studies.

Biochemical analysis: In serum, CK-MB, LDH, ALT, AST, creatinine and urea levels were estimated by using diagnostic kits. Inflammatory biomarkers in serum such as; TNF-α, IL-1β and IL-6 levels were estimated by using ELISA kits for rats. In heart, liver and kidney homogenates, TBARS and GSH levels were estimated by using ELISA kits. In post-mitochondrial supernatant (PMS) of cardiac, hepatic and renal cells, SOD, CAT, GPx and GST activities were measured by using ELISA kits for rats. The analytical procedures of all parameters are completed by following the manufacturer's instructions.

Histological study: Cross sections of the heart, livers and kidneys from control and each treatment group were preserved in 10% buffered formalin and fixed in paraffin blocks. Sections 5 μm were sliced with an American Optical rotary microtome (Leica Camera AG, Wetzlar, Germany). Hematoxylin and eosin mixture was used for staining the sections and screened the histological changes under a microscope.

Statistical analysis: Data was analyzed by using One-way ANOVA, significance levels were measured by using Student-Newman-Keuls multiple comparisons test (n = 6). Differences between groups was considered statistically significant when $p \leq 0.05$, *p<0.05, **p<0.01 and ***p<0.001. All statistics tests was conducted by using Graph Pad Prism (v. 5) software.

RESULTS

Effect on body and organ weights: In DOX group of rats, body weights were markedly (p<0.001) decreased in comparison to normal rats. The co-administration of NG and LT significantly enhanced the body weight levels compared to DOX group. Liver weights showed increased (p<0.05) in DOX treated rats and this weights found decreased in DOX+NG+LT group when compared to DOX group. Mean kidney weights are remained same in all experimental groups when compared to each other. However, heart weight ratio found increase in DOX challenged group when compared to control animals, but these values are statistically insignificant (Fig. 1).

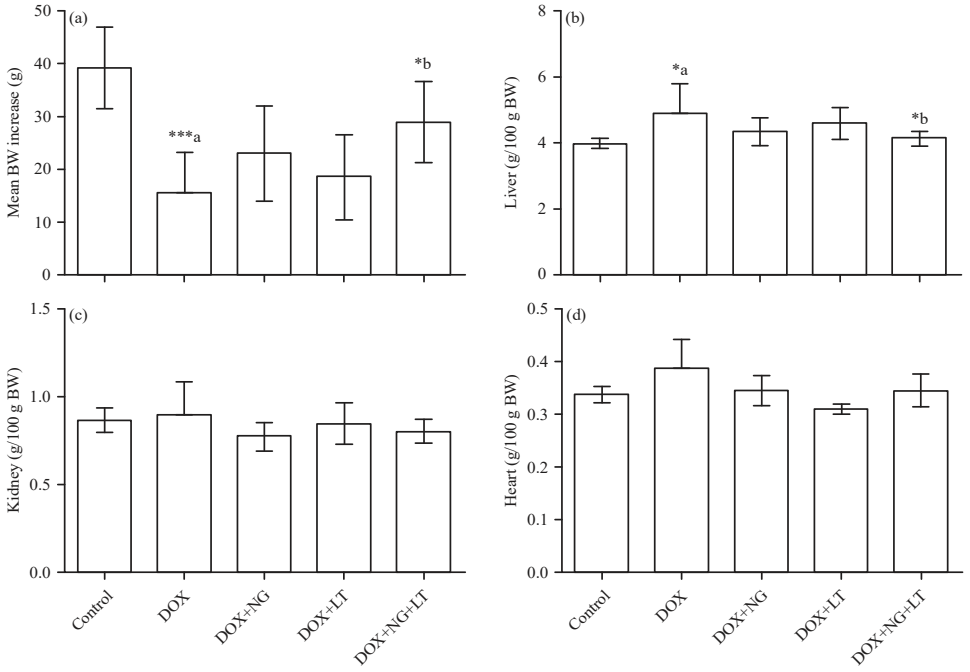

Fig. 1(a-d): Effect of naringenin (NG) or/and losartan (LT) on doxorubicin (DOX)-induced changes in (a) Body, (b) Liver, (c) Kidney and (d) Heart weights of rats

Data were expressed as Mean±SD (n = 8) and analyzed by using one-way ANOVA followed by Student Newman-Keuls as *post hoc* test. [a]Control vs. DOX group, [b]DOX vs. NG or/and LT treated groups. p-values consider significant when *p<0.05 and ***p<0.001

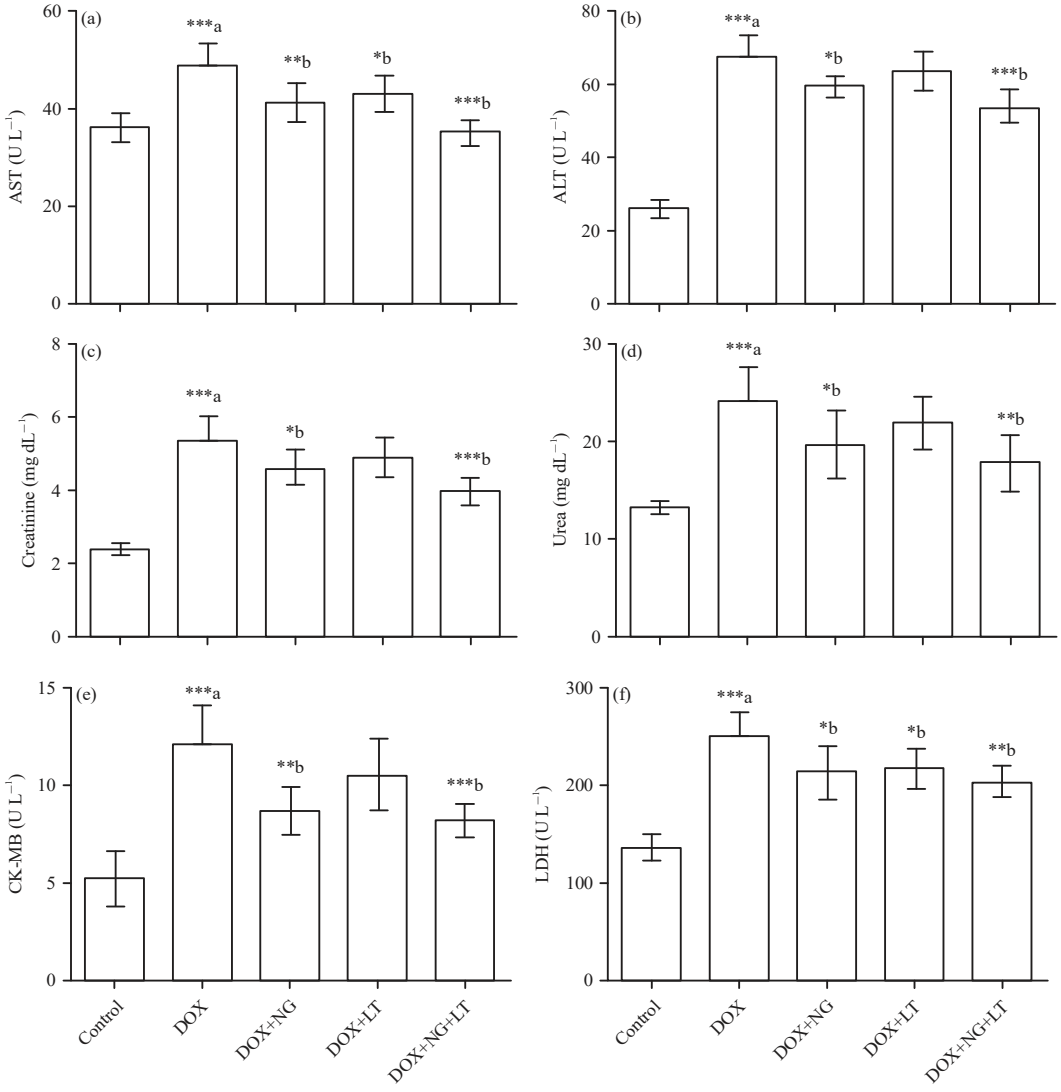

Fig. 2(a-f): Effect of naringenin (NG) or/and losartan (LT) on doxorubicin (DOX)-induced changes in serum levels of (a) Aspartate aminotransferase (AST), (b) Alanine aminotransferase (ALT), (c) Creatinine, (d) Urea, (E) Creatinine kinase-MB (CK-MB) and (f) Lactate dehydrogenase (LDH)

Data were expressed as Mean±SD (n = 8) and analyzed by using one-way ANOVA followed by Student Newman-Keuls as *post hoc* test. [a]Control vs. DOX group, [b]DOX vs. NG or/and LT treated groups. p-values consider significant when *p<0.05, **p<0.01 and ***p<0.001

Biomarkers of liver, kidney and heart assessments: Liver enzymes (AST and ALT) levels were markedly (p<0.001) enhanced by the single DOX injection in serum. These levels were found significantly inhibited in NG treated group and seen more such effect in NG and LT combined group as compared to DOX group. Renal markers (creatinine and urea) in serum were also significantly elevated in DOX challenged group compared to normal rats. The NG treatment markedly (p<0.05) inhibited these markers, which were observed highest effect by the combined NG and LT treatment. Cardiac markers like LDH and CK-MB were markedly (p<0.001) increased in serum of DOX injected rats. The combined treatment of NG and LT revealed better protective effect against DOX-induced cardiac toxicity by reducing CK-MB and LDH levels (Fig. 2).

Cytokines assessments: Serum pro-inflammatory cytokines including TNFα, IL-1β and IL-6 levels were significantly (p<0.001) increased in DOX challenged group compared to controls. These markers were found markedly inhibited in NG and LT treated groups, while the combined treatment of NG and LT produced greater inhibition as compared to DOX group (Fig. 3).

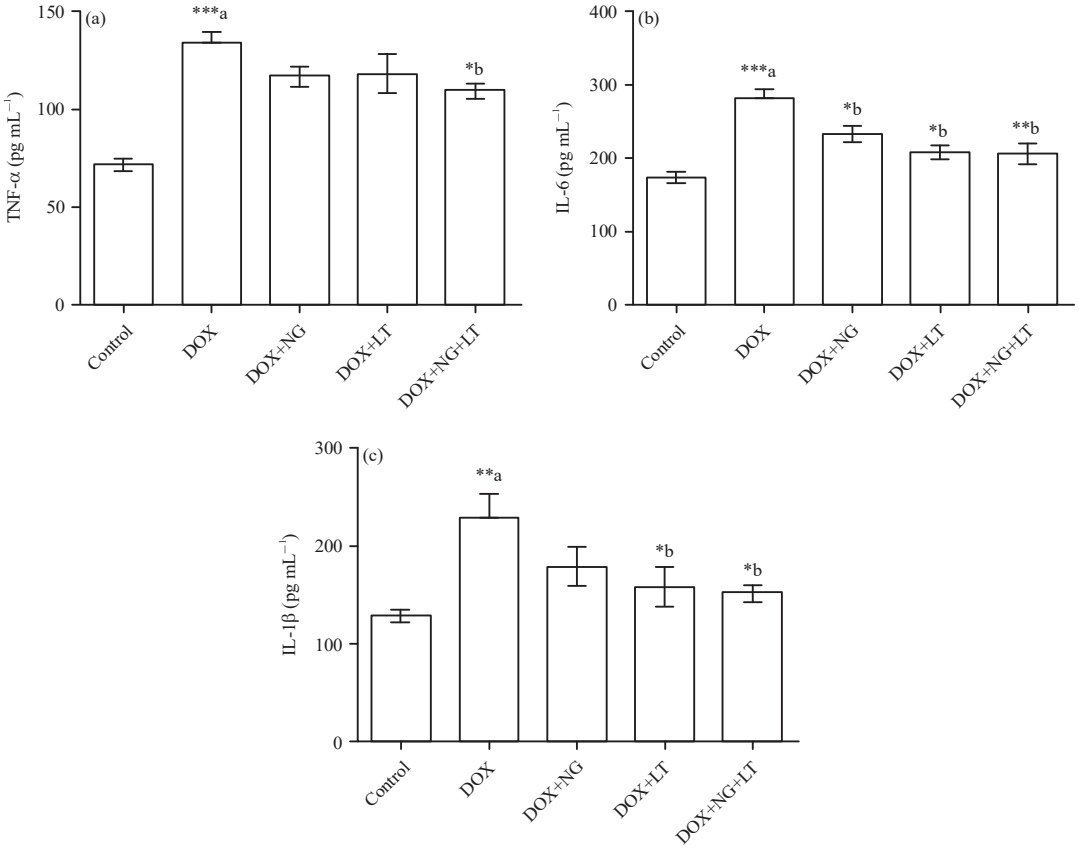

Fig. 3(a-c): Effect of naringenin (NG) or/and losartan (LT) on doxorubicin (DOX)-induced changes in serum levels of pro-inflammatory cytokines including, (a) Tumor necrosis factor-α (TNF-α), (b) Interleukin-6 (IL-6) and (c) Interleukin-1β (IL-1β)

Data were expressed as Mean±SD (n = 8) and analyzed by using one-way ANOVA followed by Student Newman-Keuls as *post hoc* test. [a]Control vs. DOX group, [b]DOX vs. NG or/and LT treated groups. p-values consider significant when *p<0.05, **p<0.01 and ***p<0.001

Oxidative stress biomarkers in hepatic, renal and cardiac cells: Single DOX injection induced oxidative stress in cardiac, hepatic and renal tissues by significantly ($p<0.001$) increasing TBARS and reducing the GSH levels. The DOX also significantly ($p<0.001$) inhibited the enzymatic activities of SOD, CAT, GPx and GST. Individual treatment of NG markedly produced corrections of DOX-induced alteration in cardiac, hepatic and renal TBARS and GSH and significantly restored the enzymatic activities in cardiac, hepatic and renal cells. The LT also markedly restored the levels and activities of TBARS ($p<0.05$) in renal tissues, GSH ($p<0.05$) in cardiac and renal tissues, SOD ($p<0.05$) in hepatic and renal tissues, GPx ($p<0.05$) in hepatic tissues and GST ($p<0.05$) in renal tissues as compared with DOX group. In combined NG and LT treatment group, found more significantly corrections against the levels and activities of TBARS, GSH, SOD, CAT, GPx and GST as compared with DOX group (Fig. 4). Finally it concluded that, the combined therapy is more beneficial than the individual use.

Histological observations in liver, kidney and heart: Histopathological photographs of heart tissues which stained by H and E are presented in Fig. 5. The control group exhibited normal appearance of myocardial cells with oval elongated nuclei, homogenous cytoplasm and normal myocardial myofibril. In the DOX group, destructed myocardial myofibril, congestion of blood capillaries and infiltration of mononuclear cells were observed. Samples of NG or LT treated groups showed mild disrupted and destructed myocardial myofibril with congested blood vessels. The LT and NG co-treated group showed less injury and normal myocardial cell. Figure 6 represented the histopathological photographs of liver samples. Control animals demonstrated normal histological architecture and regular hepatic cords. In the DOX group, fatty degeneration, vacuoles and necrotic cells were noticed. Liver samples of NG+DOX and LT+DOX groups showed partially and not complete regenerating hepatocytes with dilatation in

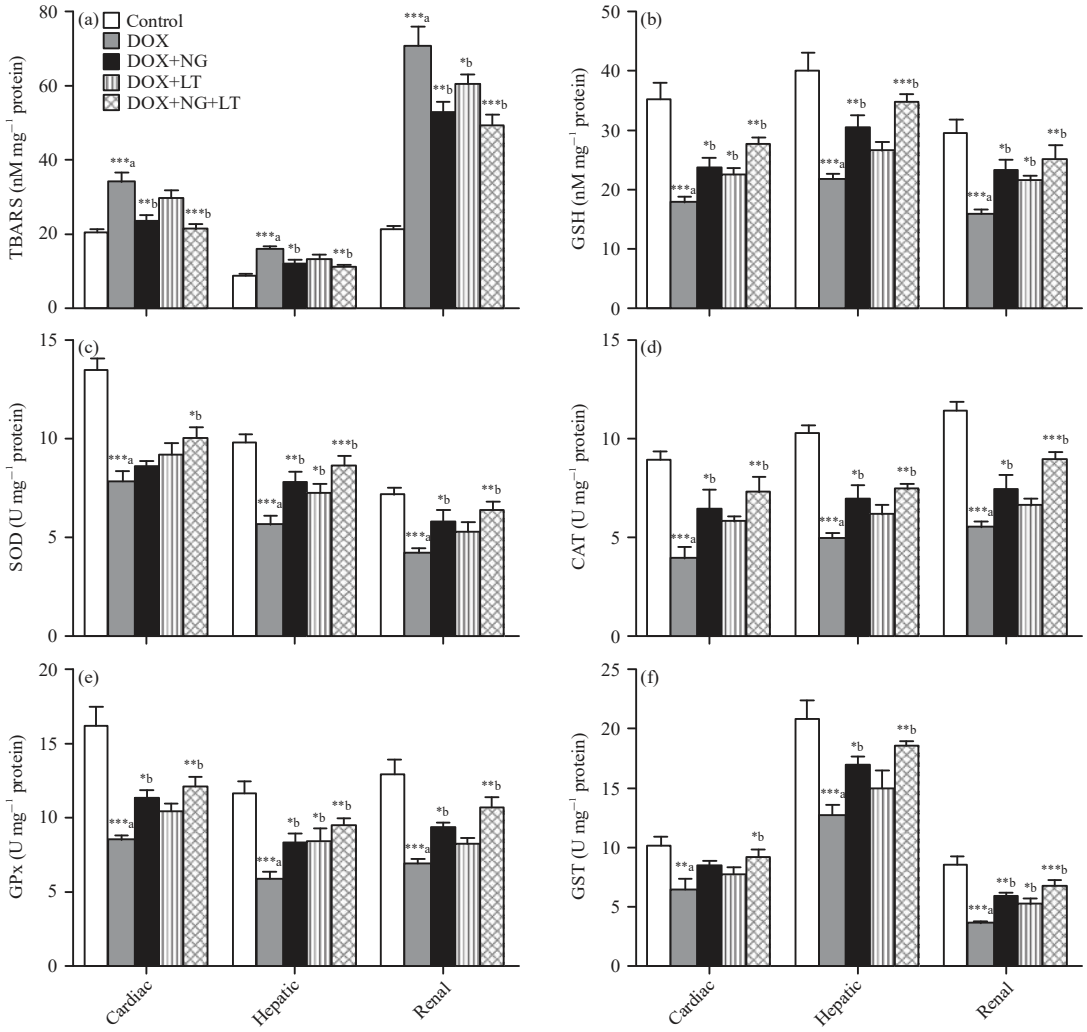

Fig. 4(a-f): Effect of NG or/and LT on DOX-induced changes in oxidative stress biomarkers including, (a) Thiobarbituric acid reaction substance (TBARS), (b) Glutathione (GSH) levels and enzymatic activities of (c) Superoxide dismutase (SOD), (d) Catalase (CAT), (e) Glutathione peroxidase (GPx) and (f) Glutathione-S-transferase (GST) in cardiac, hepatic and renal tissues

Data were expressed as Mean±SD (n = 8) and analyzed by using one-way ANOVA followed by Student Newman-Keuls as *post hoc* test. [a]Control vs. DOX group, [b]DOX vs. NG or/and LT treated groups. p-values consider significant when *p<0.05, **p<0.01 and ***p<0.001

sinusoidal spaces. The liver of LT+NG+DOX treated group showed complete regenerating hepatocytes with binuclear cells. Renal cortex samples are presented in Fig. 7. In control group of animals, histopathological changes showed quite normal proximal convoluted tubules, distal convoluted tubules, Bowman's capsule and glomerulus. In DOX group, renal damage with atrophy of some glomeruli, tubular dilatation and glomerular sclerosis were demonstrated. In NG+DOX and LT+DOX groups, mild improvements in the glomerulus and tubule interstitial lesions were noticed. Renal samples of LT+NG+DOX treated group showed distinct improvement in the architecture of glomeruli and renal tubules.

DISCUSSION

In present study, NG and LT markedly restored the cardiac, hepatic and renal antioxidant capacity in DOX treated rodents. Moreover, NG showed marked potentiation of LT anti-inflammatory properties. Evaluation of the histological architecture of cardiac, hepatic and renal tissues confirmed this co-protective value.

The DOX is a common member of anthracycline cytotoxic medications. It has multiple clinical uses as a pillar in treatment of several types of tumors. In clinical practice, DOX use showed different types of adverse effects mainly cardiovascular. Mechanisms behind these reported side

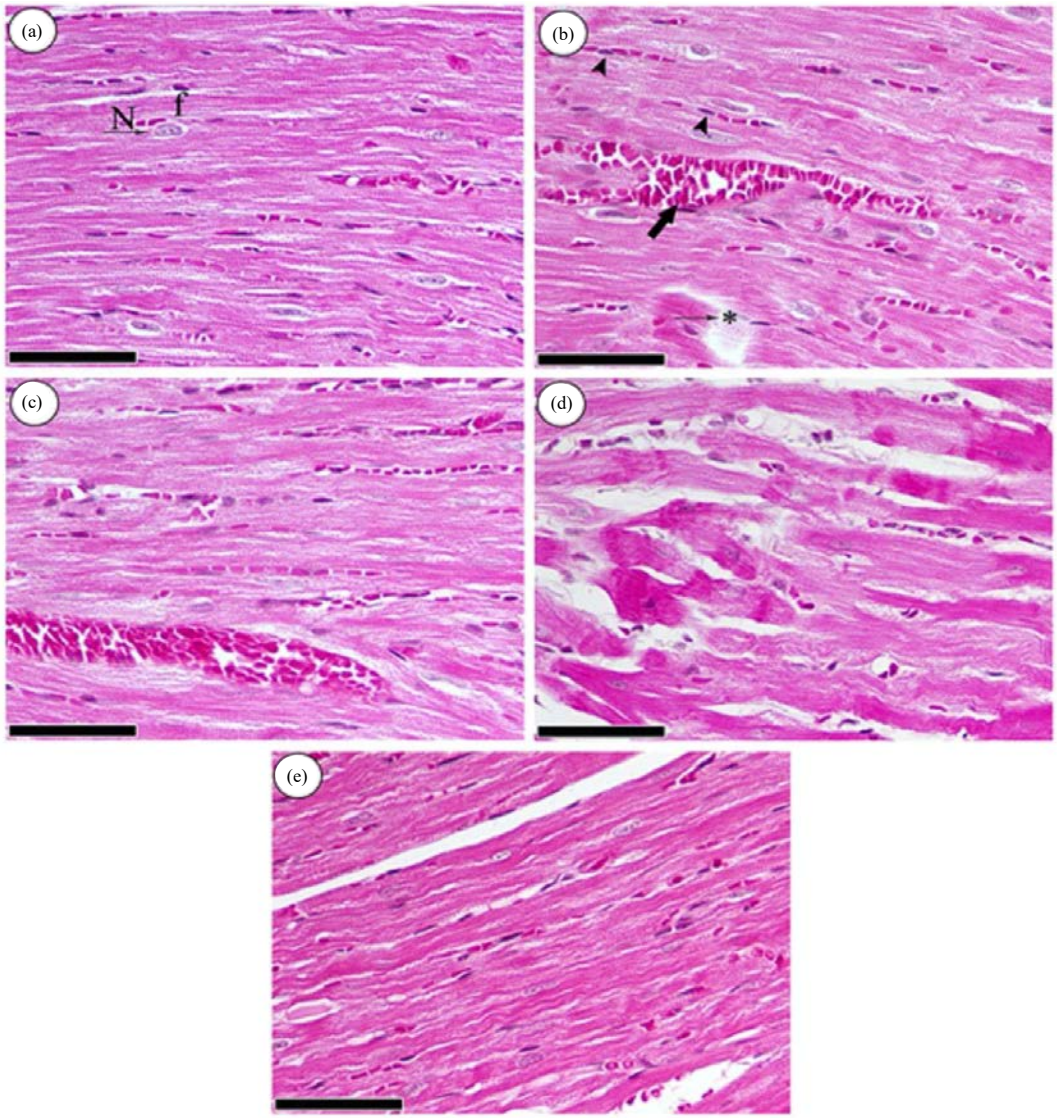

Fig. 5(a-e): Effect of naringenin (NG) or/and losartan (LT) on doxorubicin (DOX)-induced histopathological changes in cardiac tissue stained by H and E, (a) Section from control, (b) Section from DOX showing (*) destructed myocardial myofibril, (arrow) congestion of blood capillaries and (head arrows) infiltration of mononuclear cells, (c) Section from LT showing mild destructed myocardial cells, (d) Section from NG showing mild degenerative myocardial cells and (e) Section from LT and NG minor injury with usual cardiac morphology. (N) Oval elongated nuclei, (f) Homogenous cytoplasm and normal myocardial myofibril
Scale bar = 50 μm

effects were investigated in several studies. Extensive accumulation of ROS following DOX therapy was reported in different studies[14]. In addition, DOX was found to inhibit the endogenous antioxidant capability through depleting the antioxidant molecules and suppression of antioxidant enzymes[15]. In agreement with previous studies, DOX significantly reduced the activities of SOD, CAT, GPx and GST enzymes, which are known mitochondrial enzymes involved in the detoxification process of free radicals[16]. Furthermore, the architectures of cardiac, hepatic and renal tissues were considerably altered by DOX. This was combined with disturbances in serum levels of AST, ALT, creatinine, urea, CK-MB and LDH, which also indicated the cardiac, hepatic and renal damage[17]. These toxicities are explained by oxidative damage abilities of the accumulated ROS. Free radicals such as; hydroxyl radical, superoxide anion radical and hydrogen

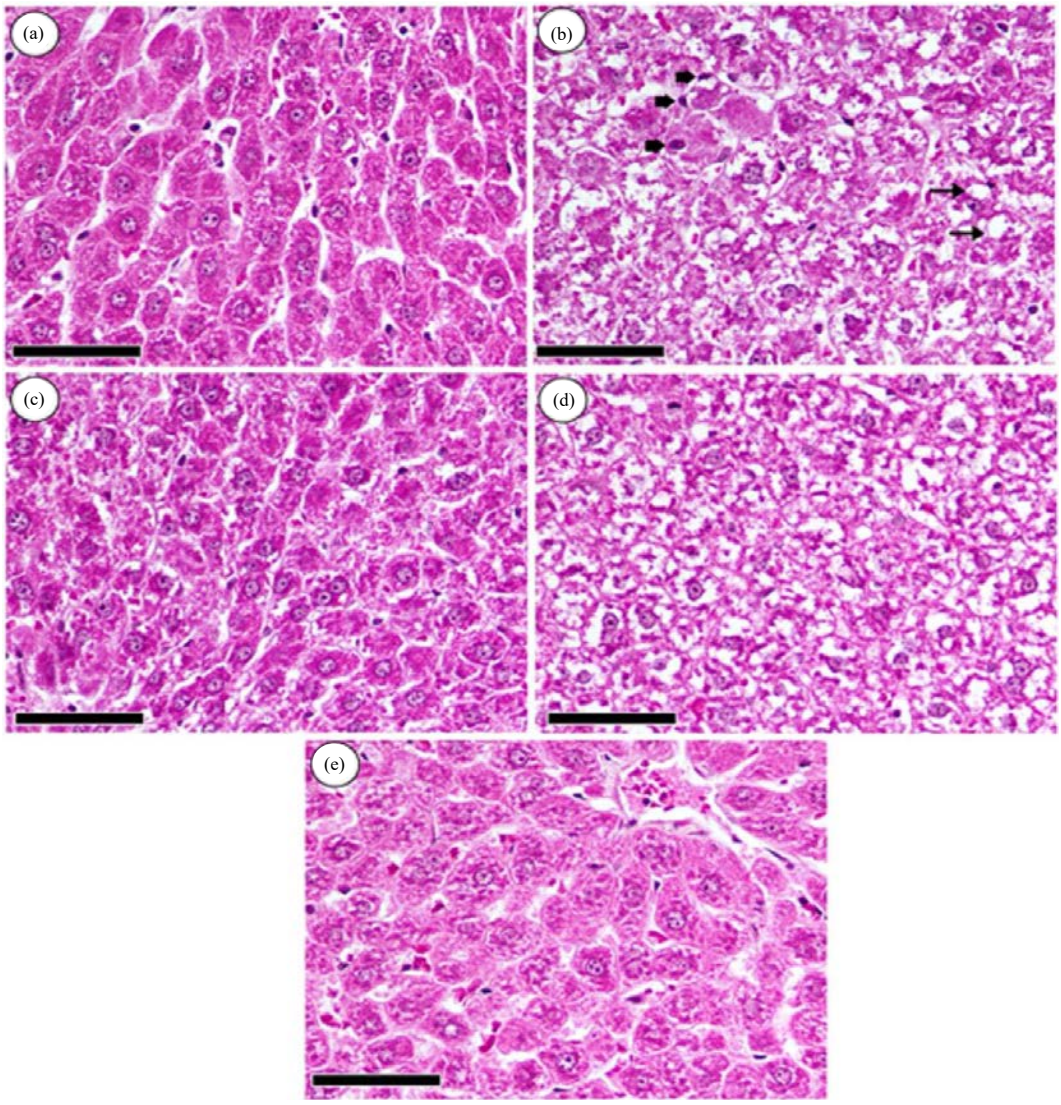

Fig. 6(a-e): Effect of naringenin (NG) or/and losartan (LT) on doxorubicin (DOX)-induced histopathological changes in hepatic tissue, (a) Section from control, (b) Section from DOX showing fatty degeneration and vacuoles (arrows) and necrotic cells (head arrows), (c) Section from LT showing partial regenerating hepatocytes, (d) Section from NG showing mild degenerative hepatic cells and (e) Section from LT and NG showing complete regenerating hepatocytes
Scale bar = 50 μm

peroxide may induce peroxidation of the lipids in the cellular membranes leading to augmented production of TBARS, which was reported in DOX treated rats[18]. Inflammation and oxidative stress are correlated. Oxidative stress was found to provoke the expression of multiple transcription factors including NF-κB resulting production of inflammatory cytokines[19]. This inflammatory response was also reported in the present study, where DOX groups exhibited higher values of TNF-α, IL-6 and IL-1β compared to LT and/or NG treated groups.

In the present study, LT therapy depressed the signs of oxidative damage and lipid peroxidation as well as preserved the cardiac, hepatic and renal functions and histological structures in DOX-treated animals. The LT also lowered the serum inflammatory cytokines after their induction by DOX. Numerous other experimental studies have reported comparable protective efficacies for AT_1 receptor antagonism against cellular oxidative and inflammatory damage induced by cytotoxic medications. In an earlier study found that AT_1 receptor blocking by irbesartan may attenuate DOX-induced

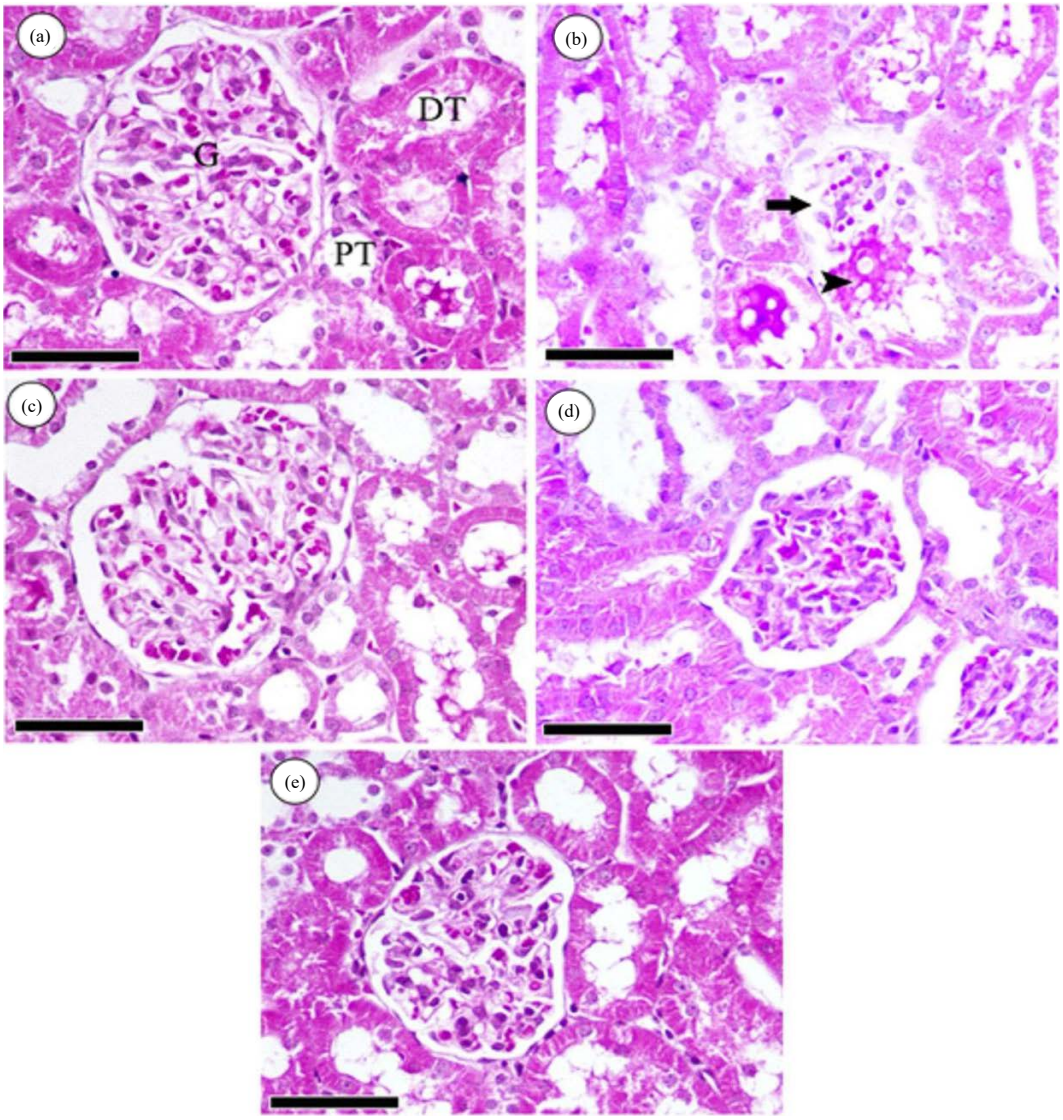

Fig. 7(a-e): Effect of naringenin (NG) or/and losartan (LT) on doxorubicin (DOX)-induced histopathological changes in renal tissue, (a) Section from control, (b) Section from DOX showing atrophy of some glomeruli (arrow), tubular dilatation and glomerulosclerosis (head arrow), (c) Section from LT showing mild improvement in the glomerulus and tubulointerstitial lesions, (d) Section from NG showing mild degenerative changes with tubular dilation and atrophy in the glomerulus and (e) Section from LT and NG showing distinct improvement in the architecture of glomeruli and renal tubules. (PT) Proximal convoluted tubules, (DT) Distal convoluted tubules, (G) Bowman's capsule and glomerulus
Scale bar = 50 μm

hepatotoxicity dose-dependently via inhibition of oxidative stress, apoptosis and inflammation in BALB/c mice[20]. Another AT_1 receptor blocker, fimasartan was reported to prevent DOX-associated cardiotoxicity in Rats[21]. In addition, olmesartan ameliorated the nephrotoxicity signs in rats challenged with DOX[22]. Studies have suggested that Ang II may provoke cellular oxidative stress through stimulation of its AT_1 receptor[23]. Therefore, blocking of this receptor by LT is associated with enhance the endogenous oxidative defensive cascades and hence, biological protective effects.

In the present study, NG markedly corrected DOX-induced cardiac, hepatic and renal oxidative damage as displayed by the histological results as well as lower serum aminotransferase, kinases, urea and creatinine levels. These protective effects may consider by the oxidative stress depressor effects of NG. In this context, NG restored the

activities of antioxidant enzymes and GSH and lowered the levels of lipid peroxidation products. Furthermore, NG reduced the systemic expression of inflammatory cytokines in DOX-treated animals. The NG showed similar protective effects against DOX in earlier studies[13,14,24]. Most of these studies suggested that the preventative role of NG against DOX-toxicities in mediated through its antioxidant and inflammatory properties. Treatment of the animals with both LT and NG showed significant preventive effects against oxidative stress, inflammation and histological impairments induced in DOX-challenged rodents.

Earlier studies have demonstrated the benefits that may arouse by combining the anti-RAS medications and natural compounds. For instance, co-administration of the well-known antioxidant, vitamin E, with telmisartan therapy would ameliorate the provoked cardiac inflammatory and oxidative response in DOX treated rats[25]. Furthermore, quercetin, which is another natural existing flavone, augmented the preventative ability of LT in DOX treated rodents through restoration of TNF-α, CK-MB, LDH, MDA and nitric oxide levels along with re-activating SOD and CAT[5]. In addition, NG and p-coumaric acid combined treatment were found to alleviate DOX-induced cardiotoxicity in rats, which highlighted the therapeutic importance of combined treatments[13]. In this study, two experimental limitations reported. First, the experiment was carried out on male rats only, which might interfere with assumption that gender difference and sex hormones may influence the metabolic and physiological response to toxicities. Secondly, the expressions of inflammatory cytokines were not evaluated in cardiac, hepatic and renal tissues. However, the systemic levels of TNF-α, IL-6 and IL-1β were determined. Furthermore, the histological assessment described the distribution of inflammatory infiltrates as an indication for tissue inflammation. These findings of the current study confirmed the remarkable therapeutic value of NG and LT particularly in their combination finally which recommended to use during chemotherapy.

CONCLUSION

Present findings revealed that NG can not only improve the cardiac, hepatic and renal toxicities of doxorubicin, but also its harmonious administration with LT provided additional benefit to the heart's, liver's and kidney's protections of the latter. The anti-inflammatory effect of NG along with its antioxidant properties may have a major role in the remarkable synergistic effect of LT.

SIGNIFICANCE STATEMENT

The current study found beneficial effects of NG and/or LT against heart, hepatic and renal toxicity caused by DOX. These effects, which are taken into account through the anti-inflammatory effect of NG with its antioxidant properties, may play a major role in the synergistic effect of LT. Thus, NG and LT-associated doxorubicin showed very promising

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