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Research Article
Potential Protective Role of Rutin and Alpha-lipoic Acid Against Cisplatin-induced Nephrotoxicity in Rats

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Abstract

Background and Objective: Cisplatin-induced nephrotoxicity is a serious complication that restricts its utilization in cancer treatment. Rutin and alpha-lipoic acid have antioxidant effectiveness, anti-inflammatory efficacy and prevent oxidative stress. Therefore, the current study planned to investigate the potential defensive impacts of rutin and alpha-lipoic acid on cisplatin-induced renal damage in rats. **Materials and Methods:** Fifty-six adult male Wistar albino rats were randomly divided into seven groups. Rats of group 1: Treated with saline as the control. Group 2: Orally received rutin daily for 2 weeks. Group 3: Rats were orally administered with alpha-lipoic acid (ALA) daily for 2 weeks. Group 4: Rats were intraperitoneal (i.p.) injected with cisplatin to develop the acute renal injury. Group 5: Rats injected with cisplatin then treated orally with RT. Group 6: Rats were injected i.p., with cisplatin then treated orally with ALA. Group 7: Rats injected with cisplatin then treated orally with RT and ALA daily for 2 weeks. **Results:** The cisplatin administration to rats induced nephrotoxicity associated with a significant increase in serum urea, creatinine, albumin and significantly reduce haemoglobin and red blood cells count. The animal treated with cisplatin showed a significant increase in the level of renal malondialdehyde associated with reduction in the levels of glutathione-s-transferase, glutathione reductase and catalase compared to control group. Moreover, cisplatin treated group recorded significant increase in nuclear factor kappa B, IL-6 and p53 levels compared to control group. Additionally, histopathological examination showed that cisplatin-induced interstitial congestion, focal mononuclear cell inflammatory, cell infiltrate and acute tubular injury. In correlation with the cisplatin group, Rutin and alpha-lipoic acid ameliorated cisplatin-induction increase in serum urea, creatinine, albumin, oxidative stress and inflammation were observed. Moreover, rutin and alpha-lipoic acid showed an enhancement in haematological and histopathological structures. **Conclusion:** These results indicated that rutin and alpha-lipoic acid showed a protective effect against cisplatin-induced nephrotoxicity in rats.

Key words: Cisplatin, nephrotoxicity, rutin, alpha-lipoic acid, renal damage, cancer treatment

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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

Functional impairment and toxic injuries of kidney have been recorded to produced by diversified therapeutic agents used in clinical pursuit and thus have greatly contributed to hospital acquired acute renal failure (ARF). The mortality rate of patients with ARF has remained in the range of about 20% despite the patients are under medical care[1]. The reason for this is that the kidney is the major organ of excretion and is exposed to large amounts of parent and active metabolites of drugs[2,3].

Cisplatin (cis-dichlorodiammineplatinum II) is an anti-tumor drug broadly used in the treatment of numerous human cancers such as malignancy of testis, head, ovarian, neck and colon[4]. Unfortunately, the clinical interest of cisplatin is usually inadequate due to the development of undesirable side effects such as nephrotoxicity, ototoxicity and cardiomyopathy, kidney, ear, gastrointestinal tract and nervous system[5] and spermiotoxicity[6].

Most anti-cancer agents cause toxicity in different organs by distributing the oxidant/antioxidant balance[6]. Cisplatin has several intracellular influences, causing direct cytotoxicity with reactive oxygen species, activating mitogen-activated protein kinases, inducing apoptosis[7] and motivating inflammation but its major dose-limiting side effect is nephrotoxicity. Various studies have revealed that the increased tissue content of inflammatory mediators together with inflammatory cell infiltration, signifying that inflammation has a significant role in cisplatin-induced renal injury[8,9]. Additionally, cisplatin-induced nephrotoxicity is closely associated with an increase in lipid peroxidation[10]. Several studies suggested that the renal injury following cisplatin treatment is associated with oxidative damage[11]. The oxidative stress mainly results from the formation of cisplatin-GSH conjugation[12]. All these mechanisms greatly encourage the using of free radical scavengers and antioxidants to counteract cisplatin-induced toxicities[13].

Rutin (RT) (3, 30, 40, 5, 7-pentahydroxyflavone-3-rhamnoglucoside) is a flavonoid constituent, which abundantly found in plant-based beverages such as buckwheat, apples, onions, tea and berries[14]. It has several pharmacological effects including anticarcinogenic, cytoprotective, hepatoprotective, cardioprotective and anticonvulsant activities[15,16]. Experimental studies have detected some renoprotective influences of rutin. Korkmaz and Kolankaya[17] demonstrated that rutin diminishes renal injury following ischemia/reperfusion (I/R) condition probably by inhibiting inducible nitric oxide synthase (iNOS)[17]. Arjumand *et al.*[18] found that pretreatment with rutin inhibits cisplatin-induced renal toxicity in rats. Furthermore, the beneficial effects of rutin on streptozotocin (STZ)-induced nephrotoxicity have been mediated by reducing reactive oxygen species (ROS) and lipid peroxidation levels[19].

Over and above, alpha-Lipoic acid (ALA), a dithiol compound is found naturally in the mitochondria and acts as an essential cofactor for mitochondrial respiratory enzymes α-ketoglutarate dehydrogenase and pyruvate dehydrogenase. It exhibits antioxidant possessions by scavenging reactive oxygen species and stimulating the synthesis of other antioxidants, such as glutathione[20]. Numerous studies have shown that ALA exerts multiple pharmacological actions in different models of diseases characterized by an increase in oxidative stress markers[21]. Moreover, ALA exerts anti-inflammatory actions by inhibiting nuclear factor-κB (NF-κB) activation and by decreasing adhesion molecule expression in endothelial cells[22].

From the above-mentioned factors, it is clear that cisplatin could be used in cancer treatment. Hence finding some natural treatments to reduce the side effect of the drug cisplatin and working to increase the efficiency of this drug in the elimination of cancer cells without affecting the healthy cells. Therefore, the current study sought to investigate the intimate mechanisms behind the efficacy of rutin and alpha-Lipoic acid in repressing cisplatin-induced nephrotoxicity in rats.

MATERIALS AND METHODS

Duration and year of study: This study was carried out from the month of January-June, 2016 at Biology Laboratory of Zoology Department-Faculty of Women for Arts, Science and Education, Ain Shams University, Egypt.

Chemicals and drugs: Cisplatin, Rutin and alpha-Lipoic acid were obtained from Sigma Chemical Co., USA. All other reagents, solvents and chemicals used for analysis met the quality criteria in accordance with international standards.

Biological assay

Animals and treatment: All the experiment involving animals and tissue samples were conducted in accordance with the principles and guidelines for the care and use of laboratory animals of the National Institute of Health (NIH) (USA). This study was approved by the Ethical Committee for animal experimentation, National Research Centre, Egypt.

Fifty-six adult male albino rats of Wistar strain weighing 120±10 g at 90 days of age were enrolled in the present

study. The animals were obtained from the Animal House Colony of the National Research Centre, Cairo, Egypt. The animals were housed throughout the experiment (8 rats/cage) in polypropylene cages under specific pathogen free (SPF) conditions with controlled illumination (12 h light/12 h dark cycle), a relative humidity (30-50%) and temperature (18-22°C). Animals were fed with standard laboratory rat diet and water provided *ad libitum*. Animals were allowed to adapt to their environment for 2 weeks before the commencement of the experiment.

After the adaptation period, the animals were divided into seven groups (n = 8): Group 1 (Con gr): Animals were treated with saline as control. Group 2 (RT gr): Rats orally received rutin (150 mg kg^{-1} b.wt.) daily for 2 weeks[18]. Group 3 (ALA gr): rats were orally administered with alpha-Lipoic acid (10 mg kg^{-1} b.wt.) dissolved in saline at an alkaline pH (7.8) daily for 2 weeks[23]. Group 4 (CP gr): Rats were intraperitoneal (i.p.) injected with CP (9.0 mg kg^{-1})[24]. This dose was divided into two doses given once a week for two weeks to develop the acute renal injury. Group 5 (CP+RT gr): Rats injected with CP (9.0 mg kg^{-1} b.wt.) as CP group and then treated orally with RT (150 mg kg^{-1} b.wt.). Group 6 (CP+ ALA gr): Rats were injected i.p., with CP (9.0 mg kg^{-1}) as CP group and then treated orally with ALA (10 mg kg^{-1} b.wt.) and Group 7 (CP+RT+ALA gr): Rats were injected with CP (9.0 mg kg^{-1}) as CP group and then treated orally with RT (150 mg kg^{-1} b.wt.) and alpha-Lipoic acid (10 mg kg^{-1} b.wt.) daily for 2 weeks.

Sample collection: At the end of the experimental period or vital blood samples were obtained from retro-orbital venous plexus using microcapillaries. Blood samples were divided into two portions, the first portion, serum samples were collected in clean dry centrifuge tubes and allowed to clot to obtain sera. Serum samples were separated by centrifugation at 1800 rpm for 10 min at 4°C. Aliquots of serum samples were frozen and stored at -20°C pending further analysis. The other portion of blood was collected in EDTA coated glass tubes for determination of hematological characteristics (Hb and RBC). Following blood collections, animals were sacrificed by cervical dislocation and a midline abdominal incision was performed and whole two kidneys of each animal were rapidly dissected out, thoroughly washed with ice-cold isotonic saline, blotted dry and then weighed. One kidney was immediately homogenized to give 10% (w/v) homogenate in ice-cold medium containing phosphate buffer (pH 7.4). The homogenate was centrifuged at 1800 rpm for 10 min at 4°C. The supernatant (10%) was separated and stored at -20°C for determination of different biochemical analysis. The second kidney was fixed in 10% buffered formalin after washing with 10% formal saline for histopathological investigation.

Biochemical determinations

Renal biomarkers: Urea was assayed by colorimetric methods using kit purchased from Biodiagnostic Co. Egypt according to Fawcett and Soctt[25]. Creatinine and albumin were determined using kit purchased from Diamond Diagnostics Co. Egypt according to Kaplan and Pesce[26] and Young and Friedman[27], respectively.

Hematological investigation: Hemoglobin and red blood cells count were measured using an automatic haematology analyzer (Mindray Hematology analyzer, BC-2300) according to Ita and Udofia[28].

Antioxidant status: Renal malondialdehyde (MDA), glutathione-s-transferase (GST), glutathione reductase (GSR) and catalase contents were determined by colorimetric methods using kits purchased from Biodiagnostic Co. (Egypt) following the methods of Satoh[29], Habig *et al.*[30], Goldberg and Spooner[31] and Aebi[32], respectively.

Inflammatory markers: Nuclear factor kappa B (NF-κB) was determined by ELISA technique using rat nuclear factor kappa B ELISA kit purchased from Glory Science Co., Ltd, USA according to the manufacturer's instruction. The IL-6 level was estimated using ELISA technique using kit purchased from Uscn life science Inc., USA according to the manufacturer's instruction. The P53 was assayed by ELISA technique according to the manufacturer's instruction of ELISA kit of rat p53 purchased from Glory Science Co., Ltd, USA.

Histopathological investigation: After fixation of kidney specimens in buffered formalin (10%) for 24 h, washing in tap water was done and then the kidney samples were subjected to ascending grades of ethyl alcohol for dehydration. Afterward, kidney specimens were cleared in xylene and embedded in paraffin wax at 56 degrees in a hot air oven for 24 h. Paraffin wax tissue blocks were prepared for sectioning at 4 µm by a sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin[33]. After staining, the slides were viewed with an Olympus CH (Japan) light microscope. The image capturing was performed with a Sony DSC-w 3 digital camera (Japan) and photomicrograph calibration was done with image J[34].

Statistical analysis: The results of the present study were expressed as mean±SE of the mean. Statistical Package for the Social Sciences (SPSS) program, version 19.0 was used to compare the significance between every 2 groups. Difference was considered significant when p<0.05. Percentage the difference representing the percent of variation with respect to the corresponding control group was calculated according to the following formula:

$$\text{Difference } (\%) = \frac{\text{Treated value} - \text{control value}}{\text{Control value}} \times 100$$

RESULTS

Renal biomarkers: The data illustrated in Table 1 revealed that serum urea, creatinine and total albumin showed the significant increase (p<0.05) in CP-challenged group compared with control group. However, treatment of CP-challenged group with RT, ALA or both of RT and ALA led to significant reduction (p<0.05) in the serum levels of urea, creatinine and albumin versus CP-challenged group.

Hematological results: Results in Table 2 showed the effect of treatment with RT, ALA and both of RT and ALA on the hematological characteristics of rats treated with cisplatin. In comparison with the control group, there was a significant decrease (p<0.05) in serum Hb level and RBC count in CP group. On the other hand, treatment of CP-challenged group with either RT, ALA or RT and ALA together resulted in a significant elevation in the level of Hb and RBC count compared with CP-challenged group.

Antioxidant status: The results in Table 3 showed the effect of RT and ALA acid treatment or both of RT and ALA together on MDA and enzymatic antioxidants contents of rats treated with cisplatin. The results revealed that renal MDA content displayed significant increase (p<0.05) in CP-challenged group versus the control group. On the contrary, treatment of CP-challenged group with either RT, ALA or RT and ALA together reverted this increase as indicated by the significant drop (p<0.05) in renal MDA content as compared with that recorded in CP-challenged group (Table 3). The contents of GST, GSR and catalase in kidney homogenate significantly decreased (p<0.05) in CP-challenged group when compared with that of the control group. A significant increase (p<0.05) in the levels of GST, GSR and catalase in kidney homogenate were recorded following the treatment of CP-challenged groups with either RT, ALA or both of RT and ALA together compared to CP-challenged group (Table 3).

Inflammatory markers: Data in Table 4 illustrated the effect of treatment with RT, ALA and both of RT and ALA together on renal NF-κB and IL-6 and P53 levels in CP-challenged group. The CP-challenged group showed significant elevation (p<0.05) in the renal levels of NF-κB, IL-6 and P53 as compared to the control group. In contrast, treatment of CP-challenged group with RT, ALA or both of RT and ALA together led to significant reduction (p<0.05) in the renal levels of NF-κB, IL-6 as compared to the untreated CP-challenged group. Furthermore, CP group treated with RT, ALA or both of RT and ALA together displayed significant reduction (p<0.05) in the level of P53 when compared with untreated CP-challenged group.

Histopathological investigation: In controls groups, the histology of the rat kidney tissue sections showed normal architecture pattern. The glomerulus and the proximal and distal convoluted tubules showed normal appearance (Fig.1a, b and c). On the contrary, kidney tissue section of rat in the CP-challenged group showed marked disruption of renal architecture, histological lesions appeared as severe congestion in the tufts of the glomeruli associated with focal haemorrhage in between the tubules in the cortex and focal inflammatory cells infiltration in the periglomerular tissue (Fig. 1d). In addition, disturbance in architecture of kidney were frequently detected as deformed glomeruli with hemorrhagic changes in between the degenerated tubules

Table 1: Effect of rutin and /or alpha-Lipoic acid on the renal function of rats treated with cisplatin

	Parameters		
Groups	Urea (mg dL^{-1})	Creatinine (mg dL^{-1})	Albumin (g dL^{-1})
Con	16.82±2.68	0.54±0.13	3.93±0.52
RT	17.53±2.92	0.61±0.12	3.60±0.42
ALA	17.23±3.84	0.59±0.13	3.49±0.47
CP-challenged	36.82±4.02[a] (118.91%)	1.21±0.24[a] (124.07%)	6.72±0.81[a] (70.99%)
CP+RT	23.24±3.05[b] (-36.88%)	0.82±0.15[b] (-32.23%)	4.63±0.60[b] (-31.10%)
CP+ALA	21.15±2.25[b] (-42.56%)	0.77±0.14[b] (-36.36%)	4.36±0.64[b] (-35.12%)
CP+RT+ALA	19.13±2.73[b] (-48.04%)	0.65±0.13[b] (-46.28%)	4.09±0.61[b] (-39.14%)

Data were represented as Mean±SE of 8 rats /group, a: Significant change at p>0.05 in comparison with control group, b: Significant change at p>0.05 in comparison with cisplatin group

Table 2: Effect of rutin and/or alpha-Lipoic acid on the haematological levels of rats treated with cisplatin

	Groups						
Parameters	Con	Con+RT	Con+ALA	CP-challenged	CP+RT	CP+ ALA	CP+RT+ALA
Hb (g dL^{-1})	13.40±2.81	13.10±2.61	13.20±3.76	7.60±1.92[a] (-43.28%)	10.50±1.82[b] (38.16%)	10.90±1.68[b] (43.42%)	12.60±1.73[b] (65.79%)
RBC (count μL^{-1})	$7.99\times10^3\pm1.12\times10^3$	$7.83\times10^3\pm1.21\times10^3$	$7.88\times10^3\pm1.17\times10^3$	$4.54\times10^3\pm1.10\times10^{3a}$ (-43.18%)	$6.27\times10^3\pm1.20\times10^{3b}$ (38.11%)	$6.51\times10^3\pm1.13\times10^{3b}$ (43.39%)	$7.52\times10^3\pm1.14\times10^{3b}$ (65.64%)

Data were represented as Mean±SE of 8 rats/group, a: Significant change at p>0.05 in comparison with control group, b: Significant change at p>0.05 in comparison with cisplatin group

Table 3: Effect of rutin and/or alpha-Lipoic acid on malondialdehyde and anti-oxidant enzymes contents of rats treated with cisplatin

	Parameters			
Groups	MDA (tissue nmol g^{-1})	GST (tissue U g^{-1})	GSR (tissue U g^{-1})	Catalase (tissue U g^{-1})
Con	0.97±0.22	1.02±0.23	3.21±0.61	1.62±0.43
Con+RT	0.91±0.21	1.07±0.24	2.82±0.69	1.53±0.33
Con+ALA	0.87±0.13	1.13±0.22	2.94±0.36	1.51±0.32
CP-challenged	3.84±0.82[a] (295.88%)	0.34±0.05[a] (-66.67%)	1.94±0.32[a] (-39.56%)	0.43±0.11[a] (-73.46%)
CP+RT	1.62±0.33[b] (-57.81%)	0.79±0.13[b] (132.35%)	2.65±0.38[b] (36.59%)	0.92±0.18[b] (113.95%)
CP+ALA	1.54±0.22[b] (-59.89%)	0.82±0.12[b] (141.18%)	2.79±0.40[b] (43.44%)	1.08±0.22[b] (151.16%)
CP+RT+ALA	1.28±0.28[b] (-66.67%)	0.92±0.14[b] (170.59%)	2.93±0.44[b] (51.03%)	1.31±0.23[b] (204.65%)

Data were represented as Mean±SE of 8 rats/group, a: Significant change at p>0.05 in comparison with control group, b: Significant change at p>0.05 in comparison with cisplatin group

Table 4: Effect of rutin and/or alpha-Lipoic acid on NF-κB, IL-6 and P53 levels of rats treated with cisplatin

	Parameters		
Groups	NF-κB (protein ng mg^{-1})	IL-6 (protein Pg mg^{-1})	P53 (protein Pg mg^{-1})
Con	85.32±8.14	71.43±6.41	58.21±7.01
Con+RT	83.41±7.82	69.82±6.22	56.81±6.42
Con+ALA	82.81±8.18	68.39±7.81	57.42±7.61
CP-challenged	136.44±10.46[a] (59.92%)	158.38±11.12[a] (121.73%)	93.11±9.21[a] (59.96%)
CP+RT	111.82±12.23[b] (-18.04%)	112.08±13.14[b] (-29.23%)	76.43±8.24[b] (-17.91%)
CP+ALA	105.35±11.88[b] (-22.79%)	103.24±10.68[b] (-34.82%)	72.42±7.81[b] (-22.22%)
CP+RT+ALA	95.40±9.63[b] (-30.08%)	92.32±10.02[b] (-41.71%)	66.64±7.62[b] (-28.43%)

Data were represented as Mean±SE of 8 rats/group, a: Significant change at p>0.05 in comparison with control group, b: Significant change at p>0.05 in comparison with cisplatin group

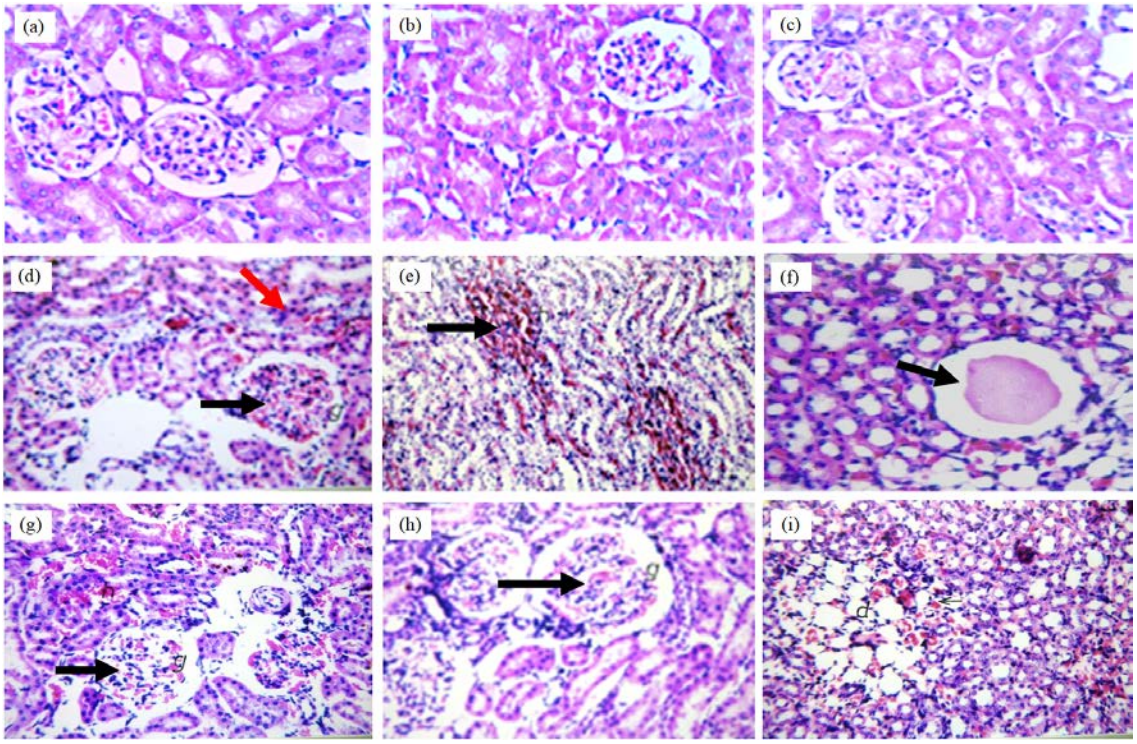

Fig. 1(a-i): Photomicrograph of kidney tissue section of rat (a) Control group showing normal histological structure (H and E×400), (b) Control group treated with RT only showing no structural alterations in renal tissues (H and E×400), (c) Groups treated with ALA only showing normal histological structure (H and E×400), (d) CP-challenged group showing sever congestion in the tufts of the glomeruli (black arrow) associated with focal haemorrhages (red arrow) in between the tubules in the cortex (H and E×400), (e) CP-challenged group showing haemorrhages (black arrow) in between the degenerated tubules in the medulla (h) (H and E×100), (f) CP-challenged group showing homogenous eosinophilic renal casts in the lumen of the degenerated tubules in the medulla (black arrow) (H and E×400), (g) CP-challenged group treated with RT showing an improvement in the congestion in the glomeruli (black arrow) (H and E×200), (h) CP-challenged group treated with ALA showing improvement in the congestion in the tufts of the glomeruli (black arrow) (H and E×200) and (i) CP-challenged group treated with RT+ALA showing partial near to normal architecture (H and E×100)

in the medulla, increased eosinophilic staining (Fig. 1e), contraction of the glomerular tuft and separation in intertubular tissue associated with the presence of homogenous eosinophilic renal casts in the lumen of the degenerated tubules in the medulla (c) were greatly noticed (Fig. 1f). In contrast, Photomicrograph of kidney tissue section of rat in CP-challenged group treated with RT showed an improvement in the congestion in the glomeruli with less focal haemorrhages between the tubules in the cortex, mild inflammatory cell invasion compared with the CP-challenged group (Fig. 1g) in addition to the reduction in the haemorrhages between the tubules in the medulla. Furthermore, Photomicrograph of kidney tissue section of rat in CP-challenged group treated with ALA showed improvement in the congestion in the tufts of the glomeruli (g) (Fig. 1h) and very few hyperchromatic deposits. Whereas, Photomicrograph of kidney tissue section of rat in CP-challenged group treated with RT+ALA revealed minimal changes in the structure of the kidney, both the glomeruli and convoluted tubules gained partial near to normal features (Fig. 1i).

DISCUSSION

The current study demonstrated that cisplatin administration caused a reduction in glomerular filtration rate, accompanied with a significant elevation in serum creatinine, urea and albumin levels. These findings come in line with those of Atessahin *et al.*[35] and Sahu *et al.*[36], who found an increment in serum creatinine level in subjects having a history of renal dysfunction or those taking nephrotoxic medications. The increased creatinine level in cisplatin

group might be due to upregulation of guanidinoacetate methyltransferase enzyme[37]. Moreover, the alterations in glomerular function in cisplatin challenged group might also be secondary to reactive oxygen species (ROS)[38] which promote mesangial cells contraction[39].

On the other side, the treatment with RT, ALA or both improved cisplatin-induced abnormalities in renal parameters (serum urea, creatinine and albumin). Rutin could improve renal dysfunction[18,40-42]. It has been reported that rutin act as anti-lipoperoxidant and free radicals scavenger agent[43]. Bhatti *et al.*[44] stated that ALA administration in alloxan diabetic rabbits notably enhances renal function. Treatment with ALA amazingly kept the diminished glomerular filtration rate, by increasing creatinine clearance. Trial proof additionally demonstrated that ALA is known to increase the creatinine clearance[45,46].

Significant diminishing in RBCs count and Hb concentration has been recorded in cisplatin-challenged group. This finding could be attributed to the repression of red blood cell synthesis via inhibition of erythropoiesis in bone marrow. Erythropoietin stimulates bone marrow to make a sufficient number of red blood cells and in case of marked renal damage, the production of erythropoietin decreases with consequent drop in red blood cells production and development of anemia[47,48]. Stuti and D'Souza[49] explained the lower Hb concentration demonstrated the lower RBC in the blood.

The significant increase in RBCs count and Hb concentration has been detected upon treatment with RT. Bao *et al.*[50] stated that rutin protects against haemoglobin oxidation inside human red blood cells via the antioxidant activity of the flavonol glycoside of rutin. This exhibits the capacity of constraining lipid peroxidation[51,52]. Similarly, treatment with ALA documented an increase in RBCs count and Hb concentration. Mirjana *et al.*[53] reported that the administration of ALA in diabetic rats plays a beneficial role in preserving the structural and functional integrity of RBC. It has been reported that ALA provides antioxidant activity by chelating Fe^{2+} and Cu^{2+} ions[53,54].

The current results showed a significant increase in renal MDA content by paralleled significant depletion in renal antioxidant enzyme activities (GST, GSR and catalase) in CP-challenged. These results are consistent with those of Erman *et al.*[55] and Noori and Mahboob[56], who reported that oxidative stress resulting from cisplatin is characterized by the increased production of oxidative stress markers (lipid peroxidation) and decreased concentrations of the antioxidants. The reduction in GSH redox cycle, i.e., the inhibition of renal GST, GSR and catalase activities were demonstrated in kidneys of rats administered with cisplatin[57]. The treatment with RT, ALA or both was found to decrease renal MDA level and modulated the values of renal antioxidant enzymes. This suggested their role in scavenging free radicals generated by cisplatin[18]. The mechanism of this effect was explained by Lopez-Revuelta *et al.*[58], who declared that flavonoids that cause a dramatic reduction in the fluidity of the membrane hydrophobic core could hinder the diffusion of free radicals, resulting in a repression of oxidative damage. Though, flavonoids, for example, rutin that especially collaborated with the membrane-surface through hydrogen bonding to the polar head groups of phospholipids, could prevent the access of molecules to the hydrophobic region of the lipid bilayers[59]. ALA plays a role as an antioxidant candidate that restricts the ROS-induced lipid peroxidation and strengthens the antioxidant system[60]. Otherwise, ALA has a strong ability to chelate metals and to scavenge free radicals such as hydroxyl radical[61]. The present data indicated that the combination of RT and ALA resulted in a significant regression in renal MDA content and enhanced the values of renal antioxidant enzymes. This could be attributed to the cooperation between RT and ALA for doubling the protection of renal tissue from ROS liberated due to CP intoxication[18,60].

Cisplatin administration has been found to produce a significant increase in renal levels of NF-κB and IL-6. NF-κB is activated in response to oxidative stress in a variety of cells[62]. Upon encouragement by a variety of deleterious inflammatory and environmental stimuli, activated NF-κB moves to the nucleus and activate target genes, including cytokines which play a role of kidney injury[63]. So *et al.*[64] advertised that cisplatin causes the secretion and expression of the pro-inflammatory cytokines (TNF-α, IL-1β and IL-6). The NF-κB activation regulates an excess of genes including pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6[65-67]. Thus, the significant increase in the renal level of NF-κB in a cisplatin-challenged group could be the cause of increasing renal IL-6 level.

The treatment with RT or ALA resulted in significant diminishing in the levels of NF-κB and IL-6 in kidney tissue. Rutin having anti-inflammatory and antioxidant activity[68] as it could inhibit the activation of transcription factor, NF-κB and IL-6. Hafez *et al.*[69] and Imam *et al.*[70] demonstrated that rutin applies its anti-inflammatory influence through the repression of the upstream NF-κB activation and pro-inflammatory cytokine secretion. Previous studies have provided important insights into the therapeutic effects of ALA in inflammatory conditions[71]. Principal amongst these are its powerful free radical scavenging, activation of anti-inflammatory pathways, prevention of NF-κB activation, reduction in the

secretion of IL-6 and initiation of Phase II antioxidant defense mechanisms[72]. The double treatment with RT and ALA caused a marked decline in the renal levels of NF-κB and IL-6. This could be attributed to the combined effect of RT and ALA as anti-inflammatory and antioxidants agents to reduce the levels of NF-κB and IL-6 in kidney tissue[68,71,72].

The level of P53 in kidney tissue was increased in cisplatin-challenged rats. The P53 pathway plays a vital role in DNA damage mediated apoptotic signals[73]. The cisplatin induced renal oxidative stress may activate cisplatin-induced p53 production[6,74,75]. The treatment with RT down regulated p53 as shown in the present study and this could be due to suppression of ROS generation because RT is an electron donor and powerful antioxidant agent. The RT could inhibit the expression of P53 and in turn its protein level in the kidney of the treated rats[76]. The present study revealed that the treatment with ALA results in a significant depletion in renal P53 level. The nephroprotective effect of ALA seems to be related to its antioxidant activity as well as with its capacity to inhibit renal inflammation and tubular cell apoptosis[77]. Tamilselvan *et al.*[78] noted that ALA supplement reduced apoptosis in elderly rats by maintaining mitochondrial membrane integrity and thus preventing further loss of cytochrome c *in vivo.* The treatment with RT plus ALA recorded a significant decrease in the renal level of P53. This could be attributed to the strong radical scavenging ability of RT and ALA. In addition, the inhibition of P53 may be due to the antiapoptotic and anti-inflammatory effects of RT and ALA which inhibit renal inflammation and tubular cell apoptosis[69,77].

The histopathological investigation of kidney tissue sections from rat in CP-challenged group showed tubular necrosis, desquamation of the tubular epithelial cells with intense granular degeneration of renal cortex accompanied by severe congestion in the tufts of the glomeruli associated with focal haemorrhages in between the tubules in the cortex. Many of the tubular epithelial cells in this area lacked well-defined cellular membrane in addition to the presence of cellular inflammatory infiltration. This finding is in agreement with Sahu *et al.*[36]. Administration of RT or/and ALA provided renal histological treatment as normal glomeruli with minimal damage of epithelial lining have been observed. These observations are consistent with Hussein *et al.*[60] and Kamel *et al.*[79]. The current results could be attributed to the role of RT and ALA as antioxidant agents to scavenge of ROS in addition to their antiapoptotic and anti-inflammatory effects[69,77].

CONCLUSION

In conclusion, the present study provided a convincing evidence for the contribution of oxidative stress and inflammation in the pathophysiological mechanism of cisplatin-induced nephrotoxicity. The present results suggested that rutin and alpha-lipoic acid have significant therapeutic benefit when administered with cisplatin treatment to overcome the nephrotoxic insult of cisplatin due to the antioxidant and anti-inflammatory properties of rutin and alpha-Lipoic acid.

SIGNIFICANCE STATEMENT

This study confirmed that cisplatin-induced nephrotoxicity. Rutin and α-lipoic acid have antioxidant effectiveness, anti-inflammatory efficacy and prevent oxidative stress. Therefore, the current study planned to investigate potential defensive impacts of rutin and α-lipoic acid on cisplatin-induced renal damage in rats.

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