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Phytomodulatory potential of lycopene from *Lycopersicum esculentum* against doxorubicin induced nephrotoxicity

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An elevated level of serum urea and creatinine was observed in doxorubicin (DOX) treated animals indicating DOX-induced nephrotoxicity. Enhanced lipid peroxidation (LPO) in the renal tissue was accompanied by a significant decrease in the levels of reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT) activities. Administration of lycopene (LycT) extracted from tomato to DOX treated mice showed a significant reduction in serum creatinine and urea levels which were associated with significantly low levels of LPO and significantly enhanced level of GSH and related antioxidant enzymes activity (GPx, GR and CAT) when compared to DOX group. Histopathological analysis revealed severe damage in the renal tissue of DOX treated animals. However, animals pretreated with LycT were observed to have reduced damage. Thus, from present results it may be inferred that lycopene may be beneficial in mitigating DOX induced nephrotoxicity in mice.

Keywords: Doxorubicin, Lycopene, Nephrotoxicity, Phytomodulation

Doxorubicin (DOX) is an anthracycline chemotherapeutic drug commonly used for the treatment of a wide variety of cancers[1]. However, optimal use of DOX is limited by a number of side-effects including cardiotoxicity[2], haematotoxicity[3] and a dose-limiting nephrotoxicity[4]. Although the exact mechanism of DOX induced nephrotoxicity is still not clear, the likely mechanisms are: formation of semiquinone free radicals by NADPH dependent reductase and non-enzymatic reaction which involves reaction of DOX with iron[5]. The semiquinone free radical further gives rise to various other free radicals and non-free radicals which include reactive oxygen species (ROS) like super oxide anion (O_2^-), hydroxyl radical (OH^-), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2) which lead to oxidative stress. Enhanced generation of ROS is responsible for causing damage to biomolecules such as proteins, DNA and lipids leading to oxidative stress[6]. Oxidative stress plays a major role in DOX induced toxicity. Altered intraglomerular hemodynamics, tubular cell toxicity, inflammation, thrombotic microangiopathy, necrosis, increased sclerosis of glomeruli and tubulointerstitial tissue have been found to be associated with drug induced nephrotoxicity[7].

Therefore, use of potent antioxidants seems to be a rational approach to counteract oxidative stress induced disorders. Dietary antioxidant supplements during chemotherapy may help in counteracting several types of toxicities[8-10]. Carotenoids like lycopene have been reported to be the most efficient singlet oxygen and free radical quencher[11]. Lycopene, an acyclic carotenoid possess high nutraceutical value and imparts red color to tomato, guava, rosehip, watermelon and pink grapefruit[12]. Dietary intake of lycopene rich food has been associated with a decrease risk of chronic diseases including cancers and cardiovascular diseases[13]. Several experimental and epidemiological studies have demonstrated the chemopreventive effect of lycopene in combating prostate cancer, mammary tumor and hepatic cancer[14,15]. Beneficial effect of lycopene against xenobiotic induced toxicities has been reported[16]. Lycopene being an antioxidant has been suggested to protect critical biomolecules including lipids, low-density lipoproteins (LDL), proteins and DNA from free radicals[17,18]. Ameliorating effect of lycopene from tomato in various toxic states/conditions related to organ like lung, liver, and kidney have been reported[19]. In fact, significant inhibitory effect of lycopene extracted from tomato has been reported in carcinogenesis where commercially available pure lycopene did not show

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the effect; it may be due to the synergistic action of other minor phytochemicals in tomato[20]. The lycopene extract from tomato has shown more strength in inhibiting lipid peroxidation, than lycopene *per se*[21].

Considering the above mentioned facts, it seems worthwhile to explore the involvement of lycopene from tomato in ameliorating DOX induced nephrotoxicity. The present investigation has been designed to induce nephrotoxicity in mice using DOX and to demonstrate the beneficial effect of lycopene from tomato in terms of renal oxidative damage, antioxidant defense system and histopathology.

Materials and Methods

Doxorubicin manufactured by Fresenius Kabi Oncology Limited, Nalagarh was used. Butylated-hydroxyltoluene (BHT), nitroblue tetrazolium (NBT) and 5-5'–dithiobis (2-thiobarbiotic acid) (DTNB) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Reduced glutathione (GSH), oxidized glutathione (GSSG), bovine serum albumin (BSA), reduced nicotinamide adenine dinucleotide (NADH), reduced nicotinamide adenine di-nucleotide phosphate (NADPH) were obtained from Sisco Research Laboratories Pvt. Ltd., India. Urea and creatinine estimation kits (ENZOPAK) were obtained from Reckon Diagnostics P. Ltd. All other chemicals utilized were obtained from reputed local firms (India) and were of the highest purity grade. Fresh tomatoes were used for lycopene extraction.

Extraction of lycopene from tomato (LycT)—Lycopene (LycT) was extracted as per Gupta *et al*[15]. Lycopene spectrum in the extracting solvents was determined by spectrophotometric measurements at room temperature in the wavelength range (350-600 nm) using UV-1800 Shimadzu UV spectrophotometer. The amount of LycT in the sample was estimated at 503 nm by using molar extinction coefficient of lycopene in hexane (17.2×10,000 $M^{-1}cm^{-1}$) as determined by Markovic *et al*[22].

In vitro determination of antioxidant activity of LycT

DNA damage inhibition efficiency assay—The assay was performed using the method of Stoewe and Prutz[23]. It is based on the formation of a fluorescent complex between double-stranded DNA and ethidium bromide (EtBr). A standard reaction mixture containing 20 m*M* Tris buffer (*p*H 7.0), 100 µg/mL calf thymus DNA, 50 µM $CuSO_4$, and 25 m*M* H_2O_2 was prepared. To determine the inhibitory effect of LycT on DNA damage, various concentrations of LycT in ethanol were prepared and added to the standard reaction mixture. The reaction was terminated by the addition of a stock solution of 0.5 *M* EDTA. Fluorescence intensity at 590 nm was measured with Perkin Elmer LS 55 Luminiscence spectrofluorometer with excitation at 510 nm. The level of DNA damage caused by free radicals generated by $CuSO_4$ and H_2O_2 (Fenton's Reagent) was calculated according to the following formula:

$$\text{DNA damage}(\%) = \left(\frac{I_c - I}{I_c}\right) \times 100$$

where I_c is the fluorescence intensity of the DNA and I is the fluorescence intensity of reaction mixture. The percentage reduction of fluorescence intensity was used as DNA damage inhibition efficiency of the test sample.

DPPH radical scavenging activity assay—The DPPH free radical scavenging activity of LycT was measured using the method of Shimada *et al*[24]. DPPH*(1, 1-Diphenyl-2-picrylhydrazyl) is a stable free radial with its characteristic absorbance maxima at 517 nm (deep violet). Scavenging of DPPH* to DPPH by antioxidants changes its absorbance and as a result its colour changes to yellow. To determine the DPPH* scavenging activity of LycT, various concentrations of LycT in ethanol were prepared and added to 0.1 m*M* DPPH* solution prepared in ethanol. After 10 min of incubation, absorbance was measured at 517 nm using UV-1800 Shimadzu UV spectrophotometer with ethanol as blank. BHT (5 mg/mL in ethanol) was used as a positive control. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The level of percentage inhibition of DPPH* was calculated according to the following formula:

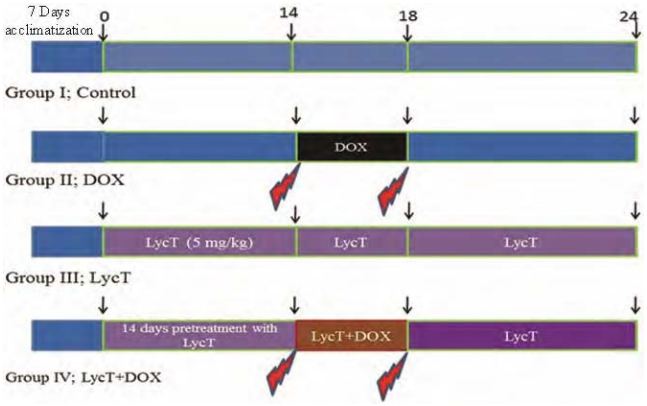
$$\text{DPPH}^* \text{ scavenged } (\%) = \left(\frac{A_c - A}{A_c}\right) \times 100$$

where A_c is the absorbance of the control (DPPH*) and A is the absorbance of sample (DPPH*+LycT). Percentage scavenging was also evaluated in BHT equivalent.

Animal model and treatment—Female Balb/c mice weighing 25-30 g each, procured from Central Animal House, Panjab University, Chandigarh, were housed in polypropylene cages, bedded with sterilized rice husk. Mice in all the groups had free access to standard animal pellet diet (Ashirwad Industries Ltd., Ropar, Panjab) and clean tap water throughout the experiment. All the experimental protocols were approved by the Institutional Ethics Committee (Panjab University, Chandigarh, India) and conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

The mice were divided into following four groups of 8-10 mice/group, on the basis of the treatment that they received: Gr. I (control), Gr. II (DOX), Gr. III (LycT) and Gr. IV (LycT+DOX) (Scheme 1). Control animals received 0.1 mL olive oil (vehicle) orally at alternate days for 24 days. Gr. II mice received 10 mg doxorubicin/kg body weight iv at 14th and 18th day of the treatment period. Gr. III animals were administered LycT, po, at a dose of 5 mg/kg body weight in olive oil on alternate days for 24 days. Animals of Gr. IV received co-treatment of LycT and DOX as described above. The first dose of DOX was given to the animals after 14 days of LycT pretreatment. The body weight was recorded weekly in all the groups throughout the experiment. Animals were sacrificed after 5 days of the last DOX dose for various biochemical and histopathology analysis.

Preparation of blood serum—Blood was withdrawn from the retro-orbital plexus (ocular vein) of the mouse eye with a capillary. Blood (500 µL) was withdrawn in microcentrifuge tubes and incubated in an upright position at 37 °C for 3 h to allow clotting. After incubation the samples were centrifuged at 2500 rpm for 15 min. The supernatants



Scheme 1—Treatment protocol in different groups of animals.

(serum) were carefully aspirated and stored at -20 °C for further use.

Estimation of serum creatinine and urea levels—Serum creatinine and urea levels were estimated using commercially available kits namely Enzopak creatinine and Enzopak urea respectively.

Preparation of sample for biochemical estimations—After completion of the treatment period, the animals were kept on an overnight fast before sacrifice. The animals were sacrificed by cervical dislocation under light ether anesthesia. The kidney tissues were obtained and perfused with cold normal saline (0.9% NaCl solution), blotted and then weighed carefully. For biochemical estimations, the kidney tissues were homogenized in 50 m*M* Tris buffer (*p*H 7.4) to obtain 10% homogenate (w/v). The aliquots of 10% homogenate were kept at 4 °C for estimation of lipid peroxidation (LPO)[25] and reduced glutathione (GSH)[26]. The remaining homogenate was subjected to cold centrifuge at 10000 *g* for 30 min. The pellet was discarded and supernatant (PMF) thus obtained was used for the estimation of glutathione reductase (GR)[27], glutathione peroxidase (GPx)[28], glutathione-S-transferase (GST)[29], catalase (CAT)[30] and superoxide dismutase (SOD)[31].

Histopathological studies—Formalin fixed tissues were processed for hematoxylin and eosin staining using the conventional laboratory procedure. Briefly, the tissues were dehydrated through ascending grades of alcohol, cleared in benzene and embedded in low melting point paraffin wax. Sections (5 µm thick) were cut, placed serially on clean glass slides and then de-paraffinized through descending grades of alcohol. Stained sections were mounted in DPX after dehydration and viewed under light microscope.

The histological slides of kidney were evaluated for semi quantitative analysis without knowledge of the treatment protocol. The changes seen were limited to the tubular interstitial and renal corpuscles area and graded as follows: 0, normal grade; I, areas of tubular atrophy, glomerular congestion, necrosis, vacuolization, tubular degeneration involving < 25% of renal cortical and medullary area; II grade, similar changes involving 25-50% of area; III grade, similar changes involving 50-75% of required area; IV grade, similar changes involving >75% of renal cortical and medullary area.

Statistical analysis—Data are presented as mean±SD and were analyzed by Student's *t*-test and one-way ANOVA followed by post hoc test.

Results

LycT Extraction and its quantification—The extracted lycopene was identified and quantified using UV-VIS spectroscopy. Characteristic peaks of trans-lycopene were obtained at λ = 444, 470 and 503 nm and these characteristic peaks were obtained in the absorption spectrum. The content of lycopene in the extract was estimated at 503 nm using molar extinction coefficient of lycopene in hexane ($17.2\times10{,}000\ M^{-1}cm^{-1}$). On an average the lycopene content was 11-15 mg/kg tomato.

In vitro antioxidant activity of LycT (Figs 1 and 2)—LycT at the concentration of 2 and 5 mg/mL caused a significant decrease in the DNA damage when compared to DNA treated only with Fenton's reagent. Moreover, LycT showed significant DPPH* scavenging activity in a concentration dependent manner. BHT was used as a positive control in above assays.

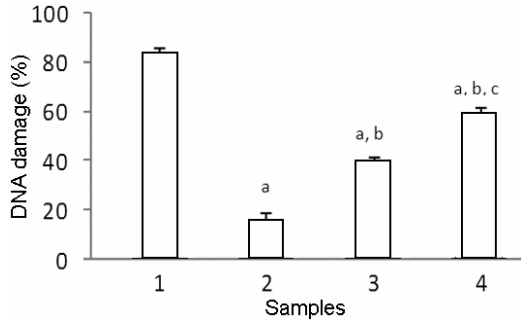


Fig. 1—Effect of LycT on DNA damage [Values are mean±SD (n=5); One-way ANOVA followed by post hoc test. $P \leq 0.005$, compared to [a]1, [b]2, [c]3, [d]4. Sample 1=DNA+$CuSO_4$+H_2O_2, sample 2=DNA+$CuSO_4$+H_2O_2+100 µL LycT (2 mg/mL), sample 3=DNA+$CuSO_4$+H_2O_2+100 µL LycT (5 mg/mL), sample 4=DNA+$CuSO_4$+H_2O_2+100 µL BHT (5 mg/mL)].

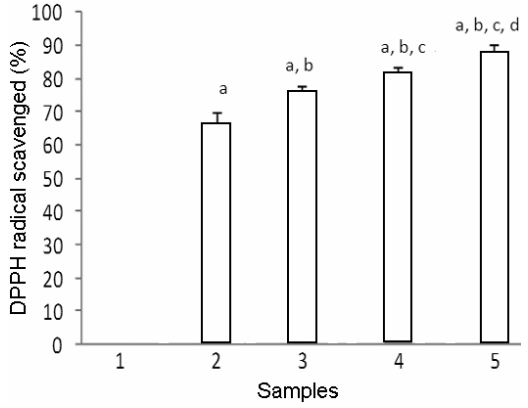


Fig. 2—Effect of LycT on DPPH* [Values are mean±SD (n=5); One-way ANOVA followed by post hoc test. $P \leq 0.005$, compared to [a]1, [b]2, [c]3, [d]4. Sample 1=ethanolic solution of DDPH*, sample 2=DPPH*+100 µL of LycT (0.5 mg/mL), sample 3=DPPH*+100 µL LycT (1.0 mg/mL), sample 4=DPPH*+100 µL LycT (2.0 mg/mL), sample 5=DPPH*+100 µL BHT (5.0 mg/mL).

Effect of DOX, LycT and co-treatment of DOX and LycT on body weight of mice—During the entire treatment, the mice were observed at weekly intervals for changes in body weight. Non-significant changes were observed in the body weight in all the groups studied as compared to their control counterparts (unpublished data).

Serum creatinine and urea levels (Fig. 3 A and B)—A significant increase in the serum creatinine and urea levels were observed in DOX treated mice when compared to control and LycT group. LycT intervention to DOX challenged mice caused a significant reduction in serum urea level when compared to DOX group. However, the decrease in serum creatinine level was found to be statistically non-significant between LycT+DOX group and DOX group. No significant alterations were observed between LycT and control group.

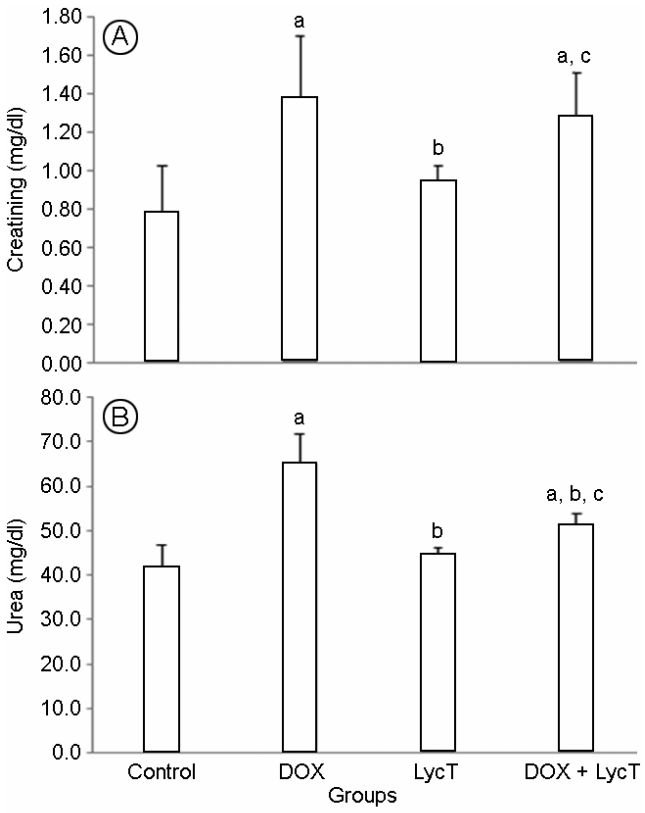


Fig. 3—Effect of DOX and/or LycT on creatinine level (A) and urea level (B) [Values are mean ± SD (n=5); One-way ANOVA followed by post hoc test. *P* values: ≤0.5, as compared to [a]control, [b]DOX group, [c]LycT group].

Lipid peroxidation and reduced glutathione (Table 1)—On DOX administration, a significant increase in the level of LPO and decrease in the levels of GSH were observed when compared to control group, whereas when LycT pre-treated mice were challenged with DOX, a significant decline in LPO levels was observed when compared to DOX treated animals. However, level of GSH was found to be increased non-significantly in the LycT+DOX group when compared to the DOX group.

Enzymatic antioxidant defense system (Table 2)—Upon DOX administration, a significant reduction in the activities of renal GR, GPx and CAT was observed when compared to the control group. LycT pre-treated mice when challenged with DOX showed a significant increase in CAT activity and non-significant increase in GR and GPx activities when compared to DOX group. DOX exposure caused a significant increase in renal GST and SOD activities when compared to control group. When LycT pretreated mice were challenged with DOX, GST and SOD activities showed a significant decline as compared to DOX group.

Histopathological observations (Figs 4 and 5)—For semi-quantitative analysis grading was performed as described. The changes seen were limited to the tubular interstitial and renal corpuscles area and graded as follows: 0, normal grade; I, areas of tubular atrophy, glomerular congestion, necrosis, vacuolization, tubular degeneration involving < 25% of renal cortical and medullary area; II, similar changes involving 25-50% of area; III, similar changes involving 50-75% of required area; IV, similar changes involving >75% of renal cortical and medullary area (Table 3). Histopathological examination of kidney sections from control and LycT animals revealed normal histoarchitecture (grade 0). A gross section of the kidney capsule revealed two distinguishable regions i.e. outer cortex and deeper medulla forming renal pyramids. Cortical labyrinth revealed proximal and distal convoluted tubules (PCT and DCT) together with renal corpuscles. Renal corpuscles consisted of an epithelial cup called Bowman's capsule enclosing a knot of capillaries and other elements called the glomerulus as shown in the (Fig. 4 A and B). Medullar region consisted of loops of Henle and collecting ducts (Fig. 5 A and B). DOX and LycT+DOX treatment resulted in alterations in the renal architecture. Renal lesions including marked glomerular sclerosis, tubular dilation, glomerular congestion with wide Bowman's space, proximal tubular degeneration, renal necrosis, focal inflammatory cells in the medulla and focal tubular atrophy were observed (Fig. 4 C-E and Fig. 5 C-D). Compared with the DOX group, LycT administration shifted the histological grade from grade III and IV to lower grade (I and II).

Table 1—Effect of DOX and/or LycT on levels of lipid peroxidation and reduced glutathione in renal tissue
[Values are expressed as: mean ± SD from 5 observations]

Groups	Lipid peroxidation (nanomole of MDA-TBA chromophore formed/mg protein)	Reduced Glutathione (nanomole of GSH/mg protein)
Control	1.14 ± 0.29	22.8 ± 4.33
DOX	2.56 ± 0.49[a]	10.4 ± 1.44[a]
LycT	1.21 ± 0.16[b]	21.7 ± 3.61[b]
LycT+DOX	1.95 ± 0.38[a,b,c]	17.7 ± 1.56[a]

P values ≤0.05 significant compared to [a]control group; [b]DOX group; [c]LycT group

Table 2—Effect of DOX and/or LycT on activity of antioxidant defense enzymes in renal tissue
[Values are expressed as: mean ± SD from 5 observations]

Groups	Glutathione reductase (µmol of NADPH consumed/min/mg protein)	Glutathione Peroxidase (µmol of NADPH consumed/min/mg protein)	Glutathione-S-transferase (µmol of GSH-CDNB conjugate formed/min/mg protein)	Catalase (mmol H_2O_2 degraded/min/mg protein)	Superoxide Dismutase (IU/mg protein)
Control	2.55 ± 0.92	3.12 ± 0.15	15.2 ± 2.56	14.9 ± 1.92	1.86 ± 0.06
DOX	1.45 ± 0.22[a]	1.45 ± 0.23[a]	26.4 ± 7.22[a]	9.5 ± 1.33[a]	3.11 ± 0.57[a]
LycT	2.07 ± 0.26	2.33 ± 0.01[a, b]	15.4 ± 2.09[b]	12.7 ± 1.55[a, b]	1.86 ± 0.21[b]
LycT+DOX	1.84 ± 0.58	2.04 ± 0.63[a]	17.0 ± 1.26[b]	11.9 ± 1.44 [a, b]	1.90 ± 0.11 [b]

P values ≤0.05 significant compared to [a]control group; [b]DOX group; [c]LycT group

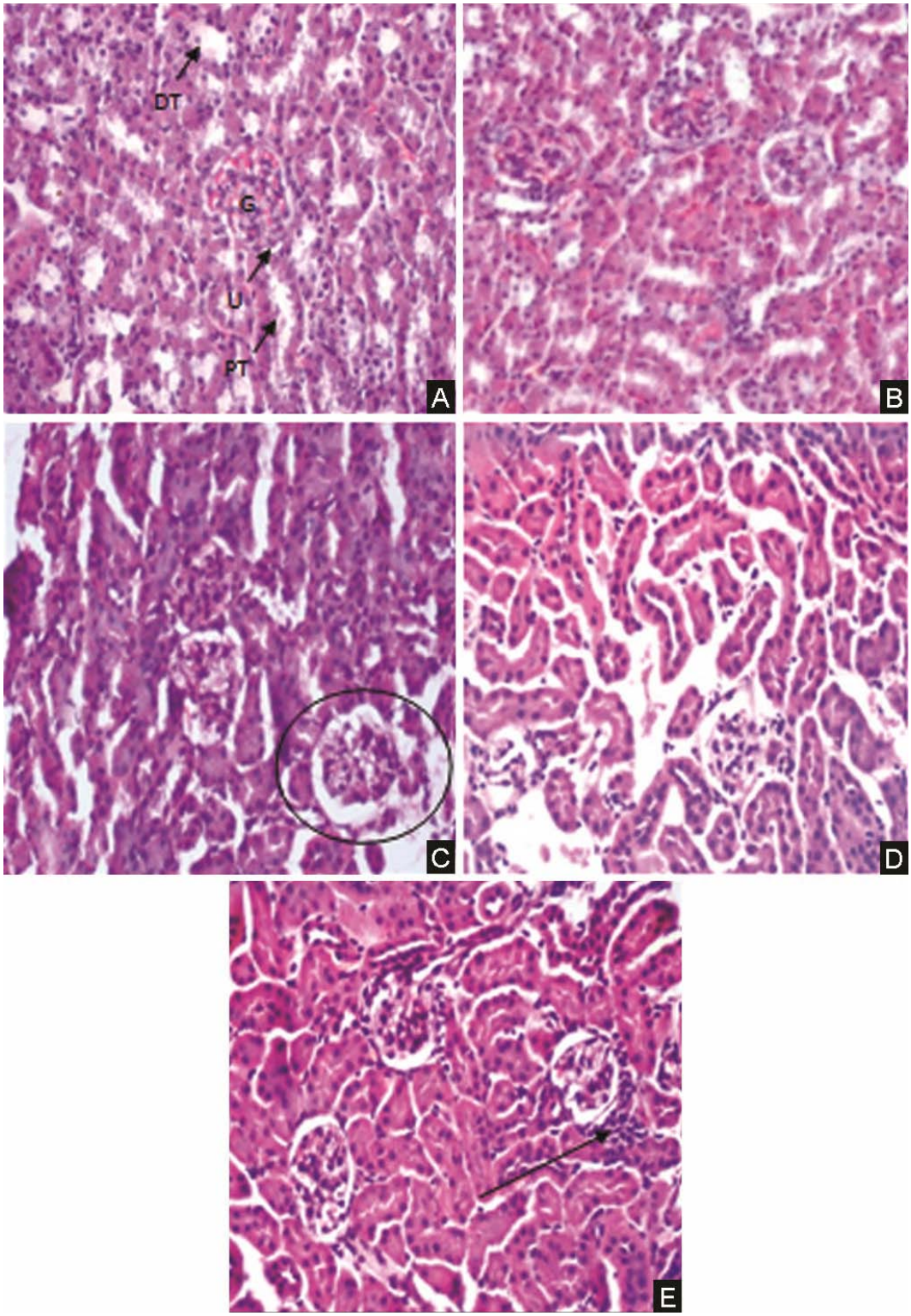


Fig. 4—Histomicrographs of kidney tissue (cortical labyrinth) from different groups [A and B: normal cortical labyrinth from control and LycT mice respectively; C and D: disrupted renal histoarchitecture from DOX group, C: renal cortex having glomerular sclerosis, congestion and vacuolization, D: renal cortex showing renal necrosis and atrophy; E: renal cortex from DOX+LycT group showing infiltrated inflammatory cells around renal corpuscle (G=glomerulus, V=vascular pole, U=urinary pole, PT=proximal convoluted tubule, DT=distal convoluted tubule; 400×].

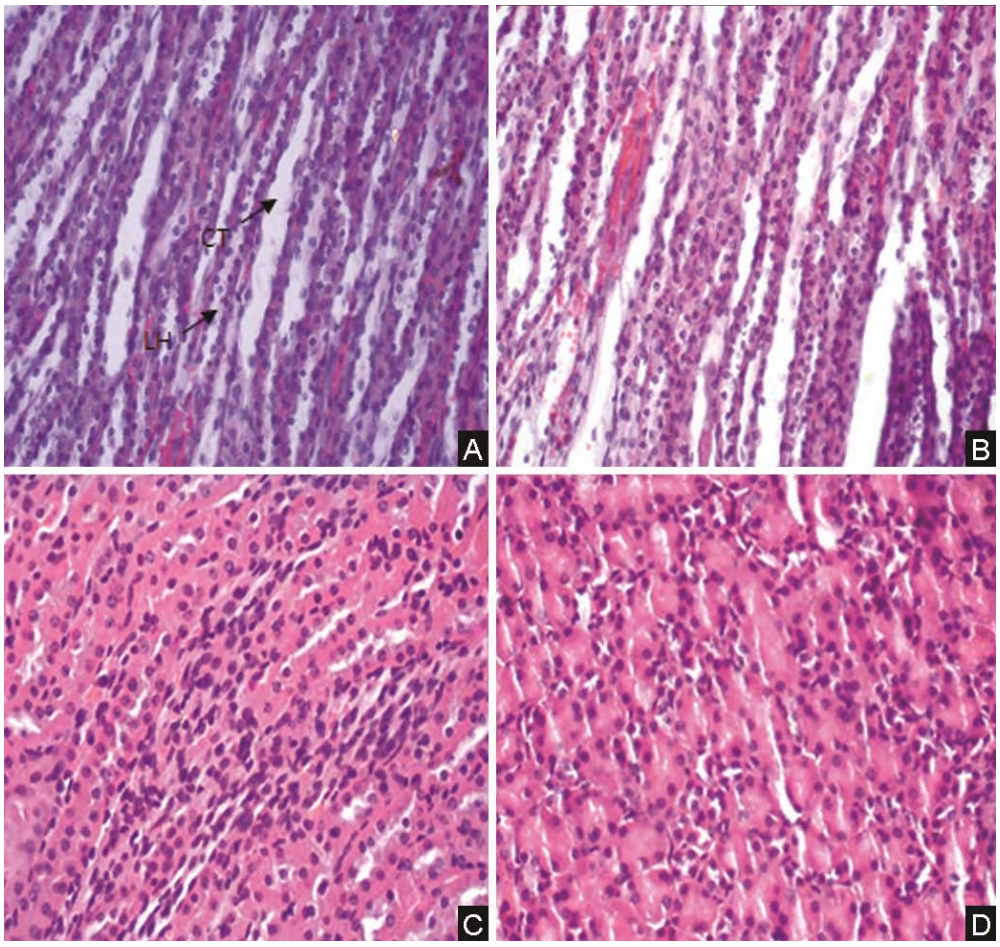


Fig. 5—Histomicrographs of kidney tissue (medulla region) from different groups [A and B: normal medulla from control and LycT mice respectively; C: distorted medulla from DOX showing tubular dilation (cells have lost their striation) and infiltrated inflammatory cells; D: medulla region showing tubular dilation from DOX+LycT group (LH=loop of Henle, CT=collecting tubule); 400×].

Table 3—Distribution of histological grades and semi-quantitative analysis of kidney damage in control, LycT, DOX and LycT+DOX groups

	Histopathological Grades				
Groups	0	I	II	III	IV
Control	+				
DOX				++	++
LycT	+				
LycT+DOX		+	++		

Distribution of histological grades and semiquantitative analysis of kidney damage in control, LycT, DOX and LycT+DOX groups (+ normal, ++ severity of damage)

Discussion

The present study was designed to determine the modulatory effect of lycopene (LycT) extracted from tomato against doxorubicin (DOX) induced nephrotoxicity. Epidemiological studies have established relationship between the usage of various anti-neoplastic drugs and nephrotoxicity[32,33]. Despite its vast utility in clinical oncology, the use of DOX is limited by a number of side effects including several organ toxicities including cardiotoxicity, hepatotoxicity and nephrotoxicity[34,35]. DOX treatment has been reported to be associated with significant reduction in antioxidant capacity in kidney during chemotherapy, which accounts for the increase in susceptibility to oxidative stress of the cellular structure leading to nephrotoxicity[36,37]. Therefore, a great interest has emerged concerning the protective functions of natural antioxidants contained in dietary plants for prevention of oxidative damage caused by free radical species. Several studies have demonstrated the ability of antioxidants and vitamins of natural origin to prevent organ toxicity[38,39]. Lycopene, a carotenoid occurring naturally in

tomatoes, is gaining considerable attention owing to its antioxidant properties[11]. Although lycopene lacks provitamin A activity, it has an exceptionally high free radicals and singlet oxygen quenching ability[11]. Lycopene exhibited ameliorative effects against several drugs and xenobiotic induced nephrotoxicity[40,41]. Yilmaz *et al.*[17] have reported that lycopene prevented adriamycin induced cardiac and renal toxicities in mice. Histopathological and ultrastructural study also revealed the protective effect of lycopene against deltamethrin-induced nephrotoxicity[42]. Among the various defense strategies, lycopene is most likely involved in the scavenging of two of the reactive oxygen species, singlet molecular oxygen ($^1O_2$), and peroxyl radicals[11]. Lycopene has immunomodulatory, anti-inflammatory, anticarcinogenic, antidiabetic and antioxidant activities[43-45].

The antioxidant property of various phytochemicals using DNA damage inhibition assay and DPPH radical scavenging assay has been demonstrated[46]. The present study in *in vitro* experiments showed that LycT has a protective capability against DNA degradation induced by ROS. The present results also revealed that low LycT concentration exhibited high protective potential towards DNA damage as compared to high concentration. This may be due to its pro-oxidant activity at high concentrations[47,48]. In DPPH* scavenging assay, LycT exerted dose dependent effect on DPPH* scavenging. Cellular macromolecules are highly reactive towards free radicals in *in vivo* conditions leading to several effects including cell damage[49]. ROS in *in vivo* can be balanced by the antioxidant action of low-molecular weight antioxidants as well as antioxidant enzymes. Among low-molecular weight antioxidants, carotenoids are known to play a key role[50]. Antioxidative potentials of phytochemicals can thus be exploited to minimize oxidative damage. Phytochemicals protecting DNA in *in-vitro* conditions have greater probability to perform similarly inside the cell.

Creatinine and urea levels in serum are generally elevated due to renal damage, and thus serve as diagnostic markers for kidney pathology[51]. In the present study a cumulative dose of 20 mg/kg body weight of DOX resulted in nephrotoxicity which had been demonstrated on the basis of significantly increased serum creatinine and urea levels. The present observations are in concordance with report of Ayla *et al*[52]. Significant reduction in creatinine and urea levels during LycT intervention to DOX challenged mice clearly indicates considerable protection against nephrotoxicity. These results are in harmony with those previously reported observations that lycopene was effective in attenuating DOX-induced cardiotoxicity and other toxicities[17,53].

ROS attack the phospholipids of cell membranes and react with polyunsaturated fatty acids to form lipid peroxides resulting in cellular injury. Increased LPO levels are considered as marker of toxicity[54,55]. In the present study, dose of 20 mg/kg of DOX resulted in increased renal LPO level indicating oxidative damage[56]. Free radical production by DOX is known to increase LPO levels in the renal tissue. In the present study, the renal MDA (index of peroxidative damage) levels were found to be decreased in LycT+DOX group indicating the protective effect of LycT against DOX-induced renal damage. Reduction in LPO levels by intervention with LycT has been reported in various toxicological studies[8,17,57]. Moreover, in *in vitro* systems, lycopene was found to inactivate hydrogen peroxide and nitrogen dioxide[58].

GSH is a ubiquitous tri-peptide, which has an essential role in maintaining cell integrity because of its reducing properties. It is involved in a protective mechanism that involves inactivation of ROS, including peroxides formed during cellular metabolism. In the present study, renal GSH level was significantly lowered in DOX-treated group. Significant reduction in the GSH level during drug induced nephrotoxicity has been reported[48,59] whereas some studies have reported increased levels of GSH[17]. DOX administration can have different effects on GSH levels, depending on the organ studied, and treatment regimen for example chronic administration causes increase in cardiac GSH levels, whereas both elevated and reduced GSH levels have been reported in acute studies. The present data indicated that LycT administration to DOX challenged mice significantly reduced the depletion of GSH levels in the kidney and thus had provided protection by boosting the antioxidant potential. The effects of lycopene on cellular GSH may be due to direct antioxidant effects, enhanced biosynthesis of GSH or increase in levels of other antioxidants[60]. In the present study increased activity of renal GST enzyme observed in the DOX group could be correlated with decreased levels of GSH. GST protect the cells from the damaging effects

of xenobiotic by catalyzing the conjugation of a variety of endogenous and exogenous compounds with the non-protein thiol, glutathione. However, the observed decrease in the activity of GST during LycT administration to DOX challenged mice could be correlated with direct scavenging of DOX metabolites by LycT.

In the present study, a significant increase in the activity of SOD was observed in the DOX group when compared to control group. Low activities of GPx and CAT enzyme are one of the early consequences of disturbance of the pro-oxidant/antioxidant balance[61,62]. Thus, in the present study declined activities of antioxidant enzymes explain the mechanism of DOX induced nephrotoxicity. LycT administration resulted in amelioration in biochemical indices of nephrotoxicity by increasing the activities of essential antioxidant enzymes, indicating its indirect contribution in uplifting the cellular antioxidant system. Such observations are in complete agreement with the previously reported literature[16,17].

Increased activity of SOD in the present study was observed in the DOX group; this may be due to high amount of semiquinone radicals production. However, there are studies reporting both increase and decrease in the activity of SOD during drug induced nephrotoxicity[61]. SOD activity has been shown to be induced in cells exposed to oxygen free radicals[63]. In LycT+DOX group, SOD activity was found to be normalized when compared with control SOD activity. However, induction in SOD activity was found during lycopene administration[64]. Studies reviewed related to several antioxidant demonstrate the occurrence of biphasic effects depending on the organ, type of free radicals and many other factors.

Normal histopathological architecture of grade 0 was observed in the renal tissue of animals of control and LycT group. However, histopathological alterations were observed in DOX and LycT+DOX groups. Tubular necrosis, atrophy, dilation, inflammation and glomerular congestion considered to be characteristic features of nephrotoxicity were observed in the DOX treated animals which is concordance with previously reported observations[61]. The severity of the toxicity was demonstrated as histopathological grade III and IV characterized by more than 75% of renal damage in DOX group. However, renal histopathology of LycT+DOX demonstrated grade I and II revealed reduced toxicity in the tissue as characterized by decrease inflammation and reduced glomerular congestion. LycT intervention in DOX challenged kidney tissue showed lesser renal damage as compared to DOX group. Disrupted structure of renal tissue is related to decreased filtration process of kidney. Acute tubular necrosis and glomerular congestion in DOX group indicated decline in renal function as was evident by acute elevated serum creatinine and urea levels. Moreover, locally infiltrated neutrophils and activated glomerular mesangial cells continue free radical production causing renal tissue damage[37]. Renal damage, sclerosis and tubular necrosis have been attributed to high LPO level in renal tissue of DOX treated animals.

Therefore, it may be inferred that LycT intervention to the DOX administered mice boosted the enzymatic and non-enzymatic antioxidant defense system which may be helpful in ameliorating DOX induced nephrotoxicity.

Acknowledgement

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