

## ABROGATION OF CISPLATIN-INDUCED NEPHROTOXICITY IN RATS BY LYCOPENE THROUGH AMELIORATING OXIDATIVE STRESS, INFLAMMATION AND APOPTOSIS

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### ABSTRACT

This study aimed to evaluate the possible protective effect of lycopene against cisplatin-induced nephrotoxicity in male rats. A single dose (6 mg/kg) of intraperitoneal (ip) injection of cisplatin caused significant increase in serum urea, creatinine, TNF- $\alpha$ , caspase-3 and lipid peroxides measured as malondialdehyde (MDA), with significant decreases in serum albumin, reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) as compared with the control group. Lycopene and vitamin E (a standard reference drug) were given orally (po) at doses of 5mg & 1g/kg, respectively for 14 days before cisplatin and 7 days after cisplatin injection produced significant decrease in serum levels of urea, creatinine and TNF- $\alpha$  as well as kidney contents of MDA and caspase-3 with marked increase in serum albumin and GSH content and activities of enzymes (SOD and CAT). In conclusion, lycopene afforded protection against cisplatin-induced nephrotoxicity through its antioxidant, antiapoptotic and anti-inflammatory activities.

**Keywords:** Antiapoptotic; Antioxidant; Cisplatin; Lycopene; Nephrotoxicity.

### INTRODUCTION

Cisplatin is one of the most efficient antineoplastic agents used in the treatment of many types of cancers<sup>1</sup>. Nevertheless, the full therapeutic effect of cisplatin is limited by its nephrotoxicity<sup>2</sup>. Nearly 28% to 36% of patients receiving an initial dose (50–100 mg/m<sup>2</sup>) of cisplatin develop acute kidney injury. The vigorous hydration has not been effective in eliminating cisplatin nephrotoxicity. The discontinuation of cisplatin remains the only option in cases of progressive acute kidney injury<sup>3</sup>. In addition to

direct tubular toxicity in the form of apoptosis, necrosis and inflammation that have been involved in the pathogenesis of cisplatin-induced nephrotoxicity<sup>4</sup>, several studies have reported that cisplatin induced oxidative stress is involved in the development of renal tubule injury<sup>5</sup>. The involvement of oxidative stress was supported by the fact that free radical scavengers and antioxidants inhibit cisplatin-induced nephrotoxicity<sup>6</sup>.

Many antioxidant substances were investigated for their protection abilities against cisplatin toxicities. Some researches recommended the use of enriched diets with natural antioxidants like vitamin E, ascorbic acid, and methionine<sup>7</sup>. Other studies reported that the use of sulfhydryl-containing drugs, such as captopril, diethyl dithiocarbamate, sodium thiosulfate, N-acetylcysteine, and lipoic acid, could also exert antioxidant activity<sup>8</sup>.

Lycopene a carotenoid, which is found mainly in tomatoes has a potent antioxidant, anti-inflammatory and chemotherapeutic effect in cardiovascular, neurodegenerative diseases and in some cancers<sup>9</sup>. The antioxidant effects of lycopene have been reported in terms of reactive oxygen species (ROS) scavenging activity, which allows lycopene to prevent lipid peroxidation and DNA damage. In addition, lycopene induces enzymes of the cellular antioxidant defense systems by activating the antioxidant response element transcription system<sup>10</sup>.

Therefore, the aim of the present study was to evaluate the possible protective effect of lycopene on cisplatin-induced nephrotoxicity in rats through inhibition of oxidative damage, inflammation and apoptosis.

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## MATERIALS AND METHODS

### Experimental

Fifty-six male adult Swiss albino rats were allocated into seven groups (eight rats each); two rats from each group were used for histopathological examination as follows:

Group 1: Received saline and served as control.

Group 2: Received DMSO 50% (as a solvent of lycopene).

Group 3: Received cisplatin in a single dose of 6 mg/kg, ip.

Group 4: Received lycopene in a dose of 5mg/kg, po for 21 consecutive days.

Group 5: Received vitamin E in a dose of 1g/kg, po for 21 consecutive days.

Group 6: Pretreated with lycopene in a dose of 5 mg/kg, po, for 14 consecutive days, followed by a single dose of cisplatin (6 mg/kg, ip) and lycopene in a daily dose of 5mg/kg, po for 7 consecutive days.

Group 7: Pretreated with vitamin E in a dose of 1g/kg, po for 14 consecutive days, followed by a single dose of cisplatin (6 mg/kg, ip) and vitamin E in a daily dose of 1g/kg, po for 7 consecutive days.

Lycopene was pretreated for 14 consecutive days before cisplatin to give sufficient period for protection and for 7 consecutive days after cisplatin as the symptoms of toxicity appear within 7 days of cisplatin injection<sup>11,12</sup>. The duration of treatment was based on the published literature<sup>13</sup> and pilot studies performed in our laboratory.

### Drugs and chemicals

Cisplatin was obtained from EIMC United Pharmaceuticals, Egypt, and given i.p. in a single dose of 6 mg/kg<sup>13</sup>. Lycopene was obtained from Abcam Biochemicals (Cambridge, UK) with purity greater than 90% and was dissolved in DMSO 50%. It was administered orally in a dose of 5 mg/kg, daily for 14 days before cisplatin injection and 7 days after cisplatin injection<sup>14</sup>. Vitamin E was purchased from El-Gomhoria Co. for chemicals (Cairo, Egypt) and was administered orally in a dose of 1g/kg, daily for 14 days before cisplatin injection and 7 days after cisplatin injection<sup>15</sup>. Ellman's reagent, thiobarbituric acid, reduced glutathione (GSH), 1,1,3,3-tetraethoxypropane, bovine serum albumin, pyrogallol, and trichloroacetic acid and dimethylsulphoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of the standard grades.

### Animals

Male adult Swiss albino rats weighing 200–225 g were obtained from the breeding colony maintained at the animal house of the Nile, Egypt. Animals were caged in seven groups, given a standard diet and water *ad libitum* and maintained at 21–24 °C and 40–60% relative humidity with 12h light–dark cycles. Animals were subjected to an adaptation period of 2 weeks in the animal house of Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt, before experiments. The experiments were conducted in accordance with the ethical guidelines for investigations in laboratory animals and comply with the Guide for the Care and Use of Laboratory Animals<sup>16</sup>.

### Serum and Tissue Preparation

On the seventh day after the cisplatin injection, all rats were weighed by electrical balance (Shimadzu, Japan) to determine the final body and kidney weights, then kidney/body weight ratio was determined according to the following equation;  

$$\frac{\text{Kidney weight}}{\text{Final body weight}} \times 1000.$$

Also blood samples were collected from retro-orbital venous plexus under light ether anesthesia in non-heparinized tubes. Serum was separated by centrifugation for 20 min at 4000g and stored at –20°C. The kidneys were rapidly isolated and washed with ice-cold isotonic saline (0.9%). Then, they were stored at –80°C until they were homogenized in ice-cold 0.15M KCl (w/v) using a Sonicator homogenizer (4710 Ultrasonic homogenizer, Cole-Parmer instrument Co., USA) to prepare 10% (w/v) homogenate. The homogenate was then divided into aliquots and used for the determination of kidney contents of caspase-3, MDA, GSH and enzymatic activities of CAT and SOD.

### Biochemical Analysis

Serum urea nitrogen, creatinine, and albumin were estimated colorimetrically according to methods of Fawcett and Scott<sup>17</sup>, Bartles et al.<sup>18</sup>, and Doumas and Peters<sup>19</sup>, respectively, using aqueous primary standard urea solution (50 mg/dL), standard creatinine (2 mg/dL), and standard albumin (4 g/dL). TNF- $\alpha$  was estimated in serum using rat TNF- $\alpha$  ELISA kit provided by Alpico® (India); this is a solid-phase sandwich ELISA that utilizes a monoclonal antibody specific for rat TNF- $\alpha$  coated on a 96-well plate and the concentrations were measured from the standard curve. The total protein content of kidney tissue is determined according to the method of Lowry et al.<sup>20</sup>. The kidney homogenate was used for the

determination of TBA reactive substances levels measured as MDA according to the method of Mihara and Uchiyama<sup>21</sup> and the concentrations were measured from the standard curve, which was constructed using serial dilutions of 1,1,3,3-tetraethoxypropane. GSH contents were assessed by the method of Ellman<sup>22</sup> and the concentrations were measured from the standard curve constructed using serial dilutions of GSH. The CAT activity was determined colorimetrically using hydrogen peroxide as a substrate according to the method of Claiborne<sup>23</sup>, and the SOD activity was determined using the method of Marklund<sup>24</sup>, which relies on the ability of the enzyme to inhibit the pyrogallol autoxidation. Caspase-3 was measured using an ELISA kit from Cusabio® Biotech (Wuhan, People's Republic of China). The concentration of caspase-3 was determined from a standard curve constructed from a set of serial dilutions of the standard.

#### Histopathological examination of the kidney

Autopsy samples were taken from the kidney of rats from different groups and fixed in 10% neutral buffered formalin for 24h. Washing was done with tap water, and then serial dilutions of alcohol (methyl, ethyl, and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56°C in a hot air oven for 24h. Paraffin wax tissue blocks were prepared for sectioning at 4µm thicknesses by a sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin and eosin, and then examination was done through the light electric microscope<sup>25</sup>.

#### Statistical analysis of data

All values were presented as means ± standard

error of the means (SEM). Statistical analysis was performed using GraphPad Prism version 5 (Graph- Pad, San Diego, CA). A comparison between different groups was carried out using one-way analysis of variance (ANOVA), followed by Tukey's test for multiple comparison between groups. The difference is considered significant when  $p$  is ≤ 0.05.

## RESULTS

Table 1 shows that injection of cisplatin (i.p.) in a single dose of 6 mg/kg caused significant increases in serum urea (241%), creatinine (334%) & TNF-α (93%) and kidney-body weight ratio (105%) as well as significant decrease in body weight (45%) and serum albumin level (38%) after 7 days of treatment as compared with the control group. Moreover, cisplatin (6 mg/kg) caused a significant increase in the caspase-3 (296%) and MDA contents (129%). Additionally, cisplatin injection induced significant decreases in the GSH renal content (61%) and the enzymatic antioxidant activities, SOD and CAT in the kidney (52% and 62% respectively) in comparison with the control group, Table 2.

In contrast, administration of lycopene for 14 days before cisplatin and 7 days after cisplatin significantly reduced the elevated levels of urea, creatinine and TNF-α in serum by 69%, 72% and 58%, respectively, as well as kidney-body weight ratio (42%), and significantly increased the body weight (27%) and the serum albumin level (35%) in comparison with the cisplatin-treated group. Furthermore, lycopene decreased the caspase-3 (74%) and MDA (36%) contents and increased GSH content (125%) as well as activities of SOD and CAT (136% and 100%, respectively) in kidney tissue in comparison with cisplatin-treated group

**Table 1. Effect of treatment of lycopene and vitamin E on serum urea, creatinine, albumin, TNF-α levels, final body weight, and kidney-body weight ratio in cisplatin-treated Rats**

Group	Urea (mg/dl)	Creatinine (mg/dl)	Albumin (g/dl)	TNF Alpha (pg/mL)	Final body weight (g)	Kidney- Body Weight Ratio (1000×)
Saline	34.5±2.5	0.76±0.07	4.2±0.13	31.0±1.51	224.5±1.8	5.9±0.14
DMSO	35.5±2.44	0.77±0.07	4.3±0.10	31.0±1.52	225.7±2.3	5.8±0.13
Cisplatin	117.7±4.0 <sup>a</sup>	3.3±0.08 <sup>a</sup>	2.6±0.12 <sup>a</sup>	59.6±2.20 <sup>a</sup>	154.9±3.5 <sup>a</sup>	12.3±0.26 <sup>a</sup>
Lycopene	28.5±1.2	0.9±0.04	4.0±0.11	32.4±1.50	237.0±5.6	7.0±0.32
Vitamin E	26.2±2.1	0.9±0.05	4.4±0.14	32.4±1.40	240.2±5.2	6.2±0.40
Lycopene+ Cisplatin	36.0±2.2 <sup>b</sup>	0.9±0.03 <sup>b</sup>	3.5±0.16 <sup>a,b</sup>	24.5±1.40 <sup>b</sup>	195.5±5.0 <sup>a,b</sup>	7.2±0.25 <sup>b</sup>
Vitamin E + Cisplatin	39.3±2.2 <sup>b</sup>	1.1±0.05 <sup>a,b</sup>	4.0±0.18 <sup>b</sup>	28.0±1.20 <sup>b</sup>	189.3±3.7 <sup>a,b</sup>	6.6±0.10 <sup>b</sup>

Data are expressed as means ± SEM of six rats per group; <sup>a</sup> significantly different from the control saline group; <sup>b</sup> significantly different from the cisplatin-treated group, using one-way ANOVA, followed by the Tukey's test for multiple comparison between groups at  $p$  ≤ 0.05.

(Table 1 & 2). Moreover, administration of vitamin E for 14 days before cisplatin and 7 days after cisplatin reduced the levels of urea, creatinine and

## DISCUSSION

Cisplatin is an antineoplastic drug used in the treatment of many solid-organ cancers<sup>26</sup>, while the

**Table 2. Effect of treatment lycopene and vitamin E on kidney contents of caspase-3, MDA, and GSH as well as activity of antioxidant enzymes (CAT and SOD) in cisplatin-treated rats**

Group	Caspase-3 (ng/g tissue)	MDA (nmol/g tissue)	GSH ( $\mu$ mol/g tissue)	SOD (U/mg protein)	CAT (U/mg protein)
Saline	0.52±0.03	20.93±0.93	6.06±0.26	21.25±1.87	6.01±0.57
DMSO	0.53±0.03	21.07±1.06	6.52±0.66	21.22±1.5	5.90±0.50
Cisplatin	2.07±0.09 <sup>a</sup>	48.01±1.37 <sup>a</sup>	2.40±0.19 <sup>a</sup>	10.23±1.10 <sup>a</sup>	2.23±0.11 <sup>a</sup>
Lycopene	0.59±0.05	24.15±1.41	6.43±0.31	27.0±3.20	6.28±0.34
Vitamin E	0.60±0.04	21.10±1.81	5.30±0.15	20.70±1.78	5.08±0.50
Lycopene + Cisplatin	0.53±0.06 <sup>b</sup>	30.10±0.60 <sup>a,b</sup>	5.40±0.40 <sup>b</sup>	24.12±2.62 <sup>b</sup>	4.60±0.31 <sup>b</sup>
Vitamin E + Cisplatin	0.67±0.04 <sup>b</sup>	28.73±0.70 <sup>a,b</sup>	5.32±0.35 <sup>b</sup>	25.64±2.33 <sup>b</sup>	4.91±0.22 <sup>b</sup>

Data are expressed as means  $\pm$  SEM of six rats per group; <sup>a</sup> significantly different from the control saline group; <sup>b</sup> significantly different from the cisplatin-treated group, using one-way ANOVA, followed by the Tukey's test for multiple comparison between groups at  $p \leq 0.05$ .

TNF- $\alpha$  in serum by 67%, 68% and 53%, respectively as well as kidney-body weight ratio (46%) and increased the body weight (22%) and the serum albumin level (53%) in comparison with cisplatin-treated group. Furthermore, vitamin E decreased the caspase-3 (67%) and MDA (40%) contents and increased GSH content (121%) as well as activities of SOD and CAT (151% and 117%, respectively) in the kidney tissue in comparison with cisplatin treated group (Table 1 & 2).

Table 3 & Figure 1 illustrate histopathological

**Table 3. Effect of treatment lycopene and vitamin E on histopathological findings of kidney tissues of cisplatin-treated rats**

	Control	Lycopene	Cisplatin	Vitamin E + Cisplatin	Lycopene + Cisplatin
Coagulation necrosis	0	0	++++	++	++
Renal casts	0	0	+++	0	0
Cystic tubules	0	0	+++	0	0
Focal hemorrhage	0	0	++	0	++

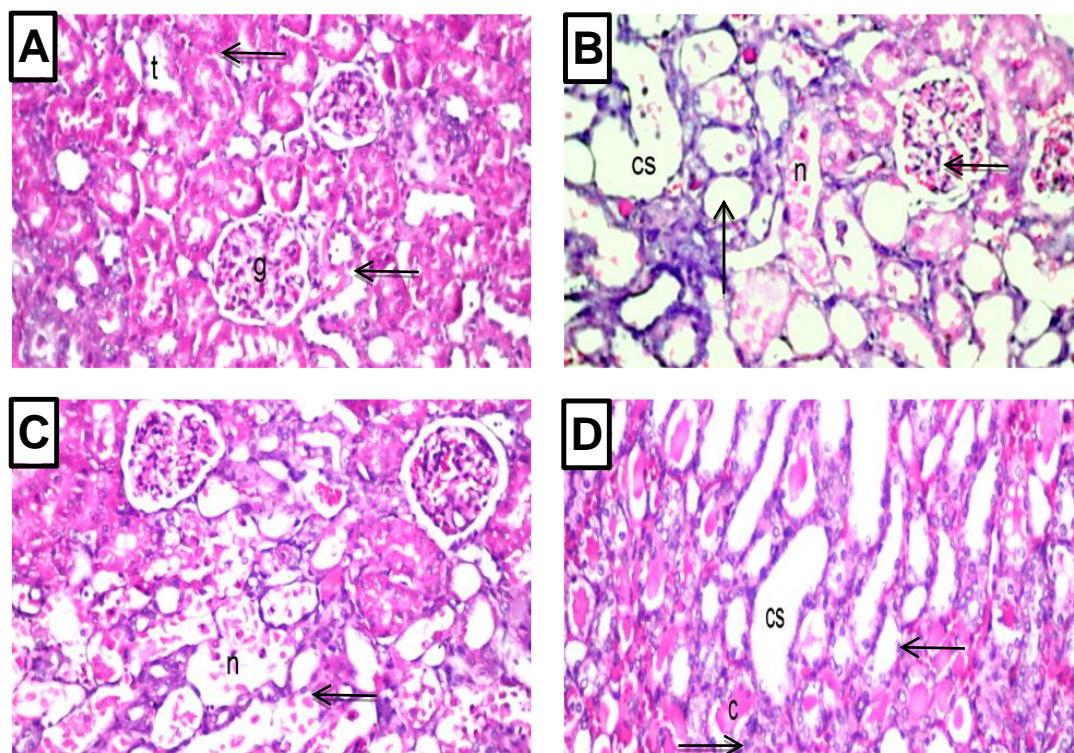
0=none; +=mild; ++=moderate; +++=severe; ++++=very severe.

findings of kidney tissues. The histopathological examination of kidney sections of the control group (saline) showed a normal histological structure (Figure A). On the other hand, administration of cisplatin to rats revealed necrosis in tubules with cystic dilatation at the cortex (Figure B). Pretreatment of the rats with vitamin E (Figure C) and lycopene (Figure D) mitigated the histopathological changes induced by cisplatin.

main dose-limiting side effect of cisplatin is nephrotoxicity<sup>27</sup>, so the strategies of ameliorating the nephrotoxicity of cisplatin are of clinical interest. The present study was conducted to evaluate the possible protective effects of lycopene against cisplatin-induced nephrotoxicity in rats.

Our results showed that cisplatin produced significant elevations in serum creatinine, urea, TNF- $\alpha$  level & kidney-body weight ratio and a significant decrease in the serum albumin level

and body weight. Elevation in serum creatinine and urea levels may be due to the decreased glomerular filtration rate or may be secondary due to the increase of the reactive oxygen species<sup>28</sup>. It was reported that, cisplatin induces cascade of inflammatory reactions with increased production of TNF- $\alpha$  which is responsible for further renal tissue injury<sup>29</sup>. The increase in the kidney-body weight ratio might be attributed to the reduction of body weight from gastrointestinal toxicity<sup>30</sup>.



**Figure 1.** Histology of kidney samples of the control (saline), cisplatin-treated group, vitamin E + cisplatin-treated group, lycopene + cisplatin-treated group. (A) *Control group*: normal histological structure of the glomeruli (g) and tubules (t); (B) *cisplatin-treated group*: necrosis in tubules (n) with cystic dilatation (cs) at the cortex; (C) *vitamin E + cisplatin-treated group*: necrosis in some tubules (n) lining epithelium at the cortex; (D) *lycopene + cisplatin-treated group*: renal eosinophilic casts (c) in the lumen with cystic dilatation (cs). Hematoxylin–eosin staining, magnifications: × 40.

Moreover, apoptosis plays an important role in the pathogenesis of a variety of renal diseases<sup>31</sup>. The results of this study revealed significant elevation of caspase-3 activity in the cisplatin-treated rats. This finding was consistent with a study showing that cisplatin-induced nephrotoxicity is mediated through caspase-3 dependent and independent apoptotic pathways<sup>32</sup>.

Our results showed that cisplatin administration exhibited significant elevation in MDA content with concomitant reduction of GSH content as well as CAT and SOD activities. These findings could be attributed to the ability of cisplatin to generate ROS and to inhibit the activity of the antioxidant enzymes in renal tissue. The increased ROS attack the cell membrane lipids leading to increased tissue lipid peroxides as manifested by increased MDA content<sup>28</sup>.

Our data showed that lycopene produced significant mitigation in the above-mentioned parameters. Our results are consistent with that of Atessahin et al.<sup>33</sup> who revealed the protective effects of lycopene against cisplatin-induced

nephrotoxicity through amelioration of the alterations in serum urea and creatinine levels and body weight. In addition, the authors revealed that lycopene produced significant reduction in MDA content, elevation of GSH content, and enhancement of CAT activity in kidney tissue. Additionally, Buyuklu et al.<sup>34</sup> reported the beneficial effects of lycopene against contrast medium-induced oxidative stress, inflammation, autophagy, and apoptosis in rat kidney.

The histopathological findings demonstrated that administration of cisplatin induced various degenerative changes in kidney cells. In contrast, the pretreatment with lycopene, obviously mitigated the histopathological changes induced by cisplatin.

## CONCLUSION

The results indicate that lycopene has a nephroprotective effect. These actions might be attributed to the antioxidant, anti-inflammatory, and antiapoptotic activities of lycopene. Therefore, lycopene represents potential candidate to prevent cisplatin-induced nephrotoxicity.

## ACKNOWLEDGEMENTS

The authors are grateful to Dr. Adel B. Kholoussy, Professor of Histology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt, for examining and interpreting the histopathological data of this study.

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