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Lycopene Ameliorate the Gentamicin Induced Nephrotoxicity in White Mice Model

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ABSTRACT

Gentamicin is an aminoglycoside antibiotic used against gram-negative bacterial infections. In the face of antibiotics, gentamicin also has a toxic effect on kidney. This study investigated the protective role of lycopene against gentamicin induced nephrotoxicity. Results of this works found that lycopene has a great protective effect on the oxidative status of the kidney challenged with gentamicin and significantly ameliorated ($P \leq 0.05$) the level of oxidative markers MDA, GPx and SOD in kidney cells. Kidney function markers (serum urea and creatinine) were ameliorated by using lycopene in comparison with mice challenged by gentamicin. Results of this work showed that lycopene decreased the pro-inflammatory cytokines (IL-6 and TNF-α) and anti-inflammatory cytokines (IL-4 and IL-10). mRNA expression rate of different investigated cytokine markers were modified significantly ($P \leq 0.05$) in lycopene treated group. Serum glucose also adjusted significantly using lycopene. Oxidative stress results were confirmed by histopathological investigations those showed a protective effect of lycopene of kidney tubules.

Key words: Cytokines, Gentamicin, Kidney, Lycopene, Oxidative stress.

INTRODUCTION

Aminoglycoside antibiotics are broadly utilized in the treatment of gram-negative bacterial infections (Chen and Kaye 2011). These anti-biotics have the potential for causing of nephrotoxicity and it has assessed that practically 25% of patients who get positive clinical effects may encounter nephrotoxicity. Gentamicin preferably accumulates in the proximal renal tubules in cortex due to membrane transporters of proteins and cations in kidney (Selby *et al*., 2009) and gives rise to vascular and tubular damages. Accumulated gentamycin activates reactive oxygen species markers like, ROS, lipid peroxidase (LPO) and serum creatinine. Moreover it was stated that gentamicin toxicity increase the pro-inflammatory cytokines (Priyamvada, *et al*., 2008). Oxidative stress produced by gentamicin leads to activation of caspase mediated apoptosis (Sanchez-Gonzalez *et al*., 2011; El-Bahr, 2015).

Lycopene is a lipid soluble carotenoid pigment with the chemical formula $C_{40}H_{56}$. This compound is responsible for the red color of many fruits such as tomato, grapes and water-melon with the highest concentration in tomatoes (Sigurdson *et al*., 2017). Lycopene was reported to have antioxidant effect due to the nature of its structure. Lycopene can keep many diseases away from the human body such as cancer, hypertension and others (Wildman 2016; Liu, 2017). The antioxidant effect of lycopene was estimated to be ten folds higher as compared to other antioxidants and may reaches up to 100 folds than that for vitamin E in diseases like cancer and in *in-vitro* studies, hence recommended as daily nutrient supplement (Ascenso, *et al*., 2013,). Long time ago, lycopene was used in traditional medicine in treatment of nephrotoxicity and hepatotoxicity as an antioxidant (Sadek *et al*., 2015; Iranshahy *et al*., 2018). Moreover it was also reported to suppress significantly proinflammatory cytokines production in *in-vitro* studies (Shyu *et al*., 2008). Results of in *in-vitro* studies recorded that Lycopene inhibits the expression of inducible nitic oxide synthase gene which consequently inhibit the production of NO induced by bacterial lipopolysaccharide (LPS) (Rafi *et al*., 2007 and Saedisomeolia *et al*., 2009). Proinflammatory cytokines such as IL-6 and tumor necrosis factor alpha (TNF-α) expression had also been reported to be inhibited by lycopene (Liu and Chen, 2016). Cadmium induced nephrotoxicity was recorded to be reduced by lycopene (Sharma and Vijaya, 2015) and adjusted the level of urea, creatinine, Lipid peroxidation and malondialdehyde (MDA) level. According to the high antioxidant effect and protective effect of lycopene and due to the rareness of studies regarding the antibiotic gentamicin, the aim of this study is to clarify the renal protective effect lycopene after challenge by gentamicin.

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MATERIALS AND METHODS

Chemicals

Gentamicin and lycopene were purchased from Sigma Aldrich Company. All chemical those used in the biochemical analysis were purchased from Human Diagnostic Worldwide

(Max-Planck-Ring 21, Wiessbaden, Germany). Antibodies for different cytokines were obtained from Biosource, USA..

Animals

In this study 32 adult male mice weighting around 25-30 g were used. Animals were obtained from Animal facility, Department of Biological Sciences, College of Science, King Faisal University. Mice were acclimatizedd in the laboratory environment of controlled temperature, light/dark cycle for two weeks before the experiment. Mice used in this experiment were supplied by standard pellet diet. Water and food were supplied three times a week. Mice care was in compliance with the guidelines of animal ethical committee of KFU.

Experimental protocol

Mice were divided into four groups (8 mice each). Group I: control group received a daily dose of corn oil for three weeks. Group II: gentamicin group received gentamicin 80 mg/kg of animal body weight for three weeks. Group III: treated group received both gentamicin and lycopene (4mg/kg and 80mg/kg), respectively for three weeks. Group IV: lycopene group received 4 mg/kg for three weeks. At the end of the experiment, animals were anesthetized and serum were collected for biochemical investigations. Kidney were collected and divided into two portions one for histopathological study and the other for the biochemical and molecular analysis.

Biochemical analysis

Different marker for kidney, creatinine and serum urea were investigated. Cytokines (IL-6, TNF-α, IL-4 and IL-10) were investigated using enzyme-linked immunosorbent assay kit (Biosource, USA) according to manufacturer protocol. Oxidative stress markers (MDA, GPx and SOD) were investigated in kidney homogenate according to the company protocol. Serum glucose was also investigated.

Semi-quantitative PCR

Total RNA from kidney tissue was extracted and mRNA purified. The obtained mRNA was reverse transcripted into cDNA using oligo-dt_{12-28} primer (GIBCO-Invitrogen, USA). cDNA stored until use in -20°C. Primers for cytokines (TNF-α, IL-6, IL-4 and IL-10) sequences as shown in (Table, 1) with annealing temperature 54, 50, 52, 51 respectively. The expected product was 438bp, 336bp, 318 and 482.

Histopathological studies

Kidney samples those taken for histopathological investigation subjected to routine hematoxylin and eosin processing. Stained slides were examined under normal light microscope.

Statistical analysis

All one way analysis of variance (ANOVA) was used to analyze all numerical data. The analysis was performed throughout mean, standard deviation, maximum and minimum range. Significant data were considered at (P value ≤ 0.05).

RESULTS AND DISCUSSION

Gentamicin is an antibiotic induces a nephrotoxicity in a great percent of the patient those receive it (Abdel-Raheem *et al*., 2009). The nephrotoxicity induced by gentamicin due to it accumulation selectively in proximal tubule cells leading to loss of cell integrity and finally cell degeneration and apoptosis occur (Liu *et al*., 2016 and Veljković *et al*., 2016). Results of kidney markers in mice serum gave an indication for nephrotoxicity where the level of both marker, urea and creatinine were recorded a highly significant increase (P≤0.05) in the serum of mice challenged with gentamicin (Fig 1). These results were supported and in agree with many previous studies such as (Bapaydin *et al*., 2017, Katary and Salahuddin, 2017). Creatinine and urea low clearance rate from the blood indicate the impaired kidney function (Calderon *et al*., 2010). Mice treated with lycopene showed a significant decrease in serum level of both kidney markers (urea and creatinine) (Fig 1).

The oxidative status of the kidney was determined by estimation of oxidative enzymes markers. Injection of gentamicin cause a significant decrease (P ≤ 0.05) of GPx and SOD in kidney tissue homogenate and increase MDA level (Fig 2). Gentamicin cause an oxidative stress (Chashmi, 2017 and Mosaad *et al*., 2016). This may be due to production of free radicals by enhancing the production

Table 1: primers sequence used in PCR amplification.

Primer name	Primer sequence	Annealing temperature
TNF-α forward	5-atccgagatgtggaactg-3	54°C
TNF-α reverse	5-cacgtagtcggggcagcc-3	
IL-6 forward	5-cttcttgggactgatgtt-3	50°C
IL-6 reverse	5-gtaagttgttcttcacaa-3	
IL-4 forward	5-ccccaccttgctgtcacc-3	52°C
IL-4 reverse	5-tgagttcagaccgctgac-3	
IL-10 forward	5-tgttgcctgccttactg-3	51°C
IL-10 reverse	5-gcagttgatgaagatgtc-3	
β-actin forward	5'-agagctacgagctgcctgac-3'	50°C
β-actin reverse	5'-agcactgtgttggcgtacag-3'	

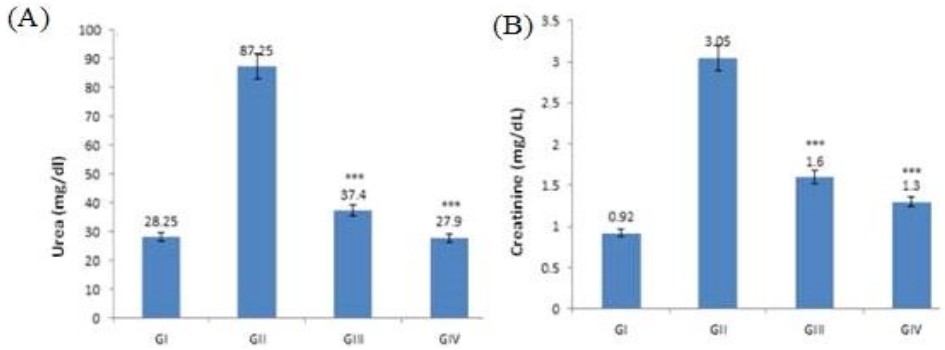

Fig 1: Level of serum urea (a) and creatinine (b). Significant considered at ($P \leq 0.05$).

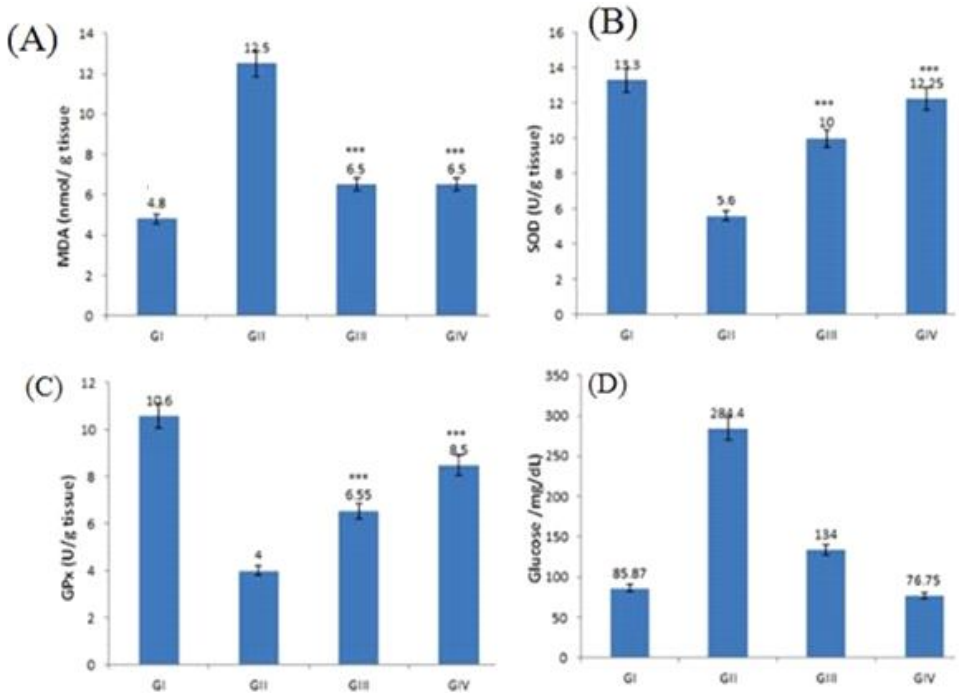

Fig 2: Level of tissue homogenate MDA (a), SOD (b), GPx (c) and serum glucose (d). Significance stated at ($P \leq 0.05$).

of mitochondrial hydrogen peroxide and other ions those leads to peroxidation of membrane lipid and finally disturbance of osmotic balance and cell degeneration. Decreasing of SOD, GPx by gentamicin injection also reported to indicate antioxidants defense which related to glomerular filtration impairment (El-Kashef *et al*., 2015 and Manikandan *et al*., 2011) leading to increasing level of serum glucose (Fig 2). Results of this study show a repairing of SOD, GPx and MDA (Fig 2) where it inhibit the production of reactive oxygen species (ROS). Lycopene is characterized by giving an electron ability which scavenge most of the free radicals induced by gentamicin.this is an agreement with Daniel *et al*., 2015. Results indicate that lycopene is a great antioxidant among the whole carotenoids where the lycopene treated group results usually more or less near to the normal group.

Results also showed that mice challenged with gentamicin showed a significant ($P \leq 0.05$) increase in the level of cytokines (TNF-α, IL-6, IL-4 and IL-10) Fig (3). Treatment of mice with lycopene at the same time results in highly significant ($P \leq 0.05$) decrease of both types of cytokines (pro-inflammatory and anti-inflammatory). Mice those received lycopene only showed a serum cytokines (both types) and glucose more or less near to that of normal group (Fig 3). Serum cytokines estimation of this work reveal a significant increase ($P \leq 0.05$) in all investigated cytokines in the gentamicin challenged mice (Fig 3). The increase of the inflammatory mediators indicate that gentamicin affect the inflammatory oxidative pathway. Inflammatory cytokines had been reported to play an important role in nephrotoxicity (Kirbas *et al*., 2015). Using lycopene treatment significantly decreases the level of both types cytokines (Fig 3). These results were confirmed by semi-quantitative PCR where the expression rate of the TNF, IL-6, IL-4 and IL-10 were changed due to treatment with lycopene (Fig 4). Results obtained by this study were supported with previous one

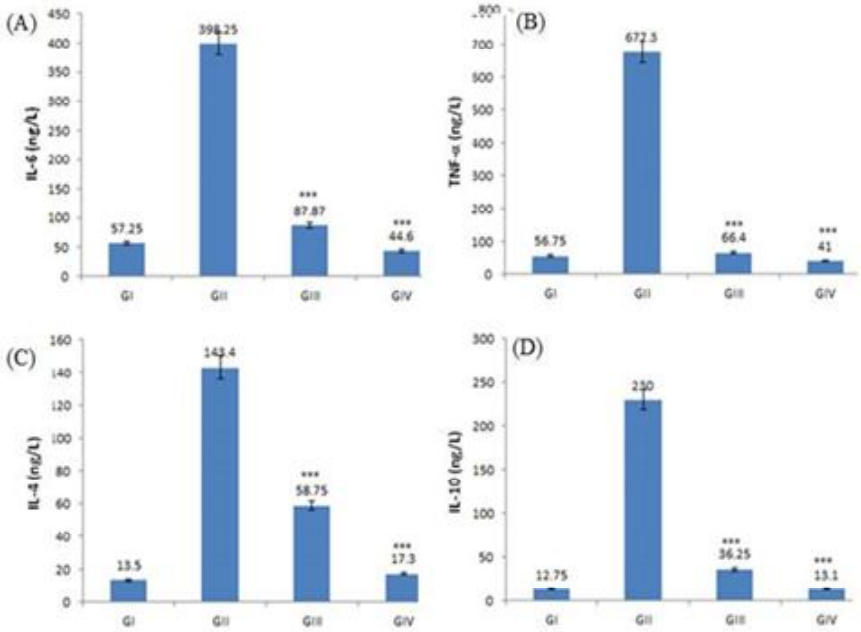

Fig 3: Level of serum proinflammatory cytokines (a and b), anti-inflammatory cytokines (c and d). ($P \leq 0.05$).

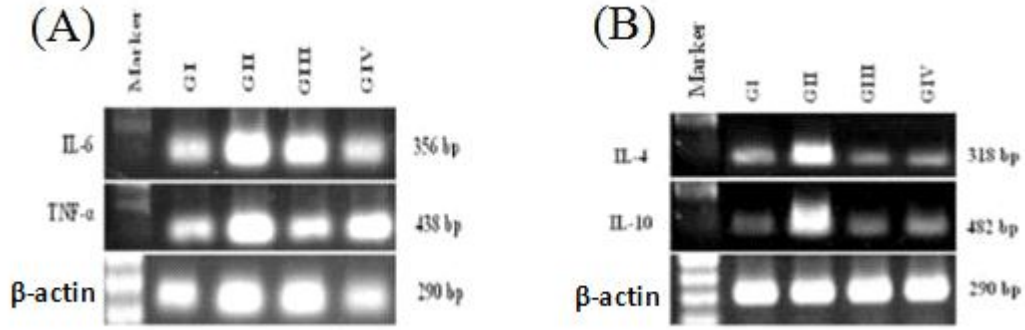

Fig 4: Expression rate of proinflammatory cytokines (a), anti-inflammatory cytokines (b) in kidney homogenate.

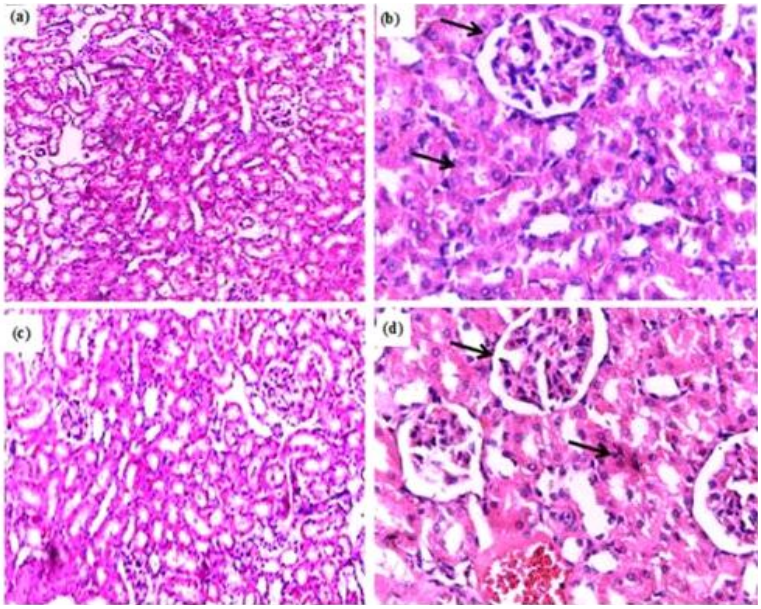

Fig 5: Hematoxylin eosin stained slides control kidney (a and b) sowing the normal architecture of the tubules and gentamicin challenged kidney (c and d) showing partial degeneration of kidney tubular cells

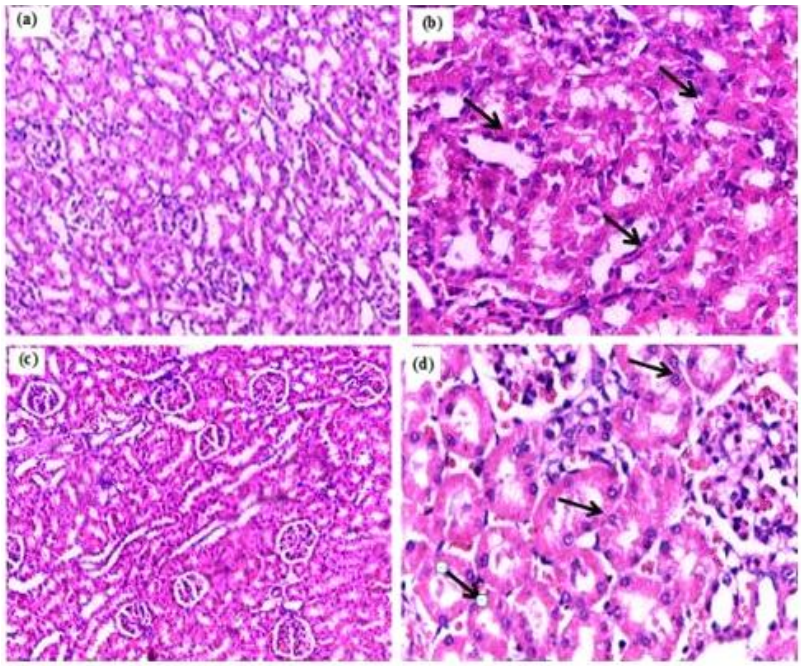

Fig 6: Hematoxylin eosin stained slides of kidney treated with lycopene and gentamicin (a and b) and lycopene only treated mice kidney (c and d) the necrotic nuclei were decreased.

where it was reported that lycopene can alleviate the inflammatory status of the kidney (Oguz *et al*., 2015).

The oxidative status of the kidney tissue induced by gentamicin and its attenuation by lycopene were confirmed by histopathological studies in this work. Stained slides showed a protective effect of lycopene on the kidney parenchyma from damage by oxidation the finally reduces nephrotoxicity (Fig 5,6). Tubular cells necrosis and tubular dilation is also reduced in lycopene treated group (Fig 5,6). Infiltration of the kidney tissue by several leucocytes indicate the inflammatory status of the kidney (Fig 5,6).

CONCLUSION

Results of this work conclude that lycopene is very powerful antioxidant that have a protective effect against the renal toxicity induced by Gentamicin. It is also indicates that lycopene alleviate the inflammatory and the oxidative stress status of the kidney cells and protect the integrity of the cell membrane of the proximal convoluted kidney tubule. So the author support using of lycopene as a protective for kidney diseases in the form of food supplementation.

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Conflict of Interest

The author declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

Abdel-Raheem I.T., Abdel-Ghany A.A., G.A. Mohamed G.A. (2009). Protective effect of quercetin against gentamicin-induced nephrotoxicity in rats. Biological and Pharmaceutical Bulletin: 32: 61- 67.

Ascenso A., Pinho S., Eleut_erio C., Praca F. G., Bentley M.V., Oliveira H., Santos C., Silva O. and Simoes S. (2013). Lycopene from tomatoes: Vesicular nanocarrier formulations for dermal delivery. Journal of Agricultural and Food Chemistry. 61(30): 7284-93.

Bapaydin Y.A., Kordali S., Terim Kapakin K.A., Yildirim F., Aktas S.E., Altin S. (2017). Effect of *Helichrysum plicatum* DC. Subsp. *plicatum* ethanol extract on gentamicin-induced nephrotoxicity in rats. Journal of Zhejiang University SCIENC B: (Biomed & Biotechnol). 18: 501-511.

Calderon R.M., Cubeddu L.X., Schiff E.R. (2010). Statin in the treatment of dyslipidemia in the presence of elevated liver aminotransferase level: a therapeutic dilemma. Mayo Clinic Proceedings. 85: 349–356.

Chashmi N.A., Emadi S., Khastar H. (2017). Protective effects of hydroxytyrosol on gentamicin induced nephrotoxicity in mice. Biochemical and Biophysical Research Communi--cations. 482(4): 1427-1429

Chen L.F. and Kaye D. (2011). Current use for old antibacterial agents: polymyxins, rifamycins and aminoglycosides. The Medical clinics of North America. 95: 819-842. viii–ix.

Daniel E. E., Mohammed A., Tanko Y., Ahmed A. (2015). Effect of lycopene on altered kidney antioxidant enzymes activity and functions in streptozotocin-induced diabetic wistar rats. Cell Biology. 3(1): 1-13.

El-Bahr S.M. (2015). Effect of curcumin on hepatic antioxidant enzymes activities and gene expressions in rats intoxicated with aflatoxin B1. Phytotherapy Research. 29: 134-140.

El-Kashef D.H., El-Kenawi A.E., Suddek G.M., Salem H.A. (2015). Protective effect of allicin against gentamicin-induced nephrotoxicity in rats. International Immunopharmaco--logy. 29: 679-686.

Iranshahy M., Iranshahi M., Abtahi S. R. and Karimi G. (2018). The role of nuclear factor erythroid 2-related factor 2 in hepato -protective activity of nat-ural products: A review. Food and Chemical Toxicology. 120: 261-276.

Katary M., Salahuddin A. (2017). Ameliorative effect of gossypin against gentamicin-induced nephrotoxicity in rats. Life Sciences. 176: 75–81.

Kirbas A., Cure M. C., Kalkan Y., Cure, E., Tumkaya L., Sahin O. Z., Yuce S., Kizilkaya B., Pergel A. (2015). Effect of infliximab on renal injury due to methotrexate in rat. Iranian Journal of Kidney Diseases: 9: 221-229.

Liu P., Feng Y., Dong D., Liu X., Chen Y., Wang Y., Zhou Y. (2016). Enhanced renoprotective effect of IGF-1 modified human umbilical cord-derived mesenchymal stem cells on gentamicin induced acute kidney injury. Scientific Reports: 6: 20287. http://dx.doi.org/10.1038/srep20287.

Liu T.Y. and Chen S.B. (2016). Sarcandra glabra combined with lycopene protect rats from lipopolysaccharide induced acute lung injury via reducing inflammatory response. Biomedicine and Pharmacotherapy. 84: 34-41.

Liu Y., H. Pu and D.W., Sun. (2017). Hyperspectral imaging technique for evaluating food quality and safety during various processes: A review of recent applications. Trends in Food Science and Technology. 69: 25–35.

Manikandan R., Beulaja M., Thiagarajan R., Priyadarsini A., Saravanan R., Arumugam M. (2011). Ameliorative effects of curcumin against renal injuries mediated by inducible nitric oxide synthase and nuclear factor kappa B during gentamicin-induced toxicity in Wistar rats. European Journal of Pharmacology. 670: 578-585.

Mosaad Y.O., Gobba N.A., Hussein M.A. (2016). Astaxanthin; A Promising protector Against gentamicin-induced nephrotoxi--city in rats. Current Pharmaceutical Biotechnology. 17: 1189-1197.

Oguz E., Kocarslan S., Tabur S., Sezen H., Yilmaz Z., Aksoy N. (2015). Effects of Lycopene Alone or Combined with Melatonin on Methotrexate-Induced Nephrotoxicity in Rats. Asian Pacific Journal of Cancer Prevention. 16(14): 6061-6066.

Priyamvada S., Priyadarshini M., Arivarasu N.A., Farooq N., Khan S., Khan S.A. (2008). Studies on the protective effect of dietary fish oil on gentamicin-induced nephrotoxicity and oxidative damage in rat kidney. Prostaglandins Leukotrienes Essential Fatty Acid. 78: 369-381

Rafi M.M., Yadav P.N. and Reyes M. (2007). Lycopene inhibits LPS-induced Proinflammatory mediator inducible nitric oxide synthase in mouse macrophage cells. Journal of Food Sciences. 72: S069–S074.

Sadek K., Abouzed T. and Nasr S. (2015). Lycopene modulates cholinergic dysfunction, Bcl-2/Bax balance and antioxidant enzymes gene transcripts in monosodium glutamate (E621) induced neurotoxicity in a rat model. Candian Journal Physiology and Pharmacology. 94: 394-401.

Saedisomeolia A., Wood L.G., Garg M.L., Gibson P.G. and Wark P.A. (2009). Lycopene enrichment of cultured airway epithelial cells decreases the inflammation induced by rhinovirus infection and lipopolysaccharide. The Journal of Nutritional Biochemistry. 20: 577-585.

Sanchez-Gonzalez P.D., Lopez-Hernandez F.J., Perez-Barriocanal, F. Morales A.I., Lopez-Novoa J.M. (2011). Quercetin reduces cisplatin nephrotoxicity in rats without compromising its anti-tumour activity. Nepherology Dialysis Transplantation. 26: 3484-3495.

Selby N.M., Shaw S.M., Woodier N., Fluck R.J., Kohle N.V. (2009). Gentamicin-associated acute kidney injury. Quarterly Journal of Medicine: 102: 873–880.

Sharma S. and Vijaya P. (2015). Ameliorating potential of lycopene against cadmium toxicity in kidney of albino mice. International journal of Advanced Research. 3: 766-770.

Shyu K.G., Huang W.C., Tai P.A., Hsiao G., Chou D.S., Lee W.L., Chen J.S. and Sheu J.R. (2008). Inhibitory effects of lycopene on the induction of NO, cytokines and mitogen-activated protein kinase expression by lipopolysaccharide in primary cultured microglia. Pharmaceutical Biology. 46: 579-586.

Sigurdson G.T., Tang P. and Giusti M.M. (2017). Natural colorants: Food colorants from natural sources. Annual Review of Food Science and Technology. 8: 261–80.

Veljković M., Pavlović D.R., Stojiljković N., Ilić S., Petrović A., Jovanović I., Radenković M. (2016). Morphological and morphometric study of protective effect of green tea in gentamicin-induced nephrotoxicity in rats. Life Sciences. 147: 85-91.

Wildman R.E. (2016). Handbook of nutraceuticals and functional foods. New York, NY: CRC Press.