The Protective Impacts of *Spriulina Platensis* Against *Cisplatin*-Induced Renal Injury Through The Regulation of Oxidative Stress, Pro-Inflammatory Cytokines and Bax/Bcl2 Expression Cascade

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Abstract

One of the main antineoplastic chemotherapy medications is *cisplatin*, nephropathy is a major side effect of cisplatin. The current study investigates the molecular protective effect of *Spirulina Platensis (SP)* on *cisplatin*-induced nephrotoxicity. Forty eight healthy male albino rats were allocated into 4 groups. Group 1 received saline intraperitoneally (IP) twice per week (normal rats). Group 2, received *SP* (100 mg/kg bw orally). Group 3 injected cisplatin (1.5mg/kg IP) twice per week. Group 4 received *SP* and at 4th day received cisplatin (1.5mg/kg IP) for 21 days. After 3 weeks of experimentation, blood and renal tissues were taken for serum analysis, gene expression using qRT-PCR and renal histopathology. *SP* significantly ameliorated the alterations in the body weight, relative kidney weight, and the disturbance in examined renal markers. Furthermore oxidative stress biomarkers (MDA, NO, SOD, and GSH) induced by cisplatin were recovered and restored by *SP*. *Cisplatin* induced upregulation in the gene expression of *TNF-α, iNOS, TGF1-β, IL-1β and IL-6* that were ameliorated by pre-administration of *SP*. Finally, *cisplatin* upregulated pro-apoptotic gene; *Bax* and downregulated anti-apoptotic gene; *Bcl2*. Of interest, *SP* mitigated this alteration in apoptosis and anti-apoptosis associated genes. Renal histopathology revealed the protective impacts of *SP* against *cisplatin* induced severe glomerular congestion, hemorrhage, inflammatory cell infiltration, degeneration and sever necrosis in renal glomeruli and tubules. In conclusion, *SP* has protective impact against cisplatin induced renal damage through the modulation of oxidative stress, anti-inflammatory, anti-necrotic and anti-apoptotic associated genes.

Introduction

*Cisplatin* is commonly used for chemotherapy to treat different cancers effectively\(^1\). The use of *cisplatin* in treatment of various diseases is restricted due to its serious side effects\(^2\). Nephrotoxicity, neurotoxicity and ototoxicity are common cisplatin side-effects. Nephrotoxicity occurs predominantly in kidney proximal tubule epithelial cells\(^3\), The cisplatin is less toxic in blood level compared to the kidneys, as cisplatin concentration in epithelial tubular cells is five times higher than in the blood\(^4\). Renal dysfunction occurs within days from the usage of cisplatin (50–120 mg/m\(^2\)) in treatment\(^5\). Proximal and distal convoluted tubules are the key targets of cisplatin effects in the kidneys, where it promotes cellular damage, oxidative stress, DNA damage, apoptosis and nephritis\(^6,7\).

Nephrotoxicity and ototoxicity induced by cisplatin are due to toxic generation of reactive oxygen species (ROS) and imbalance of antioxidant glutathione system, as cisplatin is highly reactive with molecules contain thiol such as including glutathione\(^8\). In parallel, cisplatin inhibits various antioxidant enzymes such as glutathione peroxidase (GPX), glutathione-S-transferase (GST), and superoxide dismutase (SOD)\(^9\). Conjugation of glutathione with cisplatin contribute to mitochondrial lysis, oxidative stress and lipid peroxidation causing ROS generation in the cells\(^10\). In addition to ROS, *cisplatin* increases protein expression of renal inducible nitric oxide synthase (iNOS) and the formation of nitrotyrosine that are associated with the stress response\(^11\).
Generation of ROS triggers cascades of signal transduction that results in apoptosis, necrosis. Till now, the mechanism of ROS induces cisplatin signaling pathways still unclear. Lipid peroxidation due to production of highly toxic 4-hydroxynonenal (4-HNE) is one possible pathway. Increased 4-HNE levels cause an increase in Ca\(^{2+}\) influx, and apoptosis\(^{12}\). Mitogen-activated protein kinases (MAPKs), including c-Jun N-terminal protein kinase (JNK), extracellular signal-regulated kinase (ERK), and p38 are also activated by Cisplatin. It is uncertain the pathway of cisplatin to activate MAPKs, but inhibition of MAPKs decreases cisplatin nephrotoxicity. Cisplatin activates EGFR/Src, inducing ERK and caspase-3, leading to apoptosis and nephrotoxicity \(^{13,14}\). A number of cellular stress pathways, including oxidative stress and inflammation leading to activation of p38 and JNK contribute to TNF-alpha transcriptional induction that cause nephrotoxicity \(^{15}\).

* Spirulina platensis (SP) * is a blue-green freshwater algae, naturally grows in warm climate countries and has been regarded as a human and animal food supplement. \(^{16}\) Recent studies demonstrated the protective impacts for human health \(^{17}\). SP is a unicellular filamentous blue-green cyanobacterium, because of its high nutritional value. It is very rich in minerals, essential amino acids, essential fatty acids (ω-3 and ω-6), vitamins A, C, and E, and accessory pigments such as phycobiliproteins. Therefore, it is useful as food by humans and other animals \(^{18}\). In addition, several studies showed that SP species exhibit various biological activities such as anti-inflammatory \(^{19}\) and antioxidant effects \(^{20}\). Therefore, current study was speculated to examine the possible protective impacts of SP against cisplatin induced renal toxicity at different cellular levels.

**Material And Methods**

**Chemicals.** Cisplatin was obtained from EIMC pharmaceutical industry (Cairo, Egypt) as *cisplatin* vials (50mg/ml). Isopropanol and chloroform were obtained from Sigma-Aldrich (St. Louis, MO, USA). TRizol was provided by Genetix Branid Company (Genetix Biotech Asia Pvt. Ltd.). QuantiFast SYBR Green PCR Master Mix was obtained from QIAGEN (Hilden, Germany). HiSenScript\textsuperscript{TM} cDNA synthesis kit was purchased from (iNtRON Biotechnology Company). Creatinine, urea, uric acid, and albumin kits were purchased from Spinreat, S.A with Reference No. # 1001110, 1001323, 1001010, 1001020, respectively. Lipid peroxidation (MDA), superoxide dismutase (SOD), reduced glutathione (GSH), nitric oxide (NO) kits were purchased from Biodiagnostic Company, Cairo, Egypt (Cat number 2529, 2521, GR25H, 2533, respectively). *Spirulina platensis* was from Algal Biotechnology unit, National Research Center Dokki, Cairo, Egypt

**Animals and Experimental design.** The Ethical Committee of Kafrelsheikh University has approved all used procedures and animal use for this study for project 13/2/2018. Forty eight male Wistar rats (170–200 g) were bought from Egyptian Organization for Biological Products and Vaccines (Giza, Egypt). Rats were housed Biochemistry Department, Faculty of Veterinary Medicine, Kafrelsheikh University. Rats were handled daily for 7 days. Rats lived on 12/12 hours light/dark cycle and temperature with range 24 ± 2 °C. All animals gained free access to food and water.
Animals were allotted into 4 groups. Group 1 (control group); injected intraperitoneally saline twice a week for 3 weeks. Group 2 (spirulina platensis; SP) received SP in saline (1000 mg/kg/bw, intragastric) for consecutive 3 weeks. Group 3 (cisplatin-group); rats injected intraperitoneally with cisplatin (1.5 mg/kg) twice a week for 3 weeks. Group 4 (SP + cisplatin), rats received SP as in group 2 and cisplatin as in group 3 for consecutive 3 weeks.

**Blood sampling.** Animals were anaesthetized at the end of the experiment with thiopental sodium (50 mg/kg body weight, IP) \(^{21}\). Blood samples were obtained from rats' retro-orbital venous plexus and obtained in clean and dry centrifuge tubes and held in an ice box, centrifuged for 20 minutes at 3000 rpm after coagulation. Sera was transferred to a clean dry Eppendorf tubes and processed for biochemical analysis at -80 °C.

**Tissue sampling.** Animals were subjected to mild anesthesia using thiopentone sodium of 50 mg/kg of body weight by IP injection at the end of the experiment \(^{21}\), and were sacrificed by decapitation. Kidney tissue was excised and weighed. The right kidney was split into two different sections and rapidly immersed in liquid nitrogen and deposited in 80 °C for RNA extraction quantitative real-time PCR. Part from kidney tissues was used for antioxidant estimation. For histopathology examination, left kidney were immersed in 10 % neutral buffer formalin (NBF).

**Biochemical assays.** All kidney markers such as serum creatinine was estimated utilizing using commercially available kit (Spinreat, S.A # 1001110). Serum urea was measured using commercial available urea kit (Spinreat, S.A # 1001323). Uric acid was estimated using uric acid kit (Spinreat, S.A # 1001010). Albumin was measured using albumin kit (Spinreat S.A # 1001020). MDA, SOD, GSH and NO kits were purchased from (Biodiagnostic Co, Cairo, Egypt) All methods were carried out according to the instructions provided by the manufacturer.

**Histopathology analysis.** Left kidney specimens were sectioned at a thickness of 5 μm and cleared in xylene and stained with H&E \(^{22}\). Microscopic histopathological changes were examined (Leitz DMRBE, Germany). Pictures were taken on the X200 for H&E amplification with an advanced camera (Leica DFC 295).

**RNA extraction and Real-time polymerase chain reaction (PCR).** Prior to RNA extraction, the right kidney tissues were snap-frozen in liquid nitrogen and stored at -80 °C. Following the manufacturer's protocol (GeneZoITM RNA extraction reagent), complete RNA was extracted using Trizol reagent. In RNase free water (Nanodrop 2000c, Thermo Scientific, USA). RNA was dissolved and the integrity was examined in denatured gel. Complementary DNA was blended using the HiSenScriptTM[-] cDNA synthesis kit (iNtRON Biotechnology). By mixing 10μl of 2X RT reaction solution, 1μl of enzyme mix solution, 1μg of RNA and completion of RNase free water to 20μl total amount, complementary DNA was added. The mixture was incubated for 30 minutes at 50 °C and 10 minutes at 85°C. Using real time PCR with SYBR green qRT-PCR, the gene expression levels in the kidney tissue were investigated. The primers were synthesized by Macrogen Co. Seoul, Korea, and were listed in (Table 1).
The gene expression levels in the kidney tissue were determined using real-time PCR using SYBR green. Introductory denaturation at 92 °C for 10 min, accompanied by 40 cycles of 15 seconds at 92 °C, 30 seconds at 60 °C and 30 seconds at 72 °C, according to the PCR thermal cycler machine program. The differences between the groups in gene expression were determined using the method of ΔΔCt (cycle time, Ct), normalized to β-actin, and expressed as relative levels of mRNA compared to placebo.

Data analysis. Prism software (GraphPad Software Inc., San Diego, CA, USA) was used to perform the data analysis. One-way variance analysis (ANOVA) was carried out, followed by a Bonferroni test to assess the statistically relevant differences between groups. Values of \( p \leq 0.05 \) have been deemed statistically significant.

Results

Effect of *spirulina platensis* on Body weight and kidney/Body weight. The body weight was significantly decreased (\( P<0.05 \)) in cisplatin-treated rats by 31.1% comparison with normal rats, and by 29.9% compared to SP alone treated rats. Interestingly, spirulina treatment to cisplatin-treated rats was significantly increase (\( P < 0.05 \)) by 36.8% compared to cisplatin-treated rats as shown in (Figure 1A). Furthermore, rats treated by cisplatin alone showing significantly increase (\( P<0.05 \)) kidney/body weight by 111.5% compared to normal rats and by 96.4% compared to group received spirulina alone. Interestingly, administration of spirulina to cisplatin-treated rats significantly decreased (\( P<0.05 \)) kidney/body weight by 41.5% compared to cisplatin-treated rats (Figure 1B)

Effect of *spirulina platensis* on renal biochemical parameters. Kidney function markers were shown in table (2). Cisplatin injected rats showed an increase in creatinine (\( P < 0.05 \)), which was 214% verse to normal rats and 209.2% verse to rats receiving Spirulina alone. Administration of Spirulina in cisplatin-treated rats decreased the serum creatinine level substantially (\( P < 0.05 \)) by 48.8 % compared with cisplatin group. Serum urea levels in cisplatin-treated rats were decreased significantly (\( P < 0.05 \)) by 219.8 % compared to normal rats, and by 207.5% compared to rats treated by spirulina alone. Spirulina pre-administration to cisplatin-treated rats significantly decreased serum urea by 43.5 % relative to rats treated with cisplatin group.

The level of serum uric acid was significantly increased by 114.4 % compared to normal rats, and by 108 % compared to rats treated with spirulina alone. Serum uric acid was significantly reduced by 45.6 % compared to cisplatin-treated rats by spirulina pre-administration. In cisplatin-treated rats, serum albumin decreased significantly by 57.2 % relative to normal rats, and by 59.4 % compared with group received spirulina alone. Serum albumin levels were increased (73.3%) when spirulina pre-administered to cisplatin group.

Effect of *spirulina platensis* on antioxidant enzyme activity. There was a substantial increase in lipid peroxidation in kidney tissue of cisplatin-treated rats (278.5 %) compared to normal rats and 303.9 % compared to rats treated with spirulina alone. Pre-administration of spirulina to cisplatin group, a
substantial reduction in MDA by 60.6 % was reported. In parallel, NO was a significantly increased in cisplatin-treated rats by 429.7 % compared to normal rats, and by 625.9 % compared to spirulina-treated rats. Spirulina pre-administration to cisplatin-treated rats decreased NO by 62.7 %. Interestingly, spirulina administration induced significant increase in SOD, and GSH activity by 74.1%, and 104.7% respectively compared to cisplatin-treated rats as listed in Table 3.

**Renal histopathological analysis.** Histopathological analysis of the kidney sections of normal and spirulina treated rats showed normal glomerular and tubular structures as shown in (Figure 2A-B). Cisplatin treated rats showed severe glomerular congestion, inflammatory cell infiltration inside the interstitium and serious hemorrhage occurred in the medullar and cortical regions in addition to presence of hyaline cast, sever degeneration and severe necrosis in the tubule as shown in (Figure 2C). Interestingly, the spirulina pre-administration to cisplatin treated rats showed marked decrease in all pathological changes induced by cisplatin (figure 2 D).

**Impacts of *Spirulina platensis* on gene expression analysis.** In order to investigate the impact of *Spirulina* on genes of oxidative stress biomarkers, TNF-a, iNOS and TGF1b were examined using qRT-PCR. TNF-a, iNOS and TGF1b expressions were up-regulated in cisplatin-treated rats compared to normal and *Spirulina* treated rats. Interestingly, reduction in TNF-a, iNOS and TGF1b expression were seen in cisplatin-treated rats pre-administered with *Spirulina* (Figure 3A-C).

In addition, IL-1β, IL-6 mRNA expressions in cisplatin-treated rats were significantly up-regulated compared to normal and Spirulina treated rats respectively. Pre-administration of Spirulina to cisplatin injected rats showed restoration and recovery for alter cytokines (Figure 4 A-B).

Furthermore, we investigated the effect of spirulina administration on apoptotic and anti-apoptotic genes. The result revealed that the expression level of Bax was significantly upregulated in cisplatin-treated animals compared to normal control and spirulina treated rats. *Spirulina* pre-administration for cisplatin-treated rats counteracted levels such upregulation and restored it to nearly control levels (Figure 5A). In contrast to Bax findings, Bcl2 mRNA expression was significantly downregulated in cisplatin-treated rats compared to normal control and *Spirulina* treated rats. Pre-treatment of cisplatin injected rats by *Spirulina* significantly restored and upregulated Bcl2 expression (figure 5B). Such findings confirmed that *Spirulina Platinsis* has anti-necrotic, anti-inflammatory, and anti-apoptotic properties, and confirmed the protective effect of *Spirulina* on renal damage induced by cisplatin (Figure 6).

**Discussion**

Oxidative stress plays a central role in kidney dysfunction, and cisplatin increased the production of superoxide, peroxynitrite and hydrogen peroxide, as cisplatin accumulates in proximal and distal convoluted tubules and caused oxidative stress, apoptosis and inflammation. Current study showed that cisplatin treatment induces renal marker dysfunction as indicated by a significant increase in serum creatinine, urea, uric acid, and decrease in albumin levels. It has been shown that single administration of
cisplatin induced significant renal dysfunction \(^{30,31}\). Interestingly, renal dysfunction induced by cisplatin was ameliorated by *Spirulina platensis* treatment. In a separate study, *Spirulina* dose-dependently ameliorated creatinine, urea clearance and BUN elevation levels \(^{32}\).

Significant increase in NO caused cellular ATP depletion that can inactivate enzymes of Krebs cycle and mitochondrial electron transport enzyme \(^{33}\). Lipids peroxidation, is a marker of tissue damage \(^{34}\), and the antioxidants play a critical role in the prevention of many diseases and protected against a large variety of nephrotoxic chemicals from toxicity \(^{35}\). Cisplatin-treatment increased lipid peroxidation and decreased the activity of antioxidant enzymes, such as SOD, catalase and glutathione peroxidase as reported in this study and in others \(^{36}\). It has been shown that cisplatin induced renal oxidative and nitrosative stress \(^{25,26,34}\). Interestingly, *Spirulina* pre-administration significantly ameliorated the increase in MDA and NO, and the decrease in SOD and GSH levels in cisplatin received rats, and agree with another study that reported the antioxidant activity of *Spirulina* against lead-exposed animals in the liver, lungs, brain, and kidney \(^{20,37}\). *Spirulina*-enriched diet raised cerebellar glutathione levels, decreased lipid peroxidation levels and controlled pro-inflammatory cytokines \(^{38}\). The use of *Spirulina platensis* clearly decreased NO production due to gentamicin injection that might be due to inhibition of iNOS \(^{39}\). In parallel, Spirulina platensis recovered renal damage showed by histopathology as reported by us and others for *Spirulina fusiformis* \(^{32}\).

ROS activates the transcription factor NF-kB induced by cisplatin, which increases the development of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-\(\alpha\) \(^{40}\). ROS induced by cisplatin mediates synthesis of TNF-\(\alpha\) through phosphorylation of p38 MAPK \(^{41}\). Many cytokines have been implicated in cisplatin-induced renal toxicity, among which is TGF-\(\beta\) and hemeoxygenase-1; HO-1 \(^{42}\). Similarly, cisplatin induced up-regulation of several cytokines, such as TNF-alpha, TGF-\(\beta\) and IL-1b in kidney \(^{43}\) that coincided with reported results. Interestingly, all altered cytokines were ameliorated by *Spirulina* administration due to its antioxidant and anti-inflammatory properties \(^{38}\).

Cisplatin caused an increase in the proportion of Bax/Bcl-2 protein expression. Increase in Bax (pro-apoptotic protein) and Bcl2 (anti-apoptotic protein) increases the capacity of renal cells for apoptosis \(^{44,45}\). Cisplatin therapy up regulated Bax and down regulated Bcl2 expression, and *Spirulina* administration down regulated Bax and up-regulates Bcl2 expression \(^{46}\). Collectively, *SP* showed the potential to attenuate the cisplatin nephrotoxicity. *SP* regulated oxidative stress, inflammatory cytokines, apoptosis and anti-apoptosis cascade.

**Conclusion**

Protective impacts of *Spirulina* on cisplatin-induced renal damage was confirmed. The possible mechanisms of *Spirulina* to protect the kidney against cisplatin effects involve antioxidant, anti-necrotic, anti-inflammatory, anti-apoptotic signaling pathways. Therefore, *Spirulina* administration is
recommended to hold the kidney against cisplatin. The collective impacts of spirulina against cisplatin nephrotoxicity are shown in figure 6.

Declarations

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Author Contributions

All authors equally contributed in preparation, writing, data analysis and revising all contents of this manuscript.

Competing Interest

No conflicts of interest was reported for current study.

Data Availability

Data are available from corresponding author upon request

Ethical Statement

All experimental procedures were carried out under Guidelines for the care and use of laboratory animals of National Institutes of Health. All procedures designed to minimize the suffering of animals.

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References

1 Einhorn, L. H. Curing metastatic testicular cancer. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 4592-4595, doi:10.1073/pnas.072067999 (2002).

2 Brock, P. R. *et al.* Platinum-induced ototoxicity in children: a consensus review on mechanisms, predisposition, and protection, including a new International Society of Pediatric Oncology Boston ototoxicity scale. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **30**, 2408-2417, doi:10.1200/jco.2011.39.1110 (2012).
3 Karasawa, T. & Steyger, P. S. An integrated view of cisplatin-induced nephrotoxicity and ototoxicity. *Toxicology letters* **237**, 219-227, doi:10.1016/j.toxlet.2015.06.012 (2015).

4 Kuhlmann, M. K., Burkhardt, G. & Köhler, H. Insights into potential cellular mechanisms of cisplatin nephrotoxicity and their clinical application. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* **12**, 2478-2480, doi:10.1093/ndt/12.12.2478 (1997).

5 Akcay, A., Turkmen, K., Lee, D. & Edelstein, C. L. Update on the diagnosis and management of acute kidney injury. *International journal of nephrology and renovascular disease* **3**, 129-140, doi:10.2147/ijnrd.s8641 (2010).

6 Mohan, I. K. *et al.* Protection against cisplatin-induced nephrotoxicity by Spirulina in rats. *Cancer chemotherapy and pharmacology* **58**, 802-808, doi:10.1007/s00280-006-0231-8 (2006).

7 Schaaf, G. J., Maas, R. F., de Groene, E. M. & Fink-Gremmels, J. Management of oxidative stress by heme oxygenase-1 in cisplatin-induced toxicity in renal tubular cells. *Free radical research* **36**, 835-843, doi:10.1080/1071576021000005267 (2002).

8 Fuertes, M. A., Alonso, C. & Pérez, J. M. Biochemical modulation of Cisplatin mechanisms of action: enhancement of antitumor activity and circumvention of drug resistance. *Chemical reviews* **103**, 645-662, doi:10.1021/cr020010d (2003).

9 Khynriam, D. & Prasad, S. B. Changes in glutathione-related enzymes in tumor-bearing mice after cisplatin treatment. *Cell biology and toxicology* **18**, 349-358, doi:10.1023/a:1020899221192 (2002).

10 Kruidering, M., Van de Water, B., de Heer, E., Mulder, G. J. & Nagelkerke, J. F. Cisplatin-induced nephrotoxicity in porcine proximal tubular cells: mitochondrial dysfunction by inhibition of complexes I to IV of the respiratory chain. *The Journal of pharmacology and experimental therapeutics* **280**, 638-649 (1997).

11 Mukhopadhyay, P. *et al.* Cannabinoid-2 receptor limits inflammation, oxidative/nitrosative stress, and cell death in nephropathy. *Free radical biology & medicine* **48**, 457-467, doi:10.1016/j.freeradbiomed.2009.11.022 (2010).

12 Clerici, W. J., DiMartino, D. L. & Prasad, M. R. Direct effects of reactive oxygen species on cochlear outer hair cell shape in vitro. *Hearing research* **84**, 30-40, doi:10.1016/0378-5955(95)00010-2 (1995).

13 Arany, I., Megyesi, J. K., Kaneto, H., Price, P. M. & Safirstein, R. L. Cisplatin-induced cell death is EGFR/src/ERK signaling dependent in mouse proximal tubule cells. *American journal of physiology. Renal physiology* **287**, F543-549, doi:10.1152/ajprenal.00112.2004 (2004).

14 Henson, E. S. & Gibson, S. B. Surviving cell death through epidermal growth factor (EGF) signal transduction pathways: implications for cancer therapy. *Cellular signalling* **18**, 2089-2097,
15 Mishima, K., Baba, A., Matsuo, M., Itoh, Y. & Oishi, R. Protective effect of cyclic AMP against cisplatin-induced nephrotoxicity. *Free radical biology & medicine* **40**, 1564-1577, doi:10.1016/j.freeradbiomed.2005.12.025 (2006).

16 Ruiz Flores, L. E., Madrigal-Bujaidar, E., Salazar, M. & Chamorro, G. Anticlastogenic effect of *Spirulina maxima* extract on the micronuclei induced by maleic hydrazide in Tradescantia. *Life sciences* **72**, 1345-1351, doi:10.1016/s0024-3205(02)02412-8 (2003).

17 Hirahashi, T. *et al.* Activation of the human innate immune system by *Spirulina*: augmentation of interferon production and NK cytotoxicity by oral administration of hot water extract of *Spirulina platensis*. *International immunopharmacology* **2**, 423-434, doi:10.1016/s1567-5769(01)00166-7 (2002).

18 Kumar, D., Dhar, D. W., Pabbi, S., Kumar, N. & Walia, S. Extraction and purification of C-phycocyanin from *Spirulina platensis* (CCC540). *Indian journal of plant physiology* **19**, 184-188, doi:10.1007/s40502-014-0094-7 (2014).

19 Reddy, C. M. *et al.* Selective inhibition of cyclooxygenase-2 by C-phycocyanin, a biliprotein from *Spirulina platensis*. *Biochemical and biophysical research communications* **277**, 599-603, doi:10.1006.bbrc.2000.3725 (2000).

20 Upasani, C. D. & Balaraman, R. Protective effect of *Spirulina* on lead induced deleterious changes in the lipid peroxidation and endogenous antioxidants in rats. *Phytotherapy research : PTR* **17**, 330-334, doi:10.1002/ptr.1135 (2003).

21 Chatterjee, T. Handbook of laboratory Mice and Rats. *Department of Pharmaceutical Technology, Jadavpur University*, 157 (1993).

22 Bancroft, J. D. & Layton, C. The Hematoxylin and eosin. *Theory & Practice of histological techniques. 7th ed., Churchill Livingstone of El Sevier. Philadelphia*, 173-214 (2013).

23 Matsushima, H., Yonemura, K., Ohishi, K. & Hishida, A. The role of oxygen free radicals in cisplatin-induced acute renal failure in rats. *The Journal of laboratory and clinical medicine* **131**, 518-526, doi:10.1016/s0022-2143(98)90060-9 (1998).

24 Davis, C. A., Nick, H. S. & Agarwal, A. Manganese superoxide dismutase attenuates Cisplatin-induced renal injury: importance of superoxide. *Journal of the American Society of Nephrology : JASN* **12**, 2683-2690 (2001).

25 Lind, D. S. *et al.* Nitric oxide contributes to adriamycin's antitumor effect. *The Journal of surgical research* **69**, 283-287, doi:10.1006/jsre.1997.5015 (1997).
26 Srivastava, R. C. *et al.* Evidence for the involvement of nitric oxide in cisplatin-induced toxicity in rats. *Biometals: an international journal on the role of metal ions in biology, biochemistry, and medicine* **9**, 139-142, doi:10.1007/bf00144618 (1996).

27 Baek, S. M. *et al.* Differential roles of hydrogen peroxide and hydroxyl radical in cisplatin-induced cell death in renal proximal tubular epithelial cells. *The Journal of laboratory and clinical medicine* **142**, 178-186, doi:10.1016/s0022-2143(03)00111-2 (2003).

28 Xiao, T., Choudhary, S., Zhang, W., Ansari, N. H. & Salahudeen, A. Possible involvement of oxidative stress in cisplatin-induced apoptosis in LLC-PK1 cells. *Journal of toxicology and environmental health. Part A* **66**, 469-479, doi:10.1080/15287390306449 (2003).

29 Cummings, B. S. & Schnellmann, R. G. Cisplatin-induced renal cell apoptosis: caspase 3-dependent and -independent pathways. *The Journal of pharmacology and experimental therapeutics* **302**, 8-17, doi:10.1124/jpet.302.1.8 (2002).

30 Winston, J. A. & Safirstein, R. Reduced renal blood flow in early cisplatin-induced acute renal failure in the rat. *The American journal of physiology* **249**, F490-496, doi:10.1152/ajprenal.1985.249.4.F490 (1985).

31 Farooqui, Z. *et al.* Protective effect of *Nigella sativa* oil on cisplatin induced nephrotoxicity and oxidative damage in rat kidney. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* **85**, 7-15, doi:10.1016/j.biopha.2016.11.110 (2017).

32 Kuhad, A., Tirkey, N., Pilkhwal, S. & Chopra, K. Renoprotective effect of *Spirulina fusiformis* on cisplatin-induced oxidative stress and renal dysfunction in rats. *Renal failure* **28**, 247-254, doi:10.1080/08860220600580399 (2006).

33 Förstermann, U. & Sessa, W. C. Nitric oxide synthases: regulation and function. *European heart journal* **33**, 829-837, 837a-837d, doi:10.1093/eurheartj/ehr304 (2012).

34 Kuhad, A., Tirkey, N., Pilkhwal, S. & Chopra, K. Effect of *Spirulina*, a blue green algae, on gentamicin-induced oxidative stress and renal dysfunction in rats. *Fundamental & clinical pharmacology* **20**, 121-128, doi:10.1111/j.1472-8206.2006.00396.x (2006).

35 Forman, H. J., Zhang, H. & Rinna, A. Glutathione: overview of its protective roles, measurement, and biosynthesis. *Molecular aspects of medicine* **30**, 1-12, doi:10.1016/j.mam.2008.08.006 (2009).

36 Antunes, L. M., Darin, J. D. & Bianchi, M. D. Protective effects of vitamin c against cisplatin-induced nephrotoxicity and lipid peroxidation in adult rats: a dose-dependent study. *Pharmacological research* **41**, 405-411, doi:10.1006/phrs.1999.0600 (2000).

37 Upasani, C. D., Khera, A. & Balaraman, R. Effect of lead with vitamin E, C, or *Spirulina* on malondialdehyde, conjugated dienes and hydroperoxides in rats. *Indian journal of experimental biology*
38 Gemma, C. et al. Diets enriched in foods with high antioxidant activity reverse age-induced decreases in cerebellar beta-adrenergic function and increases in proinflammatory cytokines. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* **22**, 6114-6120, doi:10.1523/jneurosci.22-14-06114.2002 (2002).

39 Avdagić, N. et al. Spirulina platensis protects against renal injury in rats with gentamicin-induced acute tubular necrosis. *Bosnian Journal of Basic Medical Sciences* **8**, 331-336, doi:10.17305/bjbms.2008.2892 (2008).

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

41 Ramesh, G. & Reeves, W. B. p38 MAP kinase inhibition ameliorates cisplatin nephrotoxicity in mice. *American Journal of Physiology-Renal Physiology* **289**, F166-F174 (2005).

42 Yao, X., Panichpisal, K., Kurtzman, N. & Nugent, K. Cisplatin nephrotoxicity: a review. *The American Journal of the Medical Sciences* **334**, 115-124 (2007).

43 Ramesh, G. & Reeves, W. B. TNF-α mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity. *The Journal of Clinical Investigation* **110**, 835-842 (2002).

44 Wada, Y. et al. Epidermal growth factor receptor inhibition with erlotinib partially prevents cisplatin-induced nephrotoxicity in rats. *PloS One* **9**, e111728 (2014).

45 Humanes, B. et al. Cilastatin protects against cisplatin-induced nephrotoxicity without compromising its anticancer efficiency in rats. *Kidney International* **82**, 652-663 (2012).

46 Zakaria, M. M. et al. Protective and curative role of Spirulina platensis extracts on cisplatin induce acute kidney injury in rats. *Egyptian Journal of Basic and Applied Sciences* **6**, 54-67 (2019).

**Tables**

Table 1. Primer sequences used for Real time PCR
| Gene   | Direction | Primer sequence               |
|--------|-----------|--------------------------------|
| TNF-a  | Sense     | AAATGGGCTCCCTCTCATCAGTTC       |
|        | Antisense | TCTGCTTGGTGGTTTGCTACGAC        |
| iNOS   | Sense     | TCTTCAAGGACCTACCTCAGGC         |
|        | Antisense | GCTAAGGCAAAGCTGCTAGGTC         |
| TGF-1β | Sense     | TCACTTGTTTTGGTGGATGC           |
|        | Antisense | TTCTGTCTCTCAAGTCCCCC           |
| IL-1β  | Sense     | CACCTCTCAAGCAGAGCAG            |
|        | Antisense | GGGTCCATGGTGAAGTCAAC           |
| IL-6   | Sense     | TCCTACCCCAACTTCAATGCTC         |
|        | Antisense | TTGGATGGTCTTGTCCTTAGCC         |
| Bax    | Sense     | CTGAGCTGACCTTGGAGC             |
|        | Antisense | GACTCCAGCCACAAAGATG            |
| Bcl2   | Sense     | GGAGGGCACTTCCTGAG              |
|        | Antisense | GCCTGGCATCAGC                  |
| β-actin| Sense     | GGACCTGACAGACTACC              |
|        | Antisense | GGCATAGAGGTCTTTACGG            |

**Table 2.** Effect of spirulina on serum biochemical parameters of cisplatin induced nephrotoxicity

|                  | Creatinine (mg/dl) | Urea (mg/dl) | Uric acid (mg/dl) | Albumin (mg/dl) |
|------------------|--------------------|--------------|-------------------|-----------------|
| Normal           | 0.64±0.10b         | 26±2.28c     | 1.94±0.14b        | 5.14±0.40a      |
| Normal + Spirulina| 0.65±0.08b         | 29.2±2.46c   | 2±0.19b           | 5.42±0.21a      |
| Cisplatin        | 2.01±0.17a         | 83.16±4.15a  | 4.16±0.28a        | 2.2±0.16c       |
| Cisplatin + Spirulina | 1.03±0.07b       | 47±4.35b     | 2.26±0.27b        | 3.82±0.17b      |
The data represent the mean ± SE (n=9). One-way ANOVA- Bonferroni's as a post hoc test and; Different liters mean significant at p < 0.05. Same liters mean insignificant at p > 0.05

Table 3: Effect of spirulina on tissue antioxidant activity in cisplatin induced nephrotoxicity

|                      | SOD (u/g) | GSH umol/g | MDA (nmol/g) | NO (umol/g) |
|----------------------|-----------|------------|--------------|-------------|
| Normal               | 77±3.94\(^a\) | 3.63±0.19\(^a\) | 0.056±0.01\(^b\) | 0.37±0.04\(^c\) |
| Normal + Spirulina    | 80.8±4.74\(^a\) | 3.34±0.14\(^a\) | 0.051±0.01\(^b\) | 0.27±0.06\(^c\) |
| Cisplatin            | 39.4±4.20\(^c\) | 1.27±0.13\(^c\) | 0.206±0.01\(^a\) | 1.96±0.08\(^a\) |
| Cisplatin + Spirulina | 68.6±3.15\(^ab\) | 2.6±0.15\(^b\) | 0.081±0.01\(^b\) | 0.73±0.08\(^b\) |

The data represent the mean ± SE (n=6). One-way ANOVA- Bonferroni's as a post hoc test and; Different liters mean significant at p < 0.05. Same liters mean insignificant at p > 0.05

**Figures**

![Body weight comparison](image1)

![Kidney/Body weight comparison](image2)
Figure 1

Effect of Spirulina platensis on (A) body weight, and (B) relative kidney weight to body weight of different studied groups. Data were presented as mean ± SEM at p < 0.05 (Bonferroni’s as a post hoc test) (n=12).
Figure 2

Kidney of normal group (A), Spirulina treatment alone group (B); showing intact glomeruli (G) and renal tubules (T). Cisplatin treated group (C); showing degenerative changes in renal tubules (Black arrow), and shrinkage, increase capsular space of renal glomeruli (arrow head), in addition to presence of hyaline cast (white arrow). Kidney of spirulina with cisplatin treated group (D); showing mild increase of capsular space of glomeruli (arrow head), furthermore intact renal tubules. H&E, X200
Figure 2

Kidney of normal group (A), Spirulina treatment alone group (B); showing intact glomeruli (G) and renal tubules (T). Cisplatin treated group (C); showing degenerative changes in renal tubules (Black arrow), and shrinkage, increase capsular space of renal glomeruli (arrow head), in addition to presence of hyaline cast (white arrow). Kidney of spirulina with cisplatin treated group (D); showing mild increase of capsular space of glomeruli (arrow head), furthermore intact renal tubules. H&E, X200
Figure 3

Effect of Spirulina platensis on gene expression of (A) TNF-α, (B) iNOS, and (C) TGF β in kidney tissue of different studied groups. Data were presented as mean ± SEM at p < 0.05 (Bonferroni’s as a post hoc test) (n=6).
Figure 4

Effect of Spirulina platensis on gene expression of (A) IL-1β and, (B) IL-6, in kidney tissue of different studied groups. Data were presented as mean ± SEM at p < 0.05 (Bonferroni's as a post hoc test) (n=6).
Figure 5

Effect of Spirulina Platensis on gene expression of (A) pro-apoptotic protein (Bax) and, (B) anti-apoptotic protein (Bcl-2) in kidney tissue of different studied groups. Data were presented as mean ± SEM at p < 0.05 (Bonferroni’s as a post hoc test) (n=6).
Figure 6

Spirulina Platensis ameliorates cisplatin-induced renal injury through the regulation of oxidative stress, pro-inflammatory cytokines and Bax/Bcl2 expression cascade (Symbol ⬇️ means inhibit).
Figure 6

Spirulina Platensis ameliorates cisplatin-induced renal injury through the regulation of oxidative stress, pro-inflammatory cytokines and Bax/Bcl2 expression cascade (Symbol $\downarrow$ means inhibit).