Original Paper

Comparative evaluation of the protective effects of garlic and ginger against cisplatin induced nephrotoxicity in a rat model

Mona Yahya[1*], Ashraf El Komy[1], Enas Farag[2], Alyaa Abou Elmaged[3]

[1] Department of Pharmacology, Faculty of Veterinary Medicine, Benha University, Toukh 13736, Egypt.

[2] Deputy of AHRI for regional laboratories. AHRI. ARC.

[3] Department of Pathology, Faculty of Medicine, Menofia University, Egypt

ARTICLE INFO

ABSTRACT

Cisplatin is one of the most frequently used drugs for cancer treatment. However, its use declined due to its dangerous side effects including nephrotoxicity. The aim of this study is to examine the garlic and/or ginger ameliorative impact on cisplatin-caused nephrotoxicity in rats. Seven groups of rats (n=10) were used. Group I was administered 0.5 ml normal saline, group II was administered garlic (200 mg /kg), and Group III was administered ginger (310 mg/kg) orally once time per day for 21 consecutive days. Group IV was administered 0.5 ml normal saline orally once daily and injected cisplatin on 16th day (10 mg/kg, intraperitoneal). Group V was received garlic plus cisplatin, group VI was administered ginger plus cisplatin, and group VII was received garlic and ginger plus cisplatin. Twenty-four hours following the last dose, the blood and tissue samples were collected for the biochemical, antioxidant, and histopathological examinations. Administration of cisplatin significantly increased serum urea level, creatinine level, and uric acid concentration. Furthermore, cisplatin significantly increased MDA level and induced a significant decrease in CAT, GSH, and SOD levels in renal tissue. Histologically, cisplatin induced marked degeneration of the renal tubular epithelium with lymphocytic cellular infiltration of the renal parenchyma. Treatment with ginger and/or garlic restores the levels of renal biochemical markers towards normalcy, improves oxidative stress and histological picture.

1. INTRODUCTION

Cisplatin is one of the efficient chemotherapeutic drugs involved in the treatment of different types of tumors such as breast cancer, head and neck cancer, testicular cancer, and lung cancer (El komy et al., 2020). Cisplatin induced nephrotoxicity by several mechanisms including release of Mitochondrial ROS, mutation of DNA, release of inflammation factor, mitochondrial dysfunction, and through direct cytotoxicity to the renal tubular epithelial cells (McSweeney et. al., 2021). Cisplatin-induced nephrotoxicity can be alleviated by diuretics and pre-hydration of patients. However, the rate of cisplatin nephrotoxicity is still high, and patients do not respond to this management (Oh et al., 2014).

Uses of herbal medicinal products and supplements has increased greatly over the past years. There were a lot of previous studies discussing the importance of these plants and they have become the attention of many researchers. Medicinal plants possess several active constituents that are responsible for their pharmacological action which are used in fungal infection, bacterial infection, rheumatic arthritis, hypertension, diabetes, cancer chemotherapy, and in traditional medicines (Nasr and Saleh, 2014 and Martinsb et al., 2016).

Garlic (*Allium sativum* L.) is one of the most aromatic herbaceous annual spices that have been used from the oldest years as traditional medicine against several common diseases such as stomach disorders, whooping cough, cold, and hypertension (Abdel-Daim et al., 2020). The main active components of garlic include organosulfur compounds, volatile oil containing sulfur compounds, and flavonoids (Abd Elghaffar et al., 2015).

Ginger (*Zingiber officinale*) is one of the safest herbal plants and is usually used as a food spice and in traditional medicine. Ginger contained numerous constituents such as zingerone, gingerdiol, gingerols, shogoals, and zingiberene which are responsible for its antioxidant action as they reduce the generation of free radicals (Al-Rekabi et al., 2019). Ginger is reported to have therapeutic effects such as antimicrobial, gastroprotective, antidiabetic, anti-hypertensive, cardioprotective, anticancer and anti-inflammatory effects (Kandemir et al., 2019).

The present study aimed to illustrate the protective effect of garlic and ginger against cisplatin induced nephrotoxicity through evaluation of serum renal biomarkers, histopathological examination, and oxidative stress estimation.

---

* Corresponding author: monatorky1983@gmail.com

2. MATERIAL AND METHODS

Chemicals:

Cisplatin was purchased from EIMC United Pharmaceuticals (Cairo, Egypt) as Unistin® vial (50 mg/50 ml). Garlic in this study was obtained from ATOS Pharma (Cairo, Egypt) as Tomex plus tablets (300 mg of dried garlic powder). Ginger was obtained from MEPACO Company (Sharquia, Egypt) as Ginger tablets (400 mg of ginger rhizome powder). All kits for biochemical analysis were purchased from Bio Diagnostics Company (Giza, Egypt).

Experimental animals:

Seventy Wister male albino rats, weighing between 160-200 gm were purchased from the Center of Laboratory Animals, Faculty of Veterinary Medicine, Benha University, Egypt and were kept in metal cages at a temperature of $36°C \pm 2°C$. Rats were given a commercial diet and and water *ad libitum.* They were left 14 days for acclimation before beginning the experiment. The study was approved by the Ethics Committee, Faculty of Veterinary Medicine, Benha University' with approval number (BUFVTM 04-01-23).

Experimental design:

Seventy adult male rats were randomly divided into seven groups of 10 animals each as follows. Group I (control) received normal saline; Group II received garlic (200 mg/kg body weight according to Arafat et. al., 2021) and Group III received ginger (310 mg/kg body weight according to Mohamed et. al., (2018) orally, daily for 21 days. Group IV received normal saline daily, orally for 21 days, and on 16th day, rats were injected intraperitoneally with cisplatin (10 mg/kg body weight according to Abo El-Magd et. al., (2021) Group V received garlic orally for 21 days plus cisplatin (I.P on 16th day). Group VI received ginger for orally 21 days plus cisplatin (I.P on 16th day) and group VII received garlic and ginger orally for 21 days plus cisplatin (I.P on 16th day).

Serum collection and tissue sampling:

Twenty-four hours following the last dose, blood samples were collected from the medial canthus of the eye. The blood sample was drawn without anticoagulants in sterilized tubes and the sera were separated and kept at -80°C until used for the bioassays. The rats of all groups were euthanized then the kidney was immediately removed then washed. The first section of the kidney was used in biochemical analysis to estimate the concentration of catalase (CAT), reduced glutathione (GSH), superoxide dismutase (SOD) and malondialdehyde (MDA) as oxidative stress markers. The second part of the kidney was then taken for histological investigations.

Serum biochemical analysis:

Assessment of renal biomarkers:

The concentration of serum urea level, creatinine level, and uric acid level were measured according to methods of Coulombe and Favreau (1963), Bartels et al., (1972), and Walker (1990) respectively. The previous biochemical tests were evaluated in accordance with data protocol provided by using commercial kits (Bio-Diagnostic Company, Giza, Egypt).

Assessment of inflammation markers:

Serum Interleukin-1β (IL-1β) and TNF- α concentrations were measured using rat-specific enzyme-linked immunosorbent assay (ELISA). Analyses were performed according to the manufacturers' instructions by using Rat Interleukin 1b Kit and Rat Tumor Necrosis Factor- α ELISA kit. Briefly, monoclonal antibody specific for rat TNF-α and IL-1β were coated onto the wells of the micro plates. The tissue homogenate, standards, and biotinylated monoclonal antibody specific and streptavidin-HRP were pipetted into these wells and then incubated at 37°C for 60 min. After washing, chromogen reagent A and chromogen reagent B were added, acting upon the bound enzyme to produce a color. It was incubated at 37°C for 10 min. Then a stop solution was added. The intensity of this colored product is directly proportional to the concentration of rat TNF-α and IL-1β present in the original specimen. At the end of the course, the well plates were read at 450 nm. The absorbance of the samples was calculated with formulas using standard graphics.

Tissue homogenate preparation for oxidative stress and antioxidant status evaluation:

The tissue was dissected and rinsed by a PBS solution (phosphate-buffered saline) consisting of 0.16 mg/ml heparin to separate any RBCs and curd. Tissues were homogenized by sonicator homogenizer using 5 ml of 5-10 ml buffer (i.e., 50 mM potassium phosphate, pH 7.5 1 mM EDTA) added each gram of tissue. Tissue homogenates aliquots were centrifuged in a cooling centrifuge (4000 rpm for 20 min) and then kept at -80°C. Next, oxidative status was done by determination of reduced-glutathione (GSH) (Sedlak and Lindsay, 1968), catalase (CAT) activity (Aebi, 1984), superoxide dismutase (SOD) according to the Procedures of Nishikimi et al., (1972) and malondialdehyde (MDA) according to Ohkawa et al., (1979) utilizing specific diagnostic kits get from the Laboratory Bio-Diagnostic Company Giza, Egypt.

Histopathological analysis:

Small tissue specimens were collected from the kidney of rats in all groups and immediately fixed in 10 % formalin. The tissue was dehydrated using gradual concentrations of ethanol, cleared with xylene, and embedded in paraffin. The blocks were cut at a thickness of 5–7 μm. These sections were stained with hematoxylin and eosin (H&E) (Bancroft et al., 2013) and examined for histopathological changes.

Statistical analysis:

The statistical analysis was performed by the SPSS (Version 20; SPSS Inc., Chicago, USA). Statistical analysis was conducted after the application of the Shapiro-Willk test for the determination of the normal distribution of the data. Comparing between groups was performed by one-way ANOVA and followed by Duncan's multiple range test. (Duncan, 1955) A p-value less than or equal to 0.05 was considered significant.

3. RESULTS

Cisplatin toxicity significantly ($P \leq 0.05$) increases the levels of serum urea, creatinine, and uric acid in comparison to their normal level in the control group. Cisplatin-intoxicated rats and pretreated with garlic or ginger showed a significant (P<0.05) decrease in serum urea, creatinine, and uric acid levels in comparison to cisplatin-intoxicated group. The combination of garlic and ginger with cisplatin-intoxicated group showed a a significant (P<0.05) decrease in serum urea, creatinine, and uric acid levels in comparison to cisplatin-intoxicated

group and revealed best results than garlic and ginger independently by restoring these parameters towards normal levels, Fig. (1).

Cisplatin induced a significant (P<0.05) decrease in the level of renal CAT, GSH, and SOD, while cisplatin significantly (P<0.05) increased renal MDA in comparison to their normal level in the control group. Pretreatment with garlic or ginger in cisplatin-intoxicated rats induced a significant (P<0.05) increase of serum CAT, GSH, and SOD levels,while pretreatment with garlic or ginger in cisplatin-intoxicated rats induced a significant (P<0.05) decreas in MDA level as compared with cisplatin-intoxicated rats. The administration of garlic and ginger in cisplatin -treated rats revealed a significant increase of serum CAT, GSH, and SOD levels towards normal levels in control group and improve lipid peroxidation by significant (P<0.05) decrease of MDA level towards normal level as in control group. Moreover, pretreatment of garlic and ginger with cisplatin-intoxicated group was more efficient than garlic and ginger alone in ameliorating oxidative stress, Fig. (2).
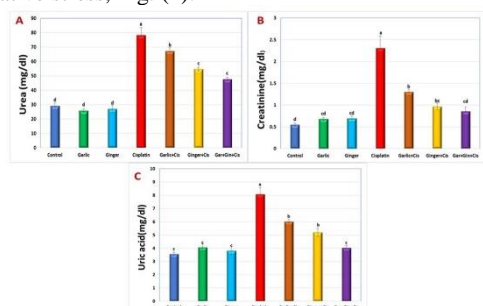

Fig (1) showing effects of garlic or/and ginger on creatinine, uric acid, and blood urea in the rat model of cisplatin induced nephrotoxicity. The mean± standard error of the mean was used to represent the values.
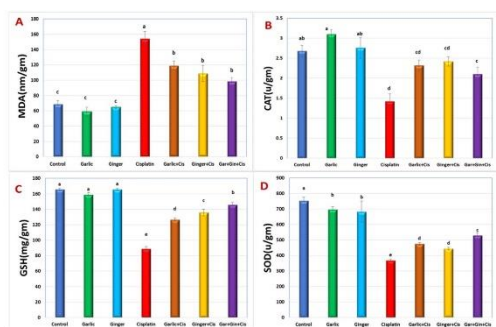

Fig. (2) showing effects of garlic or/and ginger on MDA, CAT, GSH and SOD levels in the rat model of cisplatin induced nephrotoxicity. The mean± standard error of the mean was used to represent the values.

Cisplatin induced a significant (P<0.05) increase in serum levels of inflammatory marker (IL-1β and TNF-α) in comparison to their normal level in the control group. Rats treated with garlic or ginger before administration of cisplatin showed a significant (P<0.05) decrease in IL-1β and TNF-α levels as compared with cisplatin-intoxicated group. The administration of garlic and ginger together with cisplatin induced marked decrease in IL-1β and TNF-α levels towards normal level as in control group and more efficient than garlic and ginger independently, Fig. (3).

The microscopic examination of the kidneys of rats in control, garlic and ginger rats showed normal histoarchitecture of glomeruli and renal tubules with unremarkable changes (Fig.4 A, B, C). In contrast, the examined kidneys of rats intoxicated with cisplatin

revealed degeneration of lining epithelium of renal tubules, and lymphocytic cellular infiltration (Fig 4D and E).

Meanwhile pretreatment with garlic before induction of cisplatin toxicity was partially improved the microscopic pictures of the examined kidneys where focal tubular atrophy with the presence of hyaline casts in the lumens of some renal tubules were recorded (Fig. 4F). Also, pretreatment with ginger reduced the renal cellular damage induced by cisplatin toxicity where only focal mononuclear inflammatory cellular infiltration with degeneration of the renal tubular epithelium with debris in the lumens of some tubules were detected (Fig4G). Moreover, pretreatment with both garlic and ginger was greatly enhanced the microscopic picture of the renal parenchyma where most of the renal tubules were more of less normal and only focal areas of cloudy swelling and widening of glomerular spaces of some glomeruli were found (Fig. 4H).
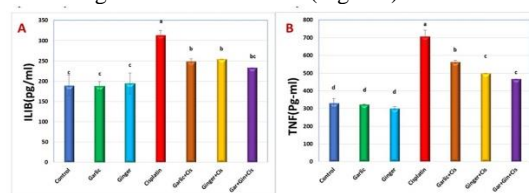

Fig. (3) showing effects of garlic or/and ginger on IL1B and TNF-α levels in induced nephrotoxicity. The mean± standard error -the rat model of cisplatin of the mean was used to represent the values.
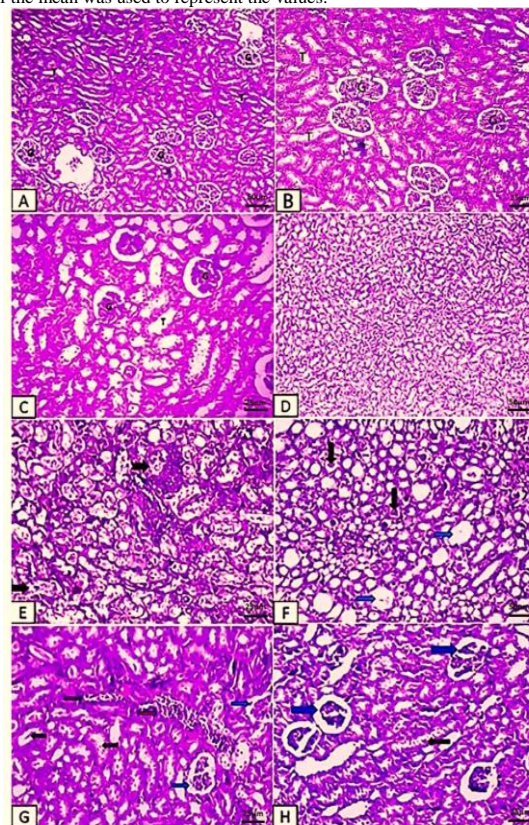

Figure (4) Photomicrographs presented histopathological changes in kidney sections between examined groups. (A): Control group (H&E x100), (B): Garlic(H&Ex200) and (C): Ginger (H&E x200) showing a normal architecture of kidney tissue. (D): Cisplatin (H&E x100) and (E): cisplatin (H&Ex200) showing degenerative renal cell and mononuclear inflammatory cellular aggregation in the interstitial tissue (F): Garlic + Cisplatin (H&E x100) and (G): ginger +cisplatin (H&Ex200) showing focal mononuclear inflammatory cellular infiltration with degeneration of the renal tubular epithelium with debris in the lumens of some tubules. (H): garlic +ginger +cisplatin (H&Ex200) showing near normal appearing renal tubules with focal cloudy swelling.

4. DISCUSSION

Cisplatin causes mitochondrial DNA damage and production of reactive oxygen species that lead to activation of both mitochondrial and non-mitochondrial pathways of apoptosis and necrosis. Mitochondrial energy is also disrupted by cisplatin and may contribute to nephrotoxicity (Miller et al., 2010 and Hajian et al., 2014) In this study cisplatin induced a significant elevation in the levels of urea, creatinine, and uric acid indicating impaired renal functions which confirmed by Salem and Salem (2016) and Sadeghi et al., (2020). Moreover, cisplatin showed a significant elevation in MDA level and a significant reduction of SOD, CAT activities and GSH levels. These results were consistent with Oh et al., (2014) and Eslamifar et al., (2021) who reported that the generation of mitochondrial reactive oxygen species (ROS) in cisplatin-treated rats caused oxidative stress which is responsible for the alteration of these parameters. The histopathological examination of rats intoxicated with cisplatin revealed degeneration of the renal tubular epithelium with lymphocytic cellular infiltration of the interstitial tissues. These lesions were also reported by Ibrahim et al., (2018) and Kandemir et al., (2019).

Cytokines are defined as a large group of peptides, extracellular soluble proteins, or glycoproteins that are secreted by the immune system and are known as a class of signaling molecules that mediate and regulate immunity, inflammation, and hematopoiesis (Duque and Descoteaux, 2014). Inflammatory cytokines including TNF-α play a central role in the inflammatory response and stimulate the production of other inflammatory chemokines and cytokines (Volarevic et al., 2019). In the present study, cisplatin toxicity induced a significant increase in serum TNF-α and IL-1β levels. These data agreed with Li et al., (2015) and Yousef and Hussien (2015).

In the current study, pre-treatment with garlic produced a significant reduction in urea, creatinine, and uric acid levels these results were in agreement with Anusuya et al., (2013), Abdel-Daim et al., (2020) and Farzaneg et al., (2020) who reported the action of garlic in improvement the renal function via its organosulfur compounds which could elevate its antioxidant effect. Garlic administration improve the renal function through a significant decrease in MDA level and a significant increase in SOD level, CAT level, and GSH level of the kidney tissues which caused an improvement in the histopathological changes in cisplatin-intoxicated rats (Nasr and Saleh 2014 and Fang et al., 2021). The treatment with garlic reduced serum levels of IL-1β, and TNF-α compared with the cisplatin-treated group. These data were consistent with El-Sebaey et al., (2019) who reported that the beneficial effect of garlic could be attributed to the powerful antioxidant activity of garlic and its ability to inhibit the production of proinflammatory cytokines.

Ginger administration decreases urea, creatinine, and uric acid levels and returns them to nearly normal levels. These results are consistent with a previous study that the zingiber contains a high concentration of alkaloids and flavonoids that acts as an antioxidant and/or free radical scavenging activity (Mani et al., 2016 and Ali et al., 2020). Furthermore, pretreatment administration of ginger was associated with a significant reduction in MDA level and a significant elevation of SOD, CAT activities, and GSH levels of the renal tissues which resulted significant reduction of cell damage in the kidneys of cisplatin-intoxicated rats. These results were in agreement with Sheriff et al., (2017) and Beagloo et al., (2019) and explained by Kandemir et al., (2019) who revealed that zingerone significantly decreased oxidative stress, inflammation, apoptosis, and histopathological alterations while elevated Aquaporin1 (AQP1) levels in the renal tissue. In addition, Ginger administration showed a significant reduction in the serum level of IL-1β and TNF-α which were consistent with Gholampour et al., (2017) and Ali et al., (2020).

5. CONCLUSION

Cisplatin induced severe alteration of renal biomarkers, lipid peroxidation biomarkers (MDA), antioxidant enzymes (CAT, SOD, and GSH), inflammatory biomarkers (IL-1β, TNF-α), and renal parenchyma. However, the pre-treatment of garlic and/or ginger provided a beneficial role to such prior changes in cisplatin- intoxicated groups through their free radical scavenging and antioxidant activities. Therefore, this study suggests garlic and ginger as useful dietary supplementary compounds for cancer patients under cisplatin treatment.

6. REFERENCES

1. Abdel-Daim, M.M., Abdel-Rahman, H.G., Dessouki, A.A., El-Far, A.H., Khodeer, D.M., Bin-Jumah, M., Alhader, M.S., Alkahtani, S. and Aleya, L., 2020. Impact of garlic (Allium sativum) oil on cisplatin-induced hepatorenal biochemical and histopathological alterations in rats. Science of the Total Environment.710, 136338. doi: 10.1016/j.scitotenv. 2019.136338.
2. Abdel-Ghaffar, S.K., Omar, H.M., Fiedan, I.O. and Ahmed, E.A., 2015. Acrylamide induced testicular toxicity in rats: Protective effect of garlic oil. Reproduction in Domestic Animals. 50, 55-55.
3. Abo El-Magd, N.F., Ebrahim, H.A., El-Sherbiny, M. and Eisa, N.H., 2021. Quinacrine ameliorates cisplatin-induced renal toxicity via modulation of sirtuin-1 pathway. International Journal of Molecular Sciences.1, 22. doi: 10.3390/ijms221910660.
4. Aebi, H. 1984. Catalase in vitro. Method. Enzymol. 105, 121–126. doi: 10.1016/ S0076-6879(84)05016-3
5. Ali, S.A., Kadhem, M.A. and Ali, H.A., 2020. Protective effect of ethanolic extract of zingiber officinale against mercuric chloride induced renal toxicity in rats. Indian Journal of Public Health Research and Development.11, 1118–1123.
6. Al-Rekabi, B.K.K., 2019.The protective role of taurine and zingiber officinale on aluminum chloride induced testicular injury and impaired spermatogenesis in adult male rabbits: Biochemical, hormonal and histopathological study. Plant Archives. 19, 1755-1767.
7. Anusuya, N., Durgadevi, P., Durgadevi, P., Dhinek, A. and Mythily, S., 2013. Nephroprotective effect of ethanolic extract of garlic (allium sativum l.) on cisplatin induced nephrotoxicity in male wistar rats. Asian Journal of Pharmaceutical and Clinical Research. 6, 97-100.
8. Arafat, E.A., Youssef, E.M.I. and Khalaf, H.A., 2021. The possible alleviating effect of garlic supplement on the neural retina in a rat model of hypercholesterolemia: a histological and immunohistochemical study. European Journal of Histochemistry.65, 352-362.
9. Bancroft, J.D., Layton, c., and Suvarna, S. K., 2013. Theory and Practice of Histological Techniques. Amsterdam, the Netheelands, Elsevier.
10. Bartels, H., Böhmer, M. and Heierli, C., 1972. Serum creatinine determination without protein precipitation. Clinica Chimica Acta; International Journal of Clinical Chemistry. 37,193–197.
11. Beagloo, I.E., Valilu, M.R., Motiei, M., Rahbar, M. and Hejazi A., 2019.The antioxidant and hepatoprotective effect of alcoholic extract of ginger against the cisplatin induced

oxidative stress in rats. Biomedical Journal of Scientificand Technical Research. 19, 14240-14245.

12. Coulombe, J.J., and Favreau, L.A., 1963. A new simple semimicro method for colorimetric determination of urea. Clinical chemistry, 9, 102-108. doi: 10.1093/clinchem/9.1.102

13. Duncan, D.B. 1955. Multiple range and multiple F tests. Biometrics 11: 1–42.

14. Duque, A.G. and Descoteaux, A., 2014. Macrophage cytokines: involvement in immunity and infectious diseases. Frontiers in Immunology. 7,491. doi: 10.3389/fimmu.2014.00491.

15. Elkomy, A., Abdelhiee, E.Y., Fadl, S.E., Emam, M.A., Gad, F.A., Sallam, A., Alarifi S., Abdel-Daim M.M. and Aboubakr, M. 2020. L-carnitine mitigates oxidative stress and disorganization of cytoskeleton intermediate filaments in cisplatin-induced hepatorenal toxicity in rats. Frontiers in Pharmacology. 11, 574441. doi: 10.3389/fphar.2020.574441

16. El-Sebaey, A.M., Abdelhamid, F.M. and Abdalla, O.A., 2019. Protective effects of garlic extract against hematological alterations, immunosuppression, hepatic oxidative stress, and renal damage induced by cyclophosphamide in rats. Environmental Science and Pollution Research. 26, 15559–15572. https://doi.org/10.1007/ s11356-019-04993-7

17. Eslamifar, Z., Moridnia, A., Sabbagh, S., Ghaffaripour, R., Jafaripour, L., and Behzadifard. M., 2021. Ameliorative effects of gallic acid on cisplatin-induced nephrotoxicity in rat variations of biochemistry, histopathology, and gene expression. BioMed Research International. 26, 2195238. doi: 10.1155/2021/ 2195238.

18. Fang, C.Y., Lou, D.Y., Zhou, L.Q., Wang, J.C., Yang, B., He, Q.J., Wang, J.J and Weng Q.J., 2021. Natural products: potential treatments for cisplatin-induced nephrotoxicity, Acta Pharmacologica Sinica. 42, 1951–1969; https://doi.org/10.1038/s41401-021-00620-9

19. Farzanegi, P., Abbaszadeh, H., Farokhi, F., Ahmadabad, S.R., Hosseini, S.A., Mazandarani, M.R., Shokrie, M., Vizvari, E., Alinejad, H. and Azarbayjani, M.A., 2020. Attenuated renal and hepatic cells apoptosis following swimming exercise supplemented with garlic extract in old rats. Clinical Interventions in Aging. 15 ,1409–1418.

20. Gholampour, F., Ghiasabadi, F., Owji, S.M. and Vatanparast J., 2017. The protective effect of hydroalcoholic extract of Ginger (Zingiber officinale Rosc.) against iron-induced functional and histological damage in rat liver and kidney. Avicenna Journal of Phytomedicine. 7, 542-553.

21. Hajian, S., Rafieian-Kopaei, M. and Nasri, H., 2014. Renoprotective effects of antioxidants against cisplatin nephrotoxicity. Journal of Nephropharmacology. 1, 39-42.

22. Ibrahim, A., Al-Hizab, F.A., Abushouk, A.I., and Abdel-Daim, M.M., 2018. Nephroprotective effects of benzyl isothiocyanate and resveratrol against cisplatin-induced oxidative stress and inflammation. Frontiers in pharmacology. 9, 1268. doi: 10.3389/fphar.2018.01268

23. Kandemir, F.M., Yildirim, S., Caglayan, C., Kucukler, S. and Eser, G., 2019. Protective effects of zingerone on cisplatin-induced nephrotoxicityin female rats. Environmental Science and Pollution Research. 26, 22562-22574.

24. Li, L., Khan, M.N., Li, Q., Chen, X., Wei, J., Wang, B., Cheng, J., Gordon, J. R., and Li, F., 2015. G31P, CXCR1/2 inhibitor, with cisplatin inhibits the growth of mice hepatocellular carcinoma and mitigates high-dose cisplatin-induced nephrotoxicity. Oncology Reports 33, 751-757.

25. Mani,V., Siddique, A.I., Arivalagan, S., Thomas, N.S. and Namasivayam, N., 2016. Zingerone ameliorates hepatic and renal damage in alcohol-induced toxicity in experimental rats. International Journal of Nutrition, Pharmacology, Neurological Diseases. 6 ,125-132.

26. Martinsb, N., Petropoulosa, S. and Ferreirab, I.C., 2016. Chemical composition and bioactive compounds of garlic (Allium sativum L.) as affected by pre- and post-harvest conditions. A Review Food Chemistry, 211, 41-50.

27. McSweeney, K. R, Gadanec, L.K., Qaradakhi, T., Ali, B.A., Zulli A., and Apostolopoulos, V., 2021. Mechanisms of cisplatin-induced acute kidney injury: pathological mechanisms, pharmacological interventions, and genetic mitigations. Cancers (Basel). 29, 1572. doi: 10.3390/cancers13071572.

28. Miller, R.P., Tadagavadi, R.K., Ramesh, G., and Reeves, W.B., 2010. Mechanisms of cisplatin nephrotoxicity. Toxins (Basel). 2, 2490-518. doi: 10.3390/toxins2112490.

29. Mohamed N.A., Hussein, J., EL-Toukhy, S.E., Mohamed, R.A. and ElKhayat, Z., 2018. Drug-induced nephrotoxicity in rats using zingiber officinale roscoe. ARC Journal of Nephrology. 3, 9-17.

30. Nasr and Saleh, 2014.Aged garlic extract protect against oxidative stress and renal changes in cisplatin-treated adult male rats, Cancer Cell International.14, 92.

31. Nishikimi, M., Appaji, N. and Yagi, K., 1972. The occurrence of superoxide anions in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochemical and Biophysical Research Communications. 46, 849–854.

32. Oh, G.S., Kim, H.J., Choi, J.H., Shen, A., Choe, S.K. and Karna, A., 2014. Pharmacological activation of NQO1 increases NAD (+) levels and attenuates cisplatin-mediated acute kidney injury in mice. Kidney international. 85, 547-560.

33. Ohkawa H., Ohishi N., Yagi K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry. 95, 351-358. doi: 10.1016/0003-2697(79)90738-3.

34. Sadeghi, H., Mansourian, M., PanahiKokhdan, E., Salehpour, Z., Sadati, I., Abbaszadeh-Goudarzi, K., Karna, A., Lee, S.H., Jo, H.J., Yang, S.H. and Kwak, T.H. 2020. Antioxidant and protective effect of Stachys pilifera Benth against nephrotoxicity induced by cisplatin in rats. Journal of Food Biochemistry. 44, e13190. doi: 10.1111/jfbc.13190

35. Salem, N.A. and Salem, E.A. 2016. Protective antioxidants of garlic against lead-induced renal and testicular toxicity in adult male rats. Journal of Heavy Metal Toxicity and Diseases. 1,15

36. Sedlak, J. and Lindsay, R.H., 1968. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Analytical Biochemistry. 25, 192–205.

37. Sheriff, M.H., Abas, A.M., and Abd- El- Rahman, B.M., 2017. protective effect of ginger extract against cisplatin-induced nephrotoxicity in rats, biochemistry letters, 12, 230-247.

38. Volarevic, V., Djokovic, B., Jankovic, M Harrell, C.R., Fellabaum, C., Djonov , V., and Arsenijevic, N.G., 2019. Molecular mechanisms of cisplatin-induced nephrotoxicity: a balance on the knife edge between renoprotection and tumor toxicity. Journal of Biomedical Science. 26, 25. https://doi.org/10.1186/ s12929-019-0518-9.

39. Walker, H.K., Hall, W.D., Hurst, J.W., 1990. Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd edition. Boston: Butterworths .

40. Yousef, M.I. and Hussien, H.M., 2015. Cisplatin-induced renal toxicity via tumor Necrosis factor a interleukin 6, tumor suppressor P53, DNA damage, xanthine oxidase, histological changes, oxidative stress and nitric oxide in rats: protective effect of ginseng. Food Chemistry and Toxicology. 78, 17–25.