Effect of Melatonin on Superoxide Dismutase (SOD) and Kidney Injury Molecule 1 (KIM-1) in Nephrotoxic Male Rat

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Abstract

Background: Melatonin (N-acetyl-5-methoxy tryptamine) is a hormone primarily secreted by the pineal gland at night also it prevents impairment of cellular mitochondrial function and dynamics, reducing the production of oxygen free radicals.

Aim of study: Investigation of melatonin effect on SOD and KIM-1.

Methods: Thirty six male rats were divided into 6 groups I: Negative control group, group II: Positive control nephrotic induced by cisplatin (5mg/kg), group III: rats treated with melatonin 5mg\kg\day after nephrotic induction by cisplatin (5mg/kg), group IV: nephrotic rats treated with melatonin 10mg/kg, Group V: nephrotic rats treated with melatonin 20 mg/kg, group VI: nephrotic rats treated with Berberis vulgaris 100mg /kg. SOD, KIM-1, urea and creatinine were also recorded.

Results: This study showed a reduction in the serum SOD level between negative and positive control groups. In nephrotic group, serum SOD levels were decrease from (43.50 ± 5.78 to 25.50 ± 3.14 pg/ml), highest mean (41.83 ±3.18 pg/ml) was found after treatment with 100 mg\kg Berberis vulgaris compared with the positive control group, also showed significant elevated between 20mg melatonin and positive groups(25.50 ±3.14 to 40.33±3.55)

Nephrotoxicity increase KIM-1 level (from 1.16 ±0.20 to 2.05 ±0.24 ng/l), significant difference were found between the use of 20 mg melatonin and positive control groups i.e. the use of 20 mg of melatonin reduce KIM-1 levels (from 2.05 ±0.24 to 1.45 ±0.28b ng/l).

Nephrotoxicity produced significant elevation in serum urea level compared to negative control, as well as melatonin and Berberis vulgaris treated group. The positive control group showed the highest mean (148.0± 6.78mg/dl) while the negative control group showed the lowest mean (30.83± 2.13mg/dl). Nephrotoxicity produced significant elevation in serum creatinine level compared to negative control as well as melatonin treated groups, and berberis vulgaris treated group. The positive control group showed the highest mean (0.84 ± 0.11mg/dl) while the negative control group showed the lowest mean (0.34833 ± .035 mg/dl).

Conclusions: Melatonin improve the levels of SOD, KIM-1, Urea and creatinine in nephrotoxicity.

Keywords: Nephrotoxicity, Melatonin, kidney injury molecules-1.

1. Introduction

Nephrotoxicity occurs when kidney-specific detoxification and excretion do not work properly due to the damage or destruction of kidney function by exogenous or endogenous toxicants. Exposure to drugs often leads to kidney toxicity. The kidney is the main control system for maintaining body homeostasis, so it is particularly exposed to xenobiotics.

Cisplatin significantly decreased hepatic superoxide dismutase (SOD). There are several molecules that play a role in finding out reactive oxygen species termed antioxidants that are obtained from both exogenous and endogenous sources to avoid oxidative stress. Superoxide dismutase (SOD) is an endogenous enzyme antioxidant that may convert O2•- to H2O2. The absence of SOD demonstrates the elevated oxidative damage that causes various diseases in vivo [1].

KIM-1 is a type I transmembrane glycoprotein and one of the families of genes that make up T-cell immunoglobulin (Tim) and is known to have an immunoglobulin-like domain consisting of an unusual six cysteine that tops a long myosin-like domain in the extracellular region. Also known as hepatitis A virus cellular receptor [2].

Melatonin (N-acetyl-5-methoxy tryptamine) is a hormone primarily secreted by the pineal gland and found in all living organisms and was initially known for its physiological role in controlling the circadian rhythm. It was found to be biosynthesized as a metabolic end product of tryptophan in pineal cells in a higher proportion at night and is easily released into the bloodstream due to its highly lipophilic nature. Melatonin and its metabolites possess free radical scavenging activity [3]. Melatonin has been shown to be a potent free radical scavenger and a stimulator of antioxidant enzymatic machinery in oxidative stress.

The role of melatonin in stimulating antioxidant enzymes was first identified as it was found to significantly increase glutathione peroxidase (GPx) activity. It has been suggested that increased glutathione peroxidase (GPx) activity is associated with increased metabolism of H2O2 to water and oxygen rather than conversion to strong free radicals through a Fenton-type reaction [4].

Primarily, GPx activity complements the antioxidant process of superoxide dismutase (SOD) and catalase (CAT) induced by melatonin. However, increased glutathione peroxidase (GPx) activity utilizes reductive

doi.org/10.31838/hiv22.02.299 Received: 12.06.22, Revised: 13.07.22, Accepted: 05.08.22

glutathione (GSH) and glutathione disulfide precipitation (GSSH) as the oxidized form [5].

Melatonin has been reported to be involved in the induction of G6PD enzymatic activity to protect the cell from oxidative damage. Besides, melatonin also attenuates the enzymatic activity of synthesized nitric oxide (NOS) and nitrogen oxide production which further provides protection from NO mediated by the production of stronger oxidized peroxynitrite. Therefore, melatonin mediates the multifaceted stimulation of antioxidant enzymes and enhances cellular protection against oxidative stress [5]. Therefore, melatonin tends to improve renal protective pathways that counterbalance cisplatin dose-related nephrotoxicity and broadens the therapeutic index of cisplatin [4, 5].

The current study aimed to study the mechanism underlying the protective effect of melatonin against cisplatin-induced renal damage in an in vivo rat model.

2. Materials and Methods

Animals and experimental design

Thirty-six albino Wister male weighing 225–275g were purchased from Iraqi center for cancer research and kept in the animal house of the College of Pharmacy/ Mustansiriyah University, where this study had been conducted. Before being employed in tests, the rats were given a ten-day environmental adaptation period. Animal groups were as follows:

Group 1 (n=6): Negative control group, rats had received normal saline (0.9%) by intraperitoneal (IP) route (10 mL/kg) for 14 consecutive days.

Group 2 (n=6): Positive control group, received normal saline (0.9%) for seven days (day 1-7) followed by a single dose of cisplatin (5mg/kg) in the 8th day, then normal saline again for six consecutive days (day 9-14), all by IP route.

Group 3 (n=6): Rats treated with melatonin 5mg/kg/day (suspended in 0.9 % saline) orally for 14 days, and cisplatin was injected IP on the 8th day.

Group 4 (n=6): Rats treated with melatonin 10mg/kg/day (suspended in 0.9 % saline) orally for 14 days, and cisplatin was injected IP on the 8th day.

Group 5 (n=6): Rats treated with melatonin 20 mg/kg/day (suspended in 0.9 % saline) orally for 14 days, and cisplatin was injected IP on the 8th day.

Group 6 (n=6): Rats treated with Berberis vulgaris 100mg/kg (suspended in 0.9 % saline) orally for 14 days, and cisplatin was injected IP on 8th day.

Serum sample collection

Blood was obtained from all animals at the end of the trail, and rats were anesthetized using the chloroform inhalation procedure. The blood samples were taken through heart puncture using a 10 ml syringe, then placed in a plain tube containing gel and allowed to clot for 15 minutes before being centrifuged at 3000x for 15 minutes to prepare the serum. For ELISA analysis, the serum was kept in Eppendorf tubes at -40°C.

Measurements

Serum KIM-1 were measured by using ELISA (Maybiosource Co., china). Serum SOD levels were measured by method described by Marklund (7).Blood urea levels were measured by spectrophotometer technique (spainreact, Moroco)

3. Result

As shown in table (1) and figure (1); cisplatin induced nephrotoxicity (G2) produced significant elevation in serum SOD level compared to negative control (G1), as well as melatonin treated groups (G4, G5, and G6). There was also significant difference in SOD levels between G3 and G1. The negative control group showed the highest mean (43.50 ± 5.79 pg/ml) while the positive control group showed the lowest mean (25.50 ± 3.17 pg/ml).

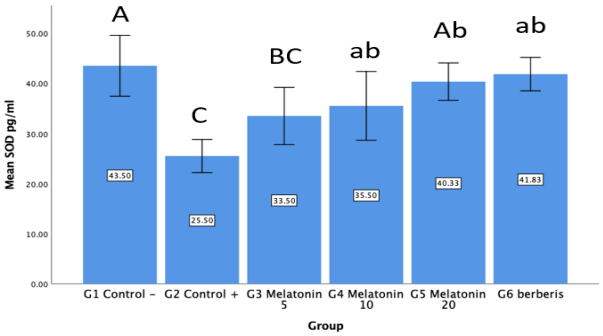

Figure (1): The Effect of melatonin and berberis vulgaris on serum superoxide dismutase levels. Results were expressed as Mean ±SD. Different lower-case letters indicate significant difference between groups (p<0.05) which was done by t test

Table (1): Effect of Melatonin and Berberis vulgaris extract on serum oxidative stress parameters in cisplatin-induced nephrotoxicity rats.

Groups	SOD (pg/ml)
G1: Negative Control, n=6	43.50 ±5.78a
G2: Positive Control, n=6	25.50 ±3.14c
G3:Cisplatin+Melatonin 5mg	33.50 ±5.43bc
G4:Cisplatin+Melatonin 10mg	35.50 ±6.53ab
G5:Cisplatin+Melatonin 20mg	40.33±3.55ab
G6: Cisplatin+ Berberis vulgaris extract	41.83 ±3.18ab
Results were expressed as Mean±SD. Different lower-case letters indicate significant difference between groups (p<0.05) which was done by t test.	

As shown in table (2) and figure (2); cisplatin induced nephrotoxicity (G2) produced significant elevation in serum KIM 1 level compared to negative control (G1), as well as melatonin treated group (G5), and berberis vulgaris treated group (G6). Significant difference was found between G6 and melatonin treated groups (G3, and G4). The positive control group showed the highest mean (2.05 ± 0.24 ng/ml) while the negative control group showed the lowest mean (1.16 ± 0.21 ng/ml).

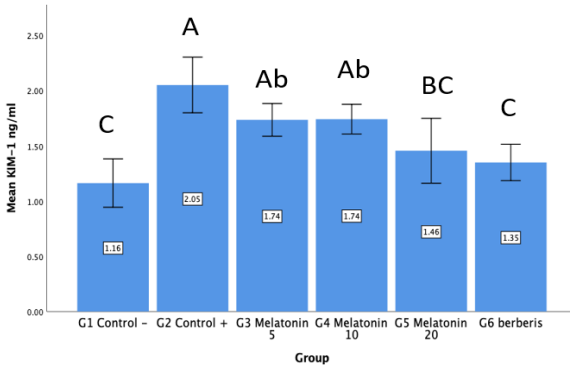

Figure (2): The Effect of melatonin and berberis vulgaris on Kidney Injury Molecule 1 levels.

Results were expressed as Mean ±SD. Different lower-case letters indicate significant difference between groups (p<0.05) which was done by t test.

Table (2): Effect of Melatonin and Berberis vulgaris extract on KIM1 level in cisplatin-induced nephrotoxicity in rats.

Groups	KIM 1 (ng/ml)
G1: Negative Control, n=6	1.16 ±0.20c
G2: Positive Control, n=6	2.05 ±0.24a
G3: Cisplatin+Melatonin 5mg	1.73 ±0.14ab
G4: Cisplatin+Melatonin 10mg	1.74 ±0.13ab
G5: Cisplatin+Melatonin 20mg	1.45 ±0.28bc
G6: Cisplatin+ Berberis vulgaris extract	1.35 ±0.16c
Results were expressed as Mean±SD. Different lower-case letters indicate significant difference between groups (p<0.05) which was done by t test.	

As seen in table (3) and figure (3); cisplatin induced nephrotoxicity (G2) produced significant elevation in serum urea level compared to negative control (G1), as well as melatonin treated groups (G3-G5), and Berberis vulgaris treated group (G6). There was also significant difference in urea level between G1 and melatonin treated groups (G3, and G4). Also, there was significant difference between G3 and G6. The positive control group showed the highest mean (148.0± 6.78mg/dl) while the negative control group showed the lowest mean (30.83± 2.13mg/dl).

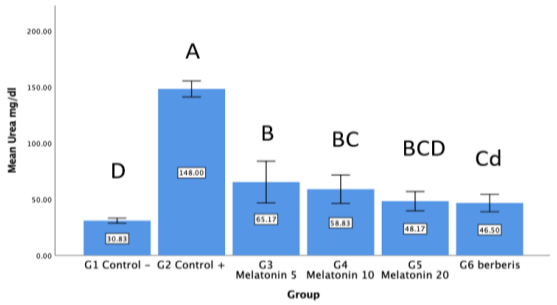

Figure (3): The Effect of melatonin and Berberis vulgaris on serum urea levels. Results were expressed as Mean ±SD. Different lower-case letters indicate significant difference between groups (p<0.05) which was done by t test.

As seen in table (3) and figure (4); cisplatin induced nephrotoxicity (G2) produced significant elevation in serum creatinine level compared to negative control (G1), as well as melatonin treated groups (G3-G5), and berberis vulgaris treated group (G6). There were no significant differences in creatinine levels among G3, G4, and G5 of melatonin nor G6 of berberis vulgaris. The positive control group showed the highest mean (0.84 ± 0.11mg/dl) while the negative control group showed the lowest mean (0.34833 ± .035 mg/dl).

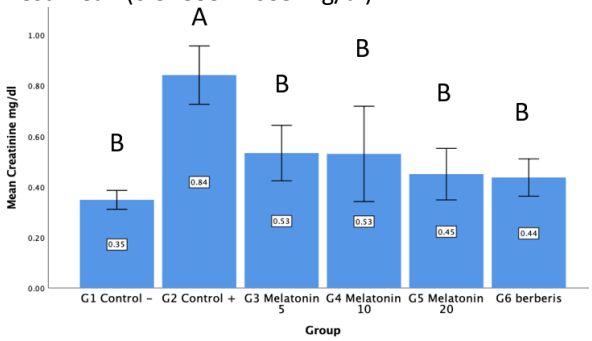

Figure (4): The Effect of melatonin and Berberis vulgaris extract on serum Creatinine levels. Results were expressed as Mean ±SD. Different lower-case letters indicate significant difference between groups (p<0.05) which was done by t test.

Table (3): Effect of Melatonin and Berberis vulgaris on Urea, Creatinine

Groups	Urea (mg/dl)	Creatinine (mg/dl)
G1: Negative Control, n=6	30.83 ±2.14d	0.348 ± 0.035b
G2: Positive Control, n=6	148.00 ±6.78a	0.84 ± 0.10a
G3:Cisplatin+Melatonin 5mg	65.16 ±17.69b	0.53 ±0.10b
G4:Cisplatin+Melatonin 10mg	58.83 ±12.06bc	0.53 ±0.18b
G5:Cisplatin+Melatonin 20mg	48.17 ±8.16bcd	0.45 ±0.097b
G6: Cisplatin +Berberis vulgaris	46.50 ±7.40cd	0.44 ± 0.07b
Results were expressed as Mean±SD. Different lower-case letters indicate significant difference between groups (p<0.05) which was done by t test.		

4. Discussion

Melatonin has been shown to have protective properties under a variety of circumstances, all of which involve the generation of free radical species. For example, melatonin reduces hepatic DNA damage of rats exposed to the carcinogen, alleviates' the free radical-induced suppression of the Ca+2-pump in cardiomyocytes, protects against paraquat-induced oxidative damage and recently melatonin was also shown to prevent lipopolysaccharide-induced oxidative injury in phenobarbital-treated rats, as well as protect against kainic acid-induced neural damage [6].

The current study recorded that SOD levels were decreased by effect of cisplatin induced nephrotoxicity while SOD was found to be increased by melatonin in dose dependent manner as well as with B. vulgaris extract.

Studies had indicated that changes in mitochondrial oxidative metabolism along with altered expression of antioxidants, including SODs, and consequent increased mitochondrial ROS; are crucial in cisplatin-induced AKI

and CKD. Thus, manipulations of mitochondrial ROS via increasing SOD activity may potentially minimize cisplatin-induced kidney injury. Protection of kidneys from cisplatin-induced injury might be achievable by combining cisplatin-based drugs with antioxidant-based therapeutic interventions that increase antioxidant levels and thus mitigate ROS damage while maintaining anti-cancer efficacy. These therapeutic approaches may enhance the tolerance to cisplatin and hence enable greater dose intensity associated with better outcomes [7, 8].

Oxidative stress plays a central role in the pathophysiology of cisplatin-evoked renal toxicity. Previous reports have displayed that cisplatin injection led to overproduction of free radicals, involving hydroxyl radical and superoxide anion, and further cause oxidative injury and lipid peroxidation (LPO) in kidneys. Additionally, it was illustrated that cisplatin damage antioxidant defense mechanisms followed by an apparent reduction in the levels of SOD, and elevation in MDA content [9, 10].

This study elucidated that cisplatin-treated rats exhibited decreased kidneys antioxidant enzymes activities such as SOD. These enzymes are metalloproteins that detoxify peroxides (–OOH), H2O2, and O2.-, respectively. These antioxidant enzymes depend on essential trace elements and prosthetic groups for their structure and activity, and therefore are the potential targets of cisplatin cytotoxicity [11, 12].

Therefore, the decreased activities of these antioxidant enzymes observed in cisplatin -treated rats indicated a failure of antioxidant defense system to overcome the influx of ROS induced by Cisplatin exposure. Furthermore, many reports depicted the inhibition of the antioxidant enzymes activities as the main mechanism of cisplatin induced cytotoxicity [13, 14].

Melatonin has been found to directly scavenge variety of free radicals such as O, OH, ONOO and H2O2. Overall oxidative stress, in aerobes, is initiated with the reduction of molecular or ground state oxygen into superoxide anion (O2−) free radical which further interact and transform into more toxic free radicals such as OH, ONOO and H2O2. Superoxide anion (O2−) is scavenged, naturally, by superoxide dismutase (SOD) into H2O2 which is further metabolized into the water and oxygen [15].

B. vulgaris extract administration increased antioxidant enzyme activities in cisplatin treated rats, which could be induced by lowering free radicals generation [15]. In this concern, B. vulgaris extract could react with free radicals or with lipid peroxidation metabolites, and may also enhance tissue thiol contents, which lead finally to the reduction of oxidative modification of enzymes, and enhancement of antioxidants and glutathione- related enzymes activities [16]. In this context, the beneficial antioxidative effect of B. vulgaris extract has been previously reported in some animal models [15, 16].

KIM-1 levels were increased by effect of cisplatin, then its level decreased by melatonin in dose dependent manner, also, KIM-1 level decreased by B. vulgaris. Elevated levels of KIM-1 in G2 of positive control due to the oxidative stress that resulted from nephrotoxicity which in turn decreases the bioavailable NO and produced signals of KIM-1. This evidence exhibits relation between KIM-1 as well as oxidative stress [17, 18].

KIM-1 is used in both preclinical and clinical studies to identify and monitor drug-induced kidney injury, therefore rapid urinary tests are currently under development. Several studies showed the role of specific biomarkers in diagnosis and follow-up of AKI patients. Among these, KIM-1 also has a possible predictive role in different pathologies alone or associated with other biomarkers. This biomarker was approved by the FDA more than a decade ago, as a nephrotoxic biomarker for different drugs in use.

The prediction ability of novel biomarkers still remains uncertain, however their utility in early diagnosis is of outmost importance. KIM-1 was isolated in atrophic epithelial cells of the proximal tubule; this biomarker shows promise in early diagnosis of renal damage. Its soluble form is a 90 kDa molecule found in the urine of both animals and humans with renal injury. Its presence was associated with AKI in several experimental studies, and some authors suggest that it may be predictive of cisplatin nephrotoxicity. Anti-KIM-1 antibodies have been developed as a potential therapy in neoplasia characterized by KIM-1 overexpression (renal, ovarian, and lung carcinomas) [19].

AKI is the most important dose-limiting side-effects of cisplatin-based chemotherapy. Thus, development of effective pharmacotherapy for cisplatin-induced AKI might have a strong clinical impact. In this study, we found that melatonin treatment significantly ameliorates acute renal failure in cisplatin-treated rats. Since cisplatin is known to induce severe tubular injuries in kidneys, we further investigated the effect of melatonin on expression of tubular injury markers. Elevated levels of KIM-1 in kidneys were found to be reduced by melatonin [27].

Recent studies focused on efficient natural antioxidants, as they may partially prevent or modulate the level of oxidative stress and subsequent inflammation that characterize cisplatin administration. Melatonin is a very well-known antioxidants and has been shown to reduce oxidative stress following cisplatin therapy. The beneficial effects of antioxidant therapy can be explained by the pathophysiological importance of ROS and subsequent mitochondrial dysfunction in the nephrotoxic effects of cisplatin [20].

Tubular cell apoptosis is recognized as a critical process in the renal side-effects of cisplatin therapy. Cisplatin can induce activation of the pro-apoptotic proteins, which form pores in the outer membrane of mitochondria, resulting in induction of cytochrome c release into cytosol and resultant activation of executioner caspases such as caspase-3. Previous studies have shown that in addition to its anti-oxidant and radical-scavenging properties, melatonin exerts an anti-apoptotic effect on various types of cells [21].

One study indicated that administration of B. vulgaris extract (20 and 40 mg/kg, orally) reduced cisplatin-

induced nephrotoxicity via antioxidant, anti-inflammatory and anti-apoptosis activities [22]. Furthermore, berberine decreased the mRNA expression of kidney injury molecule-1(KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), and NF-κB. Also, B. vulgaris extract inhibited the apoptotic effect of cisplatin by increasing the expression of Bcl-2 mRNA in kidney rat.

Acknowledgement

The authors would like to thank Mustansiriyah University (www.uomustansiriyah.edu.iq),
Baghdad, Iraq, for its support in the present work.

5. Reference

1. Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutrition journal. 2015;15(1):1-22. https://doi.org/10.1186/s12937-016-0186-5
2. Ichimura T, Brooks CR, Bonventre JV. Kim-1/Tim-1 and immune cells: shifting sands. Kidney international. 2012;81(9):809-11. https://doi.org/10.1038/ki.2012.11
3. Aulinas A. Physiology of the pineal gland and melatonin. Endotext [Internet]. 2019. Available from: https://www.ncbi.nlm.nih.gov/sites/books/NBK550972/
4. Raza Z, Naureen Z. Melatonin ameliorates the drug induced nephrotoxicity: Molecular insights. nefrologia. 2020;40(1):12-25. https://doi.org/10.1016/j.nefro.2019.06.009
5. Şener G, Şatiroglu H, Kabasakal L, Arbak S, Öner S, Ercan F, Keyer-Uysal M. The protective effect of melatonin on cisplatin nephrotoxicity. Fundamental & clinical pharmacology. 2000;14(6):553-60. https://doi.org/10.1111/j.1472-8206.2000.tb00440.x
6. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European journal of biochemistry. 1974;47(3):469-74.
7. Bonomini F, Borsani E, Favero G, Rodella LF, Rezzani R. Dietary melatonin supplementation could be a promising preventing/therapeutic approach for a variety of liver diseases. Nutrients. 2018;10(9):1135. https://doi.org/10.3390/nu10091135
8. Mapuskar KA, Wen H, Holanda DG, Rastogi P, Steinbach E, Han R, Coleman MC, Attanasio M, Riley DP, Spitz DR. Persistent increase in mitochondrial superoxide mediates cisplatin-induced chronic kidney disease. Redox biology. 2019;20:98-106. https://doi.org/10.1016/j.redox.2018.09.020
9. Singh G. A possible cellular mechanism of cisplatin-induced nephrotoxicity. Toxicology. 1989;58(1):71-80. https://doi.org/10.1016/0300-483X(89)90105-4
10. Ognjanović BI, Djordjević NZ, Matić MM, Obradović JM, Mladenović JM, Štajn AŠ, Saičić ZS. Lipid peroxidative damage on cisplatin exposure and alterations in antioxidant defense system in rat kidneys: a possible protective effect of selenium. International journal of molecular sciences. 2012;13(2):1790-803. https://doi.org/10.3390/ijms13021790
11. Santos N, Bezerra C, Martins N, Curti C, Bianchi M, Santos A. Hydroxyl radical scavenger ameliorates cisplatin-induced nephrotoxicity by preventing oxidative stress, redox state unbalance, impairment of energetic metabolism and apoptosis in rat kidney mitochondria. Cancer chemotherapy and pharmacology. 2008;61(1):145-55. https://doi.org/10.1007/s00280-007-0459-y
12. Sheth S, Mukherjea D, Rybak LP, Ramkumar V. Mechanisms of cisplatin-induced ototoxicity and otoprotection. Frontiers in cellular neuroscience. 2017;11:338.
13. Hassan HA, Edrees GM, El-Gamel EM, El-Sayed EA. Amelioration of cisplatin-induced nephrotoxicity by grape seed extract and fish oil is mediated by lowering oxidative stress and DNA damage. Cytotechnology. 2014;66(3):419-29. https://doi.org/10.1007/s10616-013-9589-8
14. Tan D. Manchester LC, Reiter RJ, Qi WB, Karbownik M, Calvo JR. Significance of melatonin in antioxidative defense system: reactions and products Biol Signals Recept. 2000;9:137-59.
15. Laamech J, El-Hilaly J, Fetoui H, Chtourou Y, Gouitaa H, Tahraoui A, Lyoussi B. Berberis vulgaris L. effects on oxidative stress and liver injury in lead-intoxicated mice. Journal of complementary and integrative medicine. 2017;14(1). https://doi.org/10.1515/jcim-2015-0079
16. Thirupurasundari CJ, Padmini R, Devaraj SN. Effect of berberine on the antioxidant status, ultrastructural modifications and protein bound carbohydrates in azoxymethane-induced colon cancer in rats. Chemico-biological interactions. 2009;177(3):190-5. https://doi.org/10.1016/j.cbi.2008.09.027
17. Edelstein CL. Biomarkers in acute kidney injury. Biomarkers of Kidney Disease. 2017:241-315. https://doi.org/10.1016/B978-0-12-803014-1.00006-6
18. Abood MK, Fadil A. Effect of Tamsulosin on Biomarkers after Ureteral Stones Lithotripsy. Al Mustansiriyah Journal of Pharmaceutical Sciences. 2015;15(2):13-20. https://doi.org/10.32947/ajps.v15i2.166
19. Miller RP, Tadagavadi RK, Ramesh G, Reeves WB. Mechanisms of cisplatin nephrotoxicity. Toxins. 2010;2(11):2490-518. https://doi.org/10.3390/toxins2112490
20. Adeeb A, Dalia A, Mustafa G. Comparison between the Effect of Melatonin and Zinc sulfate in Prevention of cisplatin induced Nephrotoxicity in Rats. AJPS. 2012;12(2):149-60.
21. Mohammadzadeh N, Mehri S, Hosseinzadeh H. Berberis vulgaris and its constituent berberine as antidotes and protective agents against natural or chemical toxicities. Iranian journal of basic medical sciences. 2017;20(5):538. https://doi.org/10.22038%2FIJBMS.2017.8678
22. Adil M, Kandhare AD, Dalvi G, Ghosh P, Venkata S, Raygude KS, Bodhankar SL. Ameliorative effect of berberine against gentamicin-induced nephrotoxicity in rats via attenuation of oxidative stress, inflammation,

apoptosis and mitochondrial dysfunction. Renal failure. 2016;38(6):996-1006.
https://doi.org/10.3109/0886022X.2016.1165120