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Role of melatonin and garlic treatment in cisplatin induced acute kidney injury in adult male rats

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Keywords

- Oxidative stress
- Apoptosis
- Cisplatin
- Melatonin
- Garlic

Abstract

Objective: assess possible role of melatonin and garlic in cisplatin-induced AKI in rats. Materials and methods: Forty adult male rats were divided into 5 groups. Group 1: control group. Group 2: cisplatin group (6 mg/kg) IP injection on the 8th day. Group 3: melatonin group (10 mg/kg/day) in saline orally for 12 days+ IP injection of cisplatin on the 8th day. Group 4: garlic group (500mg/kg/day) in saline orally for 12 days+ IP injection of cisplatin on the 8th day. Group 5: received same doses of melatonin and garlic in saline orally +IP injection of cisplatin on the 8th day. By the end of 12th day, blood samples were collected for biochemical analysis. Renal tissue was examined for oxidative stress markers and caspase3. Results: Histopathology and caspase 3 expression revealed marked damage and marked expression of caspase 3 in group 2. The use of melatonin or garlic caused marked reduction of this damage and caspase 3 expression with the best outcome in combined group. Serum creatinine and urea showed a significant increase in group 2 compared to group 1. However, this elevation was significantly reduced by melatonin in group 3 and garlic in group 4 and best outcome in group 5. KIM-1 significantly increased with cisplatin and decreased with treated groups with best outcome in group 5. The oxidative stress showed a significant improvement in group 5 compared to group 2. Conclusion: Melatonin and garlic may protect against cisplatin induced AKI in rats with best outcome in combined group.

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1. Introduction

Introduction

List of abbreviations:

AKI	Acute kidney injury
BUN	Blood urea nitrogen
Cr	Creatinine
GSH	Reduced glutathione
KIM-1	Kidney Injury Molecule 1
MDA	Malondialdehyde
MMP	Matrix metalloproteinases
NF-κB	Nuclear factor kappa B
Nrf2	nuclear factor erythroid 2-related factor 2

Cisplatin is a chemotherapeutic agent used in management of many tumors of ovary, cervix, colon, lung, and testis**.** However, its clinical usefulness has been lessened due to multiple side effects on various tissues including the liver, kidneys, inner ear, peripheral nerves, testes, and gastrointestinal tract. The kidney is more vulnerable than other tissues for cisplatin toxicity since it absorbs cisplatin at higher concentration than other tissues due to high mitochondrial density in the kidney. There is over production of reactive oxygen species (ROS) in the kidney because cisplatin can block antioxidant enzymes by conjugation with glutathione and finally ROS lead to mitochondrial dysfunction **[1]**. Cisplatin is correlated to high incidence of AKI. Even with just one dose of cisplatin, there is a risk of having AKI**[2]**. AKI is a global health problem with high morbidity and mortality rates **[3]**. Thus, we must develop an effective therapy coadministered with cisplatin to protect the kidney from its harmful impact.

Cisplatin may induce apoptosis through the extrinsic or intrinsic pathways of apoptosis, DNA damage-mediated pathway and endoplasmic reticulum stress-mediated pathway**[4].**

Therefore, it is crucial to reduce cisplatin side effects by co-administeration of powerful antioxidants that can prevent the production of free radicals and apoptosis**[5].** It has been declared that some potent antioxidants offer some protection against renal oxidative stress and apoptosis caused by cisplatin**[6].**

Melatonin; is a hormone produced in the pineal gland and has several vital functions**.** Many previous studies have reported that melatonin has multiple therapeutic properties including anti-oxidant, anti-inflammatory, anticancer and immunomodulatory effects**[7].** In addition, the anti-apoptotic effects of melatonin via mitochondrial pathways have been well documented in previous reports **[8].**

Since ancient times, people have used garlic as a medicine. Research has demonstrated that it possesses anti-inflammatory, antioxidant, anti-cancer, anti-diabetic, anti-atherosclerotic, antibacterial, renoprotective and immunological effects **[9]**. Antioxidant property of garlic is due to ability of garlic to scavenge free radicals and to conserve integrity of cells**[10] .** So the aim of this study was to assess the possible role of melatonin and garlic treatment in cisplatin-induced acute kidney injury in adult male rats.

Materials and Methods

Experimental Animals

This study was conducted on forty adult male Sprague Dawely rats of average weight, (200 – 250 g.) housed in the animal house of Medical Experimental Research Center (MERC), Faculty of

Medicine, Mansoura University. Animals were kept in controlled environmental conditions.Animal care and ethics committee of Mansoura faculty of medicine approved our experimental protocol. Code number: MS.21.02.1371.

Drugs and chemicals:

Cisplatin [1 mg / ml] was purchased from Mylan Pharmaceuticals, France. Melatonin (5mg) in the form of tablets was purchased from Puritans Pride Company. Garlic (300mg) in form of TOMEX PLUS tablet was purchased from Atos pharma company.

Experimental model of cisplatin induced acute kidney injury(AKI):

AKI was induced by single intraperitoneal (IP) injection of cisplatin (6 mg/kg) on the 8th day of experimental period which lasted for 12 days **[11].**

Experimental Groups: Forty adult male Sprague Dawley rats were divided into 5 main groups with 8 rats in each group. Group 1:received 0.9 % saline and 2% carboxymethylcellulose orally for 12 consecutive days. Group 2: received 0.9 % saline orally for 12 consecutive days and only single IP injection of cisplatin (6 mg/kg) on the 8th day **[11].** Group 3: received melatonin (10 mg/kg/day) dissolved in 0.9 % saline orally for 12 days, and only single IP injection of cisplatin on the 8th day **[11].** Group 4: received garlic powder (500mg/kg/day) dissolved in 0.9 % saline orally**[12]** for 12 days, followed by single IP injection of cisplatin on the 8th day.Garlic dose was determined by calculating the weight of all rats in group 4 which was 2 kg so the total dose of garlic was 1000 mg (500 mg multiplied by 2 kg), 1000 mg of garlic powder equals to 3.33 tablets of tomex plus which were grinded and then dissolved in 10 ml of 0.9 % saline. For example, the rat whose weight was 250 g its dose per day was 125 mg garlic powder which is equivalent to 1.25 ml saline (1000 mg garlic was dissolved in 10 ml saline so 125 mg garlic was dissolved in 1.25 ml saline given by oral gavage. Group 5: received melatonin (10 mg/kg/day) and garlic powder (500 mg/kg/day) dissolved in 0.9 % saline orally and single IP injection of cisplatin on the 8th day.

By the end of 12th day for each group, the following investigations were done:

Blood samples collection and obtaining samples of renal tissue:

Blood samples were obtained by puncturing the heart after the rats were sedated, put on a surgical board, and cleaned with ethanol. The abdomen of rat was then opened by making an incision in the center of the xiphoid process. Rats were perfused via inferior vena cava with normal saline then kidney was extracted from the abdomen.

Serum was obtained by centrifugation of blood at 2000 rounds per minute for 10 minutes. After that, sera were frozen and kept at-20ºC for biochemical analyses. One kidney was kept in formalin (10%) for histopathological and immunostaining investigations while the other kidney was kept in liquid nitrogen to measure oxidative stress markers.By the end of the study, rats were euthanized by IP injection of overdose of thiopental sodium at a dose of 800 mg /kg. After necropsy, syringes were put in safety boxes and all carcasses were placed in body bags and labeled with following information ; IACUC method used to insure death , date and initials of person disposing the carcass. After euthenesia , animals were sent to incinerator.

Biochemical assay:

Serum creatinine and serum urea levels:

Using commercial kits from Bio-diagnostic, Egypt (CAT. No. CR 12 50 for creatinine and (CAT. No. UR 21 10) for urea, serum levels of creatinine and urea were determined in accordance with instructions of manufacturer.

Serum KIM-1 assay:

Using rat KIM 1 ELISA kit purchased from EAGLE BIOSCIENCES (USA) CAT# RKM29-K01, levels of serum KIM 1 were determined under instructions of manufacturer.

Assessment of markers of oxidative stress in renal tissue:

A small part of the renal tissue was homogenized in a solution of 50 mMole potassium phosphate, 1mM EDTA, pH 7.5 and aliquoted into several ependorffs. Malondialdehyde (MDA), a lipid peroxidation marker, and reduced glutathione (GSH), an antioxidant, were assessed using colorimetric kits from Bio-Diagnostics , Egypt, in accordance with instructions of manufacturer. (CAT. No. MD 25 29) for MDA and company (CAT. No. GR 25 11) for GSH.

Histopathological examination and immunohistochemistry for caspase 3:

Kidneys were washed with saline and then fixed in 10% formalin solution. After that, renal tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin (H&E) and then examined by light microscope for histopathological evaluation.

Deparaffinization, rehydration, washing, immersion in 3% H2O2, pepsin digestion for antigen retrieval, and overnight incubation with rabbit monoclonal antibody class IgG were all performed on the tissue sections. Diaminobenzidine/peroxidase substrate produced a brown-colored signal. Phosphate buffered solution was used to replace primary antibody and adjacent sections were used as negative control. Caspase 3 was quantified by calculating the percent of renal area occupied by positive staining by use of image j software.

Computer Assisted digital image analysis (Digital morphometric study):

Olympus® digital camera installed on an Olympus® microscope with 1/2 X photo adaptor was used photograph our slides, using 40 X objective. Image analysis was performed by image j software.

Statistical analysis:

Data were entered and statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 23.Quantitative data were described as means (SD) after testing for normality by Shapiro-Wilk test. One way ANOVA (analysis of variance) test, with LSD post-hoc multiple comparisons, was used for comparison between groups. Correlation between parametric continuous variables was done using Pearson's correlation. "p value ≤ 0.05" was considered to be statistically significant.

Results

Biochemical analysis:

Table (1) showed a significant elevation in the serum levels of creatinine, urea and KIM-1 in cisplatin group in comparison to control group ($p < 0.05$) . Also, the cisplatin+melatonin and cisplatin+Garlic groups displayed a significant increase in serum creatinine, urea and KIM-1 compared to control group ($p < 0.05$). Serum creatinine, urea and KIM-1 decreased with

melatonin, garlic and combined groups respectively in comparison to cisplatin group ($p < 0.05$). Garlic and combined groups showed a decline in serum creatinine and KIM-1 levels which are better than melatonin group ($p < 0.05$). Combined group demonstrated a significant decline in serum KIM-1 compared to melatonin group and Garlic group separately ($p < 0.05$).

Oxidative stress markers:

Table (2) showed that cisplatin significantly reduced GSH level and elevated MDA level compared to the control group indicating that oxidative stress state was worsened. Both melatonin and garlic treated rats showed a significantly elevated GSH level and significantly reduced MDA level compared to cisplatin group. Combined group significantly improved oxidative state observed by significant elevation of GSH level and significant reduction of MDA level compared to cisplatin group,melatonin group and garlic group.

Table 1: Values of serum creatinine, blood urea nitrogen (BUN) and serum KIM-1 among study groups.

Measure Mean (SD)	Control group	Cisplatin group	Cisplatin& melatonin group	Cisplatin& garlic group	Cisplatin, melatonin & garlic group	Test of significance ANOVA
Creatinine (mg/dl)	0.5 (0.04)	1.8 (0.4)	0.97 (0.14)	0.75 (0.05)	0.57 (0.01)	F=53.3, p<0.001
		p1 < 0.001	p1 < 0.001 p2 < 0.001	p1 = 0.015 p2 < 0.001 p3 = 0.049	p1 = 0.447 p2 < 0.001 p3 = 0.001 p4 = 0.083	
Urea (mg/dl)	67.3 (3.4)	211.8 (32.7)	98.9 (8.3)	86.4 (4)	72.5 (6.2)	F=118, p<0.001
		p1 < 0.001	p1 < 0.001 p2 < 0.001	p1 = 0.019 p2 < 0.001 p3 = 0.116	p1 = 0.507 p2 < 0.001 p3 = 0.002 p4 = 0.083	
Kim 1(mg/dl)	0.4 (0.02)	2.4 (0.2)	1.6 (0.2)	1.2 (0.08)	0.4 (0.03)	F=396.5, p<0.001
		p1 < 0.001	p1 < 0.001 p2 < 0.001	p1 < 0.001 p2 < 0.001 p3 < 0.001	p1 = 0.727 p2 < 0.001 p3 < 0.001 p4 < 0.001	

Analysis was done by One Way ANOVA with post hoc Tukey's Test. P value < 0.05 is considered significant.
p1=significance as compared to control group
p2=significance as compared to cisplatin group
p3=significance as compared to cisplatin& melatonin group
p4=significance as compared to cisplatin& garlic group

Table 2: Values ofmarkers of tissue oxidative stress; malondialdehyde (MDA), reduced glutathione (GSH) and caspase 3 among study groups.

Measure Mean (SD)	Control group	Cisplatin group	Cisplatin& melatonin group	Cisplatin& garlic group	Cisplatin, melatonin & garlic group	Test of significance ANOVA
MDA (n.mol/g.tissue)	30.9 (6.4)	83.3 (10.6)	54.4 (6.7)	44.8 (4.2)	36.5 (6.3)	F=66.4, p<0.001
		p1 < 0.001	p1 < 0.001 p2 < 0.001	p1 < 0.001 p2 < 0.001 p3 = 0.011	p1 = 0.131 p2 < 0.001 p3 < 0.001 p4 = 0.026	
GSH (m.mol/g.tissue)	2 (0.1)	0.7 (0.2)	1.3 (0.1)	1.5 (0.08)	1.9 (0.1)	F=119.7, p<0.001
		p1 < 0.001	p1 < 0.001 p2 < 0.001	p1 < 0.001 p2 < 0.001 p3 < 0.001	p1 = 0.114 p2 < 0.001 p3 < 0.001 p4 < 0.001	
Capase 3 %	0.5 (0.02)	18.1 (1.1)	8.6 (0.6)	4.7 (0.5)	1.05 (0.02)	F=1180, p<0.001
		p1 < 0.001	p1 < 0.001 p2 < 0.001	p1 < 0.001 p2 < 0.001 p3 < 0.001	p1 = 0.066 p2 < 0.001 p3 < 0.001 p4 < 0.001	

Analysis was done by One Way ANOVA with post hoc Tukey's Test. P value < 0.05 is considered significant.
p1=significance as compared to control group
p2=significance as compared to cisplatin group
p3=significance as compared to cisplatin& melatonin group
p4=significance as compared to cisplatin& garlic group

Histopathological examination:

Fig.(1) represents the result of histopatliotogicol examination of the kidney; with normal renal tissue with intact glomeruli and tubules in control group. Cisplatin group showed marked tubular necrosis with desquamated epithelial cells, vaculation, tubular dilatation and hyaline cast. Cisplatin+melatonin group showed reduction of structural damage compared to cisplatin group by minimal hyaline cast and minimal vaculation. Cisplatin +garlic group demonstrated normal structure of most of renal tubules with few scattered hyaline cast indicating decrease of structural damage compared to cisplatin group. Combined group(cisplatin+melatonin+garlic) demonstrated normal structure of glomeruli and tubules ,no hyaline cast and absence of epithelial degeneration and that results indicate best outcome by combination of melatonin and garlic**.**

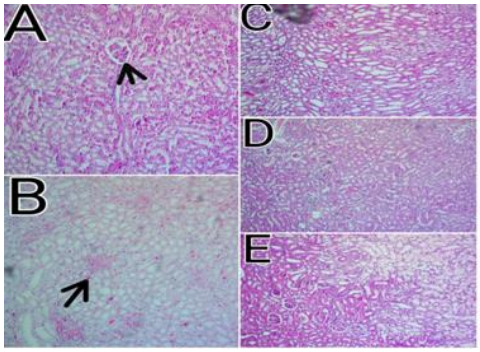
Immunohistochemistry for renal caspase 3:

Table (2) and figure (2) showed a significant increase in level of caspase 3 in cisplatin group compared to control group as well as to all other treated groups ($p < 0.05$). Level of caspase 3 in cisplatin& melatonin group showed significant increase compared to control and significant decrease compared to cisplatin group ($p < 0.05$). In cisplatin& garlic group, level of caspase 3 showed significant increase compared to control and significant decrease compared to cisplatin group and cisplatin& melatonin group .Caspase 3 level in combined group showed non-significant increase compared to control but showed significant

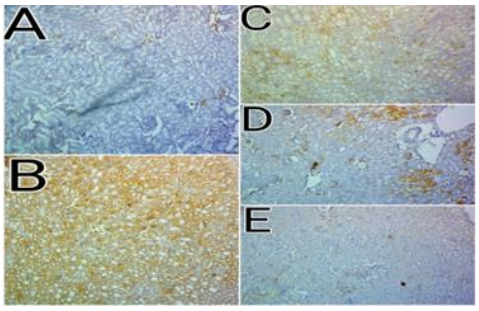
decrease compared to all other groups ($p < 0.05$).

Correlation betweenKIM-1 and kidney biomarkers:

Table (3) showed a strong significant positive correlation between KIM-1 and kidney biomarkers (serum creatinine, serum urea, MDA and caspase 3) in all study groups (Control, Cisplatin, Cisplatin& melatonin, Cisplatin& garlic, Cisplatin, melatonin & garlic). A strong significant negative correlation was found between KIM-1 and GSH .



Figure(1): Histopathological examination in different groups: (A) control group with normal renal structure and intact glomeruli and tubules.(B) cisplatin group with marked tubular necrosis desquamated epithelial cells, hyaline cast and vaculation. (C) cisplatin+melatonin group showed tubular necrosis with minimal hyaline cast. (D) cisplatin+garlic group showed normal structure of most of tubules with few scattered hyaline cast.(E)) cisplatin+melatonin+ garlic group restored normal renal structure with very few hyaline cast and without tubular vaculation.



Figure(2): Immunohistochemical staining of caspase 3 in rat kidney in diffedrent experimental groups. (A) control group showed no expression of caspase 3.(B) cisplatin group without treatment showed a significant increase in caspase 3 immunoreactivity. (C) cisplatin+melatonin group showed mild reduction in caspase 3 immunoreactivity. (D) cisplatin+garlic group demonstrated moderate reduction in immunoreactivity. (E) combined group demonstrated marked reduction in caspase 3 immunoreactivity. Brown color indicates caspase 3 positivity. X400

Table (3): Correlation between KIM-1 and kidney biomarkers:

Measure		**Control group**	**Cisplatin group**	**Cisplatin& melatonin group**	**Cisplatin& garlic group**	**Cisplatin, melatonin & garlic group**
Creatinine	r	0.959	0.973	0.988	0.97	0.924
	p	0.0001	0.0001	0.0001	0.0001	0.001
Urea	r	0.85	0.923	0.897	0.9	0.914
	p	0.007	0.001	0.003	0.002	0.001
MDA	r	0.955	0.946	0.989	0.936	0.821
	p	<0.0001	<0.0001	0.0001	0.001	0.012
GSH	r	-0.908	-0.966	-0.874	-0.961	-0.936
	p	<0.002	<0.0001	0.005	0.0001	0.001
Caspase3	r	0.951	0.989	0.926	0.771	0.962
	p	<0.001	<0.001	0.001	0.025	<0.001

(r) represent correlations coefficient. $p < 0.05$ in each correlations indicates significance

Discussion

This study is considered as a trial on the path of finding an effective defense against cisplatin induced AKI. This study explored the effect of combining melatonin and garlic to see if they would afford greater protection on cisplatin induced AKI than when they are given separately. The current study is one of the earliest studies to try the combined approach of melatonin and garlic.

The current study confirmed toxic effect of cisplatin on kidney because cisplatin group showed impaired renal function, as indicated by significant increase in serum creatinine and blood urea nitrogen (BUN) levels when compared to control and treated groups (table 1). These findings are in line with previous studies**[13, 14]**and point towards reduced glomerular filtration of creatinine and BUN which is possibly caused by injury in the renal vasculature by cisplatin, resulting in renal vasoconstriction, reduced renal blood flow and ischemic injury to the kidneys **[15].**

Melatonin was able to significantly alleviate serum creatinine and urea compared to cisplatin but still significantly elevated compared to control group and this result is coherent with **Ko et al. (2019), Ali et al. (2020)**who attributed improvement compared to cisplatin group to anti-oxidant, anti-inflammatory and anti-apoptotic role of melatonin. **[11, 16]**

Also, garlic was able to significantly decrease serum creatinine and urea compared to cisplatin but still significantly different from control and this result is coherent with Yahya et al. (2023) who reported the action of garlic in improvement of renal function via its organosulfur compounds which could elevate its antioxidant effect **[17]**. This findings are in agreement with **Abdel-Daim et al. (2020), Albrakati and Research. (2021)[18, 19].**

Cisplatin caused a significant elevation of KIM-1incisplatin group compared to control group and other treated groups. This result is consistent with **Sami et al.(2022), Ramadan et al. (2023), Ijaz et al.(2023)[20-22].** This result is attributed to shedding ofextracellular domain of KIM-1 due to cisplatin induced inflammation **[23].**

Tanase, Gosav [23], Raad, Kamel [24]stated that KIM-1 is a transmembrane protein situated in apical membrane of proximal tubule with high sensitivity and specificity than serum creatinine in identifying and monitoring early dysfunction of proximal tubules caused by nephrotoxic agents as cisplatin. Elevated KIM-1 levels is correlated with inflammation through activation of NF-κB pathway.

The use of melatonin has reduced KIM-1 level compared to cisplatin group. This result is coherent with **Kim et al. (2019), Raad et al. (2022)** and indicate antioxidant, anti-apoptotic and anti-inflamatory properties of melatonin**[24, 25].**

KIM-1 level significantly decreased after garlic supplementation comparedto cisplatin group. This result came in accordance with**Galal and Abd el-Rady. (2019)** who reported a significant decrease in Kim-1 mRNA expression after oral administration of garlic in a rat model of gentamycin induced nephrotoxicity and this decline might be attributed to decrease in ROS production with subsequent decrease in the expression of matrix metalloproteinase (MMP)-3, specific enzyme present in proximal renal tubular cells, that in turn, decreased Kim-1 expression**[26]**. Combined group showed a significant reduction in KIM-1 level compared to all other groups and non-

significant to control group suggesting melatonin and garlic combination is more potent in ameliorating nephrotoxicity than when used singly.

In the current study, serum KIM-1 showed strong, significant and positive correlation with serum creatinine, table (3) .This correlation is in harmony with **Saleh et al. (2023)** who conducted a study to evaluate possible predictive value of KIM-1 in early detection of renal diseases **[27]**. There is also strong, significant and positive correlation between KIM-1 and BUN, table (3).This correlation is in agreement with **Khan et al. (2019), Al-Kuraishy et al. (2020)** who concluded that KIM-1 levels increase with progressive kidney damage **[28, 29].**

As indicated from the results of this study, markers of oxidative stressexhibited a significant increase in the MDA level in cisplatin-intoxicated rats compared to the control group. That increase in MDA was associated with a reduction in the level of antioxidant GSH which supports the relation between oxidative stress and cisplatin-induced nephrotoxicity reported by other studies **[30-32].**

Mitochondrial dysfunction is considered as one of the possible mechanisms of cisplatin induced AKI. Mitochondrial damage leads to increased generation of reactive oxygen species (ROS) **[33-35].** ROS damage cell membranes through peroxidation of its phospholipid fatty acids, producing lipid peroxides. The lipid peroxidation degree can be quantified through the release of MDA**[36].**Kidney possess a distinguished enzymatic antioxidant system. Glutathione (GSH) system is one of the most prominent antioxidant pathways in renal tissue. GSH in its reduced form detoxifies oxygen radicals and prevents cellular damage from oxidative stress **[37].**

The current study confirms the anti-oxidant effect of melatonin against cisplatin induced AKI by significant decrease of MDA and significant increase of GSH compared to cisplatin group. The results are consistent with **Ko et al. (2019)**who found that treatment with melatonin significantly mitigated oxidative stress induced by cisplatin**[16]**

Garlic has partially regained disorganized oxidative-stress markers by cisplatin and that is evidenced by a significant decrease in MDA and a significant elevation in GSH compared to the cisplatin group. In agreement with our result, **Elkhoely, Kamel [38]** also revealed that Diallylsulfide **(**DAS), one ofactive component of garlic, significantly restored cisplatin-depleted GSH content and ameliorated cisplatin-elevated MDA level. Both results supports anti-oxidant effect of garlic. **Galal and Abd el-Rady [26]**postulated the possible antioxidant mechanism of garlic that hydrogen sulfide (H2S), the main end product in garlic, increases the expression of the nuclear factor E2-related factor-2 (Nrf-2) with consequent increase in antioxidant enzymes.

The administration of melatonin and garlic together in combined group revealed a significant increase of serum GSH and improve lipid peroxidation by significant decrease of MDA level compared to cisplatin group, cisplatin + melatonin group and cisplatin + garlic group .

In this study, serum kim-1 showed strong, significant and positive correlation with MDA, table (3). This correlation is coherent with **Galal and Abd el-Rady. (2019) [26].** This correlation may be attributed to increase in ROS as a result of

cisplatin nephrotoxicity with increase in expression of MMP-3 and consequent increase in Kim-1. On the other hand, there is strong, significant and negative correlation between kim-1 and GSH, table (3).This correlation declared that renal injury decreases with increasing antioxidant level.

Histopathological picture is in harmony with biochemical as well as immunohistochemical results of this study as cisplatin group demonstrated marked necrosis in tubules with hyaline cast, epithelial desquamation, vaculation and dilated tubules. This result is in agreement with **Ali et al. (2021), Elsayed et al. (2021), Hassan et al. (2022)[39-41]**. The possible mechanism of this result may be due to formation of ROS and activating transcription factor nuclear factor kappa b (NF-κB) which trigger transcription of genes responsible for encoding proinflammatory cytokines **[42, 43]**.

Melatonin at a dose of 10mg/kg/day for 12 days has alleviated histopathological picture of renal injury induced by cisplatin as demonstrated by minimal hyaline cast andminimal vaculation. This result is coincident with previous studies **[11, 44].**

Garlic administration at a dose of 500 mg/kg/day for 12 days has improved histopathological picture of renal injury induced by cisplatin as demonstrated by nearly normal structure of majority of renal tubules which have a sparse amount of hyaline cast enhancing better effect of garlic than melatonin and it may be attributed to high dose of garlic as evidenced by **Elkhoely et al. (2018)** who utilized 2 doses of garlic 50 and 100 mg/kg and renal damage significantly decreased at a dose of 100 compared to dose of 50**[38]**.

Combined group has demonstrated normal structure of glomeruli and tubules, no hyaline cast and absence of epithelial degeneration and that indicates both agents together afford greater protection than when used singly.

Level of caspase 3 significantly increased in cisplatin group compared to control group and other treated groups. **Taghizadeh et al. (2020), Taghizadeh et al. (2021), Usefzay et al. (2022)** have reported similar result **[45-47]**. **Kim, Jo [25]** confirmed that cisplatin induces tubular cell apoptosis through activation of the pro-apoptotic proteins BAX, which form mitochondrial outer membrane pores, with release of cytochrome c into cytoplasm and caspase-3 activation (executioner caspase).

Interestingly, treatment with melatonin caused significant reduction in caspase -3 expression when compared to cisplatin group (table 2). This may be attributed to the anti-apoptotic role of melatonin. Our results are in agreement with **Sun et al. (2022), Karvan et al. (2022) [48, 49]. Zhang et al. (2021)** has confirmed anti-apoptotic effect of melatonin on a model of AKI induced by contrast media by reducing level of caspase3 and ratio of Bax/Bcl2**[50]**.

Level of caspase 3 in garlic group illustrated significant decrease compared to cisplatin group and that confirmed anti-apoptotic effect of garlic which is declared by **Elbeltagy et al. (2022)** who claimed that that s-allyl cysteine found in garlic can suppress apoptosis by preventing lipid peroxidation and oxidative damage to DNA**[51]**.

Caspase 3 level in combined group showed non-significant increase compared to control but showed significant decrease compared to cisplatin group, cisplatin& melatonin group and cisplatin& garlic group and that establish both melatonin and garlic together give better outcome in reduction of apoptosis.

In our study, serum kim-1 showed strong, significant and positive correlation with caspase 3, table (3). This correlation is in harmony with previously mentioned results and may be attributed to increase level of caspase 3 due to inflammation and activation of NF-KB pathway which eventually leads to increase in level of kim-1.

Conclusion: Co-administration of melatonin and / or garlic with cisplatin is advisable because they attenuate nephrotoxic effect of cisplatin and they have anti-cancer property potentiating the efficacy of cisplatin as chemotherapeutic agent. Combined administration of melatonin and garlic has the best outcome regarding biochemical and histopathological changes than separate administration of melatonin or garlic with cisplatin.

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Conflict of Interest: Nil

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