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Protective Effect Of Ginger Extract Against Cisplatin-Induced Nephrotoxicity In Rats.

Mohamed H Sherif [1], Al-shimaa M Abas[2]*, and Basem M Abd- El- Rahman [2]

[1]Chemistry Department, Faculty of Science, Zagazig University, Egypt
[2]Biochemistry Division, Chemistry Department, Faculty of Science, Zagazig University, Egypt

ABSTRACT

This study was designed to evaluate the nephro protective effect of Ginger extract promoted by cisplatin. Animals were divided into7 groups as follow: Group1 (Control)**:** Animals were injected intraperitoneal with1ml single saline dose.Group2 (DMSO): Animals were administered orally with1ml of 6.5% DMSO for 19 days .Group3 (cisplatin): Animals were injected intra peritoneal with cisplatin (10 mg/kg) as single dose.Group4 (Gingeronly): Animals were administrated orally with 600 mg/Kg/day of ginger extract for 19 days. Group 5, 6and7: Animals pretreated with oral dose of ginger extract (200, 400 and 600 mg /kg/day respectively) for 2 weeks before and 5 days after single IP cisplatin (10 g/kg). Administration cisplatin caused significant elevation in serum urea, creatinine concentration and kidney tissue levels of MDA and NO. Also caused significant increase in Caspase-3 levels, cisplatin significantly decreased level of Na. it decreased K, Mg and Ca level but statistically non-significant. Also it significantly decreased the antioxidant level of Catalase. Treatment with ginger extract restored the levels of kidney functions also oxidative stress markers and also decrease Caspase-3 level. Histopathological analysis confirmed that. ginger extract have a protective role in cisplatin induced nephrotoxicity.

Keywords: Nephrotoxicity, Ginger extract, Cisplatin, Caspase-3.

Corresponding author

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INTRODUCTION

Cisplatin (cis-diammine dichloro platinum(II),CDDP) is a platinum-based drug**[1]**,compound which has a wide part toward different strong malignancies, for example, head and neck, bladder, lung, ovaries, gonads, and uterus **[2]**.The cytotoxic impact of cisplatin is considered to come about essentially from its cooperation with DNA, by the framing of covalent adducts between certain DNA bases and the platinum compound**[3],**in spite of its clinical utility, cisplatin treatment has been connected with a few harmful symptoms including ototoxicity, nephrotoxicity, myelosuppression, and peripheral neuropathy **[4]**.

Ginger (Zingiber officinale), an individual from the Zingiberaceae family, is a notable flavor utilized as a part of the everyday consume less calories in numerous Asian nations **[5].**It was accounted for that ginger likewise had hostile to anti-cancer, anticlotting, anti-inflammatory, and analgesic activities **[6].** Concentrates of the ginger are wealthy in shagaols and gingerols which show anti-inflammatory, anti-oxidant and anti-carcinogenic proprieties under ‘‘in vitro’’ and ‘‘in vivo’’systems **[7].**

MATERIALS AND METHODS

Animal management

Adult male albino rats, weighing 180 -200 g, were gotten from the Experimental Animal Care Center and were housed in metabolic confines under controlled ecological conditions (25°C and a 12 h light/dark cycle) one week before beginning the trials acclimatization period. The animals were nourished with libtium and given drinking water.

Ginger extract / dose selection:

Measurements of ginger extract (bought from Sigma– Aldrich Co) was chosen by **[8]**who expressed that study conducted in male rats at a dosages of 500, 1000 and 2000 mg/kg body weight for 35 days and results demonstrated that chronic organization of ginger was not related with any mortalities and abnormalities in general conditions, behavior, growth, and food and water consumption. Ginger extract was dissolved initially in DMSO**[9]**and further diluted in PBS with the final concentration of DMSO (6.5 %) before administering to the rats.LD50 of cisplatin in rats is 12 mg/kg body weight **[10]**. The dose of cisplatin was selected on the basis of its effectiveness in inducing nephrotoxicity **[11].**

Experimental design

To achieve the ultimate goal of this study, following an acclimatization period of one week with standard basal diet, a total of 70 adult male albino rats were divided into seven groups as follow.

Group 1(Negative control): Animals were injected intraperitoneal (i.p.) with 1 ml single saline dose and animals were sacrificed after 19 days.
Group 2(DMSO): Animals were administered orally with 1 ml of 6.5% DMSOdaily for 19 days then scarified.
Group 3(cisplatin): Animals were injected intraperitoneal (i.p.) with cisplatin (10 mg/kg) (was purchased from Sigma Chemical Co) as single dose on the 15th day of the experiment and animals were sacrificed after 5 days.
Group 4(Ginger only): Animals were administrated with oral dose of ginger extract (600 mg/kg) for 19 days.
Group 5, 6 and 7: Animals pretreated with oral dose of ginger extract (200, 400 and 600 mg /kg/day respectively) for two weeks before and 5 days after single IP cisplatin (10 mg/kg).Animals were sacrificed after 19 days.

Collection and sampling of blood

Toward the finish of experimental period (19 days), animals were fasted for 12 hours. Animals were killed by cervical execution and blood serum was gathered by a described procedure **[12].**Serum was set up by accumulation of blood in anticoagulant – free tube, at that point left for 10 minutes in water bath at 37 ºC until cluster, at that point centrifuged at 2000 rpm for 10 minutes for separation of serum which was moved into another tube and kept frozen at -20 ºC.

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Tissue processing for oxidative parameters and histopathological examination

After blood collection kidney was evacuated, washed with ice-cold phosphate-buffered saline (pH 7.4). The homogenates were centrifuged and the supernatants got were moved into eppendorf tubes, and protected until utilized for lipid peroxidation (MDA), nitric oxide levels, catalase activity and Caspase-3 concentration. The another piece of kidney was settled in 10% formalin for histopathological examination .Three µm-thick paraffin sections were stained with hematoxylin and eosin (H and E) for light microscope examination utilizing conventional protocol **[13].** Histopathological studies were performed under a light microscope. Slides were coded and analyzed by a histopathologist who was blinded to the treatment groups.

Estimation of biochemical parameters

Estimation of serum urea and creatinine were performed by colorimetric technique as indicated by**[14, 15]** respectively**.** also, Serum potassium, sodium, calcium and magnesium were performed by colorimetric technique as indicated by **[16-19]**respectively**.**

Level of kidney tissue homogenate MDA, NO and Catalase were determined according to **[20-22]**using colorimetric kit obtained from Biodiagnostic Company (Biodiagnostic, Egypt)Catalogue No. 201-11-0281. 3(Caspase-3/CPP32) ELISA Kit is used to assay the Rat cysteinyl aspartate specific proteinases 3(Caspase-3/CPP32) in the homogenate.

Statistical analysis

All results were analyzed by SPSS software (version 14). Data were expressed as mean ± SD. Comparison of mean values of studied variables among different groups was done using ANOVA test. P<0.05 was considered to be significant **[23].**

RESULTS

Kidney function tests in all studied groups

Cisplatin caused significant increase in urea and creatinine levels (P < 0.001), (P < 0.0001) respectively. Administration of ginger extract before and along with cisplatin significantly reduced their levels comparing to cisplatin group (Table 1), (Figures 1-2).

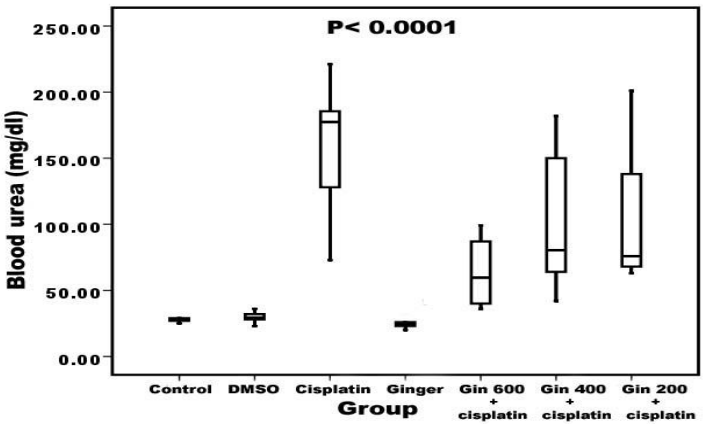

Figure 1: Box plots of blood urea for comparison between laboratory data of all groups in kidney disease. The box represents the interquartile range. The whiskers indicate the highest and lowest values, and the line across the box indicates the median value. Overall significance of differences between laboratory data of all groups was determined by ANOVA test (p < 0.0001)

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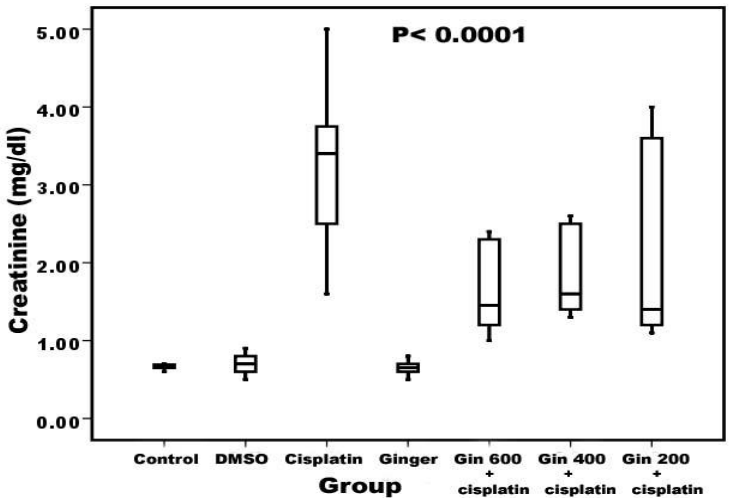

Figure 2: Box plots of creatinine for comparison between laboratory data of all groups in kidney disease. The box represents the interquartile range. The whiskers indicate the highest and lowest values, and the line across the box indicates the median value. Overall significance of differences between laboratory data of all groups was determined by ANOVA test.

Table 1: Kidney function tests in all studied groups.

Group	*Kidney functions*		
		Blood urea (mg/dl)	**Creatinine (mg/dl)**
Control	*Mean ± SE*	27.5 ± 0.6	0.66 ± 0.01
DMSO	*Mean ± SE*	29.60 ±1.76	0.70 ± 0.06
	*P **	0.921	0.925
	*% **	7.6	6.1
Cisplatin (Positive)	*Mean ± SE*	159.5 ± 16.56	3.23 ± 0.37
	*P **	< 0.001	< 0.0001
	*% **	480.0	390.5
Ginger only	*Mean ± SE*	25.3 ± 1.9	0.65 ± 0.04
	*P **	0.919	0.981
	*% **	-7.9	-1.5
Ginger 600+cisplatin	*Mean ± SE*	63.5 ± 10.8	1.6 ± 0.2
	*P **	0.096	0.026
	*% **	130.9	147.5
	*P ***	0.001	< 0.001
	*% ***	-60.2	-49.6
Ginger 400+cisplatin	*Mean ± SE*	99.8 ± 22.4	1.8 ± 0.2
	*P **	0.001	0.008
	*% **	263.0	177.8
	*P ***	0.004	0.001
	*% ***	-37.4	-43.4

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Ginger 200+cisplatin	*Mean ± SE*	103.67 ± 22.5	2.12 ± 0.5
	*P **	0.001	0.001
	*% **	277.0	220.7
	*P ***	0.007	0.007
	*% ***	-35.0	-34.6

P * = P Value compared to control group; P** =P Value compared to cisplatin (positive) group. The mean difference is significant at P< 0.05. % * = Percent of change compared to control group; % ** = Percent of change compared to cisplatin (positive) group.

Minerals levels in all studied groups

Table (2) indicated that serum levels of Ca, K, Mg and Na showed statistically non-significant difference in DMSO and ginger treated groups compared to control group (p>0.05). Also the administration of cisplatin (group 3) showed slight decrease in Ca, K, Mg and Na levels which statistically non-significant that amounted to -5.5, -0.8, -1.2 and -1.9 % respectively. While in group 5, 6 and 7 serum levels of Ca, K, Mg and Na were increased compared to cisplatin group. Group 5 (ginger 200 + cisplatin) showed statistically non-significant increase in serum Ca, K, Mg and Na of treated rats that amounted to 3.8, 0.1, 0.0 and 1.2 % respectively compared to cisplatin group. Group 6 (ginger 400 + cisplatin) showed statistically non-significant increase in serum Ca, K, Mg and Na of treated rats that amounted to 4.0, 0.4, 0.8 and 1.5 % respectively compared to cisplatin group. Group 7 (ginger 600 + cisplatin) showed statistically non-significant increase in serum K, Mg and Na of treated rats that amounted to 0.5, 0.9 and 1.7 % respectively compared to cisplatin group while significantly increased in Na level which amounted to 5.2 % compared to cisplatin group (Figures 3-6).

Table 2: Mineral levels in all studied groups

Group	*Minerals*				
		Ca (mg/dl)	**K (mmol/L)**	**Mg (mg/dl)**	**Na (mEq/L)**
Control	*Mean ± SE*	10.15 ± 0.09	6.275 ± 0.1	2.725 ± 0.04	144.0 ± 0.7
DMSO	*Mean ± SE*	10.12 ± 0.03	6.28 ± 0.14	2.72 ± 0.05	143.4 ± 2.4
	*P **	0.884	0.985	0.963	0.754
	*% **	-0.3	0.1	-0.3	-0.4
Cisplatin (Positive)	*Mean ± SE*	9.59 ± 0.08	6.225 ± 0.2	2.69 ± 0.09	141.25 ± 0.4
	*P **	0.005	0.841	0.853	0.130
	*% **	-5.5	-0.8	-1.2	-1.9
Ginger only	*Mean ± SE*	10.12 ± 0.14	6.266 ± 0.16	2.72 ± 0.07	144.3 ± 0.8
	*P **	0.871	0.975	0.986	0.862
	*% **	-0.3	-0.1	-0.1	0.2
Ginger 600+cisplatin	*Mean ± SE*	10.08 ± 0.1	6.256 ± 0.2	2.716 ± 0.2	143.7 ± 0.6
	*P **	0.745	0.945	0.966	0.862
	*% **	-0.7	-0.3	-0.3	-0.2
	*P ***	0.013	0.899	0.889	0.182
	*% ***	5.2	0.5	0.9	1.7
Ginger 400+cisplatin	*Mean ± SE*	9.97 ± 0.2	6.25 ± 0.2	2.71 ± 0.18	143.3 ± 2.1
	*P **	0.373	0.925	0.946	0.728
	*% **	-1.8	-0.4	-0.5	-0.5
	*P ***	0.054	0.920	0.911	0.249
	*% ***	4.0	0.4	0.8	1.5
Ginger 200+cisplatin	*Mean ± SE*	9.95 ± 0.23	6.23 ± 0.15	2.69 ± 0.21	143.0 ± 1.2

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	*P **	0.332	0.876	0.864	0.602
	*% **	-2.0	-0.7	-1.2	-0.7
	*P ***	0.065	0.973	0.998	0.331
	*% ***	3.8	0.1	0.0	1.2

P * =P Value compared to control group; P** =P Value compared to cisplatin (positive) group. The mean difference is significant at P< 0.05.

% * = Percent of change compared to control group; % ** = Percent of change compared to cisplatin (positive) group.

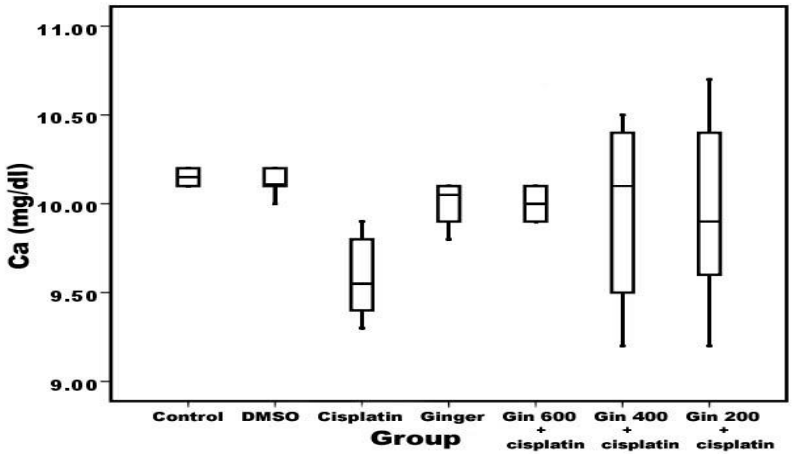

Figure 3: Box plots of Ca for comparison between laboratory data of all groups in kidney disease. The box represents the interquartile range. The whiskers indicate the highest and lowest values, and the line across the box indicates the median value. Overall significance of differences between laboratory data of all groups was determined by ANOVA test.

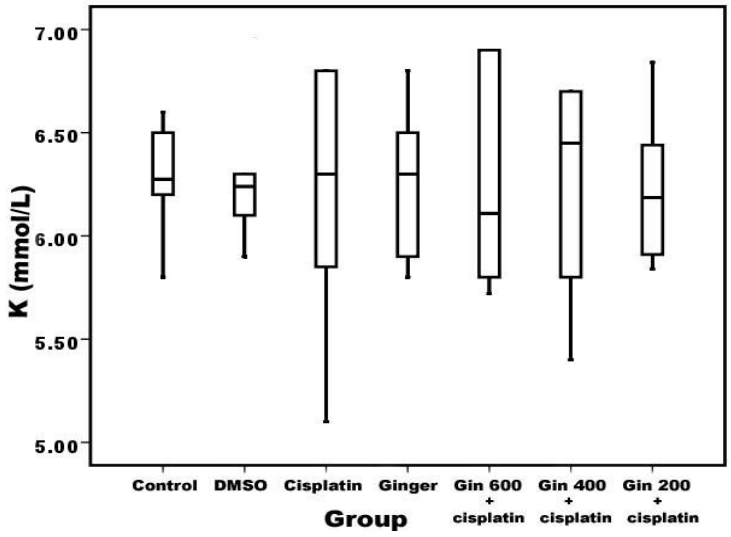

Figure 4: Box plots of K for comparison between laboratory data of all groups in kidney disease. The box represents the interquartile range. The whiskers indicate the highest and lowest values, and the line across the box indicates the median value. Overall significance of differences between laboratory data of all groups was determined by ANOVA test.

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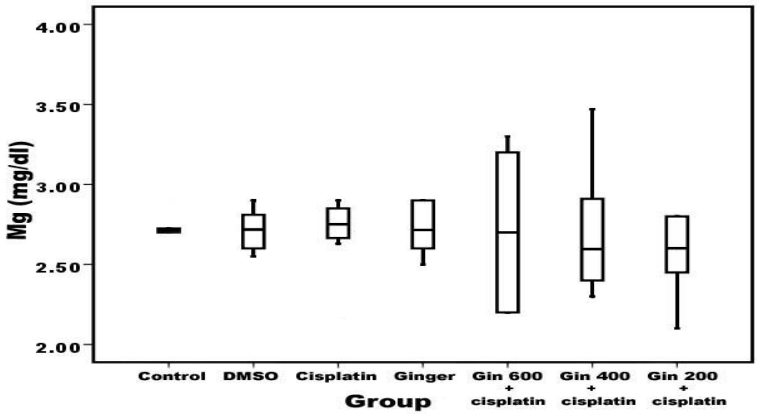

Figure 5: Box plots of Mg for comparison between laboratory data of all groups in kidney disease. The box represents the interquartile range. The whiskers indicate the highest and lowest values, and the line across the box indicates the median value. Overall significance of differences between laboratory data of all groups was determined by ANOVA test.

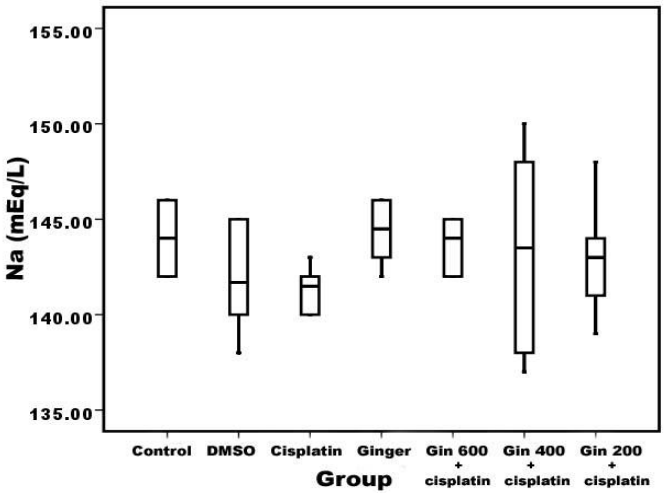

Figure 6: Box plots of Na for comparison between laboratory data of all groups in kidney disease. The box represents the interquartile range. The whiskers indicate the highest and lowest values, and the line across the box indicates the median value. Overall significance of differences between laboratory data of all groups was determined by ANOVA test.

Effect of ginger extracts on oxidative stress levels

The levels of MDA and NO were significantly increased in cisplatin group which amounted to 203.4 and 190.5 % respectively comparing to control group. Administration of ginger extract before and along with cisplatin significantly reduced the levels of No in group 5, 6 and 7comparing to cisplatin group. Also significantly reduced the levels of MDA in group 7, while showed non-significant decrease in group 5 and 6 comparing to cisplatin group(Table 3), (Figures 7-8).

Table 3: Antioxidant levels in all studied groups

Group	*Antioxidant*			
Control		**No (µmol/L)**	**MDA (nmol / g tissue)**	**Catalase (U/g)**
	Mean ± SE	18.39 ± 2.2	4.45 ± 0.4	42.9 ± 1.5
DMSO	*Mean ± SE*	18.44 ± 0.7	5.3 ± 0.3	36.2 ± 0.9
	*P **	0.994	0.591	0.246
	*% **	0.3	19.1	-15.6

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Cisplatin (Positive)	*Mean ± SE*	55.81 ± 3.86	12.9 ± 0.99	27.4 ± 1.2
	*P **	< 0.001	< 0.001	0.006
	*% **	203.4	190.5	-36.1
Ginger only	*Mean ± SE*	16.4 ± 1.4	5.60 ± 0.27	36.7 ± 0.48
	*P **	0.776	0.471	0.284
	*% **	-10.6	25.7	-14.4
Ginger 600+cisplatin	*Mean ± SE*	22.9 ± 8.6	6.09 ± 1.6	35.6 ± 9.1
	*P **	0.488	0.305	0.203
	*% **	24.8	36.7	-17.2
	*P ***	< 0.001	0.004	0.135
	*% ***	-58.9	-52.9	29.6
Ginger 400+cisplatin	*Mean ± SE*	25.1 ± 3.2	6.67 ± 0.6	31.5 ± 1.7
	*P **	0.309	0.167	0.052
	*% **	36.7	49.7	-26.6
	*P ***	< 0.001	0.092	0.448
	*% ***	-54.9	-48.5	14.9
Ginger 200+cisplatin	*Mean ± SE*	25.38 ± 6.0	7.40 ± 1.97	30.09 ± 5.2
	*P **	0.290	0.069	0.030
	*% **	38.0	66.1	-29.9
	*P ***	< 0.001	0.378	0.621
	*% ***	-54.5	-42.8	9.7

P * = P Value compared to control group; P** =P Value compared to cisplatin (positive) group. The mean difference is significant at P< 0.05.

% * = Percent of change compared to control group; % ** = Percent of change compared to cisplatin (positive) group.

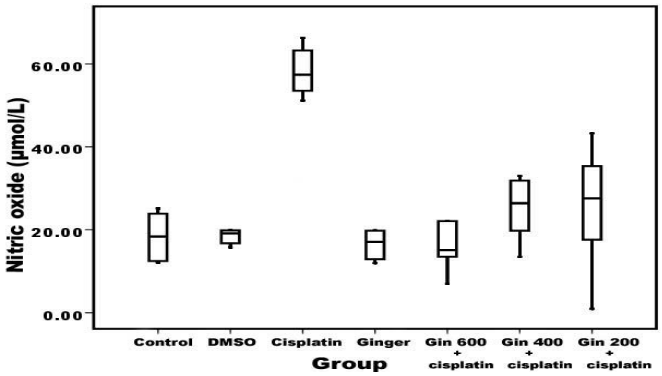

Figure 7: Box plots of nitric oxide for comparison between laboratory data of all groups in kidney disease. The box represents the interquartile range. The whiskers indicate the highest and lowest values, and the line across the box indicates the median value. Overall significance of differences between laboratory data of all groups was determined by ANOVA test.

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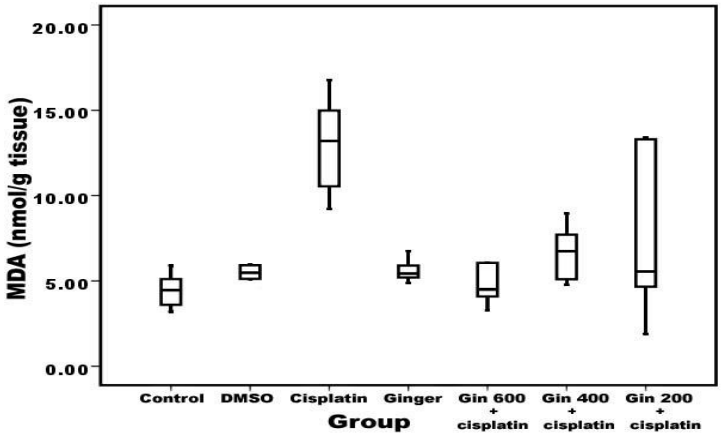

Figure 8: Box plots of MDA for comparison between laboratory data of all groups in kidney disease. The box represents the interquartile range. The whiskers indicate the highest and lowest values, and the line across the box indicates the median value. Overall significance of differences between laboratory data of all groups was determined by ANOVA test.

Effect of ginger extract on antioxidant levels

The level Catalase was significantly decreased in cisplatin group which amounted to -36.1% comparing to control group. Administration of ginger extract before and along with cisplatin increased the levels of catalasein group 5, 6 and 7 comparing to cisplatin group (Table 3), (Figures 9).

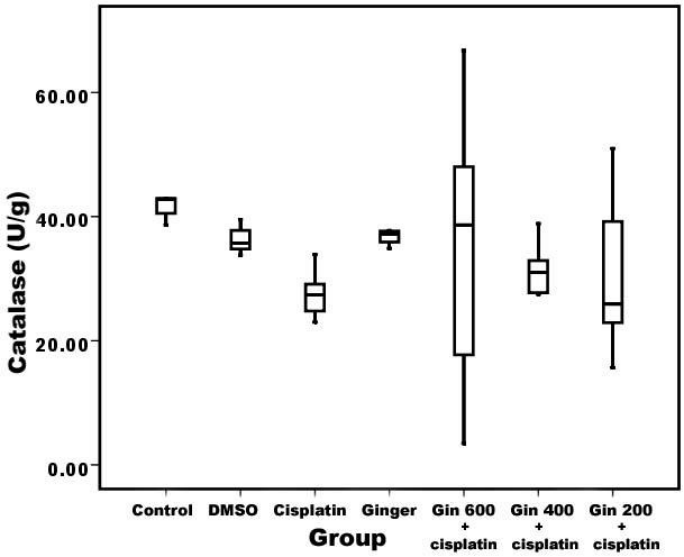

Figure 9: Box plots of catalase for comparison between laboratory data of all groups in kidney disease. The box represents the interquartile range. The whiskers indicate the highest and lowest values, and the line across the box indicates the median value. Overall significance of differences between laboratory data of all groups was determined by ANOVA test.

Effect of ginger extract on caspase - 3 levels.

The mean level of Caspase-3 showed statistically non-significant increase in DMSO and ginger only treated groups which amounted to 0.8 and 8.5 % respectively compared to control group ($p>0.05$).

In group 3 administration of cisplatin induced apoptosis by causing significant elevation in caspase-3 level in kidney tissue which amounted to 70.3 % compared to control group ($p<0.001$).

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Treatment with ginger extract reduced the elevation in caspase-3 level in group 5, 6 &7 which amounted to 29.6, 20.2 and 7.9 % respectively compared to control group (Table 4), (Figure 10).

Table4: Caspase-3 levels in all studied groups

Group	*Caspase-3*	
		Caspase-3 (ng/ml)
Control	*Mean ± SE*	2.55 ± 0.09
DMSO	*Mean ± SE*	2.575 ± 0.12
	*P **	0.914
	*% **	0.8
Cisplatin (Positive)	*Mean ± SE*	4.35 ± 0.08
	*P **	< 0.001
	*% **	70.3
Ginger only	*Mean ± SE*	2.77 ± 0.16
	*P **	0.279
	*% **	8.5
Ginger 600+cisplatin	*Mean ± SE*	2.755 ± 0.16
	*P **	0.315
	*% **	7.9
	*P ***	< 0.001
	*% ***	-36.6
Ginger 400+cisplatin	*Mean ± SE*	3.07 ± 0.21
	*P **	0.031
	*% **	20.2
	*P ***	< 0.001
	*% ***	-29.4
Ginger 200+cisplatin	*Mean ± SE*	3.31 ± 0.11
	*P **	< 0.001
	*% **	29.6
	*P ***	< 0.001
	*% ***	-23.9

P * =P Value compared to control group; P** =P Value compared to cisplatin (positive) group. The mean difference is significant at P< 0.05.

% * = Percent of change compared to control group; % ** = Percent of change compared to cisplatin (positive) group.

Histopathological examination of kidney tissue

Histological examinations of tissue sections from the kidney showed vacuolated glomeruli and degenerated of some renal tubules with luminal renal cast were observed in the group 3 (cisplatin). While in group 7 (ginger 600 + cisplatin) the kidney tissues were protected against cisplatin-induced damage and show normal glomeruli and renal tubules with no pathological changes when compared with the control heart tissue slide (Figures 11-16).

DISCUSSION

Cisplatin is an inorganic complex created by a molecule of platinum encompassed by chlorine and ammonia. One of the potential mechanisms by which cisplatin gathers in the cells is by carrier-mediated processes **[24]**. Cisplatin activated once it enters the cell. In the cytoplasm the chloride particles on cisplatin are dislodged by water atoms. This hydrolyzed product is an intense electrophile that can respond with any

nucleophile, including the sulfhydryl groups on proteins and nitrogen donor atoms on nucleic acids. Cisplatin ties to the N7 reactive focus on purine residues and in that capacitycan cause deoxyribonucleic acid (DNA) harmin cancer cells, blocking cell division and so causing apoptotic cell death **[25]**.Despite its efficacy as an antitumor drug, different reactions, including nephrotoxicity **[26]**, hepatotoxicity and cardiotoxicity.

Serum creatinine and urea levels were viewed the principle parameters that decide kidney function**[11]**. In the present study, nephrotoxicity of cisplatin was clear from the increased levels of serum urea and creat in inelevels. The rise in the serum levels of these renal biomarkers may be because of the impaired renal functions **[27]**, tubular obstruction, and/or the back-leakage of the renal tubules **[11]**. Such functional disorder in cisplatin exposed rats could show the ability of cisplat into inhibit protein synthesis in the tubular cells **[28]**or to initiate lipid peroxidation and generate free radicals in renal tubules **[29].**The cisplatin toxicity incited serious renal dys function that permits the elevated secretion of creatinine from the proximal tubules, a marker of irreversible renal tubular injury **[30]**. Renal function can also be determined by analyzing theserum urea level. During the metabolism of protein in the body, the liver produces ammonia which is converted into a byproduct called urea. However, because of renal dysfunction, urea is released into the bloodstream as serum urea. Therefore, higher serum urea level is directly proportional to harshness of renal damage **[30]**. Similar findings were already detailed in various studies **[31]**. The kidney biomarkers increased due to the direct dangerous effect of cisplatin on the glomerular and tubular structures through the generation of ROS. Constriction of the mesangial cells with subsequent alteration in the filtration surface area came about because of the generation of ROS **[32].**

Our results are in line with **[33]** who detailed that cisplatin is also harmful to renal vasculature which results in diminished blood flow leading to ischemic injury of the kidneys, showing up as a decrease in the glomerular filtration rate which is reflected as increased serum creatinine and BUN levels.

Our results are in accordance with **[34]** who revealed that cisplatin-induced nephrotoxicity was apparent by increase in serum creatinine and urea levels in the rats**.**

Treatment with ginger extract significantly reduced the elevation in blood urea and creatinine levels in treated groups. Our results are in agreement with **[35]** indicated that ginger extract markedly diminished the blood urea nitrogen concentrations in experimental mice in a non-linear fashion concerning the administered dose. None the less, little changes observed in the levels of creatinine in these animals as compared with the control group.

Minerals are inorganic substances, present in all body tissues and fluids and their presence is vital for the support of certain physicochemical processes which are basic to life. Serious disruptions in physiologic functions can be resulted from imbalance of any of the minerals **[36].**

Cisplatin is a co-ordinate metal complex with significant antineoplastic activity and the side effects including acute and chronic renal insufficiency, renal magnesium wasting, and electrolyte disturbances like hypomagnesia, hypocalcaemia, Hyponatremia and hypokalemia are common with cisplatin treatment. so routine monitoring of magnesium in plasma is recommended to stay away fromtetany **[37].**

Hypomagnesaemia is a well-known side-effect in patients receiving cisplatin; the possible mechanisms behind the cisplatin induced hypomagnesaemia might be the direct injury to magnesium reabsorption in the ascending limb of loop of henle, as well as the distal tubule **[38]**.

Hypocalcaemia is another known side effect related with cisplatin chemotherapy. Excessive urinary loss of calcium, diminished renal uptake of calcium because of the proximal tubular damage, because of low tissue response of parathyroid hormone and low serum magnesium levels, is the possible mechanism behind cisplatin induced hypocalcaemia **[39].**

Hypokalemia is a common electrolyte abnormality occurred during cisplatin treatment; it is due to increased renal reabsorption capacity observed in response to decreased intestinal absorption of potassium. Further magnesium and potassium metabolism subjected to predicable changes in intestinal absorption and renal excretion with each cisplatin treatment **[40].**

Hyponatremia is not an uncommon clinical disorder, in previous studies authors have found that both renal salt wasting syndrome and syndrome of inappropriate antidiuretic hormone secretion have been reported as the underlying mechanism for cisplatin chemotherapy induced hyponatremia **[41].**

Our results are in line with **[42]** who reported that Clinically, cisplatin nephrotoxicity is frequently observed after 10 days of cisplatin administration and is manifested as lower glomerular filtration rate and decreased serum magnesium and potassium levels.

Results are in agreement with **[43]** that Profound that hypo-magnesaemia, hypokalemia and hypocalcaemia have been reported in patients on cisplatin therapy. Also are in agreement with **[44]** who detailed that Platinum chemotherapy, especially cisplatin, is usually related with electrolyte imbalances, including hypomagnesemia, hypokalemia, hypophosphatemia, hypocalcemia and hyponatremia.

Administration of ginger extract restored the levels of Ca, K, Mg and Na to normal value and this are in agreement with**[45]**who revealed that cisplatin-induced cause excessive urinary loss of magnesium and potassium**[46]**could be partly restored by supplementation.

Our results are in accordance with **[47]** who reported that there was a significant decrease in serum sodium and calcium concentrations in ginger extract-treated groups when compared to the control group.

The oxidative stress has emerged as the principle mechanism in cisplatin-induced nephrotoxicity **[2].** It has been reported that excess reactive oxygen species (ROS) production as well as antioxidant system depletions are consequent to cisplatin administration **[48].** The likely sources of ROS during cisplatin administration include the mitochondrial electron transport chain system **[49]**, xanthine oxidase **[50],** cytochrome P450 enzymes **[51],** and NADPH oxidase **[52].** Since, ROS are highly reactive and unstable; they may attack and modify cellular components such as lipids, proteins, and DNA, resulting in cellular stress **[53].** ROS accumulation also activates important signaling pathways, including apoptotic pathway, which leads to cell death in the event of cisplatin-induced nephrotoxicity **[54].**

Our results demonstrated that cisplatin treatment caused significant increase in kidney MDA, NO levels and significant decrease in catalase activity (Table 3). Results reported by **[55]** showed that cisplatin induces an increase in oxidative stress and alters intracellular Ca^{2} concentration, including cytosolic and mitochondrial Ca^{2} in cisplatin-sensitive SKOV3 cells. Cisplatin induces mitochondrial harm and triggers the mitochondrial apoptotic pathway in cisplatin-sensitive SKOV3 cells.

[56] declared that Cisplatin induced a significant increase in malondialdehyde, and nitric oxide levels. However, glutathione, superoxide dismutase, and catalase levels were significantly decreased.

The proposed mechanism of induced nephrotoxicity of cisplatin could be clarified as in the accompanying: During the physiological process, the mitochondrial respiratory chain continuously generates ROS. Around 2% of the electrons which influxalong the respiratory chain escape from the chain and partially reduces molecular oxygen, originating superoxide anion (O2−•). Superoxide anion, the precursor of most of the reactive oxygen species generated in mitochondria as for example hydroxyl radicals HO. An effective mitochondrial antioxidant defense system maintains the balance between ROS generation and detoxification **[57]**. Cisplatin unbalances the oxidant–antioxidant ratio by (i) Augmenting ROS generation, mainly hydroxyl radical and (ii) Inhibition of the antioxidant defense system which are SOD, CAT and GSH. These radicals can evoke extensive tissue damage, reacting with membrane lipids, proteins and nucleic acids **[58]**.Our results agreed with **[59]** reported that the renal content of peroxynitrite and nitric oxide is increased in cisplatin treated rats; furthermore, cisplatin caused significant increases in the level of MDA, and impaired the activitiy of CAT.

Also our results are in accordance with**[60]**reported an increase in lipid peroxidation and decrease in the activities of antioxidant enzymes upon similar cisplatin treatment of rats.

Antioxidants have proven to be effective in ameliorating cisplatin-induced toxicity. Ginger extract is a potent antioxidant which is reported to have antitumor effect and to enhance the effect of many known

anticancer agents in addition to reducing their toxicities as well. This is in agreement with **[61]** who reported that administration of ginger extract cause increased level of catalase while decreased levels MDA and NO.

Our results are in line with **[13]**who showed that, high levels of polyphenolic and flavonoid compounds with high antioxidant activity for ginger. The presence of polyphenols and flavonoids in the Z. officinale extract might be responsible for the antioxidant and nephro protective activities.

Mitochondria play a vital role in the signal transduction of apoptosis. The intrinsic pathway included disturbance of mitochondrial membrane potential results in the modulation of Bcl-2 family of pro- and anti-apoptotic proteins and release of cytochrome c and/or apoptosis inducing factor (AIF) from the mitochondria into the cytosol. When cytochrome c is released into the cytosol, it forms an apoptosome with Apaf-1 and procaspase-9. This causes the activation of caspase-9, which additionally activates caspase-3, leading to apoptosis **[62].**

The activation of caspase is known to play a pivotal role in the initiation and execution of apoptosis induced by various stimuli. When cells are undergoing apoptosis, executioner caspase-3 triggers cellular proteins and DNA fragmentation factor, resulting in the characteristic changes of apoptosis **[63]**.

In this study administration of cisplatin induced apoptosis by causing significant rise in caspase-3 level in heart tissue which amounted to 70.3 % compared to control group (Table 4) **[29]** are in line with our results who reported that the activation of mitochondrial pathways was important in apoptosis induced by cisplatin, which leads to the release of cytochrome c, activation of caspase 3 and entry into the execution phase of apoptosis.

The ability of ginger to down regulate caspase-3 activity may be attributed to its antiglycation and antioxidant properties. This was in agreement with **[64]** who noticed the antiglycating effect of ginger in the lens of diabetic rats.

In this study histological examination of tissue showed vacuolated glomeruli and degenerated of some renal tubules with luminal renal cast were observed in the cisplatin group.

In this study, cisplatin was found to induce many histopathological alterations in the kidney including necrosis of the renal tubular cells together with karyolytic and pyknotic nuclei, degeneration of theglomeruli and widening of the urinary space. These histopathological changes are parallel to the findings of many other investigators **[65].**

Our results are in agreement with **[66]** who detailed that cisplatin-induced nephrotoxicity was assessed bychange in levels of blood urea, creatinine, and histopathology of the kidney.

While in group 7 (ginger 600 + cisplatin) the kidney tissues were protected against cisplatin-induced damage and showed normal glomeruli and renal tubules with no pathological changes when compared with the control heart tissue slide (Figure 11:16). This is in agreement with who reported that there is no histological difference between control and ginger extract groups, ginger is safe and well tolerated and co-administration of ginger extract in dose of 250 mg/kg with diclofenac sodium partially ameliorated the histological changes produced in the liver by diclofenac toxicity **[67].**

Ginger is treating kidney protector of inflammation resulting from cisplatin drug, where the histopathology results supported these findings. The results agreed with **[68]** who announced that treatment with different extracts of ginger ameliorated kidney tissues.

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