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Toxicopathological Studies On Cisplatin Toxicity In Rats And Trials For Protection Using Green Tea Extract And Coriandrum Sativum L Oil.

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ABSTRACT

This is the first study carried out to ensure the conservative effect of green tea extract and CoriandrumsativumL. oil against cisplatin-induced hepatotoxicity, cardiotoxicity and nephrotoxicity in rats. Forty two male albino rats were used and divided in to 6 groups (n=7). Group l (negative control), group 2 received green tea extract, (30 mg/kg B.W. orally for 14 days), group 3 received C.sativumoil (40 mg/kg B.W. orally for 14 days), group 4 administered single dose of cisplatin (5mg/kg B.W. i.p), groups 5 and 6 pretreated with green tea extract and C.sativumoil respectively 7days prior to cisplatin injection. Blood samples were collected from all rats at the end of the experiment for estimation of liver function tests (ALT& AST), LDH, CK, kidney function tests (Urea and Creatinine). Samples were collected from liver, heart&kidney tissues and fixed in 10% neutral buffer formalin for histopathological and immunohistochemical studies, while others were weighted and put in freeze at -20^{o}C for evaluation of oxidative stress markers (MDA-SOD). Uncommonly, pretreatment with green tea extract and C.sativumoil reduced the elevated liver& kidney function biomarkers; LDH& CK levels; oxidative stress markers and also protect the hepatic, cardiac& renal tissue from further damage induced by the cisplatin. Moreover, green tea extract and C.sativumoil inhibited the apoptotic cascade by down regulating caspase-3 and Bax gene expression. In conclusion, both green tea extract andC.sativumoil have anameliorative role against cisplatintoxicity in rats.

Keyword: Cisplatin- Green tea extract–Coriander oil-Oxidative stress- Histopathology.

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INTRODUCTION

Cisplatin have been broadly used as chemotherapy for treatment of different tumor, including liver cancer **[1, 2]**. Notwithstanding, they create ideal outcome in chemotherapy of a few tumors; but they additionally display extreme foundational harmfulness and severe systemic toxicity because of the medication's absence of target selectivity**[3,4]**. Nephrotoxicity is therefore considered as the most widely recognized and genuine reaction of cisplatin chemotherapy **[5]**and also, cisplatin treatment has been related with a few dangerous reactions including hepatotoxicity and cardiotoxicity**[6].** One of the most vital components of cisplatintoxicity is the oxidative stress **[7]**. Cisplatin stimulates production of oxidative stress markers and generates reactive oxygen species (ROS) such as superoxide anion and hydroxyl radical which induced oxidative damage within the cells **[8, 9]**.

The protective impacts of a few antioxidants on alienating the symptoms related with cisplatin have been assessed by numerous preclinical trials **[10].** Since ancient times, therapeutic plants have been utilized for the treatment of several diseases and more consideration has been paid to the defensive impact of the common natural antioxidants against chemically induced toxicities **[11]**.

Green tea (Camellia sinensisL.) is extremely powerful naturally occurring antioxidant**[12]**. Moreover, the scientists proposed that green tea might be valuable in improving the cytotoxic impacts of chemotherapeutic agents**[13]**. The principle bioactive molecules in tea are polyphenol compounds **[14]**. Polyphenol compounds which involves epigallocatechin 3- gallate (EGCG) (the most numerous cathechin in GT1), epigallocatechin (EGC), epicatechin- 3-gallate (ECG) and epicatechin (EC), are the fundamental constituents of green tea **[15].** In this way, the antioxidant ability of green tea is more noteworthy than that of different sorts **[16, 17].** The green tea refreshment is considered as a pharmaceutical due to its polyphenol content**[18]**.Those, polyphenols of green tea have turned into a core of scientific interest focused for creating novel remedial specialists **[19].**

Essential oils are generally utilized as segments of medications **[20]**. In addition, various investigations report biological effectiveness of essential oils: they show antibacterial, fungicidal and antioxidant activities **[21]**. Coriander (Coriandrumsativum L.) is a yearly herbaceous plant initially from the Mediterranean and Middle Eastern districts, developed for its culinary, aromatic and medicinal use**[22]**. The essential oil and various extracts from coriander have been shown to possess antibacterial, antimutagenic, antioxidant and free radical scavenging activities **[23]**. The antioxidant impacts of coriander oil have been described, recommending that this oil could be considered as a source of natural antioxidants and utilized as a potential substitute for synthetic antioxidants in the food industry **[24, 25]**.

A few investigations recommended the defensive impact of green tea and its phenolic extract as anti-inflammatory, antioxidant and successful scavengers of ROS **[26, 27]**, while others confirmed the hepatoprotective and antioxidant effect of coriander oil **[28]**. So far no data is accessible on the defensive impact of either green tea extract or coriander oil on cisplatin induced hepatotoxicity, cardiotoxicity& nephrotoxicity. In addition there is no any investigation look at between those two plants as an antioxidant, hepatoprotective and nephroprotective effect. In this way, this is the first study intended to explore the antioxidant and the protective impacts of both green tea extract and Coriander oil supplementation on cisplatintoxicity in rats with a view to suggest the same for remedial reason.

MATERIALS AND METHODS

Plant extraction

Green tea phenolic extraction

The plant materials were identified at the Herbarium of the Department of Botany, Faculty of Science, Cairo University. A sample was deposited in the Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Egypt. The powdered plant (200 g) was extracted and percolated in ethanol 95% for 5 to 7 times till complete extraction. The ethanol extracts was concentrated under reduced pressure using a rotary evaporator at temperature not more than 50ºC. The concentrated extract was kept at - 4 0C until use.

Extraction of the essential oil

Samples of coriander (Coriandrumsativum L.) were purchased fromlocal supermarket. Air-dried coriander finely grounded (0.5 mm). The ground coriander was subjected to steam distillation for 4 hours utilizing a Clevenger apparatus to deliver the essential oil. After extraction, the essential oil was separated using ethyl ether and was dried with anhydrous sodium sulfate. Yellowish oil was obtained and stored in a refrigerator (8 °C) until use.

Animals and experimental design

All procedures were conducted according to the protocol approved by the Institutional Animal Care and Use Committee at Cairo University (IACUC,CU-II-F-59-17), Egypt. Forty-two male albino wester rats (weighing 130-150gm) were obtained from Holding Company for Biological Products and Vaccines (VACSERA) – Helwan, Egypt. All animals were housed in plastic cages (5 or 3 rats/ cage) in a well-ventilated environment and received a daily illumination of 16 hours of light. They were fed on dry commercial standard pellets and gain access to tap water ad- libitum throughout the experimental period. They were acclimatized to the environment for 2 weeks prior to the onset of the experiment use to ensure their healthy state.

Rats were randomly divided in to six experimental groups (n=7 in each) as designed in **table (1)**. Gl (control negative group), G2 received green tea extract, (30 mg/kg B.W. oral for 14 days), G3 received C.sativumoil (40 mg/kg B.W. oral for 14 days), G4 administered single dose of cisplatin (5mg/kg B.W. i.p), G5 pretreated with green tea extract 7 days before cisplatin injections and G6 received C.sativumoil 7days prior to cisplatin injection. Doses of cisplatin, green tea extract, and C. sativumoil were selected according to previous studies**[29, 30, 31]**respectively.

Table (1): Experimental design

Group NO	Treated materials	Rout of administration	Dose (mg/kg B.W)	Dose number
1	-	-	-	-
2	Green tea extract	Oral	30	14
3	*C.sativum*oil	Oral	40	14
4	Cisplatin	i.p	5	Once
5	GTE +Cisplatin	Oral for GTE, i.p for cisplatin	30 for GTE, 5 for cisplatin	14 for GTE, 1 for cisplatin
6	C.oil+cisplatin	Oral for C.oil, i.p for cisplatin	40 for C.oil, 5 for cisplatin	14 for C.oil, 1 for cisplatin

Sampling

On completion of the experimental period, Blood sample were collected from all rats under anesthesia before euthenization. The samples were then centrifuged at 4,500 rpm for 5 min to obtain clear serum samples which separated and preserved at -20°C till used for determination of biochemical parameters. Rats were euthenized by cervical dislocation. Liver& kidney tissues were collected for oxidative stress evaluation, histopathological and immunohistochemical studies.

Liver and Kidney functions evaluation:-

Serum ALT, AST, Creatinine and Urea levels were measured according to manufacturer's instruction of the commercial kits (Bio-diagnostic Co., Cairo, Egypt)

Lactate dehydrogenase and Creatine kinase levels estimation:-

Serum LDH and CK were measured according to manufacturer's instruction of the commercial kits (Bio-diagnostic Co., Cairo, Egypt).

Preparation of tissue homogenates:-

Homogenization of liver& kidneys tissue was done in 5-10 ml cold buffer (50 mM potassium phosphate, pH7.5) per gram tissue for MDA Assay **[32],** while for SOD assay, tissue homogenization was carried out in 5-10 ml cold buffer (100Mm potassium phosphate pH 7.0 and 2Mm EDTA) **[33].** All tissue homogenates were then centrifuged at 4000 rpm for 15 minutes at 4 °c and the supernatants were aspirated for MDA, and SOD assays.

Oxidative stress evaluation:-

The malondialdehyde (the marker of lipid peroxidation)was determined in homogenates by monitoring the thiobarbituric acid reactive substance (TBARS) formation using colorimetric kits (Bio-diagnostic Co., Cairo, Egypt) as described by **Ohkawaet al., [32].** The absorbance was measured colorimetrically at wave length 534 nm by UVD-2950 and the results were expressed as nmol/g tissue. The antioxidant marker SOD was measured by a colorimetric method using commercial kits (Biodiagnostic, Co., Cairo, Egypt) according to the manufacturer procedures **[33]**. SOD activity was determined based on the ability of the enzyme to inhibit nitrobluetetrazolium (NBT) reduction by superoxide and the results were expressed as U/g tissue.

Histopathological and immunohistochemical studies

Tissue specimens from liver&kidneys were taken from all rats and preserved in 10% neutral buffer formalin (PH 7.0). The specimens were processed by convention method and cutting at 4.5µm was performed to obtain paraffin sections stained by H&E and some sections stained by immunohistochemical staining **[34].**

For grading and scoring of both hepatocellular injury and cardiotoxicity, at least five microscopic areas were assessed. The criteria for liver and cardiac muscle injury were vacuolar degeneration, necrosis, vascular congestion, edema and inflammatory cells infiltrations. Each specimen was scored as mild, moderate, and severe, on a scale of 0-4, as follows(0 = none, 1 = <25%, 2 = 25%: 50%, 3 = 50%: 75%, 4= >75%)for each criterion.

The microscopic grading and scoring of the kidney tissue section was carried out to express the degree of severity of the observed histopathological lesions according to **Baligaet al.,[35]**but with some modifications. Grading of subacute nephrotoxicity was performed according to the following parameters:- renal tubular epithelial cells degenerations, necrosis, Hyaline droplets and casts, vascular congestion, Interstitial leucocytic infiltration. The above parameters are assessed and scored as mild, moderate, and severe, on a scale of 0-4, as follows(0 = normal histology, 1 = <25%, 2 = 25% : 50%, 3 = 50% : 75%, 4= >75%).

Immunohistochemical studies were carried out for detection of Caspase-3and Bax expressions on paraffin liver&kidney sections using avidin-biotin peroxidase (DAB, Sigma Chemical Co.) according to the method described by **Hsu et al., [36]**. Briefly, tissue sections were incubated with a monoclonal antibody for caspase-3 and Bax (Dako Corp, Carpenteria) and reagents required for the avidin-biotin peroxidase (Vactastain ABC peroxidase kit, Vector Laboratories) method for the detection of the antigen–antibody complex. Each marker expression was localized by the chromogen 3, 3-diaminobenzidine tetrahydrochloride (DAB, Sigma Chemical Co.).

Statistical analysis:-

All the previous data were expressed as mean ± SD for five rats in each group. Variables were statistically analyzed by Student's test between two groups and one way analysis of variance (ANOVA) test was used to compare means of more than two groups. When differences were significant, Least Significant Difference (LSD) test was performed to find the individual differences between groups. The significance level was set as P value ≤ 0.05 significant. Statistical analysis was performed using SPSS version 16**[37].**

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RESULTS

Kidney functions tests evaluation:-

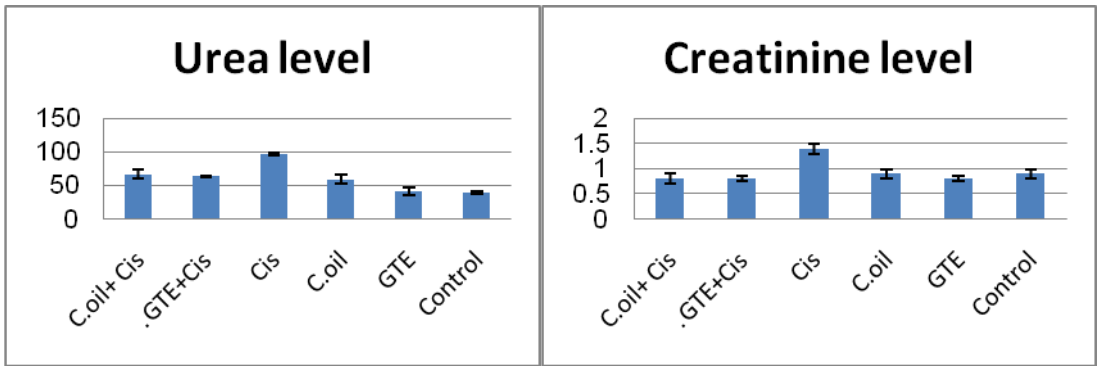
The results in **table (2)**and in **fig. 1** indicated that there was significant increase in blood urea nitrogen and creatinine levels in cisplatin administered group compared with group control. Rats pretreated with either green tea extract (GTE) or coriander oil (C.oil) exhibited nephroprotective effect reveled by diminishing the elevated blood levels of urea nitrogen when compared withcisplatin administered group. But, these levels remained high when compared with control group. On the other hand, there was no significant difference in creatinine level between pretreated groups and control groups.

Table (2): Mean values ± S.D of creatinine and urea nitrogen in control male rat and other treated groups

Parameters / Groups	Control	GTE	C.oil	Cis.	GTE+ Cis.	C.oil+ Cis.
urea nitrogen (mg/dl)	39.3± 1.5^{b}	42.3±5.5^{b}	59.3±6.6^{ab}	96.7±1.5^{a}	64.3±1.2^{ab}	67.3±6.8^{ab}
Creatinine (mg/dl)	0.9^{b}	0.8±0.05^{b}	0.9±0.1b	1.4 ±0.1^{a}	0.8±0.05^{b}	0.8±0.1^{b}

Values bearing different superscripts (a, b) are significant at P < 0.05.

Fig1: Mean values ± S.D of creatinine and urea levels in control male rat and other treated groups



Liver functions tests evaluation:-

The results in **table (3), fig. 2**indicated that there was significant increase in serum ALT & AST enzyme activity in cisplatin-injected group compared with group control. Rats pretreated with GTE& coriander oil exhibited hepatoprotective activity reveled by diminishing the elevated serum levels of AST and ALT when compared withcisplatin-injected group. But, these levels remained high when compared with control negative group.

Table (3): Mean values ± S.D of AST, ALT in control male rat and other treated groups

Parameter / Gr.	Control	GTE	C.oil	Cis.	GTE+ Cis.	C.oil+ Cis.
ALT (U/L)	46.3±1.5^{b}	45.3±0.5^{b}	43.3±3.5^{b}	30± 3.4^{a}	37.3±6.1^{ab}	30± 4.0^{a}
AST (U/L)	115.3±1.5^{b}	118±3.1^{b}	120±0.6^{ab}	148.3 ±2.2^{a}	123.3±4.6^{ab}	123.7±1.1^{ab}

Values bearing different superscripts (a, b) are significant at P < 0.05.

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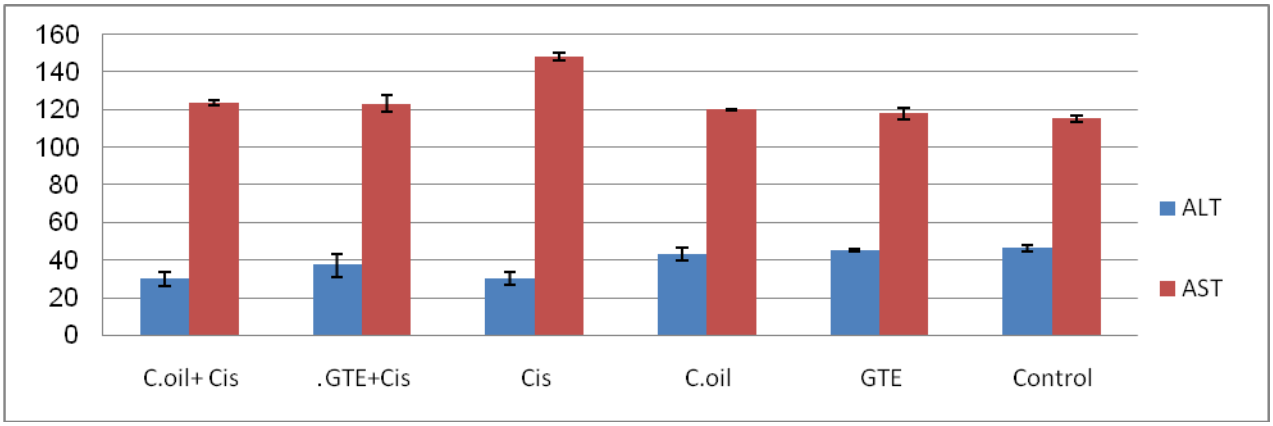
Fig 2: Mean values ± S.D of AST, ALT activity in different groups of rats



Estimation of LDH and CK levels:-

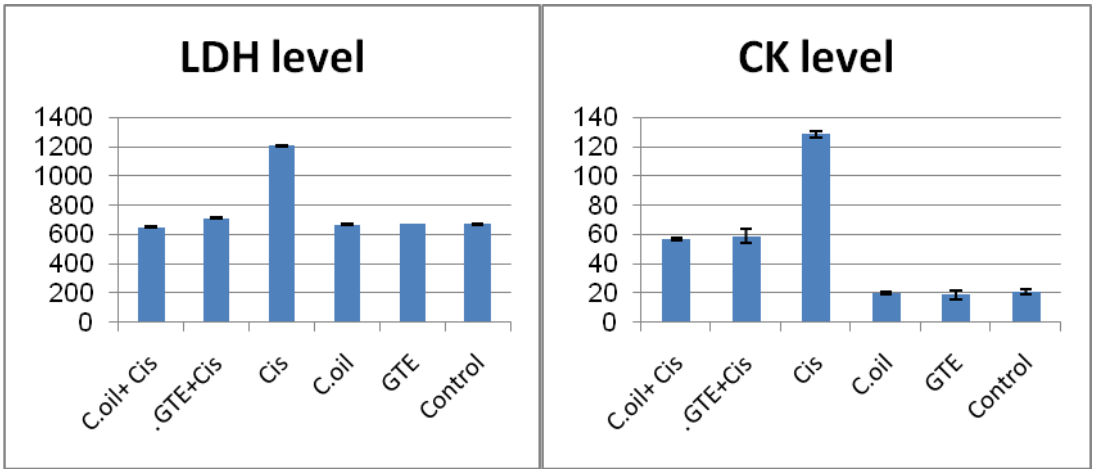
The results in **table (4), fig. 3** showed significant increase in LDH& CK levels in cisplatin administered group compared with group control. There was significant decrease in LDH& CK levels in rats pretreated with either GTE or C.oil when compared withcisplatin administered group.

Table (4): Mean values ± S.D of LDH, CKin control male rat and other treated groups

Parameters / Groups	Control	GTE	C.oil	Cis.	GTE+ Cis.	C.oil+ Cis.
LDH (U/L)	671±1.5^{b}	670±0.5^{b}	668±3.5^{b}	1206± 3.4^{a}	713±6.1^{ab}	650± 4.0^{a}
CK (U/L)	21±1.5^{b}	19±3.1^{b}	20±0.6^{b}	128.3 ±2.2^{a}	59±4.6^{ab}	57±1.1^{ab}

Values bearing different superscripts (a, b) are significant at P < 0.05.

Fig 3: Mean values ± S.D of LDH, CKactivity in control male rat and other treated groups



Oxidative stress evaluation:-

The results in **table (5& 6), fig. (4& 5)** revealed significant increase in MDA levels and significant decrease inactivity ofSOD marker in both liver and kidney tissues incisplatin injected group when compared with control group. The administration of GTE and C.oil(each alone) showed nearly similar values of the previous parameters of control group. Rats pretreated with GTE and those pretreated with C.oil prior to cisplatin injection exhibited hepatoprotective and nephroprotective activity reveled by diminishing the

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elevated MDA value and elevating the diminished serum activity of SOD when compared with cisplatin injected group.

Table (5): Mean values ± S.E of renal antioxidant parameters (MDA level & SOD activity)in control male rat and other treated groups

Parameters / Gr.	Control	GTE	C.oil	Cis.	GTE+ Cis.	C.oil+ Cis.
MDA(n mol/g)	195±3.2^{b}	180.9±2.4^{b}	206.2±2.4^{b}	360 ±2.2^{a}	181.5±1.7^{b}	211.5±1.08^{b}
SOD(U/g)	4496±2^{b}	3938±2.9^{b}	2837±1.8^{ab}	1332±1.4^{a}	3514±2.4^{b}	2466.3±0.8^{ab}

a: Significant from control group (P≤0.05).b: Significant from cisplatin group (P≤0.05).

Table (6): Mean values ± S.E of hepatic antioxidant parameters (MDA level & SOD activity) in control male rat and other treated groups

Parameters / Gr.	Control	GTE	C.oil	Cis.	GTE+ Cis.	C.oil+ Cis.
MDA(n mol/g)	157.7± 0.5^{b}	158.4±1.5^{b}	144±1^{b}	337.4±1.4^{a}	158±2.05^{b}	151.8±1.4^{b}
SOD(U/g)	3303±1.8^{b}	3061±1.7^{b}	4510±2^{ab}	904.3±1.4^{a}	2846.3±1.6^{b}	2873.3±2.1^{b}

a: Significant from control group (P≤0.05).b: Significant from cisplatin group (P≤0.05).

Fig 4: Mean values ± S.E of the renal and hepatic antioxidants parameters (superoxide dismutase) in control male rat and other treated groups

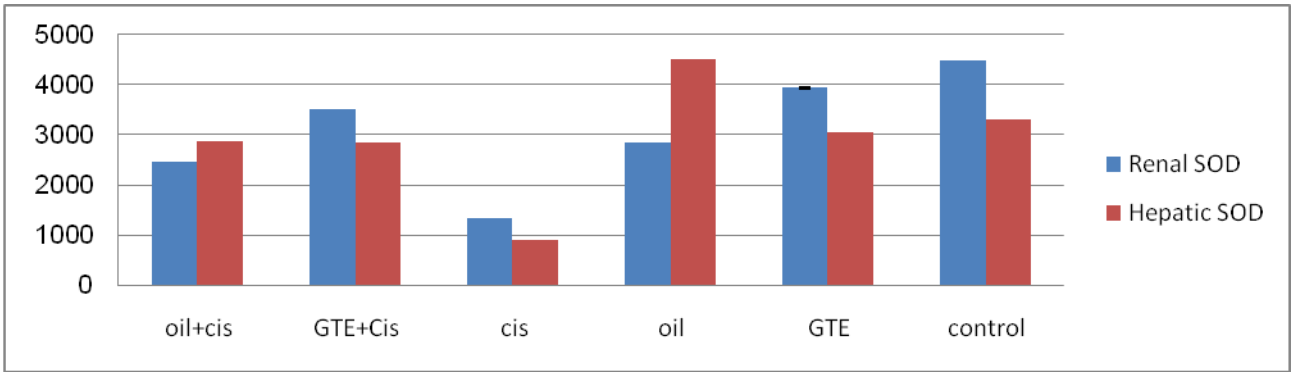
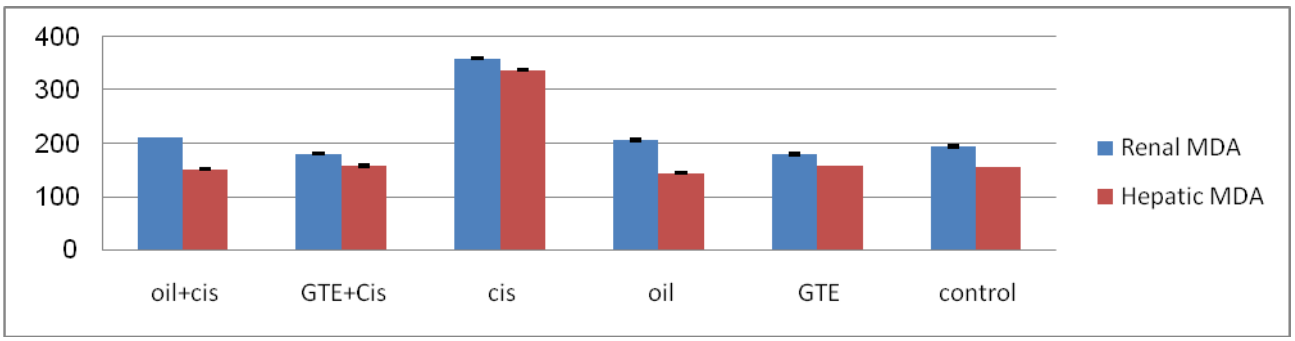


Fig 5: Mean values ± S.E of the renal and hepatic antioxidants parameters (lipid peroxide) in control male rat and other treated groups



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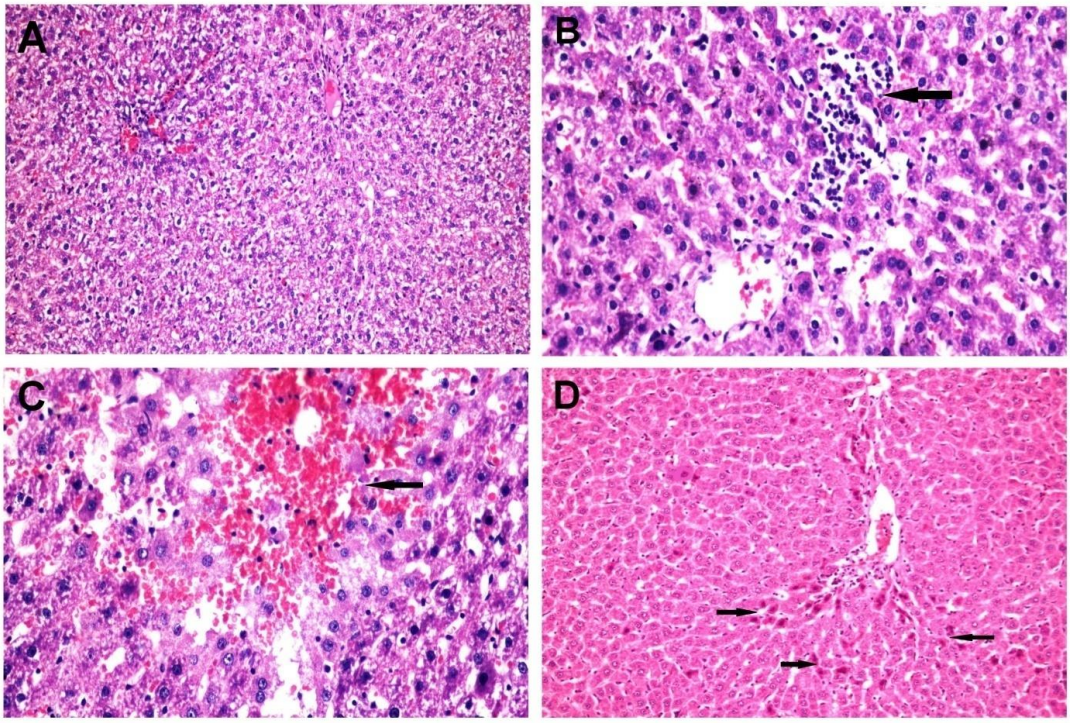
Histopathological studies:-

Liver

Microscopic examination of liver tissues from the control rats revealed normal histological structures, while those from Cis-injected groups demonstrated remarkable hepatocellular injury with noticeable diffuse vacuolar degeneration **(fig.6A).**Focal areas of hepatocellular coagulative necrosis were detected. The cytoplasm of hepatocytes appeared deeply eosinophilic with pyknotic nuclei. The necrotic hepatocytes were replaced and displaced by mononuclear inflammatory cells **(fig.6B).** Individual hepatocellular necrosis also recorded with presence of apoptotic cells and bodies. There were vascular congestion and sinusoidal dilatation in most of the examined sections. Focal and diffuse hemorrhage in the hepatic parenchyma also recorded **(fig.6C).** Hyperplasia of epithelial lining bile duct with formation of nonfunctioning bile ductules as well as portal and periportal lymphocytic cells infiltrations were noticed in most of examined cases**.** There was marked improvement in histopathological parameters in GTE and C.oil pretreated groups and the hepatic tissue returned to normal histological structure with mild degeneration **(fig.6D).**Hyperactivation of Kuppfer cells with presence of binucleated hepatocytes noticed in the pretreated groups.

Fig, 6: (A-C) Liver section from rat in cisplatin injected group showing (A)Vacuolar degeneration (H&E X 10). (B)Focal area of hepatocellular necrosis (arrow) replaced by inflammatory cells (H&E X 20). (C)Focal area of hemorrhage (arrow) in the hepatic parenchyma (H&E X 40). (D)Liver section from rat in C.oil pretreated group showing normal histological structure with separate individual hepatocellular necrosis and apoptosis (arrows) (H&E X 10)



Heart

Histological examination of tissue sections from heart muscle of the control rats revealed normal histological structures, while those of Cis-injected groups clearly showed vacuolar degeneration and necrosis of the cardiac muscle fiber cells**.**The myocardium showed congestion of blood vessels, edema and hemorrhage between muscle bundles with lymphocytes infiltrations **(fig.7A)**. GTE and C.oil may be effective in amelioratingcisplatin-induced cardiac damage and shows normaltissue structures in these groups with no significant degenerative changes when compared with Cis-injected group **(fig. 7B).**

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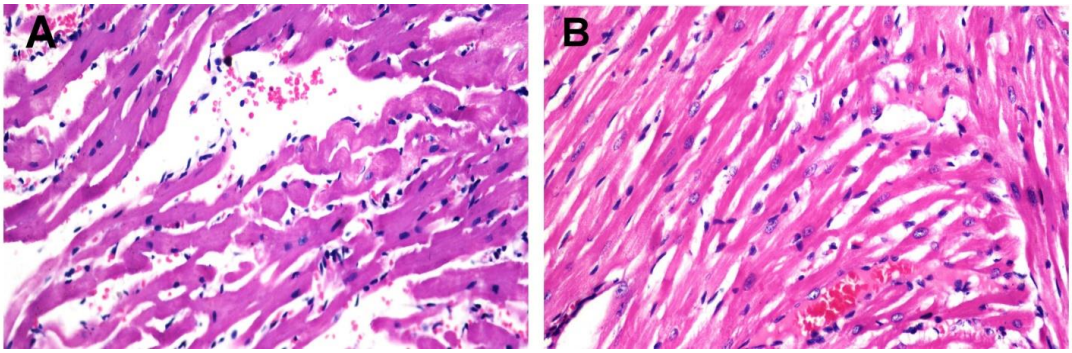
Fig, 7: (A)Heart tissue section from rat in cisplatin injected group showing edema, hemorrhage and lymphocytes infiltrations in between necrosed cardiac muscle bundle. (B)Heart tissue section from rat in GTE pretreated group showed marked improvement with normal cardiac tissue structures. (H&E X 400).



Microscopic scoring of hepatotoxicity and cardiotoxicity in rats of different groups were described in **Table (7)**and illustrated in **fig.9**. Liver and heart of rats in control negative group showed normal histology with no signs of injury (score= 0). On the other hand, liver and heart of cisplatin injected rats showed severe injury and have a higher score in all the previous parameters when compared with other groups (score= 4). The results also revealed that pretreatment of rats with either GTE or oil improved hepatic and cardiac muscle lesions and showed mild toxicity (score <1).

Table (7): Microscopical scoring of hepatocellular injury and cardiotoxicity in rats

HL / GR	control	GTE	C.oil	Cis	GTE+ Cis.	C.oil+ Cis.
HCD	0	0.2	1.4	4	0.6	0.9
HCN	0	0	0	4	0.5	0.2
HC	0	0	0	4	1	1
HEI	0	0	0.7	4	0	0
Score for HCI	0	0.05	0.5	4	0.5	0.5
CMD	0	0	0.3	4	0	0.1
CMN	0	0	0	4	0	0
CC	0	0	0.2	4	1	0.4
CME	0	0	0	4	0	0
CMI	0	0	0	4	0	0
Score for CMI	0	0	0.1	4	0.2	0.1

HCD: hepatocellular degeneration, HCN: hepatocellular necrosis, HC: hepatic congestion, HEI: hepatic edema and inflammation, CMD: cardiac muscle degeneration, CMN: cardiac muscle necrosis, CC: cardiac congestion, CME: muscular edema, CMI: mononuclear cells infiltrations between cardiac muscle.

Kidneys

Microscopical examination of kidney sections in control groups showed normal histological structures, while, kidney sections of cisplatin administered group noticed severe histopathological alterations. Renal tubules showed diffuse cellular swelling, vacuolar degeneration and extensive necrosis of its epithelial lining **(fig, 8A, 8B)**. The necrotic cells appeared more easinophilic with pyknotic nuclei or without any nuclear details. Both granular and hyaline casts mixed with mononuclear inflammatory cells were noticed in the tubular lumen. Hyaline casts were prominently observed in the renal tubular lumen **(fig, 8C)**. Hyperplasia as well as regeneration in some of renal tubular epithelial cells was also noticed **(fig, 8D)**. There were congestion of interstitial blood vessels and glomerular capillary tuft in addition to inter tubular hemorrhage in some sections. Some of the glomeruli showed hypercellularity while others noticed atrophy in the glomerular tuft. Interstitial tissue was infiltrated by mononuclear inflammatory cells mainly lymphocyte **(fig, 8E)**. There was noticeable improvement in both GTE and C.oil pretreated groups, in which the kidney sections showed minimum

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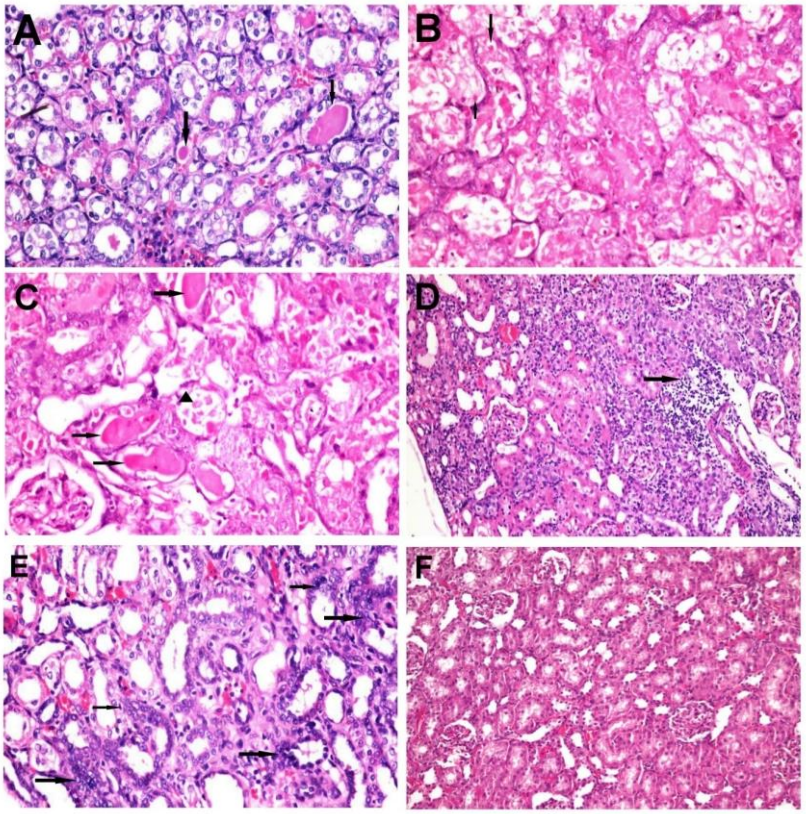
histopathological alterations. Renal tubules returned to normal with very mild degree of degeneration in epithelial lining **(fig, 8F)**. Congestion of interstitial blood vessels and glomerular capillary tuft was also detected in few sections.

Fig, 8: (A-E)Kidney section from rat in cisplatin injected group showing (A)Vacuolar degeneration in tubular epithelial cells with luminal hyaline cast (arrow) (H&E X 200). (B)Extensive necrosis in tubular epithelial cells with intracellular eosinophilic apoptotic bodies (arrows) (H&E X 400). (C)Extensive hyaline cast (arrows) and droplets (arrow head) in tubular lumen (H&E X 400). (D) Congestion of the interstitial blood vessels as well as mononuclear inflammatory cells infiltration (arrow) (H&E X100). (E)Hyperplasia (arrows) in some of tubular epithelial lining while others necrosed (H&E X 200).(F)Kidneys section from rat in green tea pretreated group showed marked improvement with mild congestion and tubular epithelial cell degeneration (H&E X 100).



Grading and scoring of nephrotoxicity in rats of different groups were described in **Table (8)**and in **fig, 9**. Kidneys of rats in control negative group showed normal renal histology with no signs of injury (score= 0). On the other hand, Kidneys of rats in cisplatin treated group showed severe renal injury and have a higher score in all renal parameters when compared with other groups (score= 4). The results also revealed that pretreatment of rats with either GTE or C.oil improved renal lesions and showed mild nephrotoxicity (score<1).

Table (8): Microscopical scoring of subacute nephrotoxicity in different groups

HL / GR	control	GTE	C.oil	Cis	GTE+ Cis.	C.oil+ Cis.
TCD	0	0.2	1.4	4	1.6	1.9
TCN	0	0	0	4	0.5	1.2
HC&HD	0	0	0	4	1	1.1
GC	0	0	0.7	4	0	1.5
GH	0	0	0.3	4	0	0.1
GA	0	0	0	4	0	0

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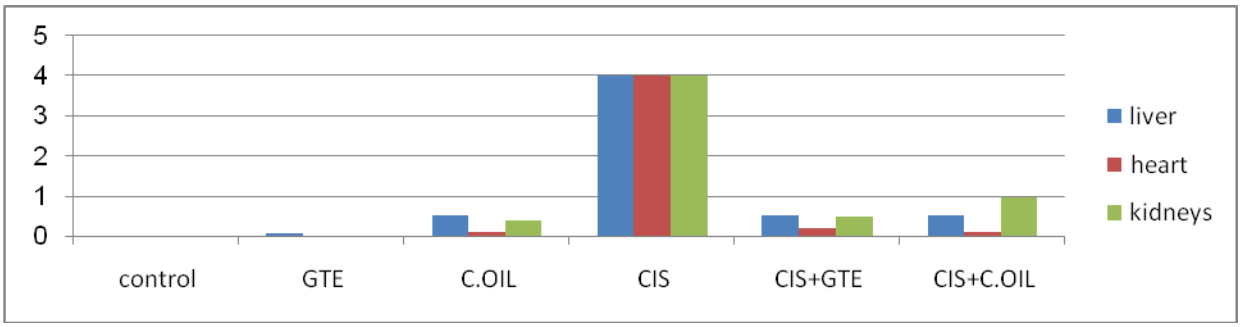
IC	0	0	1.2	4	1	1.4
IH	0	0	0	4	0	0
ICI	0	0	0	4	1	1.7
Total score	0	0.02	0.4	4	0.5	0.99

HL: histological lesions, GR: group, TCD: tubular epithelial degeneration, TCN: tubular epithelial cell necrosis, HC: hyaline cast, HD: hyaline droplets, GC: glomerular congestion, GH: glomerular hypercellularity, GA: glomerular atrophy, IC: interstitial congestion, IH: interstitial hemorrhage, ICI: interstitial mononuclear cells infiltrations.

Fig 9:-Total microscopic scoring of subacutehepato cellular, cardiac and renal toxicity in different groups



Immunohistochemical studies:

Immunohistochemistry staining of different liver**(fig, 10)** &kidney**(fig, 11)** sections revealed marked positive expression of both caspase-3 and Bax among the hepatocytes, epithelial lining bile ducts andrenal tubular epithelial cells incisplatin administrated group. An obvious decrease of both apoptotic markers expression was noticed in liver and kidney sections from GTE as well as C.oil pretreated rats.

Fig 10: Immunohistochemical staining of liver sectionsshowing strong positive Caspase-3 and Bax gene expression among hepatocytes and epithelial lining bile ducts in Cis injected group.Mild to negative gene expression among the hepatic tissues in both GTE and C.oil pretreated rats.

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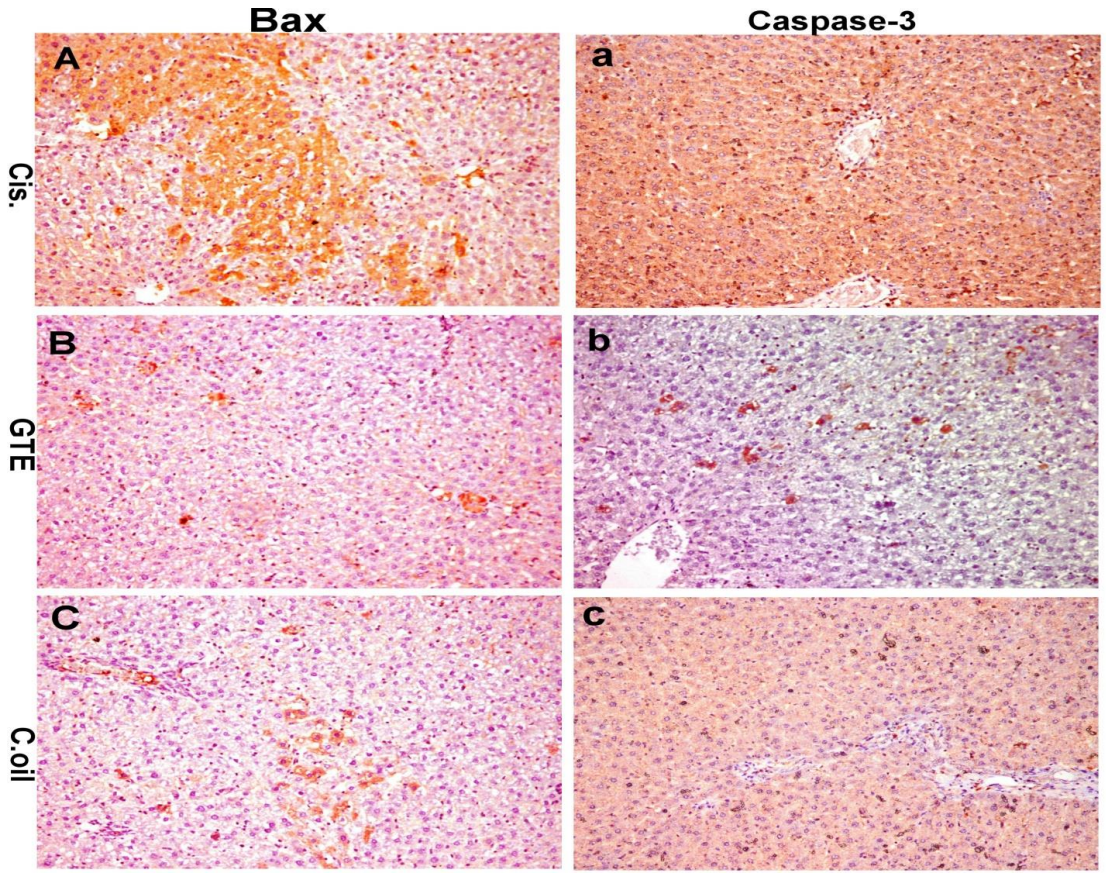

Fig, 11: Immunohistochemical staining of kidneys sectionsshowing strong positive Caspase-3 and Bax gene expression among renal tubular epithelium in Cis injected group.Mild to negative gene expression among the renal tissues in both GTE and C.oil pretreated rats.

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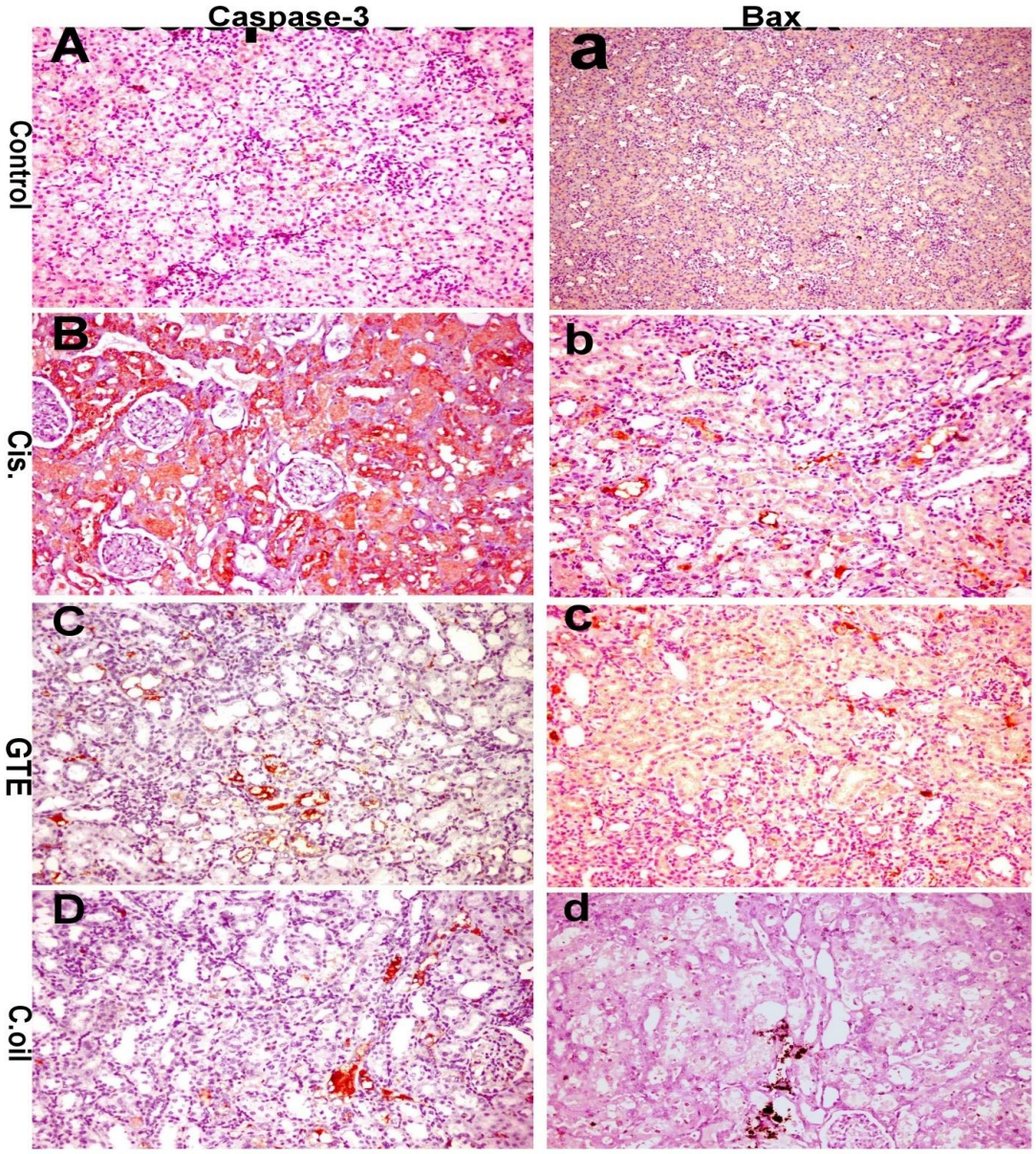

DISCUSSION

The present study is the first one confirmed the protective effect of green tea extract (GTE) and coriander oil (C.oil) against cisplatin-induced disturbance in biochemical parameters, oxidative stress markers and histopathological changes in rat's liver, cardiac muscle and kidney. In our study, there was significant increase in the levels of MDA and diminished in the SOD activity in liver and kidney of cisplatin injected group which could be attributed to the excessive generation of free radicals. This finding is in agreement with a previous report by **Valentovicet al., [38]**. It has also been reported that oxidative stress plays a pivotal role during cisplatintoxicity possibly due to depletion of glutathione and thiol groups **[10]**. Cisplatin was found to cause elevation of lipid peroxidation levels and decreased levels of antioxidant enzymes in the liver and renal tissues **[39].** In the current study, GTE and C.oil have an antioxidant effect manifested by reducing the elevated MDA levels and also increased the SOD activity inbothhepatic and renal tissues. Green tea act as scavenger of free radicals and prevent oxidative damage **[40].** This results in agreement with previous reports delivering the effects of GTE as an antioxidant **[41, 42].**This study was the first report to evaluate the preventive effect of coriander oil against cisplatin-induced toxicity in rats. It was suggested that addition of coriander to food would increase the antioxidant content and may have potential as a natural antioxidant and thus inhibit unwanted oxidation processes **[43]**.

Our results showed significant increase in serum levels of urea nitrogen and creatinine in cisplatin administered group, which indicated renal damage as well as defect in elimination of both break down products via the kidneys as recorded by **Fatima et al.[44]**.The changes observed in the kidneys biochemical parameters were confirmed by histopathological alterations. In the present study, kidneys of rats treated with cisplatin showed severe renal toxicity, mainly in the form of tubular epithelial cells degeneration and necrosis with renal casts and showed strong positive expression of Caspase-3 and Bax in the renal tubular epithelium. The mechanism for cisplatin-induced renal cell injury has been the focus of intense investigation for many years**[45],**and suggested that inflammation, oxidative stress injury, and apoptosis probably explain part of this injury. Unbound cisplatin is freely filtered at the glomerulus and taken up into renal tubular cells and partially metabolized into toxic species. Cisplatin has multiple intracellular effects, including regulating genes, causing direct cytotoxicity with reactive oxygen species, activating mitogen-activated protein kinase, inducing apoptosis, and stimulating inflammation and fibrogenesis. These events cause tubular damage and tubular dysfunction **[46].**

The improvement of different biochemical parameters and pathological changes of rats kidney in pretreated groups might be related to the antioxidant effect of the above mentioned plant extracts (GTE and C.oil). These plant extracts afforded significant protection against cisplatin induced kidney dysfunction reflected in the significant reduction of plasma creatinine and urea levels and showed marked regression in renal tissue damage. This improvement could be due to the significant elevation of SOD activity with simultaneous reduction of MDA that reflect their ability to scavenge free radicals with subsequent reduction of renal tissue damage and inflammation.Our results were in agreement with**Tavafiet al., [47]**, which indicate that administration of GTE ameliorate glomerular functions. It was suggested that green tea polyphenol participates in the elimination of uremic toxins and prevention of renal failure **[48]**. Coriander was reported previously to possess antioxidant and anti-inflammatory activity**[49, 50].** Other health benefits may help in improving kidney dysfunction such as diuretic, antihypertensive and naturetic. Coriander demonstrated diuretic and blood pressure lowering effect **[51].**

In the present study there was significant elevation in hepatic enzymes (ALT &AST) in cisplatin injected group which indicate liver damage and it had been previously reported that cisplatin administration causes deterioration in hepatic functions **[52]**. ALT &AST are located in the cytoplasm of hepatocytes and are released into the circulation after hepatocellular damage **[53]**.Histopathological changes observed in the present study including hepatocellular degeneration and necrosis withperiportal inflammatory cells infiltration are in agreement with the previous studies**[54. 55]**. Pretreatment of rats by either GTE or C.oil showed marked improvement in liver function biomarkers and in the histopathological changes of the liver tissue which returned in to normal and this indicate thehepatoprotective effect of both plants.

Our results showed significant elevation of serum LDH and CK levels in cisplatin injected group compared to the control and these results are consistent with those observed in other study**[56]** which was reported that cisplatininduced lipid peroxidation of cardiac membranes with the consequent increase in the leakage of LDH and CK from cardiac myocytes. Concerning the histological changes,the cardiac damage produced by cisplatin revealed degeneration and necrosis of cardiac muscle fiber cells with inflammatory cells infiltrations are consistent in general with findings observed by **Al-Majedet al.[57]**.

There are many evidences deal with the administration of antioxidants may be effective in ameliorating cisplatin-induced cardiotoxicity**[58]**. The cardiac protection of both GTE and C.oil is very well evident in this study, reflected in better protection; the reduction of serum enzymes levels and reversing the histological changes observed in the cardiac muscles. This improvement may be related to the antioxidant effects of both plants as discussed previously.

CONCLUSION

In conclusion, cisplatin (toxic chemotherapeutic drug) induced severe hepatotoxicity, nephrotoxicity and cardiotoxicity which is manifested by elevated renal and hepatic function, elevated LDH and CK enzymes, increased lipid peroxide level, reduced SOD activity as well as liver, cardiac muscle and kidney degenerative changes in histopathological findings. The main histopathological alteration observed was confirmed by immunohistochemical examination and concluded that cisplatin remarkably increased the gene expression of both Caspase-3 and Bax either in liver and kidneys. Pretreatment with green tea

extract and coriander oil exhibit a prominent protective effect against cisplatin toxicity in rats due to its antioxidant properties. This study recommends that intake of natural products like green tea extract and coriander oil may be beneficial for patients who suffer from cancer and received anticancer therapy (cisplatin).

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