

Plant Food Extracts as a Source of Bioactive Compounds for Prevention of Cisplatin-Induced Kidney Dysfunction in Rats

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The present study investigated the protective effect of extracts prepared from grape, coriander, roselle and fennel in a rat model of kidney dysfunction induced by intraperitoneal cisplatin. A mixture of ethanol and petroleum ether extracts was prepared from a given plant. Six groups of rats were analyzed; control healthy, cisplatin group and 4 test groups where rats were given a daily oral dose of each extract mixture before cisplatin injection. Different biochemical and cytogenetic parameters and kidney histopathology were determined. Total phenolic contents, fatty acids and unsaponifiable matter (UNSAP) were assessed in the extracts. Results showed roselle ethanol extract to have the highest phenolic content (15.584 g GAE/100 g extract). Fatty acid analysis revealed the presence of linoleic and linolenic acid in all studied plants. Coriander oil showed the highest content of unsaturated fatty acids (85%). GLC investigation of the UNSAP showed the presence of campesterol in all the studied plants. Grape oil contained the highest content of phytosterol (15.9%). Cisplatin treatment induced significant increase in plasma urea, creatinine and malondialdehyde along with significant reduction in plasma albumin, total protein, catalase activity and total antioxidant level as well as reduction in creatinine clearance compared to normal control. Histopathological examination proved the induction of kidney dysfunction by cisplatin. Chromosomal aberration and sperm-shape abnormalities were noticed after cisplatin treatment. Administration of extract mixtures produced improvements in biochemical, histopathological and cytogenetic parameters. Extracts mixture under study offered protection from cisplatin induced kidney dysfunction *via* antioxidant and possibly anti-inflammatory actions.

INTRODUCTION

Cisplatin (cis-diamminedichloroplatinum II, CDDP) is the most effective chemotherapeutic agent widely used in the treatment of a variety of malignancies [Rabik & Dolan, 2007]. However, the full clinical utility of cisplatin is limited by nephrotoxicity, the most common adverse effect, in many cancer patients [Schrier, 2002]. Approximately 28 to 36% of patients receiving an initial dose of 50–100 mg/m² from cisplatin develop acute renal failure [Schrier, 2002]. Thus, there is a pressing need to protect the kidney while administering cisplatin. Cisplatin induces nephrotoxicity and direct tubular toxicity in the form of apoptosis, necrosis [Arany & Safirstein, 2003] and inflammation [Ramesh & Reeves, 2003]. Also oxidative stress is involved in the development of this drug renal tubule injury [Chirino & Pedraza-Chaverri, 2009]. The involvement of oxidative stress is further supported by the fact that free radical scavengers and antioxidants prevented cisplatin-induced nephrotoxicity [Gulec *et al.*, 2006].

Kidneys are considered as the major control system that maintain the body haemostasis and are responsible for ex-

creting metabolic waste products [Edwards, 2010]. Oxidative stress and inflammation promote the development and progression of chronic kidney disease [Nicholas *et al.*, 2012]. Oxidative stress is a key factor linked to renal function decline with age [Li *et al.*, 2012]. Kidney disease progressing to end stage is an increasing burden to patients and society in general. The final common pathway of kidney damage often involves inflammation and fibrosis [Fassett *et al.*, 2011]. So, nutrients and bioactive compounds that can influence inflammation and have antioxidant activity, such as omega-3 polyunsaturated fatty acids, phytosterols and phenolic compounds [Davis *et al.*, 2009; Rosenbaum *et al.*, 2010], may be used in the adjuvant treatment of kidney diseases. Diets rich in fruits, vegetables and spices offer protection against the development of chronic diseases due to presence of such nutraceuticals [Brevik *et al.*, 2011]. A common factor in the etiology of chronic diseases seems to be damage to biomolecules caused by reactive oxygen species. Powerful endogenous antioxidant defenses are thought to be augmented by dietary antioxidants; so much of the protective effect of fruits and vegetables has been attributed to their high content of antioxidants [Brevik *et al.*, 2011]. The aim of the present study was to prepare and test the efficacy of bioactive compounds from different plants food extracts in preventing

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cisplatin-induced kidney dysfunction. Phytochemical analyses of the extract were included in the aim.

MATERIALS AND METHODS

MATERIALS

Plant materials

Red grape (with seeds) (*Vitis labruscana*), coriander fruits (*Coriandrum sativum* L., family umbelliferae), fennel seeds (*Foeniculum vulgare*) and roselle (*Hibiscus sabdariffa*) were purchased from local markets, Cairo, Egypt.

Chemicals

Platinol, 1 mg/mL (Cisplatin) obtained from Mayne Pharmaceuticals (Warwickshire, UK) was used for induction of kidney dysfunction.

Animals

In kidney dysfunction experiment; male Sprague–Dawley rats obtained from Animal house of National Research Centre, Cairo, Egypt were used. Average body weight of rats was 150 g. They were kept individually in metabolic cages; where water and food were given *ad-libitum*.

METHODS

Preparation of plant materials

Grape was washed by tap water and cut into small pieces. Plants' materials were dried in hot air oven at 40°C, and then they were crushed.

Preparation of plant extracts

The dried plants were extracted separately in a continuous extraction apparatus by petroleum ether (40–60°C) followed by ethanol. The solvents of the extracts were removed using rotary evaporator at low temperature (40°C).

Assessment of total phenolic contents

Total phenolic content was determined in the ethanol extracts and was expressed as gallic acid equivalent (GAE) in grams per 100 gram according to Singleton & Rossi [1965].

Determination of the profile of fatty acids, hydrocarbons and phytosterols

Fatty acids methyl esters and the unsaponifiable fraction were prepared from petroleum ether extracts [AOAC, 2000] for GLC analysis of phytosterols, hydrocarbons and fatty acids content.

The unsaponifiable fraction was assessed by GLC using the present conditions: Column: 10% OV-101 packed column; Stationary phase: Chromosorb W-HP; Detector temperature: 290°C; Injector temperature, 28°C; Carrier gas N₂; flow-rate 30 mL/min; air flow-rate: 300 mL/min; H₂ Flow-rate 30 mL/min; Detector FID; Chart speed: 0.5 cm/min; Oven program: Initial temperature, 70°C; Final temperature, 270°C; programmed 4°C/min. For 35 min at 270°C, total time, 85 min. Identification of hydrocarbons and sterols contents of the unsaponifiable matter was carried out by comparison of their

retention times with co-injected authentic reference compounds. Quantization was based on peak area integration.

Assessment of the methyl ester by GLC was carried out according to the following conditions: Stationary phase: 10% diethylene glycosuccinate (DEGS) packed column; oven temperature, 170°C; detector temperature, 300°C; injector temperature, 250°C; Carrier gas, N₂; flow-rate, 30 mL/min; air flow-rate, 350 mL/min; H₂ flow-rate, 350 mL/min; detector, FID; Chart speed, 2 cm/min. Identification of the fatty acid methyl ester was carried out by direct comparison of retention times of each of the separated compounds with authentic samples of the fatty acid methyl esters analyzed under the same conditions. Quantization was based on peak area integration.

Experimental diet

In the present research rats were fed balanced diet similar in composition to that reported by Al-Okbi *et al.* [2011]. The diet was composed of 12% casein, 10% corn oil, 22.5% sucrose, 46% maize starch, 5% fibers, 3.5% salt mixture [Briggs & Williams, 1963] and 1% vitamin mixture [Morcos, 1967].

Preparation of mixture of extracts

Petroleum ether and ethanol extracts of each plant was mixed in the same ratio of their occurrence in the parent plant. Mixtures of extracts were emulsified separately in water *via* gum acacia. The vehicle was prepared using the same amount of gum acacia in water to be administered to the control group.

Kidney dysfunction experiment

Different experimental groups were designed, each of six rats. Four test groups were given a daily oral dose of extracts mixture prepared from grape, coriander, fennel or roselle at 250 mg/kg rat body weight through gastric tube, while two control groups were given only the vehicle received by the test groups. After twenty days of treatment, each rat was given a single intraperitoneal dose of cisplatin as 7.5 mg/kg rat body weight [Yilmaz *et al.*, 2004; Atessahin *et al.*, 2005] except for rats of the control normal group (healthy group without cisplatin). Rats were fed balanced diet throughout the experiment. On the 5th day of cisplatin injection, 24-h urine was collected from each rat and blood samples were withdrawn from anaesthetized fasted animals from eye vein orbital using heparinized hematocrit tube. The blood was mixed with heparin. Plasma was separated by centrifugation at 3500rpm for determination of plasma malondialdehyde (MDA) as indicator of lipid peroxidation and oxidative stress [Satoh, 1978]. Plasma total antioxidant capacity (TAC), and catalase activity were assessed *via* colorimetry as antioxidant biomarkers [Koracevic *et al.*, 2001; Aebi, 1984, respectively]. Plasma creatinine [Houot, 1985], urea [Fawcett & Scott, 1960], total protein [Rheinhold, 1953], and albumin [Domas *et al.*, 1971] were estimated as indicators of kidney function. Creatinine was determined in the collected 24-h urine [Houot, 1985] for calculation of creatinine clearance. After blood sampling, all rats were injected intraperitoneally by 2 mL colchicine (0.2 g/100 mL saline) for cytogenetic study. After 2 hours of injection, bone marrow was separated for studying chromosomal

aberration. Also the epididymides were excised for assessing sperm-shape abnormalities. For histopathological study, kidneys were removed, placed in 10% formaldehyde, dehydrated in graded alcohol and embedded in paraffin. Fine sections were prepared, mounted on glass slides and counter-stained with hematoxylin and eosin for light microscopic analysis [Ekor *et al.*, 2010]. Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre, Cairo, Egypt, and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Cytogenetic study

For chromosomal aberrations, bone marrow metaphases were prepared [Yosida & Amano, 1965] and stained with 7% Giemsa stain in phosphate buffer (pH 6.8). Hundred well spread metaphases per animal were analyzed for chromosomal aberrations. The structural aberrations included gaps, breaks, and fragments and deletions as well as numerical aberrations; tetraploidy and polyploidy were assessed.

For sperm-shape abnormalities, the epididymides were minced in isotonic sodium citrate solution (2.2%) and fixed in acetic acid:methanol (1:3). Smears were prepared and sperms were stained with Eosin Y [Wyrobek & Bruce, 1978]. At least 1000 sperms per animal (6000/group) were assessed for morphological abnormalities of the sperm shape.

Statistical analysis

The results of rat experiments were expressed as the Mean \pm SE and they were analyzed statistically using the one-way analysis of variance ANOVA followed by Tukey test. In all cases $p < 0.05$ was used as the criterion of statistical significance. For statistical analysis of cytogenetic parameters Chi-square test (2X2 contingency table) was applied.

RESULTS

Results showed that total phenolic contents of the ethanol extracts were 6.74, 10.97, 15.58 and 12.74 g GAE/100 g for grape, coriander, roselle and fennel, respectively.

Fatty acids are shown in Table 1. Linoleic acid was present in all the studied plants, and the highest content was found in roselle (32.6%) followed by fennel oil (31.5%) then grape (18.2%) and coriander (14.9%). Coriander oil showed the highest content of n3 fatty acids represented by linolenic acid (70.1%). Linolenic acid was found in lower percentage in the other plants. The highest content of unsaturated fatty acid was present in coriander oil (85%) followed by roselle oil (68.4%) then grape oil (55.7%) and fennel oil (42.9%). The n3:n6 ratio was the highest in coriander (5:1), followed by grape (1:5), roselle (1:7) and the least in fennel. GLC investigation of the unsaponifiable matter (Table 2) showed the presence of campesterol, in all oils under investigation. Fennel oil showed the highest content of campesterol and β -sitosterol as 4.2% and 9.3%, respectively. Grape oil showed the highest content of stigmasterol (3.5%). Total phytosterol was 15.9% in grape oil, 15.2% in fennel oil, 9% in coriander oil and 1% in roselle oil. Total hydrocarbon was 23.5% in fennel, 21.2% in coriander, 18.2% in grape, and 12.4% in roselle oil.

TABLE 1. Fatty acids' content of the petroleum ether extracts of different studied plants (as percentage of total fatty acids).

Fatty acids	Grape oil	Coriander oil	Roselle oil	Fennel oil
Palmitic, C16 : 0	1.1	-	2.3	-
Palmitoleic, C16:1	9.6	-	15.6	-
Oleic, C18:1	24.2	-	15.8	9.3
Linoleic, C18 : 2	18.2	14.9	32.6	31.5
Linolenic, C18 : 3	3.7	70.1	4.4	2.1
Total saturated fatty acids	1.1	-	2.3	-
Total unsaturated fatty acids	55.7	85	68.4	42.9
n3:n6 fatty acids	1:5	5:1	1:7	1:15

Different biochemical parameters are presented in Table 3. Plasma creatinine, urea and MDA were significantly elevated while plasma total protein, albumin, catalase and TAC and creatinine clearance were significantly reduced in cisplatin group compared to control healthy group. Significant improvement of the abovementioned parameters was noticed on administration of the different nutraceuticals except for plasma total protein in case of the group given roselle. Nevertheless, the majority of improved parameters did not match those of the control healthy group. However some parameters such as plasma total protein and catalase and creatinine clearance in the group given fennel nutraceutical and TAC in the group given grape nutraceutical reached the normal levels.

Cytogenetic study

The number and percentage of different types of chromosomal aberration in different experimental groups are illustrated in Table 4. Aberration was highly significantly induced in cisplatin group when including and excluding gaps ($p < 0.001$) compared to healthy normal control. Also, number and percentage of metaphases with different types of chromosomal aberrations in cisplatin group were shown to be higher than those in control healthy rats.

Pre-treatment of rats with different extract mixtures showed significant decrease in aberration after excluding gaps ranged from 4% in case of grape to 6.2% in case of roselle compared to cisplatin group except for coriander that showed non-significant decrease (7.4%). Also significant reduction was noticed in percentage including gaps on administration of different extract mixtures compared to cisplatin group. The previous improvement did not reach that of the control. Breaks and fragments were reduced on administration of extract mixtures which reached 1.6% in case of grape group. Generally, the number and percentage of metaphases with different types of chromosomal aberrations were reduced on administration of the different extract mixtures except in very few cases. The efficiency of extract mixtures as inhibitors of total chromosomal aberrations was in the following order: grape > fennel > roselle > coriander. Table 5 demonstrates the different types of sperm-shape abnormalities. Cisplatin induced highly significant percentage of abnormal sperms, reaching 7.29% in cisplatin group com-

TABLE 2. GLC analysis of unsaponifiable matter of the different petroleum ether extracts of the plants under study (as percentage of total unsaponifiable matter).

Hydrocarbon & sterols	Grape oil	Coriander oil	Roselle oil	Fennel oil
Hydrocarbons				
C15	0.11	-	-	-
C16	1.09	-	-	0.50
C17	0.16	0.42	0.13	0.34
C18	0.37	1.65	1.52	2.50
C19	1.00	0.25	-	0.39
C20	0.33	9.94	-	-
C21	3.12	0.69	0.43	-
C22	1.88	1.20	1.07	0.81
C23	2.25	0.74	1.04	-
C24	0.69	-	-	1.63
C25	-	0.68	8.22	1.15
C27	3.95	-	-	3.69
C28	3.25	5.63	-	12.49
Total hydrocarbons	18.2	21.2	12.4	23.5
Phytosterols				
Campesterol	3.9	2.8	1.0	4.2
Stigmasterol	3.5	1.3	-	1.7
β -Sitosterol	8.5	4.9	-	9.3
Total phytosterols	15.9	9	1.0	15.2

pared to normal control. Pre-treatment with extract mixtures inhibited this percentage significantly.

Histopathology

Histopathological examination showed cisplatin group to demonstrate generalized severe necrosis of tubular epithelial cell accompanied with diffuse tubular lumina (hyalinized casts) and, diffuse intratubular ducts. Also foci of fibrosis and congested vessels were noticed. No significant pathological changes were observed in control healthy rats (Figure 1-G). Ingestion of grape extract showed protective effect in most of rats, while complete protection from the nephrotoxic effect of the cisplatin was seen in 16% of the rats. Some rats showed focal medullary intra-tubular casts (Figure 1-A). Coriander extract produced mild protective effect against cisplatin with histopathological changes in the form of clear cell changes in the collecting ducts and distal convoluted tubules and intratubular casts (Figure 1-B). Roselle produced good protective effect towards cisplatin at the histopathological level, while some changes in the form of congested vessels and few casts in the collecting ducts were noticed (Figure 1-C). Fennel extract showed little protective effect against the nephrotoxic effect of cisplatin, where congested vessels surrounded by inflammatory cells, some intratubular casts and focal clear cell changes in the collecting ducts were shown (Figure 1-D).

DISCUSSION

Cisplatin is a potent chemotherapeutic agent that has been widely used to treat many solid tumors, however neph-

TABLE 3. Biochemical parameters of different experimental groups (Mean \pm SE).

Groups	Creatinine (mg/dL)	Urea (mg/dL)	Total protein (g/dL)	Albumin (g/dL)	Catalase (u/mL)	TAC (mmol/L)	MDA (nmol/mL)	Creatinine clearance (mL/min)
Normal control (GP 1)	0.762 ^a ± 0.027	30.1 ^a ± 1.449	7.2 ^a ± 0.114	3.8 ^a ± 0.076	380.6 ^a ± 10.7	1.5 ^a ± 0.036	9.0 ^a ± 0.344	0.069 ^a ± 0.004
Cisplatin group (GP 2)	3.1 ^b ± 0.153	204.4 ^b ± 5.637	6.2 ^b ± 0.116	2.7 ^b ± 0.081	200.2 ^b ± 8.607	1.0 ^b ± 0.035	19.6 ^b ± 0.432	0.047 ^b ± 0.004
% Change	307	597	-14	-27	-47	-34	117	-32
Grape + cisplatin (GP 3)	1.14 ^c ± 0.124	76.9 ^c ± 8.563	6.6 ^c ± 0.090	2.9 ^c ± 0.037	291.3 ^c ± 8.001	1.46 ^{ac} ± 0.037	13.3 ^c ± 0.193	0.089 ^c ± 0.011
**% Change	-65	-62	6	11	46	45	-32	89
Coriander + cisplatin (GP 4)	1.3 ^c ± 0.166	108.5 ^c ± 6.587	6.6 ^c ± 0.099	3.2 ^c ± 0.062	271.2 ^c ± 9.844	1.2 ^c ± 0.028	14.8 ^c ± 0.198	0.102 ^c ± 0.011
**% Change	-58	-47	6	19	35	20	-24	117
Roselle + cisplatin (GP 5)	2.3 ^c ± 0.118	131.3 ^c ± 3.828	6.3 ^{bc} ± 0.161	2.9 ^c ± 0.062	301.4 ^c ± 12.215	1.1 ^c ± 0.032	15.1 ^c ± 0.171	0.057 ^c ± 0.003
**% Change	-26	-36	2	11	51	10	-23	21
Fennel + cisplatin (GP 6)	1.5 ^c ± 0.137	97.1 ^c ± 3.328	6.9 ^{ac} ± 0.134	3.2 ^c ± 0.081	359.4 ^{ac} ± 6.599	1.1 ^c ± 0.027	15.3 ^c ± 0.171	0.096 ^{ac} ± 0.009
**% Change	-52	-52	11	19	80	-	-22	104

In column different letters means significant difference at 0.05 probabilities. **% Change calculated in comparison to normal control rat. **% Change calculated in comparison to cisplatin group. GP1: Rat without cisplatin and without any treatment. GP2: Rats injected by cisplatin (1p) without any treatment. GP3: Rats pretreated with grape extracts mixture before cisplatin injection. GP4: Rats pretreated with coriander extracts mixture before cisplatin injection. GP5: Rats pretreated with roselle extracts mixture before cisplatin injection. GP6: Rats pretreated with fennel extracts mixture before cisplatin injection. TAC: Total antioxidant capacity. MDA: Malondialdehyde.

rotoxicity is a major and dose-limiting side effect of cisplatin [Dolin & Himmelfarb, 2008]. Renal side effects of cisplatin include acute kidney injury, Fanconi-like syndrome, distal renal tubular acidosis, hypomagnesemia, hypocalcemia, renal

salt wasting, renal concentrating defect, hyperuricemia, transient proteinuria, erythropoietin deficiency, thrombotic microangiopathy and chronic renal failure [Miller et al., 2010]. Cisplatin-induced nephrotoxicity is mainly mediated through

TABLE 4. Number and percentage of different types of chromosomal aberration in bone marrow in different experimental groups.

Groups	Total chromosomal aberrations		No. and (%) of metaphases with different types of chromosomal aberrations				
	Including gaps Mean%±SE	Excluding gaps Mean%±SE	Chromatide gap	Break and fragment	Deletion	More than one aberration	Polyploidy
Normal control (GP 1)	3.4±0.25	1.8±0.20	8(1.6)	4(0.8)	2(0.4)	-	3(0.6)
Cisplatin group (GP 2)	13.2±0.97***	9.2±1.06**	20(4.0)	27(5.4)	6(1.2)	7(1.4)	6(1.2)
Grape + cisplatin (GP 3)	6.2 ^a ±0.25*	4.0 ^a ±0.54	11(2.2)	8(1.6)	2(0.4)	6(1.2)	4(0.8)
Coriander + cisplatin (GP 4)	10.4 ^a ±0.38***	7.4±0.55**	15(3.0)	22(4.4)	5(1.0)	4(0.8)	6(1.2)
Roselle + cisplatin (GP 5)	8.8 ^a ±0.38**	6.2 ^a ±0.60**	13(2.6)	21(4.2)	6(1.2)	2(0.4)	2(0.4)
Fennel + cisplatin (GP 6)	8.8 ^a ±0.80**	5.4 ^a ±0.68*	17(3.4)	18(3.6)	4(0.8)	2(0.4)	3(0.6)

When all groups were compared with normal control: *: significant at 0.05 level **: significant at 0.01 level ***: significant at 0.001 level. When test groups were compared with cisplatin group: a: significant at p<0.05. GP1: Rat without cisplatin and without any treatment. GP2: Rats injected by cisplatin (I.p) without any treatment. GP3: Rats pretreated with grape extracts mixture before cisplatin injection. GP4: Rats pretreated with coriander extracts mixture before cisplatin injection. GP5: Rats pretreated with roselle extracts mixture before cisplatin injection. GP6: Rats pretreated with fennel extracts mixture before cisplatin injection.

TABLE 5. Number and mean percentage of different types of sperm shape abnormalities in rat sperms in different experimental groups.

Groups	No. of scored sperms	Abnormal sperms No.	No. and (%) of different types of abnormal sperms						
			Mean% ± SE	Amorphous	straight	Banana shape	Without hook	Coiled tail	Double head
Normal control (GP 1)	5040	151	2.92±0.13	32 (0.63)	72 (1.43)	26 (0.52)	21(0.42)	-	-
Cisplatin group (GP 2)	5143	375	7.29±0.21**	63(1.22)	142(2.76)	86(1.67)	53(1.03)	23(0.45)	8(0.16)
Grape + cisplatin (GP 3)	5176	260	5.02 ^a ±0.40*	31(0.60)	90(1.74)	71(1.37)	52(1.00)	8(0.15)	8(0.15)
Coriander + cisplatin (GP 4)	5047	257	5.09 ^a ±0.30*	42(0.83)	106(2.10)	56(1.11)	46(0.91)	6(0.12)	4(0.08)
Roselle + cisplatin (GP 5)	5044	255	5.06 ^a ±0.12*	33(0.65)	103(2.04)	69(1.37)	35(0.69)	12(0.24)	3(0.06)
Fennel + cisplatin (GP 6)	5084	260	5.12 ^a ±0.11*	36(0.71)	119(2.34)	69(1.36)	33(0.65)	1(0.02)	2(0.04)

All groups were compared with normal control: *: significant at 0.05 level. **: significant at 0.01 level. When test groups were compared with cisplatin group: a: significant at p<0.05. GP1: Rat without cisplatin and without any treatment. GP2: Rats injected by cisplatin (I.p) without any treatment. GP3: Rats pretreated with grape extracts mixture before cisplatin injection. GP4: Rats pretreated with coriander extracts mixture before cisplatin injection. GP5: Rats pretreated with roselle extracts mixture before cisplatin injection. GP6: Rats pretreated with fennel extracts mixture before cisplatin injection.

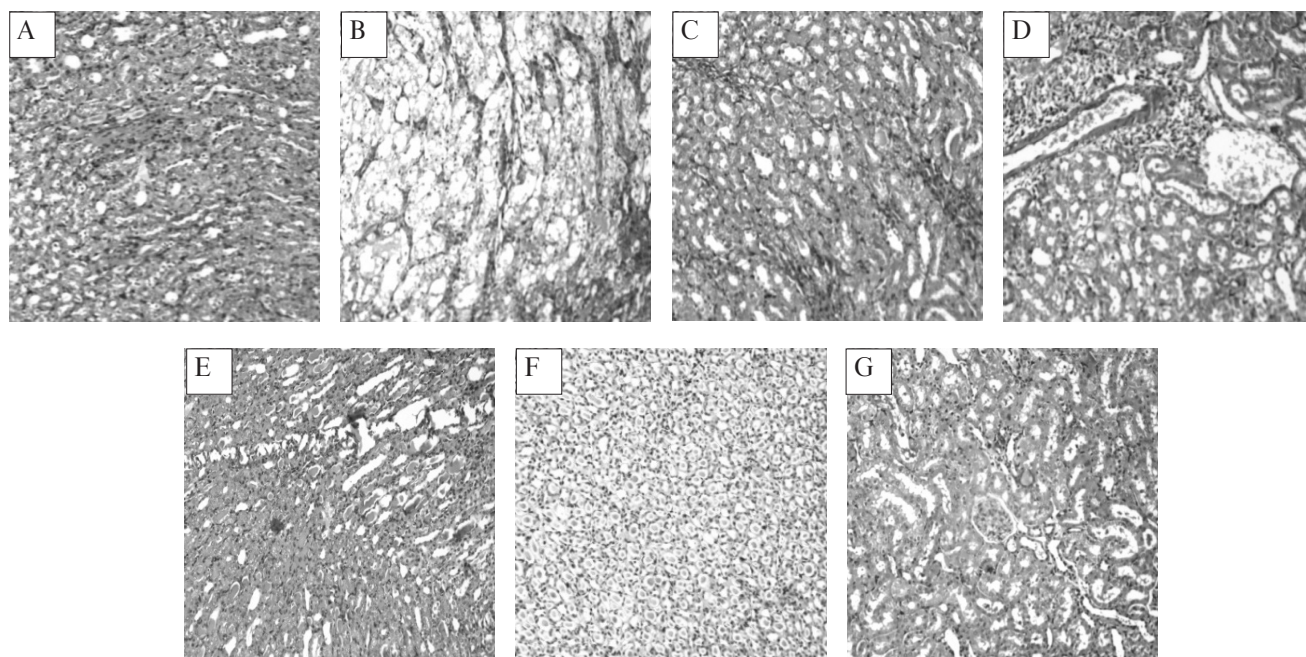


FIGURE 1. Section of rat kidney (x400) (A) grape extracts mixtures + cisplatin, (B) coriander extracts mixtures + cisplatin, (C) roselle extracts mixtures + cisplatin, (D) fennel extracts mixtures + cisplatin, (E, F) cisplatin control and (G) normal control.

drug transport into renal epithelial cells, which subsequently causes injury to nuclear and mitochondrial DNA, activation of cell apoptosis and necrosis, and stimulation of inflammatory responses [Pabla & Dong, 2008; Miller *et al.*, 2010]. Cisplatin-induced production of ROS has also been implicated in its nephrotoxicity [Davis *et al.*, 2001]. The major inflammatory factor involved in cisplatin-induced nephrotoxicity is TNF- α [Davis *et al.*, 2001; Naughton, 2008], which is highly dependent on ROS and NF- κ B activation [Ramesh & Reeves, 2005]. So in the present research extract mixtures were prepared from some edible plants rich in phytochemicals that possess antioxidant and anti-inflammatory activities such as, phenolic compounds and phytosterols, for protection from kidney dysfunction. Experimental models of kidney dysfunction are essential for discovering of new therapies that may have curative or protective effect towards such dysfunction. In the present research cisplatin was used for induction of kidney dysfunction according to Yilmaz *et al.* [2004] and Atessahin *et al.* [2005]. Extensive accumulation of cisplatin in the renal tubular cells is the major factor inducing kidney dysfunction [Ali & Al-Moundhri, 2006]. The decrease of plasma total protein and albumin that was noticed in the present study in cisplatin control group might be due to decreased reabsorption by the damaged tubules as can be observed from the histopathological changes. The increase in plasma creatinine and urea together with decreased creatinine clearance as noticed from the current results indicate the occurrence of kidney dysfunction which was supported by the histopathological study. These results are in accordance with previous researches [Mansour *et al.*, 2002; Tikoo *et al.*, 2007]. Accumulation of cisplatin preferentially affects the terminal proximal tubule and the distal nephron and can cause either apoptosis or necrosis, depending on exposure time and concentration [Ikari *et al.*, 2005].

Results from the current work further support reports from several studies that provided evidence that the cellular events in cisplatin-mediated nephrotoxicity are a consequence of reactive oxygen species (ROS) generation, which produces oxidative renal damage [Yilmaz *et al.*, 2004; Kadikoylu *et al.*, 2004]. Cisplatin in the present study produced significant reduction of the activities of plasma catalase (an antioxidant enzyme), plasma total antioxidant capacity accompanied by increased lipid peroxidation compared with the control rats. The renal oxidative stress may have resulted from the build-up of ROS such as O_2^- and H_2O_2 following the decrease in the activities of the renal antioxidant enzymes, which is typical during cisplatin treatment [Pratibha *et al.*, 2006]. The increased generation of O_2^- and H_2O_2 leads to increased production of the more reactive hydroxyl (OH \cdot) radical *via* Fenton and Haber-Weiss reactions [Stadtman, 1990]. Kidney damage due to elevated oxidative stress related to cisplatin treatment was also reported through reduction of renal reduced glutathione (GSH), superoxide (SOD), catalase, glutathione peroxidase and glutathione transferase (GST) and elevated MDA [Mansour *et al.*, 2002; Shimeda *et al.*, 2005; Ajith *et al.*, 2007].

Lipid peroxidation which produced thiobarbituric acid was induced by free radicals damage resulted from cisplatin treatment and resulted in damage in membrane lipids, pro-

teins and nucleic acids [Sato *et al.*, 2003; Conklin & Nicolson, 2008].

Grape, coriander fruits, fennel seeds and roselle were the sources of extract mixtures tested in the present study. Each extract mixture was composed of polar (ethanol extract) and non polar (petroleum extract) fraction of each plant food. From the present study it can be noticed that ethanol extract of all plants contained phenolic compounds ranging from 15.58 to 6.74 g GAE/100 g extract. It has been reported that phenolic compounds have antioxidant, anti-inflammatory and free radicals scavenging activity [Visioli *et al.*, 2011]. It was also observed that petroleum ether extract of the studied plants contained important phytosterols that have been shown previously to possess both antioxidant and anti-inflammatory activity [Islam *et al.*, 2009; Tan & Shahidi, 2011]. Also these non polar extracts were shown in the present study to contain high content of unsaturated fatty acids, from which linolenic (n3 fatty acid) that was present in the highest level in coriander and which was shown previously to possess anti-inflammatory activity [Saha & Ghosh, 2011].

The improvement of different biochemical parameters and histopathology of the kidney after administration of different extracts mixtures might be related to the synergistic effect of the abovementioned bioactive constituents. These extract mixtures afforded significant protection from cisplatin induced kidney dysfunction reflected in the significant reduction of plasma creatinine and urea and significant increase of plasma total protein and albumin and creatinine clearance. This improvement could be due to the significant elevation of TAC and catalase with simultaneous reduction of MDA that reflect their ability of scavenge free radicals with subsequent reduction of renal tissue damage and inflammation. In support to this explanation; red grape, fennel, roselle and coriander were reported previously to possess antioxidant and anti-inflammatory activity [Celik & Isik, 2008; Jabeen *et al.*, 2009; Gris *et al.*, 2011; Wu *et al.*, 2010; Deepa *et al.*, 2011; Frank *et al.*, 2012]. Other health benefits may help in improving kidney dysfunction such as diuretic, antihypertensive and naturetic. Fennel was shown previously to possess blood pressure lowering, diuretic and natriuretic effect [El Bardai *et al.*, 2001; Guimarães *et al.*, 2011]. Roselle was reported to possess different activities including diuretic, blood pressure lowering, antioxidant, attenuate nephropathy and preserve renal function [Wright *et al.*, 2007; Kao *et al.*, 2009; Ajiboye *et al.*, 2011; Olatunji *et al.*, 2012]. Coriander demonstrated diuretic and blood pressure lowering effect [Jabeen *et al.*, 2009].

The elevated oxidative stress due to treatment with cisplatin might be the cause of chromosomal aberration and sperm shape abnormalities demonstrated in the present study. The presence of antioxidants and anti-inflammatory bioactive constituent in the studied extract mixtures might be the cause of reduction of such chromosomal aberration and sperm shape abnormalities. Stability of genome might be afforded by treatment with antioxidant, anti-mutagenic, anti-carcinogenic and anti-inflammatory bioactive constituent such as phenolic compounds [Ferguson *et al.*, 2001]. Grape seed extract (GSE) significantly protected mice bone marrow chromosomes from gentamicin-induced genotoxicity by reducing the total number of aberrant cells, and different types

of structural chromosomal aberrations. So, the grape extracts mixture, in the present study, acted as a potent antioxidant preventing kidney damage and genotoxicity of bone marrow cells, which agreed with previous study [El-Ashmawy *et al.*, 2006]. GSE significantly protected mice bone marrow chromosomes from doxorubicin-induced genotoxicity by reducing the total aberrant metaphases and the frequency of structural chromosomal aberrations [Yalçın *et al.*, 2010], which go parallel with the present study. GSE treatment also decreased the frequency of micronucleus and increased the mitotic index values [Yalçın *et al.*, 2010].

CONCLUSIONS

In conclusion, extract mixtures prepared from polar and non polar fraction of red grape, coriander, roselle and fennel were shown to be efficient in prevention of cisplatin-induced kidney dysfunction reflected in improvement of oxidative stress, kidney function tests and kidney histopathology. The extract mixtures also reduced chromosomal aberration and abnormal sperm induced by cisplatin.

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