

Impact of Consuming Each of Dried Black Grape and Hot Red Pepper Alone or in Combination on Nephrotoxicity Induced by Cisplatin Injection in Rats

¹Hanan M.F. Abd El-Wahab, ¹Nagwa I.Y. Hassanin, ²Elhady M. Ahmed,
¹Asmaa R. Abd El-Monem

¹Biochemistry and Nutrition Department Women's College, Ain Shams University, Cairo, Egypt
²Nutritional Requirement and Growth Department, National Nutrition Institute Ministry of Health

Abstract: This study introduces a novel and useful strategy to elucidate the potent inhibitory effects of the active constituents of dried black grape and dried hot red pepper alone or in combination against cisplatin toxicity in rats. Eight groups, each consisting of 15 rats, Sprague dawley strain were used. All rat groups were injected *ip* with cisplatin (10 mg/kg of body weight), except for G1 which was injected *ip* by saline solution. The diets given to the different groups were as follows: G1 basal diet (-ve control) and G2 basal diet (+ve control), G3 and G4 : basal diet+ (5%) of either dried black grape or hot red pepper. G5 and G6: basal diet + (10%) of either dried black grape or hot red pepper. G7 and G8: basal diet is incorporated with total either of 5% or 10% of dried food, respectively. After 37 days, there was a significant increase in parameters of kidney function and serum nitric oxide, whilst there was a significant decrease in serum total protein and total antioxidant capacity in G2 compared to G1. Wherein, there was a significant increase in kidney tissues lipid peroxides and nitric oxide, together with a significant decrease in kidney tissues and blood SOD, GSHPx activities and blood HB was noticed in G2 compared with G1. The results revealed that, when the diet is supplemented with dried black grape and/or dried hot red pepper a remarkable modulation of these abnormalities occurred. This effect was in the following descending order: (G8 and G7), (G6 and G5) and then (G4 and G3). The conclusion is that supplementing the diet with either dried black grape and hot red pepper alone or both together can protect against nephrotoxicity induced by cisplatin.

Key words: Cisplatin; lipid peroxidation; enzymatic antioxidant; nephrotoxicity; black grape; hot red pepper.

INTRODUCTION

Cisplatin is an active cytotoxic agent or an anti-cancer ("antineoplastic") chemotherapy drug that has proved to be successful in the treatment of metastatic tumors of the testicular, ovarian, bladder, head and neck, esophageal, small and non-small cell lung, breast, cervical, stomach and prostate cancers. It also treats Hodgkin's and non-Hodgkin's lymphomas, neuroblastoma, sarcomas, multiple myeloma, melanoma, and mesothelioma. The mechanism of cisplatin induced injury can be explained through the generation of free oxygen radicals in tubular cells. Data indicates that cisplatin induces oxidative stress, lipid peroxides, DNA damage and renal damage. The drug-induced cisplatin has some toxic side effects such as joint pain, ringing in the ears, trouble in hearing, weakness, hepatotoxicity and nephrotoxicity; which is considered a major side effect (Pabla *et al.*, 2008 a).

Grapes are small round or oval berries that feature semi-translucent flesh encased by smooth skin. Some contain edible seeds while others are seedless. All types of grapes and grapes products contain several vitamins as C, B1, B6, minerals as manganese and potassium. Grapes also contain antioxidants such as resveratrol, and polyphenols called ellagitannin and acutimissin A, which is identified as an excellent candidate for use as cancer preventive agent in prostate, lung, liver and breast cancer development resulting from exposure to chemical toxins). The burning sensation that makes chili peppers so appealing to culinary thrill-seekers comes from capsaicin or more accurately a collection of compounds called capsaicinoids; these are developed in the crossribs of the fruit, which makes that part of the pepper the hottest (Osawa and Kato, 2005).

Corresponding Author: Hanan M.F. Abd El-Wahab, Biochemistry and Nutrition Department Women's College, Ain Shams University, Cairo, Egypt.
Tel: 0020125871204; Fax: 202-24157804.
E-mail: mf_nana@hotmail.com

Materska and Perucka., (2005), studied phenolics contents and antioxidant activity which exist in hot red pepper. Two fractions of phenolics, flavonoids (with phenolic acids) and capsaicinoids, were isolated from the pericarp of pepper fruit at two growth stages (green and red) and were studied for their antioxidant capacity. Both fractions from red fruits had higher activities than those from green fruits. A comparison of the capsaicinoid fraction with the flavonoid and phenolic acid fraction from red fruit, with respect to their antioxidant activity, gave similar results. The present study aims to evaluate the efficiency of dried black grape and dried hot red pepper, alone or in combination, in combating the toxicity of cisplatin. The evaluation is based on lipid peroxidation pattern and antioxidant enzyme activities.

MATERIAL AND METHODS

Material:

- 1- Cisplatin ($\text{Cl}_2\text{Pt}(\text{NH}_3)_2$) drug was obtained from local pharmacy in a liquid form (10mg/10ml).
- 2- Hot red pepper (*Capsium Salanaceae* species) and black grape (*Vitis species*) were purchased from local market.
- 3- Rats were obtained from the National Cancer Institute, Cairo, Egypt.

Preparation of the samples:

Black grape: Fresh samples with seeds, then washed with tap water followed by distilled water, put in sodium hydroxide (0.2-0.3%) for five minutes, then washed by tap water many times, and dried on stainless steel tray. They were then put in sodium meta-bisulphide (1-2%) for 5 min, and then placed in the oven till complete dryness (Shell-lab model FX 750U.S.A) at 50-60° C for 8-12 h. This was followed by grinding. This is done in National Research Center, Giza, Egypt.

Hot red pepper: Fresh samples, then washed with tap water followed by distilled water, and dried in open air on a stainless steel tray, put in the oven at 50-70 ° C for 3-5 h. (Shell-lab model 1545 U.S.A), followed by grinding. This is done in National Research Center, Giza, Egypt.

The proximate composition moisture, crude protein, fat, ash (Muffle furnace.FB-1415-M U.S.A) and crude fiber of dried black grape and hot red pepper were determined according to A.O.A.C. (2000). The carbohydrate content was calculated by subtracting the sum of percentage of moisture, fat, protein, crude fiber and ash from one hundred (Table 1).

Table 1: Proximate analysis data of dried black grape and hot red pepper (g%).

Substrate	Moisture	Crude protein	Fat	Ash	Crude fiber	Carbohydrate
Dried black grape	13.6	2.3	1.1	7.52	1.8	73.68
Dried hot red pepper	10.21	0.8	8.5	8.7	26.3	45.49

Animal experiment:

One hundred and twenty adult male albino rats (Sprague dawley strain), weighing from 80 g to 90 g were obtained from the National Cancer Institute, Cairo, Egypt were used. The animals were housed individually in stainless steel cages fitted with a wire mesh bottoms and front in a room maintained at 25-30° C with about 50% relative humidity. The room was lighted on a daily photoperiod of 12 h light and dark. Rats were maintained under identical condition for a period of one week to be adapted with the surrounding environment; food and tap water were provided ad libitum. After a period of acclimatization, animals were divided into eight groups. Group1 (-ve control) was fed on basal diet (Reeves *et al.*, 1993) and was injected *ip* with saline (10 mg/kg b.wt.), G2 (+ve control) fed on basal diet and injected *ip* with cisplatin (10 mg/kg b.wt.) (Cetin *et al.*, 2006). From group G3 to group G8, all rats were injected *ip* with cisplatin (10 mg/kg b.wt.), G3 and G4: fed on basal diet+ (5%) of either dried black grape or hot red pepper (Cetin *et al.*, 2006). G5 and G6: fed on basal diet + (10%) of either dried black grape or hot red pepper. G7 and G8: fed on basal diet and incorporated with either 5% or 10% of dried black grape and dried hot red pepper respectively.

Blood and tissue samples:

On the 37th day of the experiment and after overnight fasting, rats were sacrificed under ether anesthesia. Incisions were made into the abdomen and part of blood samples were obtained from the portal vein into tubes containing EDTA. Whole blood samples were taken for determination of antioxidative enzyme activity: Glutathione peroxidase enzyme activity (GSHPx) was determined according to Habig *et al.* (1974) and superoxide dismutase activity (SOD) was determined according to Minami and Yoshikana (1979). Also hemoglobin was determined according to Bünger *et al.*, (1981).

At the same time, the other parts of blood samples were taken into centrifuge tubes and left at 37 ° C for 15 minutes, then serum was separated by centrifugation at 3000 r. p. m. for 15 minutes, and were kept in plastic vials then frozen at -20 °C for subsequent biochemical analysis of serum total protein according to Gornal *et al.*(1949), uric acid according to *Barham and Trinder* (1972), potassium according to Sunderman and Sunderman (1958), urea according to Fawcett, and Soct.,(1960), creatinine according to Larsen,(1972), nitric oxide according to Green *et al.* (1982), total antioxidant capacity according to Rice-Evan and Miller(1994). Kidneys were excised, rinsed in saline solution, blotted on filter paper to dry and stored at -20°C until analysis. Homogenize the tissue in 4 ml of 0.2 mol/L ice cooled phosphate buffer, pH: 7.4 per one gram tissue. Centrifuge at 9000 r.p.m for 30 minutes. Remove the supernatant for assay. The analysis included the determination of Glutathione peroxidase (GSHPx); Superoxide dismutase (SOD); Nitric oxide (NO) and lipid peroxides as malondialdehyde (MDA) determined according to Ohkawa *et al* (1979).

Statistical analysis:

The data was presented as mean \pm SD. One way Analysis of Variance (ANOVA) then post hoc-lest significant difference analysis (LSD) was performed using the statistical package for social science (SPSS) version 9 to compare all the treated groups. The value of $p < 0.05$ was considered statistically significant and $p < 0.001$ was considered statistically very highly significant. Percentage of difference representing the percent of variation with respect to the control group was also calculated (Daniel., 1991).

RESULTS AND DISCUSSION

There were non-significant differences in initial body weight between groups of rats at the beginning of the experiment.

In case of cisplatin injected rats (G2) (10 mg/Kg body weight) Table 2, acute nephrotoxicity was represented by a highly significant increase ($P < 0.001$) in serum creatinine, urea level, potassium level and uric acid, together with a significant decrease ($P < 0.001$) in serum total protein (1.95 \pm 0.39 g/dl) compared to negative control group (G1). On the other hand, there were improvements in parameters of both G3 fed on the 5% dried black grape and G4 fed on the 5% dried hot red pepper. There were a highly significant decrease in the values of serum urea, uric acid ($p < 0.001$), and potassium ($p < 0.05$) in both G3 and G4 compared to G2.. Regarding serum creatinine, there was a significant decrease in case of G3 ($p < 0.05$) and non-significant decrease in G4 compared to G2. A non-significant increase in serum total protein in either G3 or G4 was detected when compared with values of positive control group (G2). Using high concentration (10%) of either dried black grape diet (G5) or dried hot red pepper diet (G6) caused a more significant decrease in serum creatinine, urea level, potassium, and uric acid respectively compared to G2. A significant increase in serum total protein was observed in rats of G5 and non significant increase in rats of G6, relative to rats serving as positive control group (G2). The results obtained for rats of G7 and G8 which were given diet incorporated with a mixture of either 5% or 10% of dried black grape and dried hot red pepper showed improvement in biochemical parameters compared to that obtained when dried black grape or dried hot red pepper were served alone.

Table 2: The effect and consequences of dried black grape and/or dried hot red pepper with an active constituents on kidney function tests of rats injected by cisplatin-induced nephrotoxicity.*

Groups	G ₁	G ₂	G ₃	G ₄	G ₅	G ₆	G ₇	G ₈
Parameters	Negative	Positive	5% B.G	5% H.R.P	10% B.G	10% H.R.P	Mix 5%	Mix 10%
Total protein (g/dl)	5.66 \pm 0.70	1.95 \pm 0.39 ^a	2.90 \pm 0.26 ^a	2.66 \pm 0.43 ^a	3.61 \pm 0.78 ^{a,b}	3.13 \pm 0.86 ^a	3.93 \pm 0.79 ^{a,b,c,d}	4.41 \pm 0.47 ^{b,d,1,3,6}
Uric acid (mg/dl)	5.15 \pm 0.43	12.28 \pm 0.8 ^a	9.41 \pm 0.31 ^{a,b}	9.83 \pm 0.23 ^{a,b}	8.06 \pm 0.77 ^{a,b,d,3}	7.45 \pm 0.57 ^{a,b,c,d}	6.66 \pm 0.34 ^{a,b,c,d,5}	5.86 \pm 0.53 ^{b,c,d,e,f}
Potassium (mmol/L)	3.85 \pm 0.37	6.68 \pm 0.43 ^a	5.62 \pm 0.44 ^{a,2}	5.70 \pm 0.41 ^{a,2}	5.12 \pm 0.31 ^{b,1}	5.18 \pm 0.80 ^{a,b}	4.61 \pm 0.63 ^{b,3,4}	4.11 \pm 0.27 ^{b,c,d,5,6}
Urea (mg/dl)	23.6 \pm 5.12	67.25 \pm 4.2 ^a	53.62 \pm 4.89 ^{a,b}	54.25 \pm 2.96 ^{a,b}	47.25 \pm 3.99 ^{a,b}	48.50 \pm 4.50 ^{a,b}	43.37 \pm 3.7 ^{a,b,d,3}	40.12 \pm 1.24 ^{a,b,c,d,6}
Creatinine (mg/dl)	0.68 \pm 0.04	3.12 \pm 0.41 ^a	2.42 \pm 0.38 ^{a,2}	2.57 \pm 0.36 ^a	2.26 \pm 0.35 ^{a,2}	2.32 \pm 0.34 ^{a,2}	2.15 \pm 0.26 ^{a,b}	1.88 \pm 0.34 ^{a,b,4}

*Values are expressed as mean \pm S.D., n =15.

Highly Significant differens ($P < 0.001$) : (a) compared to group 1,(b) to group 2, (c) to group 3, (d) to group 4, (e) to group 5, (f) to group 6 and (g) to group 7.

Significant differens ($P < 0.05$) : (1) compared to group 1, (2) to group 2, (3) to group 3, (4) to group 4, (5) to group 5, (6) to group 6 and (7) to group 7.

Comparing G3 with G5 and G4 with G6, there was a significant decrease in serum uric acid in G5 ($p < 0.05$) when compared to G3, and in G6 ($p < 0.001$) compared to G4. A non-significant decrease in serum uric acid was noticed in G8 compared to G7.

Table 3 shows that in case of rats in the positive control group (G2), there was a significant decrease ($p < 0.001$) in superoxide dismutase (SOD) and glutathione peroxidase (GSHPx) activities, and a significant increase ($p < 0.001$) in the mean values of nitric oxide level and lipid peroxide in kidney tissues compared with negative control group (G1).

The effect of different treatment on the levels of nitric oxide, lipid peroxide, SOD and GSHPx activities in kidney tissues is summarized in Table (3). The results revealed that, there were a significant gradual decrease in kidney nitric oxide and lipid peroxide levels in all treatment groups except G4 (which fed 5% of dried hot red pepper).

Table 3: The effect and consequences of dried black grape and/or dried hot red pepper with an active constituents on antioxidant status in kidney tissues of rats injected by cisplatin-induced nephrotoxicity.*

Groups	G ₁	G ₂	G ₃	G ₄	G ₅	G ₆	G ₇	G ₈
Parameters	Negative	Positive	5% B.G	5% H.R.P	10% B.G	10% H.R.P	Mix 5%	Mix 10%
Nitric oxide $\mu\text{mol/L}$	1.37 \pm 0.48	4.96 \pm 0.39 ^a	4.01 \pm 0.67 ^{a,2}	4.16 \pm 0.30 ^a	3 \pm 0.44 ^{a,b,d,3}	3.2 \pm 0.38 ^{a,3,4}	2.45 \pm 0.35 ^{b,c,d,1}	1.85 \pm 0.36 ^{b,c,d,1,5}
GSHPx mg/g	78.5 \pm 3.07	31.62 \pm 4.14 ^a	41.25 \pm 3.06 ^{a,b}	38.87 \pm 4.05 ^{a,2}	50.62 \pm 2.88 ^{a,b,d,3}	48.5 \pm 4.81 ^{a,b,d,3}	59.62 \pm 3.34 ^{a,b,c,d,1,5}	68.25 \pm 3.06 ^{a,b,c,d,e,1,7}
SOD U/g	82.87 \pm 8.0	49.0 \pm 5.21 ^a	57.25 \pm 3.65 ^a	56.87 \pm 4.64 ^a	63.00 \pm 6.30 ^{a,2}	60.0 \pm 5.80 ^a	69.5 \pm 4.95 ^{b,1,3,4}	75.75 \pm 5.09 ^{b,c,d,1,5}
Lipid Peroxide nmol/L	1.65 \pm 0.58	4.11 \pm 0.39 ^a	3.23 \pm 0.31 ^{a,2}	3.41 \pm 0.34 ^a	2.68 \pm 0.27 ^{a,b}	2.83 \pm 0.23 ^{a,b}	2.1 \pm 0.40 ^{b,c,d,6}	1.86 \pm 0.32 ^{b,c,d,5,6}

*Values are expressed as mean \pm S.D., n =15.

Highly Significant differens (P<0.001) : (a) compared to group 1,(b) to group 2, (c) to group 3, (d) to group 4, (e) to group 5, (f) to group 6 and (g) to group 7.

Significant differens (P<0.05) : (1) compared to group 1, (2) to group 2, (3) to group 3, (4) to group 4, (5) to group 5, (6) to group 6 and (7) to group 7.

On the other hand, there was a significant increase in GSHPx activity in all treatment groups as compared to G2. A significant increase in SOD activity by 28%, 41.8% and 54.6% was reported in groups (5, 7 and 8) respectively when compared with G2.

Comparing G3 (fed 5% of dried black grape) with G5(fed 10% of dried black grape), there was a significant decrease (p< 0.05) in the mean value of nitric oxide, and a significant increase (P<0.05) in GSHPx activity in G5 compared with G3.

Comparing G4 with G6 (fed 10% of dried hot red pepper), there was a significant decrease (P <0.05) in the mean value of nitric oxide in G6 compared to G4. On the other hand, there was a significant increase (P <0.001) in kidney tissue GSHPx activity in G6 compared with G4. There were non-significant differences in kidney tissues SOD activity and lipid peroxide between the two treatment groups.

Comparing G7 (fed mix 5% of dried black grape and 5% dried hot red pepper), with G8 (fed mix 10% of dried black grape and 10% dried hot red pepper), it was found that there were non-significant differences in the mean values of nitric oxide, SOD activity and lipid peroxides. On the other hand, there was a significant increase (P< 0.05) in GSHPx activity in G8 compared to G7.

Table 4 shows the effect of cisplatin on serum total antioxidant capacity and nitric oxide. There was a highly significant increase on serum nitric oxide by (290%) and a highly significant decrease on serum total antioxidant capacity by (-65%) in G2 compared with negative control group (G1).

Table 4 depicts the changes in serum total antioxidant capacity and nitric oxide that occurred in all animals during 37 days of experiment. After feeding rats with all experimental diets, serum total antioxidant capacity was significantly increased in G7(p< 0.05) and in G8 (p< 0.001) compared with G2. Also there was a significant decrease (p < 0.001) in serum nitric oxide in G5, G7, and G8 compared to G2.

From Table 4, it is clear that there was a non-significant difference on serum total antioxidant capacity and the mean values of serum nitric oxide among the different groups of rats (G3 with G5, G4 with G6, and G7 with G8)

Table 4: The effect and consequences of dried black grape and/or dried hot red pepper with an active constituents on serum total antioxidant capacity and nitric oxide of rats injected by cisplatin-induced nephrotoxicity.*

Groups	G ₁	G ₂	G ₃	G ₄	G ₅	G ₆	G ₇	G ₈
Parameters	Negative	Positive	5% B.G	5% H.R.P	10% B.G	10% H.R.P	Mix 5%	Mix 10%
T. antioxidant Capacity mM/L	2.20 \pm 0.65	0.76 \pm 0.11 ^a	1.18 \pm 0.31 ¹	1.33 \pm 0.48 ¹	1.40 \pm 0.52 ¹	1.39 \pm 0.50 ¹	1.75 \pm 0.46 ²	1.95 \pm 0.13 ^b
Nitric Oxide $\mu\text{mol/L}$	0.77 \pm 0.25	3.02 \pm 0.37 ^a	2.41 \pm 0.31 ^a	2.68 \pm 0.29 ^a	2.37 \pm 0.35 ^{a,b}	2.43 \pm 0.51 ^a	1.97 \pm 0.24 ^{a,b,4}	1.9 \pm 0.25 ^{b,d,1,3,6}

*Values are expressed as mean \pm S.D., n =15.

Highly Significant differens (P<0.001) : (a) compared to group 1,(b) to group 2, (c) to group 3, (d) to group 4, (e) to group 5, (f) to group 6 and (g) to group 7.

Significant differens (P<0.05) : (1) compared to group 1, (2) to group 2, (3) to group 3, (4) to group 4, (5) to group 5, (6) to group 6 and (7) to group 7.

Table 5, illustrates the effect of supplementing different concentration of dried black grape and/or dried hot red pepper on the hemoglobin concentration and antioxidant enzymes activities in blood. The present study revealed that there was a highly significant decrease in HB concentration, SOD and GSHPx activities by (-16%, -41%, and -65%), respectively, in G2 when compared with G1 but there was a significant increase in HB concentration in G7 (p<0.05) and G8 (p<0.001) compared to G2.

Table 5: The effect and consequences of dried black grape and/or dried hot red pepper with an active constituents on the blood SOD, GSHPx activities and HB concentration of rats injected by cisplatin-induced nephrotoxicity.*

Groups	G ₁	G ₂	G ₃	G ₄	G ₅	G ₆	G ₇	G ₈
Parameters	Negative	Positive	5% B.G	5% H.R.P	10% B.G	10% H.R.P	Mix 5%	Mix 10%
HB g/dl	10.53 \pm 0.34	8.81 \pm 0.23 ^a	9.01 \pm 0.16 ^a	8.91 \pm 0.12 ^a	9.33 \pm 0.46 ^a	9.13 \pm 0.21 ^a	9.57 \pm 0.43 ^{a,2,4}	10.10 \pm 0.33 ^{b,c,d,1,5}
SOD U/ml	85.62 \pm 4.53	50.00 \pm 7.34 ^a	64.50 \pm 5.68 ^{a,b}	60.75 \pm 4.65 ^{a,2}	68.25 \pm 3.05 ^{a,b}	65.62 \pm 3.85 ^{a,b}	70.37 \pm 5.82 ^{a,b}	76.62 \pm 4.13 ^{b,d,3,6}
GSHPx mU/ml	64.37 \pm 4.47	22.50 \pm 5.23 ^a	33.50 \pm 5.34 ^{a,2}	29.75 \pm 3.65 ^a	38.00 \pm 4.00 ^{a,b}	36.50 \pm 4.27 ^{a,b}	47.12 \pm 4.51 ^{a,b,c,d,5,6}	54.50 \pm 3.81 ^{b,c,d,e,f}

*Values are expressed as mean \pm S.D., n =15.

Highly Significant differens (P<0.001) : (a) compared to group 1,(b) to group 2, (c) to group 3, (d) to group 4, (e) to group 5, (f) to group 6 and (g) to group 7.

Significant differens (P<0.05) : (1) compared to group 1, (2) to group 2, (3) to group 3, (4) to group 4, (5) to group 5, (6) to group 6 and (7) to group 7.

Also there was a significant increase in blood SOD activity in all treatment groups as compared with G2. Generally, it was found that, there was a significant increase in blood GSHPx activity in all treatment groups except G4 as compared with G2. Whilst, there was a non-significant difference between treatment groups in the values of HB concentration, SOD and GSHPx activities compared as: (G3 with G5), (G4 with G6) and (G7 with G8).

Discussion:

Cisplatin CDDP, is a platinum-based chemotherapy drug used to treat various types of cancers, including sarcomas, some carcinomas (e.g. small cell lung cancer, and ovarian cancer), lymphomas, and germ cell tumors. It was the first member of a class of anti-cancer drugs which now also includes carboplatin and oxaliplatin. These platinum complexes react *in vivo*, binding to and causing cross linking of DNA, this coordination complex not only inhibits replication and transcription of DNA, but also ultimately triggers apoptosis (programmed cell death)), (Pabla *et al.*, 2008b)

In the present study, administration of CDDP (10 mg/kg body weight i.p.) produced a significant nephrotoxicity in rats, demonstrated by a significant increase in serum creatinine, urea level, serum potassium level and serum uric acid and a significant decrease in serum total protein in positive control group compared with negative control group. This increase in parameters of kidney function may be due to the decrease in glomerular filtration rate or may be secondary to increasing the reactive oxygen species (ROS). This result agrees with other results of Noori and Mahboob.,(2010) which confirmed that, cisplatin accumulated in mitochondria, leading to the increase of (ROS) production.

Our results, agree with that of Mohamed, (2010), who assumed that the increase of ROS which attack the cell membrane lipids leads to the increase of tissue lipid peroxidation, manifested by increased MDA. Over accumulation of lipid peroxide in tissue causes over consumption and depletion of antioxidant enzymes as SOD and GSHPx activities.

There was a significant decrease in hemoglobin concentration, blood and kidney tissues superoxide dismutase (SOD) and glutathione peroxidase (GSHPx) activities, and a significant increase ($p < 0.001$) in the mean values of nitric oxide level and lipid peroxide in kidney tissues in comparison with negative control group. On the other hand, there was a highly significant increase on serum nitric oxide and a highly significant decrease on serum total antioxidant capacity in positive control group compared with a negative control group. These results agree with that of Yilmaz *et al.*, (2005) and Yao *et al* (2007) who showed that cisplatin treatment causes acute nephrotoxicity which was demonstrated by a marked increase in serum creatinine, serum urea, also there was an increase in the level of MDA and a depletion of SOD and GSHPX activities. Karaoglu *et al.*, (2005) go on the same line with the previous findings which was accompanied by increasing Na^+ and K^+ level in kidney tissues.

Oxidative stress injury is actively involved in the pathogenesis of CDDP-induced acute kidney injury. Reactive oxygen species (ROS) directly act on the cell component, including lipids, proteins, DNA and destroy their structure. ROS are produced via xanthine-oxidase system, mitochondria, and NADPH oxidase in the cells, in the presence of CDDP; ROS are produced through all these pathways and are implicated in the pathogenesis of acute CDDP-induced renal injury. Superoxide anion, hydrogen peroxide (H_2O_2) and hydroxyl radical are increased and react with lipids of the cell membrane by peroxidation and denature membrane cell proteins (Kawai *et al.*, 2006). This explains the high level of MDA in the CDDP group, also the reduced SOD and GSHPx activities, as a result of decreased total antioxidant capacity in group 2 as compared to group 1 as shown in the present study.

Also our results are in agreement with, Gao *et al.*, (2006) who investigated the changes of serum erythropoietin (Epo) during cisplatin – inducing anemia in rats. Anemia was induced with single intravenous injection of CDDP (8 mg/kg body weight). Serum Epo, hemoglobin, blood urea nitrogen (BUN) concentration, and reticulocyte (Ret) counts were measured on 7 and 21 days after administration of the anticancer drugs. The changes in renal tissue were examined by a light microscope. A single injection of CDDP decreased Ret counts and Hb concentration and increased BUN. Serum Epo was decreased on 7 days but was increased on 21 days after CDDP treatment; however, these results suggest that, in CDDP-induced anemia, the concentration of serum Epo level was low in relation to the level of anemia, and CDDP-induced nephrotoxicity might be the main cause of changes of serum Epo.

In the present study, black grape and hot red pepper were used as a whole after drying, so it is assumed to contain different ingredients which play important role as antioxidants, for example: in the hot red pepper as capsaicin and in black grape as resveratrol, polyphenols called ellagitannin and acutimissin A which blocks the action of an important enzyme, that is essential to the development of cancerous cells, also black grape contain vitamin C.

Better results can be observed for both G3 (fed 5% of dried black grape) and G4 (fed 5% of dried hot red pepper). There were a significant decrease in the levels of serum urea, uric acid, and potassium, there was a significant decrease in serum creatinine in G3 and non significant decrease in G4 compared to G2. Whilst, there was a more significant decrease in case of using the high concentration (10%) of either dried black grape diet (G5) or dried hot red pepper diet (G6). The results from G7 and G8 which were given a diet incorporated with a mixture of either 5% or 10% of dried black grape or dried hot red pepper showed better values than that of dried black grape or dried hot red pepper alone compared to G2. By comparing G3 with G5 and G4 with G6, there was a significant decrease in serum uric acid in G5 compared to G3, and G6 ($p < 0.001$) relative to G4. This improvement reported upon supplementation with black grape may be due to the action of resveratrol present in grape, it prevents oxidative stress damage caused by free radicals. Resveratrol can inhibit the growth of liver, breast, and lung cancer, by inhibiting receptors on cells called "the Aryl Hydrocarbon Receptors" (AHR), (Tadolini *et al.*, 2000).

Holthoff *et al.*, (2010) investigated that oxidant damage from reactive oxygen species (ROS) and reactive nitrogen species (RNS) is a major contributor to the cellular damage seen in numerous types of renal injury. Resveratrol (trans-3, 4, 5-trihydroxystilbene) is a phytoalexin found naturally in many common food sources especially black grape. The anti-oxidant properties of resveratrol are of particular interest because of the fundamental role that oxidant damage plays in numerous forms of kidney injury. Resveratrol produced a concentration-dependent inhibition of cytotoxicity. To examine the mechanism of protection, resveratrol was incubated with authentic peroxynitrite and found to block nitration of bovine serum albumin, in contrast to the known RNS scavenger. The data of these study suggested that resveratrol could provide functional protection by directly scavenging peroxynitrite. The data suggests that resveratrol is able to provide functional protection of renal tubular cells, at least in part, by directly scavenging the RNS peroxynitrite. This property of resveratrol may contribute to the understanding of its anti-oxidant activities.

The results of the present study revealed that there were a significant decrease in kidney nitric oxide and lipid peroxide levels in all treatment groups except G4 as compared to G2, and a significant increase in kidney GSHPx activity in all treatment groups as compared to G2. But only a significant increase in kidney SOD activity in groups (G5, G7 and G8) when compared with G2. By comparing G3 with G5, there was a significant decrease in the mean value of nitric oxide but on the other hand there was a significant increase in kidney GSHPx activity in G5 when compared with G3. Comparing G4 with G6, there was found a significant decrease in the mean value of kidney nitric oxide in G6 compared with that value in G4. On the other hand there was a significant increase in kidney GSHPx activity in G6 compared to G4. Comparing G7 with G8, there was a significant increase in kidney GSHPx activity in G8 compared to G7.

The present results agree with the study of Cetin *et al.*, (2006), which aims to elucidate the molecular mechanism(s) of cisplatin nephrotoxicity and the possible protective effect of antioxidant food supplementation. The study showed that black grape eliminated oxidant stress induced by cisplatin by increasing antioxidant potential.

Rosa *et al.*, (2005), investigated the reduction of the protective effect of capsinoid on lipid peroxidation in rat tissues, they concluded that the levels of total lipids and concentrations of malondialdehyde (MDA) were reduced in the group that received hot red pepper. Luqman and Rizvi., (2006), investigated the antioxidative property of capsaicin (8-methyl-N-vanillyl-6-nonenamide) found in hot peppers reported reduced oxidative stress; membrane lipid peroxidation (formation of malondialdehyde) and membrane carbonyl groups in human erythrocytes.

Also Oyagbemi *et al.*, (2010), concluded that, capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) is the principal pungent ingredient of hot red pepper and chili peppers that belong to the plant genus *Capsicum* (Solanaceae). Capsaicin is a cancer-suppressing agent. It blocks the translocation of nuclear factor kappa B (NF- κ B), activator protein 1 (AP-1), and signal transducer and activator of transcription (STAT3) signaling pathways that are required for carcinogenesis. The anti-inflammatory potential of capsaicin is attributed to its inhibitory effect on inducible COX-2 mRNA expression. Cytochrome P450 2E1 mediates the activation of xenobiotics such as vinyl carbamate and dimethyl nitrosamine to their toxic metabolites. This metabolic activation of xenobiotics by Cytochrome P450 2E1 has been shown to be inhibited by capsaicin. Capsaicin also generates reactive oxygen species in cells with resultant induction of cisplatin-induced apoptosis and cell cycle arrest, which is beneficial for cancer chemoprevention. Therefore, the use of capsaicin as a chemo preventive agent is of immense benefit for cancer chemoprevention.

The results of the present study revealed that serum total antioxidant capacity was significantly increased in G7 and in G8 compared with G2. In another way, there was a significant decrease in serum nitric oxide in G5, G7, and G8 compared to G2.

Regarding the effect of dried black grape and/or dried hot red pepper on the blood SOD, GSHPx activities and HB concentration cisplatin induced nephrotoxicity in rats; there was a significant increase in SOD activity in all treatment groups compared with G2. Generally, it was found that there was a significant increase in GSHPx activity in all treatment groups except in G4 compared with G2. Hogan *et al.*, (2010), studied the antioxidant compounds found in black grape and their effect on oxidative stress and inflammation in diet-induced obesity. They concluded that, grape contains significant amount of antioxidants and that resveratrol exerted an anti-inflammatory effect. Recent study done by Liu and Nair., (2010), found that capsaicin, which is present in hot peppers, could reduce the level of lipid peroxides in rats suffering from cisplatin-induced nephrotoxicity.

Conclusion:

From our study we can conclude that eating hot red pepper and/or black grape at least 2-3 times weekly in moderate quantities can give a protection against the illness. It can protect the kidneys of cancer patients, who receive cisplatin as a drug, from damage.

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