PROTECTIVE EFFECT OF CYSTEINE AND VITAMIN E, CROCUS SATIVUS AND NIGELLA SATIVA EXTRACTS ON CISPLATIN-INDUCED TOXICITY IN RATS

EZZAT S. EL DALY*

SUMMARY: Cisplatin [cis-dichlorodiammineplatinum (II)] is a widely used chemotherapeutic drug that is toxic to the kidney. Concurrent administration of cysteine together with vitamin E, Crocus sativus and Nigella sativa reduced the toxicity of cisplatin in rats. When administered i.p. for 5 alternate days with 3 mg/kg cisplatin, cysteine (20 mg/kg) together with vitamin E (2 mg/rat) an extract of Crocus sativus stigmas (50 mg/kg) and Nigella sativa seed (50 mg/kg) significantly reduced blood urea nitrogen (BUN) and serum creatinine levels as well as cisplatin-induced serum total lipids increases. In contrast, the protective agents given together with cisplatin led to an even greater decrease in blood glucose than that seen with cisplatin alone. The serum activities of alkaline phosphatase, lactate dehydrogenase, malate dehydrogenase, aspartate aminotransferase and alanine aminotransferase of cisplatin-treated rats were significantly decreased, whereas the activities of glutathione reductase and isocitrate dehydrogenase were significantly increased. Addition of cysteine and vitamin E, Crocus sativus and Nigella sativa in combination with cisplatin partially prevented many changes in the activities of serum enzymes. In cisplatin-treated rats, the liver activities of isocitrate dehydrogenase and aspartate aminotransferase were significantly increased, whereas much greater changes were found in the kidneys, with increased activity of glucose-6-phosphate dehydrogenase and decreased activities of alkaline phosphatase, isocitrate dehydrogenase, malate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, sorbitol dehydrogenase and γ -glutamyl transferase, as well as a decreased phosphorylation to oxidation ratio in the mitochondria, indicating reduced adenosine triphosphate production. Also, administration of cysteine and vitamin E, Crocus sativus and Nigella sativa together with cisplatin partially reversed many of the kidney enzymes changes induced by cisplatin. Cysteine together with vitamin E, Crocus sativus and Nigella sativa tended to protect from cisplatin-induced falls in leucocyte counts, haemoglobin levels and mean osmotic fragility of erythrocytes and also prevented the increase in haematocrit.

The results of this study indicate a basis for the toxic effects of cisplatin, and suggest a possible way of counteracting the toxicity by introducing protective agents such as sulphydryl compounds, other antioxidants and extracts of natural products. It also appears that cells adapt to the effects of cisplatin through the induction of system that produce NADPH, which in turn compensates the decrease of free sulphydryl groups.

We conclude that cysteine and vitamin E, Crocus sativus and Nigella sativa may be promising compounds for reducing cisplatin-toxic side effects including nephrotoxicity.

Key Words: Cysteine, Vitamin E, Crocus sativus, Nigella sativa, Cisplatin.

INTRODUCTION

Cisplatin was discovered to have cytotoxic properties in the 1960s, and by the end of the 1970s it had earned a place as the key ingredient in the systemic treatment of germ cell cancers. Since the early seminal work in the preclinical and clinical development of this drug, several thousand analogues have been synthesized and tested for properties that would enhance the therapeutic index of cisplatin (50). Although renal toxicity is the dose limiting factor for the use of cisplatin, other associated toxicities include emesis, nausea, diarrhea, anorexia, hair epilation and myelosuppression (2). Mechanisms for renal toxicity range from definitive histologic changes found in the proximal convoluted tubules to physiologic and biochemical alterations involving a decrease in mitochondrial respiratory function, enzymic activity in the respiratory chain and glutathione peroxidase, and effects on cellular calcium homeostasis. Important factors related to nephrotoxicity include age, renal irradiation, and concurrent alcohol intake (3). A potential mechanism influencing the sensitivity of cells to cisplatin may result from the interaction of specific proteins with cisplatin-damaged DNA (32). Other molecules, including RNA and proteins, also react with cisplatin (29). These reactions occur either by a direct pathway in which the co-ordinated chlorides are displaced by nucleophilic entering groups or by an indirect pathway in which the entering nucleophiles react with cisplatin molecules that have already exchanged chloride groups for solvent (H_2O) (43,44). Cisplatin protein interactions are important in determining the therapeutic efficacy of the antitumour agent. Binding of cisplatin to plasma proteins significantly alters the rate of clearance of the drug from circulation (6,20). Reactions of native proteins with cisplatin may be responsible for the observed toxicity to the kidneys and gastrointestinal tract (18,40).

The effect of cisplatin on liver and kidney functions has been reported in several studies. Treskes *et al.* (49); Hanigan *et al.* (25) and Bogin *et al.* (14) showed that an injection of cisplatin changed liver and kidney enzyme activities. Also, Nair *et al.* (40) reported that body weight, haemoglobin levels and leucocyte counts were decreased after cisplatin injection in mice. On the other hand, administration of cysteine and vitamin E together with cisplatin partially reversed the uraemia and many of the biochemical changes induced by cisplatin (14).

Biochemical studies with heavy metals show that they react with free sulphydryl groups. It is postulated that the nephrotoxicity caused by several heavy metals, e.g. mercury, is related to the intracellular decrease of reduced glutathione, enzymes are then inactivated because their SH-groups are not maintained in a reduced form (19). Levi *et al.* (33) showed that following the administration of cisplatin to rats, there was a significant decrease of SH groups per gram wet weight. This change was seen prior to the elevation of blood urea nitrogen (BUN) and creatinine in the blood, suggesting that SH group depletion may be a primary event leading to the renal failure. The decrease of SH groups was due to a decrease of protein-bound SH, with the greatest decline in the cytosolic and mitochondrial fractions (33).

Ways of protecting SH groups and reducing the toxic effects caused by cisplatin have been reported (4,14,16,34).

Considerable interest has been focused on the isolation of compounds that might be administered with cisplatin to reduce the dose-limiting toxicity (2). For these compounds to be useful, they must either be selectively absorbed by non-tumour cells or administered at an appropriate time, before or after the antitumour drug, when injury to tumour cells is irreversible and reversible to non-tumour cells e.g. MPG (2-mercaptopropionyl glycine), methimazole, WR-2721 [S-2 (3-amino propylamino) ethyl phosphorothioic acid] and dithiocarbamates (27,39,45,49).

Considering the above, the current study was undertaken to examine the effects of cisplatin on various enzyme systems in the blood, liver and kidney, to relate these effects to renal failure, and to evaluate possible methods of reversing these effects by an administering compounds that protect or increase SH groups, e.g. cysteine and vitamin E. Also the effects of extracts of natural products like *Crocus sativus* dried stigmas (Saffron) and *Nigella sativa* seeds, as potential protective agents against cisplatin-induced toxicity in adult male albino rats were examined.

MATERIALS AND METHODS

Animals

Adult male albino rats (160-180 g; Animal House of the National Research Center, Doki, Giza) were housed in a con-

*From Department of Biology, Faculty of Education, At Suez, Suez Canal University, Egypt.

trolled room on a 12-hr light/dark cycle and given food and water *ad libitum*.

Chemicals and Natural Products

Cisplatin, cysteine and vitamin E were obtained from the Sigma Chemical Co. (St. Louis, MO). Crocus sativus (Saffron) dried stigmas and *Nigella sativa* seeds were purchased from the local market with a fair degree of quality assurance. All other chemicals used were of analytical grade.

10g of Saffron (*Crocus sativus* L.; Family; Irideae) was extracted with 95% ethanol (500 ml) thrice, overnight at 28°C with continuous stirring. The pooled extracts were evaporated under reduced pressure to a known volume (50 ml) and loaded on to a silica gel (Shimadzu-Japan) column and eluted with petroleum benzene, chloroform, methanol and water, successively. The water fraction (yellow colour) containing the active ingredient was separated by ascending paper chromatography using butanol/acetic acid/water (6:1:2) as the solvent system. The active spot (faint yellow colour was cut, eluted with 95% methanol overnight, dried under reduced pressure to render the product alcohol-free and stored at 2°C. Approximate content of the active compound in the saffron was 5.6% (w/w).

The dried seeds of *Nigella sativa* L. (Family; Ranunculaceae) were powdered and 25g of the powder was extracted with 95% ethanol (500 ml) thrice, overnight at 28°C with continuous stirring. The pooled extracts were evaporated under reduced pressure to a known volume (50 ml) and loaded on to a silica gel (Shimadzu-Japan) column and eluted with 95% methanol/water (9:1). The active fraction (as indicated by brown colour) was collected and separated by ascending paper chromatography using chloroform as the solvent system. The active spot at the solvent front was cut, eluted with methanol (overnight), evaporated under reduced pressure to render the product alcohol-free and stored at 2°C. Approximate content of the active compound in the seeds was 2.2% (w/w).

Trials to reduce the nephrotic and toxic effects of cisplatin were performed. The substances evaluated were:

1. Cysteine- a SH- containing amino acid with the potential to protect endogenous SH-groups, and, therefore to reduce the damage caused by cisplatin;

2. Vitamin E, a substance known for it is antioxidative activity and thus a potential agent for protection of SH-groups. Cysteine and vitamin E were used together to determine whether the combination of the two offers greater protection;

3. *Crocus sativus* dried stigmas (saffron) and *Nigella sativa* seeds, natural products used as a potential protective agents against cisplatin-induced toxicity.

The effects of cysteine together with vitamin E or *Crocus sativus* or *Nigella sativa* were studied in the absence and in the presence of cisplatin.

Schedule of Drug Administration

Cisplatin (30 mg) was dissolved in 100 ml of sterile distilled water. Vitamin E was dissolved in corn oil 1:50. The extracts of saffron (1 gm) and of *N. sativa* (1 gm) was reconstituted in 200 ml sterile physiological saline. The experiments were carried out on eight groups of adult male albino rats and each group consisted of six animals. To the first group of rats, cisplatin was given 3 mg/kg body weight i.p. for five alternate days. The second group was administered cysteine (20 mg/kg body weight i.p.) and vitamin E (2 mg/rat s.c.) 30 min before administering cisplatin (3 mg/kg) for five alternate days. The third group was given 20 mg/kg cysteine together with vitamin E (2 mg/rat s.c.) as above but without cisplatin. The fourth group was administered saffron extracts (50 mgW/kg body weight i.p.) 30 min before administering cisplatin (3 mg/kg body weight i.p.) for five alternate days. The fifth group was given 50 mg/kg saffron extracts as above but without cisplatin. In a similar way the sixth group received *N. sativa* extract (50 mg/kg body weight i.p.) and after 30 min cisplatin (3 mg/kg) for five alternate days. Group seven received only *N. sativa* extract (50 mg/kg) for five alternate days. Group eight was the control which received the same volume of normal saline for the same period.

Blood and Tissue Sampling

Animals of the different groups were killed by cervical dislocation 6 hours after the last injection. Blood from each animal was collected in two separate clean centrifuge tubes. The first tube was heparinized before use to keep blood in a fluid condition for haematological level studies. The second tube was not heparinized and blood was allowed to coagulate and the tube was centrifuged at 3000 r.p.m. for 20 min for serum separation. Serum specimens of each animal were divided into two parts in clean small tubes, the first part was kept at 4-8°C for analysis of glucose within 24 hours. The second part was quickly kept frozen at -20°C until needed for analysis.

Immediately after sacrifice, livers and kidneys were rapidly excised from the body of each animal. Accurately weighed tissues were homogenized in ice-cold bidistilled water (1:10 dilution) using a potter El-Vejhem glass homogenizer fitted with a teflon pestle. The freshly prepared homogenates were then used for determination of enzymes assays immediately or stored at -20°C till ready for biochemical studies.

Kidneys tissues were homogenized in 10-fold volumes of sucrose (0.25 mol/l, tris buffer 0.02 mol/l, pH 7.4) with a teflon-glass homogenizer. Prior to homogenization, the tissues were cut into small pieces and washed three times with the homogenizing solution. The homogenate was first centrifuged at 3000 g for 15 min to remove connective tissue, cell debris, nuclei and membranes. The supernatant obtained was recentrifuged at 18000 g for 30 min. The sediment was washed (suspension and recentrifugation in the homogenizing solution) then used as the mitochondrial fraction.

Methods

The enzymes, alkaline phosphatase, glutathione reductase, lactate dehydrogenase, isocitrate dehydrogenase, malate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, malic enzyme, sorbitol dehydrogenase, γ-glutamyl transferase and glucose-6-phosphate dehydrogenase were assyed according to Mc-Comb and Bowers (37); Bergmeyer (7); Bergmeyer and Bernt (8,9); Bernt and Bergmeyer (12); Bergmeyer *et al.* (10,11); Hus and Lardy (30); Gerlach and Hiby (24); Szasz (48) and Lohr and Waller (35) respectively.

Blood urea nitrogen (BUN) and serum creatinine concentrations were analyzed using reagent kits from Bio-Analytics Company (P.O. Box 388, Palm City, FI., USA). Glucose, total proteins, albumin and total lipids concentrations were measured using methods described by Bonder and Mead (15); Lowry *et al.* (36); Doumas *et al.* (21); and Frings *et al.* (23) respectively.

Mitochondrial fraction was prepared according to Johnson and Lardy (31). Mitochondrial oxidative phosphorylation was measured with a Gilson respirometer at 30°C according to Smith (47).

The total leucocyte counts were performed using a haemocytometer. Using 'Sahli apparatus', the haemoglobin content of the blood can be determined. Mean osmotic fragility of erythrocytes was measured on freshly withdrawn cells according to Parpart *et al.* (41).

Statistics

Data are expressed as mean ± standard error (S.E.M.). Where appropriate, analysis of variance was determined and statistical significance between groups was determined by the Student's t-test using $P<0.05$ as criteria for significance.

RESULTS

Table 1 and Figure 1 summarizes the changes observed in the enzymes activities of serum following cysteine together with vitamin E, *Crocus sativus* and *Nigella sativa* administration in adult male albino rats treated with cisplatin.

The effect of cisplatin on serum enzymes activities revealed the occurrence of significant changes in most enzymes studied. The data obtained revealed the occurrence of significant ($P<0.01$) increases in glutathione reductase and isocitrate dehydrogenase in cisplatin-treated rats. On the other hand, significant decreases were recorded in the activities of alkaline phosphatase, lactate dehydrogenase, malate dehydrogenase, aspartate aminotransferase and alanine aminotransferase after 10-days of cisplatin injection. No significant differences were seen in the level of malic enzyme.

Cysteine and vitamin E, *C. sativus* and *N. sativa* added together with cisplatin did not reverse the lowered activity of alkaline phosphatase by cisplatin. Also administration of cysteine and vitamin E alone significantly ($P<0.05$) decreased the enzyme activity in the serum. However *C. sativus* and *N. sativa* added alone caused little or no effect on serum alkaline phosphatase activity. Cysteine and vitamin E, *C. sativus* and *N. sativa* administered alone or together with cisplatin, caused little or no effect on the serum activity of glutathione reductase. Serum lactate dehydrogenase and serum alanine aminotransferase activities in rats treated with cysteine and vitamin E, *C. sativus* and *N. sativa* together with cisplatin were significantly lower from the control animals. On the other hand, cysteine and vitamin, *C. sativus* and *N. sativa* given without cisplatin did not affect lactate dehydrogenase and alanine aminotransferase activities. Cisplatin, cysteine and vitamin E, *C. sativus* and *N. sativa* in combination, led to higher serum isocitrate dehydrogenase activity. Cisplatin administered together with cysteine and vitamin E and *N. sativa* caused a significant ($P<0.01$) decrease of serum malate dehydrogenase. However the enzyme activity approached normal in the *C. sativus* treated group receiving cisplatin. The protective agents without cisplatin did not affect the malate dehydrogenase activity.

Addition of cysteine and vitamin E, *C. sativus* and *N. sativa* together with cisplatin, caused little effect on the serum activity of aspartate aminotransferase. While the activity of the same enzyme was significantly

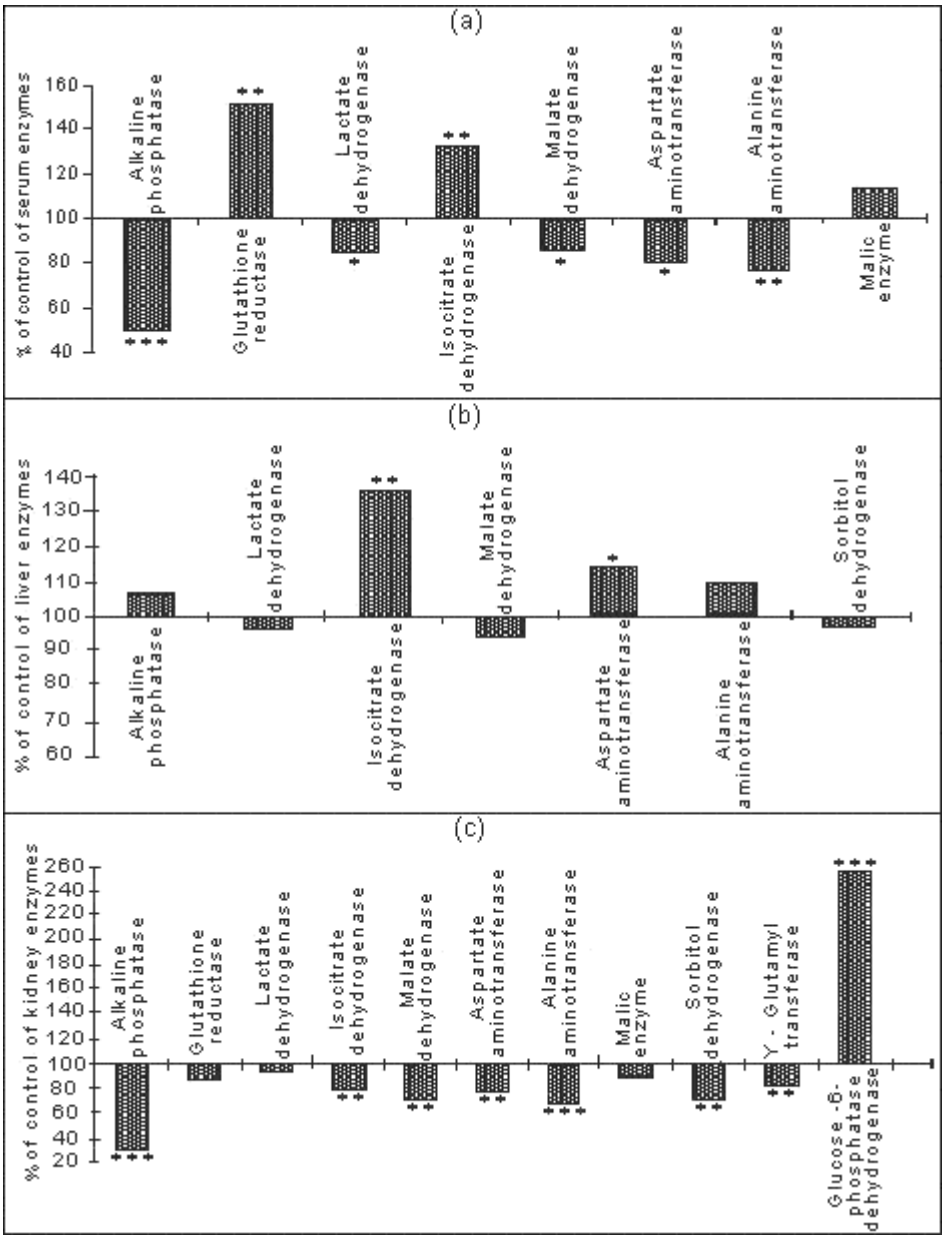
Table 1 : Effect of cysteine and vitamin E, *Crocus sativus* and *Nigella sativa* extracts on enzyme activities in the serum of adult male albino rats treated with cisplatin.

Treatment	Alkaline Phosphatase (U/100mg)	Glutathione reductase (U/100mg)	Lactate dehydrogenase (U/100mg)	Isocitrate dehydrogenase (U/100mg)	Malate dehydrogenase (U/100mg)	Aspartate amino-transferase (U/100mg)	Alanine amino-transferase (U/100mg)	Malic enzyme (U/100mg)
Control	39.1 ± 2.5	5.4 ± 0.3	329.1 ± 13.1	1.0 ± 0.1	131.7 ± 4.9	17.2 ± 0.9*	5.1 ± 0.3	5.2 ± 0.6
Cisplatin	20.1 ± 1.8 ***	8.1 ± 0.6**	284.2 ± 10.9 *	1.3 ± 0.1**	114.2 ± 5.8 *	13.9 ± 0.6	4.0 ± 0.2**	5.8 ± 0.8
Cisplatin+ cysteine + Vitamin E	21.3 ± 2.6***	5.8 ± 0.8	260.2 ± 25.4**	1.4 ± 0.2**	95.2 ± 6.1**	16.5 ± 1.2	3.3 ± 0.3**	5.4 ± 0.7
Cysteine + Vitamin E	31.2 ± 2.5 *	5.5 ± 0.7	309.3 ± 23.5	1.0 ± 0.1	125.1 ± 16.1	21.0 ± 2.0 *	4.7 ± 0.5	5.5 ± 0.4
Cisplatin+ C.sativus	25.4 ± 2.1***	5.9 ± 0.3	286.9 ± 12.17 *	1.2 ± 0.1*	123.1 ± 8.7	15.8 ± 0.7	4.4 ± 0.3 *	5.6 ± 0.5
C.sativus	36.2 ± 3.4	5.6 ± 0.4	310.8 ± 19.8	1.1 ± 0.2	133.2 ± 10.1	18.4 ± 1.2	5.3 ± 0.6	5.5 ± 0.6
Cisplatin+ N. sativa	22.1 ± 2.0***	6.2 ± 0.4	290.8 ± 12.3*	1.2 ± 0.1*	100.1 ± 8.9**	16.2 ± 1.1	3.5 ± 0.6 *	5.7 ± 0.8
N. sativa	38.7 ± 3.5	5.8 ± 0.4	340.3 ± 17.8	1.1 ± 0.1	132.9 ± 9.5	19.0 ± 1.4	5.5 ± 0.7	5.7 ± 0.7

Values represent the mean ± S.E.M of six rats per group.

Statistically significant from normal control:*P<0.05;**P<0.01; ***P<0.001.

Figure 1: Variations in the percent of control of enzymes activities in (a) serum (b) liver (c) kidney of adult male albino rats after 10 days of cisplatin administarion. Number of rats per each group = 6. Statistically significant from normal control: *p<0.05; **p<0.01; ***p<0.001.



(P<0.05) higher after administration of cysteine and vitamin E alone, the activity was not significantly different from the control when *C. sativus* and *N. sativa* were administered alone. After 10 days there was no significant difference between serum malic enzyme activity in rats receiving cisplatin and protective agents alone and those receiving the combination treatments.

Figure 2 summarizes the changes observed in proteins and metabolites of serum following cysteine together with vitamin E, *C. sativus* and *N. sativa* administration in adult male albino rats treated with cisplatin.

Animals receiving cisplatin alone showed a much greater increases in BUN and serum creatinine as compared to normal control animals indicating renal func-

Table 2 : Effect of cisplatin on enzymes activities in liver of adult male albino rats.

Treatment	Akaline Phosphatase (U/100 mg protein)	Lactate dehydrogenase (U/100 mg protein)	Isoctitrate dehydrogenase (U/100 mg protein)	Malate dehydrogenase (U/100 mg protein)	Aspartate amino transferase (U/100 mg protein)	Alanine amino transferase (U/100 mg protein)	Sorbitol dehydrogenase (U/100 mg protein)
Control	1.6 ± 0.1	361.5 ± 20.6	7.0 ± 0.4	418.3 ± 18.5	69.7 ± 5.1	49.8 ± 4.3	14.0 ± 0.7
Cisplatin	1.7 ± 0.2	348.1 ± 19.3	9.5 ± 0.6**	399.8 ± 19.5	79.1 ± 6.2*	54.0 ± 3.5	13.6 ± 0.6

Values represent the mean ± S.E.M. of six rats per group.
Statistically significant from normal control: *P<0.05;**P<0.01.

tion insufficiency. Small but significantly (P<0.05) lower concentrations of blood glucose and serum albumin and higher total lipids were seen in the sera of cisplatin-treated rats.

Cysteine and vitamin E or *C. sativus* significantly reverse some of the effects of renal failure caused by cisplatin. *N. sativa* treatment, however, protected rats from elevated blood urea nitrogen and creatinine concentrations induced by cisplatin. Cysteine and vitamin E, *C. sativus* and *N. sativa* given together with cisplatin led to an even greater decrease in blood glucose than that seen with cisplatin alone. Administration of cysteine and vitamin E, *C. sativus* and *N. sativa* together with cisplatin caused non-significant changes of serum total proteins, albumin and total lipids. The effects of the protective agents alone caused non-significant (P>0.05) changes in serum proteins and metabolites.

The effect of cisplatin on the liver enzymes are shown in Table 2 and Figure 1. Isocitrate dehydrogenase and aspartate aminotransferase activities exhibited significant (P<0.01 and P<0.05, respectively) increases in liver of cisplatin-treated rats. There were no changes in other hepatic enzymes studied.

Table 3 and Figure 1 summarizes the changes observed in the enzymes activities of the kidney following cysteine together with vitamin E, *Crocus sativus* and *Nigella sativa* administration in adult male albino rats treated with cisplatin. A mixed pattern was seen. Alkaline phosphatase, isocitrate dehydrogenase, malate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, sorbitol dehydrogenase and -glutamyl transferase showed significantly lower specific activities, while glucose-6-phosphate dehydrogenase was significantly (P<0.001) higher in the cisplatin-treated rats. No significant changes were seen in the activities of glutathione reductase, lactate dehydrogenase and malic enzyme.

Cysteine and vitamin E partially reversed, to a lesser extent, the inhibitory effect on activities of renal alkaline phosphatase, malate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, and sorbitol dehydrogenase, and the excitatory effect on the activites of glucose-6-phosphate dehydrogenase by cisplatin. Also *C. sativus* and *N. sativa* given together with cisplatin led to a significant decrease in the activities of alkaline phosphatase, isocitrate dehydrogenase, malate dehydrogenase, alanine aminotransferase, sorbitol dehydrogenase and γ-glutamyl transferase relative to the normal control animals. Administration of cysteine and vitamin E alone significantly decreased renal malate dehydrogenase and sorbitol dehydrogenase activities, while glucose-6-phosphate dehydrogenase was significantly increased than that of the normal control animals. However *C. sativus* and *N. sativa* alone led to non-significant (P>0.05) changes in the kidney enzymes activities.

Estimation of mitochondrial oxidation and phosphorylation showed that the P/O ratio was decreased in mitochondria from the kidneys of cisplatin-treated rats, i.e. these mitochondria were less efficient in the production of adenosine triphosphate. While oxidation levels were almost identical in both groups, phosphorylation was significantly (P<0.01) lower in the cisplatin-treated group, decreasing the P/O ratio by 12.5% (Table 4).

Figure 2: Effect of cysteine together with vitamin E, Crocus sativus and Nigella sativa extracts on protein and different metabolities concentrations in the serum of adult male albino rats treated with cisplatin. Each bar represents mean ± S.E.M. of six rats per group. Statistically significant from normal control: *p<0.05; **p<0.01; ***p<0.001.

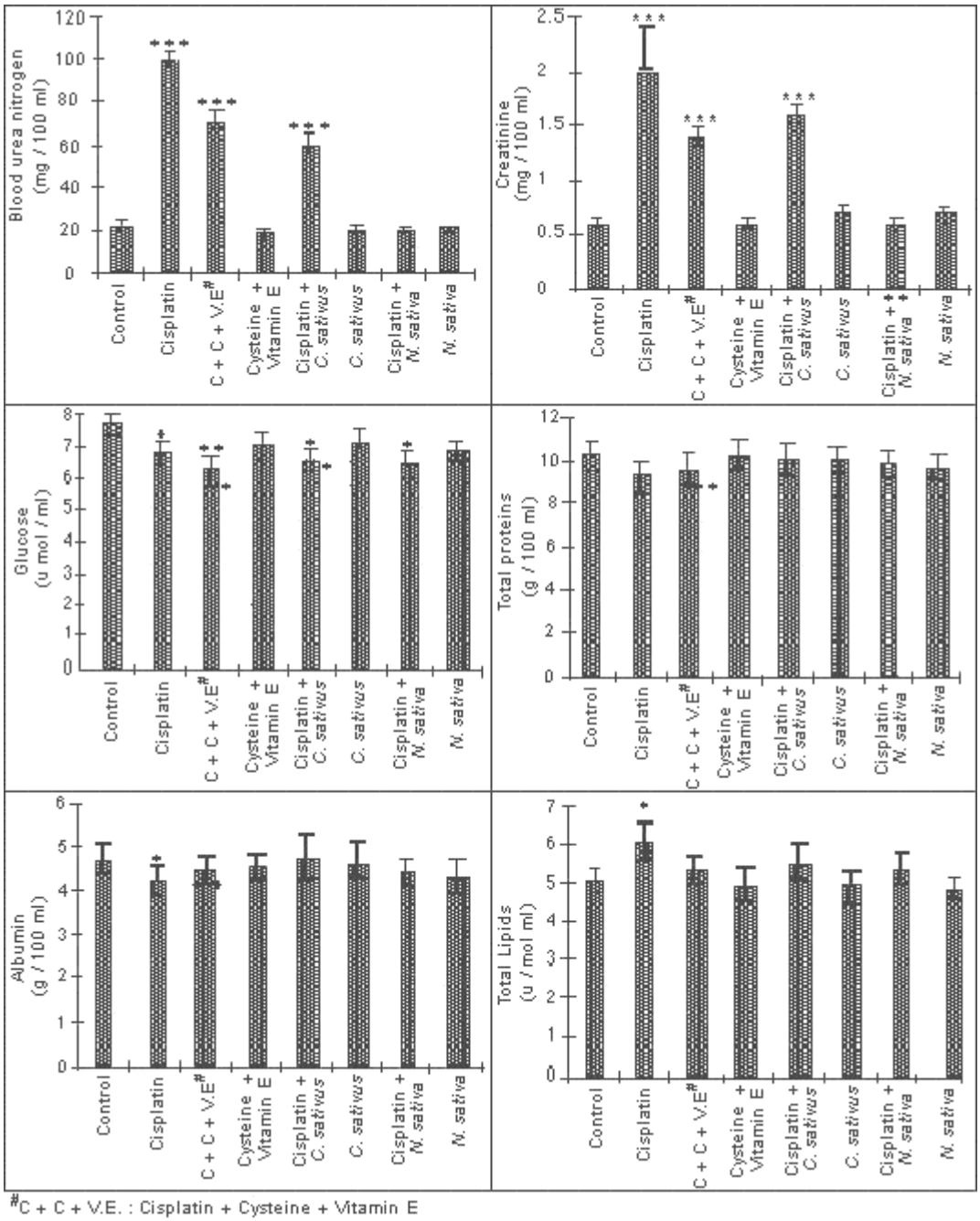


Figure 3 summarizes the changes observed in the leucocyte counts, haemoglobin levels, haematocrit and mean osmotic fragility of erythrocytes following cysteine together with vitamin E, *C. sativus* and *N. sativa* administration in adult male albino rats treated with cisplatin.

Leucocyte counts, haemoglobin levels and mean osmotic fragility were significantly decreased in the cis-

platin treated animals, while haematocrit was significantly ($P<0.05$) increased. The leucocyte counts, haemoglobin levels, haematocrit and mean osmotic fragility approached normal in both *C. sativus* and *N. sativa*-treated groups receiving cisplatin, while leucocyte counts, haemoglobin levels and mean osmotic fragility were significantly lower in the cisplatin together with cysteine and vitamin E-treated rats. Administration of cysteine and vitamin E, *C. sativus* and *N. sativa* alone caused no effect on the haematological parameters studied.

DISCUSSION

Chemotherapy and ionizing radiation affect noncancerous cells to a certain extent. Several groups have worked on chemoproctective and radioprotective agents attempting to find a preferential protection of normal tissues during cancer treatment (14,45,52). The most widely used common protective agents are sulphdryl-containing compounds like WR-2721 (38,42) and an antioxidant substances like methimazole and vitamin E (14,45). In the present study, treatment with cysteine together with vitamin E or saffron or *Nigella sativa* extracts significantly reversed the toxic effects caused by cisplatin. Most of the toxic reactions in cancer therapy with cisplatin are manifested by gastrointestinal epithelium and bone marrow, as evidenced by increased azotemia and gastrointestinal syndromes (1,22). Most of the chemotherapeutic agents used against malignant disease interfere with cell production by damaging mitotic or cell compartments of the marrow, or by slowing down cell division (51).

The results presented in this article provide evidence on the biochemical aspects, kinetics and mode of action of toxic effects caused by cisplatin.

The serum enzymes, alkaline phosphatase, lactate dehydrogenase, aspartate aminotransferase and alanine aminotransferase, as well as others, are commonly elevated following cellular damage as a result of enzymes leakage from cells to the blood. The serum catalytic concentrations of these enzymes however were significantly lower five alternate days post cisplatin administration in the treated animals, suggesting an inhibitory effect. Lower enzyme levels may be due to either lower release from the tissues to the serum, or decreased production of active enzymes and consequently lower activity in the serum.

It has been postulated that the nephrotoxic mode of action of the drug cisplatin is similar to that of other heavy metals, and is related to the decrease in the intracellular concentrations of glutathione and protein-bound SH- groups, which are required for normal cellular function (5,26,33).

The possibility that cisplatin itself is the inhibitory agent of the enzymatic reaction was excluded by the fact that *in vitro* addition of the drug to the reaction media did not affect the reaction rates (14).

The lower activities of enzymes in the serum paralleled the intracellular pattern. Administration of substances containing sulphydryl groups, or with antioxidant properties, before cisplatin, significantly reversed the inhibitory effects caused by cisplatin alone, supporting the hypothesis that the cisplatin toxicity is associated with the reduction of free sulphydryl groups.

In the present study BUN and serum creatinine levels were significantly increased after cisplatin administration. Addition of cysteine together with vitamin E or saffron or *N. sativa* significantly reversed the toxic effects caused by cisplatin. The degree of uraemia was lowered and the magnitude of the changes in the serum analyses, including glutathione reductase, were smaller. These findings may suggest that some of the toxic effects caused by cisplatin may be reduced by using cysteine and vitamin E or saffron or *N. sativa*. Similar protection effects of cysteine and vitamin E or saffron or *N. sativa* against cisplatin induced toxicity has been observed in mice and rats (14,40). This, however, needs to be further studied, in case the chemotherapeutic effect of cisplatin is also reduced.

In response to the lower SH concentrations caused by cisplatin, we observed: (a) Increased activities of the serum enzymes, NADP-isocitric dehydrogenase and glutathione reductase; (b) Significant increases in liver isocitric dehydrogenase and kidney glucose-6-phosphate dehydrogenase, all of these enzymes being associated with NADPH generation and maintenance of reduced sulphydryl groups.

By constantly preventing oxidative processes, sul-

Journal of Islamic Academy of Sciences 9:4, 105-118, 1996

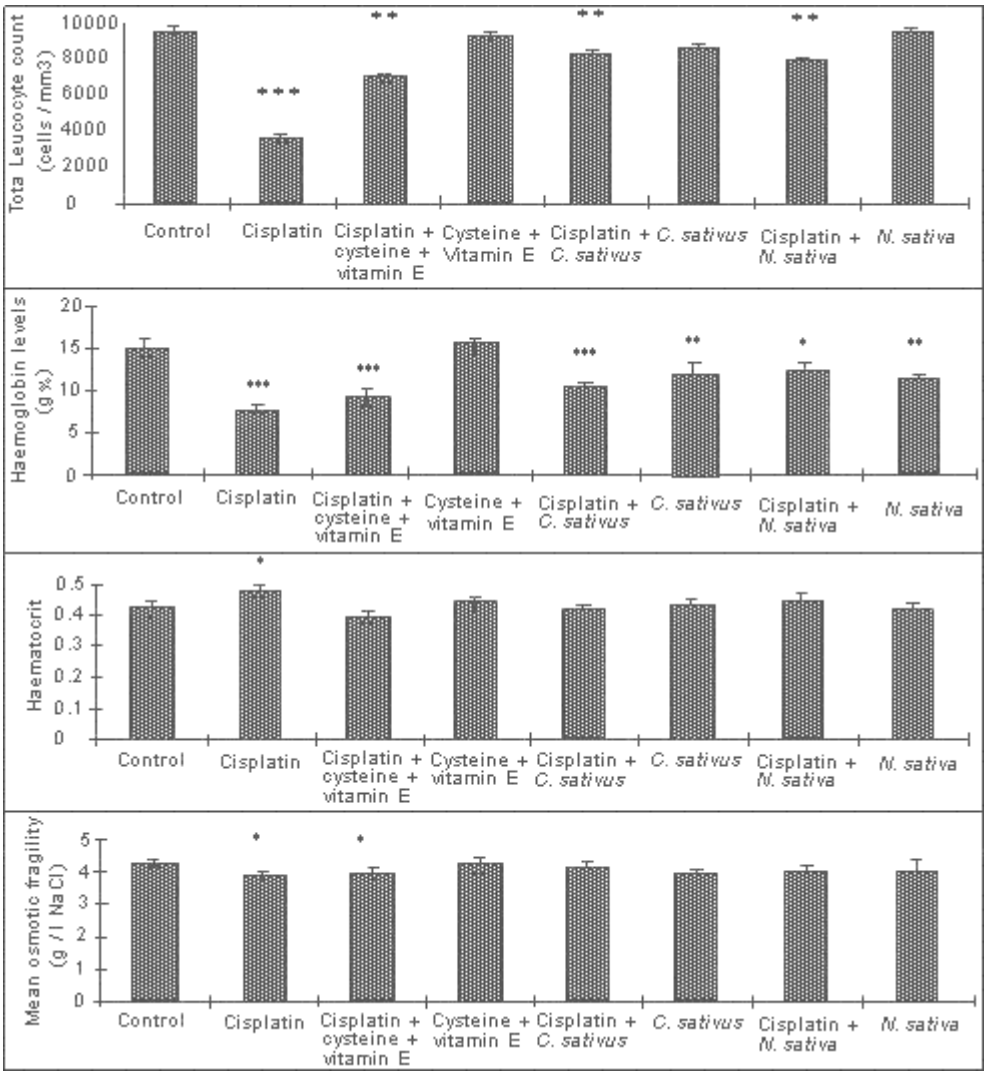
Table 3 : Effect of cysteine together with vitamin E, Crocus sativus and Nigella sativa extracts on enzyme activities in the kidney of adult male albino rats treated with cisplatin.

Treatment	Alkaline Phosphatase (U/100mg) protein	Glutathione reductase (U/100mg) protein	Lactate dehydro - genase (U/100mg) protein	Isocitrate dehydro - genase (U/100mg) protein	Malate dehydro - genase (U/100mg) protein	Aspartate amino - transferase (U/100mg) protein	Alanine amino - transferase (U/100mg) protein	Malic enzyme (U/100mg) protein	Sorbitol dehydro - genase (U/100mg) protein	g - glutamyl transferase (U/100mg) protein	Glucose - 6 - phosphate dehydro - genase (U/100mg) protein
Control	27.3 ±1.9	9.0 ±0.8	160.0 ± 7.2	2.30 ±0.4	553.2 ±19.5	50.2 ± 2.1	2.5 ±0.1	1.1 ±0.1	10.2 ±1.0	16.9 ±1.3	2.0 ±0.2
Cisplatin	8.2 ±1.0***	7.9 ± 0.7	150.0 ±8.9	1.8 ±0.2**	400.5 ±10.2**	38.4 ±1.2**	1.7 ±0.1***	1.0 ±0.1	7.3 ±0.8**	13.9 ±0.6**	5.1 ±0.3***
Cisplatin + cysteine + Vitamin E	16.0 ±2.2***	8.1 ±0.8	142.3 ±7.5	2.1 ±0.2	415.3 ±13.5**	42.9 ±1.3 *	2.2 ± 0.1*	0.9 ±0.05	7.4 ±0.9**	15.9 ±1.5	4.5 ±0.3***
Cysteine + Vitamin E	24.6 ± 2.1	8.4 ± 0.9	147.9 ± 6.9	2.2 ±0.3	450.9 ± 21.5*	46.5 ±2.5	2.3 ±0.2	1.1 ±0.1	7.5 ±0.7**	16.3 ±1.7	2.5 ± 0.2*
Cisplatin + C.sativus	10.2 ±1.0**	8.0 ± 0.5	150.3 ±10.3	2.0 ±0.2*	450.9 ± 21.6*	47.6 ±3.1	2.0 ±0.1**	1.0 ±0.05	7.8 ±0.6**	14.2 ±1.2*	4.6 ±0.3***
C.sativus	26.3 ± 2.1	9.3 ± 0.8	148.4 ±11.8	2.4 ±0.2*	560.2 ±21.7	53.2 ±3.4	2.6 ±0.2	1.1 ±0.1	9.6 ±0.9	16.0 ±1.6	2.2 ±0.1
Cisplatin + N. sativa	10.5 ±0.8***	8.1 ±0.7	160.5 ±13.0	2.0 ±0.1	460.12 ± 23.12*	45.8 ±2.9	2.2 ± 0.1*	1.0 ±0.1	7.5 ±0.8**	13.8 ±1.3**	4.7 ±0.3***
N. sativa	27.1 ±2.3	9.4 ±1.0	152.2 ±12.0	2.5 ±0.3	559.2 ± 20.14	55.1 ± 4.1	2.7 ±0.3	1.2 ±0.05	10.5 ±0.9	16.7 ±1.2	2.6 ±0.3**

Values represent the mean ± S.E.M of six rats per group.

Statistically significant from normal control:*P<0.05;**P<0.01;***P<0.001.

Figure 3: Effect of cysteine together with vitamin E, Crocus sativus and Nigella sativa extracts on the total leucocyte counts, haemoglobin levels, haematocrit and mean osmotic fragility of erythrocytes of adult male albino rats terated with cisplatin. Each bar represents mean ± S.E.M. of six rats per group. Statistically significant from normal control: *p<0.05; **p<0.01; ***p<0.001.



phydryl groups, also have an important role in the maintenance of cellular membrane integrity. The lowering of the SH level caused by cisplatin could be contributing factor to the observed decrease in mean osmotic fragility.

The effect of cisplatin was much greater on the kidney than the liver. While only two enzymes were found to change in the liver (NADP isocitrate dehydrogenase and aspartate aminotransferase increasing by 36% and 14% respectively), eight kidney enzymes showed a change in activity (seven reduced and one increased). Renal failure in rats treated with cisplatin could be a result of the lower enzymatic activities in the kidney, as well as the less efficient oxidative phosphorylation and adenosine triphosphate production of the mitochondria. The enzyme shown to be most inhibited both in the serum and kidney is alkaline phosphatase. The reason for its great sensitivity is not clear. Through its role as a membrane enzyme involved in transmembranal transport (among other functions), alkaline phosphatase may be particularly involved in the renal failure and development of uraemia.

Table 4: Effect of cisplatin on mitochondrial oxidation and phosphorylation in O_2 the kidney of adult male albino rats.

Treatment	Phosphorylation (μ mol / h mg protein)	Oxygen consumption (1/2 O_2 μ mol/h mg protein)	P/O quotient
Control	18.4 ± 0.7	11.2 ± 0.5	1.6 ± 0.1
Cisplatin	15.2 ± 0.9**	11.0 ± 0.7	1.4 ± 0.04*

Succinate was used as a substrate.
Values represent the mean ± S.E.M. of six rats per group.
Statistically significant from normal control: *P<0.05, **P<0.01.

Following the administration of cisplatin, uraemia developed and the activity of serum enzymes was significantly decreased (Table 1 and Figures 1-2). This was largely reversed by supplementation with cysteine and vitamin E or saffron or *N. sativa*, once again indicating on the possible potential of these compounds to reverse the toxic effects caused by cisplatin. Similar patterns, although of lesser magnitude, were also seen with other kidney enzymes (aspartate aminotransferase and alanine aminotransferase). It is, however, necessary to evaluate the efficiency of these compounds when given after cisplatin treatment rather than before treatment, since the supplementation of vitamin E and cysteine or saffron or *N. sativa* before cisplatin treatment may reduce the chemotherapeutic efficiency of the drug. The availability of appropriate biochemical indicators of the degree of nephrotoxicity will enable further study of these aspects of the problem.

Hopkins *et al.* (28) have reported that the decline of circulating lymphocytes reaches its lowest level on the fifth day of treatment with anticancer drugs. Protection against cyclophosphamide-induced toxicity using MPG has been reported (13). In the present work, some protective effects were exerted by both the saffron and *N. sativa* extracts considering leucocyte counts, blood haemoglobin content, haematocrit and mean osmotic fragility. Similar observations were also reported in mice (40).

The exact mechanisms by which the saffron and N. sativa extracts exert their protective effects against cisplatin-induced toxicity are not yet known. Sasen *et al.* (45) showed that cisplatin-induced nephrotoxicity was decreased when methimazole was given 30 min before and up to 4 hours after cisplatin treatment. Our findings revealed that cisplatin-induced toxicity was decreased when saffron or *N. sativa* were given 30 min before cisplatin treatment. These results may be due to the relatively slower excretion of cisplatin by the kidneys and/or the slower development of the cisplatin-induced nephrotoxicity compared to the other nephrotoxic chemicals (17).

The increased blood cell volume seen in the cisplatin treated rats could be related to the renal failure, leading to loss of body fluids and haemoconcentration and therefore an increased haematocrit. The fact that total protein was reduced could be explained by the loss of protein in the urine. Significant proteinuria was seen in the cisplatin-treated rats, confirming the results of other others (26). This is further supported by the lower serum albumin levels in the treated rats.

Evaluation of the toxicological effects of cisplatin leads to the following conclusions; (a) Blood and tissue analysis confirmed that the kidney is very sensitive to cisplatin toxicity, and more sensitive than the liver; (b) An intracellular compensatory effect was observed following cisplatin administration. This compensatory process neutralized the effects of cisplatin, by increasing the activities of enzymes associated with NADPH production and the maintenance of sulphdryl groups in their reduced state; (c) Renal failures caused by the administration of cisplatin is the result of reduced activities of several enzymes that play a role in renal function, as well as reduced adenosine triphosphate production due to uncoupling of the mitochondrial oxidative phosphorylation; (d) The toxic effects and degree of renal failure were decreased by cysteine and

vitamin E, saffron and *N. sativa* extracts, which also partially neutralized the various changes in enzyme activity, etc. caused by cisplatin.

REFERENCES

1. Allan SG, MA Cornbleet, PS Warrington, IM Golland, CF Leonard and JF Smyth : Dexamethasone and high dose efficacy in controlling cisplatin-induced nausea and vomiting. British Medical Journal. 289:878-879, 1984.

2. Allan SG, JF Smyth, FG Hay, CF Robert and CR Wolf : Protective effect of sodium-2-mercaptoethane sulfornate on the gastrointestinal toxicity of cisdiamine-dichloroplatinum. Cancer Research 46:3569-3573, 1986.

3. Anand AJ and B Bashey : Newer insights into cisplatin nephrotoxicity. Ann Pharmacother 27:1519-1525, 1993.

4. Baldew GS, CJA Hamer, G Van den Los, NPE Vermeulen, JJM de Goeji and JG McVie : Selenium induced protection againts cisplatin (II). Nephrotoxicity in mice and rats. Cancer Res. 49:3020-3023, 1989.

5. Ballantyne B, T Marrs, and P Turner : General and Applied Toxicology. M Stockton Press, 1993.

6. Bannister SJ, LA Sternson, AJ Repta and GW James : Measurement of free circulating cis-diamine-cis-dichloroplatinum (II) in plasma. Clinical Chemistry 23:2258-2262, 1977.

7. Bergmeyer HU : Glutathione reductase. In: Methods of Enzymatic Analysis. Vol 1, pp 465-466, Verlag Chemie International, Deerfield Beach, Florida. 1974.

8. Bergmeyer HU and E Bernt : Lactate dehydrogenase In: Methods of Enzymatic Analysis. Ed by HU Bergmeyer, 2nd English edition. Vol 2, pp 574-579, Verlag Chemie Weinheim. Academic press Inc New York, San Francisco and London 1974.

9. Bergmeyer HU and E Bernt : Malate dehydrogenase. In: Methods of Enzymatic Analysis. Ed by HU Bergmeyer, 2nd English Edition. Vol 2, pp 613-617, Verlag Chemie Weinheim. Academic press Inc, New York, San Francisco, London, 1974.

10. Bergmeyer HU, M Horder and R Rej : Method for the measurement of catalytic concentration of enzymes. IFCC method for aspartate aminotransferase. J Clin Chem Clin Biochem 24:497-510, 1986.

11. Bergmeyer HU, M Horder and R Rej : Method for the measurement of catalytic concentration of enzymes. IFCC method for alanine aminotransferase. J Clin Chem Clin Biochem 24:481-495, 1986.

12. Bernt E and HU Bergmeyer : Isocitrate dehydrogenase. In : Methods of Enzymatic Analaysis. Ed by HU Bergmeyer, 2nd English edition. Verlag Chemie Weinheim. Academic Press Inc, New York, San Francisco and London, pp 624-627, 1974.

13. Bhanumathy P, S Kumar and DM Vasudevan : Role of 2-MPG against toxicity of cyclophosphamide in normal and tumour bearing mice. Indian Journal of Experimental Biology 24:767-770, 1986.

14. Bogin E, M Marom and Y Levi : Changes in serum, liver and kidneys of cisplatin-treated rats; effects of antioxidants. Eur J Clin Chem Biochem 32:843-851, 1994.

15. Bonder RJL and DC Mead : Evaluation of glucose-6-phosphate dehydrogenase from Leuconostic mesenteroides in the hexokinase method for determining glucose in serum. Clin Chem 20:586-590, 1974.

16. Boogaard PJ, ELM Lempers, GJ Muller and JHN Meerman : U-Methylthiobenzoic acid reduces cisplatin nephrotoxiticy in rats without compromising anti-tumour activity. Biochem Pharmacol 41, 369-375, 1991.

17. Borch RF : The platinum antitumor drugs. In Metabolism and actions of anticancer drugs. Eds by G Powis and RA Prough, pp 163-193, Taylor and Francis, New York, 1987.

18. Borch RF and ME Pleasants : Inhibition of cisplatin nephrotoxicity by diethyldithiocarbamate rescue in a rat model. Proceedings National Academy of Science 76:6611-6614, 1979.

19. Cafruny EJ, A Farah and HS DiStefano : Effect of the mercurial diuretic mersalyl on protein-bound sulfhydryl groups in cytoplasm of rat kidney cells. J Pharmacol Exp Ther 115:390-401, 1995.

20. Deconti RC, BR Toftness, RC Lange and WA Greasey : Clinical and pharmacological studies with cis-diclhoro-diamine-dichloroplatinum (II). Cancer Research 33:1310-1315, 1973.

21. Doumas BT, WA Watson, HG Biggs : Albumin Standards and the measurement of serum albumin with bromocresolgreen. Clin Chim Acta 31:87-96, 1971.

22. Evans RG, CE Wheatley, J Nielsen and I Ciborowoski : Modification of the bone marrow toxicity of cis-diamine-dichloroplatinum (II) in mice by diethyldithiocarbamate. Cancer Reseach 44:3686-3689, 1984.

23. Frings CS, TW Fendly, RT Dunn and CA Queen : Improved determination of total serum lipids by the sulphophosphovanillin reaction. Clin Chem. 18:673-674, 1972.

24. Gerlach U and W Hiby : L-Iditiol dehydrogenase. In: Methods of Enzymatic Analysis. Ed by HU Bergmeyer, vol 1, pp 569-573, Verlag Chemie Weinheim Academic Press, New York, San Francisco and London, 1974.

25. Hanigan MH, BC Gallagher, PT Jr Taylor and MK Large : Inhibition of gamma-glutamyl transpeptidase activity by acivicin in vivo protects the kidney from cisplatin-induced toxicity. Cancer Res. 54:5925-5929, 1994.

26. Haschek WM and CG Rousseaux : Handbook of Toxicologic Pathology. Academic Press. San Diego, New York Boston, London, Sydney, Tokyo, Toronto. 1991.

27. Hidaka S, M Tsuruoka, T Funakoshi, H Shimada, M Kiyozumi and S Kojima : Protective effects of dithiocarbamates against renal toxicity of cis-diamine dichloroplatinum in rats. Renal Failure 16:337-349, 1994.

28. Hopkins HA, CJ Kovacs, WB Looney JA Wakefield and HP Morris : Cancer biochemistry. Biophysics 1:303-306, 1976.

29. Howe-Grant ME and SJ Lippard : Aqueous platinum (II) chemistry, binding to biological molecules. In: Metal Ions in Biological Systems. Marcel Decker Inc, New York, pp 63-125, 1980.

30. Hsu RY and HA Lardy : Malic enzyme. In: Methods in Enzymology. Eds by SP Colowick, and NO Kaplan, Academic Press, New York and London, vol 23, pp 230-235, 1969.

31. Johnson D and H Lardy : Isolation of liver or kidney mitochondria. In: Methods in Enzymology. Eds by RW Eastbrook, and ME Pullman, vol 10, pp 94-103, Academic Press, New York, San Francisco, London, 1967.

32. Lawrence DL, BN Engelsberg, RS Farid, EN Hughes and PC Billings : Localization of the binding region of high mobility group protein 2 to cisplatin-damaged DNA. J Biol Chem, 268:23940-23945, 1993.

33. Levi J, C Jacobs, S Kalman, M McTighe and MW Weinder : Mechanism of cis-platinum nephrotoxicity. I. Effect on SH groups in rat kidney. J Pharmacol Exp Ther. 213:545-550, 1980.

34. Litterst CL : Alterations in the toxicity of cisplatin and in tissue localization of platinum as a function of NaCI concentration in the vehicle administration. Toicol. Appl Pharmacol 61:99-108, 1981.

35. Lohr GW and HD Waller : Glucose-6-phosphate dehydrogenase. In: Methods of enzymatic analysis. Ed by HU Bergmeyer, 2nd English edition. Verlag Chemie Weinheim, Academic press Inc, New York, San Farncisco. London, vol 2, pp 636-643, 1974.

36. Lowry OH, NJ Rosebrough, AL Farr and RL Randall : Protein measurement with the folin phenol reagent. J Biol Chem. 193:265-275, 1951.

37. McComb RB and GN Bowers : Study of optimum buffer condition for measurement of alkaline phosphatase activity in human serum. Clin Chem. 18:97-104, 1972.

38. Milas L, N Hunter, BO Reid and HD Jr Thomas : Protective effect of S-2-(3-amino propylamino ethyl) phosphorothioic acid against radiation damage of normal tissues and a fibrosarcoma in mice. Cancer Research 42:1888-1897, 1982.

39. Milas L, N Hunter, H Ito and LJ Peters : Effect of tumour type, size and end point on tumour protection by WR-2721. International Journal of Radiation Oncology-Biology Physics 10:41-48, 1984.

40. Nair SC, MJ Salomi, B Panikkar and KR Panikkar : Modulatory effects of Crocus sativus and Nigella sativa extracts on cisplatin-induced toxicity in mice. J Ethnopharmacology 31:75-83, 1991.

41. Parpart AK, RB Lorenz and E Parpart : The osmotic resistance of human red cells. J Clin Invest, 26:636-640, 1947.

42. Phillips TL : Rationale for initial clinical trials and future development of radioprotectors. Cancer Clinical Trials 3:165-173, 1980.

43. Reishus JW and DS Martin : Cis-dichloro-diamineplatinum (II). Acid hydrolysis and isotopic exchange of chloride ligands: Journal of American Chemical Society 83:2457-2462, 1961.

44. Riley CM, LA Sternson, AJ Rept and SA Slyter : Reactivity of cis-dichloro-diamineplatinum (II) towards selected neucleophiles. Polyhedron 1:201-202, 1982.

45. Sausen PJ, AA Elfarra and AJ Cooly : Methimazole protection of rats against chemically induced kidney damage in vitro J Pharm Exp Ther 260:393-401, 1992.

46. Slater TF, M Ahmed and SA Ibrahim : Studies on the nephrotoxicity of cisplatin (II) and related substances. Journal of Clinical Hematology and Oncology 7:534-544, 1977.

47. Smith AL : Preparation, properties and conditions for assay of mitochondria. In: Methods in Enzymology. Ed by RW Eastbrook and ME Pullman. Academic Press, New York, San Francisco, London, vol 10, pp 81-93, 1967.

48. Szasz G : Reaction rate method for gamma glutamyltransferase activity in serum. Clin Chem 22:2051-2055, 1974.

49. Treskes M, E Boven, U Holwerda, HM Pinedo, and WJ Van Der Vijgh : Time dependence of the selective modulation of cisplatin-induced nephrotoxicity by WR 2721 in the mouse. Cancer Res 52:2257-2260, 1992.

50. Weiss RB and MC Christian : New cisplatin analogues in development. Drugs 46:360-377, 1993.

51. Wintrobe MM : Pancytopenia, aplastic anemia and 'pure red cell' aplasia. In: Clinical Hematology. Ed by MM Wintrobe, GR Lee, DR Boggs, TC Bithell, JW Athens and J Foerster. Lee and Febiger. Philadelphia, pp 1741-1776, 1974.

52. Yuhas JM, JM Spellman and F Culo : The role of WR-2721 in radiotherapy and/or chemotherapy, Cancer Clinical Trials, 3:211-216, 1980.

Correspondence:
Ezzat S. El Daly
Department of Biology,
Faculty of Education,
At Suez,
Suez Canal University,
EGYPT.