AMELIORATION OF CISPLATIN-INDUCED NEPHROTOXICITY IN DOGS BY VITAMIN C AND FISH OIL

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ABSTRACT

In a trial to study the possible prophylactic role of vitamin C and fish oil against the cisplatin induced nephrotoxicity in dogs. Forty two dogs were used, classified into seven equal groups. (I) served as control injected with physiological saline. (II) injected with a single dose of vitamin C (200 mg/kg b.wt. i.v.). (III) injected with a single dose of fish oil (2 ml/kg b.wt. i.v.). (IV) injected with a single dose of cisplatin (5 mg/kg b.wt. i.v.) (V) injected with a single dose of cisplatin plus vitamin C, (VI) injected with a single dose of cisplatin plus fish oil and (VII) injected with a single dose of cisplatin plus both vitamin C and fish oil. Dogs were sacrificed 7-days post treatment. Blood samples and kidney tissues were taken for biochemical investigations. From the obtained results, significant decreases were reported in the renal enzyme activities of catalase (CAT), glutathione peroxidase (G_{PX}) and superoxide dismutase (SOD) and reduced glutathione in cisplatin-injected dogs. In the opposite, plasma and kidney malondialdehyde (MDA), urea (BUN) and creatinine were significantly increased. Administration of vitamin C and/or fish oil concomitantly with cisplatin inhibited the increase in lipid peroxidation and inhibited renal antioxidant enzymes depletion induced by cisplatin. In conclusion, the bi-use of vitamin C beside fish oil during treatment with cisplatin improved the antioxidant defense system and produced an excellent prophylactic role against cisplatin-induced nephrotoxicity.

INTRODUCTION

Cisplatin (cis-diamminedichloroplatinum II) is a highly cytotoxic drug. It is widely used for the treatment of various malignant tumours, especially testicular and ovarian carcinoma **(Dire et al. 1990)**. Unfortunately, cisplatin use is associated with numerous side effects, particularly nephrotoxicity and neurotoxicity **(Screnci and Mckeage, 1999)**. Cisplatin accumulates within the kidney tissues and induces deterioration of renal function manifested as proximal tubular injury

(Saad and Rikabi, 2002) as well as apototic cell death in renal tubules **(Park et al., 2002)**. The toxicity of cisplatin, in non tumor cell, is induced by generating activated oxygen species which have been implicated to play an important role in renal dysfunction **(Nishikawa et al., 2001)**, and attack DNA frequently resulting in oxidative DNA damage **(De Martinis and Bianchi, 2001)**. It has been suggested that mitochondrial dysfunction is a central component of cisplatin nephrotoxicity to proximal tubules **(Brady et al., 1993)** and that cisplatin-induced toxicity to kidney slices in vitro is critically associated with oxidative damage to mitochondria **(Zhang and Lindup, 1993)** which might leads to failure of energy production or ATP depletion, or both. Moreover, cisplatin has been shown to increase the excretion of thermboxane A2 leading to alteration in membrane fatty acid composition **(Walker et al., 1989)**.

Vitamin C is a water soluble vitamin with a broad spectrum of antioxidant activities due to its ability to react with numerous aqueous free radicals and reactive oxygen species and decreases lipid peroxidation **(Greggi Antunes et al., 2000)**. Thus vitamin C forms the first line of antioxidant defence in human plasma exposed to a variety of oxidant insults **(Frei et al., 1988)**. The antioxidant activity of vitamin C is particularly relevant in a state of increased oxidative stress, which can affect endothelial cell function **(Giugliano and Ceriello, 1996)**. In addition, we have previously shown that vitamin C affect protoglycans (PG) of rat mesangial matrix quantitatively, by increasing its degree of sulphation **(McAuliffe et al., 1997)**. Such quantitatively and qualitative alterations in basement membrane and mesangial matrix farreaching effects a number of extracellular matrix functions and glomerular filtration properties. Indeed, **Beyer-Mears et al. (1996)** have reported that a decrease in the albumin filtration rate (AFR) was found in nephrotoxicity in rats after treatment with vitamin C.

Fish oil rich in omega 3-marine triglyceride decrease the production of thromboxane A2 **(Morphak et al., 1994 and Badalamenti et al., 1995)** as well as improving the recycling of GS-SG to GSH **(Yilmaz et al., 2002)** and decrease the production of free F2-isoprostanes that is thought to provide a reliable and useful assessment of lipid peroxidation in vivo. However, another study has demonstrated that ω3 fatty acid increases the susceptibility of LDL to oxidation and uptake by macrophages in vitro and has recommended dietary supplementation of a-tocopherol as antioxidant **(Suzukawa et al., 1995)**. Short term intake of ω3 fatty acids results in their incorporation into lipoprotein and membrane phospholipids, which can be observed in blood cell as erythrocytes **(Prisco et al., 1996)** prior studies indicated that changes in phospholipids composition or distribution can be associated with functional alterations in membrane erythrocytes and likely in other tissues **(Brenner, 1984)**. For instance, membrane lipid peroxidation, which depends on the membrane s fatty acid composition and antioxidant content (mainly vitamin E in cell membranes), together with protein oxidation, leads to loss of membrane integrity and function.

as occurs during hemolysis. Thus, the increased unsaturation index consequent to ω3 fatty acids incorporation into membranes could alter the red cell response to oxidative stress **(Garrido et al., 1989)**.

Active oxygen molecules such as the superoxide radical plays an important role in the inflammation process after intoxication by cisplatin **(Dehne et al., 2001)**. These radicals, which react with the cell membranes and induce lipid peroxidation, have been implicated as important pathologic mediators in many clinical disorders **(Slater, 1984)**. A major defence mechanism is the antioxidant enzymes (especially superoxide dismutase, catalase and glutathione peroxidase) which convert active oxygen molecules into non-toxic compounds. The objective of this study was to evaluate the anti-or pro-oxidant effects of vitamin C and/or fish oil in dogs submitted to cisplatin treatment, considering the antioxidant enzymes and lipid peroxidation in order to overcome a great limitation of cisplatin administration especially in patient with pre-existing renal dysfunction.

MATERIALS AND METHODS

Animals:

The experiment was carried out on 42 male dogs with an average weight of 6+1.0 kg. They received balanced diet and water was freely supplied.

Drugs:

a- Cisplatin:

It was a gift from F.H Faulding and Co limited Lexia place mulgrov Victoria 3170 Australia. It was prepared for I.v. injection.

b- Vitamin C:

It was obtained from Memphis Co. For pharm. & chemical Ind Cairo –Egypt.

c- Fish oil:

Fish oil emulsion (Omega venous 10%) was obtained from Fresenius AG, Bad Hamburg, Germany. It is prepared for intravenous injection at a dose of 2 ml/kg body weight.

Animal grouping:

Animals were divided into 7 equal groups, each group consisted of 6 animals:

Group I : The animals were injected intravenously (i.v.) with physiological saline.

Group II : The animals were injected with a single dose of vitamin C (200 mg/kg b.wt. i.v.) ac-

cording to Greggi **Antunes et al. (2000)**.

Group III : The animals were injected with a single dose of fish oil (2 ml/kg b.wt. i.v.)

Group IV : The animals were injected with a single dose of cisplatin (5 mg/kg b.wt. i.v.) according to **Daugaard et al. (1987)**.

Group V : The animals were injected with a single dose of cisplatin plus vitamin C.

Group VI : The animals were injected with a single dose of cisplatin plus fish oil.

Group VII : The animal were injected with a single dose of cisplatin, vitamin C and fish oil.

Blood sampling :

7 days post injection, food was withheld from all animals for 12 hours before blood collection. Blood samples were collected from the jagular vein, before killing the animal, and received into heparinized tube. Plasma was harvested by centrifugation of the blood at 3,000 r.p.m. for 10 minutes and kept at -20 ...C till biochemical analysis.

Tissue sampling :

The kidneys were removed and kept in an ice bath until homogenization. Tissues were immediately prepared for the enzyme activity assays as described below. They were washed out from contaminated blood with cold water. One gram was homogenized in 10-fold physiological saline, the homogenate was centrifuged at 10.000 g for 1 hour and the clear supernatant was taken for enzymatic assays and estimation of malondialdehyde level.

Experimental parameters:

a- Kidney function test. For indication of cisplatin—induced nephrotoxicity

- Blood urea nitrogen according to **Pullun and Crouch (1977)**.

- Plasma creatinine by standard colorimetric procedure **(Husdan, 1968)**.

b- Determination of lipid peroxidation products in plasma and kidney tissues expressed as level of malondialdehyde according to **Draper and Hadly (1990)**.

c- The antioxidant enzymes activity catalase, glutathione peroxidase and superoxide dismutase were measured in kidney homogenate according to **Cohen et al. (1970); Chiu et al. (1976) and Misera and Fridovich (1972)** respectively as well as reduced glutathione was measured according to **Grunet and Phillips (1951)**.

The results were statistically analyzed using analysis of variance ANOVA (F-test) and expressed as Mean + SEM, and a statistical probability of $P<0.05$ was considered to be significant **(Snedecor and Cochran, 1990)**.

RESULTS

After administration of cisplatin, clinically important changes in attitude, appetite, or hydration status were not evident. However, each dog vomited at least 12 hours of cisplatin administration.

Table (1) shows that fish oil and vitamin C were found to be safe as regarding their effect on the kidney function and lipid peroxidation products, which elucidate no change difference between the group under comparisons.

Table (2) shows the effect of intravenous administration of cisplatin (5 mg/kg body weight) on parameter of blood constituents. The urea and creatinine levels revealed significant increases after 7 days from cisplatin administration, compared to the control group, this indicates that the glomerular filtration was impaired, meanwhile the level of blood urea and creatinine in dogs given fish oil and/or vitamin C with cisplatin showed no difference compared to the control group, indicating that vitamin C and /or fish oil play in protecting against cisplatin induced nephrotoxicity .

Table (3) shows that in the cisplatin treated dogs, the plasma and kidney malondialdehyde revealed a significant increase, reflecting lipid peroxidation, whereas the level of malondialdehyde in plasma and kidney tissue remains nearly within the normal level after administration of vitamin C and/or fish oil concomitantly with cisplatin, reflecting the prophylactic role of vitamin C and fish oil against cisplatin-induced lipid peroxidation .

Table (4) shows the activity of reactive oxygen species-scavenging enzymes compared to the control group. Dogs administered cisplatin only showed significantly reduced antioxidant enzymes activities and a significant decrease in the level of reduced glutathione, whereas coadministration of fish oil and/or vitamin C, with cisplatin revealed no change in enzymatic activities and reduced glutathione level, revealing the protecting effect of vitamin C and fish oil .

DISCUSSION

The nephrotoxicity of drugs and their metabolites are often manifested by proximal tubule disorders, which result in the release of the enzymes held in the proximal tubules. In addition, it

has been reported that the major site of the renal disorder induced by cisplatin is the S3 segment of the straight part of the proximal tubule-like feature **(Weiner and Jacobs, 1983)**. As a result **(Yokozawa et al., 1999)** observed that cisplatin caused leakage of the lysosomal enzyme lactate dehydrogenase into the cellular medium increased the production of malandialdehyde, and damaged the cell membrane. It is well known that the major side-effects of cisplatin were observed in the kidney, because of the excretion of the compound **(Brady et al., 1993 and Reznik et al., 1993)**.

There are several reports attempting to elucidate the molecular mechanism leading to cisplatin nephrotoxicity **(Ajith et al., 2002 and Durak et al., 2002)**. Several researchers suggested that the free radicals play an important part in cisplatin toxicity **(Antunes et al., 2001 and Bnat et al., 2002)**. The experimental data that lazaroid afforded renal protection against cisplatin toxicity through its antioxidant capacity has supported this hypothesis **(De-martinis and Bianchi, 2001)**. However, molecular mechanisms responsible for the free radical damages in renal tissues treated with cisplatin have not yet been clarified. In this regard, thromboxane A2 was implicated in the cisplatin toxicity **(Walker et al., 1989)**. It has been suggested that excess free radical formation following the administration of cisplatin enhances the generation of eicosanoids (Thramboxane A2 and F2-isoprostanes) **(Morrow et al., 1992)**. Accordingly, marked generation of hydrogen peroxide and reduction of cytochrome P450 content in kidney increases the catalytic iron and hydroxyl radical formation accompanied by cisplatin cytotoxicity **(Liu et al., 2002)**. The ability of cisplatin to cause alteration in the activity of cytochrome P450 and some related catalytic activities suggests that cisplatin induces toxic effects in an unspecific manner.

In the present study we used cisplatin model of chronic renal failure. One single dose resulting an increase in blood urea nitrogen and creatinine, indicating dysfunction of the proximal tubule, we also noted significant decreases in catalase, superoxide dismutase, glutathione peroxidase and reduced glutathione in the kidney homogenate, meanwhile a significant increase in the level of malondialdehyde in kidney homogenate and plasma was observed, indicating lipid peroxidation. Similar results were reported by **Saad and Al-Rikabi (2002) and Yoshida et al. (2000)** who reported that rats given cisplatin intravenously showed markedly increased blood levels of urea nitrogen and creatinine in addition to significant decreases in kidney catalase, superoxide dismutase, glutathione peroxidase and reduced glutathione were observed, suggesting that the free radical scavenging system was destroyed in rats given cisplatin.

Interestingly, **Davis et al. (2001)** stated the importance of reactive cisplatin induced renal injury and specifically implicate the superoxide radical as a mediator. This is confirmed by **Dehne et al. (2001)** who stated that cisplatin toxicity is partially mediated by an iron-dependent path-

way and is associated with an enhanced formation of superoxide anion. Moreover, cisplatin-induced damage to mitochondria and proceeded depletion of ATP **(Zhang and Lindup, 1997).** Furthermore, cisplatin-induced nephrotoxicity is closely associated with an increase in lipid peroxidation and induced glutathione depletion is a determinant step in oxidative stress in kidney tissue that leads to nephrotoxicity **(Gregg, Antunes et al., 2000).**

It was demonstrated that co-administration of fish oil with cisplatin, the biochemical parameters showed amelioration in comparison to cisplatin only. Similar results were observed by **Vankatraman et al. (1994) and Hardman and Avula (2001)** who reported that CAT, Gpx and SOD activities were significantly increased in livers of mice fed fish oil. These results indicated that fish oil inhibits arachidonic acid synthesis and incorporation into phospholipids, decreases platelets production of thromboxane A_2 a potent vasocon-strictor and inducer of platelet aggregation **(Axelrod, 1989).** In this regard, **Morrow and Roberts (1997)** have reported that thromboxane A_2 and F_2-isoprostanes represents a reliable and useful assessment of lipid peroxidation and oxidant stress. However, F_2-isoprostanes are predominantly derived from the peroxidation of arachidonic acid, which was reduced following fish diets. The results therefore, reflects a true reduction in the oxidative status rather than a change resulting from a reduced supply of F_2-isoprostanes. There is evidence that fish oil reduce the leukocyte oxidative burst **(Hiramatsu and Arimori, 1988 and Vorming et al., 1995).** A decrease of superoxide production by isolated human polymorphonuclear leukocytes **(Fisher et al., 1986)** and monocytes **(Sirtori et al., 1992)** and by rat polymorphonuclear leukocytes **(Carbonell et al., 1997)** has been reported after dietary treatment with ω3 fatty acid.

Other mechanisms by which ω3 fatty acids may protects against lipid peroxidation could be related to their tight packing in complex membrane lipids and lipoproteins making the double bonds less available for free radical attack **(Applegata and Glomset, 1989).** Moreover, ω3 fatty acid inhibits the activity of phospholipase A_2 and induces the activity of hepatic antioxidant enzymes such as glutathione peroxidase **(Demoz et al., 1992).** Incorporation of ω3 fatty acids into erythrocyte membrane phospholipids stimulates glutathione peroxidase activity, together with a possible albeit not generalized, fish oil-induced stimulation of vitamin E incorporation and a trend to higher glutathione peroxidase activity **(Mabil et al., 2001).**

It is interesting to point out that administration of vitamin C with cisplatin produced good protection against cisplatin nophrotoxicity. These results were in accordance with that of **(Greggi Antunes et al., 2000 and Durak et al., 2002)** who noted that cisplatin-induced nephrotoxicity was ameliorated by vitamin C. This amelioration was manifested by preventing the deplatoin of antioxidant enzymes and inhibited lipid peroxidation. These results are explained by the action

of vitamin C as free radical scavenger **(Mayes and Weise, 1993 and Ward and Peters 1995)**. This action can antagonize the main toxic effect of cisplatin **(Durak et al., 2002)**. Vitamin C itself is metabolized to dehydroascorbate via the free radical intermediate. Trapping of the radicals prevents this radical from attacking the LDL and other lipoproteins in plasma. Since vitamin C is used-up in the course of its antioxidant action and prevents initiation of lipid peroxidation, it is considered as sacrificial and preventive antioxidant, respectively **(Balz, 1994)**.

A relationship between nephrotoxicity and oxidative stress has been confirmed in many experimental models. Administration of superoxide dismutase and antioxidant selenium significantly reduce the nephrotoxic symptoms **(Antunes et al., 2001 and Okasara, 1992)**. Recently fish oil and vitamin C has been found to have a number of direct and indirect roles in protecting the cells and the organisms from oxidative damage inflicted by highly toxic radicals generated from oxygen species **(Badalamenti et al., 1995 and Greggi Antunes et al., 2000)**.

In conclusion we have evaluated the effect of vitamin C and/or fish oil in dogs treated with cisplatin, the results might suggest that the best protection against cisplatin toxicity was achieved in the group treated with cisplatin combined with both vitamin C and fish oil. This maximum protection can be explained by the cooperation of the protective mechanism of the two drugs, so it is recommended to try both of vitamin C and fish oil with cisplatin to overcome all possible nephrotoxic mechanism of the drug.

Table (1): The effect of vitamin C and fish oil on plasma urea, creatinine, malondialdehyde and kidney malondialdehyde (MDA).

Parameters	Control	Treated groups	
		Vitamin C group	Fish oil group
Plasma urea mg/dl.	18.4±0.76[a]	16.50±0.40[a]	19.4±0.78[a]
Plasma creatinine mg/dl.	0.85±0.06[a]	0.79±0.02[a]	0.80±0.01[a]
Plasma malondialdehyde μ mol/L	20.4±0.92[a]	20.20±0.68[a]	18.90±0.46[a]
Kidney malondialdehyde n mol/gm. tissue	43.20±2.22[a]	37.72±0.98[a]	38.72±1.17[a]

Number of dogs in each group = 6

Means in the same rows without a common superscript letters are significantly (P<0.05) different, while mean with a common superscript letters are in significantly (P< 0.05) different (two way ANOVA test)

Table (2): Statistical analysis of plasma urea nitrogen and plasma creatinine in control group, cisplatin group, cisplatin plus vitamin C group, cisplatin plus fish oil group and cisplatin plus vitamin C and fish oil group.

Parameters	Control group	Treated groups			
		Cisplatin only	Cisplatin + vit. C	Cisplatin + fish oil	Cisplatin + fish oil + vit.C
Plasma urea mg/dl	18.40 ±0 76 ^{b}	38 40±1 6^{a}	20.600±0 96^{b}	21 20+1.07^{b}	20.70±0 730^{b}
Plasma creatinine mg/dl	0.85±0.069^{b}	2 02±0.33^{a}	0 93±0.042^{b}	0.95±0.038^{b}	0 89±0 064^{b}

Number of dogs in each group = 6

Means in the same rows without a common superscript letters are significantly (P<0 05) different, while mean with a common superscript letters are in significantly (P< 0 05) different (two way ANOVA test)

Table (3): Statistical analysis of plasma and kidney malondialdehyde (MAD) in control group, cisplatin group, cisplatin plus vitamin C group, cisplatin plus fish oil group and cisplatin plus vitamin C and fish oil group

Parameters	Control group	Treated groups			
		Cisplatin only	Cisplatin + vit. C	Cisplatin + fish oil	Cisplatin + fish oil + vit C
Plasma MAD μ mol/L	20.4±0.92^{b}	32.4±1.63^{a}	19.6±0 67^{b}	18 2±1.92^{b}	20.10±1 02^{b}
Kidney MAD n mol/gm tissue	43 2±2.22^{b}	64.3±2.92^{a}	39.8±0 86^{b}	38.2±1 43^{b}	40 2±1.06^{b}

Number of dogs in each group = 6

Means in the same rows without a common superscript letters are significantly (P<0.05) different, while mean with a common superscript letters are in significantly (P< 0.05) different (two way ANOVA test)

Table (4): Statistical analysis of kidney catalase (CAT), glutathione peroxidase (Gpx), superoxide dismutase (SOD) and reduced glutathione (GSH) in control group, cisplatin group, cisplatin plus vitamin C group, cisplatin plus fish oil group and cisplatin plus vitamin C and fish oil group.

Parameter	Control group	Treated group			
		Cisplatin only	Cisplatin + vit C	Cisplatin + fish oil	Cisplatin + vit C + fish oil
CAT mg/gm tissue	3.88±0 73^{a}	1.80±0 15^{b}	3.62±0.86^{a}	3.50±0 17^{a}	3.84±0.26^{a}
Gpx U/gm tissue	36.3±0.66^{a}	23.3±1.67^{c}	35.3±0.62^{a}	33.2±0 76^{b}	36.2±0 91^{a}
SOD U/gm tissue	832±34.4^{a}	536±37 09^{c}	744 0±20.39^{a}	728±40.79^{a}	752+23 32^{a}
GSH μ mol/gm tissue	20.3±0 49^{a}	9 98±0.41^{b}	18.72±1.6^{a}	17.95±1.21^{a}	19 73±1.16^{a}

Number of dogs in each group = 6

Means in the same rows without a common superscript letters are siganficantly (P<0.05) different, while mean with a common superscript letters are in significantly (P< 0 05) different (two way ANOVA test)

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الملخص العربى

الحد من إحداث التسمم الكلوى الناتج من إستخدام عقار السيسبلاتين فى الكلاب باستخدام ڤيتامين ج وزيت السمك

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فى محاولة لدراسة كيفية حدوث التسمم الكلوى بعقار السيسبلاتين والذى يستخدم كعلاج كيميائى لأمراض السرطان والدور الذى يقوم به كل من ڤيتامين ج وزيت السمك فى حماية الكلى من التأثير الضار لهذا العقار، أستخدم ٤٢ من ذكور الكلاب وتم تقسيمها إلى سبع مجاميع متساوية.

المجموعة الأولى : وكانت مجموعة ضابطة وتم حقنها بمحلول فسيولوچى وريدياً.

المجموعة الثانية : وقد تم حقنها فى الوريد بفيتامين ج بجرعة واحدة (٢٠٠ ملجم / كجم من وزن الجسم).

المجموعة الثالثة : وقد حقنت وريدياً بزيت السمك بجرعة واحدة (٢مل/كجم من وزن الجسم).

المجموعة الرابعة : وتم حقنها وريدياً بعقار السيسبلاتين بجرعة واحدة (٥ملجم / كجم من وزن الجسم).

المجموعة الخامسة : وتم حقنها بجرعة واحدة بكل من عقار السيسبلاتين وفيتامين ج بنفس الجرعات السابقة فى الوريد.

المجموعة السادسة : تم حقنها بجرعة واحدة وريدياً بكل من عقار السيسبلاتين وزيت السمك بنفس الجرعات السابقة.

المجموعة السابعة : وقد تم حقنها بجرعة واحدة بعقار السيسبلاتين وكل من ڤيتامين ج وزيت السمك بنفس الجرعات السابق ذكرها فى الوريد.

وقد تم أخذ عينات من دم الحيوانات والكلى بعد سبع أيام من التداوى وذلك لدراستها كيميائياً كالآتى :-

١- قياس وظائف الكلى (البولينا، والكرياتينين) وذلك للتأكد من حدوث تسمم كلوى بعقار السيسبلاتين من عدمه.

٢- قياس مستوى مالوندای ألدهيد فى كل من البلازما ونسيج الكلى وذلك لاستبيان مدى أكسدة الدهون.

٣- قياس الإنزيمات المضادة للأكسدة (الكاتاليز والجلوتاثيون بير أكسيديز والسوبرأكسيد ديسميوتيز) وكذلك مستوى الجلوتاثيون المختزل فى نسيج الكلى.

وقد أظهر التحليل الإحصائى للنتائج مايلى :-

أولاً : لايوجد أى تغير فى مستوى كل من البولينا والكرياتينين وأيضاً المالوندای ألدهيد فى البلازما والكلى بعد تعاطى كل من ڤيتامين ج أو زيت السمك وهذا يثبت المدى الآمن فى إستخدام كل من زيت السمك وڤيتامين ج حيث أن ليس لهما أى تأثير ضار على الكلى.

ثانياً : بعد تعاطى عقار السيسبلاتين لوحظ أن هناك تسمم كلوى تمثل فى الزيادة المعنوية لكل من البولينا والكرياتينين فى البلازما وكذلك المالوندای ألدهيد فى البلازما والنسيج الكلوى وعلى العكس وجد تناقص معنوياً للإنزيمات المضادة للأكسدة (إنزيم الكاتاليز والجلوتاثيون بيرأكسيديز والسوبر أكسيد ديسميوتيز) وكذلك كمية الجلوتاثيون المختزل، وعند تناول كل من زيت السمك أو ڤيتامين ج مع عقار السيسبلاتين لوحظ أن وظائف الكلى لاتتأثير بالعقار وكذلك الإنزيمات المضادة للأكسدة وعدم حدوث أكسدة للدهون حيث يظل نشاط الإنزيمات المضادة للأكسدة وكذلك مستوى وظائف الكلى مماثلاً كما هو فى المجموعة الضابطة مما يثبت أن كل من زيت السمك وفيتامين ج يقوى قدرة الجسم على المقاومة للأكسدة بكونهما مواد مانعة للأكسدة، وكذلك يقومان بحماية الدهون من الأكسدة أيضاً.

من هذه الدراسة يتبين لنا أن لعقار السيسبلاتين أثر سيئ على الكليتين وعلى مقاومة الجسم للأكسدة ولذلك ينصح باستخدام ڤيتامين ج أو زيت السمك أو كلاهما معاً لما لهما من تأثير مقوى لقدرة الجسم على مقاومة الأكسدة وذلك عند ضرورة إستخدام مثل هذا العقار وخاصة عند الأشخاص الذين يعانون مسبقاً من إضطرابات فى وظائف الكلى.