European Journal of Biology and Medical Science Research
Vol.1, No.4, pp.10-18, December 2013
Published by European Centre for Research and Training and Development UK (www.ea-journals.org)

POSSIBLE AMELIORATIVE ROLE OF LOW DOSE OF RADIATION AGAINST CISPLATIN INDUCED OXIDATIVE STRESS AND TISSUE DAMAGE IN MALE RATS

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***ABSTRACT**: The present study describes antioxidant effect of low dose of radiation against cisplatin induced toxicity in rats. Oxidative stress was induced in rat by a single dose of cisplatin (10 mg/kg body weight I.P), 24 hr post- cisplatin treatment, the animals exposed to 0.3 Gy single dose of gamma ray. The effect of treatments in influencing the oxidative stress as well as biochemical cisplatin changes on brain, kidney and testis were studied. The data showed significant increase in serum urea, creatinine, creatine kinase isoenzymes (CKBB) and tumor necrosis factor (TNF-α) as well as in tissue MDA levels in animals treated with cisplatin while this effects were attenuated by radiation exposure. Moreover, treatment with cisplatin caused a significant decrease in serum testosterone and tissues SOD activities, which was shown to be reversed by low dose of radiation treatment. Treatment with low dose of radiation significantly reduced the oxidative stress effects induced by cisplatin administration. It can be concluded that exposed animals to a low dose of radiation after cisplatin treatment can speed the recovery of the body during the chemotherapeutic treatment.*

KEYWORDS: Oxidative stress, Cisplatin, Brain, Kidneys, Testes, Low dose of radiation.

INTRODUCTION

Cisplatin is quite efficient in the treatment of testicular and ovarian cancers and is also excessively utilized for treating bladder, cervical, head and neck, oesophageal and small cell lung cancer. Despite its success, cisplatin has several disadvantages, which include severe side effects including nephrotoxicity and neurotoxicity (Giaccone, 2000). These toxic effects limit the dose that can be applied to patients. Although cisplatin is widely used in clinic, its application is still limited to a relatively narrow range of tumour types. Some tumours such as colorectal and non-small cell lung cancers have intrinsic resistance to cisplatin, while others such as ovarian or small cell lung cancer increase acquired resistance after the initial treatment (Fuertes *et al.*, 2003). It has emerged from the studies on the molecular mechanisms of antitumor action of cisplatin , that the biochemical mechanisms of cisplatin cytotoxicity involve the binding of the drug to DNA and non- DNA targets and further induction of cell death through apoptosis, necrosis or both within the heterogeneous population of cells that forms a tumour mass (Cvitkovic, 1998). Cisplatin is known to accumulate in mitochondria of renal tubular epithelial cells together with ROS; renal tubular cell

mitochondrial dysfunction is also important in cisplatin-induced nephrotoxicity by decreasing the activity of antioxidant enzymes and by depleting intracellular concentrations of GSH (Kawai & Gemba, 2007). A depletion of GSH can lead to increased lipid peroxidation with concomitant changes in membrane permeability and cellular damage, but an increase in GSH level enhances antioxidant protection and cellular function.

Low doses of ionizing radiation induce various effects, including radio-adaptive response (Ikushima *et al.*, 1996), an increase the antioxidant activity against environmental chemical pollutant (Yamaokak *et al*., 2004 and Gharib *et al.*, 2012), activation of immune function (Nogami *et al.*, 1993) and enhancement of resistance to high- dose radiation (Yonezawa *et al.*, 1996). Kojima *et al.* (2004) reported that low dose of radiation significantly increased total glutathione levels in organs such as liver pancreas and brain. In our previous studies, treatment with low dose of gamma rays (0.5 Gy) ameliorate harmful effects induced by TCE taking in consideration the effect of gamma radiation as a stimulant of radical detoxification (Gharib *et al.*, 2012).
In the light of above mentioned antioxidant properties of low dose of radiation, this study was passed out to investigate in how extent low dose of radiation can speed the oxidative stress recovery of the body during the chemotherapeutic treatment.

MATERIAL AND METHODS

Animals
Male albino rats weighing approximately 120-150 g were used for this experiment. They were housed in polypropylene cages in an air conditioned room with temperature maintained at 25 °C ± 3 °C, relative humidity of 50 % ± 5 % and 12 h alternating light and dark cycles. The rats were provided with a nutritionally adequate chow diet and drinking water *ad libitum* throughout the study. Experiments were begun after four-week acclimatization period.

Chemicals
Cisplatin was purchased from Central Drug House (Egypt). The chemicals used in this experiment were obtained from Sigma Chemical (USA). Kits used in this experiments were purchased from Bio-Diagnostics (UK).
Radiation process
A single dose whole body irradiation (0.3 Gy) was performed with rats, using gamma rays by Cesium 137 irradiation unit, National Center for Radiation Research and Technology (NCRRT), with the dose rate 0.74589 rad/ sec. The gamma cesium cell was calibrated by alanine dosimetry relative to a primary standard. Correction were made daily for humidity, temperature, and barometric pressure.

Experimental design
Adult male albino rats were divided into 4 groups of 6 rats each and treated as follows: *Control group,* received distilled water. *Cisplatin group*, received freshly dissolved cisplatin in 1 ml distilled water at a dose of 10 mg/ kg body weight (I.P); *low radiation dose group*, animals were exposed to a single low gamma radiation dose of 0.3 Gy and *Cisplatin+ low radiation dose group,* animals were administered with Cisplatin, 24 hr post-cisplatin administration the animals were exposed to a

single low dose of gamma rays (0.3 Gy). The dose of cisplatin was decided on the basis of Máthé *et al.* (2006). The animals were given one-day rest, killed by decapitation and the blood samples were collected. Brain, kidneys and testes were excised, homogenized in ice-cold salin and utilized for various oxidative stress and biochemical analysis.

Biochemical analysis

Blood samples were centrifuged at 3000 rpm for 15 min., clear serum were collected and stored in a refrigerator. The concentrations of urea and creatinine in serum were analyzed. Testosterone hormone in serum were assayed in duplicate using a double antibody kit (Diagnostic system Lab, Webstet, Tx, USA) with a minimum detection of 0.05/ng/ml and a range up to 25ng/ml. In addition CK BB iso-enzyme was measured using immunoassay kit which allows *in vitro* quantitative determination. The microtitr plate provided in this kit has been pre-coated with an antibody specific to CK BB. The concentration of CK BB in CK BB, biotin- conjugated Avidin will exhibit a change in colour. The enzymatic substrate reaction is terminated by the addition of sulphuric acid solution and color change is measured spectrophotometrically at wave length 450nm ± 2nm (eiaab kit). serum tumor necrosis factor (TNF- α) were determined using Elisa (quantikin R & D system, USA) according to the manufactures' instructions (Maskos *et al.*, 1998) and were carried out at the Central Laboratory, Radioisotope Dept., AEA, Giza, Egypt. Brain, kidneys and testes were minced and homogenized (10 % w/ v) in ice-cold normal saline solution. The homogenate was centrifuged at 3,000 rpm for 15-20 min at 4 °C. The resultant supernatant was used for various biochemical assays. MDA and SOD levels were measured. Urea was estimated by kits according to the method of Halled and Cook (1971). Creatinine level was measured according to the method of Henery (1974). In addition MDA concentration was measured by using Yoshioka, *et al.* (1979). SOD activity was estimated by the method of Marklund and Marklund *(*1974). All biochemical assays were performed with a Helios Thermo-Spectronic spectrophotometer (Thermo Spectronic, UK).

Statistical Analysis*:*

To assess the significant level of influence caused by low dose of radiation in cisplatine administrated rats, one way analysis of variances (ANOVA) followed by Tukey's multiple comparison test was used. Data are representative of 6 independent experiments carried out in triplicate. Statistical analysis was performed by using Graph-Pad software, San Diago, CA, USA) Differences were considered statistically significant when the P value was less than 0.05.

RESULTS

Table (1 to 4) reported the effect of cisplatin alone and the ameliorating effects of low dose of radiation individually on some biochemical variables in brain, kidneys and testes of various groups.

Table (1): Effect of low dose of radiation (0.3 Gy) on some serum biochemical variables in cisplatin exposed rats.

Biochemical Parameters	*Control*	*Cisplatin*	*Low dose of radiation*	*Cisplatin + low radiation dose*
Urea (mg/dl)	7.273± 1.451	20.93±3.364*	7.533±1.612$^{(\#)}$	7.600± 1.821$^{(\#)}$
Creatinine (mg/ dl)	1.917± 0.241	5.258± 0.953*	1.313±0.191$^{(\#)}$	2.035±0.473$^{(\#)}$
Testosterone (ng/ml)	3.984 ± 0.5778	1.582 ± 0.3558 *	3.184 ± 0.3525*$^{(\#)}$	3.400 ± 0.5292$^{(\#)}$
CKBB (ng/ml)	8.602± 0.8886	14.06± 2.272*	11.00 ± 1.077*$^{(\#)}$	9.420 ± 0.9042$^{(\#)}$
α- TNF (pg/ml)	31.14± 5.080	71.10± 11.45*	63.76± 11.18*	40.90± 5.815$^{(\#)}$

Values are Mean ± SD; n= 6, * compared with normal animals, $^{(\#)}$ compared with cisplatin exposed animals since P<0.05.

Data in table (1) shows that there are significant increases in serum urea, creatinine, CKBB and TNF- α levels that recorded (P<0.001, 188%), (P<0.0001, 174%), (P<0.0001, 63%) and (P<0.0001, 128%) respectively in cisplatin exposed rats as compared with normal control rats. Treatment with low dose of radiation significantly suppressed the increased levels of urea (P<0.0001, -63%), creatinine (P<0.0001, -1%), CKBB (P<0.0001, -33%) and TNF- α (P<0.0001, -33%) when compared with cisplatin treated animals. On the other hand, cisplatin- induced depletion in testosterone level (P<0.0001, -60%) that was significantly prevented by treatment with low dose of radiation at a dose of 0.3 Gy when compared with cisplatin treated animals.

Table (2): Antioxidant potential of low dose of radiation on lipid peroxidation and SOD activity in brain of cisplatin exposed rats.

Parameters	*Control*	*Cisplatin*	*Low radiation dose*	*Cisplatin + low radiation dose*
MDA (µM/ g tissue)	111.1± 17.34	163.3 ± 31.73*	121.9 ± 18.67$^{(\#)}$	134.4 ± 28.55
SOD (unit/ ml of tissue)	4.264 ± 0.7263	2.650 ± 0.5320*	4.033 ± 0.6831$^{(\#)}$	4.783 ± 0.9867$^{(\#)}$

Legend as in table (1).

Brain oxidative stress and antioxidant defense variables*:*
Effect of cisplatin alone and ameliorating effect of low dose of gamma radiation at a dose of 0.3 Gy individually after cisplatin administration on MDA and SOD activities in brain of various groups were assessed and presented in Table 2. The level of malonaldehyde showed non- significant increase in irradiated groups as compared with the normal control level. Cisplatin at a dose of 10 mg/kg body weight caused significant increase in the level of brain MDA. This increase was recorded (p<0.05, 47%) accompanied with a significant decrease in SOD activity (p<0.05, -38%), in comparison to control group. However, treatment with low dose of radiation after cisplatin administration caused a non-significant change in MDA level when compared with both control and cisplatin induced group. A significant increase (p<0.001, 80.5%) in the activity of SOD was observed after the treatment with low dose of gamma radiation (0.3 Gy), as compared with cisplatin exposed group.

Table (3): Antioxidant potential of low dose of radiation on lipid peroxidation and SOD activity in kidneys of cisplatin exposed rats.

Parameters	*Control*	*Cisplatin*	*Low radiation dose*	*Cisplatin + low radiation dose*
MDA (µM/ g tissue)	112.8 ± 16.68	145.6 ± 15.91*	107.5 ± 7.933$^{(\#)}$	108.8 ± 25.42$^{(\#)}$
SOD (unit/ ml of tissue)	6.195 ± 1.373	3.030 ± 0.4705*	4.622 ± 0.7176*$^{(\#)}$	4.464 ± 0.9212*

Legend as in table (1).

Renal oxidative stress and antioxidant defense variables:

Effect of cisplatin alone and ameliorating effect of low dose of gamma irradiation individually after cisplatin administration on MDA and antioxidant related parameters in kidneys of various groups were assessed and presented in Table 3.

The level of MDA was significantly higher ($p<0.05$, 29%) in cisplatin- treated animals than that of normal untreated rats. Whereas, significant decrease ($p<0.001$, -51%) in renal SOD activity, of rats were observed in cisplatin treated animals as compared with control group. After the treatment with low dose of gamma rays at a dose of 0.3Gy, a significant decrease ($p<0.05$, -25%) in the level of MDA was observed in comparison to cisplatin -treated group. In addition SOD activity in cisplatin -treated group showed insignificant increase due to radiation exposure.

Table (4): Antioxidant potential of low dose of radiation on lipid peroxidation and SOD activity in testes of cisplatin exposed rats.

Parameters	*Control*	*Cisplatin*	*Low radiation dose*	*Cisplatin + low radiation dose*
MDA (µM/ g tissue)	48.30 ± 8.134	96.58 ± 18.49*	41.15 ± 7.755$^{(\#)}$	34.48 ± 3.793$^{(\#)}$
SOD (unit/ ml of tissue)	3.333 ± 0.7737	1.800 ± 0.1897*	4.717 ± 0.9304*$^{(\#)}$	3.700 ± 0.4940$^{(\#)}$

Legend as in table (1).

Testicular oxidative stress and antioxidant defense variables*:*

Table (4) represent the effect of low dose of radiation on cisplatin induced oxidative stress in testes tissue. Cisplatin at a dose of 10 mg/kg body weight caused significant increase in the level of testes MDA. This increase was recorded ($p<0.001$, 100%) accompanied with a significant decrease in SOD activity ($p<0.05$, -46%), in comparison to control group. However, treatment with low dose of radiation after cisplatin administration showed a significant decrease in MDA level when compared with cisplatin induced group. The MDA decrease recorded -64% ($p<0.001$). However, a significant increase ($p<0.001$, 105.5%) in the activity of SOD was observed in (Cisplatin + low radiation dose) group, as compared with cisplatin exposed group.

DISCUSSION

Cisplatin, a heavy metal complex, is an effective chemotherapeutic agent for a wide variety of tumors (Park *et al.*, 2009). Despite its success, cisplatin has several disadvantages, which include severe side effects including nephrotoxicity, neurotoxicity as well as testicular damage (Salem *et al.*, 2012). cisplatin administration induces overproduction of reactive oxygen species (ROS) such as hydrogen peroxide and hydroxyl radicals, which abstract a hydrogen atom from polyunsaturated fatty acids (Kadikoylu *et al.*, 2004) and depletes the cellular antioxidant capacity (Giaccone, 2000).

Oxidative evaluation*:*
Oxidative stress, a condition of an imbalance between free radicals and antioxidant defense system, is an important factor in the pathogenesis system that has high content of polyunsaturated membrane lipid (Acar *et al.,* 2012). Recent study indicated that cisplatin led to neurotoxic effects in human and animals via induction of lipid peroxidation (Kamisli *et al.,* 2013). Our present work showed that cisplatin produced a significant increase in serum CKBB and MDA levels that induced lipid peroxidation in the brain tissues accompanied with suppression in SOD activity. According to Aksenova *et al.* (1999) the results of CK BB expression may be an early indicator of oxidative stress in neurons. On the other hand, study of Qu *et al.* (2012) and Kamisli *et al.,* (2013), explained the increase in MDA and the SOD activity decrement may be due to cisplatin and other complex producing oxidative changes in the nervous system.

On the other hand, therapeutic effects of cisplatin based on the interaction with DNA in the cell, preventing proliferation and inducing apoptosis in tumor cells. In fact, renal insufficiency is the major and most severe form of toxicity associated with use of cisplatin as a chemotherapeutic agent. Renal toxicity, which is manifested by an increase in serum urea and creatinine, can result both from doses that are higher than recommended and an accumulation of cisplatin in the body (Mukhopadhyay *et al.,* 2011). According to Mukhopadhyay *et al.* (2011); cisplatin induced mitochondrial ROS generation triggered inflammatory response, cell death and kidney dysfunction/ nephropathy. Cisplatin triggers oxidative stress in the mitochondria of kidney proximal tubuler and endothelial cells which is followed by a secondary wave of ROS/ RNS (reactive nitrogen species) generation, deterioration response (Ognjanovic *et al.,* 2012). Moreover, cisplatin induces serious inflammatory changes and enhances renal expression TNF- α (Ramesh and Reeves, 2004). TNF- α induces apoptosis that produces reactive oxygen species and coordinate the activation of large network of chemokines and cytokines in kidney (Ramesh and Reeves, 2002). In addition several studies demonstrated that cisplatin induced acute nephrotoxicity is mediated by impaired activity of SOD as well as an increase in renal lipid peroxidation (Gonzalez *et al.,* 2004). It has been suggested that cisplatin is able to generate reactive oxygen species by inducing glucose -6-phosphate dehydrogenase and hexokinase activity and inhibit the activity of antioxidant enzyme in renal tissue such as SOD, CAT, GSH- Px (Naziroglu *et al.,* 2004). In addition, according to Atasayar *et al.* (2009) the increase in MDA level in the cisplatin treated group are also responsible for the renal oxidative stress.

In the present study, testicular damage induced by cisplatin treatment was characterized by decrease in serum testosterone level and significant reduction in testis SOD activity as well as elevated MDA production compared with the untreated control animals. The decreased serum testosterone level in cisplatin treated rats could be attributed to the impairment of Leydig cells (Ilbey *et al.,* 2009). It has also been reported that the cisplatin -induced changes in testosterone are associated with decreased numbers of LH receptors on Leydig cells (Maines *et al.,* 1990). Besides, Khaki *et al.* (2009) reported that spermatozoa are highly susceptible to damage by excessive concentration of ROS in the cisplatine treated rats due to the high content of

polyunsaturated fatty acids within their plasma membrane. Lipid peroxidation destroys the structure of spermatozoa with loss of its motility and impairment of spermatogenesis (Sharma and Agarwal, 1996).

Antioxidant evaluation:

In the biological system, one of the active sources of oxygen radicals is the ionizing radiation. Cells can be injured, and even killed under the most severe condition of radiation exposure, when the content of reactive oxygen species (ROS) gets uncontrolled by the cellular antioxidant (Pathak *et al.*, 2007). On the other hand, low-dose radiation, may have different beneficial effects, it may decrease the cancer risk according to "linear hypothesis" and increase the immunity of the cells (Kojima *et al.*, 2004). The present study has investigated the efficacy of low dose of radiation exposure which is probably induced adaptive protection. The delayed and temporary adaptive protection at low doses involves damage prevention, damage repair and immune responses. They appear to operate primarily against DNA damage from non-radiation sources. According to the results recorded by Pathak *et al.*, (2007), the antioxidant enzyme activities and the reduced glutathione (GSH) content increased significantly almost in general to the generation of lipid peroxides after exposure to whole body low dose γ- radiation (0.5 Gy) (Yamaok *et al.*, 1991). This investigation was in agreement with the present work since exposure to 0.3 Gy caused a non significant increase in lipid peroxidation in brain tissue. In addition, the increase in GSH levels may be due to the activation of protective response in the organs to counteract the excessive formation of ROS. Moreover, it was interesting that this effect was observed over a range of 0.25- 0.5 Gy Kawakita *et al.* (2003). It is evident from the results of the present work that exposing to low dose of radiation after cisplatin administration protected animals from toxic effects of cisplatin in general and oxidative stress in particular. This study is in confirmation with the previous report suggested the preventive effects of low dose of radiation against exposure to chemical compound (Gharib *et al.*, 2012). In addition, according to Yamaoka *et al.* (2004) low- dose irradiation induces various stimulating outcomes such as increase in resistance toxicity, enhancement of immune function; these effects may be related to the induction of antioxidant enzymes and the degeneration of O_2^-, H_2O_2 and $^{\bullet}OH$ is inhibited by low dose irradiation.

CONCLUSION

These findings are of immense support in understanding the possible role of low radiation dose in rendering protection against cisplatin inflicted damages to Brain, Kidneys and testes tissues. Additional studies using different low dose of gamma radiation will be needed to address this issue.

REFERENCES

Acar, N., Berdeaux, O., Grégoire, S., Cabaret, S., Martine, L., Gain, P., Thuret, G., Creuzot- Garcher, C.P., Bron, A.M. and Bretillon, L. (2012) *Lipid composition of human eye: Are red blood cells a good mirror of retinal and optic nerve fatty acid?* PLOS ONE, 7(4)e35102.

Aksenova, M.V., Aksenov, M.Y., Payne, R.M., Trojanowski, J.Q., Schmidt, M.L., Carney, J.M., Butterfield, D.A. and Markesbery, W.R. (1999) *Oxidation of cytosolic proteins and expression*

of creatine kinase BB in frontal lobe in different neurodegenerative disorders. Dement Geriatr Cogn Disord, 10(2)158-65.

Atasayar, S., Gürer-Orhan, H., Orhan, H., Gürel, B., Girgin, G. and Özgüneş, H. (2009) *Preventive effect of aminoguanidine compared to vitamin E and C on cisplatin-induced nephrotoxicity in rats.* Exp. Toxicol. Pathol, 61,23–32.

Cvitkovic, E. (1998) *A historical perspective on oxaliplatin: rethinking the role of platinum compounds and learning from near misses*. Semin. Oncol., 25,1.

Fuertes, M.A., Alonso, C. and Pérez, J.M. (2003) *Biochemical modulation of cisplatin mechanism of action enhancement of antitumor activity and circumvention of lung resistance.* Chem. Rev, 103,645.

Gharib, O.A., Abd Ellatif, U.A., Abdellah, N.M. and Mohammad, M.A. (2012) *Radio-protective response on the environmental pollutant induced oxidative stress*, Adv. Biosci. Biotech, 3,989-996

Giaccone, G. (2000) *Clinical perspectives on platinum resistance.* Drugs 59(Suppl 4) 9–17.

Gonzalez, R., Borrego, A., Zamora, Z., Romay, C., Hernandez, F., Menendez, S., et al. (2004) *Reversion by ozone treatment of acute nephrotoxicity induced by cisplatin in rats.* Mediators Inflamm, 13(5-6) 307-12.

Halled, C.J. and Cook, J.G. (1971) *Reduced nicotinamide adenine dinucleotide- coupled reaction for emergency blood urea estimation.* Clin Chim Acta, 35,33-40.

Henery, R.J. (1974) *From Principle and Techniques: Clinical chemistry* 2nd edition. New York: Harper & Row,525.

Ikushima, T., Aritomi, H. and Morishita, J. (1996) *Radioadaptive: efficient repair of radiation induced DNA damage in adapted cells.* Mutant. Res, 358,193- 198.

Ilbey, Y.O., Ozbek, E., Cekmen, M., Simsek, A., Otunctemur, A. and Somay, A. (2009) *Protective effect of curcumin in cisplatin-induced oxidative injury in rat testis: mitogen-activated protein kinase and nuclear factor-kappa B signaling pathways.* Human Reproduction, 24, (7)1717-1725.

Kadikoylu, G., Bolaman, Z., Demir, S., Balkaya, M., Akalin, N. and Enli, Y. (2004) *The effects of desferrioxamine on cisplatininduced lipid peroxidation and the activities of antioxidant enzymes in rat kidneys.* Hum Exp Toxicol., 23,29-34.

Kamisli, S., Ciftci, O., Kaya, K., Cetin, A., Kamisli, O. and Ozcan, C. (2013) *Hesperidin protects brain and sciatic nerve tissues against cisplatin-induced oxidative, histological and electromyographical side effects in rats.* Toxicology and Industrial Health, [Epub ahead of print]

Kawai, Y. and Gemba, M. (2007) *Cisplatin-induced renal injury in LLC-PK1 cells*. In: Proceedings of the 6th World Congress on Alternatives & Animal Use in the Life Sciences; Tokyo, Japan.453–456.

Kawakita, Y., Ikekita, M., Kurozumi, R. and Kojima, S. (2003) *Increase of intra cellular glutathione by low dose gamma- ray irradiation is mediated by transcription factor API in RAW 264.7*. Cell Biol. Pharm. Bull., 26,19- 25.

Khaki, A., Fathiazad, F., Nouri, M., Khaki, A.A, Ozanci, C.C., Ghafari-Novin, M. and Hamadeh, M. (2009) *The effects of Ginger on spermatogenesis and sperm parameters of rat*. Iranian Journal of Reproductive Medicine, 7(1)7-12.

Kojima, S., Nakayama, K. and Ishida, H. (2004) *Low dose γ- rays activate immune function induction of glutathione and delay tumor growth*. J. Radiat. Res., 45,33-39.

Maines, M.D., Sluss, P.M. and Iscan, M. (1990) *Cis-Platinum-mediated decrease in serum testosterone is associated with depression of luteinizing hormone receptors and cytochrome P-450scc in rat testis.* Endocrinology, 126,2398-2406.

Marklund, S. and Marklund, G. (1974). *Involvement of Superoxide anion radical in the autooxidation of pyrogallol and convenient assay for SOD.* Eur. J. Biochem., 47,469-74.

Maskos, K., Fernandz- Catalan, C., Huber, R., Bourenkov, G.P., Bartun, H., Ellestad, G.A., Reddy, P., Wolfson, M.F., Rauch, C.T., Castner, B.J., Davis, R., Clarke, H.R., Petersen, M., Fitzner, J.N., Cerretti, D.P., March, C.J., Paxton, R.J., Black, R.A. and Bode, W. (1998) *Crystal structure of the catalytic domain of human tumor necrosis factor α- converting enzyme.* Proc. Nath. Acad. Sci., 95,3408- 3412.

Máthé, A., Komka, K., Forczig, M., Szabó, D., Anderlik, P. and Rozgonyi, F. (2006) *The effect of different doses of cisplatin on the pharmacokinetic parameters of cefepime in mice*. Lab Anim., 40(3),296-300.

Mukhopadhyay, P., Horváth, B., Zsengellér, Z., Zielonka, J., Tanchian, G., Holovac, E., Kechrid, M., Patel, V., Stillman, I.E., Parikh, S.M., Joseph, J., Kalyanaraman, B. and Pacher, P. (2011) *Mitochondrial-targeted antioxidants represent a promising approach for prevention of cisplatin-induced nephropathy.* Free Radic. Biol. Med., doi:10.1016/j.freeradbiomed.2011.11.001.

Naziroglu, M., Simsek, M., Simsek, H., Aydilek, N., Ozcan, Z. and Atilgan, R. (2004) *The effects of hormone replacement therapy combined with vitamin C and E antioxidants level and lipid profiles in postmenopausal woman with type 2 Diabetes.* Clin. Chem. Acta., 344,63- 71.

Nogami, M., Huang, J.T., James, S.J., Lubinski, J.M. and Makinedan, T. (1993) *Mice chronically exposed to low dose ionizing radiation possess splenocytes with enhanced proliferation capacity and elevated levels of HSP- 70 mRNA and protein.* Int. Radiat. Res., 63,775- 785.

Ognjanović, B.I., Nataša, Z.D., Miloš, M.M, Jasmina, M.O., Jelena M.M, Andraš, Š.Š and Zorica, S.S. (2012) *Lipid Peroxidative Damage on Cisplatin Exposure and Alterations in Antioxidant Defense System in Rat Kidneys: A Possible Protective Effect of Selenium.* Int. J. Mol. Sci., 13,1790-1803.

Park, H.R., Ju, E.J., Jo, S.K., Jung, U., Kim S.H., and Yee, S.T. (2009) *Enhanced antitumor efficacy of cisplatin in combination with HemoHIM in tumor-bearing mice*. BMC Cancer, 9,85-85.

Pathak**,** C., Yogesh, K., Jaiswal, and Manjula, V. (2007) *Possible involvement of queuine in regulation of cell proliferation.* BioFactors**,** 29,159-173.

Qu, J., Xu Li, X, Juan Wanga, J., Mi, W., Xie, K. and Qiu, J (2012) *Inhalation of hydrogen gas attenuates cisplatin-induced ototoxicity via reducing oxidative stress*, International Journal of Pediatric Otorhinolaryngology. 76,111–115

Ramesh, G. and Reeves, W.B. (2002) *TNF-alpha mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity.* J Clin Invest., 110,835–42.

Ramesh, G. and Reeves, WB. (2004) *Salicylate reduces cisplatin nephrotoxicity by inhibition of tumor necrosis factor-alpha.* Kidney Int., 65,490–9.

Salem, E.A., Salem, N.A., Maarouf, A.M., Serefoglu, E.C. and Hellstrom, W.J. (2012) *Selenium and lycopene attenuate cisplatin-induced testicular toxicity associated with oxidative stress in Wistar rats.* Urology, 79(5),1184.e1-6.

Sharma, R.K. and Agarwal, A. (1996) *Role of reactive oxygen species in male infertility.* Urology, 48,835-850.

Yamaoka, K., Edamatsu, R. and Mori, A, (1991) *Increased SOD activities and decreased lipid peroxide levels induced by low- dose X- irradiation in rat organs.* Free Radi Biol Med., 11, 299-306.

Yamoka, K., Kataoka, T., Nommra, T., Taguchi, T., Wang, D., Mori, S., Hanamoto, and Kira, S. (2004) *Inhibitory effects of prior low- dose X- ray irradiation on carbon tetrachloride- induced hepatopathy in Acatalasemic mice.* J. Radiat Res., 45, 89- 95.

Yomezawa, M., Misonoh, J and Hosokawa, Y. (1996) *Two types of X- ray- induced radio-resistance in mice: Presence of 4 dose ranges with distinct biological effects.* Mutat. Res. 358, 237-243.

Yoshioka, T., Kawada, K., Shimada, T. and Mori, M (1979) *Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood.* Am J Obstet Gynecol., 135(3) 372-376.