Effect of low radiation dose on cisplatin induced hepato-testicular damage in male rats.

ABSTRACT

Aim: The aim of this study was to evaluate the synergistic effect of low radiation dose with the chemotherapeutic drug in order to find possible way to lessen the harmful effects during chemo-radiotherapy.
Study Design: Randomized controlled experiment.
Place and Duration of Study: Experimental Animal Unit, Drug Radiation Research Department, National Center for Radiation Research and Technology, Cairo Egypt.
Methodology: Estimation of antioxidant activity of low radiation dose on oxidative stress induced by cisplatin administration at a dose of 10 mg/kg b. wt. in male albino rat.
Results: Results of experiment revealed that cisplatin administration caused a significant increase in serum alanine transaminase (GPT) activity (38.58±2.060) and FSH level (8.162 ± 1.424) accompanied with a decrease in serum albumin (3.492± 0.253), and Butyry Cholein Esterase (BChE) (65.35 12.61). In Liver and testis, GSH content (68.00 ± 2.391& 24.93 ± 4.778) as well as cytochromes P450 levels (0.3875 ±0.0727 & 0.2167 ± 0.0459) showed a significant decrease as compared to the normal control level respectively. In addition the level of Fe, Cu and Zn showed no significant changes in liver and appeared to be significantly decrease as in case of corresponding trace elements in testis organs. On the other hand, exposing to low dose of radiation (0.5 Gy) post-cisplatin treatment effectively prevented these alterations and maintained the antioxidant status.
Conclusion: Data from present results revealed that low radiation dose have the existence as an antioxidant and antitumor agents which may be useful to use as a synergistic agents with the chemotherapeutic drug.

Keywords: Low Radiation Dose, Cisplatin, Cytochromes P450, Butyryl Cholein Esterase.

1. INTRODUCTION

Cisplatin is one of the most potent anticancer drugs used in chemotherapy. It is highly effective in the treatment of testicular and ovarian cancers as well as bladder, cervical, head and neck, esophageal and small cell lung cancer [1]. Despite being an effective anti-proliferative agent, the clinical usage of cisplatin is limited by various side effects including nephro-toxicity, hepato-toxicity, neurotoxicity and oto-toxicity. The anticancer activity of this drug is attributed to its capacity to form covalent bond at N-7 position of Guanine residues of DNA leading to formation of 1, 2 or 1, 3 inter-strand crosslink and a lesser extent of the inter-strand crosslink (Fig. 1). This adduct cisplatin DNA disrupts the cellular replication and transcription machinery [2]. Many labs have demonstrated that cisplatin generates free radicals leading to oxidative and nitrosative stress which results into such deleterious effects *in vivo* [3, 4]. It is generally accepted that binding of cisplatin to genomic DNA (gDNA) in the cell nucleus is the main event that responsible for its antitumor properties [5]. Thus, the damage induced upon binding of cisplatin to gDNA may inhibit transcription, and/or DNA replication mechanisms. Subsequently, these alterations in DNA processing would trigger cytotoxic processes that lead to cancer cell death. The cytotoxicity of cisplatin is considered to be due to combination factors, including peroxidation of cell membrane, mitochondrial dysfunction, inhibition of protein synthesis, and DNA injury [6].

Till the last decade of the last century, radiotherapy was considered to be the only therapeutic option for patients with locally advanced cancer [7]. There were some trails of combined cisplatin and radiation therapies have been reported and it has proved to be hopeful method to improve the treatment results of invasive bladder cancer [8]. The effectiveness of the combination of cisplatin and radiation in experimental malignant tumor was reported by Wodinsky and his colleagues [9] in leukemic cell. Morris and his colleagues noted a similar effect in mouse leukemia [10]. Cyto-toxic chemotherapy has been shown to give good response rates in patients with good kidney function and no prior radiation therapy. Cisplatin is the most effective single agent [11] and it was shown to be synergistic with radiotherapy in cell lines. Mechanisms underlying the interaction between drugs and radiation may include inhibition of potentially lethal or sub-lethal damage repair, and increasing radio-sensitivity of hypoxic cells [12]. It has been widely used prior to surgery or radiotherapy with the aim of reducing tumor volume and facilitating local treatment. It may have the additional benefit of

controlling micro-metastatic disease. Combined chemo-radiotherapy seems to offer substantial benefit for women with cervical cancer. However, acute toxicity, predominantly hematological and gastrointestinal, was increased with chemo-radiation [13]. Acute side effects are generally of short duration and resolve with medical management, while the late complications of radiotherapy lead to damage which can be difficult to reverse, and permanently impair quality of life. Details of late morbidity are more poorly documented, but with the exception of one trial [14].

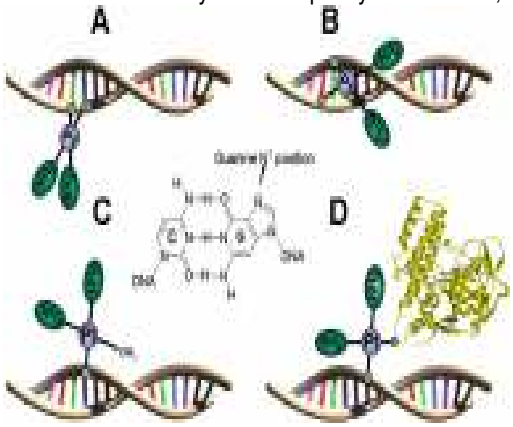

Fig. (1) Main adducts formed after binding of cisplatin to DNA.
(A) 1,2-intrastrand cross-link, (B) interstrand cross-link, (C) mono- functional adduct, and (D) protein-DNA cross-link. The main site of attack of cisplatin to DNA (N7 of guanine) is shown in the central panel [2].

Low dose of radiation induce various effects including radio-protective response [15], as well as activation of immune function [16]. It has been well documented that the immune function is linked to the release of radical oxygen species (ROS). Access amount of ROS is commonly eliminated by endogenous antioxidant system, thereby preventing injury to DNA [17]. It exerts protection against other challenges involving radicals and causing a beneficial effect by temporarily shielding the hit cell against radicals produced through endogenous processes. Low dose of radiation has been observed to stimulate the radical detoxification system, enhancement of DNA repair rates and induce immune competence that associate with an increase in number of cytotoxic lymphocytes, even causing a reduction of the incidence of metastatic cancer [18]. Our pervious study indicated that treatment with low dose of gamma rays (0.5 Gy) ameliorate harmful effects induced by TCE due to the effect of gamma radiation as a stimulant of radical detoxification [19].

According to many clinical studies, when chemotherapy and radiotherapy are concurrently administered improve effectively greater rather than at different times [20]. Combined chemo-radiotherapy presents some problems; however, with more sever adverse events, resulting in a reduced treatment compilation rate [21]. On the other hand, according to the study of Murphy and Morton [22] and Barcellos-Hoff, [23], low radiation dose administartion to entire body increased the action of the protective process in living organism including the overproduction lymphocytes that significantly prevented or impaired tumor growth. From this point of view, the current study's aim is to investigate the synergistic effect of low radiation dose in the treatment with cisplatin as a chemotherapeutic drug inducing oxidative stress.

2. MATERIAL AND METHODS

2.1. 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 a four-week acclimatization period.

2.2. 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).

2.3. Radiation process:

A single dose whole body irradiation (0.5 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.

2.4. Experimental design

Adult male albino rats were divided into 4 groups of 6 rats each and treated as follows:

Control group (normal, untreated), received distilled water. ***Cis. group***, received freshly dissolved cisplatin in 1 ml distilled water at a dose of 10 mg/ kg body weight (I.P); ***IRR group*** was exposed to a single low gamma radiation dose of 0.5 Gy and ***Cis. & IRR group*** was administered with Cisplatin, 24 hr after cisplatin administration the animals were exposed to a single low dose of gamma rays (0.5 Gy). The dose of cisplatin was decided on the basis of Máthé and his colleagues [24]. Twenty four hours after radiation exposure the animals were decapitated and the blood was collected for biochemical analysis. Liver and testes were divided into two portions. One portion were excised, homogenized in ice-cold saline and utilized for various biochemical analyses and the second portion was used for trace elements analysis.

2.5. Biochemical analysis

The blood samples were collected directly from the animals by heart puncturing. They were centrifuged at 3000 rpm for 15 min., clear sera were collected and stored in a refrigerator. The activities of GPT, BChE and FSH levels as well as concentrations of albumin in serum were analyzed. Liver and testis 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 estimation of GSH content and cytochrome P450 activity. Serum BChE activity is quantitatively measured according to the methods published in Munshaw and his colleagues [25]. FSH level was detected in serum samples using elisa kit supplied by (Kamiya, biomedical company, USA) according to manufacturer instruction. The elisa kit was read using 2100 elisa reader. Serum albumin level was determined according to the method of Doumas and his colleagues [26]. In addition GSH content in both liver and testis was measured by method of Beutler and his colleagues [27]. Liver and testis cytochrome P450 activity assayed was detected using a spectrofluorometer [28]. Serum BChE and FSH activities as well as liver and testis P450 were carried out at the Central Laboratory, Radioisotope Dept., AEA, Giza, Egypt, while serum GPT activity and Albumin concentration, as well as tissue GSH content were performed with a Helios Thermo-Spectronic spectrophotometer (Thermo Spectronic, UK).

2.6. Atomic absorption analysis

Liver and testes tissues were digested in a mixture of conc. HNO_3 and H_2O_2 (5:1). The digestion was completely by using miocro-wave oven (mls- 1200 mega) and the digested samples were diluted with de-ionized water to a fixed volume [29]. The selected element was estimated quantitatively by atomic absorption spectrophotometer. *Unicam 939* Hallow cathode lamps were used to determine Fe, Cu and Zn in air acetylene flame [30]. Concentration of elements in tissues was calculated by using calibration curve prepared from their stock solution (1mg). The concentration of elements per grams tissues could be determined by equation:

$$\text{Elemental content in the tissue } (\mu g/g) = c \,.\, v \,.\, n / m,$$

Where: c (µg/ml) is the concentration of the metal measured in the AAS sample of volume v (ml), n is the dilution factor and m (g) is the mass of the tissue taken. Wet tissue weights were used for calculating the metal concentration in tissues [31].

2.7. Statistical analysis:

To assess the significant level of influence caused by low dose of radiation in cisplatin 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.

3. RESULTS

Cisplatin is one of the most potent anticancer drugs used for the treatment of different types of cancer including testicular cancer. In spite of its significant anticancer activity, the clinical use of cisplatin is often limited by its undesirable side effect due to the influence of oxidative stress. Results indicated in fig. (2) in addition to table (1-4) evaluate the effect of cisplatin and the synergistic effect of low irradiation dose (0.5 Gy) on liver and testis tissues.

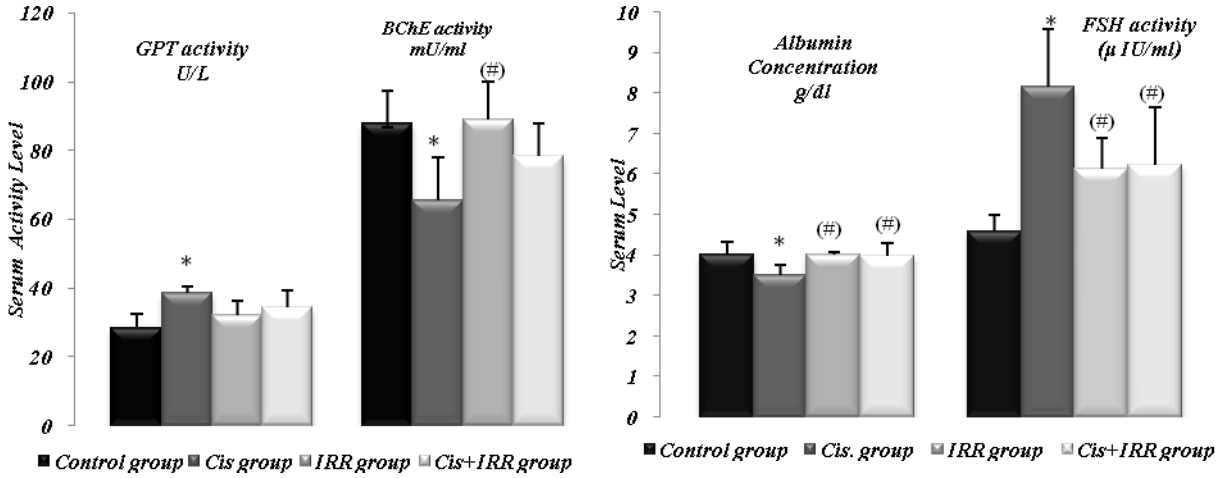

Fig. 2: Effects of cisplatin administration and/ or radiation exposure on some serum biochemical parameters 24 hours post radiation exposure (n=6)

Data are presented as mean (± SD). * Significantly different from the control group (P<0.05). (#)Significantly different from the *Cis. group* (P<0.05). IRR: Low radiation dose of 0.5 Gy gamma rays, *Cis group*: cisplatin administration.

3.1. Cisplatin demonstrated toxicity in both liver and testis:

3.1.1. Cisplatin induced liver damage:

Cisplatin administration caused a significant decrease in serum albumin concentration as well as BChE activity which recorded -12.9% and -25.6% (P<0.05) as compared to the normal control level. This decrease in serum albumin and BChE levels accompanied with a significant increase in serum GPT activity which calculated 35% comparing to the corresponding control group Fig. (2).

On the other hand, administration of cisplatin caused a significant decrease in both liver GSH (-13.9%) and P450 (-26.9%) concentration (P<0.05) as compared to the normal control level. Table (1).

The effect of cisplatin injection on liver trace metals is shown in table (3). Administration of cisplatin caused no significant change on liver Fe, Zn as well as Cu concentration when these data were compared to the normal untreated control level.

Table 1: Effects of cisplatin administration and/ or radiation exposure on liver GSH and0-cytochrome P450, 24 hours post radiation exposure (n=6)

Treatment $\bar{X}$ ± SD	*Control group*	*Cis group*	*IRR group*	*Cis & IRR group*
GSH (mg/g tissue) $\bar{X}$ ± SD	78.94 ± 2.4	68.00 ± 2.4*	86.53 ± 2.7*(#)	76.00 ± 7.6(#)
Cytochrom P450 (pmole/ml/min) $\bar{X}$ ± SD	0.5300 ±0.034	0.3875 ±0.073*	0.6025 ±0.061	0.4825 ± 0.059(#)

*Data are presented as mean (± SD). *Significantly different from the control group (P<0.05). (#)Significantly different from the Cis. group (P<0.05). IRR: Low radiation dose of 0.5 Gy gamma rays, Cis: cisplatin administration.*

3.1.2. Testicular toxicity due to cisplatin administration:

Testicular toxicity was detected by measuring the activity of FSH hormones in serum. Serum FSH showed a significant increase due to the injection of cisplatin (10mg/kg b.wt I.P), the percentage increase in serum FSH was calculated 78%, (P<0.05) when compared to the normal control level (Fig. 2).

Administration of cisplatin (10mg/kg b.wt I.P) to the animal brought about a marked reduction in both GSH content and P450 concentration in testis tissue that recorded -23%, (P<0.05) and -48% (P<0.05) as compared to the control level respectively (Table, 2).

Table (4) displays the effect of cisplatin administration on certain trace elements concentration, which indicated a significant decrease in testis Fe, Zn and Cu levels. The percentage decrease of these elements was nearly the same (-29%, -29% and -22.4%, P<0.05) comparing to the normal untreated control respectively.

Table 2: Effects of cisplatin administration and/ or radiation exposure on testis GSH and cytochrome P450, 24 hours post radiation exposure (n=6)

Treatment $\bar{X}$ ***± SD***	***Control group***	***Cis group***	***IRR group***	***Cis & IRR group***
GSH (mg/g tissue) $\bar{X}$ ± SD	32.36 ± 2.6	24.93 ± 4.8*	39.30 ± 4.2*(#)	35.38 ± 3.1(#)
Cytochrom P450 (pmole/ml/min) $\bar{X}$ ± SD	0.4160 ± 0.043	0.2167 ± 0.046*	0.3780 ± 0.05(#)	0.4027 ± 0.046 (#)

Data are presented as mean (± SD). *Significantly different from the control group (P<0.05). (#)Significantly different from the *Cis. group* (P<0.05). IRR: Low radiation dose of 0.5 Gy gamma rays, *Cis:* cisplatin administration.

3.2. Low dose of radiation exposure showed no significant change in both liver and testis:

3.2.1. Low dose of radiation (0.5 Gy) induced no change in liver function:

Exposing animals to low dose of radiation (0.5 Gy) caused no significant change in the concentration of serum albumin as well as the activities of GPT and BChE as compared to the normal control level (Fig. 2). Moreover, a significant increase was shown in the level of serum albumin and BChE recording 13% and 36% (P<0.05) comparing to the cisplatin treated group.

On the other hand, 0.5Gy dose of whole body gamma radiation detected a significant increase in liver GSH content comparing to the normal control level. This increase was calculated 9.6% and 27% as compared to both control and cisplatin treated group respectively (Table 1), while liver P450 concentration showed no significant change comparing to normal control levels accompanied by a significant increase (74%, P<0.05) in respect to cisplatin treated group.

Finally table (3) showed a non significant change in all tested trace element as compared to both control and cisplatin treated groups.

3.2.2. Testicular state after low dose of gamma radiation (0.5 Gy):

Exposing animals to low dose of radiation (0.5 Gy) caused no significant changes in serum FSH level as regard with normal control level (Fig. 1). Comparing this group with cisplatin treated one detected a significant increase by 56.5% (P<0.05) in serum FSH level (fig. 2).

In table (2) the effect of low dose of radiation showed a significant increase in testis GSH content in respect to both control untreated and cisplatin treated groups. The percentage of this increase calculated 21.44% and 57.6% (P<0.05) respectively. In addition, non-significant increase was evaluated in P450 concentration due to exposing animals to low dose of radiation with a significant increase when P450 level compared with cisplatin treated group (74%, P<0.05) table (2).

In case of trace metals concentration, testis Fe, Zn as well as Cu concentration was estimated in the group of animals exposed to the low dose of gamma radiation (0.5 Gy). Table (4) showed a non significant change on the level of Fe, Zn and Cu in low dose of radiation exposure group when these data compared to the normal untreated control level.

Table 3: Effects of cisplatin administration and/ or radiation exposure on levels of Fe, Zn and Cu in liver tissue 24 hours post radiation exposure (*n*=6)

Treatment $\bar{X}$ *± SD*	*Control group*	*Cis. Group*	*IRR group*	*Cis. & IRR group*
Fe (µg/g fresh tissue) $\bar{X}$ ± SD	205.6±35	183.1±15	186.8±17.8	190.2±21.7
Zn (µg/g fresh tissue) $\bar{X}$ ± SD	119.7±16.3	112.9±13.5	98.86±13.2	102.3±19.9
Cu (µg/g fresh tissue) $\bar{X}$ ± SD	3.450 ± 0.49	3.308 ±0.27	3.322 ± 0.3	3.087 ± 0.28

Data are presented as mean (± SD). *Significantly different from the control group (*P*<0.05). (#)Significantly different from the *Cis. group* (*P*<0.05). IRR: Low radiation dose of 0.5 Gy gamma rays, *Cis*: cisplatin administration.

3.3. The synergistic effect of low dose of radiation exposure with cisplatin administration in both liver and testis:

3.3.1. Low dose of radiation (0.5 Gy) induced a significant amelioration in liver function:

Fig. (2) showed a full restoration in serum albumin concentration as well as GPT and BChE activities in animals group exposed to low dose (0.5 Gy) gamma radiation after cisplatin treatment as regarded to the normal control value. Besides, a significant increase in both serum albumin and BChE levels was recorded 13% and 20% (*P*<0.05) as compared to the cisplatin treated group respectively. However, serum GPT activity in the previous group showed no significant increase when compared with cisplatin treated one.

According to data in table (2) the effect of low dose of gamma rays (0.5 Gy) showed a full amelioration as in case of both liver GSH and P450 concentration and their levels become more or less as the normal untreated control level. Comparing liver GSH and P450 concentration results in animals group treated with cisplatin before exposing to gamma rays together with cisplatin treated one showed a significant increase calculated11.8% and 24.5% (*P*<0.05) as in table (2).

Table (4) showed the effect of low dose of radiation after cisplatin administration in all tested trace metals concentration that indicated a non significant change in liver Fe, Zn and Cu as compared to either control untreated or cisplatin treated groups.

Table 4: Effects of cisplatin administration and/ or radiation exposure on levels of Fe, Zn and Cu in testis tissue 24 hours post radiation exposure (*n*=6)

Treatment $\bar{X}$ **± SD**	**Control group**	**Cis. Groug**	**IRR group**	**Cis. & IRR group**
Fe (µg/g fresh tissue) $\bar{X}$ ± SD	97.91±16.9	69.61±4.6*	87.09±6.4	96.38±21.2(#)
Zn (µg/g fresh tissue) $\bar{X}$ ± SD	128.8± 23.6	91.57±6.1*	106.6±13.1	116.7±21.3
Cu (µg/g fresh tissue) $\bar{X}$ ± SD	1.885±0.39	1.462±0.12*	1.603±0.26	1.603±0.17

Data are presented as mean (± SD). *Significantly different from the control group (*P*<0.05). (#)Significantly different from the *Cis. group* (*P*<0.05). IRR: Low radiation dose of 0.5 Gy gamma rays, *Cis.*: cisplatin administration.

3.3.2. Low dose of radiation (0.5 Gy) and its effect on the testis tissue after cisplatin treatment:

Exposing cisplatin treated animals to low dose of gamma radiation (0.5 Gy) caused a non- significant decrease in serum FSH activity as compared to the normal control level, which means a significant decrease (-24%, *P*<0.05) as compared to the cisplatin treated animals only (Fig. 2). In addition, the effect of whole body gamma radiation (0.5 Gy) on the animals treated with cisplatin only caused a significant increase in testis GSH content comparing to the cisplatin treated group.

This increase was calculated 42% (Table 2) haunt with a non significant change when compared to the normal untreated group, while the concentration of P450 showed no significant change comparing to normal control levels accompanied by a significant increase (68%, $P<0.05$) with regards to cisplatin treated group.

In case of trace metals concentration, testis Fe, Zn as well as Cu level was estimated in the group of animals exposing to low dose of gamma radiation (0.5 Gy) after cisplatin injection as in table (3). A significant change in the level of testisFe, Zn and Cu in this group was recorded when compared with either normal untreated control group or cisplatin treated one.

4. DISCUSSION

Cisplatin is the most widely used antitumor drug especially in the treatment for testicular cancer, but its usage is limited by toxic effects on the reproductive system [32]. Testicular damage induced by cisplatin treatment was characterized by significant decreases in plasma testosterone level [33] that may be due to the increase in FSH level as in the current work, since according to Sasson and his colleagues the gonadal failure caused by cisplatin treatment, leads to high levels of FSH and LH [34].

Once cisplatin has been administered in the body, it rapidly diffuses into tissues and is highly bound to plasma albumin and other protein [35]. This may explain why cisplatin caused a decrease in serum albumin in the present work. In addition, the decrease in both serum albumin concentration as well as butyryle cholinesterase with the increase in serum GPT activities are further evidence that anti-tumoral treatment causes liver damage [36]. According to the observation of Cepeda and his colleagues**,** there is a direct connection between the cellular concentrations of copper and platinum recommended an active transport for cisplatin through copper transporter, which reduce the uptake of each other that indicated as a significant decrease in testicular copper levels together with Zn and iron that may be due to cisplatin treatment induced oxidative stress in testis [2]. Consequently, increase in lipid peroxidation (LPO) and depletion of enzymes such as superoxide dismutase following cisplatin treatment in testis and liver clearly demonstrated failure of their antioxidant defence system [33]. It may be noted that previous investigators reported the reduction of GSH levels leads to elevation of LPO [37 & 38]. Therefore, depletion of GSH as in the present results might be inducing oxidative stress by increasing the free radical generation leading to cell death in both tested organs.

Cytochrome p450 is the catalyst enzymes that are essential for the metabolism of many medications. Suppression of P450 may be due to the consequence of inflammatory processes and can result in increased clinical toxicity of drugs with a low therapeutic index [39]. Down regulation of testicular cytochrome P450 reported by Waxman and Change and Masubuchi and his colleagues and also indicated in the present results [40 & 41]. However, exposing to low radiation dose attenuated the level of P450 after cisplatin treatments. In addition, evidence reported by Oetari and his colleagues indicates inactivation of glutathione- S- transferase accompanied by glutathione depletion and inhibition of cytochrome p450 in liver [42]. Interestingly, the cytochrome p450 and GSH content in both liver and testis showed a significant inhibition than the control group in our current study. Moreover, the active site of cytochrome p450 contain iron heme center [43], so as one could explain the decrease in both cytochrome p450 and iron in testis as in present work.

Cisplatin augments the antitumor effect of a cytotoxic T-lymphocyte-mediated immunotherapy strategy, resulting in a higher cure rate. This effect is associated with the enhanced ability of cytotoxic T lymphocytes to lyse tumor cells [44]. It seems that functional activity of lymphocytes decreased at the stage of well-developed tumor, which promoted inhibition of the lymphocyte defense properties. Cisplatin did not modify the structure and functions of lymphocytes and presumably improved their energy status [45]. So to overcome the above problems and as the evidence of Murphy and Morton [22], exposing to low radiation dose can be used as a synergistic agent with cisplatin for cancer treatment since low radiation dose administration to entire body increased the action of the protective process in living organism including the overproduction lymphocytes that significantly prevented or impaired tumor growth. Besides, an interesting observation remarked that sign of glutathione (GSH) protects against cisplatin cytotoxicity [46]. Of note is that the GSH administration achieved a superior response rate despite having a larger average tumor burden during chemotherapy [47]. According to current study animals exposed to low radiation dose alone (0.5 Gy) showed a significant increase in GSH content in both liver and testis tissue, which may explain the antioxidant effects of low radiation dose against cisplatin induced oxidative stress. These antioxidants did not interfere with chemotherapeutic effects - and mitigated cancer treatment toxicity [48].

5. CONCLUSION

In conclusion, the obtainable results proved that low radiation dose have the existence as an antioxidant and antitumor agents which may be useful to use as a synergistic agents with the chemotherapeutic drug or even in case of tumors chemo-radiotherapy. Further studies should be needed to address this issue in different biochemical views.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTEREST

Authors have declared that no competing interests exist.

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