# Evaluation of Protective Effect of Ozone Against Acute Liver Tissue Injury Induced by Irradiation in Rats

## Sıçanlarda Radyasyona Bağlı Akut Karaciğer Hasarına Karşı Ozonun Koruyucu Etkisinin Araştırılması

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**ABSTRACT Objective:** The aim of this study was to investigate radioprotective effect of ozone against liver tissue damage induced by total body irradiation in rats. **Material and Methods:** Thirty-two male Wistar rats were included in this study and divided into four equal groups: Group 1: received no treatment (control arm), group 2: only irradiated rats (RT), group 3: received ozone before and after irradiation ($O_3$RT), group 4: received ozone after irradiation (RT$O_3$). The rat weights were recorded at the beginning and the end of the experiment in each group. The ozone was administered for 4 days before irradiation and continued for 3 days after irradiation once daily by rectal application. On the 4$^{th}$ day, rats in group 2, 3 and 4 received a single whole-dose of 8 Gy. At the end of 7$^{th}$ day, blood samples were taken from the rats under ether anesthesia and then the rats were sacrificed. Liver tissues samples were taken. Oxidative stress markers, some biochemical parameters, and peripheral blood cells were measured and analyzed. **Results:** Decreases in white blood cell, neutrophile, thrombocyte counts and ALP level were detected while significiant increases AST, ALT, LDH, total bilirubin and direct bilirubin levels in radiotherapy group when compared to control group ($p< 0.05$). In addition, RT group was characterized with a significant in tissue and plasma MDA, and lower tissue CAT activities. In the groups that were administered ozone in association with irradiation, improvement was observed in some oxidative parameters, peripheral blood cells and certain other biochemical parameters. Biochemical parameters, blood cells and some oxidative parameters were markedly improved by pre-treatment groups. But pre- and post-treatment with ozone caused normalization in these parameters when compared with the radiotherapy group. **Conclusion:** The results suggest that ozone offer a remarkable protective effect against radiation-induced of liver injury.

**Key Words:** Radiation injuries, experimental; radiation protection

**ÖZET Amaç:** Bu çalışmanın amacı, sıçanlarda tüm vücut ışınlamasına bağlı oluşan akut karaciğer hasarına karşı ozonun koruyucu rolü olup olmadığının araştırılmasıdır. **Gereç ve Yöntemler:** Çalışmaya 32 adet Wistar Albino dişi sıçan alındı. Araştırmada hayvanlar 4 gruba ayrıldı; Grup 1: tedavi almayan kontrol grubu (K), grup 2: sadece ışın tedavisi verilen (RT); grup 3: ışın tedavisi öncesi ve sonrası ozon verilen ($O_3$RT); grup 4: ışın tedavisinden sonra ozon verilen grup (RT$O_3$) olarak belirlendi. Hayvanlar deney başlangıcında ve sonunda tartıldı. Deneyin 4. gününde grup 2, 3 ve 4'e 8 Gy tüm vücut ışınlaması yapıldı. Ozon, ışın tedavisinden dört gün önce ve üç gün sonra rektal yolla, günde bir defa verildi. Deneyin 7. gününde sıçanlar eter anestezisi altında iken kan numuneleri alındıktan sonra sakrifiye edildi. Karaciğer doku örnekleri alındı. Kanda toplam kan sayımı, bazı biyokimyasal paremetreler; plazma ve karaciğer dokusunda ise oksidatif stres parametrelerin analizleri yapıldı. **Bulgular:** Radyoterapi grubu, kontrol grubu ile kıyaslandığında trombosit, beyaz küre, nötrofil sayısı ve ALP düzeylerinde anlamlı düşüş olurken; biyokimyasal paremetrelerden AST, ALT, LDH, total bilirubin ve direkt bilirubin düzeylerinde istatistiksel olarak anlamlı artış görüldü ($p< 0.05$). RT grubunda doku ve plazmadaki MDA seviyesinde artış görülürken; karaciğer dokusunda CAT aktivitesinde önemli düşme ($p< 0.05$) gözlendi. Radyoterapi ile birlikte ozon verilen grup, RT grubu ile kıyaslandığında bu parametrelerde normalizasyon görüldü ($p< 0.05$). Radyoterapi öncesi ve sonrası ozon başlanılması, radyoterapi sonrası verilen ozon tedavisinden daha etkili bulundu. **Sonuç:** Yaptığımız çalışmada ozonun akut karaciğer hasarını engellemede etkili olduğu görüldü.

**Anahtar Kelimeler:** Radyasyon hasarları, deneysel; radyasyondan korunma



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Radiotherapy is used effectively in a wide variety of cancer. The main aim of radiation treatment is to apply an effective dose of ionizing radiation to eliminate tumor cells in a well-defined the tumor volume, with the minimum side effect to surrounding healthy tissue. Acute and delayed side effects on the normal tissues in the treatment field is the essential limiting factor for radiation dose that can be administered the tumor. Despite advances in modern RT technology, some acute complications have not been ameliorated in cancer patients after radiotherapy.[1-4]

Radiation is an oxidative stress for tissues. When tissue is exposed to ionizing radiation, decomposition occurs through a variety of reactive oxygen species (ROS) such as the superoxide radical, the hydrogen peroxide, and the hydroxil radical are overproduced and arise from oxidative stress. A balance between the generation of ROS and inactivation of ROS is led by the antioxidant system in organisms. The imbalance between ROS and antioxidant defense mechanism is led to injury within cellular membrane and/or intracellular molecules. ROS seems to be a major mechanism of tissue injury. One of the indices of the oxidative damage is the MDA which considered being the most significant indicator of membrane lipid peroxidation arising from the interaction of ROS with cellular membranes. All cells are equipped with protective enzymes [such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) etc.]; increased oxidative stress in cells stemming from ionizing radiation may overwhelm the protective systems, leading to the cell injury.[3-5]

Several drugs and substances may reduce the side effect of radiation. These are known as radiation protectors or dose-modifying compounds and administered short time intervals (within 30 minutes) before radiotherapy. Therapeutic doses of radioprotectors often cause side effects in patients which limit the widespread use of these agents. Therefore there is an unmet need for better and more potent compounds, especially to boost antioxidant defense.[1-3,6] One of them may be ozone in future research. Ozone has an antioxidant and free radical scavenger. The possibility that ozone could induce a useful adaptation to oxidative stress was described by Bocci.[7,8]

Ozone therapy is not officially allowed in many countries, but private medical services are using this therapy worldwide. The main ozone therapy mechanism of action is based on an extremely transitory and regulated oxidative stress imposed ex vivo. At the same time, ozone therapy acts as an efficient oxidative stress regulator stimulating the antioxidant system of the cell. Evidence that antioxidant enzymes, nitric oxide pathways, and other subcelluler activities could be modulated by low ozone doses is now proven and could support the surprising effects of ozone in many pathological conditions.[7-10] Ozone has been reported to have therapeutic and beneficial effects in several human diseases such as various neoplastic and inflammatory states, infections, heart ischemia chronic ulcers and others.[8-16]

Several studies demonstrated that ozone protected intact tissues against side effect of chemotherapy and radiotherapy, without inhibiting efficacy. These researches have reported that controlled ozone administration may promote an oxidative preconditioning or adaptation to oxidative stress that, in turn, increases antioxidant endogenous systems protecting against tissue damage.[11,14-17]

The aims of the present study were to determine whether the treatment with ozone might reduce acute liver side effect induced by irradiation and if it is so, to elucidate the role of lipid peroxidation and some antioxidant enzyme such as CAT in the protective effect.

## MATERIAL AND METHODS

### ANIMALS AND WEIGHTS OF RATS

This study was conducted at Erciyes University Experimental and Clinical Research Center and approved by the Ethics Committee of Erciyes University. All experimental procedure was conducted in accordance with the Guide to the Care and Use of Laboratory Animals. Thirty two male Wistar rats were included. They were 12 weeks-old and weighed 210-290 grams. Animals were fed

with a standard commercial diet and water ad libitum. All rats were kept in the same room with standardized air conditioning (23°C and 50-60% humidity) in 12 hours light/dark cycle. The rat weights were obtained at the beginning and the end of the experiment with in each group.

### TREATMENT CATEGORIES

Animals were divided into four groups of each including eight animals:

Group 1: Received no treatment (control),

Group 2: Only irradiated rats (RT),

Group 3: Received ozone starting from 4 days before radiation and continued for 3 days after irradiation ($O_3$RT),

Group 4: Received ozone 3 days after irradiation (RT$O_3$).

### IRRADIATION

Prior the irradiation, animals were anesthetized by subcutan injection of 30 mg/kg ketamine hydrochloride (Ketalar®; Parke Davis, İstanbul, Turkey) and 5 mg/kg xylasine (Rompun® Bayer, İstanbul, Turkey), total irradiation field was 25 x 15 x 5 cm, firmly fixed in a wooden box and four rats were irradiated simultaneously. Rats were subjected to whole body gamma-irradiation of a single dose of 8 Gy on the fourth day from the Cobalt–60 teletherapy machine (Theratron 780 C, Canada). The dose was calculated as Dmax dose at 2.5 cm given depth for SSD 80 cm (the dose rate was 3.03 cGy/MU). Control group was subjected to the same procedure but was not irradiated. Animals were returned to their home cages after irradiation.

### APPLİCATİON OF OZONE

Ozone was generated by Ozonosan α-Plus equipment manufactured by Varices & Ozon Clinic in Kayseri. Ozone was obtained from medical-grade oxygen by means of a silent electric discharge, representing only about 0.3% of the gas mixture (i.e. 0.3% ozone and 0.97% medical oxygen mixture). The ozone concentration was measured by using a UV spectrophotometer at 254 nm. The ozone dose was the product of the ozone concentration expressed as μg/mL, by the gas mixture volume (L). Group 3 and 4 received ozone treatments, 2.5-2.6 mL ozone in concentration 20 μg/mL once per day performed with a suitable polythene cannula connected to single-use silicon treated polypropylene syringes (ozone-resistant) by rectal insufflations. According to the results of the protection studies such as in the prevention of oxidative stress, ozone was selected to determine its low dose (ranging from 10-20 μg/ mL) activity. By knowing the body weight of the rat, the ozone dose and schedule were proved to be optimal in previous studies.[7,8,11,13] Rats were observed daily for the development of any signs of toxicity throughout the treatment period.

### COLLECTION OF BLOOD AND LIVER SAMPLES

At the end of experiment period, the animals were with light ether anaesthesia. Immediately, blood samples were obtained by intracardiac puncture into heparin-coated and dry tubes (3.5 mL of blood). Subsequently, the liver tissue was taken for oxidative stress markers studies.

Blood samples were centrifuged at 3000 g for 10 min for the separation of serum and plasma. Serum was used for the analyses of biochemical parameters. Plasma was obtained MDA levels. The other blood samples were collected in heparinized tubes and evaluated for hemoglobin, white blood cell, neutrophil, and platelet counts. The liver tissues were washed with ice-cold isotonic saline, blotted between two filter papers and in ice-cold 0.1 M potassium phosphate buffer, Ph 7.5 isotonic saline on ice for 10 s in the first speed level. The homogenate was centrifuged at 10.000 rpm for 60 min at 4°C. These supernatants were used for the analyses of antioxidant enzymes. The serum, plasma and supernatant were stored at-70°C until biochemical and antioxidants enzymes analysis.

### MEASUREMENT OF BIOCHEMICAL PARAMETERS IN SERUM

The measurement of serum biochemical parameters total bilirubin, direct bilirubin, gamma glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine

aminotransferase (ALT), and alkaline phosphatase (ALP) levels/activities were performed using a Konelab 60i model auto-analyser and Konelab Thermo Clinical Labsystems, Finland) label kits were used to assess.

### MEASUREMENT OF PERIPHERAL BLOOD CELLS

The other blood samples were collected in heparinized tubes and evaluated for hemoglobin, white blood cell, neutrophil, and platelet counts were measured using Johnson & Johnson label kits and determined spectrophotometrically using an automated analyzer.

### MEASUREMENT OF OXIDATIVE STRESS MARKERS OF PLASMA AND LIVER TISSUE

MDA plasma levels were measured spectrophotometrically at 532 nm as thiobarbituric acid reactants, according to Yoshiaka et al.[18] Calculations were expressed as nanomole per mililitre (nmol/mL) of plasma.

Tissue MDA and CAT measurements were performed using a spectrophotometer. Tissue MDA was measured at according to Ohkawa.[19] MDA activity was also expressed as nanomole per miligram (nmol/mg) protein of liver sediment. CAT acitivity was measured in tissue as previously described by Luck.[20] The principles of this method was based on the measurement of the decrease in absorbance at 240 nm caused by the decomposition of hydrogen peroxide, present in an appropriate buffer, by the enzyme catalese existing in the sample. CAT results were expressed as unit per miligram (U/mg-protein) of liver sediment.

### STATISTICAL ANALYSIS

Statistical analyses were performed with Statistical Package for Social Sciences 15.0 (SPSS Inc, Chicago, IL, USA). All values were expressed as mean± standard error (SEM). First, the outlier's preliminary tests for detection of error values were used. Afterward, the one-way analysis of variance (ANOVA) then homogeneity variance test (Tukey HSD) was applied. In addition, Duncan's multiple range test for the comparison of two groups were done. The level of statistical significance was set at $p< 0.05$.

## RESULTS

### WEIGHT CHANGES

Weights of rats are given in Table 1. The weight at the end of seventh day was compared to the original weight within each group. Control animals did not gain any weight during therapy. There was no significiant change in the weight of control group ($p> 0.05$). However, there was a statistically significant weight loss in group 2, 3 and 4 ($p< 0.05$). In RT group, radiation caused statistically significant decreases in the weight compared to starting weights ($p< 0.05$). The weight changes observed in the $RTO_3$ and $O_3RT$ group was compared to original weight and there were also significant differences ($p< 0.05$).

### BIOCHEMICAL FINDINGS

The effect of irradiation and ozone on serum biochemical parameters are depicted in Table 2 and 3. Liver functional parameters of rats were significiantly different among groups.

**TABLE 1:** Weight of the rats characteristics belonging to each group. The rat weights were obtained at the beginning of the experiment and the end of the experiment with in each group (n: 8).

| Groups | Weight 1 (Mean ± SD) | Weight 2 (Mean ± SD) | P< 0.05 |
|---|---|---|---|
| Group 1 | 272.75 ± 14.95 | 271.62 ± 13.96 | NS |
| Group 2 | 266.12 ± 11.01 | 249.50 ± 14.41 | * |
| Group 3 | 271.50 ± 9.94 | 254.62 ± 14.81 | * |
| Group 4 | 268.00 ± 25.30 | 248.37 ± 21.53 | * |

Results have been represent as mean±SD (Mean ± Standard Deviation); NS, no significant; *, Statistically significant ($p< 0.05$); Group 1, control; Group 2, RT; Group 3, O3RT; Group 4, $RTO_3$.

**TABLE 2:** Serum AST, ALT, ALP and GGT activities of the control and trial group.

| Groups | AST (U/L) (Mean ± SD) | ALT (U/L) (Mean ± SD) | ALP (U/L) (Mean ± SD) | GGT (U/L) (Mean ± SD) |
|---|---|---|---|---|
| Groups 1 | 160.87 ± 32.04 | 37.12 ± 5.25 | 182.87 ± 39.16 | 1.25 ± 0.88 |
| Groups 2 | 191.12 ± 47.64 | 52.75 ± 7.06* | 150.90 ± 45.61 | 2.0 ± 0.53 |
| Groups 3 | 78.37 ± 12.51* | 27.25 ± 1.66* | 88.87 ± 23.91* | 2.00 ± 0.00 |
| Groups 4 | 111.87 ± 39.74* | 29.50 ± 3.16* | 87.00 ± 7.92* | 1.87 ± 0.64 |

Results have been represent as Mean±SD (Mean ± Standard Deviation); *, Statistically significant ($p< 0.05$); Group 1, control; Group 2, RT; Group 3, O3RT; Group 4, $RTO_3$.

**TABLE 3:** Serum total bilirubin, direct bilirubin, and LDH levels of the control and trial groups.

| Groups | Total Bilirubin (mg/dL)(Mean ± SD) | Direct Bilirubin (mg/dL)(Mean ± SD) | LDH (U/L)(Mean ± SD) |
|---|---|---|---|
| Group 1 | 0.11 ± 0.03 | 0.01 ± 0.01 | 1568.75 ± 189.96 |
| Group 2 | 0.15 ± 0.01 | 0.03 ± 0.01 | 1828.87 ± 92.74* |
| Group 3 | 0.11 ± 0.02 | 0.01 ± 0.01* | 587.37 ± 189.69* |
| Group 4 | 0.10 ± 0.02* | 0.01 ± 0.01 | 880.25 ± 189.51* |

Results have been represent as Mean±SD (Mean ± Standard Deviation); *, Statistically significant (p< 0.05); Group 1, control; Group 2, RT; Group 3, O3RT; Group 4, $RTO_3$.

Serum AST level: The serum AST level of the group that was administered irradiation alone was determined to be elevated when compared to the control group. In comparison to the control group, values pertaining to the group that received ozone were determined to be significantly reduced.

Serum ALT level: A statistically significant difference was determined to exist among the groups. The serum ALT level of the group that was administered irradiation alone was determined to be elevated. This increased found to be significant, in comparison to the control group. Furthermore, a statistically significant decrease was determined in the enzyme activity of the group that received ozone plus irradiation.

Serum ALP level: Statistically significant differences were determined in the group 3 and 4, in comparison to the control group. Compared to the control, these differences were determined to be in the form of decrease in the experimental groups.

Serum GGT level: The GGT level of the RT group and that received $O_3RT$ were determined to be similar. Furthermore, values pertaining to the group that was administered ozone in association with irradiation and irradiation alone group were determined to be higher than those of the control group.

Serum LDH level: The serum LDH level of the group that was irradiated alone was determined to be elevated when compared to the control group. This increase was found to be significant, in comparison to the control group. On the other hand, values pertaining to the group that received ozone in association with irradiation displayed significant decrease and were determined to fall below the values of the control groups.

Serum total bilirubin and direct bilirubin: The serum total bilirubin and direct bilirubin level of the group that was received irradiation alone was determined to be increased. When compared $RTO_3$ this decrease was determined to be significant.

## MEASUREMENT OF BLOOD CHEMISTRY

White blood cell, hemoglobin, neutrophil, and thrombocyte counts are shown in Table 4. After irradiation, a significant drop in white blood cell and neutrophil was seen in all irradiated animals in comparison to control groups. A statistically significant difference was found when control groups were compared to all groups. Rats that had been treated with ozone after irradiation demonstrated significiantly higher neutrophil and white blood cell levels when compared with rats subjected to irradiation alone. No significant changes were noticed in hemoglobin levels counts after irradiation. Rats subjected to radiation-induced injury showed decreased thrombocyte counts in serum when

**TABLE 4:** Hemoglobin, white blood cell, neutrophil, and trombocyte of the control and trial groups.

| Groups | Hemoglobin (g/dL)(Mean ± SD) | White blood cell ($10^3/mm^3$)(Mean ± SD) | Neutropfil ($10^6/mm^3$) (Mean ± SD) | Trombocyte ($10^3/mm^3$)( Mean ± SD) |
|---|---|---|---|---|
| Group 1 | 15.03 ± 1.88 | 7.51 ± 1.78 | 1.14 ± 0.62 | 515.87 ± 177.42 |
| Group 2 | 14.88 ± 0.93 | 3.20 ± 0.57* | 0.45 ± 0.71* | 388.87 ± 59.08 |
| Group 3 | 15.47 ± 0.96 | 4.56 ± 0.81* | 0.51 ± 0.26* | 573.12 ± 132.68* |
| Group 4 | 15.37 ± 0.75 | 5.07 ± 0.98* | 0.63 ± 0.35 | 573.12 ± 132.68* |

Results have been represent as Mean±SD (Mean ± Standard Deviation); *, Statistically significant (p< 0.05); Group 1, control; Group 2, RT; Group 3, O3RT; Group 4, $RTO_3$.

compared to control group. That decrease was found to be significant, in comparison to the control group. Ozone appeared to have synergistic effect in increasing thrombocyte counts ($p< 0.05$).

### ANTIOXIDANT FINDINGS

As shown in Table 5, MDA content as an index of lipid peroxidation was significantly higher in rats subjected to irradiation induced when compared to control group. This increase induced by irradiation was significiantly attenuated by ozone. CAT in liver tissue decreased strongly in rats subjected to irradiation injury compared with control. This decrease was found to be significant. On the other hand, values pertaining to the group that received ozone displayed significant increase. Ozone appeared to have synergistic effect in further inhibiting the decrease of CAT.

## DISCUSSION

Radiation therapy is widely used to treat and prevent the recurrence in many tumors, but acute and delayed side effects on the normal tissues limit the effectiveness of radiotherapy. Tissue toxicity is the major limiting factor of radiation therapy, arising mainly from oxidative damage. Ionizing radiation has several mechanisms that induce cellular damage. The cellular biomolecules are altered free oxygen radical production. Cell injuries are caused by free oxygen radicals who contribute to the tissue damage. As lipid peroxidation is the main pathway for tissue radical damage, blocking of this pathway appears to be an attractive strategy to protect the tissue from reactive oxygen species mediated damage.[1-5] Many of the drugs and/or substances are used to reduce the side effect of radiation. They increase the efficiency of the therapy without any side effects on the normal cells.[1-3,6] One of them may be ozone in future research.

Ozone is normally present as very a reactive gas. Some people, including a number of doctors and biochemist, believe ozone remarkable healing properties. Others have argued that it is nonscientific and has no proven benefits. For many years ozone medical value or non-value has been the subject of controversial and emotional debate. Ozone therapy is a well established alternative and complementary therapy in most mainland European countries where health authorities have tolerated its practice.[7-10]

Clinical studies on the effects of ozone on the tissues have shown that ozone can be either therapeutic or toxic depending upon its concentration and cumulative dose. Ozone can exert therapeutic effects without toxicity which have so-called "therapeutic window" that is the range of ozone concentrations (expressed as mg/mL of gas per mL of blood). The range of ozone concentrations is surprisingly wide. It changes in a range of 10-15 mg/mL as a minimum and 80 mg/mL as a maximum. Most recently published scientific data emphasised and focused on the use of low ozone doses, as a pro-drug, could be modulated antioxidant enzymes and other subcellular activities. The threshold level varies in the range between 15 and 20 mg/mL, depending upon the individual antioxidant capacity. Several techniques of administering ozone (i.e. including topically by intraarterial, local irrigation, intravenous, intramuscular, subcutaneous, intraarticular, and rectal) are being promoted as a treatment for disease.[7-17]

**TABLE 5:** Plasma and tissue MDA, tissue CAT of the control and trial groups.

| Groups | Plasma MDA(nmol/ml)(Mean ± SD) | Tissue CAT(U/mg-protein)(Mean ± SD) | Tissue MDA(nmol/mg protein)(Mean ± SD) |
|---|---|---|---|
| Group 1 | 7.84 ± 0.86 | 0.16 ± 0.01 | 0.15 ± 0.01 |
| Group 2 | 9.21 ± 0.45* | 0.11 ± 0.01* | 0.25 ± 0.01* |
| Group 3 | 7.45 ± 0.26* | 0.27 ± 0.01* | 0.13 ± 0.01* |
| Group 4 | 8.48 ± 0.71* | 0.22 ± 0.01* | 0.15 ± 0.01 |

Results have been represent as Mean±SD (Mean ± Standard Deviation); *, Statistically significant ($p< 0.05$); Group 1, control; Group 2, RT; Group 3, O3RT; Group 4, $RTO_3$.

Systemic ozone therapy has been proven to be effective to modulate immune system by inducing the production of cytokines from peripheral blood mononuclear cells and to regulate the oxidative stress by inducing an increase of cellular antioxidant system. This mechanism has been ascribed to ozone therapy's protection against free radical damage of heart, prevention of renal and hepatic disorders.[9,12]

Small amount of research of ozone in the treatment of cancer has been done. In 1980, Sweet et al discovered ozone inhibited growth of lung, breast and uterine cancer cells in a dose dependent manner while healthy tissues were not damaged by ozone.[16] Recent researchers have shown ozone therapy can improve oxygenation in hypoxic tumors. In 2005 Oxford University reported of a Spanish cancer research institutes on human trial of ozone therapy. Incurable head and neck tumors (involving 19 patients) received tegafur and radiotherapy, plus either chemotherapy (12 patients) or ozone therapy (7 patients). Those received ozone intravenously during radiotherapy where on average 10 years older their tumors significiantly more abundant and progressed than the chemotherapy group. But on average the ozone group survived slightly longer than those receiving chemotherapy. They conclude these results warrant further researcher of ozone as a treatment of cancer.[22]

Currently, emerging evidence from a few research suggested that ozone as an antioxidant, anti-inflammatory and a cytoprotective agent could be useful in treatment the debilitating side effects of radiotherapy and/or chemotherapy.[8,10,11,14,15,23] Gonzalez et al. following the administration of Cisplatin (i.e. anticancer drug) which is known to cause acute kidney damage, and additionally ozone, have demonstrated ozone to exhibit protective effect extreme toxicity and acute damage in the kidneys of rats. Ozone may reduce chemotoxicity by inducing the antioxidant response.[11] So that, these researchers reports that ozone may be useful in treating the debilitating side effects of cancer treatment.

A radiation-induced injury has been successfully treated with ozone application.[14,22,24] Clavo et al. have reported that involvement of ozone therapy diminished the incidence and degree of radiotherapy side effects, improvement of the quality of life, immunological parameters, and significally increase the activity of antioxidant defense system.[22] Hernuss et al have reported that the potential improvement in the effects of radiotherapy by ozone therapy in advanced gynecological tumors.[24]

Bocci has reported that most of the patients with metastatic cancer resistant to radiotherapy and chemotherapy report a striking improvement of the quality of the life with prolonged (twice weekly for months) ozone treatments.[15,17] Zanker et al have reported that ozone has a potential positive effect during chemo-radiotherapy as being due to the effects on ischemia-hypoxia.[25]

Any study involved in the effects of ozone on irradiation-induced liver damage has not been reported yet. Ozone is a simple and harmless method that provides a new tool to protect organs from radiation-induced liver injury. However, there is a limited amount of information in the literature regarding liver ozone therapy. In a previous study, Peralte et al demonstrated that ozone protected rats against liver injury. In addition, ozone has protective effects on carbon tetrachloride and hepatic ischemia-reperfusion induced liver damage in rats with a probable mechanism by stimulation of antioxidant endogenous systems.[12]

The results of this study clearly show a significant reduction in radiation induced liver damage in rats treated with ozone (20 μg/mL) used as reference drug. In addition, it has been demonstrated that this protection is dose and schedule dependent.[7,8,11,13] The basis of the schedule dependency-i.e., the observation that ozone protection is greatest when ozone is the drug in which peak levels of ozone increased with repeated administration is given before irradiation. Our study showed that whole-body irradiation caused oxidative tissue damage in the liver of the rats and also caused a marked decrease in the weight. Ozone was able to protect against the acute irradiation-induced weight loss.

In our study, ozone protected total body irradiated rats against radiation induced myelosup-

pression. This finding is in agreement with recent studies demonstrating that overexpression of antioxidant (for example $Mn^{2+}$ superoxid dismutase) protects haemopoietic cells following irradiation by inhibition of radiation induced apopitosis.[9,12,21]

ALT, AST, total bilirubin, direct bilirubin and GGT were increased in liver damage but ALT was an enzyme that spesific to liver damage. In the present study, when compared to the control group, the ozone decreased in the serum ALT, AST, total bilirubin, direct bilirubin and GGT levels of rats irradiated; demonstrates radiation induced liver damage to have developed. LDH activities were markers of generalized tissue damage. LDH levels in RT group were different than the control group. Furthermore, when compared to RT group, the LDH levels that received ozone were demonstrated to have significiantly decrease than the RT group. The most relevant articles recently published on the effects induced by low doses on various biochemical pathways linked to common and rare human pathologies.[4,12,17,26,27]

After 7 sessions of treatment (i.e. $O_3$RT group), blood analysis repeated and compared with the first one ($RTO_3$ group), in order to determine the individual specific changes after ozone treatment. There was a slight decrease in the prooxidant state, given by a high level of TBARS and a small increase CAT. After 7 treatment sessions, ozone increased significantly in the antioxidant parameters CAT. The actions of ozone could exert protective effects, by upregulation of the antioxidant system and the reduction in reactive oxygen species. It might propose that ozone is an activator of antioxidant enzymes. Ozone therapy had a protective effect on liver tissue. As it was reported before, the ozone therapy effect, with the stimulation of the antioxidant defense system, protected the tissues against the oxidative stress shown in ionizing radiation.[7-11,23,24] The efficacy of ozone was a protector which has been used with good results. For that reason, controlled ozone administration would promote an oxidative preconditioning preventing the tissues damage by free radicals. However, combined use of precondition and postcondition ozone plus irradiation interestingly exhibited an additive radioprotective effect. Thus, we could speculate that the inability of the postcondition ozone to provite to protection may indicate that they were sufficiently and effectively available to damaged liver tissues. In any event, our results indicated that postcondition ozone may partially protect against irradiation-induced injury to the liver tissue.

Conclusion: Our results suggest that repeated administration of ozone at low doses might play a role in the control of radiation induced liver injury with no side effects. When ozone was used in therapeutic fashion it may promote an oxidative preconditioning through the increase and preservation of antioxidant endogenous systems, a novel property that is hardly achievable with another therapeutic approach.

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