

## RESEARCH ARTICLE

Editorial Process: Submission:01/16/2025 Acceptance:09/01/2025 Published:09/13/2025

# The Effect of Resistance Training on Serum Levels of Lipid Profile and Liver Enzymes in Male Rat Undergoing Radiotherapy

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### Abstract

**Background:** This study investigated the effects of resistance training on lipid profiles and liver enzyme levels in male rats exposed to x-ray radiation. **Methods:** In this experimental study, 24 eight-week-old Sprague Dawley rats (200-250g) were randomly assigned into four groups: healthy control, irradiated control, healthy resistance training, and irradiated resistance training. Irradiation was induced at a dose of 4 Gy on the whole body. The resistance training protocol was performed for ten weeks. Finally, blood serum was used to assess AST, ALT, ALP and testosterone, and lipid profile. Data were analyzed using ANOVA and Tukey's post hoc test using SPSS-16 software at a 0.05 level of significance. **Results:** The results showed that radiation significantly increased serum levels of AST ( $169.3 \pm 2.4$  vs  $131.1 \pm 2.4$ ,  $P=0.001$ ), ALT ( $75.6 \pm 3.6$  vs  $65.1 \pm 3.1$ ,  $P=0.002$ ), and ALP ( $503.5 \pm 83.1$  vs  $370.3 \pm 66.9$ ,  $P=0.034$ ) between radiation control and healthy control groups. Also, no significant difference was observed between serum levels of AST ( $P=1.000$ ) ALT ( $P=0.942$ ), and ALP ( $P=0.925$ ) in radiation resistance training and the healthy control groups. In addition, a significant increase was observed between radiation control and radiation resistance training groups in lipid profiles ( $P \geq 0.05$ ). Also, no significant difference was observed between serum levels of TG ( $P=0.212$ ) LDL-C ( $P=0.931$ ), and HDL-C ( $P=0.992$ ) in radiation resistance training and the healthy control groups. **Conclusions:** Our findings suggest that resistance training may improve lipid profiles and liver enzyme levels in male rats exposed to x-ray radiation.

**Keywords:** Exercise training- Lipid profile- Liver enzymes- Radiotherapy

*Asian Pac J Cancer Prev*, 26 (9), 3259-3264

### Introduction

Radiotherapy (RT) serves as an effective therapeutic approach for controlling liver cancer metastases. It is particularly beneficial when liver tumors are either too extensive for ablation therapy or ineligible for surgical resection. Recent technical advancements have led to a rapid increase in radiotherapy applications for liver tumors. However, cumulative radiation exposure is associated with liver cirrhosis, presenting a significant challenge in radiation oncology. Hepatic radiation may induce cellular damage, altered biochemical markers, and clinical manifestations of liver failure. This condition, known as radiation-induced liver disease (RILD), typically manifests 4 to 8 weeks post-treatment and is characterized by fatigue, rapid weight gain, and ascites (pathological abdominal fluid accumulation) [1]. In most cases, the disease is permanent or transient, but some patients develop sudden liver failure and treatment-related mortality. Radiotherapy is one of the main treatments that

is sometimes used alone and sometimes in combination with other treatments. Despite the invasion and removal of cancer cells, this treatment also damages normal cells [2]. It has been observed that radiotherapy in patients treated not only kills malignant tumors but also damages normal tissues, central nervous system, gastrointestinal tract, liver, and kidney [3]. The risk of injury depends on the size, number, frequency, volume, duration of treatment, and method of radiotherapy [4].

Radiation-induced liver damage (RILD) represents a life-threatening complication of radiotherapy for primary hepatic malignancies, for which no effective treatment currently exists. Most patients experiencing radiation-induced hepatic injury develop liver failure within a short period. The most effective preventive strategy involves restricting radiation to the minimal effective dose while employing various approaches to mitigate treatment-related adverse effects [5]. Increases in liver enzymes alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT), aspartate aminotransferase

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(AST), and alkaline phosphatase (ALP) are predictors of liver tissue damage. Studies have shown that plasma concentrations of these enzymes are the best indicator for assessing the condition of the liver because with damage to liver cells, their amount in the blood increases [6]. There is currently no non-pharmacological treatment to eliminate radiotherapy-induced liver disease, and methods to minimize its complications appear to be necessary [7]. In the other words, some existing studies show that the patient's physical activity level significantly decreases after the diagnosis of cancer, and even after a period of treatment, they do only a small amount [8]. Studies have shown that resistance training can reduce the serum levels of liver enzymes after a period of resistance training, which seems to be a Benefit aspect of resistance training for diabetics [9].

Overall, the evidence suggests that radiotherapy through mechanisms of dysfunction and damage to the liver tissue of rats exposed to radiation therapy. Alternatively, the main goal of drug treatments is to improve the condition of the liver, which does not seem to be an effective method. Currently available drugs, in addition to high costs, have side effects including hypoglycemia, hepatotoxicity, and dyslipidemia [10], and the only hope to reduce such responses is long-term use and adaptation. Therefore, using low-cost and no side effects methods such as proper physical activity to reduce these side effects can be a good goal. Therefore, the research hypothesis is that participation in exercise programs with resistance training methods by modulating and improving liver enzymes will probably improve inflammation of liver tissue in rats undergoing radiation therapy.

## Materials and Methods

### Experimental animals and protocols

24 eight-week-old male Sprague-Dawley rats (200-250g) were housed in cages in groups of controlled temperature ( $22\pm 2^{\circ}\text{C}$ ) and light/dark (12/12h) conditions with free access to water and rat chow. All experimental procedures complied with the regulations approved by the ministry of public health, medical care, and medical education. The rats were randomly divided into four groups. The groups were treated according to the experimental protocol for 60 days. These groups were as follows: healthy control (HC) (n=6), Radiotherapy control (RC) (n=6), healthy resistance training (HRT) (n=6), and radiotherapy resistance training (RRT) (n=6) groups (Table 2). All experiments were operated at the Medical Science University of Arak.

### Irradiation induction

The animals were treated with a single dose of X radiation of 400 rads (4 Gy). The source of radiation was Elekta Precise used in the Radiotherapy department of the

Khansari Hospital, Arak, Iran. The animals were kept in well-ventilated cages and their movements were restricted. The animals have been exposed to whole-body radiation at a rate of 300 mu/min in a field size of about  $35\text{cm} \times 35\text{cm}$  and a distance of 60 cm from the source.

### Resistance training protocol

The resistance training program consisted of climbing a 1 meters long ladder, set at a 90-degree angle with a weight (resistance) attached to the animals' tails. The length of the ladder required the animals to make 26 dynamic movements per climb. Each exercise session included 3 sets of 4 repetitions, at 2 minutes rest between sets and about 10 seconds rest between repetitions. At the start of the program, the rats were familiarized with how to climb the ladder for 1 week. Resistance applied to the rats was 30% of their total weights in the second week. Each week, some weights were added gradually so that in the final week of the training, the total resistance applied to each animal was 200% of its total weight (Table 1) [11].

### Sampling and analysis of liver enzymes and lipid profile

28 hours after the last exercise session, all of the rats were anesthetized by the injection of chloroform and sacrificed. Blood samples were collected by cardiac puncture (5cc) and centrifuged at 3500rpm for 5 min and the serum samples were stored at  $-80^{\circ}\text{C}$  for future analysis. The serum concentration of Alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglycerides (TG), total cholesterol (TC), and high-density lipoprotein (HDL) were measured enzymatically using commercial kits (Pars Azemoun, Tehran, Iran) by a spectrophotometer (JENWAY 6505, Europe Union). The serum low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) were calculated by the Friedewald formula (24) as follows:  $\text{VLDL}(\text{mg/dL}) = \text{TC}(\text{mg/dL}) - \text{HDL}(\text{mg/dL}) - \text{TG}(\text{mg/dL})/5$  and  $\text{VLDL} = \text{TG}/5$ . It is noteworthy that the initial and final FBG, as well as body weight of all rats, was determined at a 12-hour fasting condition.

### Statistical Analysis

A Shapiro-Wilk test was applied to determine the normality of the distribution of measures that were found to be normally distributed. Then a Leven test indicated that the variances were homogeneous. A one-way analysis of variance (ANOVA) was performed to determine the presence of differences among the groups. Significant differences between groups were identified using the post hoc Tukey test for data with equal variance and post hoc Dunnett's T3 test for data with unequal variance. The data were expressed as means and standard error mean (SEM) of two replicates for six rats in each group. A P value  $< 0.05$  was considered statistically significant. All statistical analyses were performed with GraphPad Prism software (Version 6.00).

Table 1. Resistance Training in 3 Sets of 4 Repetitions Load (Percent of Body Weight)

Week	1	2	3	4	5	6	7	8	9	10
Load	Familiarity	30%	80%	100%	120%	140%	160%	180%	190%	200%

Table 2. Means ( $\pm$ SD) General Characteristics of Rats

Groups	Body Weight (g)		Liver weight (g)	Liver volume (mm <sup>3</sup> )
	Pre test	Post-test	Final	Final
HC	228.8 ( $\pm$ 30)	275.3 ( $\pm$ 22)	9.64 ( $\pm$ 1.58)	893 ( $\pm$ 141)
RC	265.6 ( $\pm$ 44)	281.3 ( $\pm$ 37)	9.99 ( $\pm$ 1.27)	911 ( $\pm$ 121)
RRT	288.1 ( $\pm$ 35)	318.1 ( $\pm$ 28)	10.66 ( $\pm$ 1.65)	989 ( $\pm$ 146)
HRT	244.1 ( $\pm$ 15)	389.6 ( $\pm$ 11)	10.19 ( $\pm$ 1.17)	948 ( $\pm$ 108)

Abbreviations: HC, Healthy Control group; RC, Radiation Control group; RRT, Radiation Resistance Training group; HRT, Healthy Resistance Training group.

## Results

### Bodyweight, Liver weight, and left Liver volume

The body weight of rats in different groups was recorded at the beginning and end of the exercise training protocol. A comparison of liver weight and volume in the post-test showed no significant difference between all groups.

### Serum Aspartate Aminotransferase (AST) Level

These results were assessed at the end of the tenth week of training administration in all groups (Figure 1). The difference between serum AST levels of the HC rats [ $131.1 \pm 24.8$ ,  $n=6$ ] and RC rats [ $169.3 \pm 24.2$ ,  $n=6$ ] was significant ( $P=0.001$ ). The effect of training on changes in serum AST levels in radiation rats, such that was no significant difference in serum AST levels of RRT rats [ $129.6 \pm 33.8$ ,  $n=6$ ] with HC rats [ $131.1 \pm 24.8$ ,  $n=6$ ] ( $P=1.000$ ).

### Serum Alanine Aminotransferase (ALT) Level

These results were assessed at the end of the tenth week of training administration in all groups (Figure 2). The difference between serum ALT levels of the HC rats [ $65.1 \pm 3.1$ ,  $n=6$ ] and RC rats [ $75.6 \pm 3.6$ ,  $n=6$ ] was significant ( $P=0.002$ ). The effect of training on changes in serum ALT levels in radiation rats, such that was no significant difference in serum ALT levels of RRT rats [ $59.3 \pm 17.2$ ,  $n=6$ ] with HC rats [ $65.1 \pm 3.1$ ,  $n=6$ ] ( $P=0.942$ ).

### Serum Alkaline Phosphatase (ALP) Level

These results were assessed at the end of the tenth week of training administration in all groups (Figure 3). The difference between serum ALP levels of the HC rats [ $370.3 \pm 66.9$ ,  $n=6$ ] and RC rats [ $503.5 \pm 83.1$ ,  $n=6$ ] was significant ( $P=0.034$ ). The effect of training on changes in serum ALP levels in radiation rats, such that was no significant difference in serum ALP levels of RRT rats [ $342.8 \pm 94.4$ ,  $n=6$ ] with HC rats [ $370.3 \pm 66.9$ ,  $n=6$ ] ( $P=0.925$ ).

### Serum lipid profile levels

The effect of resistance training on lipid profile was shown in table 3. The triglyceride, total cholesterol, LDL, and HDL levels in the serum were significantly higher in RC than in the HC rats ( $P<0.05$ ). The administration of resistance training significantly decreased these serum triglyceride and LDL levels in RRC rats, so that was no significant difference between the mean serum triglyceride ( $P=0.212$ ) and LDL ( $P=0.931$ ) in the RRC and HC. Also, The administration of resistance training significantly increased serum HDL levels in RRC rats, so that was a significant difference between the mean of serum HDL ( $P=0.001$ ) in the RRC and RC.

## Discussion

Exposure to radiation in the workplace Operators and patients receiving radiation therapy may be exposed

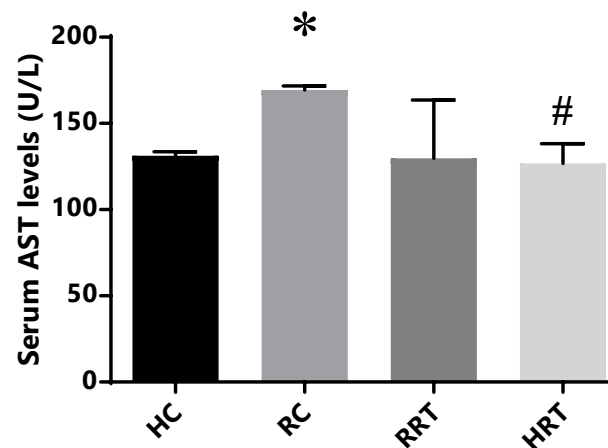


Figure 1. The Effect of Resistance Training on Serum Aspartate Aminotransferase (AST) Level in Male Rats Undergoing Radiation.

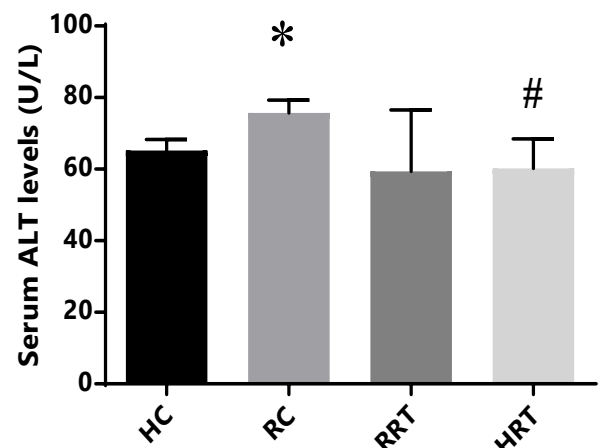


Figure 2. The Effect of Resistance Training on Serum Alanine Aminotransferase (ALT) Level in Male Rats Undergoing Radiation.

Table 3. Mean ( $\pm$ SD) of Cholesterol, Triglycerides, Low-Density Lipoprotein Cholesterol (LDL-C), Very Low-Density Lipoprotein Cholesterol (VLDL-C), and High-Density Lipoprotein Cholesterol (HDL-C) in Different Groups of Rats

Groups	Cholesterol (mg/dL)	Triglycerides (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)	HDL-C (mg/dL)
HC	54.68 ( $\pm$ 1.63)	76.66 ( $\pm$ 12.5)	10.5 ( $\pm$ 1.04)	4.55 ( $\pm$ 1.9)	34.8 ( $\pm$ 2.05)
RC	65.01 ( $\pm$ 4.69) <sup>a</sup>	112.1 ( $\pm$ 8.64) <sup>a</sup>	13.5 ( $\pm$ 1.64) <sup>a</sup>	13.63 ( $\pm$ 7.8) <sup>a</sup>	29.1 ( $\pm$ 2.45) <sup>a</sup>
RRT	68.98 ( $\pm$ 4.43) <sup>a</sup>	92.16 ( $\pm$ 15.5)	11.1 ( $\pm$ 1.41) <sup>b</sup>	15.41 ( $\pm$ 2.67) <sup>a</sup>	35.1 ( $\pm$ 2.21) <sup>b</sup>
HRT	57.06 ( $\pm$ 1.81) <sup>bc</sup>	83.5 ( $\pm$ 15.1) <sup>b</sup>	10.83 ( $\pm$ 1.60) <sup>b</sup>	4.33 ( $\pm$ 1.15) <sup>bc</sup>	36.03 ( $\pm$ 1.01) <sup>b</sup>

a. The significant difference with a healthy control group ( $p < 0.05$ ). b. The significant difference with a radiation control group ( $p < 0.05$ ). c. The significant difference with the Radiation Resistance Training group ( $p < 0.05$ ). Abbreviations: HC, Healthy Control group; RC, Radiation Control group; RRT, Radiation Resistance Training group; HRT, Healthy Resistance Training group.

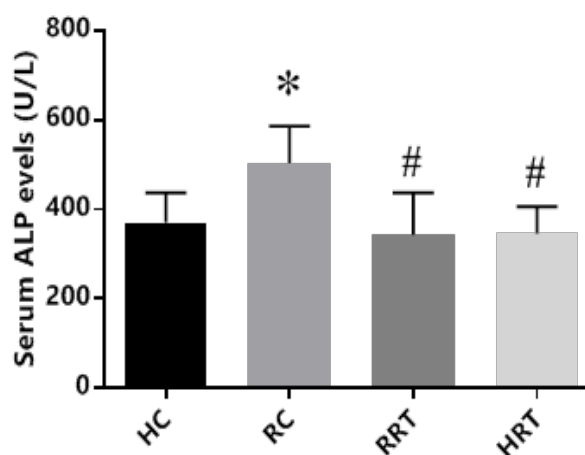


Figure 3. The Effect of Resistance Training on Serum Alkaline Phosphatase (ALP) Level in Male Rats Undergoing Radiation.

to radiation damage. While radiation therapy plays an important role in cancer treatment, it may damage normal tissues [12]. The common view is that damage to natural tissue due to ionizing radiation is mainly due to large-scale damage to biological molecules due to a large amount of ROS accumulation due to the interaction between radiation and tissue [13]. Therefore, the factors that protect against these rays are emphasized. Given that previous studies have studied the protective effects of several antioxidants on normal tissue during radiation therapy [14, 15], the present study aimed to determine the protective effect of exercise training on liver injury indices induced by radiation therapy in mice. It was desert. The results of the present study demonstrated that the serum levels of ALT, AST, and ALP in response to X-ray radiation increased significantly compared to the control group. Also, 8 weeks of resistance training could significantly reduce the serum levels of ALT, AST, and ALP in the X-ray irradiated group with resistance training compared to the irradiated control group. These changes indicate liver tissue damage after X-rays, which 8 weeks of resistance training were able to regenerate these enzymes to normal levels. According to studies performed so far, no study has been performed to evaluate changes in liver enzymes that are an indicator of liver tissue damage following resistance training in patients undergoing radiation therapy. This seems to be the first study. Which has been done in this study field.

Although there is no comprehensive consensus on changes in liver enzymes that are indicative of liver

tissue damage in response to exercise, it has been has been demonstrated that liver enzyme activity is affected by type, duration, intensity, and the way of sports activity changes [16].

The mechanism of the destructive effect of X-ray radiation is related to the production of reactive oxygen species (ROS), which causes apoptosis of tumor cells. Ionizing radiation is associated with biological systems through the generation of free radicals, which indirectly play an important role in inducing oxidative stress and lead to cell damage and organ dysfunction. Oxidative stress is the cause of some diseases and disorders of lipid and protein metabolism [17]. Serum levels of ALT, AST, and ALP enzymes are diagnostic markers of liver damage and their serum levels increase rapidly with liver damage [18]. X-ray radiation therapy with increased oxidative stress and decreased antioxidant capacity leads to liver dysfunction and increased liver enzymes that in confirmation of this content in the study of Qi Liu et al. Subsequent increase in serum levels and hepatic malondialdehyde (MDA) increases serum levels of ALT, AST, and ALP in irradiated mice [12]. This increase may be due to damage to the cell membrane of the liver cells, which in turn leads to increased permeability of the cell membrane, which facilitates the passage of cytoplasmic enzymes out of the cell and which can lead to Increases serum aminotransferase activity [19].

In this regard, studies show that exercise training eliminates this increase in the amount of enzymes by adapting. Also, Zelber et al. [20] in their study investigated the effect of resistance training on non-alcoholic fatty liver and the results of this study demonstrated that resistance training for three months reduces the amount of liver fat content and significantly lowers the mean values of the amount of AST and ALT enzymes.

A key finding of this study was that eight weeks of continuous resistance activity reduced serum levels of ALT, AST, and ALP in irradiated mice. Consistent with the findings of the present study, Ruhee et al. reported that regular exercise significantly reduced serum ALT and AST [21]. Regular exercise also promotes adaptations such as reduced hepatic ROS production. Rodrigues et al. [22] observed that 10 weeks of regular resistance training three times a week increased antioxidant enzymes and decreased ROS in the liver of trained rats. Consistent with the present study, Parastesh et al. [23] observed that a period of resistance training improved liver enzymes in diabetic and healthy rats.

Other results of the present study demonstrated that



exposing the whole body of Sprague-Dawley rats to X-ray (4 Gy) radiation significantly increased serum levels of TC, TG, LDL, and VLDL and decreased serum HDL. These results are consistent with the findings of Ahmed et al. [24] who examined the effect of gamma radiation on the lipid profile of albino male mice. Agreeing with the results of the present study, Abou-Zeid et al. [25] found that radiation following an increase in serum levels of liver enzymes leads to an increase in lipid profile in rats. The lipid profile is greatly affected by exposure to radiation because cholesterol is produced almost identically from the exogenous and endogenous diets of acetyl-coenzyme A in a series of biosynthetic reactions. In addition, radiation-induced hypercholesterolemia may be due to the activation of the enzyme hydroxymethylglutaryl reductase coenzyme A (HMG-CoA), the major enzyme that regulates cholesterol synthesis [26]. Table 3 shows that performing a period of resistance training significantly reduces the effect of X-ray risk on the lipid profile of irradiated rats. Table 3 shows that performing a course of resistance training significantly reduces the effect of X-ray risk on the lipid profile of irradiated rats, which reflects the effect of hypolipidemia of resistance training on these rats. Changes in blood lipids, including triglycerides and LDL-C, can be attributed to the response of lipoprotein lipase (LPL) to exercise. Triglycerides are rich in lipoproteins. It can be said that exercise increases the activity of the LPL enzyme and decreases hepatic triglyceride lipase (HTGL). Whereas increased LPL activity increases the catabolism of triglyceride-rich lipoproteins; Thus, LDL-C levels decrease with physical activity [27].

In summary, the relevant findings and limitations reported in this paper are as follows. The resistance training program used in this study was able to reduce the indicators of liver damage caused by single radiation. One of the limitations of the present study was the lack of measurement of oxidative stress and antioxidant enzymes in liver tissue of rats under X-ray to confirm the positive effects of resistance training on biomarkers of oxidative stress in the liver. It seems that further studies should be performed to elucidate the metabolic effects of this resistance training protocol in mice and humans under X-ray.

In conclusions, according to the results of the present study, it can be stated that performing a period of resistance training in irradiated rats creates favorable adaptations to changes in serum lipid profile, which in turn improves liver enzymes in irradiated mice.

### Perspective

It seems that resistance training by reducing serum LDL levels and increasing HDL has improved the lipid profile and ultimately improved the indicators of liver inflammation.

### Author Contribution Statement

Seif F, Bayatiani RM, and Parastesh M designed the research project and performed the experiments. Parastesh M supervised the analysis of experimental results.

Yuosofvand Z and Abili N performed the experiments.

### Acknowledgements

The present research is based on the research project approved by the Arak University of Medical Sciences [Grant number: 3446].

The code of ethics is also included in the description (IR.Arakmu.rec.1398.161) in the ethics committee of the research projects of Arak University of Medical Sciences.

### Conflict of interest

There is not any conflict of interest.

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