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Evaluation of the Therapeutic Role of the Low Level Laser on the Gamma Irradiated Teeth and the Antimicrobial Salivary Biomarkers in Rat's Model: an Experimental Study

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ABSTRACT

Purpose: The aim of the study was to examine the impact of Low-level laser therapy (LLLT) on gamma-irradiated teeth regarding tooth structure hardness, salivary pH and salivary IgA. **Material and Methods:** Thirty adult male albino rats were separated into three groups, which were control group, irradiated group (animals irradiated with a dose of 6 Gy) and gamma irradiated + Laser (the rats wear exposed to low level laser after three days of gamma radiation). **Results:** The obtained results revealed that the combination group (Gamma + Laser) recorded the highest salivary IgA followed by Control group while Gamma group recorded the lowest salivary IgA. Also, it was found that the Control group recorded the highest salivary pH followed by the combination group while Gamma group recorded the lowest salivary pH. The combination group (Gamma + Laser) recorded the highest tooth hardness followed by Gamma group while the control group recorded the lowest tooth hardness. **Conclusion:** With the limitations of this study, it was conducted that the Low-level laser therapy post gamma irradiation reflected a significant increase in salivary pH, salivary IgA and tooth hardness compared to both the control and the gamma-irradiated groups.

KEYWORDS

Low level laser therapy, γ-rays, Salivary IgA, Salivary PH.

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INTRODUCTION

Radiotherapy is a therapeutic approach based on ionizing radiation that is commonly used for treatment of cancer patients. It may cause detrimental effects in the oral cavity as mucositis, osteoradionecrosis, dry mouth (xerostomia), enamel abrasion, delamination, and damage to the dentinoenamel junction [(1)]. Low level laser therapy (LLLT) is a type of light therapy that makes biological alterations inside the cells. Photons are immersed by cellular receptors, causing positive biochemical changes to the human tissues. LLLT has been documented to treat ache for long time, which utilize low- continuous wave laser of 600 to 1000 nm wavelengths for pain decline and healing process. Several studies have confirmed analgesic and anti-inflammatory impacts resulted from photobiomodulation in the experimental and clinical judgments [(2)]. The present work is proposed to investigate impact of gamma-radiation on teeth structure hardness, salivary PH and salivary IGA with and without low level laser exposure.

MATERIAL AND METHODS

Experimental animals

Adult male Swiss albino rats (110-120g), n=30 were received from the Egyptian Organization for Biological Product and Vaccines, Giza, Egypt. The rats were provided with typical food and water. The animals were kept under typical conditions of humidity (50-60%), temperature (20-24°C), and 12-h light-dark cycle, and handled gently and as little as possible to minimize noises, vibrations and stress.

Animals were prevented from food, but not water, during the night before samples collection.

The animals were followed the rules and regulations of animal experimental studies approved by ethical committee of Faculty of Dental Medicine for Girls, Al-Azhar University- Girls' branch, including their facilities diet and method of scarification. Ethical committee code is REC17-027.

Experimental design

Animal grouping

Thirty rats were separated into three groups (n = 10). Control group, Group1: rats were not subjected to any treatment. Group2 (Gamma group): the rats' head and neck were exposed to gamma radiation (6GY). Group3 (Gamma + Laser): the rats wear exposed to low level laser after three days of gamma radiation.

Irradiation

It was made by using gamma cell-40 (Cesium^{137}) to be found at National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt. Rats were irradiated with a single dose of 6 Gy γ-rays, at a dose rate of 0.42 Gy/min during the period of experimentation. Animals were not anesthetized pre- irradiation. The animals were under the rules and regulations of the animal experimental studies approved by ethical committee of Faculty of Dental Medicine for Girls, AL-Azhar University including their facilities diet and method of scarification. Ethical committee code is REC 15- 81.

Laser Irradiation Technique:

807 nm infra-red semiconductor GaAlAs-diode low level laser was utilized. The unit has a contact probe with a laser beam diameter of 0.5 cm. Power output was maintained stable at 330 mW in the continuous wave (cw) mode. The irradiation time was 60 seconds with energy of 19.8 J/Cm^2[(3)].

The laser irradiation was used bilaterally in non-contact mode on parotid, submandibular glands, and intra-orally to the sublingual gland and the teeth.

Specimen's collection and preparation

Collection of saliva: Rats were ventrally applied in a box, and their heads stayed out of it. Under their mouth, there was a plastic graduated tube kept in ice. Stimulated whole saliva was obtained after an intra-peritoneal injection of pilocarpine (7.5 mg/kg body weight).

Collection of teeth: The rats were sacrificed by decapitation then the mandible and maxilla were dissected and the teeth were extracted and preserved in saline.

After sample collection: Samples were refrigerated immediately and freezed at -20°C (household freezer) (within hours of sample collection).

Micro hardness assessment: Surface microhardness of the specimens was determined using DigitalDisplay Vickers Microhardness Tester (model: Shimadzu HMV) with a Vickers diamond indentor and a 20X objective lens. 200 gm was applied to the buccal surface of the samples for 20 seconds. Three indentations were equally placed over a cycle of 1 mm diameter at the buccal surface of the enamel and the dentin of the specimens. The diamond shaped indentations were observed under the microscope. Image analysis software allowed accurate digital measurements of their diagonals. Microhardness Vickers values were converted into microhardness values MHV following this equation: [4] MHV=1.854 P/d2. Where; MHV Vickers microhardness values in Kgf/mm2, P the load in Kgf, D the length of the diagonals in mm. The hardness was measured at baseline, before and after the PH cycling.

IgA evaluation

Rat Immunoglobulin A was determined using Rat IgA ELISA Kit Cat. No. CSB-E07987r (Cusabio). This method is based on the competitive inhibition enzyme immunoassay system. The microtiter plate had been pre-coated with rat IgA. Standards and serum samples were put in the wells with an antibody specific for rat IgA (Horseradish Peroxidase (HRP). Following a wash to avoid any unbound reagent, a substrate solution was put in the wells and the color was proportional to the amount of IgA in the samples and the intensity of the color is determined.

PH evaluation: The PH was evaluated using litmus paper strip.

Statistical analysis

All the data were presented as mean ± SD. Data was analyzed by one way analysis of variance (ANOVA) and then by Newman-Keuls test as post ANOVA test.

Student t-test was done to detect significance between groups utilizing Graphpad Prism (version 5.03) software and differences were significant at probability levels $P \leq 0.05$. All figures were plotted by Microsoft Excel version 2013.

RESULTS

Table (1) and Figure (1) showed that irradiated rats revealed a marked decrease ($p \leq 0.0001$) in the level of salivary IgA, compared to their values of control group, while treatment with LLL post- irradiation revealed a significant increase ($p \leq 0.0001$) in the level of salivary IgA, compared to their values in the irradiated group.

Table (1) Comparison of IgA mean values as function of irradiation protocol

Variable		Mean ± SD	Rank	ANOVA Statistics (P value)
Experimental groups	Control	3.84 ± 0.5621	A	< 0.0001*
	Gamma	1.88 ± 0.625	B	
	Gamma+Laser	4.09 ± 0.3843	A	

*Same letter in the same column indicating non statistically significant difference (Newman-Keuls test; p>0.05). ns; non-significant (p>0.05) *; significant (p ≤ 0.05)*

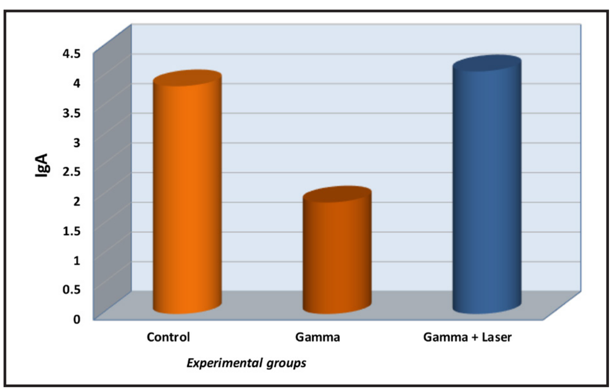

Figure (1) A column chart of IgA mean values of the experimental rats

Rats exposed to gamma irradiation (6 Gy) showed a significant decrease ($p \leq 0.0001$)in the level of salivary pH, compared to their values in the control, while irradiated rats treated with LLL displayed a marked elevation ($p \leq 0.0001$), compared to the values of irradiated group only **(Table 2)** and (Fig. 2).

Table (2) *Comparison of pH mean values as function of irradiation protocol*

Variable		Mean ± SD	Rank	ANOVA Statistics (P value)
Experimental groups	Control	7.3 ± 0.216	A	< 0.0001*
	Gamma	4.743 ± 0.1718	B	
	Gamma+Laser	6.3 ± 0.216	C	

*Same letter in the same column indicating non statistically significant difference (Newman-Keuls test; p> 0.05). ns; non-significant (p> 0.05) *; significant (p ≤ 0.05)*

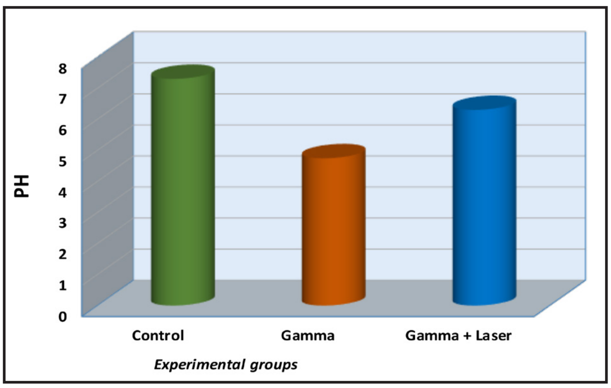

Figure (2) A column chart of pH mean values of the experimental rats

Moreover, Table (3) and Figure (3) showed a noticeable decrease ($p \leq 0.001$) in the teeth hardness of irradiated rats, compared to control group, while the treatment with LLL to irradiated rats revealed a significant increase ($p \leq 0.001$), compared to irradiated rats

Table (3) *Comparison of hardness mean values as function of irradiation protocol*

Variable		Mean ± SD	Rank	ANOVA Statistics (P value)
Experimental groups	Control	10.99 ± 1.042	A	0.0032*
	Gamma	11.83 ± 2.481	A	
	Gamma+Laser	14.09 ± 1.466	B	

*Same letter in the same column indicating non statistically significant difference (Newman-Keuls test; p> 0.05). ns; non-significant (p> 0.05) *; significant (p ≤ 0.05)*

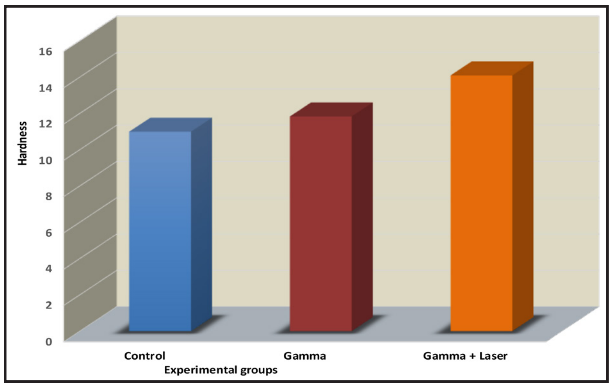

Figure (3) A column chart of Hardness mean values of the experimental rats

DISCUSSION

Radiation caries is known as one of radiation hazards as a result of the head and neck region radiotherapy with a high potential for destruction of the dentition [5]. Scientific proof reports that patients bear a lifetime danger of promoting radiation caries subsequent to radiation therapy [6]. This problem is difficult and causes high damage of the tooth enamel and dentin, and also has harmful effects on their quality of life [7]. Ionizing radiation may cause reformation in the crystal structures of tissues minerals and alter their physical properties together with the structural micro-hardness [8]. Furthermore radiation may reduce water in the cells and tissue dehydration that resulted in augmented organic matrix stiffness and, therefore leading to increased micro-hardness [9].

In contrast to the present study, another study showed no alteration in micro-hardness as a purpose of radiation [10], while a similar study [11] found a decrease in micro-hardness of teeth after gamma exposure. Moreover, irradiated rats revealed a significant depletion in the salivary PH and salivary IgA, compared to control group.

Salivary gland injury accompanied with structural modification and functional constraint is a result of radiotherapy in the head and neck area, leading to complications, including mucositis, xerostomia and hyposalivation, where it is not potential to avoid salivary glands from the treatment field [12, 13].

In spite of salivary glands being considered as radio-resistant due to their highly differentiated cellular state, they display a high sensitivity to radiation, which is manifested by a decrease in salivary flow rate, irreparable and advanced lack of glandular weight and acinar cells, and morphological alterations in gland structure[14,15]. The secretory cells of the salivary glands are the most radiosensitive, especially the serous secretors.

In the present study, it was found that rat's teeth hardness was increased in LLLT (807 nm infra red semiconductor GaAlAs-diode low level laser) irradiated group, compared to the groups of the control and irradiation. In agreement with our result [16] showed that LLLT decreases the demineralization; which is one of the hazardous effects of radiotherapy. Moreover, Lasers have also been used to prevent the enamel demineralization caused by dental caries and have shown good result [17].

Phototherapy with LLL usually used in several regions of biological fields to help cells rejuvenation in the tissues damage [18, 19]. This treatment acts as analgesic, anti-inflammatory and bio-modulatory impacts [20, 21].

In our work, it was found that LLLT (807 nm infra red semiconductor GaAlAs-diode low level laser) irradiated group reflected a significant increase in salivary PH and salivary IgA, compared to the groups of the control and irradiation. It has been demonstrated that a LLLT can elevate injury healing [22], augment bone and collagen formation [23] and stimulate anti-inflammatory effects. Data from the results are confirmed by in vitro examinations indicating that low-level laser irradiation markedly elevates cell proliferation [24].

In accordance with our result, previous study displayed that marked salivation development quantitatively and qualitatively, i.e. an enhance in the amount of saliva and sIgA was obtained upon treatment of the major salivary glands of xerostomic patients' with LLL on 10 occasions[25]. Moreover, one study stated that the effects of LLLT on salivary glands are not only motivating, but also restore the glandular response to the same quantity of applied laser therapy[26]. Another study found that LLLT could markedly increase salivary excretion and ameliorate antimicrobial features of secreted saliva (elevated level of sIgA)[27].

Although the clinical efficacy of the LLLT as stimulator of salivary gland has been previously studied using helium-neon laser in sialadenitis [28], GaAs 904 nm wavelength laser and GaAlAs 820 nm wavelength laser was manifested clinically by much lower incidence of xerostomia[29].

CONCLUSIONS

1. The Low-level laser therapy after gamma-irradiation showed a remarkable increase in salivary IgA, salivary PH, compared to both the control and the gamma-irradiated groups.
2. The low-level laser therapy post-irradiation reflected a significant increase in the tooth hardness, compared to both the control and the gamma-irradiated groups.

RECOMMENDATIONS

1. From our study we advise using low level laser radiation for head and neck cancer patients that receive radiotherapy treatment
2. Low level laser improves the mechanical properties of tooth structure that altered by radiotherapy.

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DECLARATION OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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