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Role of low level laser in ameliorating the damaging effects of gamma irradiation on mice liver

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► ***Original article***

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ABSTRACT

Background: Exposure to ionizing radiation is inevitable. Using of low-level laser therapy (LLLT) stimulates tissue repair and reduces inflammation. The objective of the present study aimed at evaluating the therapeutic efficacy of Helium-Neon (He-Ne) laser in stimulating the reparative processes in the liver of mice after whole body gamma-irradiation (WBγ-I). ***Materials and Methods***: Two hundred and sixty four female mice were divided into 6 groups: Control, Laser irradiated, One shot gamma irradiated group, One shot + laser irradiated, Cumulative gamma irradiated and Cumulative + laser irradiated. Ionizing radiation was performed using a Cesium-137 source. Two modes of exposure were used, 1- Mice were irradiated with a single shot sublethal dose of 5 Gy. 2- The same dose was given in fractionated mode daily installations of 1 Gy. Laser treatment was carried out using a computerized scanner emitting He-Ne (CW). The assessment of serum transferases (AST & ALT) was performed along with histopathological (HP) assessment of liver biopsies. ***Results:*** There was a significant increase in serum transaminases above the control levels in gamma irradiated groups. Laser therapy of these groups was accompanied by a significant decrease in the elevated levels of transaminases. HP changes in the liver of the shot gamma-irradiated group showed that the main brunt of damage was on the liver cells. Meanwhile, in the cumulative gamma-irradiated group the main brunt was on the vascular system including the central veins and the portal blood vessels. ***Conclusion:*** It could be concluded that mice exposed to WBγ-I suffered from aggravated HP changes in the liver tissues accompanied by disturbances in the level of liver enzymes. These undesirable alterations were ameliorated by the treatment of the experimental mice by He-Ne laser before being irreversibly damaged.

Keywords: *Gamma-irradiation, mice, liver, He-Ne laser.*

INTRODUCTION

The mechanisms of laser photobiomodution are complex, but essentially rely upon the absorption of particular visible red and near infrared wave lengths in photoreceptors within sub-cellular components, particularly the electron transport (respiratory) chain within the membranes of mitochondria [1]. The hypothesis of activation of photo-receptors within the electron transport chain of mitochondria was also proposed by Suresh *et al.* [2]. An effective dose is that portion of photons that is absorbed in the tissues depths where disease is located [3]. El-Batanouny [4] used He-Ne laser therapy with a wavelength of 632.8 nm for enhancing healing of chronic leg ulcers. Andrade *et al.* [5] stated that He-Ne laser (632.8 nm) belonged to the most common devices used in LLLT and that doses ranging from 3 to 6 J/cm^2 appeared to be the most effective while doses above10 J/cm^2 were associated with deleterious effects. Wagner *et al.* [6] and de Loura Santana *et al.* [7] used visible red laser (660 nm) for enhancement of the tissue

repair process. Wagner *et al.* [8] reported that more significant results regarding cytokine modulation, faster and more organized re-epithelialisation and tissue healing of the oral mucosa were achieved with an energy density of 4 J/cm^2 in comparison to 20 J/cm^2]**.** Bakshi *et al.* [9] state that tens of thousands of people are exposed daily to environmental low-dose gamma radiation. They add that epidemiological data indicate that such low radiation doses may negatively affect liver function and result in the development of liver disease and significant long -term alterations in lipid metabolism with increased liver inflammation.

The present work was undertaken to investigate the biochemical and histopathological changes induced by WBγ-I of mice using a sublethal dose of gamma rays choosing a relatively radio-resistant organ such as the liver. The novelty of the present work is shown in the effectiveness of low level laser level in ameliorating the damaging effects of gamma-irradiation on mice liver

MATERIALS AND METHODS

Irradiation facilities:

a) **Gamma rays unit:** The source of ionizing radiation was a Gamma cell-40 (Cesium-137) irradiator (Best Theratonics ltd., Ottawa, ontario, Canada). Dose rate of Cesium source was 0.67 Gy/min. Irradiation source belonged to the National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA). The irradiation tray had ventilation holes on its side, which aligned with the ventilation parts through the main shield of the unit to ensure animals aeration. Two modes of exposure were used; with a shot modality where the mice were irradiated with a single sublethal dose of 5 Gy. In the other mode, the sublethal dose was given in fractionated daily installations of 1 Gy.

b) **Laser irradiation:** The laser irradiation unit was a computerized scanner (level Laser M-300, Italy) emitting continuous wave (CW) helium-neon with wavelength 632.8 nm and the fluence of 5J/cm^2. The unit was a class 4 laser with output power of 10 mw (Time X Power/cm2 were 5 J/cm2). Time of laser irradiation was 500 sec per session. The irradiated material was placed at 30 cm from the laser source to have sufficient scanning area and to be suitable to the size of the animal slightly exceeding the latter. This unit belongs to National Institute of Laser Enhanced Sciences (NILES), Cairo University. Laser treatment started immediately after exposure to the 5 Gy gamma radiation (either by the shot or by the cummulative mode), and was repeated every other day throughout four weeks (12 sessions).

c) Subcutaneous injection of ketamine was followed by laser irradiation transcutaneously to the shaved epigastric surface of the mice abdomen. Uniform laser exposure was maintained by the use of an attached scanner.

It goes without saying that the study protocol and experimental procedures were carried out according to the International Guidelines for Animal Experiments approved by the National Institute of Health (NH No. 85:23, revised 1996) and in compliance with the regulations of the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority (AEA), Egypt.

Sacrification Schedule:

Six mice from the first two groups (Non irradiated control group &Laser irradiated group) were sacrificed by the end of each week for four weeks then the biochemical and histopathological studies were carried out.

Three mice were sacrificed every other day starting one day after irradiation, from the other 4 groups(Shot gamma irradiated group, Shot and laser irradiated group, Cummulative gamma irradiated group, cummulative and laser irradiated group). This number was increased at the end of each week to reach six mice so as enough serum would be available for biochemical assessment. After each sacrification, the histopathological studies were carried out.

Animals and expermintal design:

Two hundred and sixty four female mice of the same colony, aged approximately four months and weighed 25± 5 gram were used in the suggestyed study. The mice were kept under good ventilation and illumination conditions and were maintained on a well balnced standard diet and free water supply. The mice were divided into sex groups.

Group(1): (Non irradiated control group) This group consisted of 24 mice, recieved neither laser nor gamma irradiation.

Group(2): (Laser Irradiated group) (24mice) received He-Ne Laser every other day for a period of 4 weeks.

Group(3) was classified as group 3.1(54 mice) whole body gamma irradiated (WBI) at a single sublethal dose of 5 Gy.While group 3.2 (54 mice) were WBI as group (3.1), then on the same day started receiving laser radiation three times per week till the end of the experimental period (4 weeks).

Group (4) was classified as group 4.1 (54 mice) were WBγ-I with a daily fractionated dose level of 1 Gy for successive 5 days to collecte an overall dose level 5 Gy. While the other group 4.2 (54 mice) received cumulative (fractionated) dose level as group 4.1, then started treatment with laser every other day till the end of the 4th week.

Chemicals:

All chemicals used in analytical procedures were purchased from (Sigma-Aldrich Corp.).

Biochemical assays:

The biochemical assessments were conducted on six animals from the six groups at the end of each week throughout the whole experimental period. Serum activity of Aspartate transaminase (AST) and Alanine transaminase (ALT) was estimated according to the procedure described by Reitman & Frankel 1957 [10].

Histological Examination:

Parts of the excised liver were fixed in 10% neutral buffered formalin for 48 hours, then transferred to 70% ethyl alcohol, processed and embedded in paraffin blocks. Sections of 5-6 um thickness were stained by hematoxylin and eosin stains (H&E) and Masson trichrome [11] then examined histologically with the light microscope as.

Statistical analysis of data:

All mean values are reported as the mean ± standard error (SE). Data were analyzed using a one-way analysis of variance (ANOVA). The level of significance between mean values was set at $p < 0.05$ and $p < 0.01$ (significant and highly significant, respectively). All statistical analyses were performed by using SPSS software (version 20.0).

RESULTS

Experimental investigations were carried out along two main lines:

1. **Assessment of liver enzymes:** AST (table 1) and ALT (table 2). Laser irradiated (group 2) did not show significant difference in serum AST and ALT levels compared to those of the non-irradiated (control group) throughout the whole experimental periods.
2. Shot (Group 3.1) & Cum (Group 4.1) ionizing radiation groups exhibited a significant increase in serum AST and ALT above the control level throughout the whole four weeks.
3. He-Ne laser biostimulation resulted in a gradual progressive significant attenuation of the effect of gamma irradiation on the above mentioned enzymes level in shot + laser (Group 3.2) and cum + laser (Group 4.2).

A- Histopathological (HP) investigations of the liver:

Group (1) Non irradiated control group:

The parenchymal tissue of the liver was seen built up of hepatic cells disposed in the form of radiating plates or strands. Each strand was characterized by one or two cell thickness. These strands formulated a network structure around the central vein. They were alternated with narrow blood sinusoids converging

towards the central vein. Individual hepatic cell (hepatocytes) exhibited a polyhedral appearance with a relatively large size, prominent centrally located nucleus. The blood sinusoids had an endothelial lining formed of two kinds of cells; rather flattened cells having darkly stained elongated nuclei and Kupffer cells exhibiting an irregular appearance (figure.1).

Table 1. Mean value ± SE of serum AST (U/ml) of control and irradiated mice.

Experimental groups	Time intervals post irradiation			
	1 W	2 W	3 W	4 W
Gr1 (n=6)				
[1]	26.67	29.50	27.33	28.33
[2]	0.31	0.44	0.47	0.43
[3]	C	B and C	B and C	C and D
G 2 (n=6)				
[1]	26.83	28.67	25.83	25.17
[2]	0.48	0.78	0.78	0.89
[3]	C	B and C	B and C	D
G 3.1 (n=6)				
[1]	57.5	52.33	50.67	47.33
[2]	0.96	0.99	0.84	0.46
[3]	A **	A **	A **	A **
G 3.2 (n=6)				
[1]	40.50	36.33	34.67	34.0
[2]	0.55	0.81	0.81	0.36
[3]	B *	B *	B *	B and C *
G 4.1 (n=6)				
[1]	55.0	49.67	48.0	45.67
[2]	0.92	0.93	1.13	0.5
[3]	A **	A **	A **	A **
G 4.2				
[1]	38.83	36.83	35.0	33.83
[2]	0.55	0.22	0.25	0.36
[3]	B 8	B *	B *	B and C *

Duncan Multiple range tests (All possible pair comparison between the groups). Each value represents Mean ± SE of 6 determinations. Means with the same latter are not significantly different.
(*) significant from the group 1 (control) as P < 0.01.
(**) high significant at P < 0.001. (***) very high significant at P < 0.0001.
[1] Mean
[2] Standard error (SE)
[3]Duncan grouping

Table 2. Mean value ± SE of serum ALT (U/ml) of control and irradiated mice.

Experimental groups	Time intervals post irradiation			
	1 W	2 W	3 W	4 W
Gr1 (n=6)				
[1]	17.33	20.33	18.33	17.67
[2]	0.33	0.30	0.30	0.28
[3]	C	C and B	C and B	C
G 2 (n=6)				
[1]	13.67	14.33	12.83	11.5
[2]	0.23	0.26	0.27	0.28
[3]	C	C	C	C and D
G 3.1 (n=6)				
[1]	34.83	34.17	32.5	30.33
[2]	0.41	0.69	0.67	0.48
[3]	A *	A **	A *	A **
G 3.2 (n=6)				
[1]	26.5	23.0	21	23.83
[2]	0.38	0.39	0.39	0.22
[3]	B *	B *	B *	B *
G 4.1 (n=6)				
[1]	32.83	31.83	30.5	28.66
[2]	0.41	0.71	0.67	0.48
[3]	A **	A **	A **	A and B **
G 4.2				
[1]	15.5	16.67	14.67	13.50
[2]	0.22	0.27	0.27	0.28
[3]	C	C and B	C and B	C and D

Duncan Multiple range tests (All possible pair comparison between the groups). Each value represents Mean ± SE of 6 determinations. Means with the same latter are not significantly different.
(*) significant from the group 1 (control) as P < 0.01.
(**) high significant at P < 0.001.
(***) very high significant at P < 0.0001.
[1] Mean
[2] Standard error (SE)
[3]Duncan grouping

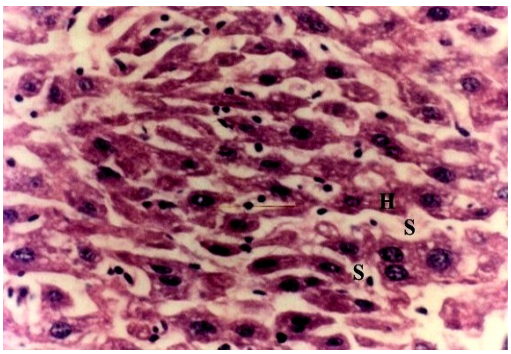

Figure 1. Light micrograph of a section in the liver of a mouse in Group (1) showing strands of normal hepatocytes (H) alternating with normal blood simusoids (S). (HE. X 400).

Group (2): Non Ionizing (Laser) radiation group

During the first three weeks no structural changes in the liver lobules or portal vessel could be seen. By the end of the fourth week the portal blood vessels (figure.2A) and the central vein (figure.2B) showed mild degree of dilation with preservation of normal liver configuration and healthy hepatocytes. No fibrosis was observed by Masson trichrome in any of the slides examined (figure.2C&D)

Group (3.1): shot group

The inflammatory changes induced by γ-rays were seen from the 1st day post irradiation as collections of lobular inflammatory cell infiltrates (figure. 3). In the portal tract there were increased in size and cellularity with moderate PNLs and mononuclear cell infiltrates (figure. 3).

Cellular changes were evident starting from the 3rd day and included areas of focal atrophy as well as, areas of focal necrosis. There was also an observable increase in Kupffer cells (figure. 4).

At the end of the first week Post γ-irradiation hepatic necrosis became massive. In these areas of massive coagulative necrosis, the basic structural outline of the cells was preserved and they looked pink, glassy and homogenous. Angomatiods were clearly seen across the necrotic areas (figure. 5). No evidence of fibrous tissue accumulation could be observed by the trichrome stain in all slides examined.

Spontaneous healing after single dose of 5 Gy γ-rays was nearly complete by the end of the fourth week with mild increase in number of pigmented Kupffer cells (figure.6).

The semiquantitative analysis of HP changes within the liver lobules in the shot group (3.1) is shown in table (3), while that of the portal tract of the same group is illustrated in table (4).

Group (3.2) Shot +Laser group: Liver biopsies carried out during the first 7 days revealed insignificant changes in the liver lobules and/or portal tract. At the end of the first week (3 episodes) large numbers of mononuclear cell infiltrations in some areas of liver parenchyma and portal tract (figure. 7) were still present.

At the end of the second week (6 episodes) Liver configuration was restored and the portal tract returned completely normal with complete disappearance of angiomatoids from all liver section (figure.8).

Group (4.1): Cumulative group: Starting from the first day post γ-irradiation hepatocytic atrophy was very evident. Diffuse liver cell shrinkage with increased nuclear glycogen predominated in some lobules while others showed diffuse hydropic changes and lytic necrosis (figure. 9).

Starting from the 2nd week up to the end of the 4th week; the central vein was dilated and congested. It's endothelial lining was partially lost in some areas while in other the loss was complete (figure. 10). There was no evidence of fibrosis by trichrome stain.

Many branching angomatoids could be seen even after regaining normal liver architecture (figure. 11).

The semiquantitative analysis of HP changes within the liver lobules in the shot group (4.1) is shown in table (5), while that of the portal tract of the same group, is illustrated in table (6).

Group (4.2) Cumulative +Laser group: On the 8th day of He-Ne irradiation (4 episodes): Atrophic and degenerated liver cells were replaced by newly formed hepatocytes that regained the pre-exposure arrangement in the form of strands radiating around the central vein and alternating with normal blood sinusoids, with disappearance of the necrotic areas. The central vein regained its normal size and pigmented Kupffer cells were observed in increased numbers (figure 12). No fibrosis was also evident at this stage in the liver parenchyma.. As regards to the portal tract, although there was an evident decrease in the amount of edema fluid and cellularity, yet mononuclear cells were still seen infiltrating the bile ducts and portal blood vessels. Returning to normality of the portal arterioles was more rapid than the venules, that were still mildly dilated and congested (figure 13).

At the end of the second week of starting treatment (7 episodes): No indication of pathological changes could be detected in either the liver lobules or in the portal tract.

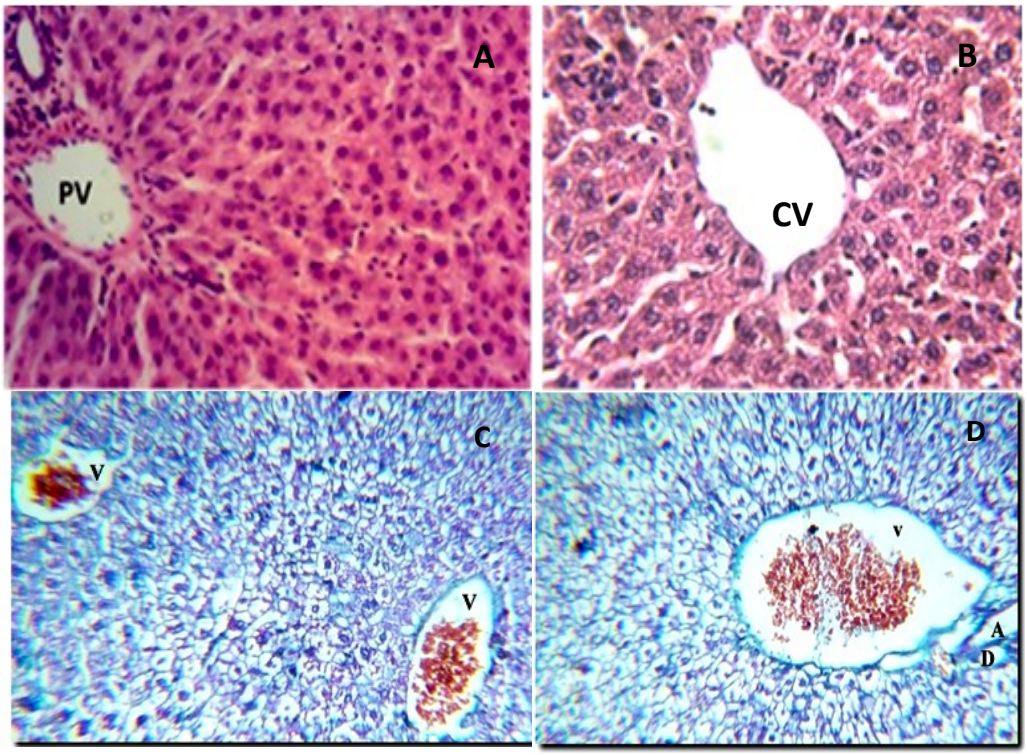

Figure 2. Light micrograph of a section in the liver of a mouse in Group 2 showing: mild degree of dilation of the portal blood vessels **(PV) (2A)** and the central vein **(CV)** (2B). (HE. X 200). Dilatation and congestion of central veins **(V)** with no parenchymal fibrosis **(2C)** and portal area also devoid of fibrosis showing portal vein **(v)** artery **(A)** and bile duct **(D)** (Masson trichrome X100).

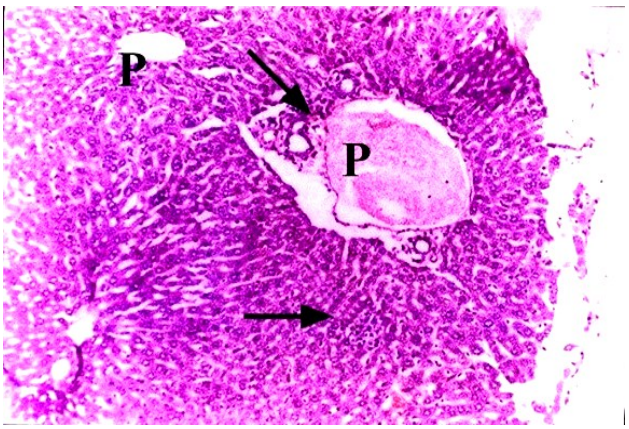

Figure 3. Light micrograph of a section in the liver of a mouse in Group (3.1) showing portal tract with mononuclear cell infiltration **(P)**. Arrows pointing at collections of inflammatory cells both lobular lower arrow and portal upper arrow. (HE. X 100).

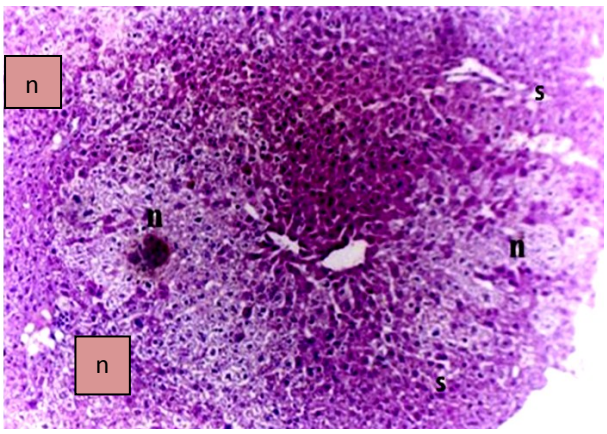

Figure 4. Light micrograph of a section in the liver of a mouse in Group (3.1) showing focal necrosis **(n)**, dilated blood sinusoids **(S)** (HE. X100).

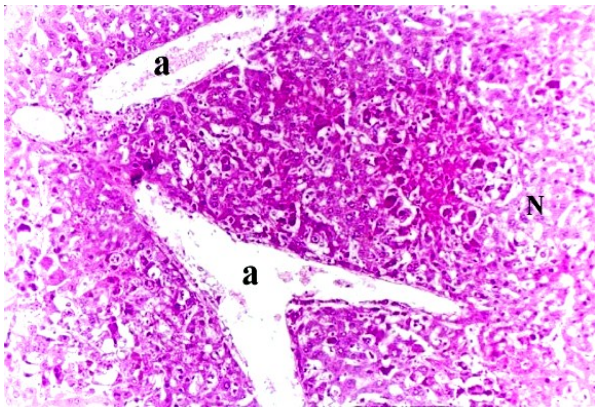

Figure 5. Light micrograph of a section in the liver of a mouse in Group (3.1) showing massive coagulative necrosis **(N)** with few anogmatoids **(a)**. (HE. X 200)

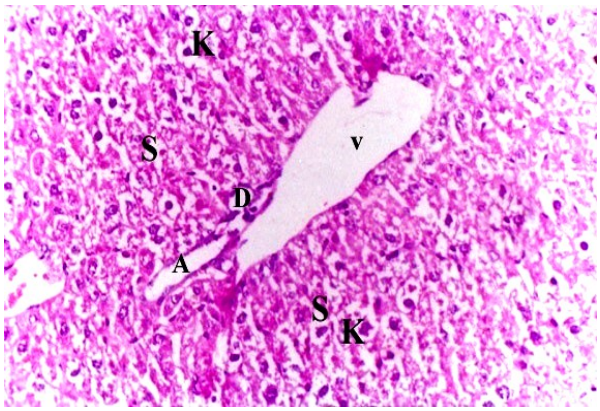

Figure 6. Light micrograph of a section in the liver of a mouse in Group (3.1) showing complete healing of the portal tract (v-vein, A-artery, D-duct) with normal blood sinusoids **(S)** in which there is an increased number of Kupffer cells **(K)**. (HE. X200).

Table 3. Showing the HP changes in the liver lobules of group 3.1 at different periods post whole body γ-irradiation.

Time interval post irradiation	Cell degeneration	Atrophy	Necrosis	Nuclear changes	Central vein	Sinusoids	Kupffer cells	Cell infiltrate
1st day	**diffuse ballooning	-	-	-	* D and C	** Compressed	few	** lobular
3th day	* diffuse ballooning	** Focal	**Lytic ** Focal	**	* D and C	** D and C	increased	** lobular
8th day	** focal	** peripheral	*** massive coagulative	***	* D and C	** D and C	average	** lobular
During 2nd week	* focal	* peripheral	** peripheral	**	* D and C	* D and C	decreased	* lobular
During 3rd week	variable	variable	Variable	variable	-	*D	* increase in number and pigmentation	-
During 4th week	variable	variable	variable	variable	-	* D	* increase in number and pigmentation	-

D and C: Dilated and congested
*: mild
**: moderate
***: severe

Table 3. Showing the HP changes in the liver lobules of group 3.1 at different periods post whole body γ-irradiation.

Time interval post IR	Size	Inflammatory cells	Bile ducts	Artery	Vein	New Vessels (Angomatoids)
1st day	*	*PNL and Mononuclears	Nl	Nl	N	-
4th day	**	**PNL and Mononuclears	N	** D and C	** D and C	**
8th day	***	**PNL and Mononuclears	N	** D and C	** D and C	***
During the 2nd week	**	** Mononuclears	N	* D and C	* D and C	*
During the 3rd week	*	* Mononuclears	N	* D	*D	-
During the 4th week	N	-	N	N	N	-

PNL : Polymorphonuclear leucocytes
D and C : Dilated and congested
N : Normal
* : Mild
** : moderate
*** : severe

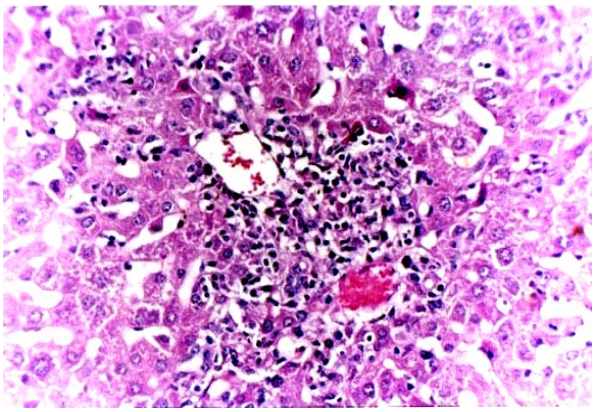

Figure 7. Light micrograph of a section in the liver of a mouse in Group (3.2) showing portal tract with mononuclear cell infiltrate (HE. X 400).

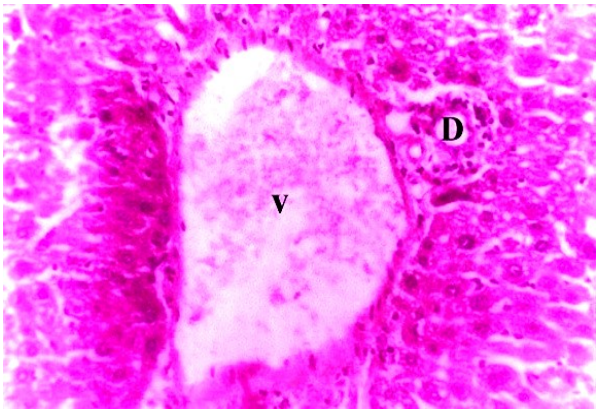

Figure 8. Light micrograph of a section in the liver of a mouse in Group (3.2) showing complete healing of liver cells and normal portal tract. v- portal vein and D- bile duct (HE. X 400).

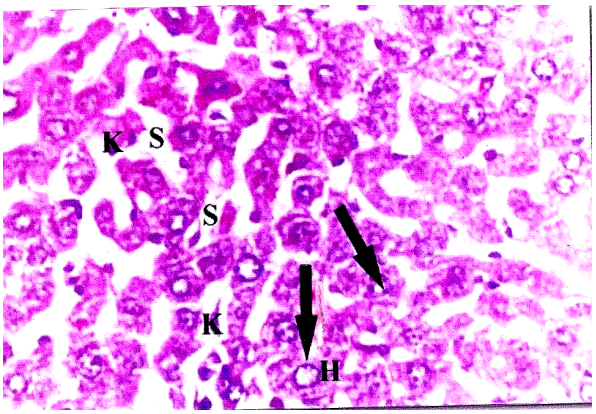

Figure 9. Light micrograph of a section in the liver of a mouse in Group (4.1) showing complete atrophic liver cells (**A),** increased nuclear glycogen (arrows), hydropic hepatocytes **(H)** and dilated blood sinusoids in which pigmented Kupffer cells **(K)** are increased in number (HE. X 400).

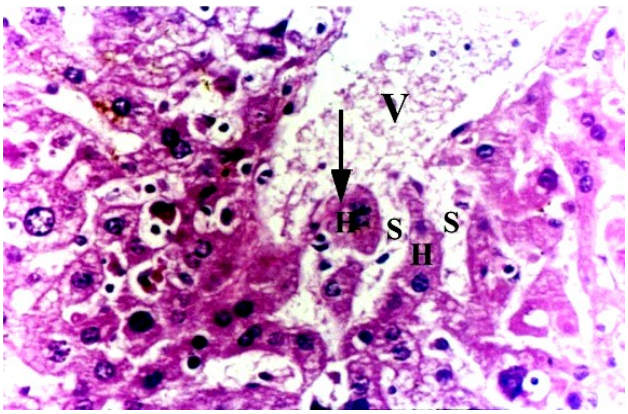

Figure 10. Light micrograph of a section in the liver of a mouse in Group (4.1) showing dilated sinusoids **(S)**, complete loss of the wall of the central vein **(V)** and hemorrhage into parenchyma (arrow& H). (HE. X 400).

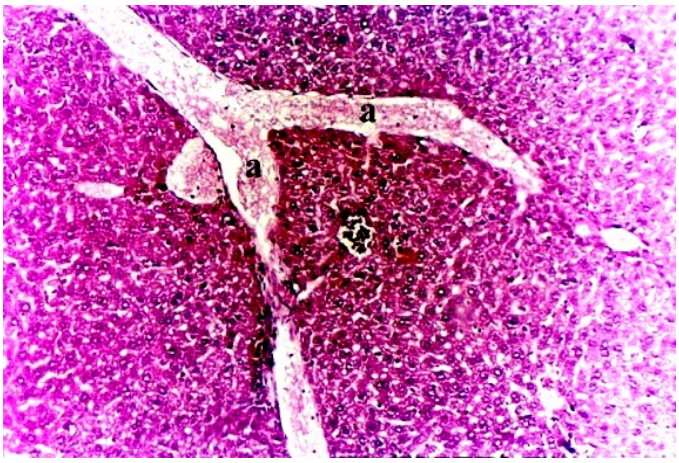

Figure 11. Light micrograph of a section in the liver of a mouse in Group (4.1) showing complete spontaneous regaining of normal liver architecture but with many branching angomatoids **(a)**. (HE. X 100).

Table 5. Showing the HP changes in the liver lobules of group 4.1 at different periods post whole body γ-irradiation.

Time interval post irradiation	Cell degeneration	Atrophy	Necrosis	Nuclear changes	Central vein	Sinusoids	Kupffer cells	Cell infilterate
1st day	** diffuse hydropic	*** diffuse	*** lytic * massive coagulative	*	** D and C	** D and C	* Increase with pigmentation	* PNL
3rd day	** diffuse hydropic	** mid zone	** lytic ** massive coagulative	**	*** D and C PLL	** D and C	Lost	** PNL
8th day	** diffuse hydropic	** mid zone	** Lytic ** massive coagulative	**	D *** D and C PLL	** D and C	Lost	** PNL
During 2nd week	** diffuse hydropic	** mid zone	** lytic * peripheral coagulative	*	*** D and C with CLL	*** D and C	** increase in number with intensive pigmentation	** Mononuclears
During 3rd week	-	** peripheral	-	-	** PLL in some areas while in others CLL	** D and C	* increase in number but with less pigmentation	* Mononuclears
During 4th week	-	* peripheral	-	-	* PLL in some areas while in others CLL	* D and C	-	-

PNL : Polymorphonuclear leucocytes
D and C : Dilated and congested
PLL : Partial lining loss
CLL : Complete lining loss
* : mild
** : moderate
*** : severe

Table 6. Showing the HP changes in the Portal tracts of group 4.1 at different periods post whole body γ-irradiation.

Time interval post IR	Size	Inflammatory cells	Bile ducts	Artery	Vein	New Vessels (Angomatoids)
1st day	* N	-	preserved	* D and C	** D and C	-
3rd day	* Increased * Edema	* PNL s around bile ducts	*Damage	* D and C * PLL	** D and C with PLL	-
8th day	** Increased ** Edema	** Mononuclears around bile ducts	** Damaged	* D and C * PLL	** D and C with PLL	*
During the 2nd week	** Increased ** Edema	** Mononuclears around bile ducts	** Duct-penia	** D and C with PLL	*** D and C with CLL	**
During the 3rd week	* Increased * Edema	-	* New ducts	* D and C	** D and C	*** Branching
During the 4th week	* Increased * Edema	-	** New dutcs	-	* D abd C	** Branching

PNL: Polymorphonuclear leucocytes
D and C: Dilated and congested
PLL: Partial lining loss
CLL: Complete lining loss
N: Normal
*: Mild
** Moderate
*** Severe

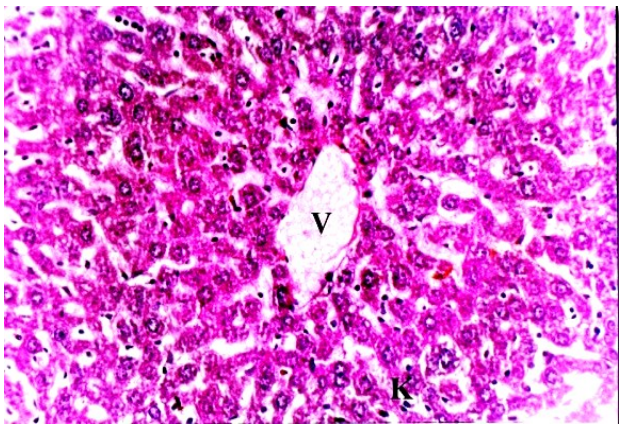

Figure 12. Light micrograph of a section in the liver of a mouse in Group (4.2) showing that the liver lobule resumed its normal architecture with increased Kupffer cells **(K)**. (HE. X 400).

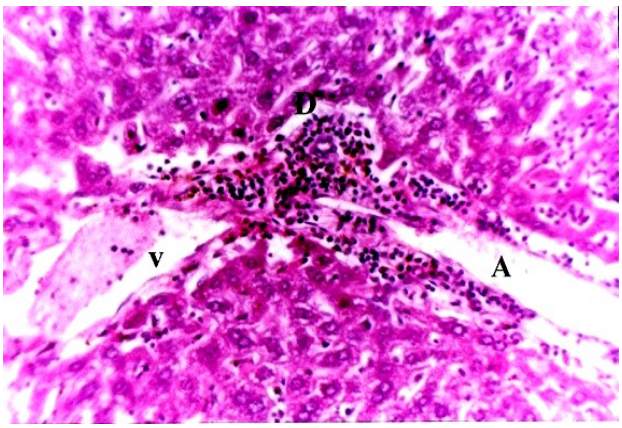

Figure 13. Light micrograph of a section in the liver of a mouse in Group (4.2) showing mononuclear cell infiltration of the portal vein, portal artery **(A)** and bile duct **(D)**. (HE. X 400).

DISCUSSION

Laser photo modulation in the present study was done using He-Ne Laser with the fluence of 5 J/cm^{2}and wavelength 632.8 nm. In a study done by one of the authors Salem (2011) [12] to assess the capacity of different laser energy densities on the liver tissue repair in gamma irradiated mice, low energy densities 3,4 and 5 J/cm^2 were more effective than higher doses (6

and 10 J/cm^2) and the most effective dose was 5 J/cm^2. On the contrary, Castro-e-Silva *et al.* (2007) [13] came to conclusion that the energy density of 10 J/cm^2 is the most effective dose in stimulating early stage of liver regeneration in rats submitted to partial hepatectomy.

Barbosa *et al.* 2011 [14] used He-Ne visible/ red laser at a wavelength of 660 nm in stimulating liver regeneration in rats after partial hepatectomy. During the present study laser irradiation was applied on alternative days for 4 weeks. Ng *et al.* (2004) [15] found that the multiple application treatment program of laser on alternate days seem to be more effective compared to either using a single treatment or in successive days. On the other hand, Andrade *et al.* (2014) [5] documented that with the use of equal daily doses of 5 J/ cm^2 better wound healing can be achieved. The current study was concerned with two modes of human exposure to IR, the 1st mode occurs in case of radiation accidents. This type was represented by irradiating the mice by one shot of γ-rays at a sublethal dose of 5 Gy. On the other hand, radiation occupational workers as well as patients receiving radiotherapy could be exposed to cumulative doses. This mode was represented in the current study by exposing mice to sublethal dose of γ-rays in fractionated daily installations of 1 Gy.

The radiation-induced alteration of liver in mice by the dose of 5 Gy was also studied by Khan *et al.* (2015) [16]. Kurland *et al.* (2015) [17] also, reported that 10 Gy WB γ-I is a lethal dose and induced acute response.

The present results revealed that exposure of mice to IR either by the shot or cumulative modes caused a high significant increase in serum transaminases compared to the control level throughout the whole experimental four weeks. These results agree with those of Sridharan and Shyamaldevi (2002) [18] who reported that WBI of rats to γ- rays caused an increase in serum AST and ALT. They postulated that, excessive production of free radicals and lipid peroxides might have caused the leakage of these cytosolic enzymes. The increase in serum AST and ALT levels under the effect of γ- irradiation was also reported by Nwozo *et al.* (2013) [19].

The present results revealed that He-Ne laser treatment of the two (IR) groups caused a gradual progressive amelioration of the harmful effect of γ- irradiation on serum transaminases.

During the course of the present study, the destructive effects on the liver due to the whole body irradiation of mice (two IR groups) were evident by the occurrence of the reversible changes including, inflammation, degeneration and liver cell atrophy as well as irreversible HP changes including hepatic necrosis. HP changes suggestive of inflammatory responses where the liver lobules and the portal tracts showed mononuclear cell infiltration, were evident post shot γ-irradiation. Moreover, intensive edema and massive cellular infiltration of the portal tracts were observed post cumulative γ-irradiation. Ibe *et al.* (2005) [20] stated that chronic inflammation is a lengthy process, in which active inflammation, tissue destruction, and attempts at healing occur in a simultaneous manner and is characterized by the infiltration of mononuclear inflammatory cells, tissue destruction, and attempts at healing by fibrosis and angiogenesis. The present results showed that He-Ne treatment of both γ-irradiated groups caused resolution of inflammation in the shot irradiated group as well as in the cumulative irradiated group. Piva *et al.* (2011) [21] documented that reduction in the inflammatory process by LLL is through modulating inflammatory mediators (IL-1b, IL-6) and inflammatory cells (macrophages and neutrophils). The efficacy of LLL in the dose of 5J/cm^2 as an anti-inflammatory modality was proposed by Silveir *et al.* (2016) [22]. Coagulative necrosis was seen in the current experiment within the shot γ-irradiation and with cumulative γ-irradiation. Mansoub and Sarvestani (2011) [23] came to the conclusion that radiation exposure induced coagulative necrosis which was due to small artery injury and thrombotic occlusion.

The mechanistic explanation of the ability of laser to counteract the inflammation and

coagulative necrosis induced by IR in this study could be attributed to the significant increase in antioxidants such as superoxide dismutase and glutathione dehydrogenase with significant decrease in oxidants such as malondialdehyde as well as significant repair in DNA and increase in platelet derived growth factors [4].

During the course of the present study progressive vascular changes in the commutative γ- irradiated group in the form of dilation and congestion were observed in central vein as well as in the portal blood vessels. Kurland *et al.* (2015) [17] stated that progressive dilation of the vessels in the liver of WB irradiated mice could be considered as a reactive change that may be related to the inhibitory effect of gamma irradiation on vascular smooth muscle which induced relaxation and consequent vasodilatation. The ability of laser photomodulation to ameliorate the vascular damage induced by ionizing radiation could be explained on the basis of the findings of El-Batanony (2009) [4]. The author [4] found a significant expression of nitric oxide synthase in human polymorphonuclear lecucytes when exposed to low level laser.

In the present experimental work, loss of endothelial lining in the central veins and portal blood vessels was evident in the cumulative group. Endothelial dysfunction after IR exposure was considered one of the important mechanisms of vascular damage associated with both acute and prolonged exposure to IR [24]. It is worthy to indicate that laser therapy in the current study resulted in dramatic restoration of the endothelial lining of the central vein and blood sinusoids in the shot γ-irradiation and with cumulative γ-irradiation. Góralczyk *et al.* (2015) [25] came to believe that the use of laser radiation with a wavelength of 635 nm was associated with a statistically significant increase in the proliferation of endothelial cells. It is worth mentioning that an increase in the number and pigmentation of KCs was noticed in the inflammatory phases in the liver of mice exposed to shot and to commutative γ-irradiation. Also, this increase was noticed in spontaneous healing in the shot γ-irradiated group. The results of the present study showed the biostimulatory effect of LLL on KCs during the healing process of the liver after cumulative γ-irradiation, where laser therapy was associated with a marked increase in KCs. The dual role of KCs in liver inflammation as well as in the liver's response to stresses has been discussed by many researchers [26, 27, 28, 29] as well as [30]. Data of the present study proves the biostimulatory effects of He-Ne laser on the hepatocytes, as laser radiation was able to reduce the time required by the hepatocytes to regain its normal arrangements and size with restoration of liver configuration to nearly half the time needed for spontaneous healing.

CONCLUSION

It is possible to conclude that in acute WB γ exposure of the mice with a single sublethal dose of 5 Gy dose the main brunt was on the liver cells while with exposure to the same dose in fractionated daily installations of 1 Gy the main brunt was on the vascular system including the central veins and portal blood vessels. The present results also emphasize the cellular photobiomodulatory effect of LLL on hepatocytes, KCs and endothelial cells. Results of the study highlight the potential role of laser photobiomodulation in combating the destructive effect of ionizing radiation on the liver. The way to clinical application of such a modality needs further studies in larger sized animals to avoid the problem of laser beam penetration.

***Conflicts of interest*:** *Declared none.*

REFERENCES

1. Karu TI and Kolykov SF (2005) Exact action spectra for cellular responses relevant to phototherapy. Photomed. *laser Surg,* ***23 (4)****: 355 - 61.*
2. Suresh S, Merugu S, Mithradas Sivasankari N (2015) Low-

level laser therapy: A biostimulation therapy in periodontics. *SRM J Res Dent Sci,* ***6****: 53-6*

3. Mester A (2013) Laser biostimulation photomedicine and laser *surgery. June,* ***31(6)****: 237-239.*
4. El-Batanony M (2009) Low level laser therapy for wound healing: Clinical and Biochemical assessments. Laser in medicine science and Practice in medicine surgery. *Dentistry and Veterinary, Ed. Z. Simunovic, pp 722-741.*
5. Andrade FDO, Clark RM, Ferreira ML (2014) Effects of low-level laser therapy on wound healing. *Rev. Col. Bras. Cir. Mar. /Apr.* ***41(2)****: 129-33.*
6. Wagner VP, Maurer L, Martins MA, Danilevicz CK *et al.* (2013) Influence of different energy densities of laser phototherapy on oral wound healing. *J Biomed Opt,* ***18 (12)****: 128002.*
7. de Loura Santana C, de Silva F, Deana AM, Prates RA *et al.* (2015) Tissue responses to postoperative laser therapy in diabetic rats submitted to excisional wounds. *PLoS One. Apr 24;* ***10 (4)****:* e0122042. doi: 10.1371/journal.pone.0122042. eCollection 2015.
8. Wagner VP, Curra M, Webber LP, Nör C, *et al.* (2016) Photobiomodulation regulates cytokine release and new blood vessel formation during oral wound healing in rats. *Lasers Med Sci, Feb* ***11***
9. Bakshi MV, Azimzadeh O, Barjaktarovic Z., Kempf SJ, Merl-Pham J, Hauck SM, Buratovic S (2015) Total body exposure to low-dose ionizing radiation induces long-term alterations to the liver proteome of neonatally exposed mice. *J Proteome Res,* ***14(1)****: 366-73.*
10. Reitman S and Frankel S (1957) A colorimetric method for the determination of serum glutamic oxaloacetic acid and glutamic pyruvic transaminases. *Am J Clin Pathol,* ***??****: 28-56.*
11. Bancroft JD, Stevens A, Turner DR (1996) Theory and practice of histological techniques. 4th ed. Churchil Livingstone, New York, London, San Francisco, Tokyo.
12. Salem ES (2011) Assessment of the capacity of different laser energy densities on the liver tissue repair in γ- irradiated mice. *J Rad Res Appl Sci,* ***4****: 2(B) 669-689.*
13. Castro - e - Silva, S Zucoloto OJ, Marcassa LC (2007) Spectral response for laser enhancement in hepatic regeneration for hepatoectomized rats. *Lasers Surg Med,* ***32 (1)****: 50 - 3.*
14. Barbosa AJ, Santana AD, Castro e Silva. T, Kurachi C, *et al.* (2011) Effect of laser on the remnant liver after the first 24 hours following 70% hepatectomy in rats. *Acta Cirúrgica Brasileira , Vol. 26 (6).*
15. Ng GY, Fung DT, Leung MC, Guo X (2004) Comparison of single and multiple applications of GaAlAs Laser on rat medial collateral Ligament repair. *Lasers Surg Med,* ***34(3)****: 285-9.*
16. Khan, K, Manna DK, Das SB Kesh M. Sinha *et al.* (2015) Gossypetin ameliorates ionizing radiation- induced oxidative stress in mice liver—a molecular approach. *Free Radical Research V* ***49****: Issue 10*
17. Kurland IJ, Broin PO, Golden A, Su G, Meng F, Liu L, *et al.* (2015) Integrative metabolic signatures for hepatic radiation injury. *PLoS One,* ***10(6)****: e0124795.*
18. Sridharan SO and. Shyamaldevi CS (2002) Protective effect of N-acetylcysteine against gamma rays induced damages in rats-biochemical evaluations. *Indian J exp Biol Feb,* ***40(2)****: 181-6.*
19. Nwozo SO, Yakubu OF, Oyinloye BE (2013) Protective effect of aqueous extracts of aframomum melegueta on γ -radiation-induced liver damage in male Wistar rats. *Mil Med Sci Lett, (Voj. Zdrav. Listy),* ***82(3)****: 126-132.*
20. Ibe O, Erin Morency E, Sosa P, Burkow-Heikkinen L (2015) The role of near- infrared light-emitting diodes in aging adults related to inflammation Healthy *Aging Research , 4 24.*
21. Piva JAC, Abreu EM, Silva V, Nicolau RA (2011) Effect of low-level laser therapy on the initial stages of tissue repair: basic principles. *An. Bras. Dermatol.* ***86*** *(5) Rio de Janeiro Sept./Oct.*
22. Silveira PC, Scheffer DD, Glaser V, Remor AP, *et al.* (2016) Low-level laser therapy attenuates the acute inflammatory response induced by muscle traumatic injury. *Free Radic Res May;* ***50(5)****: 503-513.*
23. Mansoub HN and Sarvestani HA (2011) Effects of gamma irradiation on histomorphology of different organs in rats. *Annals of Biological Research,* ***2(6)****: 580-585.*
24. Soloviev AI, Tishkin SM, Parashikov AV, Ivanova IV, Goncharov EV, Gurney AM, (2003) Mechanisms of endothelial dysfunction after ionized radiation: selective impairment of nitric oxide component of endothelium-dependent vasodilation. *Br J pharmacol,* ***138(5)****: 837-44.*
25. Góralczyk K, Szymańska J, Łukowicz M, Drela E *et al.* (2015) Effect of LLLT on endothelial cells culture. *Lasers Med Sci,* ***30 (1)****: 273-8.*
26. Kmieć Z (2001) Cooperation of liver cells in health and disease. *Adv Anat Embryol Cell Biol,* ***161****: III- XIII 1-151.*
27. Bilzer M, Roggel F, Gerbes AL (2006) Role of Kupffer cells in host defense and liver disease. *Liver Int,* ***26 (10)****: 1175-86.*
28. Nguyen-Lefebvre AT and Horuzsko A (2015) Kupffer Cell. Metabolism and Function. *J Enzymol Metab,* ***1(1)****: 101.*
29. Tan Q, Hu J, Yu X, Lu WGH, Yu Y (2016) The Role of IL-1 Family Members and Kupffer Cells in Liver Regeneration. *BioMed Research International, Volume, 6 pages.*
30. Wynn TA and Vannella KM (2016) Macrophages in tissue repair, regeneration, and fibrosis. *Immunity,* ***44 (3)****: 450-462*,