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Experimental Study of the Role of Soft Laser in the Production of Probable Histological Alterations in the Architecture of Mouse Hepatocyte

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Abstract

Our investigational present work was carried out using twenty four healthy male Swiss albino mice (age, 7-9 weeks; weight range, 24-28g). Mice were divided into 4 groups (n=6/group). The first group comprised control group (nonirradiated) of mice, and the other three groups served as irradiated groups. The upper right quadrant of the abdomen of the mice in the second, third, and fourth groups were irradiated by gallium arsenide laser (GaAs) for 15, 20, and 25 minutes once daily during the entire period of irradiation (7 days) respectively.

The laser caused various degrees of alterations in the architecture of the hepatocytes in both third and fourth irradiated groups of mice. In the third irradiated group of mice, the architecture of the hepatocyte revealed abundant alterations such as cleavage of the cytoplasm and multiplication of the nucleus, whereas in the fourth irradiated group of mice, the architecture of the hepatocyte revealed more abundant alterations such as cleavage of the cytoplasm and the multiplication of the nucleus is undistinguished because of the dark appearance of the cytoplasm as compared with the hepatocyte of the third irradiated group of mice. In the second irradiated group of mice, the architecture of the hepatocyte remained changeless as compared with the architecture of the hepatocyte in the first control group (nonirradiated) of mice.

Keywords: liver, laser, histological alterations, mice

1. Introduction

The liver is considered as the largest internal organ of the body (Mescher 2013; Ramadori *et al*. 2008) weighing about 1.5 Kg in adults or comprising 2% of the body weight (Mescher 2013), which located just below the diaphragm, in the right upper quadrant of the abdominal cavity (Mescher 2013). The liver is composed mainly of an important cells called hepatocytes which constitute 70-80% of the cytoplasmic mass of the liver (Grisham *et al*. 1975). Hepatocyte is involved in the formation and secretion of bile (Grisham *et al*. 1975). The hepatocytes are arranged in plates separated by vascular channels called sinusoids. The span of life of the hepatocyte averages 5 months, and they have the ability to regenerate (Grisham *et al*. 1975). There is another cell called Kupffer cell located inside the liver sinusoid which phagocytose aged erythrocytes (Haubrich 2004).

Moreover, Kupffer cells represent the specialized macrophages of the liver (hepatic macrophage). In 1876, these cells were firstly observed by Karl Wilhelm von Kupffer (Haubrich 2004). In 1898, after many years of research, a Polish scientist called Tadeusz Browicz, identified these cells accurately as macrophages (Szymanska & Schmidt-Pospula 1979; Stachura & Galazka 2003). The beginning of their growth is in the bone marrow with the genesis of promonocytes and monoblasts into monocytes and then on to monocytes of peripheral blood reaching to an end of their differentiation into Kupffer cells (Naito *et al*. 1997). Kupffer cells account nearly 15% of the total liver cell population (Bouwens *et al*. 1986). They are playing an important role in removing bacteria and foreign proteins from the blood reaching to an end of their differentiation into Kupffer cells (Naito *et al*. 1997). Kupffer cells account nearly 15% of the total liver cell population (Bouwens *et al*. 1986). They are playing an important role in removing bacteria and foreign proteins from the blood which is finally essential to the primary function of the liver, that is purifying the blood of foreign materials and toxic substances. In the absence of foreign materials (e.g., bacteria or bacteria products), Kupffer cells are free from activity (Kolios *et al*. 2006; Wheeler 2003).

There are several types of disorders of the liver (liver diseases) consisting of viral hepatitis, toxic and alcoholic liver diseases, and autoimmune hepatitis representing dangerous worldwide health problem in the humans (Tu *et al*. 2013; Verma & Thuluvath 2007; Czaja 2014).

Moreover, it is very essential to use histological examination including conventional histology in the diagnosis of most liver diseases (Wang *et al*. 2014).

Fibrosis of liver can be explained by an abnormality in accumulation of extracellular matrix in the liver. Advanced state of fibrosis in the liver bring about cirrhosis, liver failure, and portal hypertension and often needs liver transplantation (Ramadori & Saile 2004; Saile & Ramadori 2007; Ramadori & Saile 2004).

There are terms used to describe low-level laser (or light) therapy (LLLT) such as "cold laser", "soft laser", "biostimulation" or "photobiomodulation", (Roelandts 2002). Particularly, low-level laser therapy has considered as treatment for tissue repair or pain control (Enwemeka *et al*. 2004).

Many types of mammalian and microbial cells gave an evidence that they can respond to LLLT (Tiphlova & Karu 1991). For studies of wound healing type, these cells are suitable to be endothelial cells (Moore *et al*. 2005), fibroblasts (Hawkins & Abrahamse 2005), keratinocytes (Yu *et al*. 2003) and probably some types of leukocytes such as macrophages (Young *et al*. 1989) and neutrophils (Fujimaki *et al*. 2003). For studies of nerve regrowth and pain relief these cells will be neurons (Chen *et al*. 2005; Miloro *et al*. 2002; Balaban *et al*. 1992) and glial cells (Bymes *et al*. 2005).

For the applications of anti- inflammatory and anti-edema the cell types will be macrophages (Young *et al*. 1989), mast-cells (el Sayed & Dyson 1996), neutrophils (Lopes-Martins *et al*. 2005), lymphocytes (Agaiby *et al*. 2000).

It must be noted that the mouse is a good model for any experimental study of liver tissue for the reason that the mouse, just as in human, has a liver composed of four major lobes, and a gall bladder (Baheti *et al.* 2003; Helegbe *et al.* 2011; Aidoo *et al.* 2012).

So, we designed this experimental study for the purpose of proving the presence or absence probable histological alterations caused by soft laser in the architecture of the mouse hepatocyte.

2. Materials & Methods

Our investigational work was carried out using twenty four healthy male Swiss albino mice (age, 7-9 weeks; weight range, 24-28g). Because of the importance and speciality of the experiment, all the mice were housed under particular circumstances such as a 12h light/dark cycle, provided with standard laboratory food and water, and at controlled temperature (22-25°C).

Mice were divided into 4 groups (n=6/group), as the following:

- First Nonirradiated Group: Nonirradiated control mice.
- Second Irradiated Group.
- Third Irradiated Group.
- Fourth Irradiated Group.

The upper right quadrant of the abdomen of the mice of the second, third, and fourth groups were irradiated by gallium arsenide laser (GaAs) for 15, 20, and 25 minutes once daily during the entire period of irradiation (7 days) respectively. The laser was provided at a distance of 1 cm from the target abdomen of the mice. The wavelength of the laser was (lambda = 890nm), spot size of 1 cm^3, and power density of 10 mJ/cm^3. At the end of the irradiation period, the mice were sacrificed 24 hr after last irradiation, the livers were processed by using routine histological methods. Also, the livers of the control group of mice were processed by using the same method for the purpose of comparison with the livers of the other three irradiated groups of mice. Sections were prepared from liver tissue embedded in paraffin and stained with hematoxylin and eosin stain for histopathological examination by using light microscopy at distinct magnifications.

3. Statistical Analysis

By using Statistical Package for Social Scientists (SPSS) V.16 for statistical analysis of the histological alterations in the hepatocyte which includes:

3.1 Descriptive Statistic:

By using excel program for statistical analysis which represents the percentage ratios of the histological alterations in the hepatocyte caused by soft laser (Figure 1).

3.2 Inertial Statistic:

By using Kolmogorov- Smirnov test P-value ($P<0.01$)

4. Results

The results of our present experimental study proved that gallium arsenide (890nm) laser caused alterations in the architecture of the mouse hepatocyte. Our histological findings of the examinations of the sections of mice livers revealed as the following:

4.1 First Group: Control Group of Mice (nonirradiated)
Showed normal architecture in the hepatocyte (Figure 2).

4.2 Second Irradiated Group
Mice were irradiated for 15 minutes once daily during the entire period of irradiation (7 days) produced no histological alterations in the hepatocyte in spite of laser exposure (Figure 3).

4.3 Third Irradiated Group
Mice were irradiated for 20 minutes once daily during the entire period of irradiation (7 days). The histological alterations in the hepatocyte were observed obviously for example, cleavage of the cytoplasm and multiplication of the nucleus (Figure 4) as compared with the second irradiated group of mice which produced no histological alterations in the hepatocytes (Figure 3).

4.4 Fourth Irradiated Group

Mice were irradiated for 25 minutes once daily during the entire period of irradiation (7 days). The histological alterations in the hepatocyte were more obvious as compared with the third irradiated group of mice (Figure 4) for example, cleavage of the cytoplasm and multiplication of the nucleus is undistinguished because of the dark appearance of the cytoplasm of the hepatocyte due to laser irradiation (Figure 5). It would be helpful giving the chief points of overall results of our experimental work as the following table:

Table: Histological Alterations in the Hepatocyte Caused by Soft Laser

Number of Mice Per Group = 6			
Group	**Time of Irradiation**	**Duration time**	**Histological Alterations in the Hepatocyte**
First (control)	_____	_____	Normal architecture of the hepatocyte
Second (irradiated)	15 minutes once daily	7 days	No histological alterations were produced in the hepatocyte in spite of laser exposure
Third (irradiated)	20 minutes once daily	7 days	• Cleavage of the cytoplasm of the hepatocyte • Multiplication of the nucleus of the hepatocyte
Fourth (irradiated)	25 minutes once daily	7 days	• Cleavage of the cytoplasm of the hepatocyte • Multiplication of the nucleus of the hepatocyte is undistinguished • Dark appearance of the cytoplasm of the hepatocyte due to laser irradiation (increasing in time irradiation)

* K.S = 33.62, P < 0.01 H.S = High significant, the histological alterations caused by soft laser in the hepatocyte are of high significant between both first control and the second irradiated groups of mice and both third and fourth irradiated groups of mice.

5. Discussion

In order to improve our deep insight into the laser and its uses, we hypothesized that there were histological alterations that may occur or not caused by soft laser in the mouse hepatocyte.

The hypothesis of our experimental work has become a fact for the reason that it provided a strong evidence in accordance with our results that soft laser affected the hepatocytes and caused various degrees of alterations in the architecture of the mouse hepatocyte. In the first control group of mice, as observed by light microscopy, the hepatocyte has normal architecture (Figure 2). In the second irradiated group of mice, as observed by light microscopy, the architecture of the hepatocyte showed absence of histological alterations in spite of laser exposure (Figure 3). That was owing to the time of irradiation which might be insufficient to alter the characteristics of the hepatocyte, whereas the architecture of the hepatocyte in the third irradiated group of mice as observed by light microscopy proved presence of abundant alterations such as cleavage of the cytoplasm and multiplication of the nucleus (Figure 4) as compared with the architecture of the hepatocyte in the second irradiated group of mice (Figure 3). That was due to the increasing in time of irradiation of laser from 15 to 20 minutes respectively. And, so on, with the increasing in time of irradiation which became 25 minutes, the architecture of the hepatocyte in the fourth irradiated group of mice revealed more abundant alterations as observed by light microscopy such as cleavage of the cytoplasm and the multiplication of the nucleus is undistinguished because of the dark appearance of the cytoplasm of the hepatocyte (Figure 5) as compared with the architecture of the hepatocyte in the third irradiated group of mice (Figure 4). This was due to the sufficient time of irradiation that made the laser more effective. The results of our experimental paper could be considered as positive results because of the observed alterations in the hepatocyte as a result of laser activity or could be said laser stimulation according to the responsive ability of the hepatocyte towards the laser which may lead to increase the ability of the hepatocyte to regenerate consecutively if there was an injury in the liver tissue.

Our investigational paper demonstrated sufficient evidence justifying the use of soft laser as an active and valuable tool in the same time to stimulate the hepatocyte and alter its architecture. Few papers have been published consisting of the role of soft laser or low level laser in altering the architecture of the hepatocyte and that coincides with what reported previously by De Castro e Silva *et al*. (2001) that few works have been done to investigate the use of laser for liver regeneration. Moreover, current literature indicated that there is no experimental paper has been conducted including the use of our soft laser and its parameters such as wavelength and power density in altering the architecture of mouse hepatocyte exclusively.

Finally, we hope to achieve more and more with our colleagues further experimental investigations about the role of the laser in the fields of medicine especially in the field of histopathology in order to put ourselves in the right direction to the scientific achievements for the benefit of humanity if circumstances permit.

6. Conclusion

Our paper has provided the following conclusive remarks:

- Soft laser played a role in eliciting a stimulatory effect in order to alter the architecture of the hepatocyte.
- The observed alterations in the hepatocyte caused by soft laser could affect liver functions positively.
- Soft laser appears to be a promising method for regeneration approaches for different tissues in the future.
- We feel strongly that in spite of many reports that showed positive effects of the laser in general, but in the same time the laser and its uses have given rise to much controversy.

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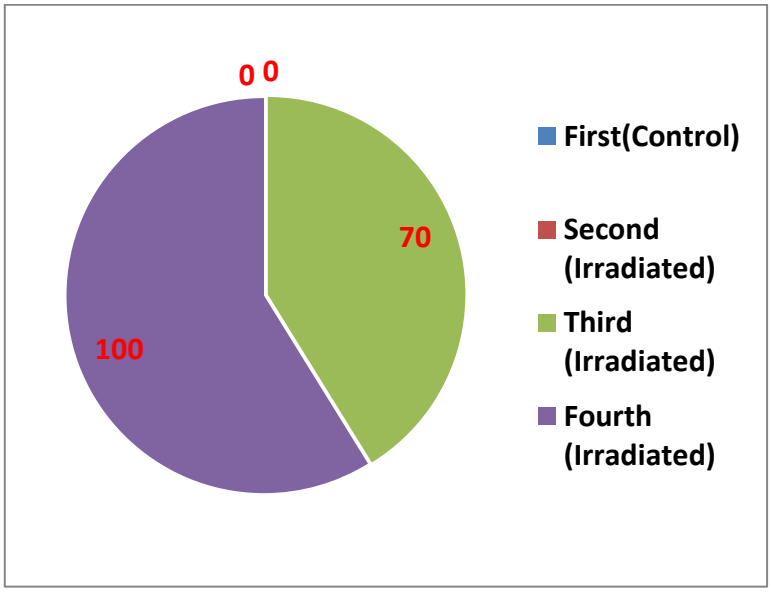

Figure (1): Representation of the percentage ratios of the hitological alterations in the hepatocyte caused by soft laser by using excel program.

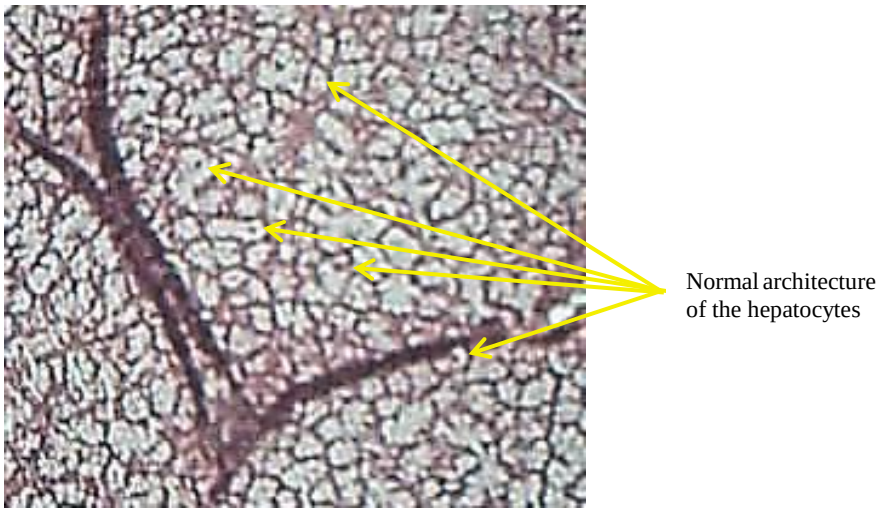

Figure (2): Light micrograph of the liver tissue in the first control group of mice representing normal architecture of the hepatocytes. (H & E X 10).

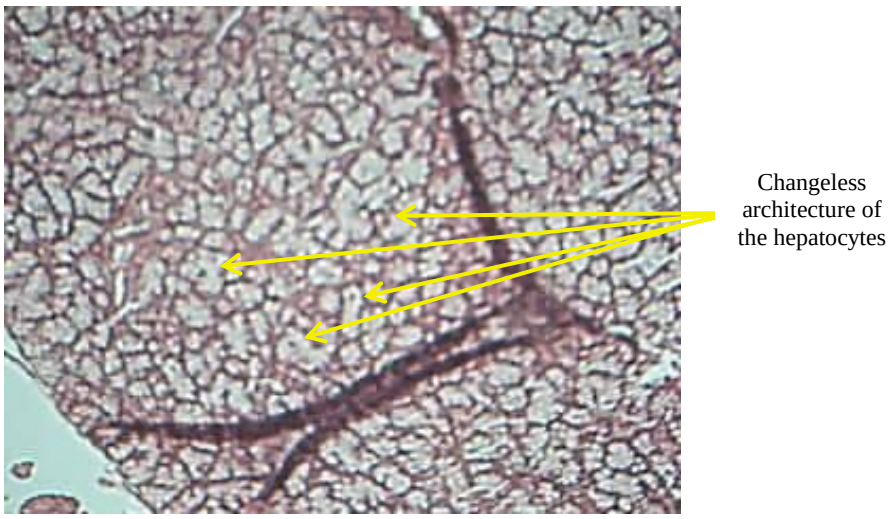

Figure (3): Light micrograph of the liver tissue in the second irradiated group of mice representing changeless architecture of the hepatocytes in spite of laser exposure. (H & E X 10).

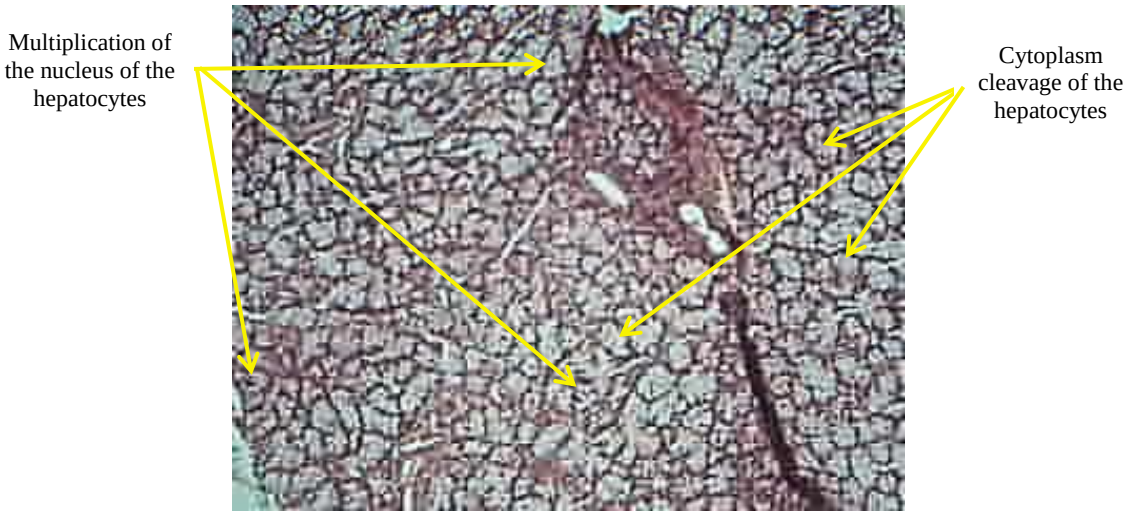

Figure (4): Light micrograph of the liver tissue in the third irradiated group of mice representing abundant alterations in the architecture of the hepatocytes. (H & E X 10).

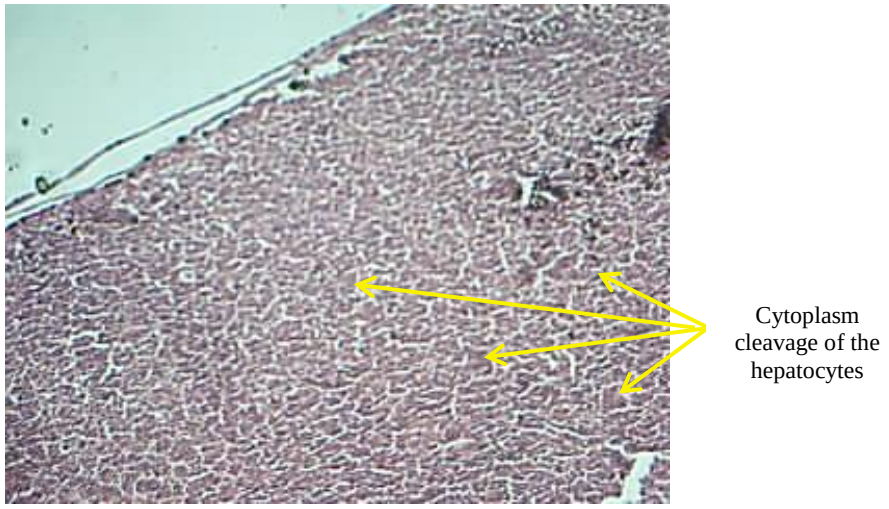

Figure (5): Light micrograph of the liver tissue in the fourth irradiated group of mice representing more abundant alterations in the architecture of the hepatocytes. (H & E X 10).