Eurasian J Med 2016; 48: 76-83 The Eurasian Journal of Medicine **Original Article**

Once the Light Touch to the Brain: Cytotoxic Effects of Low-Dose Gamma-Ray, Laser Light, and Visible Light on Rat Neuronal Cell Culture

Işık Bir Kez Beyne Değmeye Görsün: Sıçan Nöronal Hücre Kültürü Üzerinde Düşük Doz Gama-Işını, Lazer Işığı ve Görünür Işığın Sitotoksik Etkileri

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Abstract

Objective: We aimed to evaluate the effects of gamma-ray, laser light, and visible light, which neurons are commonly exposed to during treatment of various cranial diseases, on the viability of neurons.

Materials and Methods: Neuronal cell culture was prepared from the frontal cortex of 9 newborn rats. Cultured cells were irradiated with gamma-ray for 1-10 min by ^{152}Eu, ^{241}Am, and ^{132}Ba isotopes, visible light for 1-160 min, and laser light for 0.2-2 seconds. The MTT tetrazolium reduction assay was used to assess the number of viable cells in the neuronal cell cultures. Wavelength dispersive X-ray fluorescence spectrometer was used to determine Na, K, and Ca levels in cellular fluid obtained from neuronal cell culture plaques.

Results: Under low-dose radiation with ^{152}Eu, ^{241}Am, and ^{132}Ba isotopes, cell viability insignificantly decreased with time ($p>0.05$). On the other hand, exposure to visible light produced statistically significant decrease in cell viability at both short- (1-10 min) and long-term (20-160 min). Cell viability did not change with 2 seconds of laser exposure. Na, K, and Ca levels significantly decreased with gamma-ray and visible light. The level of oxidative stress markers significantly changed with gamma-ray.

Conclusion: In conclusion, while low dose gamma-ray has slight to moderate apoptotic effect in neuronal cell cultures by oxidative stress, long-term visible light induces remarkable apoptosis and cell death. Laser light has no significant effect on neurons. Further genetic studies are needed to clarify the chronic effect of visible light on neuronal development and functions.

Keywords: Neuron, cytotoxicity, cell culture, apoptosis, laser light, radiation

Öz

Amaç: Amacımız, çeşitli kranial hastalıkların tedavisi esnasında nöronların sıklıkla maruz kaldığı gama ışını, laser ışığı ve görünür ışığın etkilerinin değerlendirilmesidir.

Gereç ve Yöntem: Nöronal hücre kültürü 9 yeni doğan sıçan ön korteksinde hazırlandı. Kültür hücreleri ^{152}Eu, ^{241}Am ve ^{132}Ba izotoplarının gama ışınları ile 1-10 dakika boyunca, görünür ışık ile 1-160 dakika boyunca ve lazer ışını ile 0,2-2 saniye boyunca ışınlandı. MTT tetrazolium azalmasının ölçülmesi, nöronal hücre kültürlerindeki canlı hücre sayısını değerlendirmek için kullanıldı. Dalgaboyu dağılımlı X-ışını floresans spektrometresi, nöronal hücre kültürü plakalardan sağlanan hücresel sıvı Na, K, Ca ve seviyelerini belirlemek için kullanıldı.

Bulgular: Hücre canlılığı, ^{152}Eu, ^{241}Am ve ^{132}Ba izotoplar ile düşük dozda radyasyon altında, istatiksel olarak anlamsız bir, zamanla azaldı. Öte yandan, görünür ışığa maruziyet hücre canlılığında hem kısa süreli (1–10 dk) hem de uzun süreli (20-160 dk) maruziyette anlamlı derecede düşüşe sebep olur. Hücre canlılığı lazere 2 saniye maruz kalma ile değişmedi. Na, K ve Ca seviyeleri gama ışını ve görünür ışık maruziyeti ile önemli ölçüde azalmıştır. Oksidatif stres belirteçleri seviyesi gama-ışını ile önemli ölçüde değişti.

Sonuç: Düşük doz gama ışını oksidatif strese bağlı nöronal hücre kültürlerindeki apoptotik etkisi hafif ve orta düzeydeyken, uzun süreli görünür ışık dikkate değer bir apoptoz ve hücre ölümüne neden oldu. Lazer ışığı nöronlar üzerinde önemli bir etkiye sahip değildir. Görünür ışığın nöron gelişimi ve işlevleri üzerinde kronik etkisini açıklamak için ileri genetik çalışmalar yapılmasına ihtiyaç vardır.

Anahtar Kelimeler: Nöron, sitotoksisite, hücre kültürü, apoptoz, laser ışığı, radyasyon

Received: December 22, 2015 / **Accepted:** April 14, 2016
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DOI: 10.5152/eurasianjmed.2015.0304

Introduction

Since neurons are typically postmitotic cells, they are vulnerable to external and internal damaging factors which may lead to cell death and clinically evident loss of neurological function [1]. Radiation is an external factor that significantly affect neurogenesis and cognitive functions [2].

Radiation has two major components in electromagnetic spectrum: ionizing and non-ionizing radiation [3]. Ionizing radiation has high energy to remove electrons from atoms creating ions. Ionizing radiation is effective and commonly used for the treatment of intracranial tumors, but it also induces potential adverse effects in the normal brain tissue which is associated with serious morphologic and functional deficits [4-6]. X-ray and gamma-ray are typical examples of ionizing radiation widely used for medical purposes such as diagnostic radiology and treatment of cancer [7]. Non-ionizing radiation, such as visible light, microwave, and radiowaves has lower energy than ionizing radiation, and commonly used in daily life for various purposes [3]. Unlike other types of radiation, Light Amplification by Stimulated Emission of Radiation (laser) light has a single wavelength that can be focused in a very narrow beam, which makes it powerful and precise. Laser light is used to shrink or destroy tumors or precancerous growths, to relieve certain symptoms of cancer, such as bleeding or obstruction, or for very precise surgical work or for cutting through tissue [8]. In addition to laser light, neural tissue may expose to visible light in close distance for long durations during cranial microneurosurgical operations performed under microscopy.

Ionizing radiation hydrolyze water into hydroxyl and hydrogen free radicals, which cause oxidative stress leading to cell injury and eventually cell death. Radiation injury also directly damage the DNA, which is repairable if the damage is not too severe, but when the damage is too severe to be corrected, the cell triggers its intrinsic suicide program and dies by apoptosis [9]. As a result of apoptosis processes, extracellular deposition of calcium is often associated with cell death. Yang et al. [10] showed that gamma-ray cause cytotoxicity in a dose-dependent manner in hippocampal cultured neurons by oxidative stress. Okamoto et al. [11] suggested that radiation induces structural defects which inhibits neural development in addition to its apoptotic effect. However, there is still limited data in literature to conclude on the direct biological effects of radiation on immature neurons [10, 11].

Although few neuroprotective agents, which interfere with pro-apoptotic signaling pathway or chemical process of apoptosis, were suggested to protect neuronal tissue from cytotoxic effect of radiation, the best approach to minimize the damage of irradiation seems currently to reduce the radiation dose and duration as much as possible [12-15]. For this aim, it is necessary to know the dose and time limits for damaging effect of different types of radiation.

In this study, we aimed to contribute to the present literature on the effect of low-dose ionizing radiation on neurons in cell culture and also to evaluate the cytotoxic effect of non-ionizing radiation including high-dose visible light and laser light on neuron cells, which has not been extensively studied before.

Materials and Methods

Neuronal culture

This experimental animal study was performed in Atatürk University Pharmacology Laboratory with the approval of the Institutional Ethics Committees for Animal Studies. Nine newborn Sprague-Dawley (<24-hour) rats were sacrificed by decapitation under sterile conditions. After removing skin and skull, meninges were separated and frontal cortex was isolated. Cortex particles were placed in Hanks Balaced Salt Solution (HBSS, Sigma Co., St. Louis, USA) solution and incubated. The settled cortex material was then placed on steril petri dishes and minced finely with a 20 size razor blade. HBSS (1.5 mL) and trypsin (0.3 mL) (Sigma-Aldrich Co., UK) was added over cortex material, which was then transferred into 15-mL tubes by a syringe and incubated for 35 minutes at 37°C. To inactivate trypsin, trypsin/tissue mixture was put into DNase I and 10% fetal serum calf (FCS, Biol. Ind.) containing media for 10 min. HBBS (6 mL) was then added and the tube was spinned at 800 rpm for 10 min. HBSS and 10 mL of neuronal base medium was added onto the precipitated material. As a supplement to neuronal base medium, B27 at 1/50 ratio and then penicillin at 1/1000 ratio was poured over the mixture.

A 150 µL of the mixture was put on each well of 96-well plates (Corning Incorp.) and incubated at 37°C in 5% CO_2 containing incubator for one week. Then, the mixture of neurobasal medium+B27 was added onto the each of the wells containing cells at a rate of ½ of cell volume and was waited until neuronal cells lines the base of the plate and shows branching that can be seen under a microscope.

Irradiation of neuronal cell cultures

Cultured cells on 96-well plates were irradiated with gamma-rays for 1 min, 2 min, 5 min, and 10 min; visible light for 1 min, 2 min, 5 min, 10 min, 20 min, 40 min, 80 min, and 160 min; and laser light for 0.2 s, 0.5 s, 1 s, and 2 s. Each plate was divided into five sections, each of which was given different duration of radiation. The sections that was not exposed to radiation were isolated by using the lead plates. All experiments were repeated twice on the second set of cell culture plates under the same conditions, and the result was given as the mean of two measurements.

Table 1. Radiation levels (μGy) measured at two chips located in each time colon of irradiation

Isotope	Time	Radiation measured at 1st chip (μGy)	Radiation measured at 2nd chip (μGy)
^{152}Eu	1 min	542.39	369.9
	2 min	403.19	402.65
	5 min	549.13	650.84
	10 min	576.96	621.33
^{241}Am	1 min	716.53	485.8
	2 min	467.68	510.54
	5 min	922	1323.6
	10 min	974.71	1007.1
^{132}Ba	1 min	597.41	481.68
	2 min	466.59	427.2
	5 min	669.79	884.84
	10 min	778.29	744.09

μGy: microgray; Eu: Europium; Am: Americium; Ba: Barium

Gamma-rays were produced by using Americium-241 (^{241}Am), Europium-152 (^{152}Eu), and Barium-132 (^{132}Ba) isotopes. One plate (control) was not irradiated. Visible light was obtained from the fiber optical illuminator (World Precision Instruments, Model Cls. 7063.1502, 50 Hz 21w/150w). Two radiation chips were located at the back of each plate to measure the level of radiation.

Cell viability assay

The MTT (3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide) tetrazolium reduction assay was used to assess the number of viable cells in the neuronal cell cultures [16, 17]. To prepare MTT reagent, MTT was dissolved in Dulbecco's Phosphate Buffered Saline pH=7.4 to 5 mg/mL to produce a clear golden-yellow solution. The MTT solution was filter-sterilized through a 0.2 μm filter into a sterile, light protected container and stored at -20°C for long-term usage. For the assay, 10 μL MTT solution was added per well to achieve a final concentration of 0.45 mg/mL, and incubated 1-4 hours at 37°C. Solubilization solution was added to each well and mixed. The absorbance was recorded at 570 nm.

Element analysis

Wavelength dispersive X-ray fluorescence spectrometer (WDXRF) was used to determine sodium (Na), potassium (K), and calcium (Ca) levels as μg/cm^2 in cellular fluid obtained from neuronal cell culture plaques. The medium in plates exposed to radiation was collected with micropipette and put into 1.5-cc test tubes in which element analysis was made.

P value of Na, K and Ca concentrations (μg/cm2) by WDXRF spectrometer element analysis of cellular fluid obtained from cell culture plaques following irradiation with gamma-rays or short- term visible light and long-term visible light or laser light are $^{b}p<0.001$ and $^{c}p<0.05$ for comparison with control cell cultures.

P value of TAS and TOS levels in neuronal cell cultures exposed to gamma-rays and short-term visible light and is $^{b}p<0.05$ and $^{c}p<0.001$ for comparison with control cell cultures.

P value of TAS and TOS levels in neuronal cell cultures exposed to long-term visible light or laser light is $^{b}p<0.05$ for comparison with control cell cultures.

Oxidative stress markers

Total antioxidant status (TAS) and total oxidant status (TOS) were determined in the irradiated neuronal cell cultures. TAS was measured with a photometric method as described by Tomasch et al. [18]. TOS was quantified with a fully-automated calorimetric measurement method. TAS and TOS levels were expressed as mmol Trolox Equiv/L.

Statistical analysis

Statistical analysis was performed by the SPSS software package for Windows (Statistical Package for Social Sciences, version 15.0, SPSS Inc.; Chicago, Illinois, USA). Study data were summarized with descriptive statistics (e.g., mean±standard deviation), and irradiation and time groups were compared with one way analysis of variance (ANOVA). Significant results obtained by ANOVA were further evaluated with LSD test for post-hoc comparisons. Statistical level of significance was set to $p<0.05$.

Results

Average radiation dose measured at two chips located cell culture plates showed that radiation dose induced by ^{152}Eu, ^{241}Am, and ^{132}Ba isotopes increased steadily with time (Table 1).

Cell viability

Number of viable cells in neuron culture media determined by MTT assay was summarized in Table 2. In the first experiment performed under low-dose radiation with ^{152}Eu, ^{241}Am, and ^{132}Ba isotopes, cell viability insignificantly decreased with time ($p>0.05$). (Table 2). On the other hand, exposure to visible light produced statistically significant decrease in cell viability at both short- (1–10 min) and long-term (20–160 min) (Tables 2 and 3). Cell viability did not change with 2 seconds of laser exposure (Table 3).

Element analysis

The outcome of element analysis by WDXRF spectrometer of cellular fluid obtained from cell culture plaques following irradiation was summarized in Table 4. The element analysis revealed that Na, K, and Ca levels significantly decreased starting after 2-min of irradiation with ^{152}Eu and ^{132}Ba isotopes, and even after 1-min of irradiation with ^{241}Am isotope and visible light (Table 4). Irradiation with visible light for long-term (20-160 min) also caused significant decrease in the levels of all three elements (Table 5). However, laser light significantly decreased Na level after 0.2-2 seconds of irradiation and K levels after only 0.2 and 0.5 seconds. Ca level did not affected by exposure to laser light (Table 5).

Oxidative stress markers

TAS levels decreased significantly on cell culture materials exposed to ^{152}Eu and ^{132}Ba, this decrease started earlier with ^{132}Ba irradiation (Table 6). TAS level increased after exposure to ^{241}Am and visible light, however this increase was not statistically significant except the TAS level after 5 min exposure to visible light, which was significantly higher than TAS level of control samples ($p<0.05$, Table 6). TOS levels significantly increased with ^{132}Ba and ^{241}Am, but significantly decreased

Table 2. Number of viable cells by MTT assay in neuronal cell cultures exposed to gamma-rays or short-term visible light

	^{152}Eu	^{132}Ba	^{241}Am	Visible light
Control[a]	0.175±0.047	1.04±0.29	0.122±0.02	0.145±0.023
1 min	0.168±0.052	1.315±0.18[b]	0.186±0.22	0.126±0.021[b]
2 min	0.174±0.071	0.956±0.186	0.169±0.098	0.125±0.019[b]
5 min	0.162±0.084	0.961±0.223	0.19±0.136	0.13±0.021
10 min	0.144±0.067	1.175±0.331	0.104±0.022	0.126±0.025

[a]No radiation was applied to control cell cultures.
[b]$p<0.001$ for comparison with control cell cultures.
MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

Table 3. Number of viable cells by MTT assay in neuronal cell cultures exposed to long-term visible light or laser light

Visible light		Laser light	
Radiation duration	Number of viable cells	Radiation duration	Number of viable cells
Control[a]	1.006±0.32	Control[a]	1.265±0.245
20 min	0.855±0.152	0.2 s	1.063±0.308
40 min	[b]0.755±0.164	0.5 s	1.597±0.445
80 min	[c]0.43±0.147	1 s	1.55±0.374
160 min	[c]0.385±0.193	2 s	1.428±0.401

[a]No radiation was applied to control cell cultures.
[b]$p<0.001$ and [c]$p<0.05$ for comparison with control cell cultures.
MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

Table 4. Na, K and Ca concentrations (μg/cm²) by WDXRF spectrometer element analysis of cellular fluid obtained from cell culture plaques following irradiation with gamma-rays or short-term visible light

Radiation duration	^{152}Eu			^{132}Ba			^{241}Am			Visible light		
	Na	K	Ca	Na	K	Ca	Na	K	Ca	Na	K	Ca
Control[a]	0.543± 0.121	0.787± 0.228	0.361± 0.005	0.599± 0.010	0.9± 0.020	0.45± 0.020	0.424± 0.082	0.649± 0.134	0.315± 0.070	0.499± 0.020	0.68± 0.010	0.349± 0.020
1 min	0.524± 0.253	0.771± 0.283	0.382± 0.135	0.62± 0.020	0.91± 0.020	0.475± 0.013[c]	0.248 ±0.005[b]	0.414± 0.027[c]	0.214± 0.030[c]	0.249± 0.018[b]	0.44± 0.020[b]	0.201± 0.020[b]
2 min	0.258± 0.018[b]	0.387± 0.070[b]	0.172± 0.047[b]	0.44± 0.009[b]	0.61± 0.020[b]	0.3± 0.010[b]	0.3± 0.033[c]	0.46± 0.039[c]	0.207± 0.016[c]	0.621± 0.020[b]	0.76± 0.020[b]	0.349± 0.020
5 min	0.21± 0.037[b]	0.357± 0.142[b]	0.163± 0.070[b]	0.331± 0.020[b]	0.48± 0.020[b]	0.231± 0.010[b]	0.158± 0.094[b]	0.258± 0.189[b]	0.12± 0.094[b]	0.109± 0.010[b]	0.13± 0.020[b]	0.051± 0.010[b]
10 min	0.37± 0.019[c]	0.584± 0.005	0.344± 0.071	0.35± 0.008[b]	0.58± 0.015[b]	0.277± 0.008[b]	0.287± 0.080[c]	0.432± 0.187[c]	0.218± 0.055[c]	0.058± 0.008[b]	0.07± 0.010[b]	0.03± 0.010[b]

[a]No radiation was applied to control cell cultures.
[b]p<0.001 and [c]p<0.05 for comparison with control cell cultures.
WDXRF: Wavelength dispersive X-ray fluorescence spectrometer

Table 5. Na, K and Ca concentrations (μg/cm²) by WDXRF spectrometer element analysis of cellular fluid obtained from cell culture plaques following irradiation with gamma-rays or short-term visible light

Visible light				Laser light			
Radiation duration	Na	K	Ca	Radiation duration	Na	K	Ca
Control[a]	0.47±0.054	0.715±0.108	0.44±0.056	Control[a]	0.51±0.020	0.821±0.021	0.406±0.014
20 min	[b]0.619±0.106	[b]0.965±0.186	0.406±0.091	0.2 s	[b]0.593±0.030	[b]0.921±0.020	0.429±0.020
40 min	[b]0.297±0.001	[c]0.525±0.025	[b]0.243±0.020	0.5 s	[c]0.474±0.001	[b]0.73±0.020	0.39±0.020
80 min	[b]0.316±0.031	[c]0.51±0.056	[b]0.261±0.061	1 s	[c]0.559±0.008	0.809±0.020	0.409±0.020
160 min	[b]0.321±0.053	[c]0.539±0.033	[b]0.308±0.024	2 s	[b]0.699±0.020	0.829±0.020	0.409±0.020

[a]No radiation was applied to control cell cultures.
[b]p<0.001 and [c]p<0.05 for comparison with control cell cultures.
WDXRF: Wavelength dispersive X-ray fluorescence spectrometer

with ^{152}Eu irradiation (Table 6). Visible light had no effect on TOS levels (Tables 6, 7). In long-term exposure to visible light, TAS showed decreasing tendency, which was significant at 40 and 80 min (p<0.05, Table 7). Laser light decreased TAS level at 0.5 and 2 s (p<0.05), but had no effect on TOS level (Table 7).

Discussion

In the present study, we evaluated the effects of gamma-ray, laser light, and visible light, which neurons are commonly exposed to during treatment of various cranial diseases and microneurosurgical procedures, on the viability of neurons. Our findings showed that while low dose gamma-ray has slight to moderate apoptotic effect in neuronal cell cultures, long-term visible light induces remarkable apoptosis and cell death. Laser light has no significant effect on neurons.

For the evaluation of cytotoxic effect of ionizing and non-ionizing radiation *in vitro*, studies commonly use the assays determining the viability of cells directly and/or the measurement of intra- and extracellular enzymes, reactive oxygen species or elements showing indirectly the viability or death of cells [10]. In the present study, along with viable cell counting in neuronal cell cultures exposed to irradiation, we also determined the concentration of Na, K, and Ca ions in the extracellular fluid to estimate the range of cell death, and TAS and TOS levels to evaluate the role of oxidative stress in radiation-induced cytotoxicity. MTT assay, which we used for

Table 6. TAS and TOS levels in neuronal cell cultures exposed to gamma-rays and short-term visible light

Radiation duration	152Eu		132Ba		241Am		Visible Light	
	TAS	TOS	TAS	TOS	TAS	TOS	TAS	TOS
Control[a]	3.94±0.14	65.16±7.00	3.94±0.20	81.47±6.89	3.94±0.17	85.08±4.72	3.94±0.18	55.25±4.35
1 min	3.69±0.17	60.02±5.42	[b]3.48±0.12	[b]99.06±5.57	3.59±0.16	[b]94.72±5.50	4.25±0.15	54.55±2.42
2 min	3.81±0.18	[b]46.43±5.12	[b]3.35±0.08	91.74±5.25	3.62±0.22	[b]89.38±6.84	4.2±0.11	54.56±1.97
5 min	3.85±0.09	[b]53.28±3.99	[b]3.56±0.13	77.43±4.66	3.7±0.2	[b]95.31±5.11	[b]4.44±0.11	49.83±5.45
10 min	[b]3.13±0.13	[b]46.62±2.72	3.65±0.08	[b]100.47±4.61	4.05±0.2	[c]124.04±8.32	4.23±0.22	61.94±3.93

[a]No radiation was applied to control cell cultures.
[b]p<0.05 and [c]p<0.001 for comparison with control cell cultures.
TAS: total antioxidant status; TOS: total oxidant status

Table 7. TAS and TOS levels in neuronal cell cultures exposed to long-term visible light or laser light

Visible light			Laser light		
Radiation duration	TAS	TOS	Radiation duration	TAS	TOS
Control[a]	3.94±0.11	5123.21±57.29	Control[a]	3.94±0.17	288.76±9.21
20 min	3.68±0.12	5083.16±87.48	0.2 s	3.93±0.09	307.22±3.09
40 min	3.59±0.09[b]	5193.13±94.61	0.5 s	3.63±0.09[b]	296.76±8.98
80 min	3.59±0.12[b]	5198.88±44.61	1 s	3.87±0.16	287.62±8.69
160 min	3.69±0.09	5300.8±101.98	2 s	3.76±0.21[b]	276.58±6.61

[a]No radiation was applied to control cell cultures.
[b]p<0.05 for comparison with control cell cultures.
TAS: total antioxidant status; TOS: total oxidant status

the assessment of neuronal cell viability in the present study, sensitively shows the function of labile mitochondrial enzymes that lose activity during the process leading to cell death [19].

Radiation is widely used for treatment of various cancers. Radiation also emerges from other radioactive sources (e.g., nuclear power plants). In addition its advantages, radiation has many negative effects on human beings. Radiotherapy applications in the cranial region has been known to have cognitive side effects affecting the patient's quality of life [20]. Irradiation of frontal lobe may cause severe memory, cognitive, and speech disorders at long-term, and even lead to diffuse leukoencephalopathy and cerebral atrophy [20]. Eight years after the Chernobyl nuclear power plant accident, those who participated in the rescue operations and forest workers exposed to 13-63 cGy radiation showed remarkable loss in cognitive functions [21]. The sensitivity of young neurons to radiation is high, and this sensitivity decreases after birth [22]. Children who receive cranial radiation show long-term side effects including memory and learning disorders, which is suggested to result from the radiation-induced damage or death of neural precursor cells within the subgranular layer of the dentate gyrus of the hippocampus [13]. Indeed, irradiation of human neuronal progenitor cell line by gamma- and X-ray induces dose- and time-dependent apoptosis [23]. Monje et al. [24] showed that neurogenic cells are significantly reduced after treatment of malignant brain tumors with irradiation.

As high doses of radiation causes macroscopic changes in living tissue such as radionecrosis, diffuse leukoencephalopathy, or white matter damage, low doses may also damage living tissue and cells at molecular level. In particular, gamma-ray and X-ray have enough energy to break chemical bonds causing to disruption of DNA, which can lead to the point mutations or death of the cell [25].

In the literature, numerous studies showed cytotoxicity of radiation at cellular level [10, 11, 26- 32). Yang et al. [10] reported that gamma-ray (0-4 Gy for 12 hours) cause CASPACE dependent cytotoxicity through oxidative stress on immature rat hippocampal neuronal cell culture. Michelin et al. [30] also confirmed that CASPACE 3 enzyme plays role in gamma-ray induced neuron death and apoptosis. Kim et al. [28] revealed apoptosis in hippocampal neurons after 6

hours-14 days of whole body gamm-rays (0-4 Gy) irradiation of the mice. Furthermore, Moore et al. [31] have been put forward that irradiation of the cerebral tissue of mice causes disruption o blood-brain barrier, secretion of inflammatory cytokines, vascular collapse and consequently cerebral edema via cyclooxygenase-2 enzyme in long-term. In their study of immature and mature neuronal cell cultures irradiated with 0-4 Gy gamma-ray, Song et al. [32] proved that the number of viable cells were reduced by DNA fragmentation and apoptosis in both cell lines with more remarkable cytotoxicity in immature cells. In addition to gamma- and X-rays, carbon ion beams was shown to cause cell death by apoptosis in hippocampal cell cultures from embryonic rats [33]. Al-Jahdarl et al. [26] also showed that carbon ion beams causes neuronal apoptosis and narrowing of growth zone in cell cultures obtained from dorsal root and sympatic ganglions of 8-16 day chickens. It has been also shown on that ionic radiation inhibits neural growth in addition to its cytotoxic and apoptotic effect [11, 27].

In the present study, while we evaluated the effect of low-dose gamma-ray on neuronal cell culture, as primary focus of the study we assessed the impact of high-dose visible light and laser light on neurons, which has been seldomly reported in literature. Our findings indicated that visible light significantly decreases neuron cell viability.

We recorded that low-dose gamma-ray has no remarkable effect on viability of neurons, while they decrease cellular Na, K, and Ca concentrations significantly. This can be explained by the well-known apoptosis inducing effect of low-dose ionizing radiation, which does not disrupt cellular wall but DNA. Disruption of DNA leads to increase in intracellular free radicals and dysfunction of Na-K pump and cellular permeability. The overall effect of these changes in response to radiation is induction of apoptosis process along with increased ions [34-36]. Accordingly, we recorded increased Na, K, and Ca concentrations in cellular fluid without significant cytotoxicity in response to low-dose radiation. TAS and TOS levels also changed with gamma-ray, which also prove that gamma-ray produce cytotoxicity through oxidative stress. However, visible light had limited effect on oxidative stress markers indicating a different mechanism for cytotoxicity induced by visible light, which needs to be evaluated in further studies.

The visible light irradiation, which constitutes the essential part of our study, decreased cell viability in parallel with the decrease in concentration of elements in cellular fluid. This shows that at the applied wavelength, the visible light induces intense apoptosis in neurons. However, our findings indicated that laser light at treatment dose has no effect on viability of neurons or on element concentration of cellular fluid.

Although numerous studies have established that ionizing radiation damages DNA of neurons inducing apoptosis and cellular death [10, 11, 26-33], there are limited number of studies, which reported conflicting results on the effect of visible light on neurons [37, 38]. Ultraviolet light, which is a form of visible light, and phototherapy light have been shown to damage DNA in vitro and in vivo [37-42]. However, some studies reported that phototherapy light has no significant impact on nuclear material [43, 44].

In conclusion, although low-dose gamma-ray induce limited apoptosis in neurons, long-term exposure to visible light induces decrease in viability of neurons, which is a finding supported by the change in element concentration of cellular fluid. This suggests that visible light is able to disrupt genetic material of neurons inducing apoptosis and cell death. Further genetic studies are needed to clarify the chronic effect of visible light on neuronal development and functions. These studies will guide to develop alternative treatment methods in the surgical and medical management of central nervous system diseases that use other form of lights which are both effective and safe.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Atatürk University Pharmacology Laboratory with the approval of the Institutional Ethics Committees for Animal Studies (12/07/2013-68).

Informed Consent: Informed consent was not required because the study was performed on animals.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - M.C., A.C.; Design - M.C., A.C., C.C.; Supervision - M.C.; Resources - A.C., S.S., M.E.S.; Materials - N.T., A.H.; Data Collection and/or Processing - M.C., C.C., A.C.; Analysis and/or Interpretation - H.H.K., S.S., A.H.; Literature Search - M.C., A.C., H.H.K.; Writting Manuscript - M.C., A.C.; Critical Review - H.H.K.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

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