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Study of Mitotic Index and DNA profile when exposure to He-Ne laser and UVC radiation in mice

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Abstract: *In vitro, He-Ne laser show a modifying response of cells to ionizing radiations. So there is a need to show the effect of He-Ne laser (632.8nm), Ultraviolet radiation UVC (250nm) and He-Ne laser pre and post irradiation against the UVC radiation of Mitotic index of femur and in vivo to DNA of testis in Mice.*

In this study 100 albino male mice were divided into five groups, the first group Control which have (10) number of mice, the second group Laser which have (27) number of mice were divided into three groups different time periods (5, 10, 15 min), the third group Ultraviolet radiation (UVC) which have (9) number of mice and duration of exposure one hour, the fourth group laser (5, 10 and 15 min) + UVC (1h) which have (27) number of mice, with ½ hour time interval between the two irradiations and the finally group UVC (1h) + laser (5, 10, 15 min) which have (27) number of mice, with ½ hour time interval between the two irradiations was monitor the effect of radiation on mice according to the classification totals above after various time periods (7, 14, 21 days).

Mitotic index as shown increase the percentage of Mononucleus and less increase of Dinucleus after exposure of the radiation according to the classification totals above.

The He-Ne laser per-irradiation show a protection properties, which appeared the DNA damage against UVC light irradiation. But the He-Ne laser pre-irradiation against UVC irradiation farther more reduce the DNA testis damaging.

UVC shows a damaging effect on the DNA. This damage was reduced by the He-Ne laser pre-irradiation. Thus Laser pre-irradiation may be attributed to the induction of endogenous of radio protectors or which may be involved in DNA damage repair.

Key words: *Mitotic Index , DNA profile, He-Ne laser , UVC , radiation and mice.*

I. Introduction:

Laser is a widely used device in the medical field. In vivo effect of singular and repeated exposure of laser beam on a mammalian model was studied to ascertain any possible effect on mammalian germ cells. Since agents considered to be mutagenic affect sperm head shape, sperm morphology study may be an applicable screen for laser effects on germ cells [1].

Low level laser therapy (LLLT) is a light source treatment that generates light of a single wavelength. LLLT emits no heat, sound, or vibration. Instead of producing a thermal effect, LLLT may act via non thermal or photochemical reactions in the cells, also referred to as photobiology or biostimulation [2].

In other hand (LLLT) is the application of light (usually a low power laser or LED in the range of 1mW – 500mW) to a pathology to promote tissue regeneration, reduce inflammation and relieve pain. The light is typically of narrow spectral width in the red or near infrared (NIR) spectrum (600nm-1000nm), light is absorbed and exerts a chemical change [3].

UV radiation has clear effects on organisms, it causes several biological reaction, through the generation of the free radicals and the reactive oxygen species (ROS), formed by various photochemical processes. The free radicals induces a sequences of events including lipid peroxidation, proteins denaturation and DNA changes [4], “the deoxyribonucleic acid (DNA) changes are the single strand breaks (ssB) and the double strand breaks (dsB)” or cell damage [5].

Scientists classify UV radiation into three types or bands—UVA, UVB, and UVC. The ozone layer absorbs some, but not all, of these types of UV radiation [6]:-

- UVA: Wavelength: 320-400 nm. Not absorbed by the ozone layer.
- UVB: Wavelength: 290-320 nm. Mostly absorbed by the ozone layer, but some does the reach the Earth’s surface.
- UVC: Wavelength: 100-290 nm. Completely absorbed by the ozone layer and atmosphere.

The UV-light causes the formation of pyrimidine dimmers on DNA that provokes changes in the conformation of DNA, thus impairing its genomic contribution in the egg during the fertilization [7, 8]. Although ionizing irradiation continues being used for irradiating eggs, UV-irradiation has become the most common method used for sperm DNA inactivation. Optimal UV-irradiation avoids producing chromosome fragments (minichromosomes) [9].

He-Ne laser irradiation leads to non-lethal oxidative damage to the cells which triggers DNA repair processes, helping the pre-exposed cells to better repair the UV-induced damage to DNA

II. Materials and Methods:

In the current study, the He-Ne laser, UVC doses, and the separation time between the two irradiation are kept fixed. The UVC lamp from Industrial Fiber Optics of 250 nm wavelength, and a power of 20 Watt (W) was used. The He-Ne laser of wavelength 632.8 nm with a maximum output power of 1.0 mW (Industrial Fiber Optics IF-HIV) was employed.

Animals experiments:

In order to assess the influence of He-Ne laser plus the UVC irradiation on testis of Mice the following experiments were performed. 100 adult of albino male mice, were used in this study and were divided into five groups and irradiation as table -1-.

1-First group: the animals normal, which was control group.

2-Second group: was used to characterize the degree of UVC (250nm) irradiation on mice testis. The UVC source placed at 30 cm above the mouse cage, where the final UVC power at the mouse skin surface was 1.2mW, for 1hr.

3-Third group: was employed to study the influence of He-Ne laser irradiation. A continuous He-Ne laser of wavelength 632.8 nm with a maximum output power of 1.0 mW, and a beam diameter of 3mm, was employed. The laser beam was directed on testis for a period of (5, 10, 15 min), which equal to energy dose of (4.2 J/cm^2, 8.4 J/cm^2 and 12.6 J/m²).

4- Fourth group: was pre-irradiated by He-Ne laser (4.2 J/cm^2, 8.4 J/cm^2 and 12.6 J/cm²) for (5, 10, 15min) followed by UVC irradiation, with one hour and wait for ½ hr time (for UVC) interval between the two irradiations.

5- Fifth group: was pre-irradiated by UVC light with one hour wait for ½ hr time, this group was irradiated by He-Ne (5, 10, 15 min) laser of (4.2 J/cm^2, 8.4 J/cm^2 and 12.6 J/cm²), the laser beam
was directed on testis.

Table 1 the animals which used in this study.

NO	Type of Radiation	Number of animals after 7 days	Number of animals after 14days	Number of animals after 21 days
1-	Control	10		
2-	UVC (1h)	3	3	3
3-	a- Laser (5min)	3	3	3
	b- Laser (10min)	3	3	3
	c- Laser (15min)	3	3	3
4-	a- Laser (5min) + UVC (1h)	3	3	3
	b- Laser (10min) + UVC (1h)	3	3	3
	c- Laser (15min) + UVC (1h)	3	3	3
5-	a- UVC (1h) + Laser (5min)	3	3	3
	b- UVC (1h) + Laser (10min)	3	3	3
	c- UVC (1h) + Laser (15min)	3	3	3

Mitotic Index (MI) test:-

This examination was according to [12, 13]:

DNA extraction:

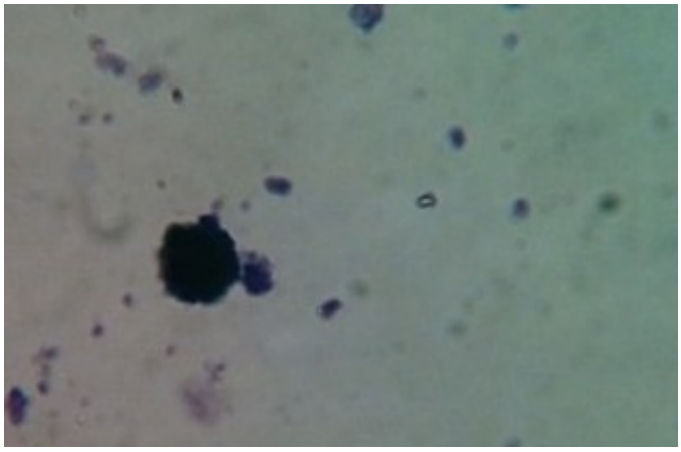
DNA was extracted from testis of mice by using Bioneer-Korea Kit. The DNA was electrophoresed using 1% agrose gel electrophoresis.

III. Results:

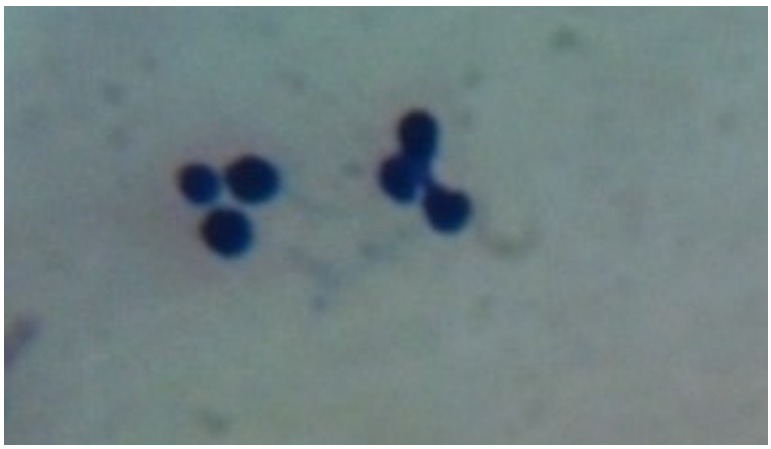
Features found in this study are: the percentage of Mitotic index are summarized in the table 2. And types of Mitotic index illustrated in Fig.1. DNA electrophoresis results are demonstrated in Fig. 2.

Table (2) The percentage of all type of Mitotic index in (7, 14, 21) days

Type of Radiation	After 7 days			After 14 days			After 21 days		
	Normal %	Mononucleus %	Dinucleus %	Normal %	Mononucleus %	Dinucleus %	Normal %	Mononucleus %	Dinucleus %
Control	100	0	0	100	0	0	100	0	0
UVC(1h)	90	10	0	64	35	1	64	35	1
Laser(5min)	80	20	0	85	15	0	97	3	0
Laser(10min)	88	12	0	82	18	0	90	10	0
Laser(15min)	98	2	0	80	20	0	85	15	0
Laser(5min)+ UVC(1h)	85	15	0	95	5	0	94	5	1
Laser(10min)+ UVC(1h)	95	5	0	65	35	0	88	12	0
Laser(15min)+ UVC(1h)	70	30	0	90	10	0	90	10	0
UVC(1h)+Laser(5min)	80	10	0	80	20	0	95	5	0
UVC(1h)+Laser(10min)	90	10	0	90	9	1	74	25	1
UVC(1h)+Laser(15min)	95	5	0	95	5	0	94	5	1



a- Mononucleus



b- Dinucleus

Figure (1) Types of Mitotic index

a- Mononucleus

b- Dinucleus

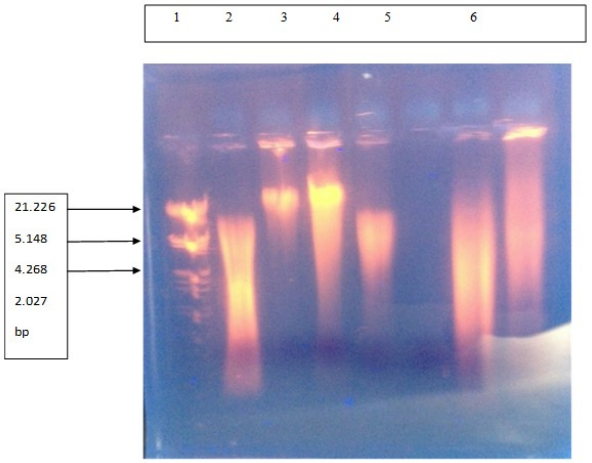


Figure 2: The pattern of Agarose gel electrophoresis of testis DNA columns and bands show different cells treatment lanes from left to right

Lane -1- DNA marker (Lambda DNA/EcoR I+Hind III)

Lane -2-Laser (15 min)

Lane -3-UVC (1h) + Laser (15min)

Lane -4- UVC (1h)

Lane -5- Normal

Lane -6- Laser (15min) + UVC (1h).

IV. Discussion:

Mitotic Index:-

Table 2, and Fig.1 indicated that there are two type of Mitotic index Mononucleus and Dinucleus. The radiation has clear effect of the organisms, it causes several biological reaction, through the generation of the free radicals and the reactive oxygen species (ROS), formed by various photochemical processes. The used UVC (1hr) radiation in this work the percentage of the normal Mitotic index after (7, 14, 21) days (90%, 64%, 64%) the percentage decrease of the control, after (14, 21) days decrease of the 7 days, percentage of the Mononucleus (10%, 35%, 35%) increase of the control, after (14, 21) days the percentage increase of the after 7 days and percentage of the Dinucleus less increase after (14, 21) days of the control and after 7 days (0%, 1%, 1%). The used He-Ne laser of the same dose but in different exposure time (5, 10, 15 min) in this work reveled a toxic effect but the percentage of the Mitotic index when exposure He-Ne laser (5 min) in normal after (7, 14, 21) days (80%, 85%, 97%) the percentage decrease of the control, after 14 days the percentage decrease but increase after 21 days of the after 7 days, in Mononucleus (20%, 15%, 3%) the percentage increase of the control, after (14, 21) days decrease of the after 7 days, in Dinucleus (0%, 0%, 0%) the percentage still constant of the control and after (7, 14, 21) days. In laser (10 min) the percentage of normal after (7, 14, 21) days (88%, 82%, 90%) the percentage decrease of the control, after 14 days the percentage decrease but increase after 21 days of the after 7 days, in Mononucleus (12%, 18%, 10%) the percentage increase of the control, after 14 days increase but decrease after 21 days of the after 7 days, in Dinucleus (0%, 0%, 0%) the percentage still constant of the control and after (7, 14, 21) days. In laser (15 min) the percentage of normal after (7, 14, 21) days (98%, 80%, 85%) the percentage decrease of the control, after 14 days the percentage decrease but increase after 21 days of the after 7 days, in Mononucleus (2%, 20%, 15%) the percentage increase of the control, after 14 days increase and decrease after 21 days of the after 7 days and in Dinucleus (0%, 0%, 0%) the percentage still constant of the control and after (7, 14, 21) days. He-Ne laser (5, 10, 15 min) pre-irradiation against UVC (1hr) irradiation, the percentage of Laser (5 min) + UVC (1hr) after (7, 14, 21) days (85%, 95%, 94%) the percentage decrease of the control, after (14, 21) days the percentage increase of the after 7 days, in Mononucleus (15%, 5%, 5%) the percentage increase of the control, after (14, 21) days the percentage decrease of the after 7 days, in Dinucleus (0%, 0%, 1%) the percentage still constant of the control but less increase after 21 days of the control and after (7,14) days, the percentage of Laser (10min) + UVC (1hr) after (7, 14, 21) days in normal (95%, 65%, 88%) the percentage decrease of the control, after 14 days decrease but increase after 21 days of the after 7 days, in Mononucleus (5%, 35%, 12%) the percentage increase of the control, after 14 days increase but decrease after 21 days of the after 7 days, in Dinucleus (0%, 0%, 0%) the percentage still constant of the control and after (7, 14, 21) days, the percentage of Laser (15min) + UVC (1hr) after (7, 14, 21) days in normal (70%, 90%, 90%) the percentage decrease of the control, after (14, 21) days the percentage increase of the after 7 days, in Mononucleus (30%, 10%, 10%) the percentage increase of the control, after (14, 21) days the percentage decrease of the after 7 days, in Dinucleus (0%, 0%, 0%) the percentage still constant of the control and after (7,

14, 21) days. When He-Ne laser (5, 10, 15 min) performed post UVC (1hr) irradiation the percentage of UVC (1hr) + laser (5 min) after (7, 14, 21) days in normal (80%, 80%, 95%) the percentage decrease of the control, increase after 21 days of the after (7, 14) days, in Mononucleus (10%, 20%, 5%) the percentage increase of the control, after 14 days increase but decrease after 21 days of the after 7 days, in Dinucleus (0%, 0%, 0%) the percentage still constant of the control and after (7, 14, 21) days. In UVC (1hr) + laser (10min) after (7, 14, 21) days in normal (90%, 90%, 74%) the percentage decrease of the control, the percentage after 21 days decrease of the after (7, 14) days, in Mononucleus (10%, 9%, 25%) the percentage increase of the control, after 14 days the percentage less decrease but increase after 21 days of the after 7 days, in Dinucleus (0%, 1%, 1%) the percentage still constant of the control after (7) days but less increase after (14, 21) days of the control and 7days and the percentage of UVC (1hr) + laser (15min) after (7, 14, 21) days in normal (95%, 95%, 94%) the percentage decrease of the control and still constant after (7, 14) but less decrease after (21) days of the (7, 14) days, in Mononucleus (5%, 5%, 5%) the percentage increase of the control and still constant after (7, 14. 21) days and Dinucleus (0%, 0%, 1%) the percentage still constant of the control after (7, 14) days but less increase after (21) days of the control and (7, 14) days.

Laser light when performed after the exposure to ionizing radiation causes an increase in mitotic activity of cells [14, 15] and tissue regeneration [16, 17]. But the photoprotective effect of He-Ne laser light is more significant when cell irradiation is performed prior to the exposure of ionizing radiation, such as UV light [18, 11], X-ray [15], and IR light [19]. Agreement with this work

Pre-exposure to He-Ne laser irradiation may lead to modulate the damaging effects of ionizing radiation and decreasing in radiation damage, by the induction of antioxidant defence mechanisms and accelerated repair or altered cell cycle progression [20].

Recently many workers showed that He-Ne laser pre-irradiation lead to increase cell survival against subsequent UV-light exposure [18, 11]. However, the mechanism for this pre-illumination induced protection against UV-light radiation is not understood. But it was concluded that visible light besides its possible effect might be stimulating other cellular responses [21]. They also noticed that the protection effect of laser pre-irradiation was more pronounced when UV-light induced less cell damaging. They summarized that the photoprotection depend the He-Ne laser exposure time (dose) and period of incubation between He-Ne laser exposure and subsequent UV-light irradiation [10].

DNA (deoxyribonucleic acid):-

DNA was extracted from testis for mice using Bioneer-Korea Kit, the DNA electrophoresis results are shown in the (fig. 2). In the Lane 1 DNA marker was (Lambda DNA / EcoR I + Hind III) which has 4 band (21.226, 5.148, 4.268, 2.027 bp), Laser (15 min) has one band (21.226 bp) with long smear (which was in lane 2) compare with DNA marker, UVC (1h) + Laser (15 min) has one at the level of UVC (1h) with long smear (which was in lane 3) compare with DNA marker, UVC (1h) with long smear (which was in lane 4) has one band nearest to the wells compare with DNA, in Normal mice the DNA (which was in lane 5) has one band was approximately (21.226 bp) compare with DNA marker and Laser (15 min) + UVC (1h) has on band (21.226 bp) with short smear (which was in lane 6) compare with DNA marker.

These results indicate that low power He-Ne laser can improve cell survival for cell damaged with UVC radiation and this in agreement with [11, 22].

From the DNA electrophoresis (Fig. 3.10), it can be seen that UVC (1h), and UVC (1h) +Laser (15min) irradiation give a smear of DNA extraction, but only Laser and also Laser (15min) + UVC (1h) give one band. This results are similar to those reported by [23].

Important mechanisms in human cells to avoid the potential mutation in UV-induced-damaged sites are to completely repair the damage by nucleotide excision repair (NER) before replication on synthesize DNA using post replication repair specific DNA polymerase which is free from error. NER is a highly conserved strategy for repairing a variety of bulky DNA damages such as CPDs and (6-4) pp [24, 25].

The toxic effect of UV light on cells is well known, however the UV light with a shorter wavelength (UVC) is the most potent inducer of cell death [26], because UV irradiation activates a p53-gene which cause DNA-damage [27].

The UV-light causes the formation of pyrimidine (Thymine dimer) on DNA that provokes changes in the conformation of DNA, thus impairing it's genomic contribution in the egg during the fertilization [7, 8]. UVC radiation continually being used for irradiation eggs, to establish the appropriate dose to be used for complete sperm DNA inactivation [9].

This results are similar to those reported by Ridha *et al.,* working on lymphocyte cells in vitro [28]. Recently, researchers observed that He-Ne laser pre-irradiation leads to increase cell survival and reduce the DNA damage against ionizing radiations. The mechanism of the He-Ne laser induce protection appears to be a sort of adaptive response. He-Ne laser irradiation has been reported to lead to the generation of single Oxygen species and also increase the activity of the antioxidant enzymes [29, 30].

As conclusion UVC shows a damaging effect on the DNA. This damage was reduced by the He-Ne laser pre- irradiation. Thus Laser pre-irradiation may be attributed to the induction of endogenous of radio protectors or which may be involved in DNA damage repair.

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