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Protective Effect of Orignm vulgara Against UV Irradiation on DNA

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Abstract.
This research aimed to study the effects of different radiations sources in the DNA structure, the irradiations used in this study is Ultraviolet and this is as a stimulation model for the Iraqi environment after the long exposure for different weapons in the repeated wars that happened, and chemical pollutants from car exhaust as well as from factories activities.The white blood cells were exposed to Ultraviolet ray for different time intervals. Also we study the effects of *Orignm vulgara* fractionation organic extract as a protective method for the destructive effects of irradiations by using DNA fragmentation test.The results showed that short time exposure to Ultraviolet ray for (5, 10, 15 min) at (30, 0 cm) highest have no effect on DNA structure, while the exposure to Ultraviolet ray for (3 hour, 0 cm) produce DNA decay. To counteract this destructive effect of irradiation the *Orignm vulgara* organic extract was added to the cells suspension before irradiation.
Key words: UV Irradiation, DNA fragmentation test, fractionation organic extract.

1. Introduction

It is known that exposure to high levels of air pollution increase the risk of cancer and this risk derived from chemicals that causes cancer that are specific within the complex mixture of air pollution. These chemicals were discovered through the use of molecular techniques, epidemiological, and measurements of biomarkers. Assessment and epidemiological cancer has been the application of these techniques involved in the past with polycyclic aromatic hydrocarbons carcinogens that are associated with particulate air pollutants, has shown clear evidence of the toxic effects on genes, such as DNA and chromosomal aberrations, (Rao *et al*., 2000).

Exposure to Ultraviolet light that comes within sunlight ray is environmentally factor carcinogen for humans. Toxic effect of Ultraviolet rays emerging from sunlight or artificial lamps treatment both of which are dangerous to human health, serious effects of radiation on the skin include inflammation, burns, skin infections and weaken the body's immune system, (Matsumura and Ananthaswamy, 2004).

Ultraviolet radiation can be classified into long UVA and UVB and short average UVC. All of them can destroy the collagen protein fibers and thus speeds up the aging of the skin. Long UVA rays and medium UVB can destroy vitamin A found in the skin (Torma, *et al* 1988).

Ultraviolet photon hits the DNA molecule of living cells in a number of ways, frequent reconfiguration links base thymine to thymine causing swelling of the DNA double helix.

Long-wave UV A, has been considered in the past as less serious, but today it is defined as the factor that speedup skin cancer through indirect sabotage of nuclear DNA. UVA spread deeply but do not cause sunburn and redness of the skin. Since they do not damage DNA directly like middle and short rays but they can generate the center of a highly reactive chemical, such as the roots of hydroxyl and oxygen, which in turn contributes to the destruction of DNA. Scientists have shed some blame that serious skin disease that hit users of solar condoms caused by the lack of filters for the specific wave in condoms (Autier, *et al* 1995).

The wave UVB is causing skin cancer and destroy the collagen fibers, but at a slower pace than UVA, through direct sabotage of irritates the DNA molecules in skin cells, where it causes abnormal covalent bonds are formed between bases cytosine and productive bilateral units. When DNA polymerase comes to increase the priming of this part of the DNA, they read the unit bilateral "AA" and not reading the original "CC", causing the restructuring of links bases thymine to binary thymidine which distorts the shape of a spiral DNA and stop reincarnation and shows gaps and prevent the merger (Svobodová, *et al* 2012). This could lead to genetic mutations and causing cancerous growth. One of the mechanisms of resistance to ultraviolet radiation is occur when the body tends to tan when exposed to a reasonable level of UVA radiation (depending on skin type) and becomes dark brown in color. This mechanism stops

the spread of the UV also prevents strong vandalism to the body's vulnerable tissues.

2. Materials and Methods

2.1. Isolation of white blood cells

The WBCs has been isolated by break down all the red blood cells in the sample by using RBCs break down fluid supplied by (Promega/USA). 900µl of the above fluid was added to 300µl of blood, then they mixed well and left for 10 min. to break down all the RBCs, after that the mixture was centrifuged at 13000 rpm/min. for 20 sec., then the supernatant was removed very carefully and the participant has been washed with PBS buffer (8gm of NaCl, 0.0015gm of Na2HPO4, 0.2gm of KCl, 0.2gm of KH_2PO_4(Sambrook, *et al*, 2001)) the pH was adjusted to 7.3 in room temperature and pH 7.3, the washing step was repeated for two times, and the final step is the re-suspending of cells with 1ml of PBS.

2.2. Preparation of plant extract

The plant extract was prepared by fragmentation method according to (Chunyan, *et al*, 2009), the desired weighted of plant was weighted and mixed with hexane solvent with shacking for about 15 min. after that the mixed(plant plus hexane) was filtered and dried using oven with a temperature setting 50 C^0 for 24 hours. The left over precipitate from the first step was used by mixing it with Ethyl acetate solvent and applying the same procedure, hot water has been added to the supernatant and the precipitate and the whole solution was filtered and the supernatant was dried to obtain the hot water extract.

2.3. Experimental design

2.3.1. Evaluation of exposure time:

For choosing suitable time to exposure WBC to UV light three time used (5 min, 10 min, 15 min, 3horus), then DNA was extracted.

For choosing the highest of the sample from UV source; used [30 cm, 0 cm (touch)].

When the time and highest of UV detected, WBC treated by plant extract as the fallowing below:

1- Plant extract (100mg/ml): 100mg of each plant extract has been dissolved in 1ml of distill water, the mixture has been centrifuged in 10000rpm for 4 min. and the supernatant was removed and stored in a dark environment.

2- Exactly 100µl from each type of plant extract (hexane and hot water plant extract) has been added to each tube that contains WBCs suspension.

A- Group no; 1 was exposed to UV in the presence of hexane in the tube.

B- Group no; 2 was not exposed to UV but the hexane was already added and this group is the first positive control group.

C- Group no; 3 was exposed to UV in the presence of plant extract (extracted by Ethyl Acetate).

D- Group no; 4 was not exposed to UV but the plant extract (extracted by using Ethyl Acetate) was already added and this group is the second positive control group.

E- Group no; 5 was exposed to UV in the presence of plan extract (extracted by using hot water).

F- Group no; 6 was not exposed to UV but the plant extract (extracted by using hot water) was already added and this group is the third positive control group.

G- Group no; 7 was exposed to UV with any additives.

3- All the groups have been exposed to UV ray with 312nm wave length and the source of radiation was placed about 15cm from the tube holder, the exposure time was set to 3 hours. After the end of the UV exposure step the UV source was shut down and the samples was taken for DNA extraction.

2.4.1 DNA extraction from Blood samples.

The manufacturer protocol (Promega/USA) was followed for extraction DNA from Blood samples.

DNA analysis was detected by compare with DNA ladder according to (Al-Terehi**,** 2012)

2.4.2. Agarose Gel Electrophoresis

The procedure of agarose gel electrophoresis for DNA molecule (Prifer, 1984) was done with some modifications.

1- Exactly 1gm of agarose has been dissolved in 100ml of TBE buffer (0.5X) and a hot plate was used for boiling the mixture until the bubbles start to appear.

2- The agarose gel was left to cool down to about 40C^0, and then about 1 drop of ethidum bromide (5mg/ml) has been added.

3- The casting try was assembled and the comb was placed at one end of the try, after that the agarose gel was poured to the desired level.

4- After the polymerization of agarose gel, the comb was removed and the try was placed at the electrophoresis tank and filled with TBE buffer until the buffer has covered the gel to about 1cm on the top of the gel.
5- All the samples have been loaded in the agarose wells after the mixing of 12µl of DNA with 3µl of loading buffer.
6- The samples have been electrophoresed on agarose by applying 70V, 20mA for about 1-2 hours.
7- After the end of electrophoresis the power supply was shut down and the gel has been placed on photo documentation system.

3. Results

Results show that WBCs was irradiated by different highest and interval time of UV light different effect on its DNA, at 30 cm and different time UV don't effect on DNA as show in figure (1), while irradiated WBCs for different time (3 hours, 5, 10, 15 min) touch with UV source; 3 hours only effected on DNA but the others time no effected as show in figure (2).
When WBCs treated by different plant extract (hot water, hexan, ethyl acetate) these extract don't effect on DNA as show in figure(3), also when its used as protective against UV radiation its appeared that its protect DNA from the effect of irradiation as in figure(3).

4. Discussion

The exposure of WBCs to UV for different 3 times at 30 cm from UV source was don't effect on DNA as show in figure (1), this may be because that UV causes simple damage on DNA or micro lesion such as point mutation, thymine dimer, apurinic and test detected macro lesion or chromosome mutation or accumulated micro lesion that causes by exposure to UV or other radiation as low and frequent doses for long time.
When WBC exposure to 3 hours in touch with UV source, this led to clear destruction effects on DNA molecule as showed in (Fig 2, lane 1), this destruction is because of the direct interaction between the UV waves with DNA double helix and this interaction can causes a great damage in the DNA molecule either in the single or double strand. Sinha and hiader (2002) clarified that UV radiation induces two of the most abundant mutagenic and cytotoxic DNA lesions such as cyclobutane–pyrimidine dimers (CPDs) and 6–4 photoproducts (6–4PPs) and their Dewar valence isomers.
The lyses in DNA may be because the indirect effect of ionic waves on DNA molecule, because these waves can ionized water molecule in living cells, and this phenomena can produce free radicals, the reactive oxygen species (ROS) such as Hydrogen Peroxide (H_2O_2), which can a great effect on DNA molecule by producing the compound known as 8-OXOdG and this compound destroy and break down the DNA double helix. These effects could be reversible if the damage is minor because each cells housing a different types of repair systems that can repair theses minor damages happened due to the exposure to UV (Pizarro et al., 2009) .
If the cells have exposed to UV repeatedly or to a single but high dose of UV waves or to a low dose but for a long time of exposure, then the repair systems will be useless and cannot counteract the destructive effects of the UV waves. The final results will be the cumulative of mutations and the increase rate of diseases (Farrell *et al*, 2011).
The most important damage that happened because of UV is the formation of dithymine dinucleotide (TT) between each two adjacent thymine nucleotide on the DNA double helix. This will cause a great challenge to the cell especially in transcription of DNA and in replication of T-G (Sinha 2002).
Also Farrell *et al* (2011) clarify the destruction in mammalian cell DNA causes by apoptosis which triggered by many endogenous and exogenous factors such as UV radiation.
The *Orignm vulgara* is one of important medical plant that use in different application such as antibacterial, anti-fungal, anti-oxidant and anti-radical activity.
Treating WBCs with *Orignm vulgara* plant extracts have decreased the destructive effects of UV as show in figure (3), this may be because *Orignm vulgara* extract contain different natural compound that can work as a protective agents against the UV waves by making a barrier that prevents the UV from entering the cells or it could re-act with the target areas before the UV waves hit these areas.
Phytochemical studies indicate that *O. vulgara* plant contains polyphenols. Such as arbutin, 6-O-4-hydroxybenzoyl arbutin, and 2-hydroxy-3-(3, 4- dihydroxyphenyl) propionic acid were isolated as antioxidants (Nakatani, 2000). Vagi *et al* (2005) detected anti-oxidant compound such as carnosic acid, ursolic acid, and carnosol by the HPLC in plant that used in present study. Also other study found a methanol extract from leaves inhibited rat intestinal

α-glucosidase, 6-hydroxyapigenin, scutellarein, 6-hydroxyapigenin-7-O-β- d-glucopyranoside, 6-hydroxy luteolin-7-O-β-d-glucopy- ranoside, 6-hydroxyapigenin-7-O-(6-O-feruloyl)-β-d-glu-copyranoside, and 6-hydroxylutcolin-7-O-(6-O-feruloyl)-β- d-glucopyranoside were isolated as active principles and related compounds(Kawabata, 2003).

The hexane plant extract that didn't show the same results for the other type of plant extract because The plant extract using hexane contain low polar compounds . hexane is an organic solvent with low polar activity and for this purpose it extract all the low polar compounds, while plant extract (using Ethyl Acetate) is contain only median polar compounds because of the activity of Ethyl Acetate that can only extract the median polar compounds from *Orignm marjorana*.

The plant extract by hexane or Ethyl Acetate can diminish the effects of UV because it contain low and median polar compounds, these compounds have the ability to help repair mechanisms in the cells to be fully functional. Chakraborty (2004) suggested the mechanisms of some phytochemichal compound that include curcumin, resveratrol, indole-3-carbinol, and ellagic acid--to modify the DNA damaging ability of the alkylating carcinogen N-methyl-N'-nitro-N-nitrosoguanidine in cultured Chinese hamster lung fibroblast cells (CH V-79) that these agents are chemopreventive by virtue of their ability to protect DNA as well as to induce DNA repair.

Different plant have the ability to reduce the destructive effect of different agents on genetic materials, these plants contain different compounds that can work as an antioxidant agents which reduces the formation of free radicals by either react with these free radicals to prevent them or react with the target molecule and stop the free radical from destroying these targets such as licorice, Al-Terehi *et al* (2012) clarified the antioxidant and antimutagenicity of licorice root extract against cyclophosphomid by decreased chromosomes aberration and lyses of DNA level in rat. Also Al-Ameri *et al* (2012) found that O*lea europaea* extract decreased the cytotoxic effect of high dose of vitamin A *in vivo*.

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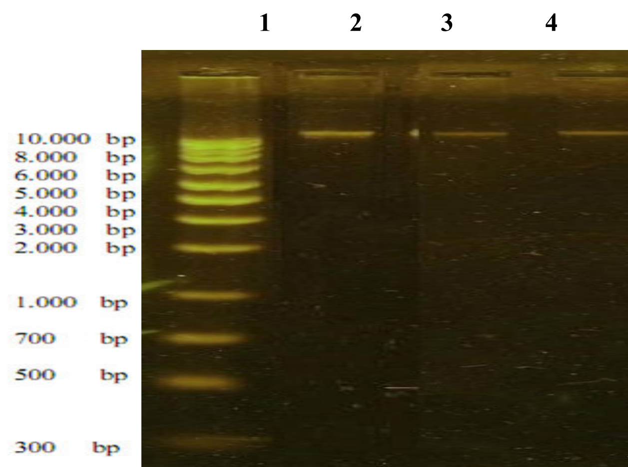

Figure (1). The effect of UV irradiation on human WBC DNA exposure for 3 intervals on 30 cm high.

1- Lane 1: DNA ladder (10kb – 300 bp).
2- Lane 2: DNA extracted from WBC after exposure to UV irradiation for 5 min.
3- Lane 3: DNA extracted from WBC after exposure to UV irradiation for 10 min.
4- Lane 4: DNA extracted from WBC after exposure to UV irradiation for 15 min.

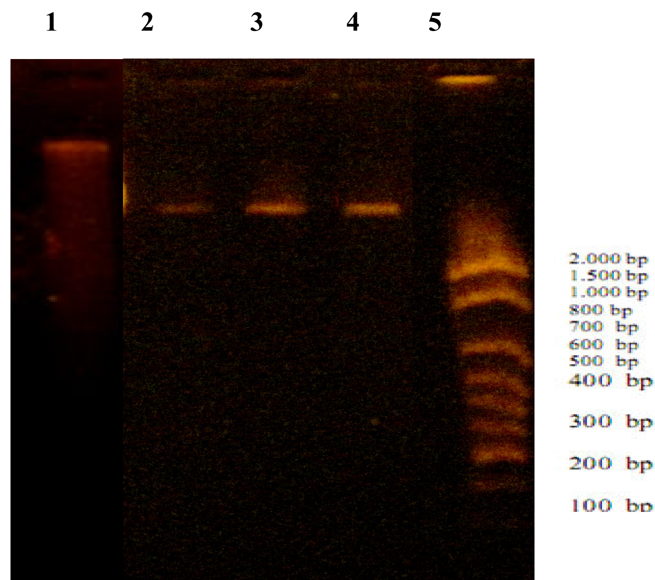

Figure (2). The effect of UV irradiation on human WBC DNA exposure for 3 intervals with no space between the sample and the UV source.

1- Lane 1: DNA extracted from WBC after exposure to UV irradiation for 3 hours.
2- Lane 2: DNA extracted from WBC after exposure to UV irradiation for 5 min.
3- Lane 3: DNA extracted from WBC after exposure to UV irradiation for 10 min.
4- Lane 4: DNA extracted from WBC after exposure to UV irradiation for 15 min.
5- Lane 5: DNA ladder (2kb – 100 bp).

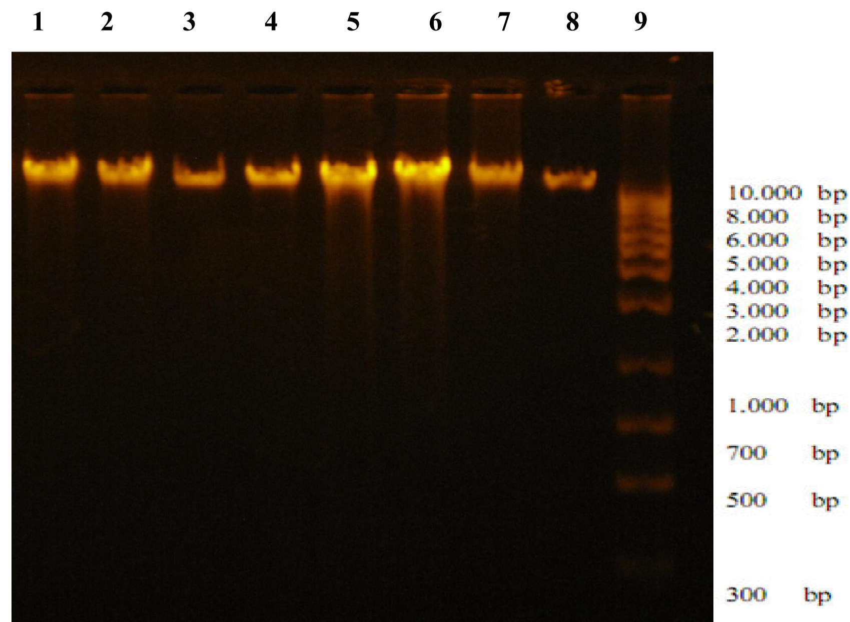

Figure (3): Electrophoresis pattern of DNA molecule extracted from WBCs, exposed to UV irradiation.

1- Lane 1: DNA of WBCs treated with plant extract (hot water extract).
2- Lane 2: DNA of WBCs treated with plant extract (Hexane extract).
3- Lane 3: DNA of WBCs treated with plant extract (Ethyl Acetate extract).
4- Lane 4: DNA of WBCs exposed to UV in the presence of plant extract (hot water extract).
5- Lane 5: DNA of WBCs exposed to UV in the presence of plant extract (Hexane extract).
6- Lane 6: DNA of WBCs exposed to UV light without any additive.
7- Lane 7: DNA of WBCs exposed to UV in the presence of plant extract (Ethyl Acetate Extract).
8- Lane 8: DNA of WBCs exposed to visible light.
9- Lane 9: DNA ladder (10 kb – 300 bp).

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