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UV rays induced DNA damage: Protection by polyphenols enriched extract of mint leaves

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Abstract

DNA of live cells is damaged when exposed to harmful Ultraviolet rays. If the cells are exposed to UV rays exposure time more duration of time, more thymine dimers are formed in the DNA and the greater the risk of an incorrect repair or a missed dimer. To analyze the effect of UV rays Calf thymus DNA and prevention by polyphenol enriched extract of Mint leaves and also its non toxic nature. The above study was done by using submarine gel electrophoresis where UV rays are used to damage the DNA and BHA (400 μ M) used as positive control. The polyphenol enriched extract was taken at 15 μ g concentration prevent UV rays induced DNA fragmentation in submarine agarose gel electrophoresis which provides same protection when compared to standard antioxidant BHA (400 μ M). The cytotoxicity studies showed that, the extract and BHA provides a protection of 54% whereas, the extract of Mint leaves extract showed 72%. In conclusion, the Mint leaves extract showed a promising DNA protectant activity against UV rays induced DNA damage.

Keywords: *Mint leaves extract*; DNA damage; DNA protectant; Cytotoxicity; UV rays; Polyphenols

1. Introduction

Ultraviolet rays are a part of electromagnetic radiation that makes black-light posters glow, and is responsible for summer tans and sunburns [1]. When the exposure to UV radiation is too, it will damage to living tissue [2]. The spectrum of electromagnetic radiations is generally divided into seven regions and are radio waves, microwaves, infrared (IR), visible light, ultraviolet (UV), X-rays and gamma-rays [3]. The sun emits energy in the form of radiations in a wide range of wavelengths. UV radiation is having wavelength range of 290 and 400 nm [4]. With the difference in its wavelength, UV radiation are having both harmful effects as well as the useful ones. In detail, UV rays are of in the range 320-400 nm plays useful role in formation of Vitamin D by the skin, and show harmful effect at the

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range of 290-320 nm like damage at the molecular level to the building block of life called DNA [5-7]. It is reported that, wide UV radiations induces oxidative stress resulting in DNA damage [8-9].

2. Material and methods

Calf thymus DNA (CT DNA), BHA, Agarose, Ethidium bromide was from Sigma Chemical company USA. BHA from HIMEDIA, India. All the other chemicals were of Anal. R grade. Fresh Mint leaves (*Mentha L*) were procured from authentic source.

2.1. Extraction

Leaves are washed thoroughly with water and rinsed in 0.5% KMnO₄ for five minutes and again washed in double distilled water to remove if any microbes present. Further, leaves were shade dried, powdered, sieved and stored in a dry glass container for further use. The polyphenol enriched extract from Mint leaves powder (25 g) was done by mixing with 250mL of methanol solvent for 72 h using Soxhlet extractor. After completion of extraction, the excess methanol was evaporated and the polyphenol enriched crude extract was obtained. Further the extract was suspended in 60 mL of water. The above crude extract further extracted with different organic solvents like hexane, chloroform, ethyl acetate and butanol and at the end all crude extracts were mixed, filtered [10].

2.2. Phytochemical analysis

The phytochemical analysis was done to ensure the presence of bioactive in the extract using standard protocols [11-14].

The protein estimation was carried according to Bradford's method [15] using BSA as standard and absorbance was read at 535nm. Total phenolics content was determined according to the method of Folin Ciocalteu reaction [16] where Gallic acid used as a standard and absorbance was read using UV visible spectrophotometer at 750 nm. Ascorbic acid estimation was carried out according to Sadasivam S., Manickam [17] and the absorbance was read against a reagent blank at 540nm. Total sugar estimation was done according to Dubois method [18] and the absorbance was read at 520 nm. Flavonoids estimation was done according to Cheon et al [19] by using Quercetin as a standard and the absorbance was measured at 415 nm. In the above analysis, standard curve was used to compare.

2.3. Isolation of human peripheral lymphocyte

10ml of venous blood drawn from young healthy donors to isolate human peripheral lymphocytes according to the method Smith et al [20]. In brief, the blood was collected in 5:1 ratio of 85mM citric acid-71mM trisodium citrate-165mM D-glucose (ACD). Four volumes of hemolyzing buffer-150mM NH₄Cl in 10mM Tris buffer, pH 7.4 was added, incubated at 4°C for 30 min. Further the pellet containing cells were washed thrice with 10 ml of 250mM m-inositol in 10mM phosphate buffer pH 7.4 and suspended in same solution. The cell viability was determined by Trypan dye blue exclusion method [21] where 10µl of lymphocyte sample added to 0.02% of 10µl of Trypan blue and the cells were loaded to Neuberg's chamber and the cell number was counted. The survival rate of lymphocytes was determined at time intervals 20th, 40th and 60th minutes of incubation. Viability was tested by Trypan blue exclusion and exceeded 96% in each isolation. Percentage viability was calculated by the formula.

$$\% \text{ viability} = \frac{\text{Total no. of viable cells}}{\text{Total no. of viable cells} + \text{dead cells}} \times 100$$

2.4. Time course study of the effect of UV rays and protection antioxidants on the viability of lymphocytes

The time course study of the effect of U.V. on the viability of lymphocytes and protection antioxidants was done according to the method of Phillips, 1973 with minor modifications as explained in methods [22].

2.5. Submarine agarose gel electrophoresis

DNA submarine gel electrophoresis was carried out using 0.8% agarose prepared in TAE (40mM Tris, 20mM Sodium acetate, 18mM NaCl, 2mM EDTA, pH 8.0) buffer containing 0.2µg/ml of Ethidium Bromide. Electrophoresis was carried out using TAE buffer. Bands visualized under U.V transilluminator.

2.6. UV rays induced DNA damage: Protection by Polyphenols enriched extract of Mint leaves (*Mentha L*) and other standard antioxidant

Finely sheared Calf thymus DNA was exposed to UV radiation (345nm) in presence and absence of antioxidants using germicidal UV lamp (Hanovia Lamp) for 60min at 37°C in 20mM, PBS, pH 7.4. At regular time interval, 200µl of the reaction mixture was drawn and mixed with Ethidium Bromide solution which was prepared using 0.5µg/ml trisodium phosphate buffer, 20mM, 100µM EDTA, pH 11.8. The fluorescence of the solution were measure at 520nm excitation and 590nm [23]. Appropriate blanks and controls were included to rule out non-specific quenching of fluorescence. The reaction mixture corresponding to 3µg of calf thymus DNA drawn at regular intervals of time, run on a 0.8% agarose gel and bands visualized under U.V Transilluminator to determine the protection offered by antioxidants.

2.7. Statistical analysis

All the results were represented as Mean \pm SD. The significance of the experimental observation was checked by students t-test and the value of p value <0.05 was considered significant.

3. Results and discussion

Table 1 Phyto-chemical analysis of *Mint* leaves extract

Phyto-chemicals in Mint leaves	Water extract
Carbohydrates	+
Protein	+
Polyphenols	+
Flavonoids	+
Ascorbic acid	+

Values are means \pm SD of triplicates



Figure 1 UV rays induced DNA damage and its protection by Mint leaves extract and other antioxidants

Lane A: Calf thymus DNA sheared (10 µg)

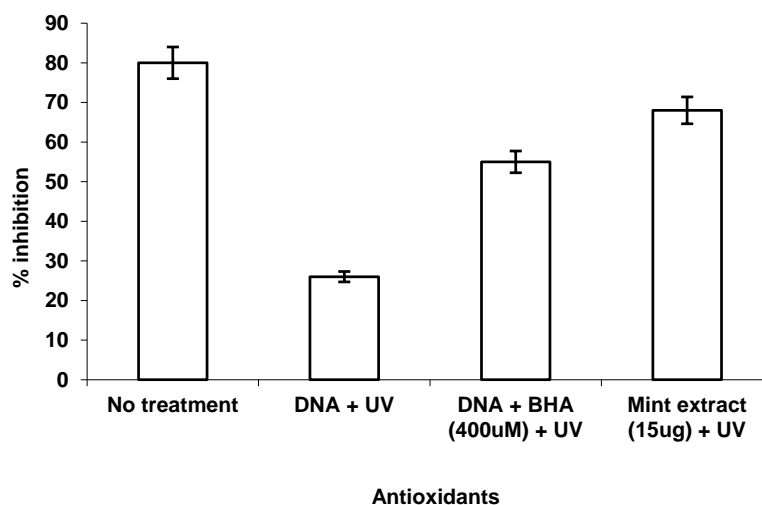
Lane B: DNA + UV radiation + *Mint* leaves extract (15µg)

Lane C: DNA + UV radiation

Lane D: DNA + UV radiation + BHA (400µM)

Sheared Calf Thymus DNA (10 μ g) with and without *Mint* leaves extract (15 μ g) / BHA (400 μ M) in 100 μ l of 20mM Phosphate Buffer Saline (PBS) pH-7.4, exposed to UV radiation (345nm) 37°C for 60min. Reaction mixture of 4 μ g DNA loaded on to 0.8% agarose gel.

Figure 2 Study of cell toxicity induced by UV rays and protection by *Mint* leaves extract and other antioxidants



Lymphocytes (10⁶cells) pretreated with or without antioxidants at indicated concentrations in 0.5ml HBSS pH 7.4, incubated at 37°C for 20min., then exposed to UV rays for 60 minutes in final volume of 1ml HBSS, pH 7.4. After the desired incubation time (60 minutes), viability of the cells was determined by Tryphan blue exclusion and the percentage of viable cells was calculated as mentioned in methods.

It is reported that, extensive exposure to UV rays induces skin cancer As explained in the materials and methods, the polyphenol enriched extract of Mint leaves subjected to proximate analysis. The results showed that, carbohydrates, proteins, Flavonoids and Ascorbic acid are present in a negligible concentration when compared to Polyphenols, Further, the extract subjected to Polyphenols rich extract using different polar and non-polar solvents. The obtained extract was thick dense, dark coloured polyphenol enriched. It was reported that, the UV rays induces sugar breakdown and double strand break in DNA [24-25]. The DNA submarine gel electrophoresis was done as explained in methods. In Figure-1 Lane A shows sheared Calf thymus DNA (10 μ g). Lane B shows, DNA damage caused by UV radiation and protection by Mint extract, Lane C shows that DNA damage caused by UV rays, Lane D showed protection given by BHA against DNA damage caused by UV at 360nm. In the cytotoxicity study, the result shows that, the polyphenol enriched Mint leaves extract is non-toxic. We also studied the protective effects of extract against UV rays induced lymphocyte cell death. The viability of lymphocytes on simultaneous pretreatment of UV rays a time course study was done. The isolated lymphocytes (10⁶cells) pretreated with or without antioxidants at indicated concentrations in 0.5ml HBSS pH 7.4, incubated at 37°C for 20min., then exposed to UV rays for 60 minutes in final volume of 1ml HBSS, pH 7.4. After the desired incubation time (60 minutes), viability of the cells was determined by Tryphan blue exclusion and the percentage of viable cells was calculated as mentioned in methods. These results indicate that the efficiency of the each antioxidant tested exhibits efficient protection against UV rays.

4. Conclusion

These preliminary results showed that, the protective effect of Polyphenols enriched extract of Mint leaves against UV rays induced DNA damage and proved that, it is non-toxic to cells.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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