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UV RAYS INDUCED DNA DAMAGE: PROTECTION BY AQUEOUS EXTRACT OF *MUNTINGIA CALABURA* PLANT ROOT

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ABSTRACT

Ultraviolet rays kill cells by damaging their DNA. If the UV rays exposure time is more the more thymine dimers are formed in the DNA and the greater the risk of an incorrect repair or a missed dimer. Herein, UV rays are used to damage the Calf thymus DNA and *Muntingia Calabura* plant root aqueous extract was used to prevent the DNA damage induced by UV rays and also the non toxic nature of the extract was studies in cytotoxicity studies, where BHA(400µM) and Ascorbic acid (400µM) are used as positive control. The aqueous extract at 15µg concentration was used against UV rays induced DNA fragmentation in agarose gel electrophoresis which provides same protection like other standard antioxidant BHA (400µM) and Ascorbic acid (400µM). The cytotoxicity studies showed that, the extract and BHA and Ascorbic acid provides a protection of 42% and 58% respectively whereas, the extract of Muntingia Calabura root showed 64%. In other words, the *Muntingia Calabura* plant root aqeuous extract showed a better DNA protectant activity against UV rays induced DNA damage.

KEYWORDS: *Muntingia Calabura* plant root, DNA damage, Cytoxocity, UV rays.

INTRODUCTION

Ultraviolet (UV) radiation of sunlight may induce skin cancer (Gentile et al, 2003, De Gruijl FR, 1999, Armstrong and Kricker, 2001), whereas, the longer UV wavelengths radiations induces oxidative stress, denaturing of protein and the short wavelength UV radiations causes predominantly DNA damage to cells in the form of pyrimidine dimers and 6-4 photoproducts (De Gruijl et al., 2001, Ravanat e al., 2001). DNA lesions kills cell and cause mutations if are not properly repaired. When cells are exposed to UV radiation results in formation of the two most common lesions, the cyclobutane pyrimidine dimer (CPD)1 and the 6-4 pyrimidinepyrimidone photoproduct (6-4PP) at adjacent pyrimidines (Satoshi Nakajima et al., 2004, Friedberg et, al, 1995). Herbs and spices have been used in time immemorial as food, medicines and cosmetics. Their potential to treat different types of skin diseases, and to improve the skin appearance is well-known. As extensive exposure to ultraviolet radiation can cause sunburns, tanning of skin, wrinkles, premature aging, and cancer, there is a demand for protection from UV radiation and prevention from their side effects. Herbs and medicinal plant preparations have a high potential due to their antioxidant activity, primarily. Antioxidants such as vitamins (vitamin C, vitamin E), flavonoids, and phenolic acids play the main role in fighting against free radical species that are the main cause of numerous negative skin changes. Earlier, it was reported that, the studies on the root, leaves and fruit aqueous extracts of Muntingia Calabura showed good antioxidant activity, anti diabetic activity (Mohamed Azmathulla Khan et al., 2015 – Accepted and in Press) and hence, the root of the plant was chosen to investigate its potency towards protection against UV rays.

MATERIALS AND METHODS

Calf thymus DNA (CT DNA), BHA, Agarose, Ethidium bromide was from Sigma Chemical company USA. BHA and Ascorbic acid was from HIMEDIA, India. All the other chemicals were of Anal. R grade. All organic solvents were distilled prior to use. *Muntingia Calabura* plant roots are collected from authentic source. Roots are washed thoroughly with water and rinsed in 1% $KMnO_4$ for five minutes and again washed in double distilled water. These roots are crushed and shade dried and powdered. 10 g of root powder mixed with 200 ml of double distilled water and vortexed for five hours.

Centrifuged at 10000 rpm, the supernatant was separated and freeze dried to reduce the volume and stored at -10°C for further studies.

Phytochemicals analysis

The extracts of roots of *Muntingia Calabura* were subjected to phytochemical analysis to check the presence of bioactive compounds by using standard protocols (Mohamed Azmathulla Khan et al., 2015).

The protein estimation was carried according to Bradford's method (Bradford MM, 1976) using BSA as standard. Absorbance was read at 535nm. Concentration of protein was calculated accordingly using standard graph. Total phenolics was determined according to the method of Folin Ciocalteu reaction (Kujala et al., 2000) using gallic acid as a standard. Absorbance was read at 750 nm and the concentration was calculated using the standard graph accordingly. Ascorbic estimation was carried out according to Sadasivam S., Manickam (Sadasivan and Manickam, 1997). The absorbance was read against a reagent blank at 540nm. The concentration was calculated on the basis of the standard curve. Sugar estimation was done according to Dubois method (Dubois et al., 1956). The absorbance was read at 520 nm. The amount of total sugar present in the given unknown sample solution was calculated using the standard calibration curve. Flavonoids estimation was done according to Cheon et al (Cheon et al., 2000) by using Quercetin as a standard. The absorbance was measured at 415 nm and the concentration was calculated accordingly.

Isolation of human peripheral lymphocyte

Human peripheral lymphocytes were isolated from 10ml of venous blood drawn from young, healthydonors. Blood was collected in ACD (85mM citric acid-71mM trisodium citrate-165mM D-glucose) in the ratio of 5:1. 4 volumes of solution A (hemolyzing buffer-150mM NH_4Cl in 10mM Tris buffer, pH 7.4) was added, mixed well, incubated at 4°C for 30 min. Centrifuged at 1200 rpm for 12 min, the supernatant (hemolysate) was discarded, pellet was washed again with 5ml of hemolyzing buffer and the pellet containing cells were washed thrice with 10 ml of solution B (250mM m-inositol in 10mM phosphate buffer pH 7.4) and suspended in same solution. The cell viability was determined by Tryphan dye blue exclusion method (Phillips, 1973). To 10µl of lymphocyte sample added 10µl of Tryphan blue (0.02%) and the cells were charged to Neuberg's chamber and the cell number was counted. The dead cells being permeable to Tryphan blue appear blue against white color of the viable cells. The survival rate of lymphocytes was determined at time intervals 20th, 40^{th} and 60^{th} minutes of incubation. Viability was tested by Trypan blue exclusion and exceeded 96% in each isolation. Percentage viability was calculated by the formula.

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

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.

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

UV rays induced DNA damage: Protection by aqueous extract of *Muntingia Calabura* plant root and other antioxidants

Calf thymus DNA was sheared 100 times using 21gauge needle and DNA (1mg/ 1ml) was subjected to UV radiation (345nm) in presence and absence of antioxidants using germicidal UV lamp (Hanovia Lamp) for 60min at 37^{o}c in 20mM, PBS, pH 7.4. At regular time interval, 200µl of the reaction mixture was drawn and mixed with Ethidium Bromide solution (0.5µg/ml trisodium phosphate buffer, 20mM, 100µM EDTA, pH 11.8) fluorescence of the solution were measure at 520nm excitation and 590nm (Dinesha and Leela Srinivas, 2015). 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.

Statistical Analysis

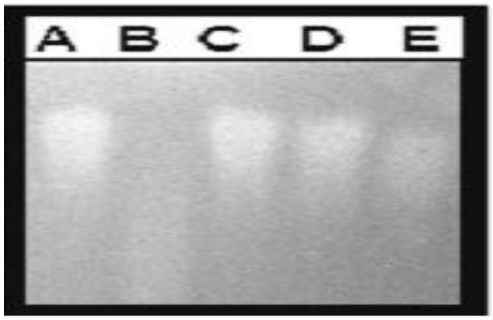
All the results were represented as Mean ± SD. The significance of the experimental observation was checked by students t-test and the value of p value <0.05 was considered significant.

RESULTS AND DISCUSSION

Table -1: Phyto-chemical analysis of *Muntingia calabura* root aqueous extract.

Phytochemicals (mg/g)	Double distilled water extract
Carbohydrates	136.0 ± 1.12
Protein	2.61±0.05
Polyphenols	13.04±0.31
Flavonoids	13.54±0.02
Ascorbic acid	09.02±0.03

Values are means ± SD of triplicates



Lane A: Calf thymus DNA sheared (10 µg)
Lane B: DNA + UV radiation
Lane C: DNA + UV radiation + *Muntingia Calabura* root extract (15µg)
Lane D : DNA + UV radiation + BHA (400µM)
Lane E: DNA + UV radiation + Ascorbic acid (400µM)
Figure-1: U.V. rays induced DNA damage and its protection by *Muntingia Calabura* root extract and other antioxidants

Sheared Calf Thymus DNA (10µg) with and without *Muntingia Calabura* root extract (15µg) / BHA (400µM) / Ascorbic acid (400µM) in 100µl of 20mM PBS pH-7.4, subjected to UV radiation (345nm) 37°C for 60min. Reaction mixture of 4µg DNA loaded on to 0.8% agarose gel.

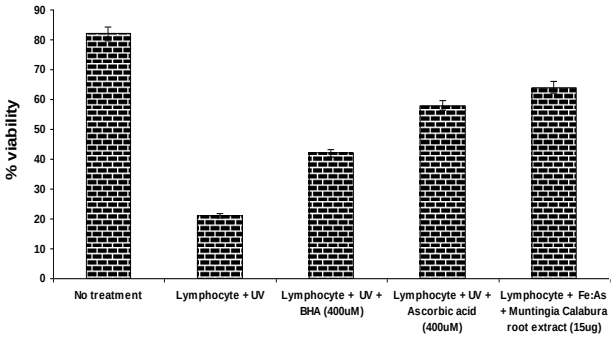


Figure-2: Study of cell toxicity induced by UV rays and protection by *Muntingia Calabura* root extract and other antioxidants

Lymphocytes (10^6cells) 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.

The aqueous extract of the Muntingia Calabura plant root was done as explained in the methods. The phytochemicals anaysis of the root extract was done and the results are as shown the Table-1. The extract was rich with Carbonydrates (136mg/g), Polyphenols (13mg/g), Flavonoids (13mg/g), Ascorbic acid (6mg/ml) and proteins (2.61mg/ml). It was reported that, the extract of Muntingia Calabura plant root has good antioxidant activity (Mohamed Azmathulla Khan et al., 2015, Mohamed Azmathulla Khan et al., 2015). It was reported that, the UV rays induces sugar breakdown and double strand break in DNA (Kitazawa M, 1997). The DNA submarine gel electrophoresis was done as explained in methods. In Figure-1 Lane A shows sheared Calf thymus DNA (10µg). Lane B shows, DNA damage caused by UV radiation, Lane C shows that the protection given by *Muntingia Calabura* plant root extract, Lane D and Lane E showed protection given by BHA and Ascorbic acid against DNA damage caused by UV at 360nm. To confirm *Muntingia Calabura* plant root extract is itself non toxic, We also investigated the protective effects of *Muntingia Calabura* plant root extract against UV rays induced lymphocyte cell death. The viability of lymphocytes on simultaneous pre treatment of UV rays a time course study was done. The isolated lymphocytes (10^6cells) 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.

CONCLUSION

These preliminary results showed that, the protective effect of an *Muntingia Calabura* plant root extract against UV rays induced DNA damage and proved that, it is non toxic to cells.

REFERENCE

1. Armstrong BK and Kricker A, The epidemiology of UV induced skin cancer. *J. Photochem. Photobiol. B*, 2001; 63: 8–18.
2. Bradford MM; A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein. *Analytical Biochemistry.*, 1976; 72; 248-54.
3. Cheon BS, Kim YH, Son KS, Chan HW, Kang SS, Kim HP; Effects of prenylated flavonoids and bioflavonoids on lipopolysaccharide-induced nitric oxide production from the mouse macrophage cell line RAW. *Plant Medicine*, 2000: 66; 596-600.
4. De Gruijl FR, Skin cancer and solar UV radiation. *Eur. J. Cancer*, 1999; 35, 2003–2009.
5. De Gruijl FR, Van Kranen HJ and Mullenders LH, UV-induced DNA damage, repair, mutations and oncogenic pathways in skin cancer. *J. Photochem. Photobiol. B*, 2001; 63, 19–27.
6. Dubois M, Gilles, KA, Hamilton JK, Rebers PA and Smith F; Colorimetric method for determination of

sugar and related substances. *Analytical Chemistry*., 1956; 28: 350-356.
7. Friedberg EC, Walker, GC, Siede W. (1995) DNA Repair and Mutagenesis, ASM Press, Washington, D.C, 1995; 24 –30.
8. Gentile M, Latonen L, Laiho M. Cell cycle arrest and apoptosis provoked by UV radiation-induced DNA damage are transcriptionally highly divergent responses. *Nucleic Acids Research*., 2003; 31(16): 4779-4790.
9. Kitazawa M, Podda M, Thiele JJ, Traber MG, Iwasaki K, Sakamoto K and Packer, L. Interactions between vitamin E homologues and ascorbate free radicals in murine skin homogenates irradiated with utraviolet light. *Photochem photobiol.*, 1997; 355-365.
10. Kujala TS, loponen JM, Klika KD and Pihaja K; Phenolics and betacyanins in red beetroot (Beta Vulgaris) root: description and effect of cold storage on the content of total phenolics and three individual compounds. *Journal of Agricultural Food Chemistry*, 2000; 48: 5338-5342.
11. Mohamed Azmathulla Khan Y, Dinesha Ramadas, Subhas Chandrappa Mundasada, Santosh Kumar N, Chikkanna D, Antioxidant and free radical scavenging activities of root extracts of Muntingia Calabura, *Scholars Journal of Applied Medical Sciences (SJAMS)*, 2015; 3(6B): 2309-2312,
12. Mohamed Azmathulla Khan Y, Dinesha Ramadas, Subhas Chandrappa Mundasada, Santosh Kumar N, Chikkanna D, *In vitro* antidiabetic activity of *Muntingia Calabura* root proteins, *World Journal of Pharmacy and Pharmaceutical Sciences*, 2015 – Accepted and in Press
13. Mohamed Azmathulla Khan, Subhas Chandrappa Mundasada, Dinesha Ramadas, Antioxidant Activity: Root, Leaves and Fruits Aqueous Extracts of *Muntingia Calabura*, *JIPBS*, 2015; Vol 2(4): 363-368.
14. Phillips HJ, Dye exlusion test for cell viability. In: Tissue culture methods (Krus, P.F., Patterson, MK Eds)., 1973; 406-408.
15. Ravanat JL, Douki T and Cadet J, Direct and indirect effects of UV radiation on DNA and its components. *J. Photochem. Photobiol. B*, 2001; 63: 88–102.
16. Sadasivam S and Manickam A; In. Biochemical Methods. *New Age Internationals*, 1997; 184-186.
17. Satoshi Nakajima, Li Lan, Shin-ichiro Kanno, Masashi Takao, Kazuo Yamamoto, Andre P. M. Eker, and Akira Yasui, UV Light-induced DNA Damage and Tolerance for the Survival of Nucleotide Excision Repair-deficient Human Cells, *The journal of Biological Chemistry*, 2004; 279(45): 46674 –46677.