



# ULTRAVIOLET-RAYS INDUCED DNA DAMAGE: PROTECTION BY POLYPHENOLS ENRICHED EXTRACT OF *MORINGA OLEIFERA* PLANT LEAVES

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## Abstract

Cellular DNA may be damaged when exposed to harmful Ultraviolet rays for long duration. More energetic UV-B rays cause harm to cells by producing chemical changes in molecules, especially proteins and nucleic acids. To analyze the effect of UV rays on calf thymus DNA and prevention by polyphenol enriched extract of Drum stick (*Moringa oleifera*) 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 Ascorbic acid (400µM) used as positive control. The polyphenol enriched extract was taken at 15µg concentration to prevent UV rays induced DNA fragmentation in submarine agarose gel electrophoresis which provides protection when compared to standard antioxidant Ascorbic acid (400µM). The cytotoxicity studies showed that the extract and Ascorbic acid provide a protection of 59% whereas, the extract of Drum stick leaves showed 69%. In conclusion, the Drum stick leaves extract showed a promising DNA protectant activity against UV rays induced DNA damage.

**Index terms:** Drum stick (*Moringa oleifera*) leaves extract, Ascorbic acid, DNA damage, DNA protectant, Cytotoxicity, UV rays, Polyphenols

## I INTRODUCTION

Electromagnetic radiations are bunch of energetic radiations which includes Ultraviolet rays (Robbins, 2013). Exposure to low intensity UV radiation helps in the synthesis of Vitamin D. Reports says that, extensive exposure to UV rays are harmful to living species (Dobrynin et al., 2009, Rinnan et al, 2009). The long term effects of UV on living things are caused by changes in the genetic material, DNA (Herndon et al., 2018). Large doses of UV may kill a cell outright by creating too much damage in its DNA. Smaller doses may have lasting effects by causing mutations (changes in the DNA sequence) or recombination between DNA molecules. Genetic changes such as mutation or recombination can affect the growth of cells, in some cases, leading to uncontrolled growth or cancer (Bello & Gbolagade, 2017; Williams, 2018; Robson et al., 2015). Herein we studied the DNA damage induced by UV rays and protection by Polyphenols enriched Drum stick plant (*Moringa oleifera*) leaves. It was reported that, it is easily available, inexpensive, resourceful antioxidant and also rich with Vitamin A (Abonyi et al., 2018; Sivasankari et al., 2014).

## II MATERIALS AND METHODS

Calf thymus DNA (CT DNA), Ascorbic acid, Agarose, Ethidium bromide was from Sigma Chemical company USA. Ascorbic acid from HIMEDIA, India. All the other chemicals were of Anal. R grade. Fresh Drum stick leaves (*Moringa oleifera*) were procured from authentic source.

### 2.1 Extraction

Leaves are washed thoroughly with water and rinsed in 0.5% KMnO<sub>4</sub> 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. Polyphenol enriched extraction was done by mixing 25g of Drum stick leaves powder was mixed with 250mL of methanol, followed with Soxhlet extractor for 72 h. Later, the excess methanol solvent was evaporated. In the same way, the extraction was done with other solvents like hexane, chloroform, ethyl acetate and butanol to obtain hexane, ethyl acetate, chloroform-butanol and residual methanol fractions, respectively. Finally, all crude extracts were mixed, filtered. The combined extracts were concentrated and dried by using rotary evaporator under reduced pressure (Hossain et al., 2014).

### 2.2 Phytochemical analysis:

The extract was subjected to phytochemical analysis to check the presence of bioactive compounds by using standard protocols (Dinesha, R. & Leela Srinivas, 2010; Mylarappa et al., 2008; Dinesha, R. & Leela Srinivas, 2011, Mohamed et al., 2015). The protein estimation was carried out (Bradford, 1976) using BSA as standard and absorbance was read at 535nm. Total phenolics was determined according to the method of Folin Ciocalteu reaction (Kujala et al., 2000) using gallic acid as a standard and absorbance was read at 750 nm. Ascorbic acid estimation was carried out (Sadasivam & Manickam, 1997) and the absorbance was read against a reagent blank at 540nm. Total sugar estimation was done according to Dubois method (Dubois et al, 1956) and the absorbance was read at 520 nm. Flavonoids estimation was done (Cheon et al., 2000) 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 (Roudafshani et al., 2019). 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 NH<sub>4</sub>Cl 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 (Kamiloglu et al., 2020) 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 20<sup>th</sup>, 40<sup>th</sup> and 60<sup>th</sup> 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 (Yamashita and Tokunaga, 2020).

#### 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 Drum stick leaves (*Moringa oleifera*) and Ascorbic acid

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 (Dinesha Ramadas et al., 2017). 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.

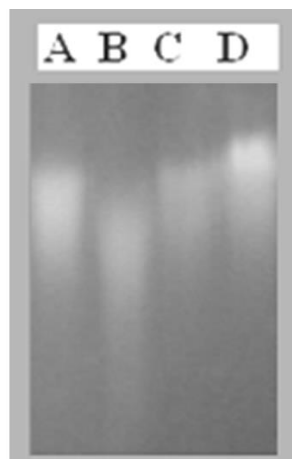
### III RESULTS AND DISCUSSION

**Table -1: Phyto-chemical analysis of *Drum stick* leaves extract**

Phyto-chemicals in Drum stick leaves	Aqueous extract
Carbohydrates	+
Protein	+
Polyphenols	+
Flavonoids	+

Values are means ± SD of triplicates

**Figure-1: Ultraviolet rays induced DNA damage and its protection by *Drum stick* leaves extract and other antioxidants**



**Lane A: Calf thymus DNA sheared (10 µg)**

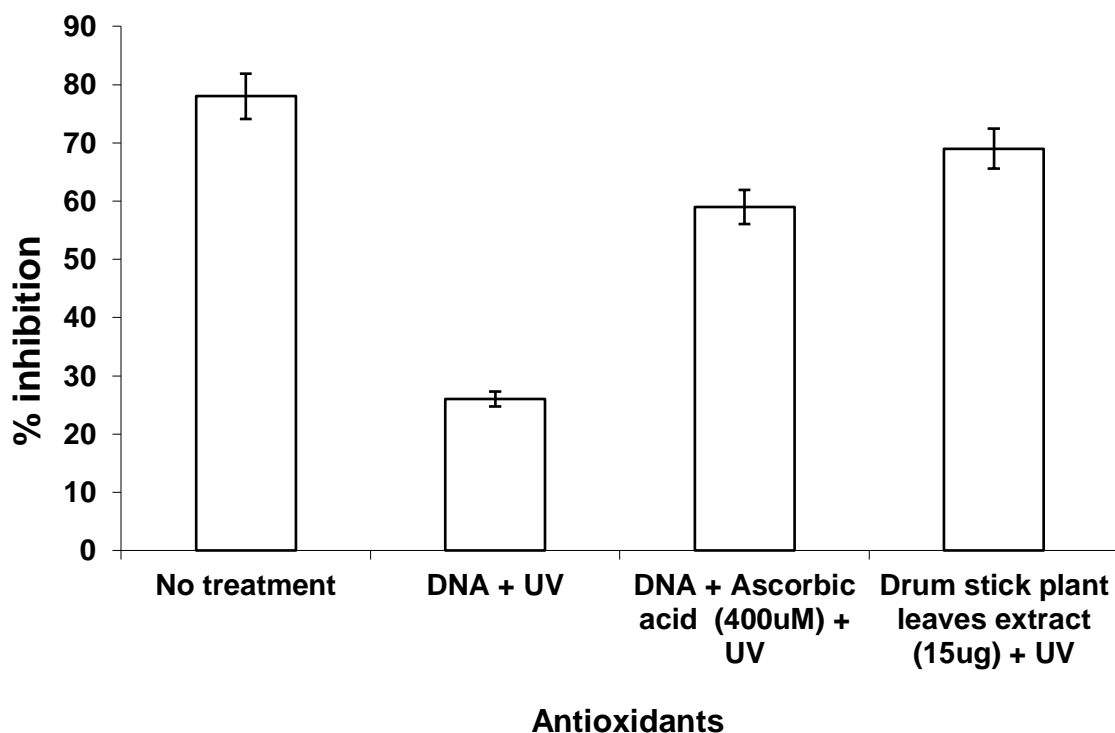
**Lane B: DNA + UV radiation**

**Lane C: DNA + UV radiation + Ascorbic acid (400µM)**

**Lane D: DNA + UV radiation + *Drum stick* leaves extract (15µg)**

Sheared Calf Thymus DNA (10µg) with and without *Drum stick* leaves extract (15µg) / 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 *Drum stick* leaves extract and Ascorbic acid**



Lymphocytes ( $10^6$  cells) 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 Trypan blue exclusion and the percentage of viable cells was calculated as mentioned in methods.

UV rays of different energies have various effects on DNA. Among these, the most important damage to DNA is the formation of pyrimidine dimers. In this pyrimidine dimer, two adjacent pyrimidine bases--Cytosine (C) and/or thymine (T) are linked in an abnormal structure which distorts the shape of the DNA double helix and blocks its copying by the DNA replication or RNA transcription machinery. The above changes leads to death of cells or cellular transformation (Kitazawa et al., 1997; Molinero et al., 2020). As explained in the materials and methods, the polyphenol enriched extract of Drum stick 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. The obtained extract was polyphenol enriched. The DNA submarine gel electrophoresis was done as explained in methods. In Figure-1 Lane A shows sheared Calf thymus DNA ( $10\mu\text{g}$ ). Lane B shows, DNA damage caused by UV radiation, Lane C shows that DNA damage caused by UV rays and protection given by Ascorbic acid, Lane D showed against DNA damage caused by UV and protection by Drum stick leaves extract. The cyto-toxicity studies showed that, the polyphenol enriched Drum stick leaves extract is non toxic. We evaluated the protective effects of 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 ( $10^6$  cells) 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 Trypan blue exclusion and the percentage of viable cells was calculated as mentioned in methods. The results indicate that, the viability of untreated cells is about 78%, the effect of UV on these cells reduced the viability of cells to 26%. But when the cells were treated with well known standard antioxidant Ascorbic acid along with UV rays, the viability raised to 59% whereas the cells treated with Polyphenols enriched drum stick leaves extracts, the viability of cells raised to 69%. The above results clearly indicates that, the drum stick extract and Ascorbic acid providing protection against UV rays to lymphocytes.

### Conclusion

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

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### Conflict of interest

The authors declare no conflict of interest.



## IV REFERENCES

- [1]. Abonyi, D.O., Eze PM., Abba, C.C., Ujam, N.T., Proksch, P., Okoye, F.B. & Esimone, C.O. (2018) Biologically active phenolic acids produced by *Aspergillus* sp., an endophyte of *Moringa oleifera*. *European Journal of Biological Research*. 28:8(3):157-67.
- [2]. Bello, H.K. & Gbolagade, K.A. (2017) A Survey of Human Deoxyribonucleic Acid. *Current Journal of Applied Science and Technology* 13:1-10.
- [3]. Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein. *Analytical Biochemistry*. 72: 248-54.
- [4]. Cheon, B.S., Kim, Y.H., Son, K.S., Chan, H.W., Kang, S.S. & Kim, H.P. (2000). Effects of prenylated flavonoids and bioflavonoids on lipopolysaccharide-induced nitric oxide production from the mouse macrophage cell line RAW. *Plant Medicine*. 66:596-600.
- [5]. Dinesha Ramadas & Leela Srinivas. Antioxidant effects of 28kda Protein from Turmeric (*Curcuma Longa* L). *Asian Journal of Pharmaceutical and Clinical Research* 2011;4(1):75-79.
- [6]. Dinesha Ramadas., Harsha, R Kashyap., Chikkanna, D. & Santosh Kumar, S. (2017). UV rays induced DNA damage: Protection by *Curcuma aromatica*, *SAS Journal of Medicine*. 3(11):323-327.
- [7]. Dinesha, R. & Leela Srinivas. (2010). BGS-Haridrin – A new antioxidant glycoprotein of Turmeric (*Curcuma longa* L), *Ind. J. Nutr. Diet.*47:118-121.
- [8]. Dobrynin, D., Fridman, G., Friedman, G. & Fridman, A. (2009). Physical and biological mechanisms of direct plasma interaction with living tissue. *New Journal of Physics* 26:11(11):115020.
- [9]. Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. & Smith, F. (1956). Colorimetric method for determination of sugar and related substances. *Analytical Chemistry*. 28:350-356.
- [10]. Herndon, J.M., Hoisington, R.D & Whiteside, M. (2018) Deadly ultraviolet UV-C and UV-B penetration to Earth's surface: Human and environmental health implications. *J Geog Environ Earth Sci Intn* 14(2):1-1.
- [11]. Hossain, M.A., Al-Hdhrami, S.S., Weli, A.M., Al-Riyami, Q. & Al-Sabahi, J.N. (2014) Isolation, fractionation and identification of chemical constituents from the leaves crude extracts of *Mentha piperita* L grown in Sultanate of Oman, *Asian Pacific Journal of Tropical Biomedicine*. 4(1):S368-S372.
- [12]. Kamiloglu, S., Sari, G., Ozdal, T. & Capanoglu E. (2020). Guidelines for cell viability assays. *Food Frontiers*. 1(3):332-49.
- [13]. Kitazawa, M., Podda, M., Thiele, J.J., Traber, M.G., Iwasaki, K., Sakamoto, K. & Packer, L. (1997) Interactions between vitamin E homologues and ascorbate free radicals in murine skin homogenates irradiated with Ultraviolet light. *Photochem photobiol* 355-365.
- [14]. Kujala, T.S., Ioponen, J.M., Klika, K.D. & Pihaja, K. (2000). 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*. 48:5338-5342.
- [15]. Mohamed A.K.Y., Dinesha Ramadas., Chandrappa, M., Santosh Kumar, N., Harsha R Kashyap. & Chikkanna, D. (2015). UV rays induced DNA damage: Protection by aqueous extract of *Muntingia calabura* plant root, *European Journal of Pharmaceutical and Medical Research*. 2(6):182-185.
- [16]. Molinero, D.D., Paulano, M.R., Ruiz, F.R., Sánchez, N.B., de Gálvez Aranda, M.V., de Castro Maqueda, G. & de Troya Martín, M. (2020). Sun Protection Behaviour and Sunburns in Spanish Cyclists. *Journal of Cancer Education*. 27:1-8.
- [17]. Mylarappa, B.N., Ramadas Dinesha & Leela Srinivas. (2008). Antioxidant and free radical scavenging activities of polyphenol-enriched curry leaf extract (*Murraya koenigii* L.), *Food Chemistry*. 106: 720-728
- [18]. Rinnan, Å., Van Den Berg, F. & Engelsen, S.B. (2009) Review of the most common pre-processing techniques for near-infrared spectra. *TrAC Trends in Analytical Chemistry*. 1:28(10):1201-22.
- [19]. Robbins, M.A. (2013). The collector's book of fluorescent minerals. Springer Science & Business Media.
- [20]. Robson, T.M., Klem, K., Urban, O. & Jansen, M.A. (2015) Re-interpreting plant morphological responses to UV-B radiation. *Plant, cell & environment* 38(5):856-66.
- [21]. Roudafshani, Z., Jazayeri, M.H., Mahmoudi, A.R., Nedaeinia, R., Safari, E. & Jazayeri, A. (2019) Evaluation of the frequency of CD5+ B cells as natural immunoglobulin M producers and circulating soluble CD5 in patients with bladder cancer. *Molecular biology reports*. 1;46(6):6405-11.
- [22]. Sadasivam S, & Manickam, A. (1997). In. Biochemical Methods. *New Age Internationals* 184-186.

- [23].Sivasankari, B., Anandharaj, M. & Gunasekaran, P. (2014). An ethnobotanical study of indigenous knowledge on medicinal plants used by the village peoples of Thoppampatti, Dindigul district, Tamilnadu, India. *Journal of ethnopharmacology*. 28:153(2):408-23.
- [24].Williams, J. (2018). The ozone hole: a story of healing and hope. *Weather-wise*. 2:71(1):12-7.
- [25].Yamashita, K. & Tokunaga, E. (2020). Noninvasive and safe cell viability assay for Paramecium using natural pigment extracted from food. *Scientific reports*. 3;10(1):1-1.

