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UV rays induced DNA damage: Protection by *Curcuma aromatica*

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Abstract: Excessive Ultraviolet rays are detrimental to cells by damaging DNA. The time of exposure is more resulting in formation of more thymine dimers in the DNA and the greater the risk of an incorrect repair or a missed dimer. The antioxidant ability of Curcuma aromatica extract was analyzed in most reliable method like DPPH radical scavenging activity. The extract showed a promising result (65%) towards inhibiting DPPH radicals in comparison with other standard antioxidants like Ascorbic acid and Alpha tocopherol (71%) and (48%) at a highest dosage of □□□g. Here, UV rays are used to damage the Calf thymus DNA and Ethanol: water extract of *Curcuma aromatica* was used to prevent the DNA damage induced by UV rays and also studied the non toxic nature of the extract in cytotoxicity studies, where alpha-tocopherol (400µM) and Ascorbic acid (400µM) are used as positive control. The extract at 15µl concentration was used against UV rays induced DNA fragmentation in agarose gel electrophoresis which provides same protection like other standard antioxidant alpha-tocopherol (400µM) and Ascorbic acid (400µM). The cytotoxicity studies showed that, the extract Ascorbic acid and alpha-tocopherol provides a protection of 50% and 70% respectively whereas, the extract of *Curcuma aromatica* 64%. In other words, the ethanol: water extract of *Curcuma aromatica* showed a better DNA protectant activity against UV rays induced DNA damage.
Keywords: *Curcuma aromatica*, DNA damage, Cytoxocity, UV rays

INTRODUCTION

The excessive releasing of CFC and air pollution damaging the protective layer of earth – Ozone and allowed the more Ultraviolet (UV) radiation of sunlight to reach earth and may induce skin cancer [1,2]. Detailed studies find a reliable like exposure to UV radiation and formation of non-melanoma skin cancer [3]. Studies also revealed that, longer UV radiations induces oxidative stress leads to denaturing of protein and the short wavelength UV radiations causes DNA damage to cells in the form of pyrimidine dimers and 6-4 photoproducts [4]. Antioxidants plays a vital role in prevention of oxidation induced by factors like UV rays, oxidative stressing factors like Hydrogen peroxide, t-BOOH, Ferrous sulphate: ascorbate mixture etc [5]. These antioxidants are may synthetic like BHA, BHT or natural resources, dietary resources, herbs and spices [6]. The added factor about safety of natural antioxidants are non toxic nature even one consume in milligram quantity [7]. In India, it is time immemorial practice of using dietary sources, herbs and spices as food, medicines and cosmetics [8]. Their potential to treat different types of skin diseases and to improve the skin appearance is well-known [9]. As extensive exposure to ultraviolet radiation can cause sunburns, tanning of skin, wrinkles, premature aging, and cancer, hence, there is a demand for protection from UV radiation and prevention from their side effects [10]. 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 [11]. Lot of studies has been done during different solvent extracts of Curcuma aromatics and hence, the extract was chosen to investigate its potency towards protection against UV rays [12]. In this study, authors made an attempt to find DNA damage protecting activity of Curcuma aromatica ethanol-water extract against UV rays induced DNA damage.

MATERIALS AND METHODS

Calf thymus DNA (CT DNA), BHA, Agarose, Ethidium bromide was from Sigma Chemical company USA. Alpha-tocopherol and Ascorbic acid was from

HIMEDIA, India. All the other chemicals were of Anal. R grade. All organic solvents were distilled prior to use. *Curcuma aromatica rhizomes* are collected from authentic source. Rhizomes are washed thoroughly with water and rinsed in 1% $KMnO_4$ for five minutes and again washed in double distilled water. These are shade dried and powdered. 10 g of powder mixed with 200 ml of 1:1 ethanol: water and vortexed for five hours, filtered using glass wool, the supernatant was kept in water bath for evaporation, reduce the volume and stored for further studies.

Phytochemicals analysis

The extracts of Curcuma aromatica were subjected to phytochemical analysis to check the presence of bioactive compounds by using standard protocols. The protein estimation was carried according to Bradford MM, 1976 [13]. Total phenolics were determined according to Kujala *et al*., 2000 [14]. Ascorbic estimation was carried out according to Sadasivam S., Manickam, 1997 [15]. Sugar estimation was done according to Dubois *et al*., 1956 [16]. Flavonoids estimation was done according to Cheon *et al*, 2000 [17].

DPPH radical scavenging activity of partially purified proteins of *Curcuma aromatica*

DPPH radical scavenging activity was assessed according to the method of Shimada *et al*. [18] with minor modifications. The ethanol and water extract of *Curcuma aromatica* at concentrations ranging from 2 µg to 14 µg was mixed in 1 ml of freshly prepared 0.5 mM DPPH ethanolic solution and 2 ml of 0.1 M acetate buffer pH 5.5. The resulting solutions were then incubated at 37°C for 30 min and measured spectrophotometrically at 517 nm. Ascorbic acid and alpha *tocopherol* (2 to 14 µg) were used as positive control under the same assay conditions. Negative control was without any inhibitor or *Curcuma aromatica* extract. Lower absorbance at 517 nm represents higher DPPH scavenging activity. The % DPPH radical scavenging activity of extract was calculated from the decrease in absorbance at 517 nm in comparison with negative control.

Isolation of human peripheral lymphocyte

Human peripheral lymphocytes were isolated from 10ml of fresh venous blood drawn from young, healthy donors. Blood was collected in ACD (85mM citric acid-71mM trisodium citrate-165mM D-glucose) in the ratio of 5:1. Four 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 [19]. To 10□l of lymphocyte sample added 10µl of Tryphan blue (0.02%) and the cells were placed in 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 10^{th}, 20^{th} and 30^{th} minutes of incubation. Viability of cells was tested by Tryphan blue exclusion and exceeded 96% in each isolation. Percentage viability was calculated by using following formula.

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

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 as explained in methods [19].

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 extract of *Curcuma aromatica* and other antioxidants

Calf thymus DNA was sheared 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°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 [20]. 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: Phytochemical analysis of *Curcuma aromatica* water - ethanol extract

Phytochemicals	Presence (+) / absence (-)
Carbohydrates	+
Protein	+
Polyphenols	+
Flavonoids	+
Ascorbic acid	+
Alkaloids	+

Values are means ± SD of triplicates

Table -2: Study of cell toxicity induced by UV rays and protection by *Curcuma aromatica* extract and other antioxidants

Lymphocytes	% viability
Lymphocytes alone (10µl)	83
Lymphocytes (10µl) + UV radiation for 60 minutes	22
Lymphocytes + UV + Curcuma aromatica (15µl)	64
Lymphocytes + UV + Ascorbic acid (400µM)	50
Lymphocytes + UV + α-tocopherol (400µM)	70

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, viability of the cells was determined by Tryphan blue exclusion method and the percentage of viable cells was calculated as mentioned in methods.

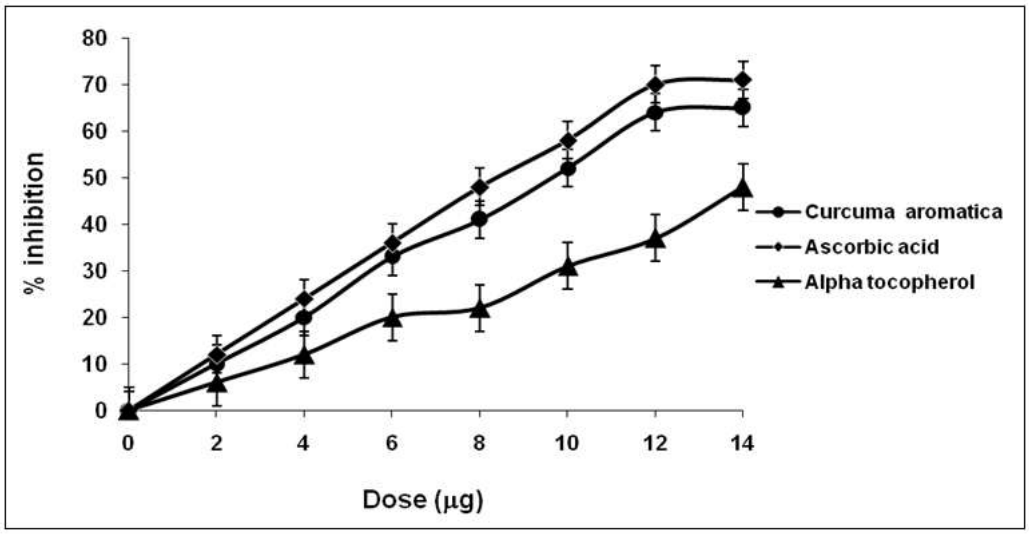

Fig-1: DPPH radical scavenging activity by *Curcuma aromatica* extract

Dose-dependent DPPH radical scavenging activity of Curcuma aromatica extract. The control was without Curcuma aromatica or Ascorbic acid or Alpha tocopherol. The DDPH radical scavenging activity was calculated accordingly as described in methods. Results are shown as mean ± SD (n = 3). Sheared Calf Thymus DNA (10µg) with and without *Curcuma aromatica* extract (15µl) / α-tocopherol (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.

Fig-2: U.V. rays induced DNA damage and its protection by *Curcuma aromatica* extract and other antioxidants

Lane A: Calf thymus DNA sheared (10 µg)
Lane B: DNA + UV radiation
Lane C: DNA + UV radiation + *Curcuma aromatica* extract (15µl)
Lane D: DNA + UV radiation + α-tocopherol (400µM)
Lane E: DNA + UV radiation + Ascorbic acid (400µM)

Spices and herbs are rich sources of powerful antioxidants. Spices and herbs have been used for flavour, colour and aroma for more than 2000 years [21]. Studies have been done on spices and herbs extensively in different parts of world because of their useful antioxidant activities and their beneficial effects on human health [22]. The antioxidant activity of extract of the spice Curcuma aromatica (wild turmeric), having a wide range of pharmacological and cosmetological applications is studied [23]. The ethanol - aqueous extract of the *Curcuma aromatica* was done as explained in the methods. The phytochemicals analysis of the *Curcuma aromatica* was done and the results are as shown the Table-1. The extract showed the presence of Carbohydrates, Polyphenols, Flavonoids, Ascorbic acid and proteins. As shown in Fig. 1, the dose dependent DPPH radical scavenging activity of *Curcuma aromatica* extract showed that, Curcuma aromatica extract showed maximum inhibition of 65% where as standard antioxidants like Ascorbic acid and α-tocopherol showed 71% and 48% respectively at a dosage of 14µg. It was reported that, the UV rays induces sugar breakdown and double strand break in DNA [24]. The DNA submarine gel electrophoresis was done as explained in methods. In Fig. 2, 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 *Curcuma aromatica* extract, Lane D and Lane E showed protection given by α-tocopherol and Ascorbic acid against DNA damage caused by UV at 360nm. As shown in table-2, the cytotoxicity study was conducted using freshly isolated lymphocytes as explained in methods to examine the non toxic nature of Curcuma aromatica extract, also investigated the protective effects of *Curcuma aromatica 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 (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 and non toxic nature of *Curcuma aromatica* extract as antioxidant and providing protection against UV rays induced DNA damage.

CONCLUSION

These preliminary results showed that, the antioxidative and DNA protective nature of an *Curcuma aromatica* extract against UV rays induced DNA damage and also proved that, it is non toxic to cells.

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