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Research Article

The Effect of Ultraviolet-B Radiation Exposure on *Hibiscus cannabinus* Linn with its Phytochemical and Pharmacological Responses

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ABSTRACT

Aim: Exposure of UV-B Radiation on*Hibiscus cannabinus* Linn and to evaluate both Phytochemical and Pharmacological responses. **Objective:** UV-B can increase the nutrients contents from plants, plants become tastier and increases the yield and UV-will make plants resistant to fungal infections and stimulate the production of phytomarkers. **Material and Methods**: *Hibiscus cannabinus* Linn seeds were soaked with 50% H_2O_2 for 12 hours and then inoculated with the Azospirillum. UV-B radiation cabinet was provided by UV lamps which are above 15 cm from control maintained. Normal daylight exposed control group Seedlings irradiated for 2 hour per day (12.30 pm to 2.30 pm) for 20 days. **Results and Discussion:** 20 days treatment increases total phenolic contents and sugars content were decreased in uncovered leaf tissue. Protein content was at first diminished but expanded on the 20 day of UV-B treatment. Also, perform pharmacological studies no toxic elements are observed 20 days treatment and pharmacological activity by using anticancer activity by using human colon HT-29 cancer cell line. UV light exposed plants shows good anticancer activity when compared with non UV exposed Plant.

Key words: *Hibiscus cannabinus* Linn, UV-B radiation, Cancer cells line HT-29, Total phenolic contents.

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INTRODUCTION

An ultraviolet ray is that incorporate the electromagnetic spectrum they are generally found inside the 400 and 10-nanometer wavelength. The Earth's is shielded from short-wave UV by an ozone layer in the stratosphere. Around 95 percent of this UV radiation is consumed by the Ozone layer.[1] UV can expand the nutrients from plants, therefore, plants will be more delectable. UV-will makes plants progressively impervious to contagious contaminations. Ultra-violet light are amazingly hurtful to these microbes and more often than not prompt their demise. In spite of the fact that it can slaughter off unsafe microorganisms, it likewise impacts the helpful ones, subsequently annihilating any common connection between the plants and the microbes.[2]

Damage of the stratospheric ozone layer the level of ultraviolet radiation reaching the biosphere, especially in the range of UV-B (280 – 320 nm) react with oxygen to convert ozone its compound protective mechanism of ozone layer.[3-5]

The limitation of herbal plant research less yield, diseases state of plants and using fertiliser produces the toxic effect of plants as well as human so new techniques are used to increases the yield of medicinal plants, reduced fertiliser usage and increases biomarkers.[6]

MATERIALS AND METHODS

Collection of plant sample

Hibiscus cannabinus Linn seeds were collected from Kaviya Garden Chennai. Surface-sterilised seeds were first soaked in H_2O_2 for 12 hours and then inoculated with the Azospirillum. Hydrogen peroxide concentrations of 50% were prepared with distilled water and then replaced to garden soil in trays, containing red earth, sand and farmyard manure in (1:2:1). UV-B radiation cabinet was provided by UV lamps which are above 15cm from control maintained. Normal daylight exposed to control group. Seedlings irradiated for 1 hour per day (12.30 pm to 1.30 pm) for 20 days (Figures 1 and 2).

Chemicals

Analytical grade 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethylsulfoxide (DMSO), doxorubicin and other chemicals purchased from Himedia Laboratories private limited, Mumbai, Reagent D, Reagent E, Folin–Ciocalteu Phenol reagent.

Estimation of biochemical constituents

Estimation of protein

Protein content determined by Lowry *et al.* method. 0.5 gm of plants was smashed with 80% Acetone. This

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Figure 1: Effect of UV-B radiation for 20 days exposed Hibiscus cannabinus Linn.

Figure 2: Effect of Non-UV-B radiation 20 days exposed Hibiscus cannabinus Linn.

extract used as a sample. 0.3 ml of extract diluted to 1 ml. Then added 2 ml of Reagent D and kept in for 10 minutes at room temperature. 0.6 Ml of Reagent E added and placed in 20 minutes at room temperature. Optical density was read at 660 nm with bovine serum albumin as a standard.[7,8]

Estimation of chlorophylls

Two gram of leaf was finely cut and gently mixed with a clean mortar and pestle. To this add 20 ml of 80% acetone and 0.5 gm $MgCO_3$. The materials were further grinded gently. The sample was then put into a refrigerator at 40 C for 5 hours. Thereafter, the sample was centrifuged at 500 rpm for 5 minutes. The supernatant was transferred to 100 ml volumetric flak. The final volume was made up to 100 ml with addition of 80% acetone. The color absorbance of the solution was estimated by a spectrophotometer using 645 and 663 nm wavelength against the solvent. Acetone (80%) was used as a blank.[9,10]

Estimation of total sugars

Total sugars estimated by a phenol-sulphuric acid method. 0.5 gm of plants smashed with 80% Acetone. This extract was used as a sample.0.3 ml of extract was diluted to 1 ml and to that 0.5 ml of 80% phenol, 4 ml of con.H_2O_4 added and absorbance read at 490 nm. Concentration of total sugars was determined using glucose as standard.[11]

Estimation of total phenolic content

The total phenol content was determined spectrophotometrically using the Folin–Ciocalteu method. Singleton and Rossi (1967) is oxidation / reduction method for phenolic compounds.0.2 ml of the sample diluted to 1 ml.0.7ml of Folin–Ciocalteu reagent was added to it and incubated 5 minutes at 220°C. Then 1 ml of Na_2CO_3 was added and again kept at 220° C for 90 minutes. The absorbance was measured at 725 nm.[12]

Estimation of total proline content

Proline contents determined by Plants treated with sulphosalicylic acid. The extract was used for estimating proline contents with help of spectrophotometric analysis.[13]

Anticancer activity against human colon (HT-29) cancer cell line

Cell culture

Colorectal cancer cell lines HT-29 collected from Texas Southern University. Cultured Cells by using RPMI 1640 medium add 10% FBS with antibiotics. Cells were kept at 37° C in a well humidified condition of 5% carbon dioxide in 95% sterilized air. HT-29 cells were added with 100 nM of phorbol myristate acetate for 72 hours to induce differentiation into macrophages. After differentiation, non-reacted cells were separated by aspiration and adherent macrophages were washed with RPMI 1640 medium 3 times and then kept in cell culture medium at 37°C.[14]

Cell viability

The effect of plants extract of both UV and non UV treated plants extract on cell proliferation in HT-29 cells was determine by using Cell viability 96 well plate assay. The viable cells seeded at a thickness of 5 × 104 (100 µ/well) in 96-well plates and brooded in a humidified environment to frame a cell monolayer. After 24 hr, the supernatant

Table 1: Effect of UV radiation and Non-UV radiation on phytochemical parameters.

S.No	Phytochemical Parameters	Non-UV-B Treatment	UV Treatment
1	Total chlorophylls	2.834 ± 0.0025	0.383 ± 0.0025
2	Carotenoid	0.273 ± 0.0034	0.193 ± 0.0024
3	Protein	0.816 ± 0.160	0.65 ± 0.001
4	Proline	0.588 ± 0.102	2.09 ± 0.01
5	Total Flavonoids	23.36 ± 1.27	50.88 ± 2.78

SD n=3

Table 2: Effect of percentage viable cell UV treated plant extract of *Hibiscus cannabinus* Linn.

S.NO	Concentration (mg/ml)	Control	Percentage Viable Cell T/C (cell control) X100	
			UV	Non-UV
1	0.3	1.14	98	84
2	0.6	1.14	79	66
3	1.2	1.14	65	58
4	2.5	1.14	52	37
5	5	1.14	35	17

on the monolayer suctioned, and 100 ʯL of normal and changing log concentrations of concentrate (100-700 µg/mL) was included and brooded for 24, 48, and 72 hr time focuses. After the particular occasions of introduction to the concentrate, 20 ʯL of 5 mg/mL MTT in PBS was added to each well and brooded for 3 h at 37°C in a 5% CO2 environment. Supernatants are evacuated and 150 µL of isopropanol included and the plates were tenderly shaken for 15 min to solubilise the formazan gems and absorbance was estimated at 560 nm utilizing Bio Tex ELX 800 plate peruser. In another examination, the impacts of Chromolaenaodorata alone and with mitogens (VEGF and ET-1) on colorectal malignant growth cell lines (HT29) feasibility following 24 and 48 hours exploring.[15]

RESULTS AND DISCUSSION

Growth characteristics

Hibiscus cannabinus Linn treated under UV-B radiation showed various growth characteristics, photosynthetic pigments and antioxidant enzymes. The irradiation caused morphological changes such as leaf curling and foliar damage. After 20 days of UV-B treatments growth characteristics such as root length, the total length of the seedlings.

In UV-B exposed plants maximum chlorophyll content were initially increased on the 3rd day then gradually reduced and significant decrease in total chlorophyll content of plants as compared to non UV exposed plants. Protein contents were decreased compared with the control level and Proline and total flavonoids increased with increasing of level UV of radiation.

Pharmacological responses

After 72 hours MTT assay showed that extract caused a similar effect of both non UV and UV light exposed plant. Indicate no toxic elements are produce during UV light treatment.

CONCLUSION

The penetration of ultraviolet radiation varies among different plant species and may reflect their sensitivity. we found the effects of UV-B on *Hibiscus cannabinus* Linn showed the 15 days of UV-B treatment affected the growth parameters and photosynthetic pigments. Chlorophyll and carotenoid content were reduced thus photosynthetic pigments were destroyed by UV radiation. Significant changes found in the biochemical constituents such as proteins, total sugars and total phenols. Total sugar content decreased, but phenolic content and proteins accumulated in UV-B exposed plant.

The results indicate that both UV treated and Untreated plant extracts show cytotoxicity against the human colon cancer (HT-29) cell lines. Interestingly, the extract from UV treated plant shows much higher cytotoxicity for the same concentration when compared to extract from natural plants indicating enhancement of bioactive compounds in the UV treated plants.

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GRAPHICAL ABSTRACT

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