E.coli: As an Alternate Model for Phototoxicity Assessment of Drugs

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Abstract- Phototoxic and photoallergic reactions represent skin reactions to the sun, in the presence of photoactive chemicals applied on the skin or taken systemically. They have a highly polymorphic clinical presentation – photo contact urticaria, eczema on sun-exposed areas sometimes with erythema multiforme, exaggerated sunburn, linear phytophotodermatitis, pseudoporphyria, photoonycholysis, dyschromia, and lupus erythematosus. Also, skin cancers are increasingly associated with exposure to photoactive chemicals. There is a geographical and timely variation in the responsible agents, but they are mostly furocumarins from plants, UV filters in sunscreens and cosmetics, and drugs (NSAIDs, antimicrobials, phenothiazines, amiodarone, etc.) Three drugs like Chloramphenicol, Norflox, and Kucil(Fluorouracil) were tested for Phototoxic effect procured from local market and check the ph and absorption spectra and note and solubility of test drug was tried out through different solvents viz. DW (Distill water), petroleum ether, ethanol, DMSO. The Phototoxic activity was studied against the bacterial culture *Escherichia coli (*DH5 α).The Results of this study revealed that these drugs seem to be phototoxic effect on Microbial culture.

Index Terms- E.coli, Alternate Model, Phototoxicity,Drugs

I. INTRODUCTION

Phototoxicity is a chemically induced skin irritation requiring light (photo irritation). The skin response resembles exaggerated sunburn. The involved chemical may enter into the skin by topical administration or it may reach the skin via systemic circulation following ingestion or parenteral administration. Sulpha-derived drugs (sulphonamide antibacterial, hypoglycemic, diuretics) have been well-known causes of photosensitivity reaction since 1939. Phototoxicity is the result of direct cellular damage caused by an inflammatory non immunological mechanism, which is initiated by a phototoxic agent and subsequent irradiation. In contrast, photoallergic reactions represent delayed or cell-mediated or type four (4) hypersensitivity responses, which require the specific sensitization of a given human individual to a photoactivated drug. The Commonly applied methods of checking of phototoxic effect of drug are based on the agar diffusion and results were shown as visible zones of growth inhibition. The present work was framed to study the Phototoxic activity of three drugs against the bacterial test organisms.

II. MATERIALS AND METHODS

Collection and Isolation of Bacterial Culture

E.coli were collected from fecal samples using sterile Whirl-Pac bag and tongue depressor. The collected sample were keep at 40C until processed. Process samples within 6 hours. Analyze sample for fecal coliform on mFC agar by membrane filtration method pick isolated colonies from mFC plate and re-streak to 100x15 mm mFC agar plates using sterile toothpicks. Incubate at 44.50C for 24 hours. The Idientification of E.coli samples were achieved using standard monograph.

Preparation of Drug Extract

Procured the Drug viz. Chloramphenicol, Norflox and Kucil from the local market. Weight all the drug and then crush the drug with the help of Mortar & Pestle and checked the solubility of the drug in different chemicals and mixed in the specific soluble medium of these drugs if the drug is not mix well then it will be mixed in DMSO which is universal soluble

After that check the pH of the drugs and absorption spectra of drugs and note. Then calculated the dose of the drug in normal person and convert it into ppm.

After that selected the standard ppm for the bacterial culture which is below of the dose of normal person and prepared the stock for further experiment. Used many plates for the selection of the ppm on which the zone of inhibition was very less and selected for experiment.

The calculation of the drug was as follows-

Dose in normal person was 500 mg in 5000 ml of blood. Then calculate it in 1 ml of blood which was 10 mg/ml So 10 mg drug is dissolved in1 ml of blood now convert it into ppm as 1mg/ml= 1000 ppm. So the drug dose in 1 ml blood was 10000 ppm. So I have to take the dose below this concentration. This step was done with the entire drug which was used in this experiment like:-

For Norflox 400 mg was 12.5 mg/ml in blood. After conversion into ppm the dose was 12500 ppm in 1 ml of blood.

***In Vitro* phototoxic potential assessment of routinely used drugs**

The commonly used drugs viz., Chloramphenicol, Kucil and Norflox were selected to assess their phototoxic potential in the cultured E.coli i.e., DH5α strain. All the drugs selected in the study are generally prescribed to the patients visited in Out Patient Department, so the possibilities of photodynamic induced systemic damages through sunlight cannot be ignored. Rose Bengal is selected as a positive control and run in the

experiments under identical conditions. Based on the results of the earlier experiments, non-toxic intensities of UV-A (1.4 mW/cm2 or 5.04 J/cm2), UV-B (0.6 mW/cm2 or 2.16 J/cm2) and sunlight (1.4×105 Lux) and exposure period of 5 min were selected as irradiation dose to assess the phototoxic potential, if any, in the drugs tested in the study. Prior to start the experiments of phototxicity assessment, different concentration like (20,40,60,100 µg/ml, and different conc. of all drugs were screened in dark and UV exposure to assist any effect on these intensities.

The Rose Bengal shows positive result so it is selected as a positive control in this experiment.

III. RESULTS

In this study, 3 Drugs (Chloramphenicol,Kucil and Norflox) are as follows-

Result of Chloramphenicol on 20,40,60 and 100ppm

The Drugs shows absorption maxima on UV-B so selected the UV-B for exposure on drug and the following results are appear as-

The Chloramphenicol shows the inhibition zone at concentration of 20 ppm in UV-B is 11 mm and in Dark is 9.0 mm and on 40 ppm in UV-B is 14 mm and in dark 10 mm.

On 60 ppm in UV-B 16 mm and in dark 12 mm.

On 100 ppm in UV-B 21 mm and in dark 14 mm.

Result of Chloramphenicol on 50,100,150, and 200 ppm

The Chloramphenicol shows the inhibition zone at concentration of 50 ppm in UV-B is 16 mm and in Dark is 8.0 mm and on 100 ppm in UV-B is 18 mm and in dark 10 mm. On 150 ppm in UV-B 20 mm and in dark 12 mm.

On 200 ppm in UV-B 21 mm and in dark 14 mm.

Result of Norflox on 50,100,150, and 200 ppm

The Norflox shows the inhibition zone at concentration of 50 ppm in UV-B is 14 mm and in Dark is 8.0 mm and on 100 ppm in UV-B is 17 mm and in dark 10 mm.

On 150 ppm in UV-B 20 mm and in dark 12 mm.

On 200 ppm in UV-B 18 mm and in dark 13 mm.

Result of Kucil on 3,5,8, and 10 ppm

The Kucil does not shows the inhibition zone at concentration of 3 & 5 ppm in UV-B and in dark. On 8 ppm in UV-B 7 mm and in dark no zone appeared.

On 10 ppm in UV-B 10 mm and in dark no zone appeared.

As shown in result that with increases of the concentration of drug and exposure of UV-B the zones are increases and the number of colonies are decreases so the drugs are showing phototoxic potential.

IV. DISCUSSION

Various drugs are being used by human beings at an alarming rate due to concomitant increase in deseases all over the world. These drugs may be toxic to human beings and their toxicity may increase further under exposure to UVR/sunlight. Thus an assessment of their phototoxic response is a matter of great concern as most of the patients may be exposed to solar radiation after intake of the chemicals.

We observed that photoexcited Chloramphenicol, Norflox and Kucil produce significant amount of singlet oxygen (1O2) at the levels of their usage. The generation of reactive oxygen species (ROS) by these drugs was dependent upon the exposure periods under ambient intensities of UV-B and solar radiation. It is well known that ROS produced by various compounds under the influence of ultraviolet/ visible radiation, are responsible to alter the normal function of cellular constituents (de gruijl et al., 2003) and skin photosensitization causing erythema or edema (Ichihashi et al., 2003; Dornelles et al., 2004)

Moreover, some photoexcited chemicals are also known to produce phototoxicity to biomolecules (Bertoloni et al., 2000; Viola et al., 2000; Miolo et al., 2002). The earlier studies of our laboratory also supported the view point (Ray et al., 2002; Ray et al., 2005).

Phototoxicity caused by illuminated may be further induced in many folds by an increase of UV radiation in sunlight due to depletion of ozone layer (Sweden, 1990; Fredrick, 1993; Winker and Trepte, 2004).The UVR/sunlight testing is very necessary during production of new drug (Fahim akhtar., 2012).

Therefore an exposure to UVR/sunlight should be avoided after intake of the phototoxic drug viz- Chloramphenicol, Norflox, Kucil.

Chloramphenicol, Norflox is a bacteriostatic antimicrobial and has gram-positive and gram-negative bactericidal activities while Kucil is an Anti-cancer drug . All the drugs shows maximum absorption at 280nm in UV-B irradiance.

V. CONCLUSIONS

We have shown the absorption spectrum of Chloramphenicol, Norflox, and Kucil, which lies in the UV region. This indicates that the chemicals are absorbing UV radiation. The phototoxicity of drug is expected on the basis of absorption spectra and testing on E.coli. It is conceivable that the therapy with these drugs represents an additional risk factor for skin photo carcinogenesis. The work performed over the E.coli DH5α strain can be used satisfactorily for in vitro phototoxicity assessment of drugs. The study suggests that the drug tested may be phototoxic in presence of increasing UVR (specially) the UV-B fraction) due to O3 depletion. Patients, who are using the photosensitive drugs and are fair skinned, would be more vulnerable for harmful effects of UVR. So the people using drugs should avoid sun exposure.

From this point of view, these environmental pollutants and drugs has always been a matter of concern to scientists. In this present study, we have shown that these widely spread pollutants and drugs becomes toxic to both Prokaryotic (E.coli sp.) and Eukaryotic when they comes in contact with the sunlight (mainly in UV Radiation). However, in vivo studies are required to further asses its potential before any recommendations. These drugs are very common to use but has strong phototoxic ability. It may cause skin and other diseases in human beings.

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COLONY FORMING UNIT (C.F.U) After incubation period, the growth of bacterial colonies on plate was counted:- 94×104 SURVIBILITY --

TABLE- 1. Effect of UV-B Radiation (0.4mW/cm2) exposure on E.coli growth

Sl.No.	Exposure	No. of Colonies
1.	0	94×104
2.	5 minutes	70×104
3.	10 minutes	35×104

AGAR DIFFUSION METHOD

TABLE-2. Inhibition zone at different concentration of Rose Bengal on E.coli

Exposure (dose)	50 ppm	100 ppm	150 ppm	200ppm
UV-B (0.4mw/cm2)	1.2	2.5	4.6	5.4
Dark	No	No	No	No

TABLE-3. Inhibition zone at 20, 40, 60, & 100 ppm concentration of Chloramphenicol.

Exposure (Dose)	20 ppm	40 ppm	60 ppm	100 ppm
UV-B (0.4mw/cm2)	11	14	16	21
Dark	9.0	10	12	14

Table-4. Inhibition zone at 50,100,150 & 200 ppm concentration of Chloramphenicol

Exposure (Dose)	50 ppm	100 ppm	150 ppm	200 ppm
UV-B (0.4mw/cm2)	16	18	20	24
Dark	8.0	10	12	14

Table-5. Inhibition zone at 50,100,150 & 200 ppm concentration of Norflox

Exposure (Dose)	50 ppm	100 ppm	150 ppm	200 ppm
UV-B (0.4mw/cm2)	14	16	17	18
Dark	8.0	10	11	13

Table-6. Inhibition zone at 3,5,8 & 10 ppm concentration of Kucil (Flourouracil)

Exposure (Dose)	3 ppm	5 ppm	8 ppm	10 ppm
UV-B (0.4mw/cm2)	No	No	7	10
Dark	No	No	No	No

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