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Development of Alternate Model for Phototoxicity Assessment of Drugs by using Bacterial strains

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Abstract: In this study the phototoxicity of drugs Ofloxacin, Doxycycline and Torsemide was determined using two bacterial strains *S.epidermidis* & *E.coli* (Dh5-α). It was found that *Staphylococcus epidermidis* was resistant against reactive oxygen species generated by drugs on exposure to UV-A while *E.coli* strain Dh5-α was affected by the phototoxic effect of these drugs which was shown by the increase in inhibition zones after exposing them under UV-B radiation. Thus, the study showed that *E.coli* can be used as a reference to determine the phototoxic effect of drugs whereas *S.epidermidis* cannot be used for this purpose. The study also suggests the phototoxic potential of drugs, Ofloxacin, Doxycyclin and Torsemide, in presence of UV radiation (especially UV-B fraction). Among nine selected drugs only above three drugs had their absorption maxima in UV-B region. After analysis it produce a phototoxic effect on exposure to UV-B radiation.

Keywords: Phototoxicity; drugs; *E.coli*; Ozone; UV-Radiation.

I. NTRODUCTION

Phototoxicity and photoallergy are different conditions of an abnormal skin reactions caused by exposure to light. Theses allergic conditions can be enhanced by use of some endogenous or exogenous substances that are selectively activated by solar radiation. Use of artificial light source (sun lamps used for aesthetic or therapeutic purposes or ultraviolet (UV) sources in occupational settings) can produce these effects but they mostly occur on sun exposure. From the solar spectrum that reaches the earth, UV radiation, and particularly UVA (320–400 nm), is responsible for most cases of photosensitivity. Even though some chromophores absorb in the UVB (290–320 nm) and UVB is more energetic, UVA penetrates the skin more deeply and, particularly for systemic chromophores, this is certainly the most important spectrum for inducing photodermatosis. [1]

Phototoxic reactions occur because of the damaging effects of light-activated cell membrane compounds and DNA. These reactions are more common in individuals exposed to sufficient amounts of light and an exogenous agent, and usually appear as an exaggerated sunburn response. Phototoxic reactions result from direct tissue damage caused by a photo-activated compound. Many compounds have the potential to cause phototoxicity and have at least one resonating double bond or an aromatic ring that can absorb radiation energy. The most common causative agents are furocoumarins, acridinic dyes or eosine. **[2]**

Photosensitivity develops when an abnormal chromophore, or a normal chromophore in exaggerated amounts, is present in the skin. When excited by a photon, these molecules suffer changes within the molecule itself, often also within neighbouring molecules, in a cascade of events that result in skin damage and inflammation. This can occur through the direct molecular modification (isomerisation, breaking of double bounds, oxidation) or production of free radicals, dependent or not on oxygen, which modify unsaturated lipids of cell membranes, aromatic amino acids of proteins, or DNA or RNA bases of nucleic acids. If the repair mechanisms do not act immediately, there is damage and/or death of skin cells and inflammatory mediators are produced (prostaglandins, IL-1, 6, 8, other cytokines, and chemokines) with consequent skin lesions [1].

An endogenous molecule can act as chromophore to cause a photosensitive reaction. One such molecule is porphyrin that accumulates in the skin due to an inborn metabolic error, or it can be an exogenous molecule that is applied on the skin or reaches the skin through the systemic circulation. . In some cases, the chromophore absorbs the energy of the photon to transform itself into a new molecule (photoproduct) or to bind an endogenous peptide and form a hapten or an allergen. Such allergens can be recognized by the skin immune system therefore developing a photo allergic reaction.

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Photosensitivity from topical agents, once frequent and often associated with persistent reactions to light, is now becoming rare, [3],[4] as the main topical photosensitizers are removed from the market, or may be photosensitivity is underreported or underdiagnosed [5].

Due to the phototoxicity problems few in vitro test systems have been developed e.g. 3T3 Neutral Red Phototoxicity Test. But most of these test system are based on animal cell cultures and are tedious to perform. Thus the present investigation was conducted, which was aimed to develop alternate test models which were based on the bacteria *E.coli* and *S.epidermidis*. For this investigation some known phototoxic drugs were used. These drugs were exposed to UVR and the ROS (Reactive oxygen species) were produced and the effect of the ROS was observed on both the bacteria.

II. MATERIAL AND METHOD

A. Isolation of Test strains:

Staphylococcus epidermidis and *E.coli* Dh-5α were received from CytoGene Research and Development Laboratory and reconfirmed by Gram staining, Mannitol salt agar test, Coagulase test, catalase test and subculturing in appropriate selective media.

B. Test drug solubility

Solubility of test drugs was tried out through different solvents viz. DW (Distilled water), Acetone, ethanol, DMSO. In this study distilled water was used as the solvent as the drugs were soluble in distilled water

C. Absorption Spectra of drug

The absorption spectra of drug were drawn in between 200 to 700 nm, covering ultraviolet and visible spectrum. The base line was set with the solvent of the test compound. The spectrum of drug was drawn using spectrophotometer.

D. Photodegradation study: Determination of Reactive Oxygen Species (ROS) - Singlet Oxygen ($^1O_2$) and Superoxide anion radical (O_2^-)

The stock solution of drug (1mg/ml) was prepared in solvent of favoured solubility. For photodegradation study, the drug solution was taken in 10 ml solvent in petridish and irradiated under UV-B (1.1mW/cm2) for varying period of time (1 hour). The evolution of reaction was monitored by recording absorption maxima of irradiated samples.

E. Determination of Zone of Inhibition

The freshly prepared inoculum was spread onto the nutrient agar plates. Wells of 6mm diameter were bored in the medium with the help of sterile cork-borer having 6mm diameter and were labeled properly and twenty micro-liters of the selected drugs of different concentrations were filled in the wells with the help of micropipette. Then plates were exposed to UV-B (1.1W/cm2) for 1 hr.All plates were incubated for 24 hrs at 37˚C and then the plates were observed for zone of inhibition.

III. RESULT

Various drugs were used for phototoxicity assessment. These drugs namely Ketoconaozole, Clindamycin, Amitryptiline, Griseofulvin, Furosemide, Torsemide, Doxycyclin, and Ofloxacin were selected to assess their phototoxic potential by using cultured *E.coli* and *Staphylococcus epidermidis* bacteria. The selected drugs showed absorption maxima in UV-B and since solar UV-C does not reach at the earth surface therefore phototoxicity study was carried out under UV-B radiation. The photochemical generation of singlet oxygen and superoxide at various concentrations of Drugs exposed to 1.1mW/cm2 intensity of UV-B was studied respectively. Rose Bengal was selected as positive control (100ppm). Generation of ROS (Reactive oxygen species) was monitored at 20, 40, 60, 80 and 100ppm concentration of drugs.

The Drugs Ketoconaozole, Clindamycin, Amitryptiline, Griseofulvin and Furosemide showed their absoption maxima in UV-C and since UV-C radiation do not reach earth they didn't showed ROS generation.

A. Ofloxacin: At 20, 40, 60, 80 and 100 ppm the drug showed absorption maxima in UV-B so UV-B was selected for ROS generation for Ofloxacin drug and the following results appeared –

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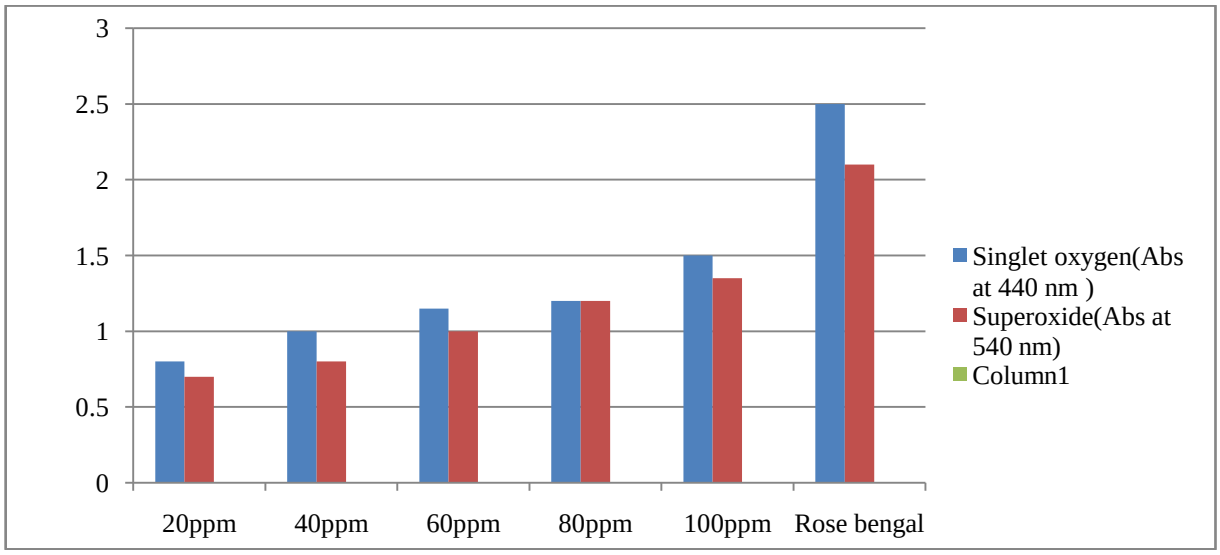

Fig 1: Generation of superoxide and Singlet Oxygen by Ofloxacin under UV-B (1.1mW/cm^2)

Table 1: Zone of inhibition of *E.coli* at different concentrations of Ofloxacin.

Ofloxacin	20ppm	40ppm	60ppm	80ppm	100ppm	Rose Bengal(100ppm)
Exposed (ZOI)	12mm	16mm	18mm	19mm	20mm	11mm
Unexposed (ZOI)	7mm	1.0mm	1.0mm	1.1mm	1.2mm	9mm

B. Doxycyclin: At 20, 40, 60, 80 and 100 ppm the drug showed absorption maxima in UV-B so UV-B was selected for ROS generation for Doxycyclin drug and the following results appeared –

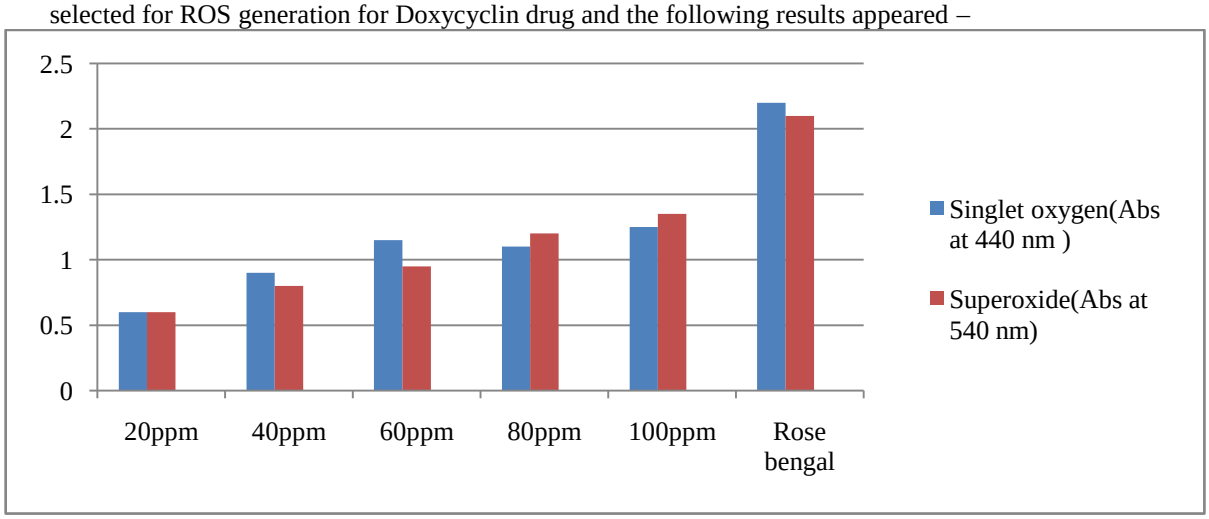

Fig 2: Generation of superoxide and Singlet Oxygen by Doxycyclin under UV-B (1.1mW/cm^2)

TABLE 2: Zone of inhibition of *E.coli* at different concentrations of Doxycyclin.

Doxycyclin	20ppm	40ppm	60ppm	80ppm	100ppm	Rose Bengal(100ppm)
Exposed (ZOI)	10mm	12mm	13mm	14mm	15mm	11mm
Unexposed (ZOI)	8mm	11mm	12mm	12mm	13mm	9mm

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C. Torsemide: At 20, 40, 60, 80 and 100 ppm the drug showed absorption maxima in UV-B so UV-B was selected for ROS generation for Torsemide drug and the following results appeared –

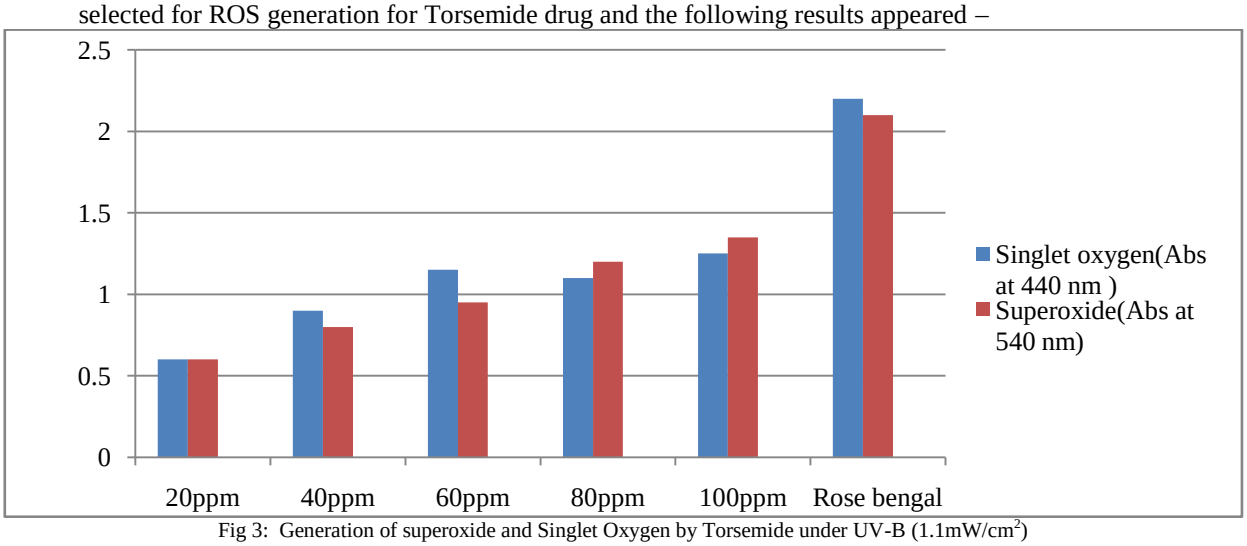

Fig 3: Generation of superoxide and Singlet Oxygen by Torsemide under UV-B (1.1mW/cm^2)

TABLE 3: Zone of inhibition of *E.coli* at different concentrations of Torsemide.

Torsemide	20ppm	40ppm	60ppm	80ppm	100ppm	Rose Bengal(100ppm)
Exposed (ZOI)	Nil	Nil	8mm	8mm	10mm	11mm
Unexposed (ZOI)	Nil	Nil	Nil	Nil	Nil	9mm

IV. DISCUSSION

Use of drugs has been greatly increased by human beings in recent years. These chemical compounds can produce phototoxicity under exposure to UVA and UVB because these two radiations can penetrate the earth atmosphere and can reach the earth surface. The photo-excited form of various drugs is known to produce phototoxic responses to cellular biomolecules. Possible increase of UV radiation in sunlight due to depletion of ozone layer in stratosphere by chemical agents like CFCs may be more detrimental to different forms of life on earth. The main cause for the onset of the phototoxicity is generation of ROS such as singlet oxygen ($^1O_2$), superoxide anion (O_2-) and hydroxyl radical (OH) in photosensitization reactions. Interconversion of these ROS leads to formation of hydrogen per oxide (H_2O_2). Thus there is an urgent need for the assessment of photoxicity of drugs because these are used in a bulk all over the world.

In this study *E.coli* and *S.epidermidis* is used as an in vitro test system for assessment of the phototoxic potential of various drugs. Most of the drugs showed their absorption maxima in UV-B Radiation range of the solar spectrum. Therefore these may be excited by absorbing UV-B Radiation. In this study out of nine drugs, only four showed absorption maxima in UV-B region and thus generated ROS on exposure to UV-B radiation. *E.coli* as model gave the reproducible, reliable and rapid results. These photoexicted drugs showed great inhibition zone than unexcited drugs showing the effect of photoxicty on E *coli*. But the *S.epidermidis* didn't show any difference in the inhibition zone of exposed drug and unexposed drugs.

V. CONCLUSION

E.coli can be used efficiently for phototoxicity assessment of drugs. Using *E.coli* as an alternate model is very effective as well as rapid in vitro test for phototoxicity assessment. *S.epidermidis* cannot be as a model for phototoxicity assessment because it didn't show any difference in inhibition zone of exposed and unexposed plates. It may be because of the strain of *S.epidermidis* used for phototoxicity assessment was resistant to ROS as it has been found that the *S.aureus* which is close relative to *S.epidermidis* is resistant to ROS because of a number of enzymes produced by it which modifies ROS into non-harmful forms. Therefore it may be possible that *S.epidermidis* strain used in the investigation might also be producing the same enzymes like that of *S.aureus*.

Also the drugs Beclomethasome, Ketoconaozole, Clindamycin, Amitryptiline, Griseofulvin and Furosemide showed absorption in the UV-C region which makes them safe to use because UV-C does not reaches to earth as they cannot penetrate the earth's atmosphere. Drugs Doxycyclin, Torsemide and Ofloxacin showed absorption in UV-B region and

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hence generated ROS. Thus they show photoxicity after consumption, on exposure to sunlight.

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