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# EFFECT OF VISIBLE RANGE ELECTROMAGNETIC RADIATIONS ON *ESCHERICHIA COLI*

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## Abstract

**Background:** *Escherichia coli* is the agent responsible for a range of clinical diseases. With emerging antimicrobial resistance, other treatment options including solar/photo-therapy are becoming increasingly common. Visible Range Radiation Therapy/Colour Therapy is an emerging technique in the field of energy/vibrational medicine that uses visible spectrum of Electromagnetic Radiations to cure different diseases. In this study, our goal was to understand the effect of Visible Range Electromagnetic Radiations on E. coli (in vitro) and therefore find out the most appropriate visible range radiation for the treatment of diseases caused by E. coli.
**Materials and Methods**: A total of 6 non-repetitive E. coli isolates were obtained from urine samples obtained from hospitalized patients with UTI. Single colony of E. coli was inoculated in 3 ml of Lysogeny Broth (LB) and 40 µl of this E. coli suspension was poured into each of the plastic tubes which were then irradiated with six different wavelengths in the visible region (Table. 1) after 18 hours with one acting as a control. The Optical Densities of these irradiated samples were then measured. Furthermore, scanning electron microscopy (TEFCAN ZEGA3) was carried out.
**Results:** The analysis of the microscopic and SEM images of irradiated E. coli samples with six different visible range radiations is representative of The fact that E. coli responded differently to every applied radiation in the visible region and the most profound inhibitory effects were that of 538nm Visible Range Radiation (Green) which proved to be bactericidal and 590nm Visible Range Radiation (yellow) which was bacteriostatic. The enhanced growth of E. coli with varying degrees was clearly observed in 610nm (orange), 644nm (red), 464nm (Purple) and 453nm (blue).
**Conclusion:** It can be concluded that 538nm (Green) and 590nm (Yellow) can effectively be used for treating E. coli borne diseases.

**Keywords:** Visible Range Radiation Therapy, E. coli, UTI, Alternative Treatment, Colour Therapy

## Introduction

Escherichia Coli (E. coli) is a gram-negative bacterium capable of causing a range of diseases including dysentery, gram-negative rod sepsis, infections of the urinary tract, and diarrhoea with complications leading to haemolytic-uremic syndrome. These diseases continue to affect hundreds of millions of people annually worldwide (Croxen and Finlay 2010; Donnenberg 2002). The most common diseases encountered in clinical practice caused primarily by E. coli are Urinary tract infections (UTIs) and intestinal infections (Azeemi et al. 2008a; Chakupurakal et al. 2010; Murugan et al. 2012) There is an increasing pattern of antibiotic resistance in diseases caused by E. coli which directly represents the problem of anti-microbial resistance (Mukherjee et al. 2013). This brings us to the search for an alternative therapy that can effectively be used to treat E. coli borne diseases.

Phototherapy and Visible Range Radiation Therapy are one of the oldest therapeutic systems used successfully to treat various health conditions but are recently emerging with serious scientific background (Azeemi and Raza 2005). Previously, Solar therapy was rediscovered by Niels Ryberg Finsen, a Danish physician and scientist who won in 1903 the Nobel Prize in Physiology / Medicine in recognition of his contribution to the treatment of diseases, notably lupus vulgaris. Phototherapy involving the use of an artificial irradiation source was born (Roelandts 2005). Therapeutic benefits of visible and near-infrared wavelengths were uncovered only many years later (Azeemi and Raza 2005; Barolet 2008).

The visual colours, referred to as a narrow band in electromagnetic energy spectrum, with their unique wavelength and oscillations generate electrical impulses and magnetic currents or fields of energy that are prime activators of the biochemical and hormonal processes (Azeemi 1999). Previously, Visible Range Radiation Therapy has

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also been shown to profoundly affect parasitic growth. (Azeemi et al. 2011; Samina. T. Yousuf Azeemi et al. 2011). In this study, we aimed to understand the effect of Visible Range Electromagnetic Radiations on E. coli (in vitro). This will help us in better determining the role of Visible Range Radiation Therapy as a viable therapeutic system for treating E. coli borne diseases.

**Materials and Methods**

A total of 6 non-repetitive E. coli isolates were obtained from urine samples from hospitalized patients with a UTI in CITI Lab, Lahore during March 2016. The urine samples were inoculated onto MacConkey agar (Oxoid) and CLED agar and incubated at 37 C overnight. Positive urine cultures were defined by the growth of a single colony morphotype of E. coli with counts $>10^5$ CFU/ml. E. coli was identified by standard laboratory methods involving morphological characteristics and biochemical tests including indole and triple sugar iron. Citrate utilization test was also positive and gram staining revealed gram negative rods. Furthermore, API 20E system confirmed API 5144572 E. coli.

Then single colony of E. coli was inoculated in 3 ml of Lysogeny Broth (LB) and 40 µl of this E. coli suspension was poured into each of the plastic tubes which were then irradiated with six different wavelengths in the visible region (Table. 1) for 18 hours with one acting as a control. They were kept in shaker-incubator at 37 C. The Optical Densities of these irradiated samples were then measured using Smart Spec Plus Spectrophotometer and slides were prepared for gram-staining. The experiment was repeated twice.

Furthermore, scanning electron microscopy (TEFCAN ZEGA3) was carried out to investigate the effects of visible range radiations on E. coli.

**Table 1**: Dominant wavelength of monochromatic light measured by Hitachi U-2000 UV-Vis double beam spectrophotometer, spectral bandwidth 0.1nm and ordinates selected 10.

| | Color | Dominant Wavelength (nm) | Hue | Purity (%) | Transmission (%) |
|---|---|---|---|---|---|
| 1 | Purple | 464 | Violet | 36% | 32% |
| 2 | Blue | 483 | Blue Green | 52% | 52% |
| 3 | Green | 538 | Greenish Yellow | 15% | 37% |
| 4 | Yellow | 590 | Reddish Yellow | 40% | 82% |
| 5 | Orange | 610 | Orange | 43% | 47% |
| 6 | Red | 644 | Red | 41% | 51% |

**Results**

The measured Optical Densities (OD) for the 18 hrs cultured samples on Lysogeny Broth (LB) are shown in Figure 2. the colony counts that were determined from the prepared MacConkey agar culture plates are shown in Figure.3. Lowest colony count was observed in the 538 nm (Green Colour) irradiated sample. In contrast to this, the greatest number of colonies were observed in 453nm (Blue Colour) irradiated sample.

The statistical analysis was carried out on IBM SPSS Statistics – Version 20. A Pearson product-moment correlation coefficient was computed to assess the relationship between the optical densities of the irradiated samples and the number of colonies. There was a strong, positive correlation between the two variables, $r = 0.974$, $n = 8$, $p = 0$. The scatter plot for the results is shown in Fig. 4.

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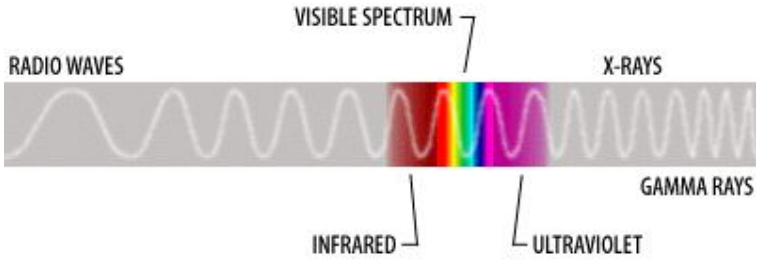


**Figure1:** shows the narrow Visible Spectrum in Electromagnetic Radiations.

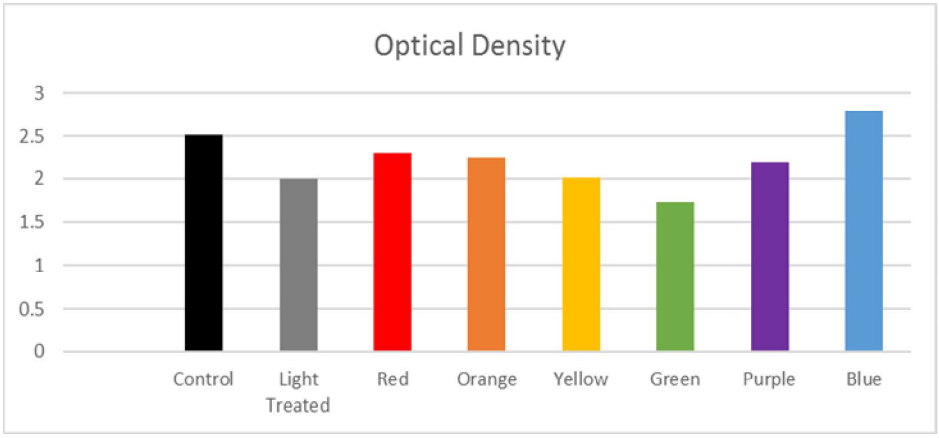


**Figure 2:** shows the optical densities measured through Smart Spec Plus Spectrophotometer for each of the irradiated samples along with the control.

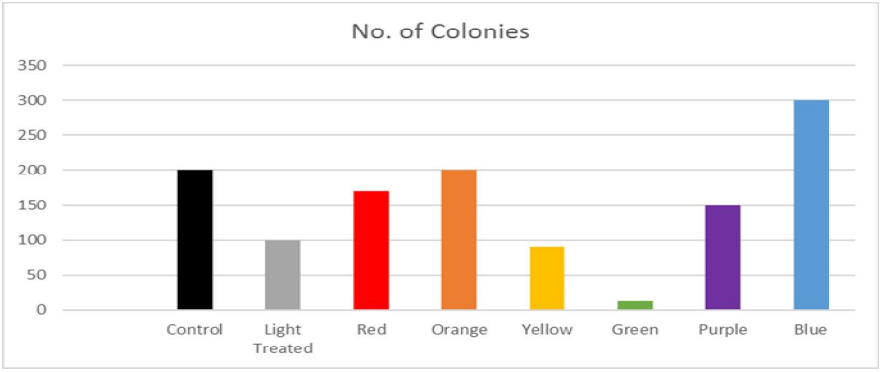


**Figure 3:** shows the counted No. of Colonies for each of the irradiated samples along with the control.

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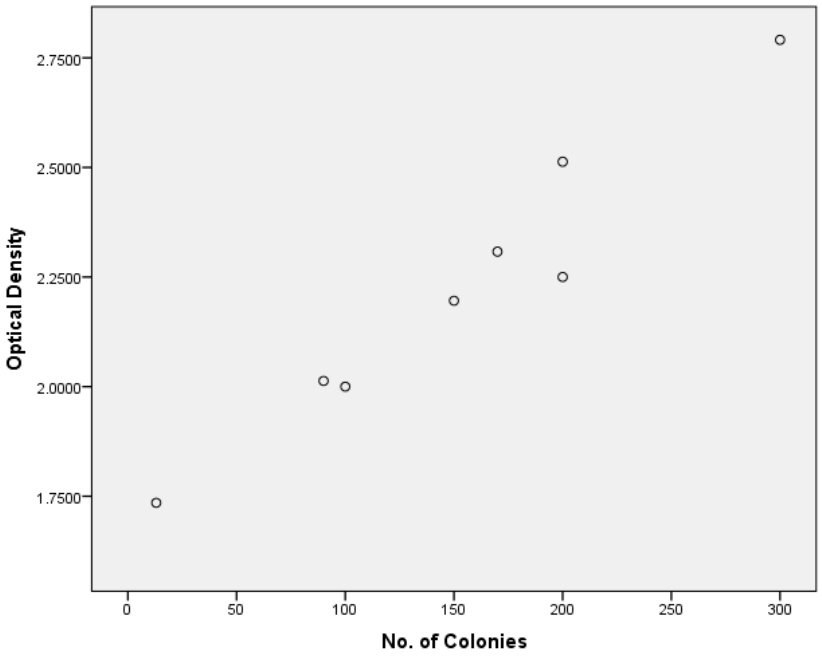


**Figure 4:** shows the scatter-plot.

**Table 2:** Correlations

| | | Optical Density | No. of Colonies |
|---|---|---|---|
| Optical Density | Pearson Correlation | 1 | .974** |
| | Sig. (2-tailed) | | .000 |
| | N | 8 | 8 |
| No. of Colonies | Pearson Correlation | .974** | 1 |
| | Sig. (2-tailed) | .000 | |
| | N | 8 | 8 |

**. Correlation is significant at the 0.01 level (2-tailed).

The gram-stained slides were viewed under OLYMPUS BX51 microscope (Fig 5-12).

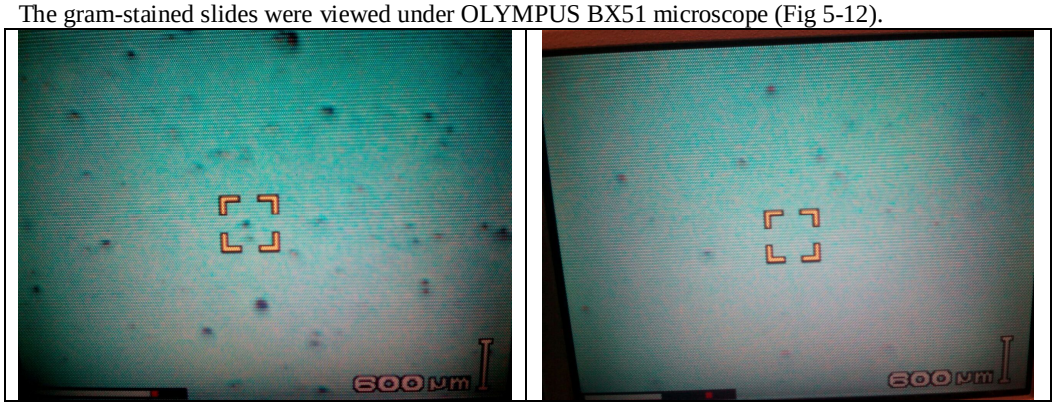

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**Figure 5:** Light Treated

**Figure 6:** E. coli sample irradiated with 464nm (Purple) radiation

**Figure7:** E. coli sample irradiated with 644nm (Red) radiation

**Figure 8:** E. coli sample irradiated with 453nm (Blue) radiation

**Figure 9:** E. coli sample irradiated with 538nm (Green) radiation

**Figure 10** E. coli sample irradiated with 610nm (Orange) radiation

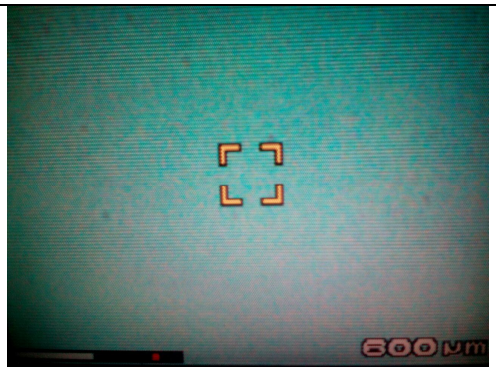

**Figure 11:** E. coli sample irradiated with 590nm (Yellow) radiation

**Figure 12:** Control

Scanning Electron Microscope (SEM) images of these slides are shown in Figures 13-20 showing that Visible range radiations significantly affected the morphology of E. coli in comparison with the control sample, The Light Treated E. coli became flattened and circular in shape (Fig. 13). The 464nm (Purple) treated E. coli were rough but elongated with mosaic pattern (Fig.14). In 644 nm (red) irradiated samples, the E. coli clearly lost its original bacillary shape becoming more flattened and wrinkled (Fig.15). The 453nm (Blue) irradiated sample were elongated and some of the E. coli showed unusual increase in their length with greatest deviation from their original size (Fig.16). The 538 nm (Green Colour) irradiated E. coli deflated owing to leakage of cell solutes (Fig. 17). In 610nm (orange) irradiated sample, the E. coli maintained their original elongated shape and were well-formed (Fig.18). In Fig.19, it is observed that 590nm (yellow) radiation disrupted the E. coli with deflated cell walls. The Control Sample is shown in Fig. 20.

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The analysis of the microscopic and SEM images together with the optical densities and counts is representative of the fact that E. coli responded differently to every colour and the most profound inhibitory effects were that of 538nm and 590 nm Visible Range Radiations. From its morphological analysis and as supported by the counts, it is clearly shown that 538 nm (Green) visible range radiation proved to be bactericidal and 590nm Visible Range Radiation (yellow) was bacteriostatic. The enhanced growth of E. coli with varying degrees was clearly observed in 610nm (orange), 644nm (red), 464nm (Purple) and 453nm (blue).

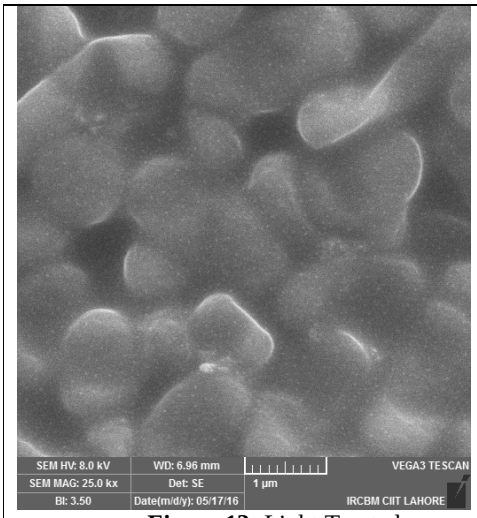


**Figure 13:** Light Treated

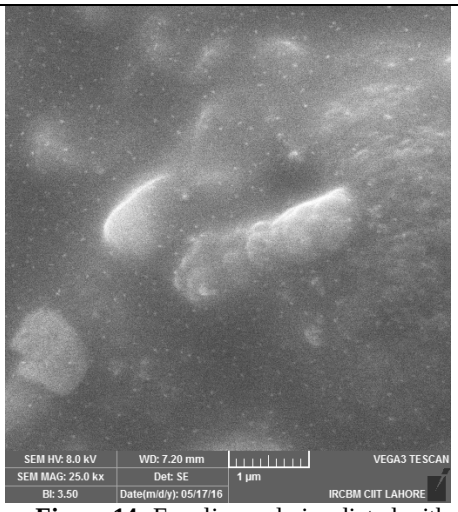


**Figure 14:** E. coli sample irradiated with 464nm (Purple) radiation

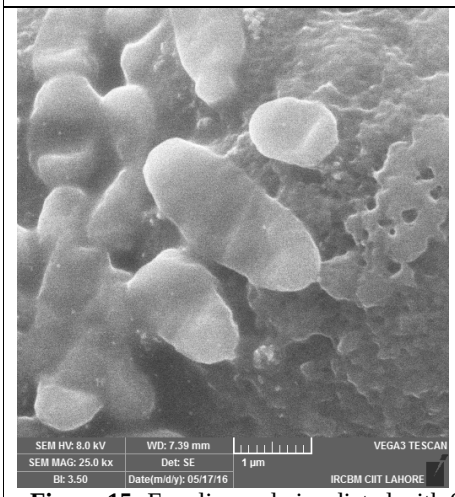


**Figure 15:** E. coli sample irradiated with 644nm (Red) radiation

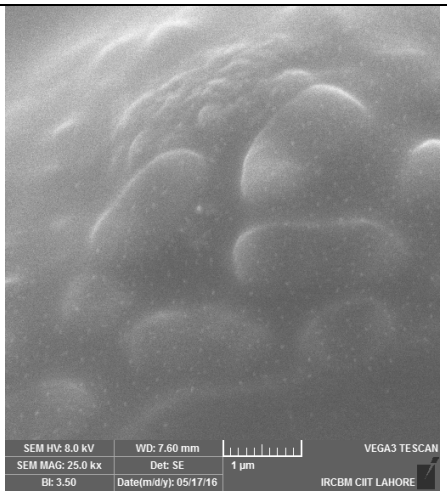


**Figure 16:** E. coli sample irradiated with 453nm (Blue) radiation

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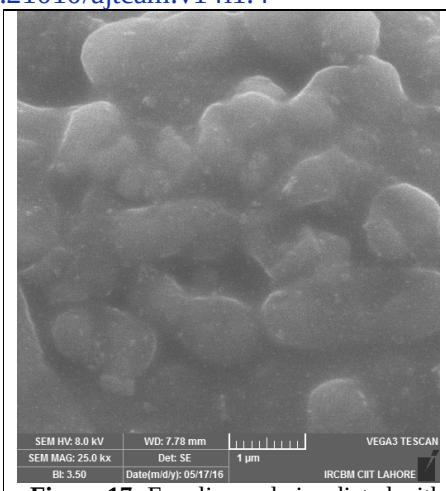


**Figure 17:** E. coli sample irradiated with 538nm (Green) radiation

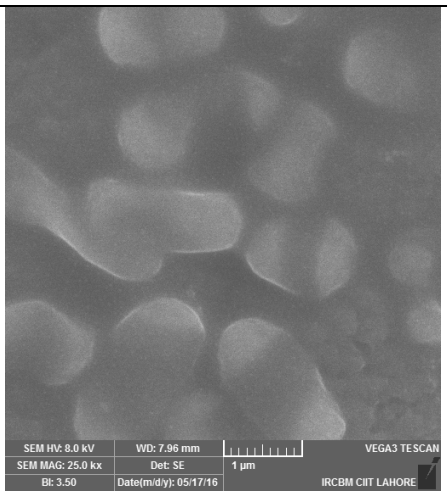


**Figure 18:** E. coli sample irradiated with 610nm (Orange) radiation

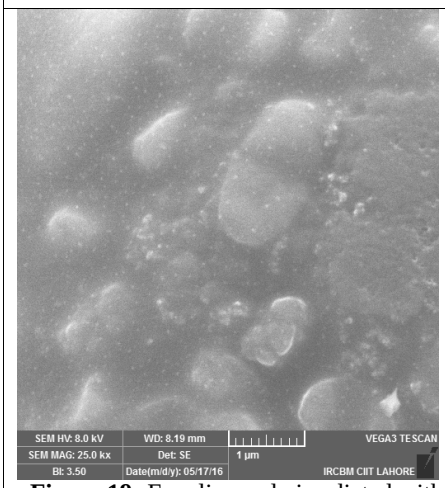


**Figure 19:** E. coli sample irradiated with 590nm (Yellow) radiation

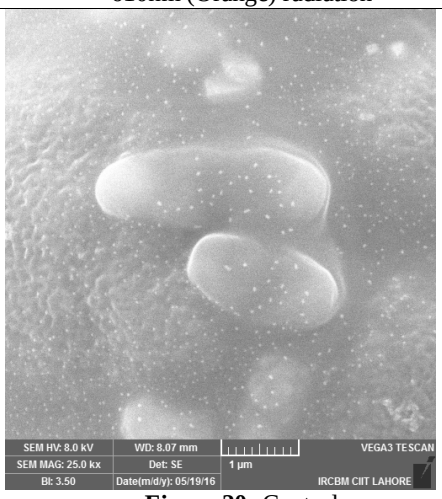


**Figure 20:** Control

## Discussion

Modern health sciences are based on biochemistry. For the greater part, the electrical half of human systems has been completely ignored. Physiology reveals that an electrical signal or an electrochemical reaction releases most of the natural chemicals of the body. Electromagnetic energy fields constituting of microwaves, radio-frequencies, the visible light spectrum (Table. 2) and even acoustic frequencies, have been shown to profoundly impact every aspect of biological regulation (Candace Pert and Oschman 2000). Specific frequencies of electromagnetic radiation affect; cell division; DNA, RNA and syntheses; protein synthesis, function; and nerve conduction and growth. If enzymes and cells can be affected by electromagnetic fields(Azeemi et al. 2008b; Azeemi et al. 2009), there is no reason not to expect electromagnetic radiations to act as a therapeutic agent. The high specificity of electromagnetic signals may result in the "direct targeting" of activity in the cell, without many of the side effects common to pharmaceutical substances (Candace Pert and Oschman 2000).

Interaction of biological object with light is primarily photochemical in nature. Photobiomodulation characteristically induces photo-biological processes in cells. The relationship between these biological responses and the radiation wavelength points to the existence of a photo-acceptor. Strong evidence suggests that Photobiomodulation mechanism is accredited to the activation of mitochondrial respiratory chain components resulting in the initiation of a cascade of cellular reactions (Barolet 2008). It has been found that there are photo-receptors at the molecular-cellular level which, when activated, induce a range of biological reactions: DNA/RNA synthesis, increased cytosolic cAMP levels, protein and collagen synthesis and cellular proliferation (Karu 1998).

Now, the detailed study conducted on the effects of Visible Rage Radiations on *Escherichia Coli* shows that different visible range radiations (Colours) have had a significant effect on the growth and morphology of E. coli. From

the six radiations applied, 590nm (yellow) and 538 nm(green) have shown very interesting results (*in vitro*) clearly showing the low colony counts, less optical density as well a significant change in their morphology that has been indicated by SEM results. These radiations can be very helpful in curing diseases (*in vivo)* caused by the above mentioned strain of E. coli e.g. intestinal infections and urinary tract infections which can be effectively treated with 590nm (Yellow) and 538nm (Green). One of the techniques used in the treatment of diseases by visible range radiations is using medium (water, juices, milk, oils etc.) for absorbed radiant energy (Azeemi et al. 2008c). Previously, Quantum Mechanics of Absorbed Radiant Energy into water samples has already shown unique and effective results (Azeemi et al. 2008a).

**Conclusion**

Thus, it can be concluded that radiations in the visible region i.e., 538nm (green), 590 nm (yellow) can effectively be used for treating E. coli borne diseases.

**Recommendations**

The effects of Visible Range Radiations on E. coli clearly show that different wavelengths affected E. coli differently. All these results indicate that some genetic changes are leading to particular morphological behaviours. Hence, genetic study is pertinent in order to further explore morphological and bio chemical changes in e coli after irradiating them with visible range radiations.

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