EFFECT OF VISIBLE RANGE ELECTROMAGNETIC RADIATION ON SALMONELLA (CAUSATIVE AGENT OF TYPHOID)

S. T. Y. Azeemi¹, S. Azeemi², M. Ari³, K. Mahmood⁴, T. B. Qasim⁵, R. Qasim⁶, S. F. Shaukat⁷, R. Adnan⁸, J. Khalid⁹, M. Paul¹ and A. Rashid⁹

¹ Professor (Physics), Govt. Postgraduate College For Women, Samanabad, Lahore.
² House Officer, Punjab Rangers Teaching Hospital, Lahore.
³ Researcher, CITI Research Lab, Lahore.
⁴ Professor, University of Lahore, Lahore.
⁵ House Officer, CMH, Lahore.
⁶ Professor, University of Lahore, Lahore.
⁷ Director, COMSATS, Vehari Campus, Lahore.
⁸ House Officer, Shalamar Medical College, Lahore.
⁹ BS Physics Student, Govt. Postgraduate College For Women, Gulberg, Lahore.

Background: Salmonella is the agent responsible for a range of clinical diseases. With emerging antimicrobial resistance, alternative treatment options, including visible range radiation therapy are becoming increasingly common. Visible Range Radiation Therapy/Color Therapy is an emerging technique in the field of energy/vibrational medicine that uses visible spectrum of Electromagnetic Radiation to cure different diseases. In this study, our goal was to understand the effect of Visible Range Electromagnetic Radiation on Salmonella (in vitro) and therefore find out the most appropriate visible range radiation for the treatment of diseases caused by Salmonella (in vivo).

Materials and Methods: A total of 8 non-repetitive Salmonella isolates were obtained from blood samples obtained from 45 years young male. A single colony of Salmonella was incubated in 25ml Tip Tip Soi broth (TTSB) and 200ul of this Salmonella suspension was poured into each of the test tubes which were then irradiated with eight different wavelengths in the visible region, after 24 hours with one acting as a control. The colony count was then measured. Furthermore, Scanning Electron Microscopy (SEM) was carried out.

Results: The analysis of the microscopic and SEM images of irradiated Salmonella samples with eight different visible range radiation is representative of the fact that Salmonella responded differently to every applied radiation in the visible region and most profound inhibitory effects were that of 610nm (Orange) Visible Range Radiation which proved to be bactericidal. The enhanced growth of Salmonella with varying degree was clearly observed in 590nm (Yellow), 547nm (Green), 480nm (Blue), 644nm (Red), 464nm (Purple), and 700nm (Black).

Conclusion: It can be concluded that 610nm can effectively be used for inhibition of Salmonella (in vitro) and has the potential to be used for treatment of Salmonella borne diseases (in vivo).

Keywords: Visible Range Radiation Therapy, Salmonella, Alternative Treatment, Color Therapy.

(Received 16.09.2023 Accepted 21.11.2023)

INTRODUCTION

Salmonella is a rod-shaped, Gram-negative facultative anaerobe that belongs to the family Enterobacteriaceae [1]. It is a major worldwide public health concern, accounting for 93.8 million foodborne illnesses and 155,000 deaths per year [2]. Salmonella can cause a variety of diseases, such as gastroenteritis, enteric fever (typhoid), and bacteraemia, depending on the serotype and host [3]. The emergence of multi-drug-resistant (MDR) Salmonella serotypes is having a great impact on the efficacy of antibiotic treatment, and an increasing prevalence of MDR strains may lead to an increase in mortality rates of Salmonella infections [1]. Therefore, there is a need for alternative therapies that can effectively treat Salmonella-borne diseases.

Phototherapy and Visible Range Radiation Therapy are alternative therapeutic systems that have a long history of use for various health conditions, but have recently gained more scientific support through various studies in a number of conditions including Insomnia [4], Diabetes [5], Seasonal Affective Disorder (SAD) [6], Immunity [7], Hyperacidity [8], Dengue Fever [9], Cutaneous wound healing [10], Chronic joint disorders [11] and Inflammation [12]. The pioneer of phototherapy...
using artificial light sources was Niels Ryberg Finsen, a Danish physician and scientist who received the Nobel Prize in Physiology / Medicine in 1903 for his contribution to the treatment of diseases, especially lupus vulgaris. The visible colors, which are a narrow band in the electromagnetic spectrum, have unique wavelengths and oscillations that generate electrical impulses that activate the biochemical and hormonal processes.

Previous studies have also shown that Visible Range Radiation Therapy can affect the growth of bacteria. Visible light can be used as a disinfectant to kill or inactivate bacteria by generating reactive oxygen species that damage the bacterial cells [13, 14]. This process is called photoinactivation and it depends on the wavelength, intensity, and duration of the light exposure, as well as the type and concentration of the bacteria. In this study, we aim to investigate the effect of Visible Range Electromagnetic Radiations on Salmonella in vitro. Therefore, we designed this study to explore the impact of different wavelengths and intensities of visible light on the growth, survival, and virulence of Salmonella in vitro. We hypothesized that visible light could be used as a novel and effective anti-Salmonella agent.

**MATERIAL AND METHODS**

We obtained 8 distinct Salmonella isolates from the blood samples of a 45-year-old male patient with suspected Salmonella infection at CITI Lab, Lahore in November. The blood samples were enriched in 25 ml of Tip Tip Soibroth (TTSB) and then streaked on Macconkey agar (Oxide) and CLED agar. The plates were incubated at 37°C for 18 hours. Salmonella isolates were identified by colony morphology and biochemical tests, such as indole production, triple sugar iron reaction, and citrate utilization. Gram staining was also performed and gram-negative rods were observed.

A single colony of Salmonella was inoculated in 60 ml of NB and 200 µl aliquots of this culture were transferred to test tubes. The test tubes were exposed to eight different wavelengths of visible light (Table 1) for 24, 36, 48, and 86 hours, respectively, while one test tube was kept as a control. The test tubes were incubated at 37°C during the exposure. The optical densities of the irradiated cultures were measured using a UV-visible spectrophotometer at 600 nm. Gram staining was also performed on the irradiated cultures. The experiment was performed in duplicate.

The effect of visible light on the morphology of Salmonella was examined by SEM. Aliquots of 0.2 ml of the Salmonella culture were exposed to the same eight wavelengths of visible light for 24 hours and their optical densities were measured. The irradiated cultures were then fixed and processed for SEM analysis.

### Table 1: Dominant wavelength of monochromatic light as measured by a spectrophotometer. The spectral bandwidth was set to 0.1 nm and the ordinates were set to 10. The spectrophotometer used was a Hitachi U-2000 UV-Vis double beam model.

<table>
<thead>
<tr>
<th>Color</th>
<th>Dominant Wavelength (n)</th>
<th>Hue (m)</th>
<th>Purity (%)</th>
<th>Transmission (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purple</td>
<td>464</td>
<td>Violet</td>
<td>36%</td>
<td>32%</td>
</tr>
<tr>
<td>Blue</td>
<td>483</td>
<td>Blue Green</td>
<td>52%</td>
<td>52%</td>
</tr>
<tr>
<td>Green</td>
<td>538</td>
<td>Greenish Yellow</td>
<td>15%</td>
<td>37%</td>
</tr>
<tr>
<td>Yellow</td>
<td>590</td>
<td>Reddish Yellow</td>
<td>40%</td>
<td>82%</td>
</tr>
<tr>
<td>Orange</td>
<td>610</td>
<td>Orange</td>
<td>43%</td>
<td>47%</td>
</tr>
<tr>
<td>Red</td>
<td>644</td>
<td>Red</td>
<td>41%</td>
<td>51%</td>
</tr>
<tr>
<td>Black</td>
<td>700</td>
<td>Black</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**RESULTS**

We count the colonies that grew on the Macconkey agar plates after exposure to different wavelengths of visible light (Figure 2). The lowest colony count was observed in the sample exposed to 610 nm (orange color), while the highest colony count was observed in the sample exposed to 700 nm (black color). The colony count of orange color is less than that of control sample, suggesting that orange light has an inhibitory effect on Salmonella. Interestingly, for purple (464nm) and green (538nm), the colony count is higher than control, suggesting stimulatory effect of these radiation on Salmonella.

Scanning Electron Microscope (SEM) images of the irradiated slides were carried out (Fig. 5 to 12). The images show that Visible Range Radiation significantly affected the morphology of Salmonella in comparison with the control sample. The light treated Salmonella became flattened and circular in shape. The 483nm (Blue) irradiated Salmonella deflated owing to leakage of cell solutes. The 644nm (Red) irradiated sample was elongated and increased in their size. In 538nm (Green) irradiated samples, the Salmonella clearly lost its original bacillary shape becoming circular. The 610nm (Orange) irradiated sample was wrinkled. In 590nm (Yellow) irradiated sample, the Salmonella maintained their original elongated shape and were well-founded. The
464nm (Purple) irradiated sample was elongated and some of the Salmonella showed an unusual increase in their length with the greatest deviation from their original size. The 700nm (Black) treated Salmonella was rough, but elongated with mosaic pattern.

Figure 3: No. of colonies for the samples irradiated with seven different radiation along with the control

Figure 5: Light Treated Sample (Control)

Figure 6: Salmonella sample irradiated with 483nm (Blue) radiation

Figure 7: Salmonella sample irradiated with 644nm (Red) radiation

Figure 8: Salmonella sample irradiated with 538nm (Green) radiation
DISCUSSION

Our work investigated the effects of visible light on Salmonella Typhi in vitro. Eight wavelengths of light were applied and their impact on the bacterial growth and morphology was assessed. The results showed that orange (610 nm) radiation significantly reduced the colony counts of the bacterial culture, as well as induced morphological changes that were observed by scanning electron microscopy (SEM). This wavelength could be potential candidates for phototherapy of infections caused by Salmonella Typhi in vivo, such as enteric and diarrheal diseases. One possible modality is to use water or other media as carriers of radiant energy to the infected site, also known as Hydrochromotherapy [15, 16].

The mechanism of photobiomodulation by visible light on Salmonella Typhi is not fully understood, but some possible explanations have been proposed. One hypothesis is that visible light interacts with photoacceptors in the bacterial membrane, such as cytochrome c oxidase, flavins, or porphyrins, and alters their redox state and electron transfer. This could affect the production of reactive oxygen species (ROS) and the cellular energy metabolism, leading to oxidative stress and cell death. Another hypothesis is that visible light induces changes in the membrane potential and permeability, resulting in the leakage of ions and molecules and the disruption of cellular homeostasis. These hypotheses need to be tested by further experiments to elucidate the molecular mechanisms of visible light on Salmonella Typhi.

Future Work: The study revealed that visible light had distinct effects on Salmonella Typhi depending on the wavelength. These effects suggested that the bacteria
experienced genetic modifications that influenced their morphology. Therefore, a genetic investigation is warranted to further elucidate the molecular and biochemical mechanisms of Salmonella Typhi after exposure to visible light.

REFERENCES

8. Azeemi, S.T. and R. Yousuf, EFFECT OF CHROMOTHERAPY IN HYPERACIDITY DURING PREGNANCY.