Ultraviolet-C haematogenous oxidation therapy of lipopolysaccharide-induced endotoxemia in a rabbit model: A biochemical study

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Systemic inflammatory reaction – due to severe response to toxins of infection associated with immune inhibition – leads to multi-organ dysfunctions and high mortality. Ultraviolet (UV) blood is used for its therapeutic effects when moving across the cells. This study aims to evaluate the impact of UV-C Haematogenous Oxidation Therapy (HOT) in Lipopolysaccharide (LPS)-induced endotoxemia of rabbit model. A total of 40 rabbits randomly divided into four groups, including normal control (NC). LPS and LPS+UV-C HOT groups received 0.1 mg/kg LPS toxin of E. coli, UV-C HOT and LPS+UV-C HOT groups subjected to UV-C HOT treatments once weekly for five times. Blood collected, perfused with oxygen, UV-C directly irradiated into blood, and then auto-transfused. Rabbits were sacrificed after five weeks; blood and serum were collected for analysis. The survival rate, liver, kidney, lipid profile, and blood ions were assessed in treated rabbits. Mortality was 40% in the LPS group, while other groups showed no death. UV-C HOT enhanced critical pH, base deficit, blood gases, hypomagnesemia, hyperlactatemia, and concurrent acidosis. Besides, TNF-α, nitrite, and nitrate were suppressed in response to UV-C HOT. Moreover, UV-C HOT reduced liver and kidney enzymes, improved lipid metabolism, and ameliorated electrolytes homeostasis. Despite that, UV-C HOT performance in ICU for human and animal endotoxemic or septic patients should be evaluated and considered.

Keywords: Lactate, Lipopolysaccharide (LPS), Magnesium, Metabolic acidosis, Sepsis, Rabbits, Ultraviolet Haematogenous Oxidation Therapy (UV-C HOT)

Systemic inflammatory endotoxemia is a life-threatening condition initiated by a disturbance in an immune system in response to infection results in multi-organ injuries and death. The condition still challenges the healthcare providers because of the high percentage of morbidity and mortality in septic patients. Hospitalized patients often get supportive treatment, but the death rate is high1,2.

Lipopolysaccharide (LPS), a significant part of the Gram-negative bacterial outer membrane, is a critical pathogenic stimulator for the tissues dysfunction. LPS can also induce non-immune cells and initiate the inflammatory process leading to severe inflammatory responses, which are usually fatal; it is widely used to produce endotoxemia and sepsis models3,4. Numerous inflammatory mediators and cytokines such as tumor necrosis factor α (TNF-α) and superoxide anion have known to be released in response to LPS-systemic inflammatory induction. Besides, the blood partial oxygen concentration is significantly decreased as reported by recent studies5,6. Although advanced studies were done, yet no radical treatment available for septic patients7.

Ultraviolet (UV) Therapy (photo-oxidation therapy) is a biological healing modality utilizing ultraviolet irradiation of the blood (with UV-C light) to produce beneficial photochemical reactions. Ultraviolet blood irradiation (UBI) was hailed as a miracle therapy for treating severe infections in the mid of 19th century. However, histrically, that period coincided with the widespread introduction of penicillin antibiotics, which were rapidly found to be an even bigger miracle therapy.
Moreover, another major success of UBI, which has been used to treat polio, was also eclipsed by the introduction of the Salk vaccine. Starting in the 1960s, UBI fell into disuse in the West and has now been called “the cure that time forgot”\(^8\). Besides its effects on animals, but also UV and gamma radiation potentiate pharmacological properties of plants\(^9,10\). Recently, biological influences due to ionizing radiation in low dose have been investigated, and irradiated cells transfer information via physical methods known as electromagnetic signals (bio-photon-mediated effect), which is well documented in biophysics research\(^11-13\).

Knott and his co-workers have successfully treated dog infection with Staphylococcus aureus and Hemolytic streptococcus as follow: blood (5-7%) collected from infected dogs, treated with UV radiation, and reinfused into the same animals. The infected dogs showed complete recovery\(^9\). Generally, radiation biology is becoming ubiquitous for its in vitro antimicrobial effects\(^14\). Therefore, the purpose of this study presented the use of UV-C haematogenous oxidation therapy (HOT) in LPS-induced systemic inflammatory reaction.

**Materials and Methods**

**Animals**

Forty male New-Zealand rabbits weighing (2-2.5 kg) were used, purchased from Samtako Biokorea, Daejeon, Korea. They were kept in cages maintained at 23±2°C and 50±5 % humidity in a 12 h light/dark cycle with free access to water and feed. Bodyweight was taken weekly throughout the study. Rabbits randomly divided into four groups, Normal Control (NC), LPS control, only UV-C HOT treated group and LPS+UV-C HOT group. The experimental protocols were concurred by the committee on the care of laboratory animal resources, Chonbuk National University, and were completed as per the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication no. 85-23, revised 1996).

**Induction of endotoxemia**

After one week of acclimation, bacterial LPS endotoxin of *E. coli* was used to induce endotoxemia reactions. The rabbits of LPS and LPS+UV-C HOT groups were made by injecting 0.1 mg/kg BW of LPS (*E. coli* O127: B8; Sigma, St. Louis, MO, USA) in 1 mL normal saline intraperitoneally (i.p) before starting UV-C HOT treatment\(^15\). 1 mL normal saline was injected into NC rabbits. All rabbits received 5 mL of normal saline i.p to avoid any complications. UV-C HOT treatment was started one h after endotoxemia induction.

**UV-C HOT device and procedure**

In this study, we have made the UV-C HOT device to identify effects of UBI on the blood in a diabetic animal model. Fig. 1 shows a simple drawing of the HOT device. In brief, a fixing holder for quartz cuvette is installed in the center, and UV lamps are installed on both sides of the holder. The UV lamps are designed to adjust the distance (5 ~ 120 mm) from the holder in the center. Ultraviolet blood irradiation is performed in the cuvette. The blood was collected in sodium citrate solution (BOIN ACDA SOLN, SBD Co. Ltd., Shenzhen, China) containing an anticoagulant, perfused with oxygen for 10 sec. UV light with the intensity of 10.290 J/cm\(^2\) is irradiated to the blood, which passes at a 1.25 mL/min flow rate using the infusion pump in the UV-C HOT device. After the UV-C HOT was performed, the blood was transfused back to the original rabbit.

![Fig. 1 — Schematic illustration of the UV-C HOT device](image-url)
Measurement of blood ions, metabolites, and enzymes
Blood was collected from marginal ear before sacrifice. Rabbits anesthetized with Rompun and Zolitel mixture at 30 and 5 mg/kg BW, respectively. Nova Stat Profile 8 CRT (NOVA Biomedical Corp, Waltham, MA, USA) was used for measuring pH, base excess blood (BE-b), base excess extracellular fluid (BE-ecf), partial pressures of carbon dioxide (pCO₂), and oxygen (pO₂), bicarbonate (HCO₃⁻), lactate, hematocrit (Hct), hemoglobin (Hb), and the concentrations of sodium (Na⁺), potassium (K⁺), calcium (Ca²⁺), magnesium (Mg²⁺), chloride (Cl⁻), and anion gap (AG).

Serum biochemical analysis
After clotting, blood serum was separated by centrifugation at 3000 rpm for 20 min. The levels of calcium, magnesium, glucose, enzymes, and proteins were analyzed using a model 7020 autoanalyzer (Hitachi, Tokyo, Japan).

Determination of net survival percentage
The survival rate was calculated according to the following equation:

\[
\text{Survival \%} = \left( \frac{\text{total no.} - \text{dead no.}}{\text{total no.}} \right) \times 100
\]

Determination of antioxidant enzymes, TNF-α, and nitrite/nitrate concentration. Serum was used for the determination of antioxidant enzymes. Commercial ELISA kits were used to quantify the concentration of catalase (Thermo Fisher Scientific, USA), TNF-α (MyBioSource, USA), nitrite/nitrate (Sigma, USA), according to manufacturer’s protocol.

Statistical analysis
The Prism 5.03 software (Graph Pad Software Inc., San Diego, CA, USA) was used for the statistical analysis of the data and making graphs. Results are expressed as mean ± standard error of the mean (SEM). Unpaired Student’s t-test correlated with the Spearman’s rank correlation coefficient was used to evaluate differences between specified groups. The level of significance was set at \( P < 0.05 \).

Results
Effect of UV-C HOT treatment on body weight, organ weight, survival percentage. Table 1 showed the bodyweight of experimental groups. LPS caused a substantial decrease in the body compared to the NC group. However, UV-C HOT and LPS+UV-C HOT almost kept the same bodyweight as the normal group. Liver, lung, and kidney weight were increased in the LPS group compared to NC rabbits but the weight of these organs remained lower in the UV-C HOT and LPS+UV-C HOT group compared to that of septic rabbits.

The survival rate is shown in the four groups. No deaths in NC, UV-C HOT, and LPS+UV-C HOT groups were observed. The survival rate was 60% in the LPS group at week five after endotoxemia induction. The death occurred as follows: two rabbits died one night after the LPS injection, whereas the other two rabbits weakly lived for one week and died. The LPS group rabbits were emaciated, lethargic, and weak. The result proposes that UV-C HOT treatment is safe and reduced the morbidity, then the survival rate was increased in treated groups.

Effect of UV-C HOT treatment on catalase, tumor necrosis factor-α, Nitrite, and Nitrate
To assess the ameliorative effect of UV-C HOT on oxidative stress and inflammation induced by LPS, we measure catalase, TNF-α, Nitrite, and Nitrate concentrations in serum. Catalase concentration in the serum was significantly decreased in the LPS group compared to the NC rabbits. However, catalase concentration in both UV-C HOT and LPS+UV-C HOT group was similarly low compared to LPS group. TNF-α, Nitrite, and Nitrate concentration in the LPS group were significantly increased compared to the normal group. However, TNF-α, Nitrite, and Nitrate concentration in UV-C HOT and LPS+UV-C HOT

| Table 1 — Effect of UV-C HOT treatment on survival rate, bodyweight, and organs/ body weight and ratio |
|-------------------------------|---------|---------|---------|---------|
| Survival (%)                  | NC      | UV-C HOT| LPS     | LPS+UV-C HOT |
| BW (kg)                       | 3.2±0.9 | 3.0±0.5 | 2.7±0.8* | 3.0±0.8*     |
| Lung/BW                       | 3.2±0.1 | 3.2±0.1 | 5.0±0.2**| 3.7±0.3***  |
| Liver/BW                      | 29.8±1.3| 29.4±1.0| 41.8±1.7***| 33.7±1.8**  |
| Kidney/BW                     | 5.0±0.2 | 4.9±0.2 | 6.3±0.2** | 5.5±0.2*    |

The data of body weight (BW) and organs/BW ratio (g/kg) were given as mean ± SEM (n = survived animal). *: \( P < 0.05 \); **: \( P < 0.01 \); and ***: \( P < 0.001 \), unpaired student’s t-test: normal control (NC) vs Ultraviolet-C haematogenous oxidation therapy(UV-C HOT) and lipopolysaccharide group (LPS), \( \# P < 0.05; \#\# P < 0.01; \) and \#\#\#: \( P < 0.001 \), unpaired student’s t-test: LPS vs LPS+UV-C HOT group.
group were significantly reduced compared to the LPS group, as shown in (Fig. 2).

**Effect of UV-C HOT treatment on blood acid-base balance, base excess and gases**

To examine the efficacy of UV-C HOT on acidosis, blood pH and gases were measured. A significant decrease in the levels of pH, HCO$_3^-$, pO$_2$, and pCO$_2$ in the LPS group was observed compared to normal rabbits. Interestingly, these parameters were substantially improved after UV-C HOT, as shown in (Fig. 3). The UV-C HOT group either showed the same result as NC or even a better result due to oxygenation properties of UV in blood and tissues discussed in this investigation.

**Effect of UV-C HOT treatment on blood magnesium, lactate, hemoglobin, and hematocrit**

To evaluate the effects of UV-C HOT on ICU critical indicators, blood parameters were measured immediately after sacrifice. Fig. 4 demonstrated that hypomagnesemia, hyperlactatemia, low Hb, and Hct were induced in the LPS group. More importantly, in UV-C HOT-treated rabbits, Mg$^{2+}$ and lactate levels were significantly increased and decreased, subsequently, whereas Hb and Hct were considerably increased compared to those of LPS group.

**Effect of UV-C HOT treatment on liver and kidney function**

Results of liver function tests, such as serum aspartate aminotransferase (AST), alanine amino transaminase (ALT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) to examine the efficacy of the UV-C HOT treatment on the septic in rabbit model as demonstrated in (Fig. 5). In the LPS group, AST, ALT, LDH, and ALP levels were significantly increased compared to those of normal rabbits. However, in the UV-C HOT group, these levels were significantly decreased compared to those of septic rabbits without treatment. Furthermore, in septic rabbits undergoing UV-C HOT treatment, all measured values were reduced compared to those of the LPS group.

Results of renal function tests, such as serum creatinine (CRE), blood urea nitrogen (BUN), uric acid (UA), and creatine kinase (CK), were analyzed to examine the efficacy of UV-C HOT on the sepsis. In septic rabbits, the levels of CRE, BUN, UA, and CK levels were significantly increased compared to those of normal rabbits.

![Fig. 2 — Effects of UV-C HOT on (A) catalase; (B) tumor necrosis factor-α (TNF-α); (C) nitrite; and (D) nitrate concentration. The Data are represented as mean ± SEM (n = survived animal). *: P < 0.05; **: P < 0.01; and ***: P < 0.001, unpaired student’s t-test: normal control (NC) vs Ultraviolet-C haematogenous oxidation therapy (UV-C HOT) and lipopolysaccharide group (LPS); #: P < 0.05; ##: P < 0.01; and ###: P < 0.001, unpaired student’s t-test: LPS vs LPS+UV-C HOT group](image-url)
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normal rabbits. However, in the UV-C HOT group, these levels are significantly decreased when compared to septic rabbits without treatment. In addition, in septic rabbits undergoing UV-C HOT treatment, all measured values were reduced compared to the LPS group.

Effect of UV-C HOT treatment on lipid and protein profile
Lipid and protein metabolism were evaluated. Total cholesterol (T-CHO), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TG), total protein (T-PRO) and Albumin (Alb) levels
in the blood were measured to examine the efficacy of UV-C HOT on endotoxemia as exhibited in (Table 2). Except for HDL, which significantly decreased, all other values were increased in septic rabbits compared to those of normal rabbits. However, in UV-C HOT group T-CHO, LDL, TG, PRO-T, and Alb were significantly decreased, but HDL was significantly increased compared to those of the LPS group. Similarly, in LPS+UV-C HOT group T-CHO, LDL, TG, PRO-T, and Alb were significantly decreased, but HDL was significantly increased compared to those of the LPS group.

**Effect of UV-C HOT treatment on blood ions**

As listed in (Table 3), Ca\(^{2+}\), Na\(^{+}\), K\(^{+}\), Cl\(^{-}\), and AG in blood were significantly reduced in the LPS group compared to those of normal rabbits. However, in rabbits subjected to UV-C HOT, these values showed a significant enhancement compared to those of the LPS group.

**Table 2 — Effect of UV-C HOT treatment on serum lipid and protein profile**

<table>
<thead>
<tr>
<th></th>
<th>NC</th>
<th>UV-C HOT</th>
<th>LPS</th>
<th>LPS+UV-C HOT</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-CHO (mg/dL)</td>
<td>29.3±1.8</td>
<td>29.6±2.4</td>
<td>53.5±2.5***</td>
<td>41.8±2.4##</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>148.5±7.4</td>
<td>139.0±6.4</td>
<td>77.5±0.4***</td>
<td>124.0±5.1###</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>8.0±0.4</td>
<td>11.4±0.7**</td>
<td>29.5±0.5***</td>
<td>24.0±0.4####</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>39.0±1.9</td>
<td>46.0±2.9</td>
<td>147.3±2.7***</td>
<td>86.2±3.4####</td>
</tr>
<tr>
<td>T-PRO (g/dL)</td>
<td>7.8±0.6</td>
<td>8.6±0.1***</td>
<td>5.8±0.5**</td>
<td>7.4±0.2#</td>
</tr>
<tr>
<td>Alb (g/dL)</td>
<td>5.4±0.1</td>
<td>5.3±0.1</td>
<td>3.6±0.4***</td>
<td>5.1±0.1##</td>
</tr>
</tbody>
</table>

Total cholesterol (T-CHO), high density lipoprotein (HDL), low density lipoprotein (LDL), triglyceride (TG), total protein (T-PRO), and albumin (Alb). The data were calculated as mean ± SEM (n = survived animal). *: P < 0.05; **: P < 0.01; and ***: P < 0.001, unpaired student’s t-test: normal control (NC) vs UV-C HOT and lipopolysaccharide group (LPS); #: P < 0.05; ##: P < 0.01; and ###: P < 0.001, unpaired student’s t-test: LPS vs LPS+UV-C HOT group.
Calcium (Ca\(^{2+}\)), sodium (Na\(^{+}\)), potassium (K\(^{+}\)), chloride (Cl\(^{-}\)), and anion gap (AG). The data were represented as mean ± SEM \((n = \text{survived animal})\). \(*: P < 0.05; \#: P < 0.01; \text{and} \quad **: P < 0.001, \text{unpaired student’s } t\text{-test: normal control (NC)} \text{ vs} \text{ UV-C HOT and lipopolysaccharide group (LPS);} \quad \# P < 0.05, \text{##: } P < 0.01; \text{and} \quad ###: P < 0.001, \text{unpaired student’s } t\text{-test: LPS vs LPS+UV-C HOT group}}

**Table 3 — Effect of UV-C HOT treatment on blood ions**

<table>
<thead>
<tr>
<th></th>
<th>NC</th>
<th>UV-C HOT</th>
<th>LPS</th>
<th>LPS+UV-C HOT</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{Ca}^{2+}) (mmol/dL)</td>
<td>1.57±0.01</td>
<td>1.62±0.03</td>
<td>1.38±0.02**</td>
<td>1.60±0.07###</td>
</tr>
<tr>
<td>(\text{Na}^{+}) (mmol/dL)</td>
<td>142.1±0.4</td>
<td>141.3±0.9</td>
<td>137.9±0.5**</td>
<td>139.8±0.3###</td>
</tr>
<tr>
<td>(\text{K}^{+}) (mmol/dL)</td>
<td>5.05±0.32</td>
<td>4.57±0.07</td>
<td>4.28±0.17</td>
<td>4.56±0.08</td>
</tr>
<tr>
<td>(\text{Cl}) (mmol/dL)</td>
<td>104.0±0.2</td>
<td>104.4±0.6</td>
<td>108.8±0.3**</td>
<td>103.4±0.3###</td>
</tr>
<tr>
<td>(\text{AG}) (mmol/dL)</td>
<td>17.9±0.4</td>
<td>17.2±0.6</td>
<td>20.4±0.3**</td>
<td>16.3±0.5###</td>
</tr>
</tbody>
</table>

**Discussion**

The importance of endotoxemia, sepsis, and systemic inflammatory reaction emerge from their low survival rate. While patients with cancer survive for more than 60 months, sepsis patient life ends in not more a month, so still, stand out as the significant determinants of death\(^{16,17}\). The present investigation indicates that the treatment of rabbits subjected to LPS toxicity with UV-C HOT for five times reduces endotoxemia-critical signs, organ dysfunctions, morbidity, and mortality. The survival percentage was low in LPS group compared to other groups, that could be due to as a direct deterioration in vital tissue and organs of the body, Fleischmann and his co-workers\(^{18}\) have reported that the death occurred in more than 50% in septic patients. Although the body weight was reduced in our LPS group, the liver, lung, and kidney weight were increased in the LPS group. Malmezat and co-workers observed similar result that rats injected with LPS showed a reduced body weight, reduction in body weight has been attributed to low food intake and reduction in skeletal muscles\(^{19,20}\), increase in lung weight has been linked to lung toxicity\(^{21}\). UV-C HOT-treated rabbit’s data indicate that organs weight and body weight were significantly reduced and increased, respectively, which is due to anti-inflammatory properties of UV-C HOT\(^{22}\).

In response to alveolar damage, \(\text{PO}_{2}\), \(\text{PCO}_{2}\), and \(\text{HCO}_{3}\) were significantly deteriorated in blood, as shown in the result, this is due to cytokines release following lung injury\(^{22}\). As anticipated, UV-C HOT-treated rabbits have shown significant enhancement in gases condition, UV irradiation is evidenced to activate cardio-circulatory oxygenation after few minutes of treatment\(^{23}\).

Montassier and others observed that there is a proportional correlation between lactate and base excess, which is easily measured in emergency room\(^{24}\). Our result emphasized this observation, we confirmed that high levels of base excess and lactate in septic rabbits. This can be attributed to the pathogenicity of LPS in kidney and lung.

Hypomagnesemia in endotoxemia and septic patients is well established, and associated with high mortality\(^{25,26}\); it has been thought to be due to incurable kidney dysfunction\(^{27}\). Therefore, kidney treatment can improve magnesium level in septic patients if treated with UV-C HOT. Ashurov et al. reported that acute glomeronephritis and hepatitis respond to complete recovery in the patient with peritonitis subjected to UBI treatment\(^{28}\). The data also indicate that lactate level of LPS group, which is incomparable with normal rabbits, was markedly increased, hyperlactatemia is an emergency-intervention required assign and relevant to lower prognosis of sepsis\(^{29}\). Hematocrit and Hb are significantly reduced in septic rabbits, attributed to septicemia induced by LPS causing severe hypovolemia and anemia\(^{30,31}\); UBI treatment improves tissue oxygen supply, increases blood viscosity, reduces platelet aggregation, and improve vascular circulation within a short time after treatment\(^{23}\). Our data are agreed with this observation, improvement of cardiovascular circulation, and increase of oxygen supply as can be reflected by increases in the level of Hb.

Our result shows high levels of TNF-\(\alpha\), Nitrite, and Nitrate in the LPS group, but catalase, a potent free radical mitigator, was significantly declined. Mediators release, such as TNF-\(\alpha\), lipid peroxidation, and free radicals spike were reported to increase in septicemia. Furthermore, an antioxidant shortage increased levels of Nitrite, and Nitrate was observed in systemic inflammation such as endotoxemia\(^{32,33}\).

The liver plays an essential role in response to a systemic infection via mechanisms, such as toxin clearance, cytokine secretion, and metabolically adapt to inflammation. Therefore, it is directly insulted by sepsis-related damages, such as hypoxic hepatitis because of ischemia, cholestasis because of disturbed
bile utilization, hepatocellular damage as a result of overpowering inflammation. Kidney failure and lung injury, including alveolar damage, are another common consequence of systemic inflammatory reactions leading to the high probability of death. That is why in our result (Fig. 5), markers of liver damage, such as AST, ALT, LDH, an ALP were crucially increased in the LPS group; kidney damage markers were also significantly disturbed in the LPS group compared to the normal rabbit.

Turmoil in pH and blood electrolytes of LPS toxicity has been investigated extensively, for instance, Carausu and his co-researchers observed that levels of Na⁺, K⁺, Cl⁻, and pH were significantly decreased in septic patients. Moreover, the acid-base disturbance is recognized, authors concluded that clinical intervention could be based on electrolytes and acid-base balance follow up. Thongprayoon and his team also reported a connection between systemic reaction due to LPS and hypomagnesemia. Electrolytes and protein imbalances in our results, as shown in (Table 3) can be attributed to renal dysfunction and considered a proof for necrotic inflammation.

Although UV has a wide range of spectra, this study was done with UV-C irradiation because of its potency among UV spectra. Owusu-Ofori and others prove UV light treatment implied in our UV-C HOT protocol can reduce the infectivity of a blood-borne pathogen when used to sterilize blood in blood transfusion unit. This technique was confirmed to be safe in terms of toxicity and teratogenicity, which agrees with our result throughout the study in UV-C HOT- treated groups, as no adverse effects were observed. Their single UV-irradiation study result also indicated that Hb, Na⁺, K⁺, lactate, HCO₃⁻, PO₂, and PCO₂ were 11.6±1.3, 9, 3.1±0.5, 4.01±1.39, 16.5±2.0, 52±31.7, and 64.1±9.9, respectively, which is more or less similar to our result after five times treatment. However, our result indicated a significant enhancement in the levels of HCO₃⁻, PO₂, and PCO₂ in normal rabbits treated with UV-C HOT, which might be because the beneficial effects UBI.

UV-C HOT-treated groups showed an increased level of catalase, which can be interpreted by the positive effect of UV-C HOT on lipid peroxidation, as catalase is well known for its essential role in peroxide amelioration, we also interestingly noticed that catalase increase was not as high as to the level that harms the body as confirmed by a previous study. This notice may also explain the result in (Fig. 3), TNF-α, nitrite, and nitrate were decreased in the UV-C HOT-treated group. However, our data contradict with other investigation, which showed that protein synthesis has increased in response to septicemia and systemic influence of toxin, which might be referred to asthe type of toxin used. A previous investigation reported that UBI without oxygenation could lead to a rise in hydrogen peroxide. Accordingly, in this study, we first oxygenated the blood before treatment with UV light.

To better understand the wide range of ailments and dysfunctions covered by UBI treatment, we need to recall the biophysics and molecular biology. According to the law of quantum physics, the electrons arranging around the shell of nuclei can be excited by less-ionizing energy-carrying photons, this excitement allows electrons to move towards the higher orbit. Thereby, when the blood irradiated with UV, an incoming treated photon of the blood transfused back to the animals can excite an electron to travel to higher orbit in atoms in a process termed as secondary irradiation (SI), then the electron itself emits a photon, the new bio-photon is finally free to go on through the same process of SI. The benefits gained from UBI are attributed to this secondary irradiation. Three patterns were hypothesized after photon absorption: direct utilization, stabilization, and degradation, the latter is the most reaction in which an excited molecule transfers its energy to rotation and vibrations of other molecules and finally to other parts of living tissue.

Photons enter organisms by a process called photon sucking, thought to regulate cell growth and development as reported to be under the control of these bio-photons and may go further to monitor the entire process of life of organisms. Fels reported that yeast cells respond positively to UV-bio-photons emitted from neighboring cells, tissue cells symmetrically arranged themselves matching cells on the opposite sides. Therefore, we propose that bio-photons emitted from irradiated blood transfused back to animals rearrange the function of the cells at the fundamental unit carrying corrective information from sound to disturbed cells, so detection of bio-photons after treatment will assist in confirming and understanding the efficacy of the UV-C HOT procedure.

**Conclusion**

To sum up, the purpose of this experiment was to examine the therapeutic effects of UV-C HOT on
rabbids exposed to LPS toxicity. The result showed that weekly application of UV-C HOT in LPS-induced inflammatory reaction for five times possess beneficial effects, which are due to anti-inflammatory, tissue oxygenation, and antioxidant qualities of UV-C HOT treatment. Our result is in line with the trend of using UBI for its antibacterial and anti-inflammatory qualities. UV-C HOT practice in endotoxemia and septic human and animal patients should be considered and evaluated for its molecular background.

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