CONTROL OF *STAPHYLOCOCCUS AUREUS* ACTIVITY IN RATS USING ELECTROMAGNETIC SIGNALS AT RESONANCE FREQUENCY "IN VIVO STUDY

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Abstract

In this work, the frequency of electric impulses that interfere with the bioelectric signals generated during *staphylococcus aureus* (*S.aureus*) cellular division is investigated. The experiment was expanded to in vivo study for the obtained data in which rats were infected with *S.aureus* and then whole body exposure were exposed to square electric pulses (SEP) that causes inhibition to the microbial cellular growth. Another group of animals was infected by previously inhibited bacteria with SEP then the histological and molecular structures of the liver were investigated for all the animal groups, Dielectric relaxation studies for the liver in the frequency range 42 KHz-5MHz was used to determined molecular structure changes.

The results indicated a highly significant inhibition of cellular growth for *S.aureus* in addition to pronounced changes in the cellular morphology after the exposure of the micro-organism to the resonance frequency of 0.8 Hz SEP for 120 minutes. From the histological and dielectric relaxation measurements and results it was indicated that the liver for animals infected by *S.aureus* and then exposed to SEP showed significant improvement in their health state as compared with infected and non exposed group. Moreover, the liver for the animals infected with previously treated bacteria with SEP showed highly significant decrease in cellular damage as

compared with untreated bacteria. It was concluded that treatment of *S.aureus* by 0.8 Hz SEP acts on the structure and biological activity of the bacteria and it is a promising methodology to control *S.aureus* activity in vivo and in vitro applications.

Keywords: *Staphylococcus aureus,* Microbial Inhibition, electric Field, resonance frequency

Introduction

S. aureus is an extremely flexible organism,it can be commensal but also dangerous pathogen, causing skin abscesses wound infections, endocarditis osteomyelitis, pneumonia, and toxic shock syndrome, bacteremia and septic arthritis (Nawras,2011). *S.aureus* is facultitatively anaerobic gram positive coccus and is the most common cause of staph infections (Subhankari et al, 2011) which is responsible for many human diseases including food poisoning soft-tissue infections, pneumonia and osteomyelitis: S.aureus can asymptomatically colonize human skin and mucous membrane including the lid and conjunctiva of eye (Balzli et al., 2010). *S. aureus* has been known to spread widely in the world and caused any anomalies of the skin and the mucous membranes of animals and human beings (Todar., 2002). All tissues can possibly be infected by *S.aureus* and cause disease with typical signs of inflammation, necrosis and abscess formation (Anonymous., 1994). According (Joklik et al., 1992) *S. aureus* is also a major pathogen in humans that cause many diseases associated with toxic shock syndrome (TSS) as a result of food poisoning. In addition, S.aureus is responsible for 80% of suppurative disease (Ahmad et al.,2012) Over the last few years, efforts had been devoted to control bacterial growth through exposure to electromagnetic fields(Liang et al.,2006 & Ayse et al., 2011)However the medical applicability of this technique is limited due to the need of very high field strengths of several KV/cm and very high temperatures. In recent work, carried by our group, efforts were devoted to control cellular activities by using electromagnetic waves of very low field intensity and frequencies which resonates with bioelectric signals generated during a particular metabolic activity (Fadel et al., 2012).

Several trials succeeded to control the growth of Ehrlich tumors in mice (Fadel et al., 2005& 2010) and fungi (Fadel et al., 2009) in a more recent work (Fadel et al., 2012), it was possible to control *S. aureus* cellular division and cause changes in the structure of the DNA after the exposure0.8 Hz square amplitude modulated waves (SAMW) for 120 min. In the present work, a trials was made to find out the resonance frequency of SEP that can inhibit the activity of S.aureus and to investigate the changes

that may occur at the molecular and cellular levels . Moreover, to carry some structural and histological studies for the liver of whole body exposed infected rats by *S.aureus* to SEP at resonance frequency.

Materials &Method

2.1 Bacterial strain

S.aureus were prepared from microbiological laboratory, Alexandria university over night (18 hours) cultures of bacterial inoculate were prepared by inoculating a single colony from brain heart infusion agar plate into 50ml of trypticase soy broth. Cultures were centrifuged for 10min at 3000rpm and then decanted (Nawras, 2011). The pellet was resuspended and washed with sterile normal saline the absorbance was measured at 600nm the bacterial sediment was resuspended in a volume of sterile normal saline equal to the discarded supernatant (Hari et al., 2011). Serial ten fold dilution in normal saline was performed from each inoculum. to get a final concentration 1.0×10^{10}Cfu/mL of bacterial suspension (Chkraborty et al., 2011, Hattie et al., 2000, Hari et al., 2011).

2.2 Experimental design

50 adult albino male rats, 60 days age and average weight 170± 5g., purchased from the Faculty of Medical Research Institute, Alexandria University were used. The animals were housed in plastic cages and feed with constant balanced diet and tap water. After death or being sacrificed the animals were excluded through coordination with the Holding Company for Biological Products and Vaccines.

The experimental animals kept in the same condition for 2 weeks for adaptation, after that divided randomly into five groups namely G1, G2, G3, G4, and G5. G1 group was of five animals used as control, which did not receive any treatment.G2 was of five animals and exposed to 0.8 Hz square electric pulse (SEP) for 120min. G3; of 15 animals; was infected orally by 10^{10}Cfu/mL of *S.aureus*.G4 of 10 animals; was infected orally by dose 10^{10}Cfu/mL of *S.aureus* and after 5 days of infection was treated with 0.8 Hz SEP for 120 min. G5; of 15 animals; was infected orally by 10^{10} Cfu/ml SEP treated bacteria. The experimental period for the present study was 15 day (Chkraborty et al., 2011, Hattie et al., 2000, Hari et al., 2011).At day 15 post infection of G3, G4, and G5 animals were sacrificed then livers were removed and prepared for histopathological and dielectric relaxation studies.

2.3 Dielectric relaxation studies for the liver

Animals were sacrificed then the liver was immediately excised and placed between a pair of 1cm diameter black platinum circular electrodes for dielectric measurements distance between the electrodes was measured

through the use of a micrometer as shown in Figure.1, while the liver sample was filling the whole volume between the electrodes. Dielectric measurements were made in the frequency range from 42 KHz to 5 MHz using a loss factor meter type Hioki, 3532, LCR Hitester Japan (Figure.2). During measurements, the sample between the electrodes was kept at a constant pressure of 24 ± 0.1°C. The capacitance (C) of the tissue was measured at each frequency and the resistance (R) was recorded,each run was repeated three times. The relative permittivity of the sample was calculated for each frequency using the relation,

)1(

Where, ε_o permittivity of free space, d is the inter-electrode distance in meter, A area of electrode in m^2 .The loss tangent (tanδ), the dielectric loss ε″, the AC conductivity σ were calculated from the relations

)2($\tan\delta = 1/2\pi fRC = \varepsilon''/\acute{\varepsilon}$

)3 $(\sigma = 2\pi f \varepsilon''\varepsilon_o$

The value of the dielectric constant έ falls from high value $\acute{\varepsilon}_s$ to έ as the frequency increases through the dispersion region where έ is the real part of the complex permittivity. The dielectric dispersion (Δέ) was calculated by applying the relation, $\Delta\acute{\varepsilon} = \acute{\varepsilon}_s - \acute{\varepsilon}$ (4)

The relaxation time (τ) was calculated from the equation,

(5)

Where f_c is the critical frequ $\tau = \frac{1}{2\pi f_c}$ onding to the midpoint of the dispersion curve. All the mea ıd been done for animals of all groups.

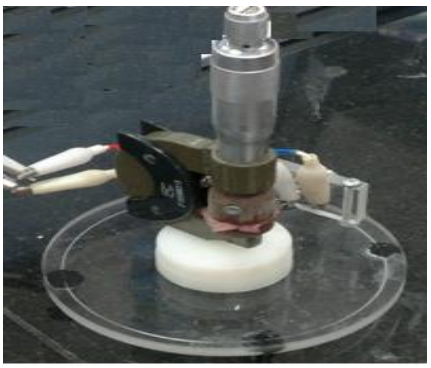

Figure.1. Cell used for liver dielectric measurement **Figure. 2.**Hioki 3532-50 LCR Hitester Bridge.

Histopathological investigations.2.4

Specimens of liver tissues were taken from all groups and prepared for the histopathological sections following Banchroft and Stevens work, 2006 (Bancroft et al .,2006) and examined by light microscope**.**

2.6. Statistical Analysis

Data from bacterial growth studies were compared for statistical significance using Student t-test and ANOVA analyses, the level of significance was set at $P<0.05$.

3. Results

Figure.3a reveal the growth curve characteristics for control and treated *S.aureus* by 0.8Hz SEP. The difference from control was significant ($P < 0.05$). Figure .3b shows the change in cellular growth (relative to control) of the microorganism, measured in the saturation region as a function of the applied SEP frequency. The results indicate a resonance frequency of the SEP that cause maximum inhibition of the growth of *S.aureus* at 0.8 Hz after exposure for 120 min. .

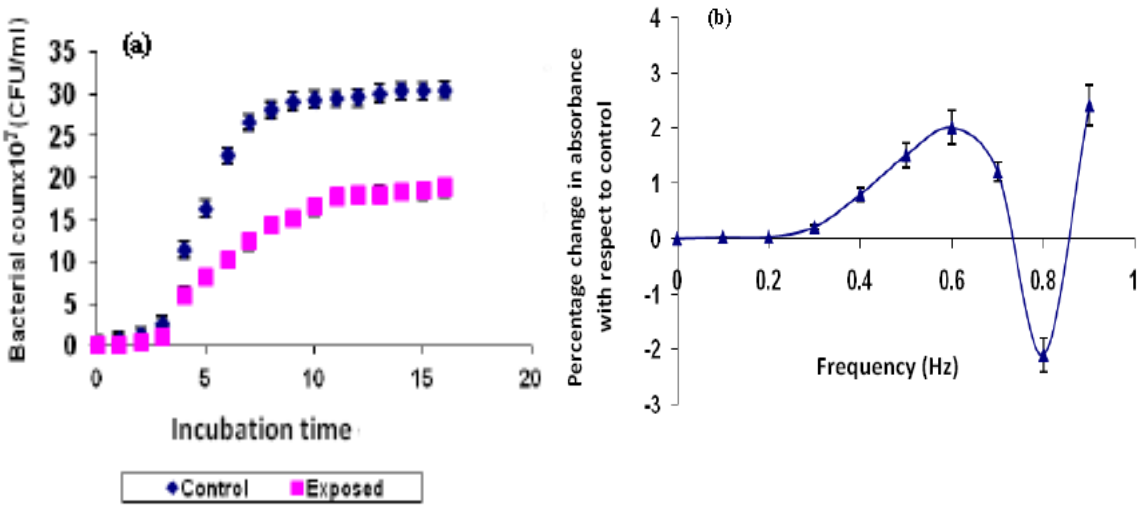

Figure. 3. (a): growth curve characteristics for control and treated S.aureus by 0.8 Hz SEP for 120 min. (b): the change in cellular growth (relative to control) of the microorganism, measured in the saturation region as a function of the applied SEP frequency.

3.1. Effects of electric field on the molecular structure

3.1.1. Dielectric properties of the liver

Figure.4. (a, b, c, d and e) illustrate the dielectric relaxation curves for the livers from groups G1, G2, G3, G4 and G5 respectively, as measured in the range of (42 KHz-5 MHz). The results indicate a dielectric dispersion in the frequency range demonstrated. The data also indicated that any decrease of the dielectric loss for the sample is associated with an increase of the electrical conductivity; this yields a consistency test for the data as reported by Kramers-Kronig relations (Polk et al., 1996). The results

indicate a dielectric dispersion for the liver for all the animals from all groups. It can also be noticed that the formation of two dispersion regions in this frequency range for control animals and those infected with the bacteria.

The second dispersion started at frequencies higher than one megahertz. The values of τ, and σ were calculated from the curves for all the livers from all groups as given in Table(1).The results indicate high significant decrease (P<0.0001) in the value of σ for animals infected by the bacteria.

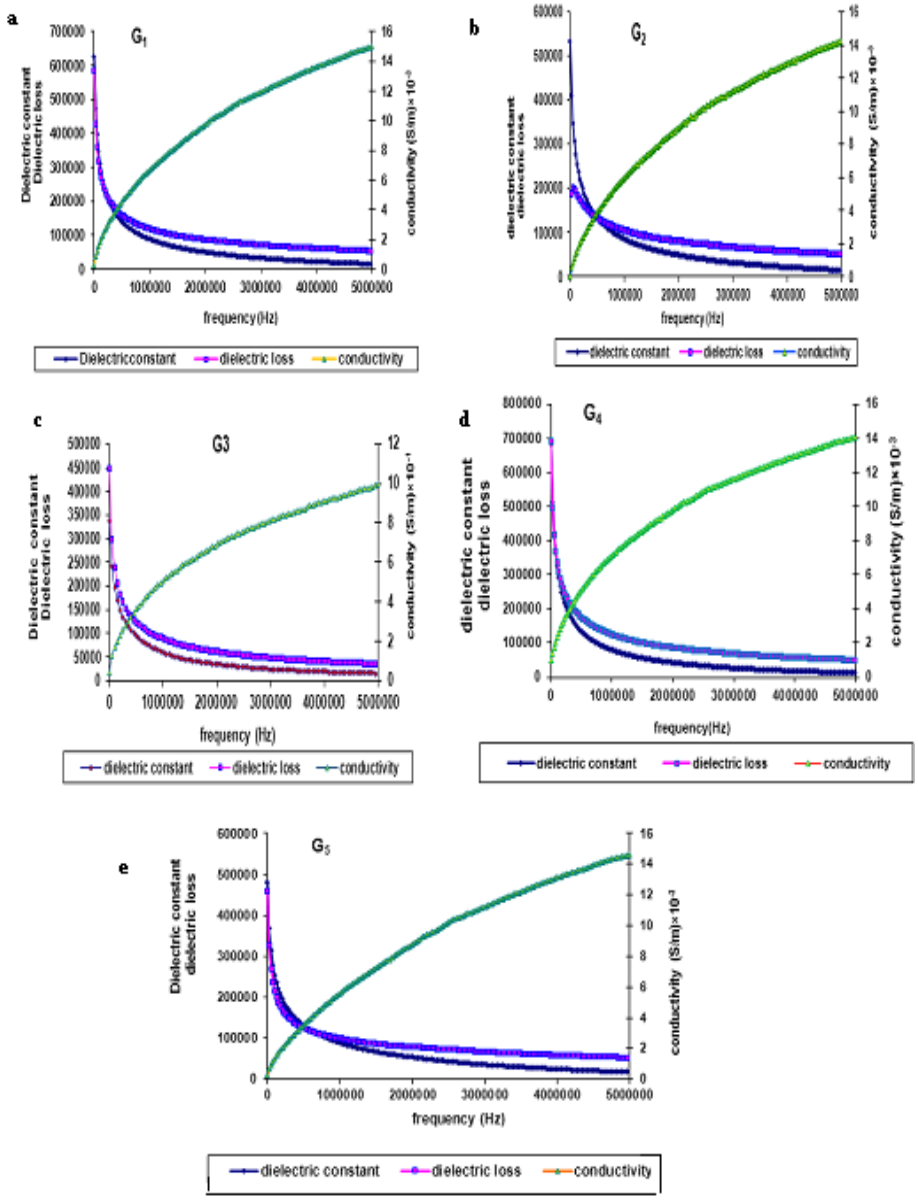

Figure.4. (a, b, c, d and e): illustrate the variation of the relative permittivity(dielectric constant) έ , the dielectric loss ε̋ and the electric conductivity σ(S/m) as a function of the frequency(Hz) for the livers from groups: Control (G1), exposed to SEP (G2), infected orally by $)10^{10}$ cfu/ml of S.aureus (G3), infected and then treated by SEP (G4) and infected with treated bacteria (G5) respectively.

Sample	Dielectric increament $\Delta\acute{\varepsilon} = \acute{\varepsilon}_s - \acute{\varepsilon}$	conductivity σ (s/m) , at 5MHz
G1	3.5×10^{6}	$(14.9\pm0.036)\times10^{-3}$
G2	5×10^{6}	$(14.22\pm0.025)\times10^{-3}$*
G3	8×10^{4}	$(10.1\pm0.39)\times10^{-1}$**
G4	1.2×10^{6}	$(13.9\pm0.241)\times10^{-3}$*
G5	2×10^{6}	$(14.6\pm0.036)\times10^{-3}$*

Table 1. Dielectric parameters for of the samples exposed to 0.8 Hz, 120 min SEP (G2), infected Orally (G3), infected then treated (G4) and infected with treated bacteria (G5) as compared with control(G1).

***The difference is not significant (NS) (p > 0.05)**

****** The difference is highly significant (HS) (P<0.0001)**

3.1.2.Histopathological Investigations

On microscopic level; Histological observations showed liver cells which was constiluted with radier and the nucleus which contained average polyhedral and sinuosoids as on normal architectures of liver tissues, normal central vein, liver cells(hepatocytes),normal kuffer cells Figure (5a). Figure (5b), showed approximately normal structure for the liver of the rats at 15 days post exposure to SEP only at resonance frequency. Figur(5a&5b) liver histopathology was in conformity with microscopic image of the normal liver as reported by (Dellmann & Brown., 1992, Ahmad Fauzi et al., 2012). Figure(5c), showed the liver of rat which were infected by S.aureus via intraperitoneal route have visible necrosis of hepatocytes cells, the infiltration of inflammatory cells, increased inflammatory reaction and polymorphonuclear cells, this agreement with (Shulman, 1994) that *S.aureus* infection can cause inflammation of liver, in which two bacterial wall components , peptidoglycos and lipoteichoic acid from *S.aureus*, work together to cause systemic inflammation and multiplr system failure associated with gram - positive organisms (Kimpe et al., 1995). Peptidoglycan of *S.aureus* causes organ injury/dysfunction, organinflammation, and systemic inflammation in the rat, also causes dilation of the bile ducts, congestion of the blood vessels, area of steatosis (fatty liver), inflammation in kuffer cells and infilterated inflammatory cells, also showed enlargement in the nuclei, thickening in some cell membranes may be due to fibrosis, enlargement in the cytoplasm of the hepatocytes and depletion in their chromatin material for the liver tissues of the rats after

15days of infection.Fig.(5d), liver histopathology of rat which were infected then exposed to SEP at 0.8Hz showed recovery in most cells after 15 day of infection, in which the treated with SEP at resonance frequency improve the capability of the rat to repair liver tissue, the liver showed structure with normal sinusoids and its cells contain radier more twards the central vein, decreased inflammation, slight congestion, little steatosin, signs of a poptotic figures (as pyknosis). This frequency could be able to induce the body's defense system, in this study, S.aureus can make damage to liver and the exposed of rats as whole body exposure to 0.8 Hz SEP can improve the immune system and body's defense system can restore the liver damage cause by infection to be normal. Rats from G5 which were infected with treated microbe for 15 days showed nearly normal, normal central vein, liver cells (hepatocytes), normal kuffer cells and sinusoid, Figure (5e).

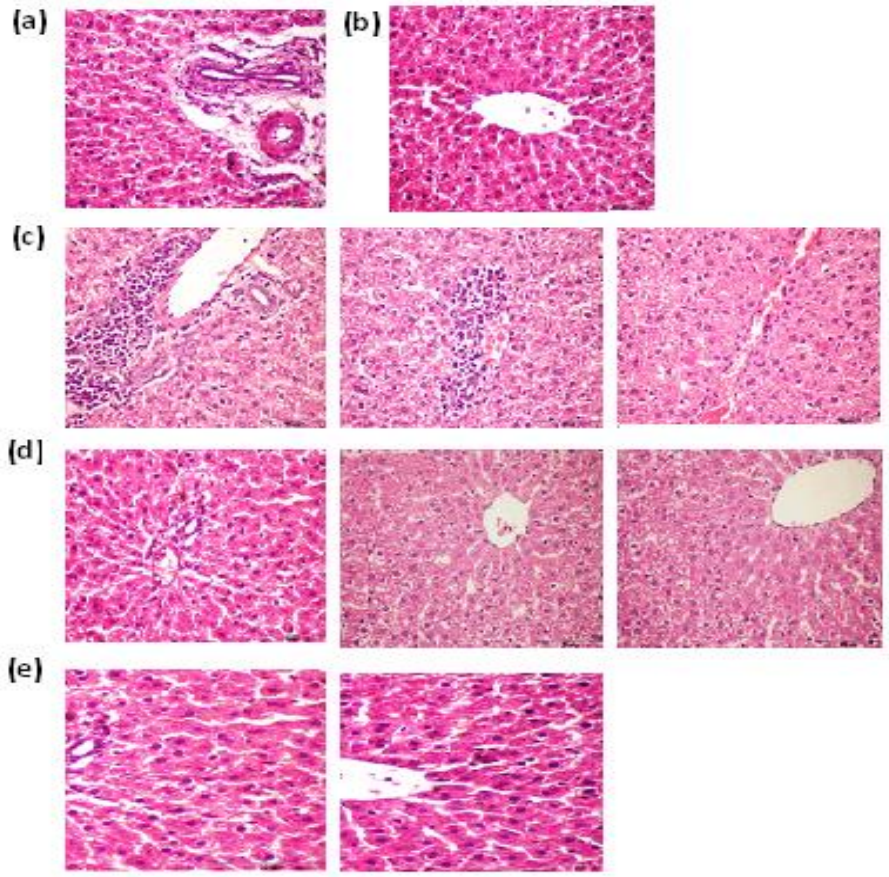

Fig.5. Photomicrographs of liver sections for Control rat, (G1) (**a**). Rats exposed to 0.8 Hz for 120 min SEP, (G2) (b). Rats infected orally by 10^{10} cfu/ml of S.aureus, (G3) (c). Rats infected orally by 10^{10}cfu/ml of S.aureus, and after 5 days of infection was treated with 0.8 Hz SEP for 120 min (d). Rats infected orally by 0.8 Hz SEP treated bacteria for (H & E 400 X) (magnification x20000).

4. Discussion

In this work; the resonance frequency of SEP that inhibits cellular division was determined. The experiment was then expanded to in vivo study where the microbe was used to infect rats and the injuries in the liver resulting from the toxins secreted were evaluated for animals did not receive any further treatment and those whole bodies exposed to the inhibiting electric pulses and animals infected with previously irradiated bacteria.

It is well known that living biological systems run their metabolic mechanisms through ionic motion. The rate of movement of these ionic charges forms ionic currents which result in ionicpotentials. The wave form and frequency of these bioelectric potentials represent the running physiological process which can be understood as the finger print of the organ. These bioelectric currents generate loops of biomagnetic fields which perturb the metabolic functions of the neighboring cells. From the basic understanding of physical concepts, energy can be only transferred from one oscillating system to another when both systems are at resonance i.e. they have the same frequency.

Based on the Metabolic Bio-magnetic Resonance Model (BMRM), suggested by Fadel (1988) an external applied electromagnetic signal can interfere with a bioelectric signal when they are at resonance. The resultant of the interference is the algebraic summation of the two waves which may be instructive or destructive, i.e enhancement or inhibition, respectively, for the running process. Based on these bases the present work was planned.

The damage in liver due to infecting the animals with *S.aureus* indicated as noticed in the histological sections is due to the toxins secreted by the microorganism which caused the observed damage. The damage thus occurred can be analyzed depending on the interaction mechanism of electric fields with biological systems. The improvement of the health state of the animals infected with *S.aureus* and treated with SEP, as can be noticed from the histological sections of the liver, is a marker for the success of this method for the treatments of infections with *S.aureus.*

4. Conclusion

It may be concluded from the present findings that the advantage of this technique on destructive, non expensive, safe and fast, where only 120 min are needed for the exposure of the infected region to stop the microbial activity and to avoid secondary harms used by prolonged treatments with antibiotics and health complications that follow treatments.

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