Electromagnetic Therapy Control the Effect of Bacteria on Liver Tissue: Histopathological, Histochemical and Immunohistochemical Studies

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ABSTRACT— ***Staphylococcus aureus (St. au.) is responsible for many human disorders including food poisoning, soft tissue infection, pneumonia and osteomylitis. The primary objective of this study is to reveal the role of electromagnetic waves (EMW) in the amelioration of the changes induced by (St. au.) infection either in rat liver tissue or its peripheral blood smears. 25 male rats were divided into 3 groups (G). G1: 5 control rats, G2: 10 experimental rats that were orally infected with St. au. by 10^8 cfu/ml, while G3: 10 experimental rats exposed after the incubation period (5days) to 0.8 Hz square magnetic pulses for 75 minutes. Our results revealed that some alterations included dilation of bile ducts, congestion of blood vessels; area of steatosis, increased infiltrated inflammatory cells, Kupffer cells inflammation and increased number of different apoptotic forms. Also abnormal shapes of RBCs, irregular shapes of monocytes, increased number of the pathogen and engulfed pathogen inside neutrophils were noticed. The intensity of collagen fibers was increased particularly at area of inflammation, around large vessels as well as sinusoids. The positivity of caspase 3 increased to be strong as compared with G1. Respecting G3, rats treated with EMW showed regression which was illustrated as reduced congestion of blood vessels, inflammation and steatosis, as well as reduction in apoptotic figures. The reappearance of normal biconcave RBCs and different types of WBCs was noticed except that there were no neutrophils. In addition, monocytes have delicate chromatin and there was no pathogen in the films, and the distribution of collagen fibers was similar to that of G1. Also the positivity of caspase 3 was more or less like that of G1. In conclusion: The noticed fast recovery of infected rats after their exposure to EMW in the present study opens a new avenue in the treatment of bacterial infection with dangerous strains like St.au. We expect that in the following years more approaches based on control of the different forms of cell death via EMW will enter the clinical practice.***

Keywords— electromagnetic waves, bacteria, apoptosis, collagen, caspase3, liver

1. INTRODUCTION

Staphylococcus aureus (*St. au*.) is responsible for many human disorders including food poisoning, soft tissue infection, pneumonia and osteomylitis [(1)]. Long-term treatment of diseases with antibiotics has its untoward effects on human health especially for pregnant women. In addition, the discriminate use of these antibiotics has led to emergence of multidrug resistance strains of the pathogen [(2)].

Electromagnetic waves (EMW) are produced by the motion of electrically charged particles. They can travel through empty spaces as well as through air and other substances. At low frequencies, they are called electromagnetic field while at high frequencies they are called electromagnetic radiation [(1)].

While the positive aspect of technologic innovations makes the life easier, it may also involve components that impair the quality of life via its certain negative effects. Resulting from that, people are exposed to EMW at levels much higher than those present in the nature [(3)].

Apoptosis is a number of different forms of cell death [(1)]. Caspase dependent-apoptosis is characterized by the activation of pathways leading to the activation of family of proteases caspases resulting in an order disruption of the cell without leakage of cellular components and induction of inflammation [(4)].

The primary objective of this study is to reveal the role of electromagnetic waves in the amelioration of the changes induced by *Staphylococcus aureus* infection either in liver tissue of rats or in their peripheral blood smears.

2. Materials and Methods

This study was carried out on 25 male rats weighing 150gms ± 5gms. Animals are purchased from Medical Research Institute, Alexandria University. These animals were kept under the standard conditions including light, humidity, temperature, ventilation and food and water *ad libitum*. Ethical protocol of Medical Research Institute concerning the use of animal in research works was strictly followed. The strains of *St. au.* was obtained from Microbiological Department, Medical Research Institute and the exposure of rats to EMW was carried out in Biophysical Department, Medical Resaerch Institute, Alexandria University. Finally, rats are divided into three groups:

1. Group 1 (G1): 5 rat are served as control group.
2. Group 2 (G2): 10 rats are served as experimental group where they were orally infected with *St. au.* by 10^8 cfu/ml[5].
3. Group 3 (G3): 10 rats were served as experimental treated group where rats are infected with *St. au.* by 10^8 cfu/ml then after the incubation period (5days), they were exposed to 0.8 Hz square magnetic pulses for 75 minutes [6].

Rats of all groups were sacrificed after 15 days of infection and liver and blood samples were prepared for histopathological, histochemical and immunohistochemical investigations.

The histopathological study was carried out via the routine Haematoxylin and Eosin stains (H & E).While the morphological stain for blood film was demonstrated by using Leishman's stain [7]. The stain of trichrome was applied to perform the histochemical study of collagen fibers in liver tissue [8].Collagen deposits in liver were demonstrated quantitatively via measuring the tissue levels of hydroxyproline (the end product of collagen metabolism) by using software (Leica-Q 500) and measuring area % of 5 randomly-chosen areas.

Immunohistochemical examination of Caspase 3 was subjected to qualitative evaluation, taking into account the intensity of color reaction at the antigen-antibody site[9]. The quantitative evaluation was analysed using the same software (Leica-Q 500). The surface area of cells with a positive reaction (+) was calculated. All quantitative results were presented as means and standard deviation of the mean using Kruskal Wallis test, P was Statistically significant at $p \leq 0.05$. 3.25% and 4.58% error risk were accepted for collagen and caspase3 quantification**.**

3. RESULTS

3.1 Histopathological findings

H & E stains revealed normal liver tissue architecture of G1 with its lobules including their central veins, portal tracts, sinusoids, Kupffer cells and hepatocytes that arrange in cords. Some hepatocytes have more than one nucleus (Fig 1). In G2, rats infected with the pathogen showed some alterations as compared with G1. These changes included dilation of bile ducts, congestion of blood vessels, area of steatosis, increased infiltrated inflammatory cells, Kupffer cells inflammation and increased number of different apoptotic forms (Figs 2,3). Respecting G3, rats treated with EMW after the incubation period of the pathogen revealed obvious regression of the previous changes that recorded in G2. This regression was illustrated in reduced congestion of blood vessels, inflammation and steatosis, as well as reduction in apoptotic figures (Fig 4).

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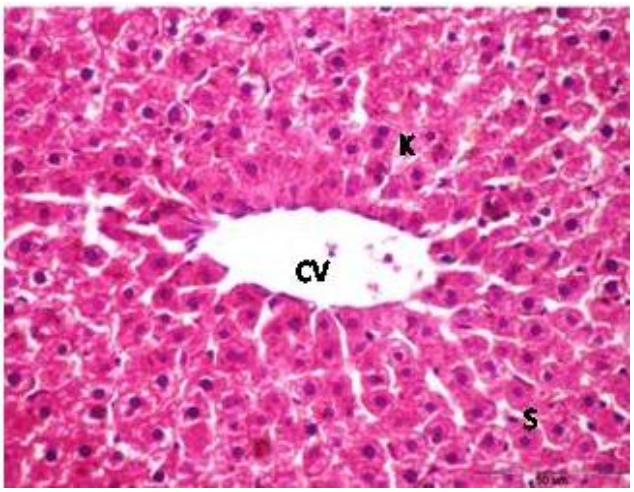

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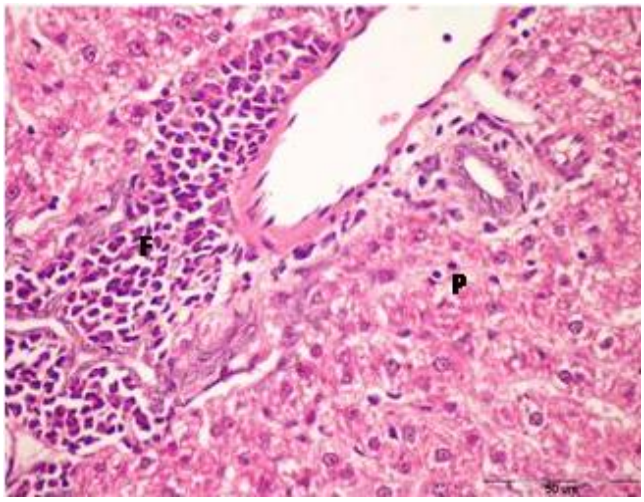

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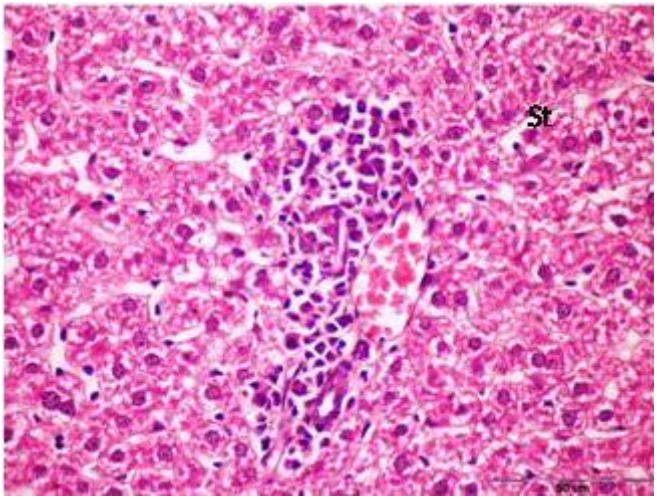

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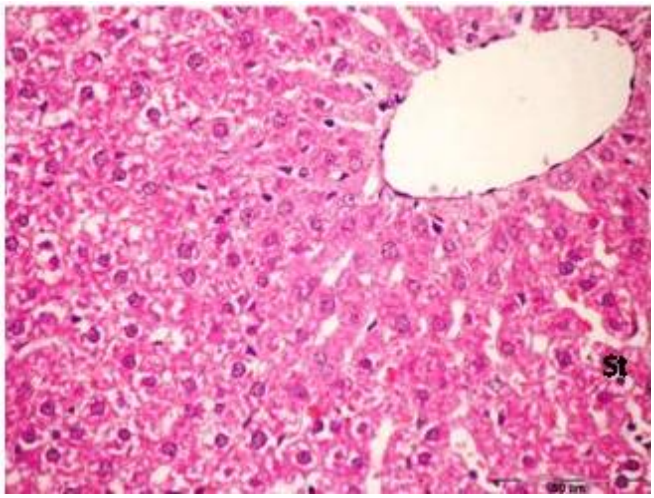


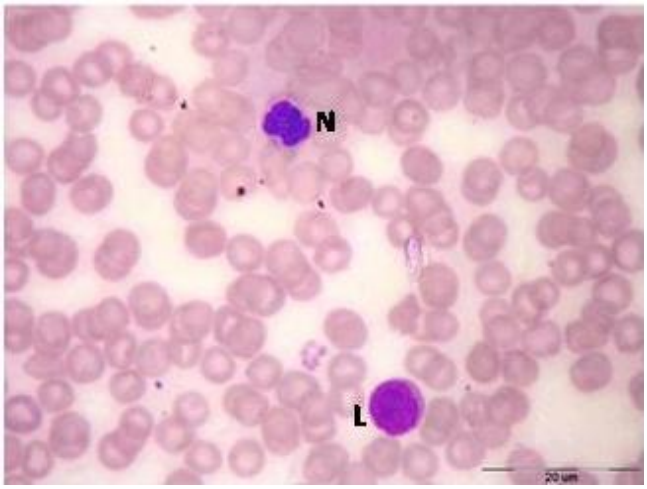
Fig.1: Control rat liver sections showing normal architecture of lobules with central vein (CV), sinusoids(S) and Kupffer cells (K). H&E stains. Bar=50

Figs. 2&3: Infected rat liver sections showing dilation of bile duct, congestion of blood vessels, area of steatosis (St), increased infiltrated inflammatory cells (F), inflammation of Kupffer cells and increased no. of apoptotic forms (P). H&E stains. Bar=50

Fig. 4: Treated sections with EMW, showing reduced congestion of blood vessels, inflammation and steatosis (St), also reduction in apoptotic figures were seen. H&E stains. Bar=50

In case of blood smears, the control films of G1 showed well developed biconcave red blood corpuscles (RBCs) and different types of white blood cells (WBCs) as monocytes, lymphocytes and other segmented cells particularly neutrophils (Figs 5, 6). In G2, the blood films of rats infected with the pathogen showed severe changes including abnormal shapes of RBCs, irregular shapes of monocytes, increased number of the pathogen and engulfed pathogen inside neutrophils (Figs 7, 8). In G3, the blood films of rats treated with EMW after the incubation period of the pathogen showed obvious improvement in the blood films. These events concerned with the reappearance of normal biconcave RBCs and different types of WBCs except that there was no neutrophils. In addition, monocytes have delicate chromatin and there was no pathogen in the films (Fig 9).

(5)



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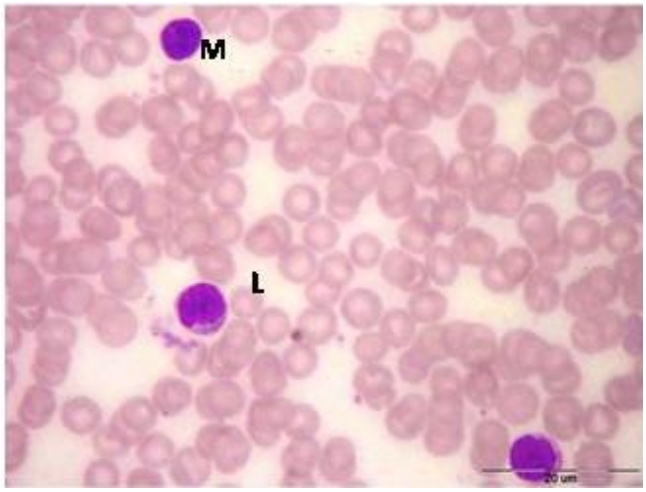

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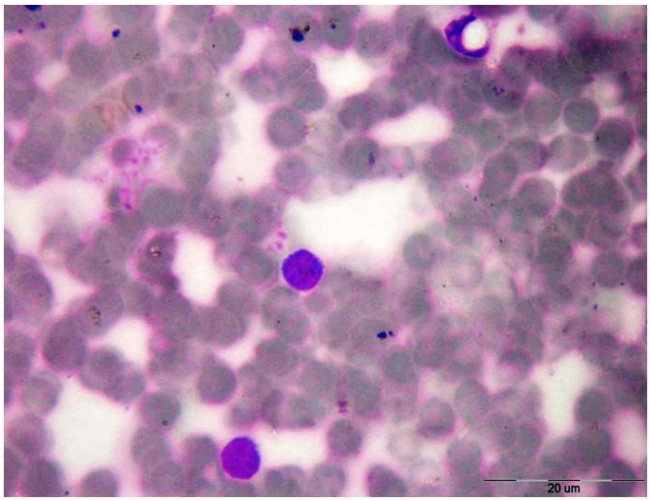

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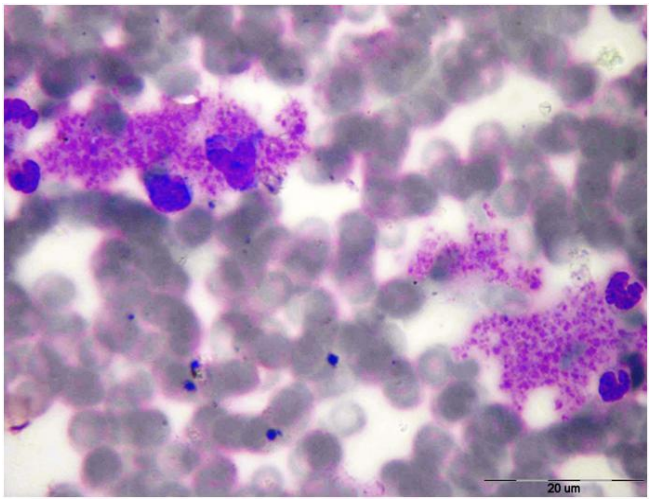

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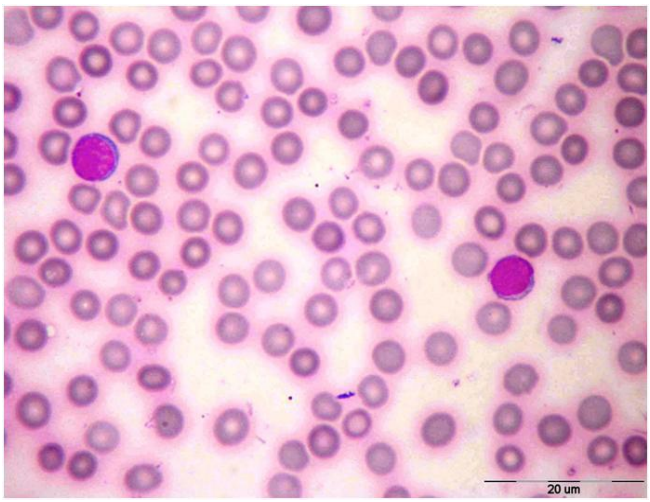


Figs.5&6: Control blood smears of rats showing normal biconcave red blood corbuscles (RBCs) and different white blood cells (WBCs) as Monocytes (M), Lymphocytes (L) and Neutrophils (N).(Leishman's Method. Bar = 50)

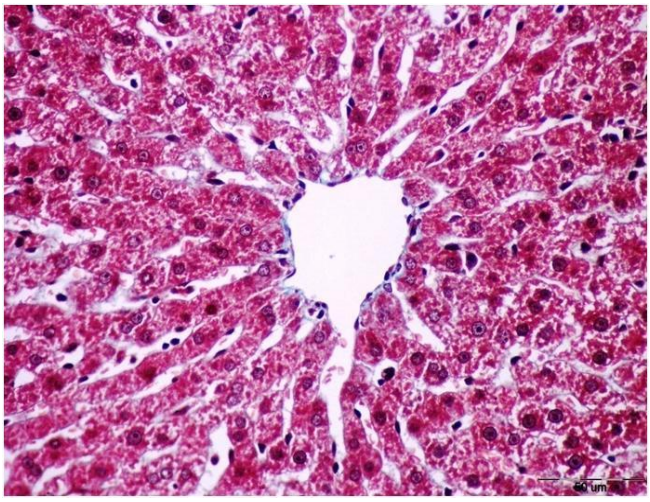
Figs.7&8: Infected smears showing abnormal shapes of RBCs, irregular shapes of Monocytes (M), increased no. of pathogen and engulfed pathogen inside neutrophils. (Leishman's Method. Bar=50).

Fig.9: Treated smears showing normal biconcave RBCs and different types of WBCs, Also monocytes have delicate chromatin. (Leishman's Method. Bar = 50).

3.2. Histochemical findings

Trichrome stain in G1 revealed collagen fibers distribution as blue color especially around the large blood vessels (Fig 10). In G2, the intensity of collagen fibers was increased particularly at area of inflammation, around large vessels as well as sinusoids (Fig 11). In G3 EMW can restore the distribution of collagen fibers to be similar to that of G1 (Fig 12).

(10)



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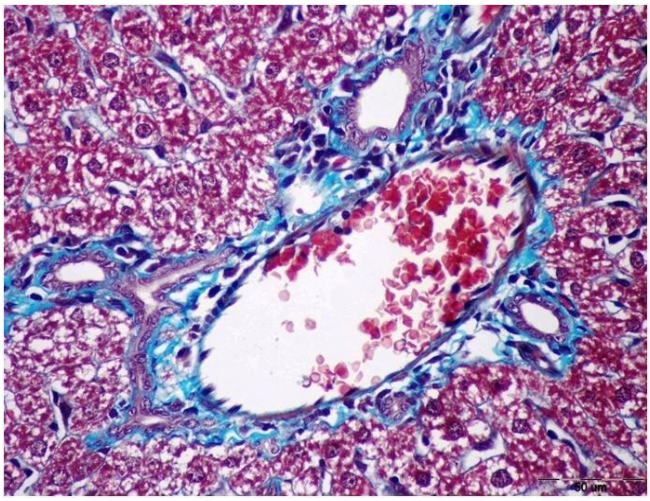

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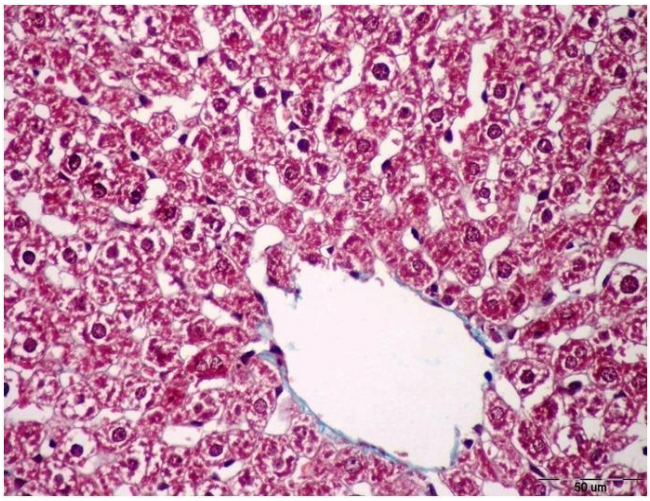


Fig.10: Control section showing collagen fibers as blue color especially around blood vessels. (Masson Trichrome stain. Bar=50).

Fig.11: Infected sections showing increased intensity of collagen fibers at area of inflammation around large vessels as well as sinusoids. (Masson Trichrome stain. Bar=50).

Fig.12: Treated sections showing similar distribution of collagen fibers to that of control group. (Masson Trichrome stain. Bar=50).

Table (2): Comparison between the three studied groups according to collagen

	Group I (n=5)	Group II (n=5)	Group III (n=5)	p
Collagen				
Min. – Max.	3.26 – 5.61	15.35 – 43.14	1.07 – 7.71	
Mean ± SD.	4.75 ± 1.18	27.98 ± 9.93	5.06 ± 3.06	0.008*
Median	5.61	26.76	6.24	
p_1		0.008*	0.596	
p_2		0.009*		

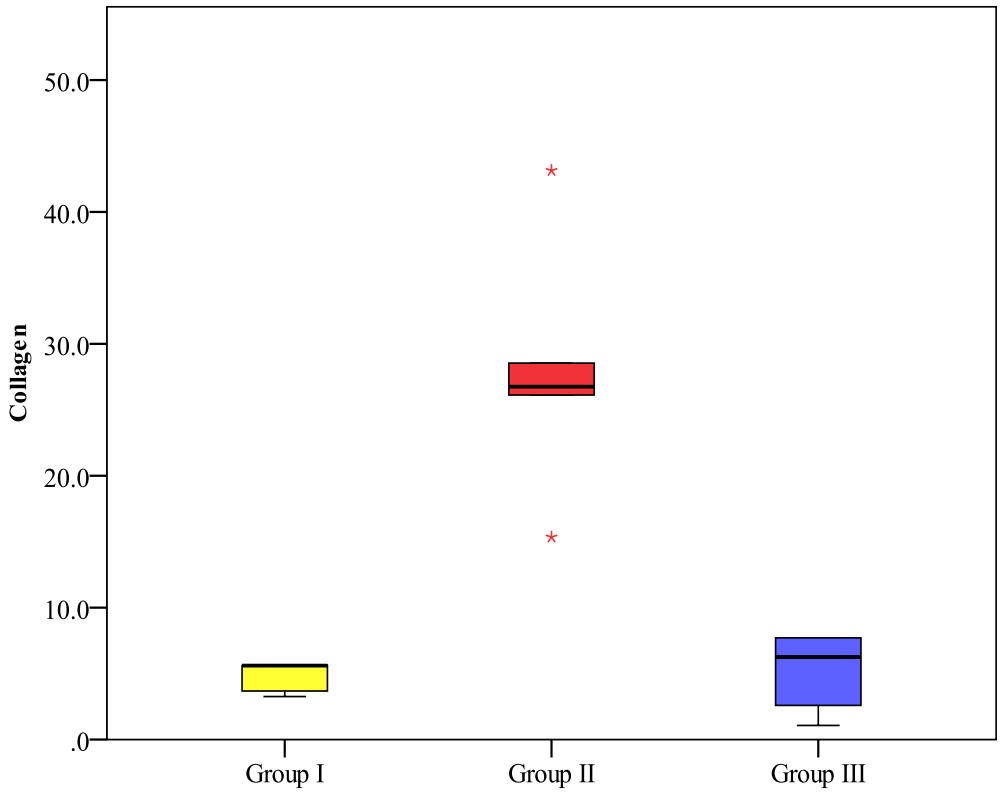
p: p value for Kruskal Wallis test

p_1 : p value for Mann Whitney test for comparing between group I and each other group

p_2 : p value for Mann Whitney test for comparing between group II and group III

*: Statistically significant at $p \leq 0.05$

We could summarize the following: Collagen deposits in G2 (27.98 ± 9.93) was significantly higher than the other groups. G1 (4.75 ± 1.18) and G3 (5.06 ± 3.06).

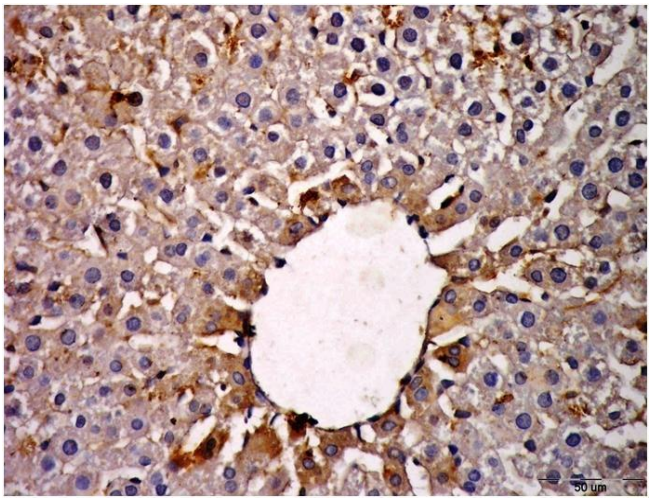


Box plot (1): Comparison between the three studied groups according to collagen

3.3. Immunohistochemical findings

Caspase 3 was illustrated as brown deposits in all liver histological features. In G1, it had mild positive expression (Fig 13). In G2, the infection with pathogen increased the positivity of caspase 3 to be strong as compared with G1 (Fig 14). In case of EMW treatment, G3 showed the positivity of caspase 3 more or less like that of G1 (Fig 15).

(13)



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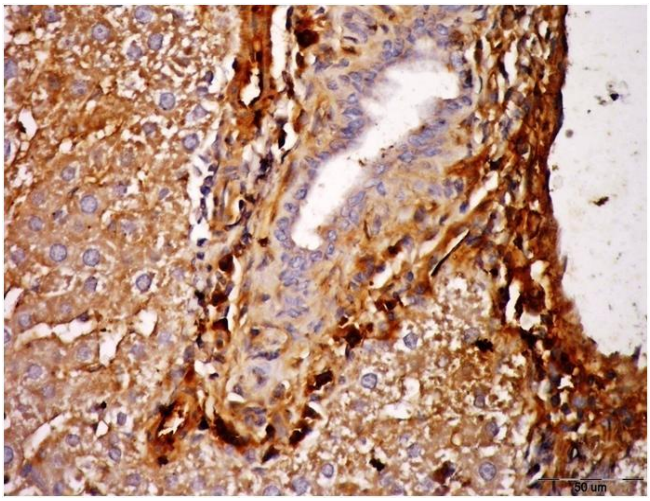

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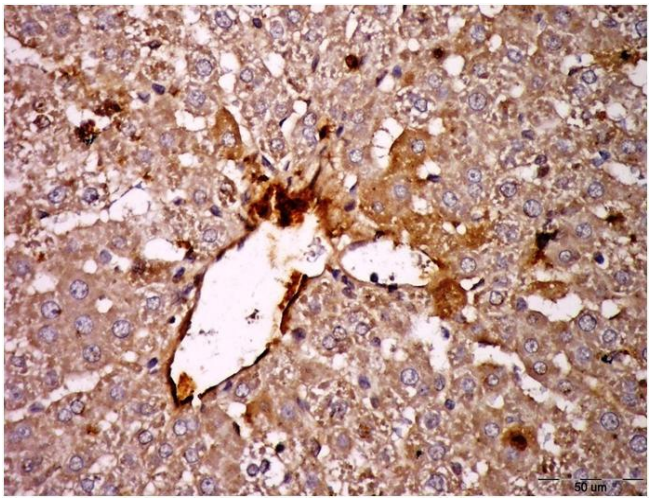


Fig.13: Control sections showing mild positive expression of caspase 3 as brown deposites in all liver histological features.(Biotin streptavidin peroxidase(BSP) method. Bar=50).

Fig. 14: Infected sections showing strong positivity of caspase 3. (BSP method. Bar=50).

Fig. 15: Treated sections showing that the positivity of caspase 3 was more or less like that control group (BSP method. Bar=50).

Table (2): Comparison between the three studied groups according to Caspase 3

	Group I (n=5)	**Group II (n=5)**	**Group III (n=5)**	**p**
Caspase 3				
Min. – Max.	0.45 – 16.92	25.11 - 59.38	1.85 – 33.42	
Mean ± SD.	6.32 ± 6.86	38.80 ± 14.19	15.31 ± 12.20	0.014*
Median	4.57	31.05	16.41	
p_1		0.009*	0.175	
p_2		0.047*		

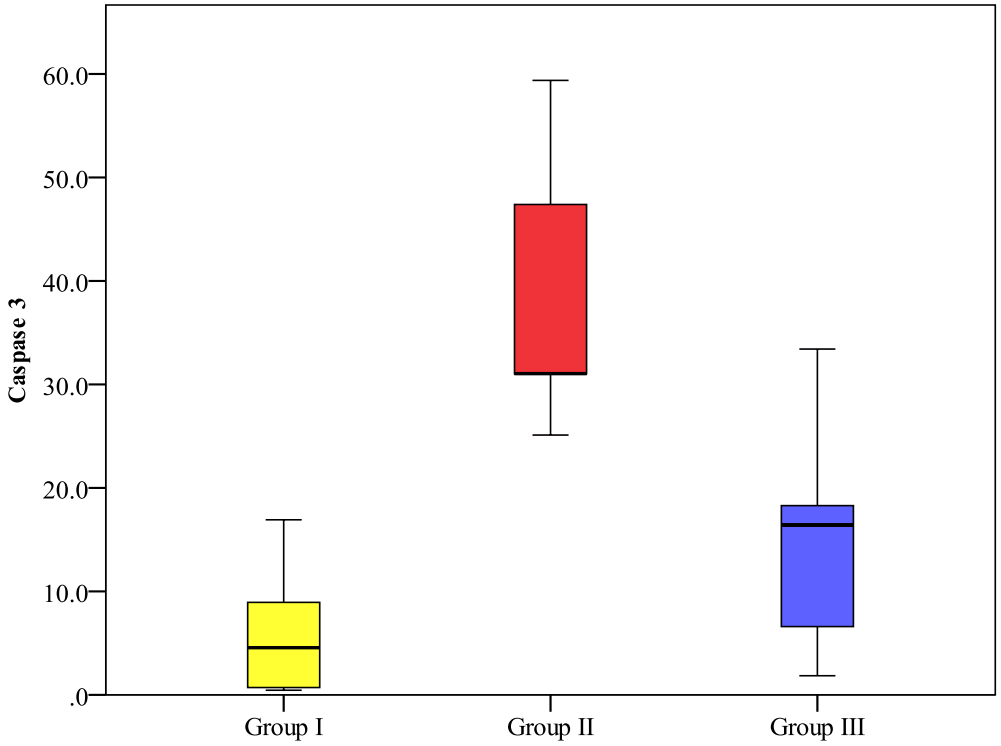
p: p value for Kruskal Wallis test

p_1 : p value for Mann Whitney test for comparing between group I and each other group

p_2 : p value for Mann Whitney test for comparing between group II and group III

*: Statistically significant at $p \leq 0.05$

We noticed that the number of caspase 3 positivity in G2 (38.80 ± 14.19) was significantly higher than the other groups. G1 (6.32 ± 6.86) and G3 (15.31 ± 12.20) .



Box plot (2): Comparison between the three studied groups according to caspase 3

4. DISCUSSION

Along with the widespread use of technological products in daily life, the biological effects of EMW started to be discussed. Studies at cellular levels which use EMW were contradictory. While some studies revealed for example, damage of DNA[2, 10, 11], others showed penetration into deeper tissues that may have an effect on several functions such as cell proliferation and differentiation [12] indicating the effect of EMW in arresting the growth of cells. This in turn can be used in therapeutic purposes [13]. Efforts have been devoted to control bacterial growth through exposure to EMF [14, 15].

St.au is ubiquitous bacterium and is a part of the human skin and mucosal flora. It can cause engender a variety of diseases and it is one of the most common causes of hospital infections. It also secretes several virulence factors, exotoxins and enzymes involved in the pathogenic mechanisms of the bacterium [16].

In the present study, infection with pathogen caused several changes at cellular levels either in liver tissue or in peripheral blood. In liver, they include increased inflammatory reactions, dilation of bile ducts, congestion of blood vessels and steatosis. These alterations reflect the unwanted effect of the bacterium. These changes were coincided with the findings of Al-Nakeeb[5] who studied the pathogenesis of *St.au.* in rabbits. His results revealed a hydrophobic degeneration in liver and lung tissues as well as necrosis and hemorrhage. As regarding EMW treated group (G3), we can show amelioration in the previous bad changes. This improvement included a reduction in inflammation, slight congestion of blood vessels, little steatosis and few apoptotic figures. These results were in accordance with the results of Ali et al [6]. They found that EMF caused a highly significant improvement in the health state of rats as compared with infected counterparts with Salmonella strain.

As regarding blood films, the changed recorded were irregular shaped monocytes, abnormal shape of RBCs and increased bacteria that majority of them were engulfed by neutrophils. Our findings were in agreement with Esen et al [16]. They showed that enterotoxins of *St.au*. caused activation-induced cell death. Moreover, α-toxins of this bacterium were hemolysin that caused pore formation in host cell membrane. After EMW treatment, these changes in the blood of rats were reduced significantly where there were reduced number of bacteria, regain of normal biconcave shape of RBCs, delicate chromatin of monocytes and little number of neutrophils. This reduction in neutrophils was coincided with the death and removal of neutrophils from blood stream after they engulf any toxins. The improvement of the changes after EMW exposure may indicate the direct effect of these waves in triggering cells towards normal homeostasis. The present findings were coincided with the findings of Atasoy et al [17] who referred to electromagnetic signals that could affect the functional capacity of the peripheral blood mononuclear cells by changing their adhesion ability which in turn reflect the sign of immune system modulation. In addition, Akan et al [18] evaluated the immune response of monocyte-derived macrophages to pathogens in extremely low frequency EMFs. Another hypothesis can explain the beneficial role of

EMW that these waves can change the free radicals levels. In supporting, Coskum et al [19] exposed Guinea pigs to 50Hz, 1.5 mT EMF for 4 days and they recorded elevated values for malondialdehyde, nitric oxide while glutathione transferase level was decreased.

With respect to collagen fibers, the present study showed increased collagen fibers in G2 more than both G1 and G3. These changes reflected the role of collagen in defense mechanism and inflammatory changes resulting due to infection. On the other hand, they reflected the indirect role of EMW in reducing the intensity of collagen fibers through their potential beneficial effect on the pathogen. In a recent study coincided with our findings, Yin et al [20] showed that *St.au* infection in mice was implicated in enhancement of collagen deposition.

As regarding caspase 3, it is one of a family that is the final effectors of both extrinsic and intrinsic apoptosis [21]. Apoptosis depends on activation of caspases that will then cleave a number of substrates resulting in biochemical and morphological changes typical of this form of death [22]. Our present results showed elevation of caspase 3 after infection of the pathogen while EMW treatment declines this elevation to reach more or less similar to that of G1. These changes are in agreement with Akan et al [18] who showed that EMW affected the response of immune system through suppression of caspase 9. However, Esen et al [16] showed that *St.au* α-toxins triggered apoptosis because they are hemolysin that caused pore formation in the host cell membranes and the consequent disruption of Na^+/ K^+ balance.

In conclusion, over many years of research in the field of cell death have clarified many aspects of this fundamental process and brought to the attention of scientist its role in a large number of different diseases. However, exploitation of this knowledge in therapy is only at its early steps. The noticed fast recovery of infected rats after their exposure to EMW in the present study opens a new avenue in the treatment of bacterial infection with dangerous strains like *St.au*. We expect that in the following years more approaches based on control of the different forms of cell death via EMW will enter the clinical practice.

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