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PREVENTION, CONTROL, INHIBITION, AND THERAPY OF HCV GT 4A VIRUS INFECTION IN EGYPT

Fadel M. Ali[1], Hala M. Ahmed*[2], A. Elmaghraby[3] and A. Amin[3]

[1]Faculty of Science-Cairo University.

[2]Faculty of Applied Medical Sciences-October 6 University.

[3]Engineering Authority of Armed Forces.

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***Corresponding Author**
Dr. Hala M. Ahmed
Faculty of Science-Cairo University.

ABSTRACT

Objective: Electric Field (ELF-EMFs) has great potential for modern biotechnological and new medical applications. This paper shows the effect of Electric Field (ELF-EMFs) on HCV GT 4a, which could imply a durable change in Clinical chemistry parameters and virological testing. **Methods:** Hepatitis C virus (HCV) infection has acute and chronic forms with most of the morbidity through the development of chronic liver disease. For this purpose, HCVGT 4a activities in Huh-7 and HepG-2 cell line media were exposed to different frequencies of amplitude modulated (AM) waves in the frequency range (from 1 Hz to 10 Hz) for a period of 30 minutes /day along four successive days no measurable effect of the square amplitude modulated waves (QAMW) was noticed after the experiment in the Huh-7 and Hep G-2 cells. At day (1, 2, 3, 4, 5, and 6) of the (PBMCs), supernatant was collected for each group then the liver enzymes (ALT), Measurement of complete blood count, determination the virological testing, Cultures were daily examined using the inverted microscope (Hund-Germany) for detection of cytopathic effect (CPE). **The results:** The data highly significant growth inhibition occurred in HCVGT 4a injected with Huh-7 and Hep G-2 cell line after exposure to 5.2 Hz QAMW for 30 minutes/day along four successive days. The mean ALT level was slightly higher in patients infected with HCV GT 4a non exposed Electromagnetic waves (410 U/L) than those infected with HCV GT 4a exposed Electromagnetic waves (90 U/L). There was a decrease of mean ALT levels during the course of treatment in both groups. Hemoglobin levels between the two groups decreased slightly during treatment the follow-up period. Neutrophil counts between the two groups decreased slightly during treatment but increased again during the follow-up period. The median

baseline serum HCV RNA load was $9.7x10^5$ IU/ml in patients with HCV GT 4 exposed infection ($p<0.001$), and $2.0x10^5$ IU/ml in patients with HCV GT 4a non exposed infection ($p<0.02$).The viral isolates showed a gradually elevated replication activity on Huh-7 and Hep G-2 cells, where the cytological changes (90% CPE) could be detected within 1-6 days post viral inoculation compared with the negative control. **Conclusions:** The results tend to show that Electric Field (ELF-EMFs) are able to inactivate viral growth and have probably no serious impact in HCVGT 4a. The results indicated that the HCVGT 4a has frequencies with the bioelectric signals generated during cellular division.

KEYWORDS: HCVGT 4a, Electromagnetic field (ELF-EMFs), Huh-7 and Hep G-2 cells.

INTRODUCTION

Several studies have been performed to verify direct effects exerted by extremely low frequency (ELF) electromagnetic fields (EMFs) on cell functions. Different variety of cell responses have been observed involving proliferation and differentiation (Lisi, *et al.*, 2006, Vianale *et al.*, 2008), gene expression (de Mattei *et al.*, 2005, Goodman, *et al.*, 2009), modulation of the membrane receptors functionality (Bersani *et al.*, 1997, Fadel *et al.*, 2018), alteration in ion homeostasis (Grassi *et al.*, 2004, Piacentini *et al.*, 2008), and free radicals generation (Morabito *et al.*, 2010, Wolf *et al.*, 2005). In particular, it has been demonstrated that bacterial exposure to (ELF-EMF) can alter its viability and growth rate with frequency and amplitude dependency (Moshe *et al.*, 2008), antibiotic susceptibility (Segatore *et al.*, 2012), and structural shape (Fadel *et al.*, 2018).The bacteria have also been used in the studies to evaluate the effects of (ELF-EMF) on some of its functional parameters as cell growth, antibiotic sensitivity, and cell morphology (Cellini *et al.*, 2008, Ayse *et al.*, 2011, Belyaev, 2011).Of note, higher frequency fields are truly electromagnetic in that the electric and magnetic fields propagate together, whereas at low frequencies the fields can be effectively separated as alternating electric or magnetic fields. The consistently low thresholds at which EMF stimulates biological processes indicate that they require little energy and its frequency response is most effective at certain values that coincide with the natural rhythms of the processes affected (Martin and Reba, 2004). Among several studies of the last years, bacteria were subjected to many experimental procedures to evaluate how such unicellular systems may respond to EMFs (Pothakamury *et al.*, 1996; Jeantet *et al.*, 1999; Fojt *et al.*, 2004; Amiali *et al.*, 2007; Jaegu *et al.*, 2008; Ji *et al.*, 2009; Tagourti *et al.*, 2010). However, most of this experimental works used electromagnetic fields of high field intensity

in the range from 10 KV/cm up to several mV/m (Cserhalmi *et al*., 2002; Fleischman *et al.*, 2004; Fox *et al*., 2008; Ruiz-Gomez *et al*., 2010) with the aid of very high temperature for inactivation of bacterial growth. Biological systems, *in vivo*, generate electric currents and fields that associated with magnetic fields, as a result of the running physiological mechanisms. In these mechanisms ionic motions are involved which are responsible for all bioelectric signals generated. The flow rate, period and direction of flow of the ions will generate an electric impulse with a specific frequency, shape and amplitude (Fadel *et al.*, 2014).

In Egypt, HCV is one of the top five leading causes of death (Mohamed M.K. and El-Said Aoun, 2002). Most of infected individuals are not aware of their infective status and are not clinically ill but they are a source of infection to others. No protective vaccine against HCV is available currently (Simmods P., 2004; Brass V. *et al.*, 2006). The treatment is costly, requires long-term medical support and follow-up and has serious side effects. Modern therapies are not affordable for the majority of HCV carriers worldwide. In Egypt, the prevalence rate of HCV infections has been reported as high as 20% (El-Ahmady O. *et al.*, 1994).The HCV genome consists of an RNA surrounded by an icosahedral protein capsid and a lipid envelope (Op De Beek A. *et al.*, 2003). Two viral envelope glycoproteins, E1 and E2, are embedded in the lipid envelope (Figure.1). HCV genotype 4 was found to be predominant with 91% in Egypt The distribution and the source of HCV isolates in each of the 15 Egyptian governorates (Stauber. *et al*., 2008).

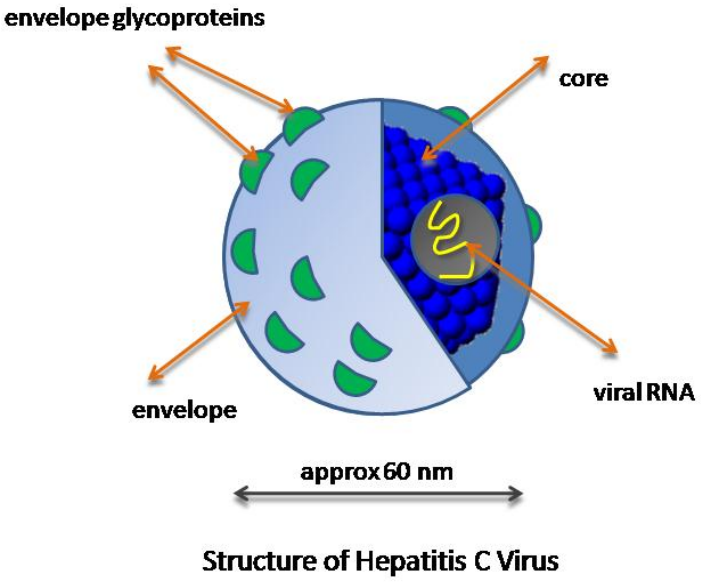

Figure (1): Structure of Hepatitis C Virus.

AIM OF THE WORK

The main objective of this work is to find the resonance frequency of (ELF-EMFs) the control viral cell growth where the resonance of the extremely high and low frequency electromagnetic fields (ELF-EMFs) that can enhance and inhibit the activity of HCV GT4a in plasma and its ability to make activity of the virus. Moreover, Biochemical examination were determined by evaluating various cellular activities (ALT) in serum, measurement of Complete blood count (Neutrophil counts and Hemoglobin) and virological testing findings that may occur as a result of exposure to the enhancement and inhibiting resonance frequency of the (ELF-EMFs)

MATERIALS AND METHODS

1-Sample collection

A total of 43 patients were enrolled from Al-Qaser El-Ainy University Hospital, Cairo University from Oct 2009- to Oct 2014. All subjects participated in the study signed written informed consents. Aliquots of 10-15 ml blood were withdrawn from all enrolled subjects. Plasma from 35 (HCV GT 4a infected) and 8 controls were collected. All subjects participated in the study signed written informed consents. The study was approved by the research ethics committee of Cairo University, Egypt. Inclusion criteria of the HCV GT 4a-infected participants were based on sero positivity for HCV antibodies, HCV RNA GT 4a as assessed by PCR, elevated aminotransferase levels for 6 days.

2-Study Design

Blood samples were centrifuged for 15 min. at 200 xg and plasma was collected for measuring HCV-RNA and measurement of liver enzyme (ALT) on fresh samples. HCV-RNA was measured before treatment, after 1, 2,3,4,5 and 6 days, at the end of treatment, and at the last follow-up visit by a quantitative reverse-transcription–polymerase-chain-reaction (RT-PCR) assay. Samples were clearly divided into two groups: First group, where the viral titer was reduced from the base line. Second group, the samples were under detection limit for HCV-RNA. To assess the viral infectivity in the supplied samples, selected group from both positive and negative groups were cultured on Huh-7 and HepG-2 cell lines as described at (Zekri *et al.*, 2011&2009).

3-Isolation of Peripheral Blood Mononuclear Cells (PBMCs)

Approximately (5-8 mL) of whole blood was drawn into an ethylene diamine tetra acetic acid vacutainer tube (BD), and Peripheral blood mononuclear cells (PBMCs) were isolated by

Ficoll-Hypaque density gradient centrifugation, replicates of ($2x10^5$ (PBMCs)) per well of a 24-wells flat-bottom plate dimensions (127.8x85.5x20.0 mm)(Costar, Cambridge, Massachusetts) were directly subjected to a 5-day proliferation assay in the presence of either (1 mg/ml) HCV GT 4a protein, (50 mg/ml) tetanus toxoid (Behring, Marburg, Germany), phytohemagglutinin (PHA), medium alone or control buffer (Al-Sherbiny M *et al.*, 2005). The stimulation index was calculated as the ratio of the average number of counts per minute of five replicate cultures in the presence of antigen as compared to control buffer or medium.as described elsewhere (Shata MT *et al.*, 2007).

4-Cell lines and cell culture

Cell monolayers of the human hepatoma cell line Huh-7 and HepG-2 were purchased from VACSERA [Giza,Egypt]. Huh-7 and HepG-2 cells were subculture in a 75 cm^2 flasks in DMEM supplemented with 2 mM L-glutamine [Biochrom], penicillin [Biochrom], streptomycin [Biochrom] and 10% heat inactivated fetal bovine serum [HyClone, UK] at 37ºC under a humidified atmosphere containing 5.2%CO_2 saturated atmosphere until confluent monolayer detected and maintained in an exponential growth state. The adherent cells were collected by 0.25% trypsin according to a previously published method (Paino IM *et al.*, 2010). Inoculated bottles were daily microscopically observed for 7 days for detection of cellular changes and development of cytopathic effect (CPE). Flasks developed CPE were freeze and thawed three times for virus extraction. (Bussereau *et al.*, 1982).

5- Electric Field (ELF-EMFs)

In this experiment, samples were exposed to electromagnetic waves, generated from 2 generators. The carrier wave was 10 MHz sine wave with amplitude ± 2 Vpp generated from a synthesized arbitrary waveform generator type Thurbly Thander Instruments (TTi TGA 1230) manufactured by Huntingdon Cams England. This wave was square amplitude modulated (AM) by a second wave generator model AFG 310 manufactured by Sony tectonics, Japan with a modulation the amplitude of the wave carrier was 20 V and the modulating depth was ±2V. (Fadel *et al.*, 2009). The output of the second generator that carries QAMW was connected to two parallel copper mesh electrodes of separation distance (1.5cm) and area ($180cm^2$). During the samples exposure the (24 wells) plate were housed between the parallel capacitance electrodes as shown in (Figure 2). The irradiation facilities of the samples were done in the Biophysics Department, Faculty of science, Cairo University.

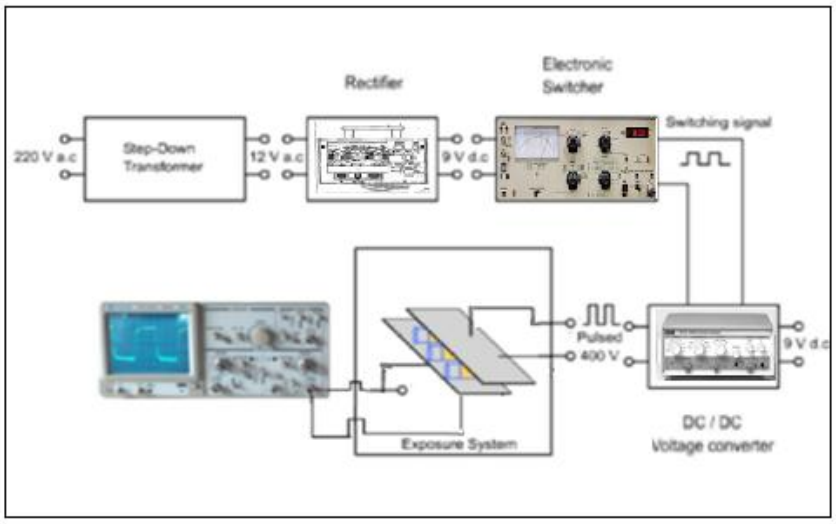

Figure (2): Experimental scheme of the Electric Field (ELF-EMFs) measurements.

6-Determination of Resonance Frequency of Growth Inhibition

Hus-7 and HepG-2 cells were harvested, washed adjusted at concentration ($5x10^4$ cells/well) growth medium and cultured in 12 well plates with 1ml medium per well. After 24 hrs, the culture medium was replaced with 1ml of complete medium containing HCV GT 4a samples plates were incubated at (37°C) in (5.2%CO_2) for (24 hrs) and the virus containing media was removed. The cells were washed twice with Phosphate buffer saline (PBS) and (2 ml) of complete culture medium was added to each well plates were incubated and the cells were harvested at the indicated times for viral samples exposure**,** the suspension of the sample ($5x10^4$ cells/well Hus-7 or Hep G-2/$5x10^4$ RNA/ml HCV GT 4a). The samples were exposed to different modulating frequencies in the (0.1,0.2,0.3,0.4,0.5,0.6,0.7,0.8,0.9 and 1 Hz) for 30 minutes each, to find out the enhancing and inhibiting frequency of the viral growth as in figure (3).In order to determine if the 10 MHz wave carrier of Electric Field (ELF-EMFs) has a role in growth inhibition process, a pulsed magnetic field at the same resonance frequency of growth inhibition that determined through Electric Field (ELF-EMFs) was used (Zare *et al*., 2007; Khaki AA *et al*., 2006)

7-Clinical chemistry parameters

A liver enzyme (ALT) was measured using ALCYON 3000 i analyzer, Abbott laboratories, USA/Canada. Spinreact kits (Ctra, Santa Coloma, Espana) was used in the measurement of ALT according to the method described by (Bergmeyer *et al.*, 1978).

8-Measurement of Complete blood Count

Complete blood count (Neutrophil counts and Hemoglobin) were measured using ADVIA analyzer, Holland.

9-Virological Testing

Reverse-transcription polymerase chain reaction (RT-PCR) (Roche Molecular Systems, Inc., Branchburg, NJ), was performed on serum samples for detection and amplification of HCV RNA using an in-house nested RT-PCR assay developed at the Al-Qaser El-Ainy University Hospital, Cairo University. RT-PCR was performed on serum samples from anti-HCV-positive patients and on serum samples from anti-HCV-negative patients. This assay has a lower limit of detection of 15 IU/ml. Positive results below this HCV RNA concentration are referred to as "low-positive". HCV genotypes and subtypes were determined by using the TruGene HCV 5´NC Genotyping Kit (Bayer Health care LLC, Tarrytown, NY) according to the manufacturer's package insert instructions. Amplification products generated by the COBAS Amplicor HCV Monitor Test (Roche) were used.

10-Isolation of Cell lines and cell culture

Isolation was carried out by inoculating specimens onto monolayer of Huh-7 and HepG-2 cell lines (American Type Culture Collection ATCC-clone CCL-81). Tissue culture flasks (Griener-Germany) were incubated at (37°C) for an adsorption period of (1-1.5 hr) with gentle mixing at (15 min) time interval for well virus distribution. Maintenance medium was dispensed to cell culture flasks post adsorption time (DMEM supplemented with 2% FBS) was added without removing the inoculums according to (Joseph and Thomas, 1994). Negative control of non-infected cells was considered. Cultures were daily examined using the inverted microscope (Hund-Germany) for detection of cytopathic effect (CPE).

11-Comparison studies

The serum HCV GT 4a exposed were compared to those with non-exposed. The ALT level, the hemoglobin concentration (HB), and the neutrophil count were compared at the baseline (BL) and at days (2, 3, 4, 5 and 6) of the treatment and day 4 of follow up. The serum HCV RNA load was tested at days 2,3,4,5 and 6 of the treatment and day 4 of follow up. The EVR, the end of treatment response (EOTR), and the SVR were compared.

12-Statistical analysis

Statistical significance was determined using Student's t test, using SPSS 13.0. $P < 0.05$ was considered to indicate a statistically significant result.

RESULTS

1-Characteristics of HCV GT 4a patients

Characteristics of patients are summarized in Table 1. Samples infected with either HCV GT 4a exposed or non-exposed had a comparable ALT, HB and neutrophils.

Table 1: Results for clinical chemistry parameters for samples infected with HCV GT 4a non exposed ELF-EMFs.

	Baseline (BL)	Day (2)	Day (4)	Day (6)
ALT (U/L)	204	306	366	469
HB (mg/L)	16	12	12	10
Neutrophils	3.2	3.3	3.5	3.5

Table 2: Results for clinical chemistry parameters for samples infected with HCV GT 4a exposed ELF-EMFs at (4.9 Hz).

	Baseline (BL)	Day (2)	Day (4)	Day (6)
ALT (U/L)	204	199	100	40
HB (mg/L)	16	12	12	12
Neutrophils	3.2	1.4	1.5	2.2

Bl, baseline; HB, hemoglobin

2- Resonance curve for exposed samples to ELF-EMFs at different frequencies

HCV GT 4a suspensions were exposed for a duration time of (30 minutes) of the virus on its growth characteristics studied to different frequencies of(ELF-EMFs) in the range of (1-10 Hz) to determine the resonance frequency of growth inhibition then the TCID 50 of the samples was measured. Then different groups of HCV GT 4a suspension were exposed to QAMW at resonance frequency of growth inhibition for period of 30 minutes in order to determine the optimum exposure period and according to treatment schedule of once/day were used in the experiments and wave emission continuous and the growth characteristics for control and exposed groups were studied .The results of exposure of HCV GT 4a and HCV GT 4a to (ELF-EMFs) for 30 minutes at frequencies (1 to 10 Hz), the data indicate a non-significant variation of in the activity of the HCV GT 4a and Huh-7 and HepG-2 cell lines after exposure to (1 to 10 Hz) (ELF-EMFs) for 30 minutes when compared to HCV GT 4a and Huh-7 and HepG-2 cell lines non exposed. The data in the figure (3) indicate a highly

significant growth enhancement occurred in HCV GT 4a injected with Huh-7 and HepG-2 cell lines after exposure to (4.9 Hz) (ELF-EMFs) for 30 minutes ($P \leq 0.001$).In contrast, The data in the figure indicate a highly significant growth inhibition occurred in HCV GT 4a injected with Huh-7 and HepG-2 cell lines after exposure to (4.9 Hz) (ELF-EMFs) for 30 minutes ($P \leq 0.002$) when compared to HCV GT 4a injected with Huh-7 and HepG-2 cell lines non-exposed. These results reflect that the (ELF-EMFs) (4.9 Hz) is the resonance frequency of growth inhibition for HCV GT 4a.

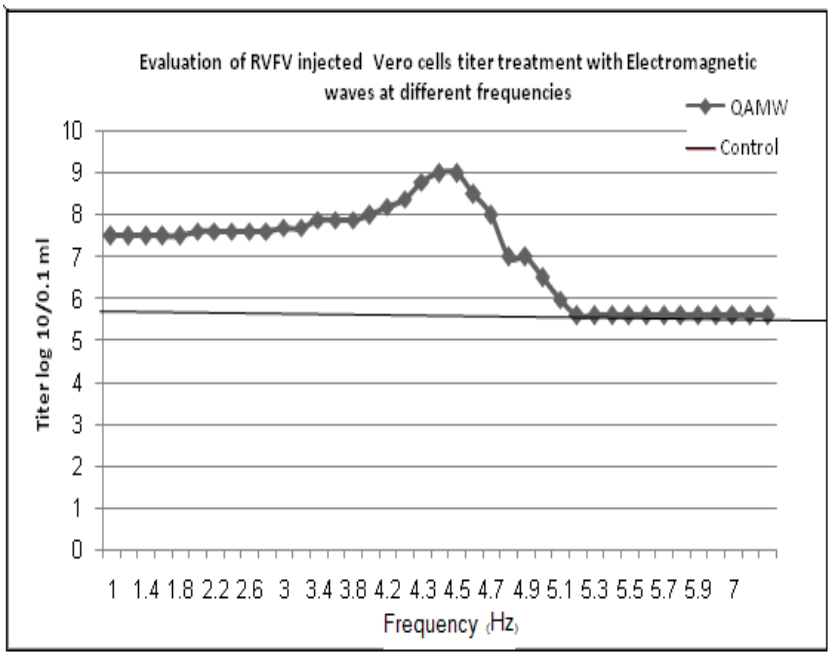

Figure (3): The variation of the HCV GT 4a on Huh-7 and HepG-2 cell lines cells exposed for 30 minutes to (ELF-EMFs) at different frequencies showing the inhibition at (4.9 Hz). The data are expressed as mean ± S.E.

3-Effect of exposures to electric field (ELF-EMFs) on (PBMCs) viability of HCV GT 4a

At BL, the mean ALT level was slightly higher in patients infected with HCV GT 4a non exposed Electromagnetic waves (410 U/L) than those infected with HCV GT 4a exposed Electromagnetic waves (90 U/L). There was a decrease of mean ALT levels during the course of treatment in both groups (Figure. 4).

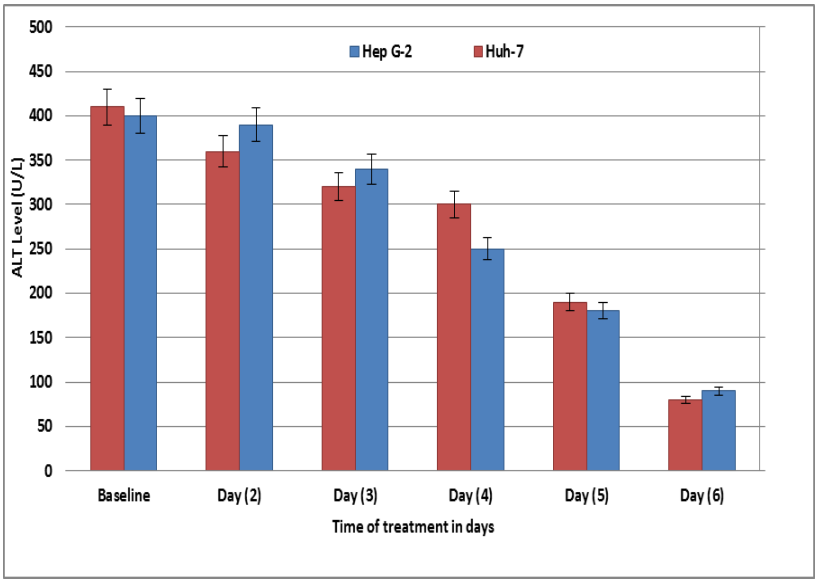

Figure (4): Mean ALT levels in samples with HCV GT 4a infection exposed or non-exposed (ELF-EMFs).

ALT (alanin aminotransferase)

There was almost difference in hemoglobin levels between the two groups. Hemoglobin levels decreased slightly during treatment the follow-up period (Figure. 5).

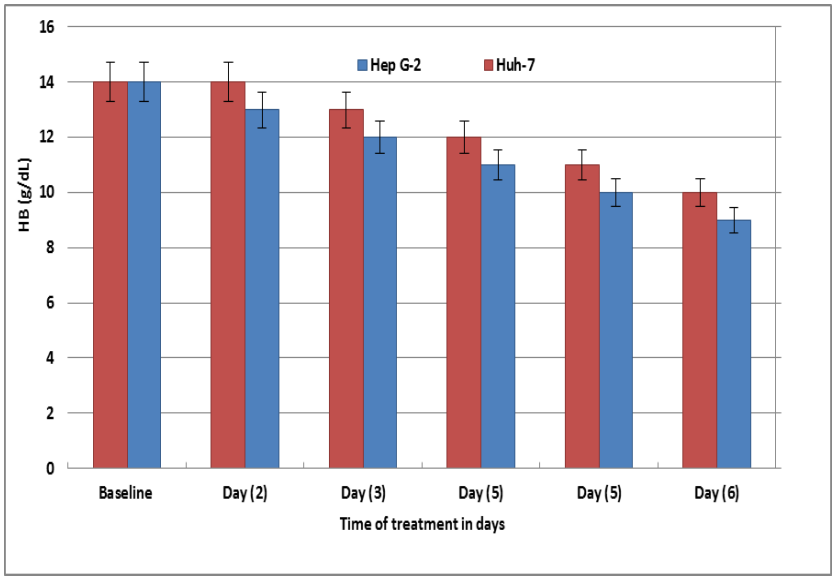

Figure (5): Mean hemoglobin levels in samples with HCV GT 4a infection exposed or non-exposed (ELF-EMFs).

There were almost no difference neutrophil counts between the two groups. Neutrophil counts decreased slightly during treatment but increased again during the follow-up period (Fig. 6).

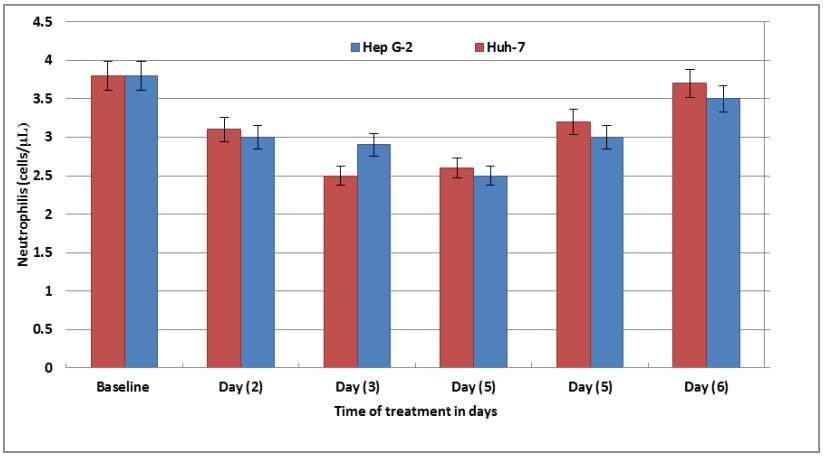

Figure (6): Mean neutrophil counts in samples with HCV GT 4a infection exposed or non-exposed (ELF-EMFs).

3-Response to anti-HCV treatment

The median baseline serum HCV RNA load was $9.7x10^5$ IU/ml in patients with HCV GT 4 exposed infection and $2.0x10^5$ IU/ml in patients with HCV GT 4anon exposed infection. The early virological response rate (EVR), the end of treatment response rate (EOTR), and the sustained virological response rate (SVR) were found to be significantly higher in patients with HCV GT 4a exposed than those with HCV GT 4a non-exposed (Fig. 7). In the HCV GT 4 a exposed group, 27 of 43 samples achieved EVR compared to 16 of 43 samples with HCV GT 4a non exposed (p <0.02). In the HCV GT 4a exposed group, 27 of 43 samples achieved EOTR compared to 16 of 43 samples with HCV GT 4a non-exposed (p<0.001). Corresponding data for SVR were 26 of 43 samples in the HCV GT 4a exposed group and 17 of 43 samples in the HCV GT 4a non-exposed group (p<0.01).

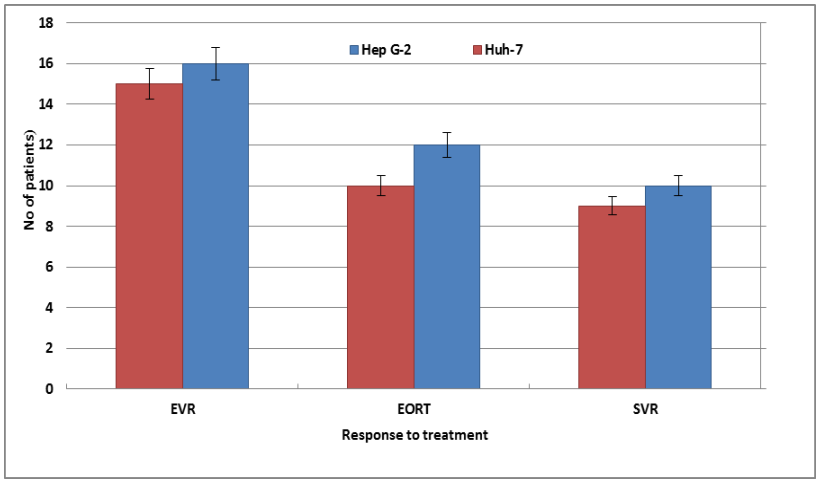

Figure (7): Response to combination therapy with (ELF-EMFs) in samples with HCV GT 4a infection.

4-Isolation Cell lines and cell culture

8 out of 43 (18.6%) specimens showed characteristic cytopathic effect (CPE) on cultured Huh-7 and HepG-2 cell lines. All viral isolates were obtained from specimen, while none was obtained from swab specimen. The four isolates showed first cytological changes on the 4th - 7th day post inoculation till the development of 90% CPE on the 4-5 day as shown in Fig. (6).On further passaging, the viral isolates showed a gradually elevated replication activity on Huh-7 and HepG-2 cells, where the cytological changes (90% CPE) could be detected within 1-6 days post viral inoculation Figure (8) compared with the negative control Figure. (8).

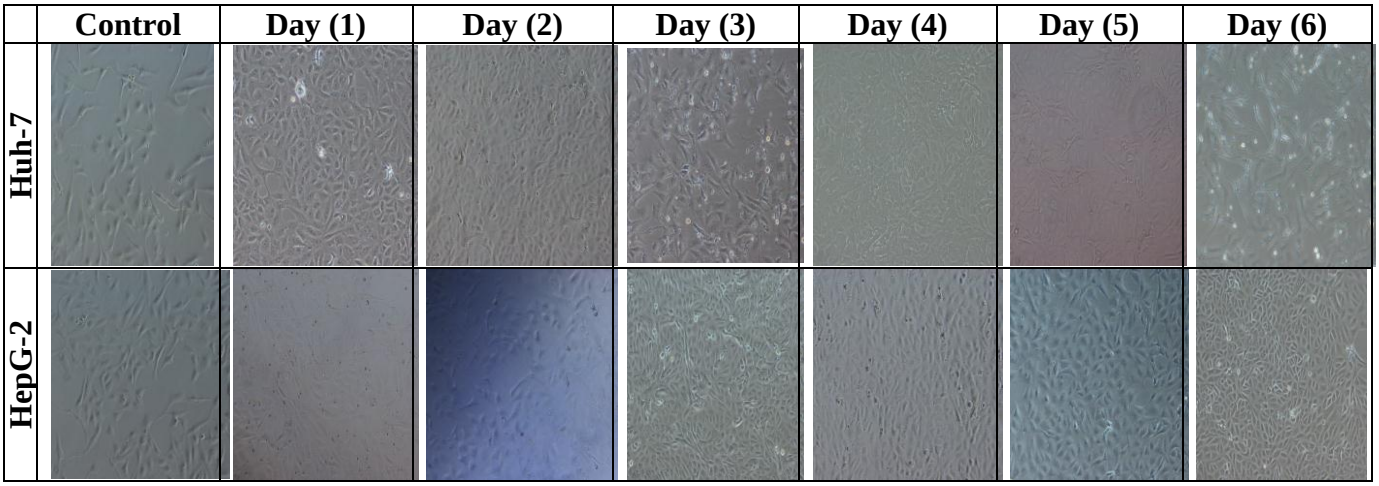

Figure (8): Cytological changes in Huh-7 and Hep G-2 cells line detected within 1-6 days cells that were swollen, retractile and Distinct from transparent cell sheet.

DISCUSSION

In the present work the prevention, control, inhibition, and therapy of HCV GT 4a virus infection in Egypt. In activity by electric field (ELF-EMFs) was studied. The procedure of treatments of the viral growth was based on the resonance interference of applied electric fields with the bioelectricity signals generated during cellular division. Our data suggest that electric field (ELF-EMFs) had direct effects on HCVGT 4a; including the inhibition of growth culture. The electric field (ELF-EMFs) deliveries on HCVGT 4a suspension induced a viral inactivation (around one log10 after 500 pulses). (Fadel 1998, Fadel *et al.*, 2009), to interfere with biological electrical signals generated during metabolic activities or in activity of cells it is necessary to apply on these cells external electric waves of the same frequency.

In this work, a wave carrier of 10 MHz was used. The results indicated that the HCV GT 4a has two resonance frequencies with the bioelectric signals generated during cellular division; these frequencies are at (4.9 Hz) as was shown in figure (3). In a previous study on the effect

of extremely low frequency electromagnetic fields on the Rift valley fever virus done by (Fadel *et al* 2018) showed that there is an inhibiting effect for cell multiplication of HCV GT 4a virus at 5.2 Hz QAMW. The inhibiting effect at 4.9 Hz QAMW may be due to destructive interference of the applied wave with the bio-waves generated during cell multiplication.

This study showed a significantly higher in sample infected with HCV GT 4a with Huh-7 than in those infected with HCV GT 4a with HepG-2. Although the (ELF-EMFs) therapy has lead to a significant progress in the management of HCV GT 4a, the response to therapy is still variable and depends on host characteristics and virological factors. To get a better insight into the interaction mechanism of the electromagnetic field with the biological systems the understanding of the bioelectrical signals resulting from the biological system during metabolic activity is required. (Mohamed *et al.*, 2002) reported that the bioelectrical signals from the microorganism were normally carried out through bending of their cellular membranes which generate an electric impulse through phenomena known as flux electricity. The amplitude and the frequency of these impulses depend on the magnitude and frequency of bending. These impulses travel through the medium separating the microorganisms and are received by the signal receptors at the surface and that impeded the cell membrane. Therefore, the flexibility of the membrane is the most important parameter for generation of these signals. There is also mentioned that the bio magnetic field from the biological system associated to the bioelectrical signals from the membrane of the cells through its metabolic function is very weak in nano Gauss range (20×10^{-8} G). When the biological systems exposed to an external magnetic field whose strength is very large relative to the bio magnetic field of the cells, a disturbance in their metabolic function will be expected which leads to death of the cells or increases their cell division, (Fadel *et al*. 2014)

In the present work, we studied the effects of the infection with HCV GT 4a virus on ALT before and after exposure to (ELF-EMFs) waves. Also, a recovery study was carried out after two months from stopping the exposure to (ELF-EMFs) waves. The changes in liver enzymes are shown in Figure (4).The values of ALT from the infected group showed significant higher values ($p < 0.05$) depending as compared to values for the control group. The obtained data showed that ELF EM waves produced alteration in biochemical parameters of the liver transaminases ALT which have been widely utilized in mammalian toxicology as biomarkers of specific organ dysfunction. In general the increase in transaminases activity is usually associated with hepatocyte damage.

The results in this work indicated that the measurement of complete blood count (Neutrophil counts and Hemoglobin) and virological testing decreased for treated liver by (4.9 Hz) QAMW waves (Huh-7 and Hep G-2 cells). The mechanism of interaction of these electromagnetic fields with the virus at this frequency may be the resonance destructive interference with the electric impulses generated from ionic motions in the virus cell division resulting in growth inhibition (Fadel et al., 2014). There was almost difference in hemoglobin levels between the two groups. Hemoglobin levels decreased slightly during treatment the follow-up period. (Figure 5). There was almost no difference neutrophil counts between the two groups. Neutrophil counts decreased slightly during treatment but increased again during the follow-up period (Figure. 6).

The delayed detection of cytological changes may be attributed to the low viral load in collected specimens, recording 90% CPE on the 12^{th}-16^{th} day post infection. Alternating passaging in cell culture could be a supporting factor to maximize the viral load, where on the 5^{th} passage the cytological changes could be detected within 2-4 days post viral infection showed a gradual increase in the mean viral infectivity titer relatively to time recording 0.56 log (10) / 24 hr, 0.4 log (10) / 24 hr, 0.429 log (10) / 24 hr and 0.543 log (10) / 24 hr for the 4 isolates respectively. These data were supported by the reports recorded by Marin *et al.*, (2000), Arvin (2001), Moretti *et al.*, (2002) and Schmutzhard *et al.*, (2004) where they noted that the infective varicella zoster virus could be isolated using cell culture and typical cytopathic effect (CPE) was observed within 3 days to 3 weeks according to the viral load.

CONCLUSION

The present study demonstrated that the inhibiting resonance frequency of ELF-EM waves that deteriorates HCV GT 4a growth will be promising method for the treatment of HCV GT 4a infection. This technique is non-destructive, non-expensive, and safe and fast, where only 30 min are needed for the exposure of viral to stop its ability to make cell division. Treatment of injected mice by HCV GT 4a with (4.9 Hz) seems successful and applicable.

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