Effect of Short Wave Diathermy (SWD) on Primary Dermal Fibroblast (Normal Human Adult (HDFA))

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Abstract:-

Objectives: Effect of shortwave diathermy (SWD) on Primary Dermal Fibroblast (Normal Human Adult (HDFA)) cell proliferation rates by constructed and implemented exposure device was investigated in this study. Materials &Methods: Primary Dermal Fibroblast (Normal Human Adult (HDFA)) cells were plated at known concentrations and incubated for 5 days. Exposure to (SWD), on days (2, 3, & 4) twice daily. After crystal violet staining (day 5), through measuring the optical density and cell count was determined spectro-photometrically. Another part of the sample was used for dielectric relaxation measurements in the range (100 kHz-10 MHz). Results:(SWD) given at mean power of 48W for 10 minutes, increased Primary Dermal Fibroblast (Normal Human Adult (HDFA)) group compared with control group (P<0.001).The optimal mean power for proliferation was estimated to be 12W There was a relationship between cell proliferations and the amount of energy given (P<0.001).While keeping mean power constant at 6W, pulse repetition and altering pulse duration rate dosage parameters did not have a significant effect on proliferation (P<0.508). The dielectric increment $\Delta\acute{\varepsilon}$, and conductivity σ (S/m) were found to be higher in group B than that of group A. Moreover the data indicated important role of duty cycle on the acceleration mechanism. Group B showed more improvement than group A as indicated by their survival periods, dielectric spectra. Conclusion :(SWD) is associated with increased rates of Primary Dermal Fibroblast (Normal Human Adult (HDFA)) proliferation in vitro, which is dose dependent. These in vitro results contribute to an understanding of the underlying cellular the physiologic mechanism for the therapeutic effects of (SWD).

Key Words: Diathermy- Electric stimulation therapy-Primary Dermal Fibroblast (Normal Human Adult (HDFA)) - Dielectric measurements**.**

Introduction:-

Electric currents can pass through a biological tissue, when metallic electrodes having a potential difference are applied directly upon the tissue. The electric current within the tissue will be carried through ionic or molecular motion or vibrations [1]. The mode of electric conduction in tissue will depend on type as structure of the tissue and the frequency of the applied current. In order to apply low frequency electric field with very high electric impedance in a biological tissue, high frequency currents in form of amplitude modulated wave (AMW) have been used to overcome the impedance encountered by such fields. Since the impedance Z is given by

$$Z = 1 / 2\pi f c$$

Where, f is the frequency and c is the capacitance.

Diathermy is a form of therapy which utilizes electromagnetic radiation to induce heat inside the patient's body. The use of non-ionizing electromagnetic energy from the radio as therapeutic agent. This leads to increased blood circulation which is believed to induce faster healing [2].The medical efficacy of this treatment, has been the subject of some controversy, while the potential hazards posed by the high power (> 200W) electromagnetic radiation emitted

by a diathermy machine are certainly not to be neglected. Physiologic effects to (SWD) have been shown in the laboratory [3]-[4].

In all of the cited studies, the investigators note that healing time is shorter in subjects exposed to (SWD) than it is in untreated controls. The common suggestion is that (SWD) influences Fibroblast cell proliferation rates. However, although this proposed cellular mechanism for (SWD) has been widely acclaimed in the subsequent literature, in vitro studies to confirm its direct effects on Fibroblast cell lines or other closely related cells, such as chondrocytes, could not be identified [5].

Electric dipole moment (μ): Most biological molecules possess an electric dipole moment, which arise from the presence of a negative and a positive charge (q) existing at a distance (r) between each other [6]-[7]. If the sum of q_i and r_i is not zero, then the molecule has finite dipole moment the dipole moment is the given by:

$$\mu = \Sigma q_i r_i$$

The dielectric constant (ε) or permittivity and conductivity (σ) of a material are, respectively, the charge and current densities induced in response to an applied electric field of unit amplitude. Polk C *et al* [8] illustrated these definitions by the example of the parallel plate capacitor of area (A) and separation (d), which contain the material under investigation. If an electric field is applied, there will be a tendency for the molecules to orient themselves in the direction of the electric field and a charge density (P_s) will be induced on the plates from The polarization of charges within the material, where the polarization is the measure of the magnitude of this average orientation and is expressed as the dipole moment per unit volume. The total charge density (D) is proportional to the applied electric field (E) can be written as:

$$D = \varepsilon_o E + P_s$$

Where ε_o is the permittivity of free space = 8.85 x 10^{-12} F/m. The capacitance (C) and conductance (G) are given by:

$$C = \frac{\varepsilon_s \varepsilon_o A}{d}$$

$$G = \frac{\sigma_s A}{d}$$

Where ε_s and σ_s are the static permittivity and conductivity of the material, respectively. In case of an applied alternating field, the dielectric properties (ε, σ) will vary with frequency [8].

Aim of the work:-

This study was designed to investigate the hypothesis that (SWD) directly accelerates Primary Dermal Fibroblast (Normal Human Adult (HDFA)) cell proliferation rates in vitro and to establish the influences of different applied dosage variables. We also studied the influence of (SWD) on Primary Dermal Fibroblast (Normal Human Adult (HDFA)) to assess whether this growth and protein synthesis was cell specific.

Method:-

1-Experimental Setup:-

The exposure device was constructed in the Faculty of Sciences Cairo University Research Lab, to produce short waves diathermy (SWD) fig (1). The peak pulse power of 150W was kept constant throughout all experiments because SWD units come with this variable preset in the machine. For samples exposure, the suspension of the sample (100 ml Primary Dermal Fibroblast (Normal Human Adult (HDFA)) cells) was exposed to (SWD) from the generators

through the use of two parallel plate electrodes placed on the outer surface of the baby flask. The Primary Dermal Fibroblast (Normal Human Adult (HDFA)) cells group was exposed to SWD treatment was performed on days 2, 3, and 4 at twice daily to Jonathan Hill *et al.* [15].

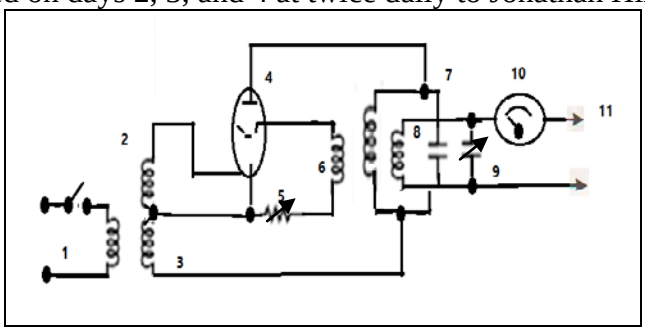

Fig (1): Circuits Diagram Short Waves Diathermy (1: mains,2:Temperature $1T_1$, E.H.T,4;Powe tube,5:Risistor Variable,6:Indicator,7: Temperature $1T_1$,8:Capacitor,9:Capacitor variable,10:Tuning indicator,11:Two sample electrodes.

2-Cell lines and culture:-

Primary Dermal Primary Dermal Fibroblast (Normal Human Adult (HDFA)) (Normal Human Adult (HDFA)) cell lines were purchased from VACSERA, EGYPT. The cell lines were maintained in Dulbecco's minimal essential medium with 10% fetal calf serum at 37°C in a humidified atmosphere with 5.2% CO_2. The cells were dispersed from nearly confluent cultures by 0.025% trypsin in 0.02% ethylene diaminetetraacetic acid solution. Cells were then spun in a centrifuge at 1000rpm for 5 minutes, after adding equal quantities of Dulbecco's minimal essential medium. A measured volume of growth medium was used to resuspend the cell pellets. Cells were then plated out in triplicate at known concentrations (1000 cells) into the 96-wells, flat-bottomed micro titer plates and incubated at 37°C for 3 days to let them proliferate [9].

3-Effect of (SWD) on Primary Dermal Fibroblast (Normal Human Adult (HDFA)) Proliferation:-

(SWD) effect on Primary Dermal Fibroblast (Normal Human Adult (HDFA)) group, we compared the optical density of a control group not exposed to (SWD), with an exposed Primary Dermal Fibroblast (Normal Human Adult (HDFA)) group (n=12 wells / group). For the treated Primary Dermal Fibroblast (Normal Human Adult (HDFA)) group, the (SWD) dosage was 48W (8Hz) mean power, for 10 minutes duration.

4-Effect of Energy on Primary Dermal Fibroblast (Normal Human Adult (HDFA))Proliferation:-

To investigate whether different patterns of (SWD) energy influenced Primary Dermal Fibroblast (Normal Human Adult (HDFA)) proliferation rate, the pulse repetition rate and pulse duration were varied while a constant mean power was maintained. A dosage of 8W mean power was selected because this power was easily reproduced with (SWD) that had been calibrated for this study. The energy used was as follows: control group (4Hz) and Primary Dermal Fibroblast (Normal Human Adult (HDFA)) group (2Hz) (n=12 wells / group). Treatment time was 10 minutes [10].

5-Dielectric Measurements for the Primary Dermal Fibroblast (Normal Human Adult (HDFA)) Proliferation:-

The dielectric measurements were carried out for the samples in the frequency range 0.1-10 MHz using a Loss Factor Meter type HIOKI 3532 LCR Hi TESTER, version 1.02,1999, Japan, with a sample cell type PW 9510/60, manufactured by Philips. The sample cell has two

squared platinum black electrodes of 0.64 cm^2 area and separated by 1 cm apart. During measurements, the sample between the electrodes was kept at a constant temperature of 24 ± 0.1 ^oC. The capacitance of the samples was measured at each frequency and the resistance was recorded. Each run was taken three times and the average was considered. The relative permittivity $(\grave{\varepsilon})$ of the sample was calculated for each frequency using the relation:

$$\grave{\varepsilon} = \frac{C\,d}{\varepsilon_o\,A}$$

Where C is the capacitance of the sample at a given frequency in Farad (F), A is the surface area of the electrodes in (m^2) and ε_o is the permittivity of free space which equals to 8.85 x 10^{-12} (F/m),d is the separation distance between the two electrodes in (m) .The loss tangent "tan δ" was calculated from the relation:

$$tan\ \delta = 1/2\pi fRC$$

Where, f is the frequency in (Hz) and R is the resistance of the sample in Ohm (Ω). The dielectric loss $\grave{\varepsilon}$ was calculated from the relation:

$$\grave{\varepsilon} = \grave{\varepsilon}\ tan\ \delta$$

The conductivity σ in (S/m) was calculated from the relation:

$$\sigma = 2\pi f \grave{\varepsilon} \varepsilon_o$$

Using all the obtained data, a relation between ε′ and f was plotted, from which the relaxation time (τ) can be calculated for each curve from:

$$\tau = 1/2\pi f_c$$

Where f_c is the critical frequency which corresponding to the mid-point of dispersion curves.

4-Statistical Analysis:-

Statistical significance was set at *P* less than 0.05 ($p < 0.05$) (2 tailed) and analysis was performed by using SPSS, version 9.0, for Windows 95. The Student's t-test and other statistical analysis were performed using statistical SPSS -12 programs.

Results:-

1-Effect of (SWD) on Primary Dermal Fibroblast (Normal Human Adult (HDFA)) Proliferation:-

(SWD) given at mean power dosage of 48W for 10 minutes, twice daily, was significantly associated with Primary Dermal Fibroblast (Normal Human Adult (HDFA)) cell division rate in vitro ($P< 0.001$) (fig 2). The median optical density was (0 .54) in the control group and (0 .83) in the Primary Dermal Fibroblast (Normal Human Adult (HDFA)) group.

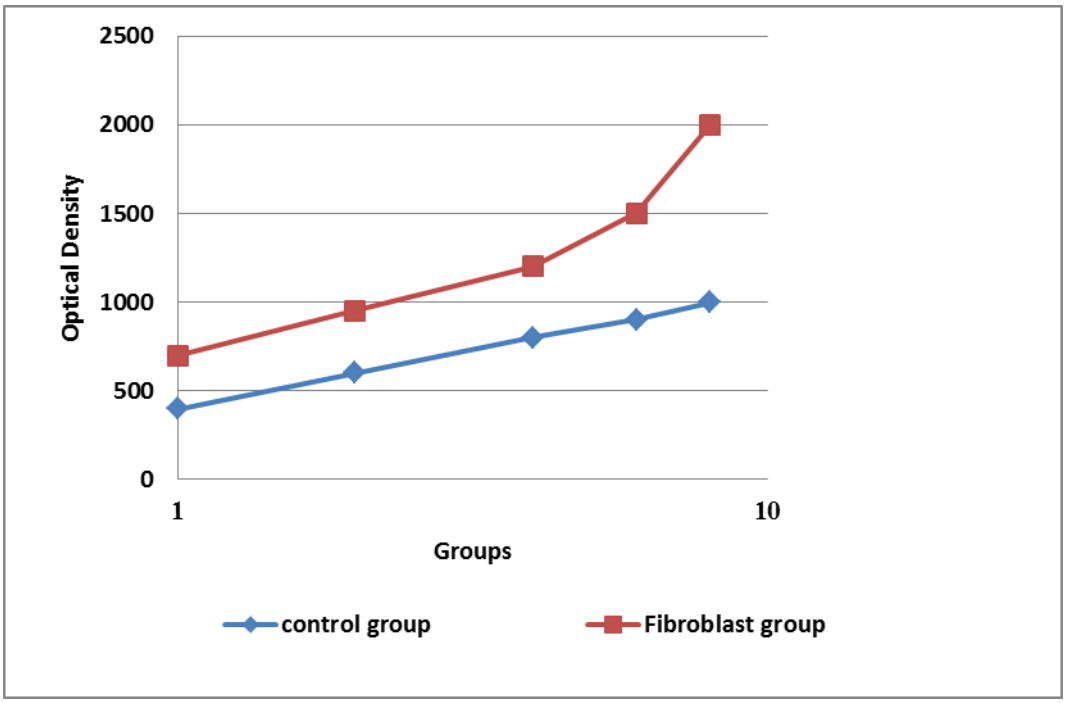

Fig (2): Effect of the optical density on Primary Dermal Fibroblast (Normal Human Adult (HDFA)) group and control group

2-Effect of Energy on Primary Dermal Fibroblast (Normal Human Adult (HDFA)) Proliferation:-

The pulse duration and pulse repetition rate while keeping the mean power constant at 8W did not affect cell proliferation rate (*P*=0.519). The median optical density was 0.12 at 6 min and 2Hz and 0.13 at 4min and 2Hz. (fig 3).

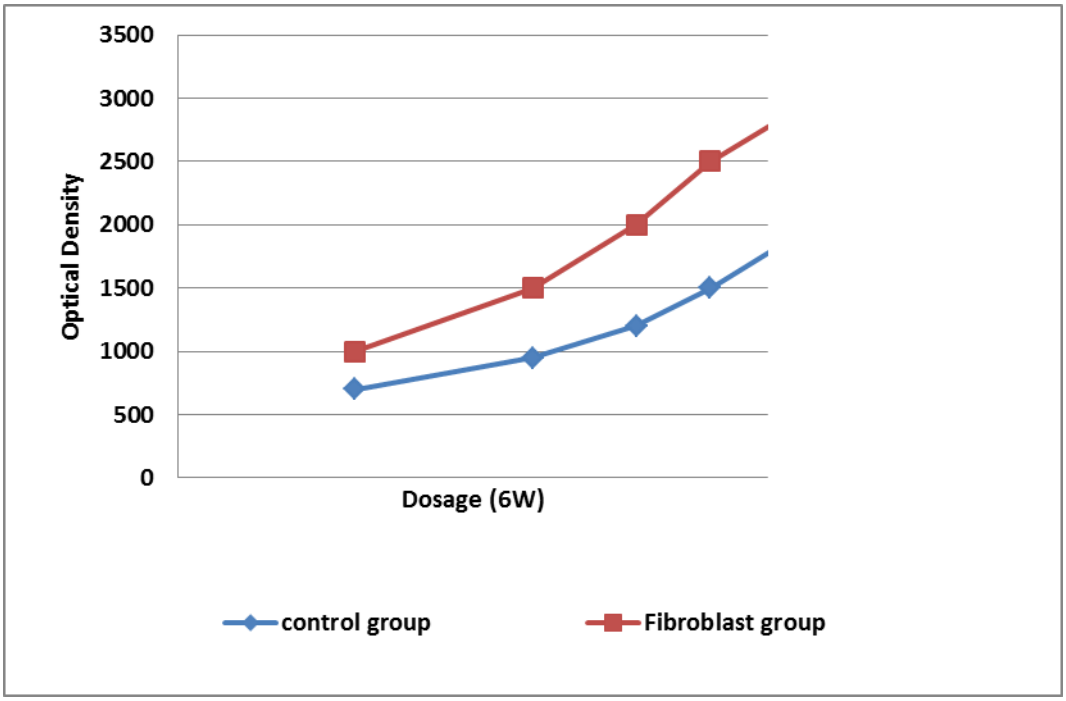

Fig (3). The relation between the optical density of Primary Dermal Fibroblast (Normal Human Adult (HDFA)) and (SWD) dosage factorsgiven a constant power of 6W.

3-Dielectric Relaxation Results:-

Figure (4) illustrates the variations of the dielectric constant, ε′ and the dielectric loss, ε″, plotted on the right y-axis as a function of the applied frequencies field in the range of 100 KHz up to 10 MHz for control group. The results indicate a dielectric dispersion in the frequency range demonstrated. The conductivity on the field frequency shows a mirror image to the ε′ dependence which is considered as a consistency test for the data as given by Kramers-Kronig relations [8].

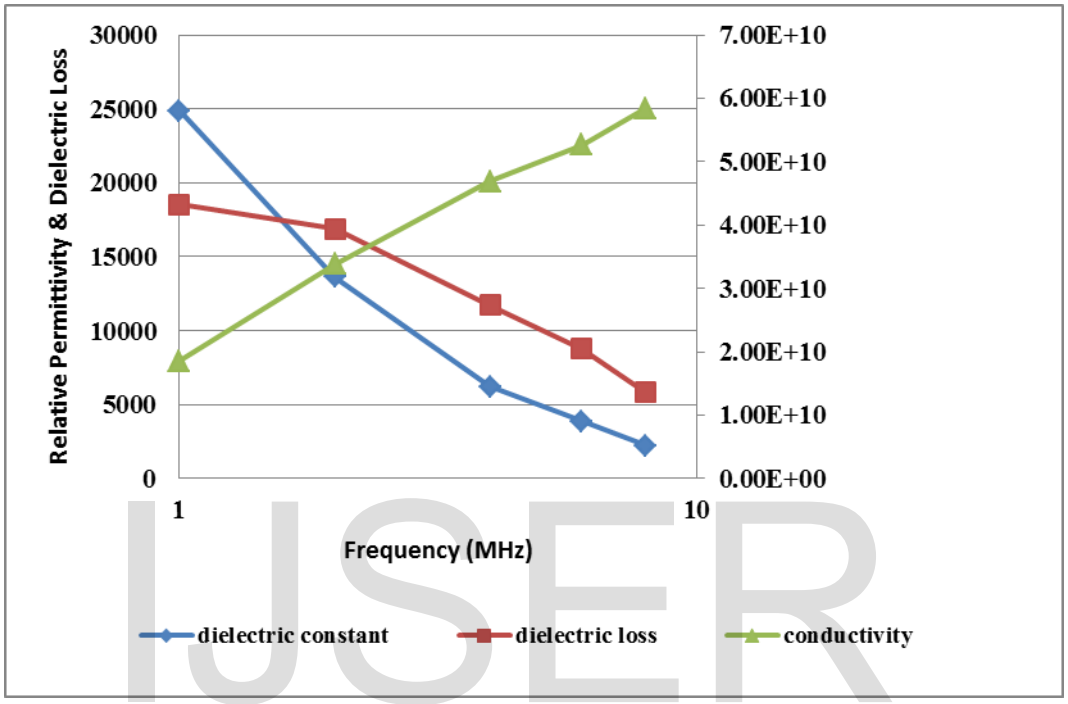

Fig. (4): The relative permittivity, ε′ , the dielectric loss, ε″ and the electric conductivity, σ as functions of frequency in the range, 0.1-10 MHz for the control group.

Figure (5) illustrates the dielectric relaxation spectra for the samples from group of Primary Dermal Fibroblast (Normal Human Adult (HDFA)) group exposed to 10 Hz compared with control group. It is clear from the curves that ε′, ε″ and conductivity σ have a higher values for the Primary Dermal Fibroblast (Normal Human Adult (HDFA)) group as compared with control group but lower than the dielectric properties of Primary Dermal Fibroblast (Normal Human Adult (HDFA)) group.

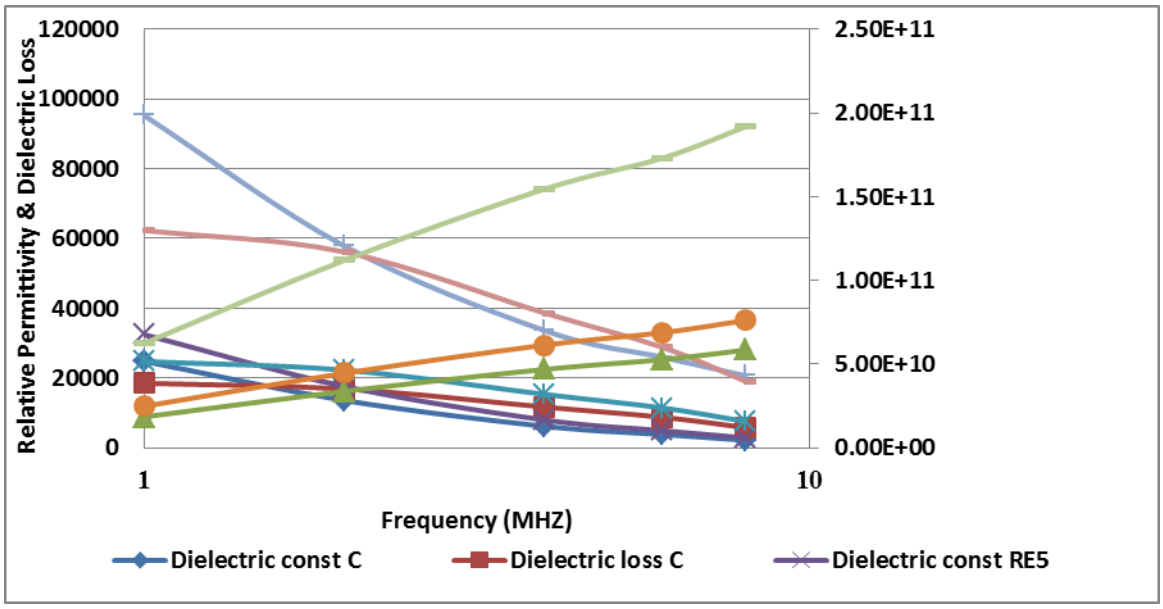

Fig. (5): The relative permittivity, ε′ , the dielectric loss, ε″ and the electric conductivity, σ as functions of frequency in the range, 0.1-10 MHz for the Primary Dermal Fibroblast (Normal Human Adult (HDFA)) group exposed to 5.2 Hz compared with control group.

Table (1) represents the values of the relaxation time (τ), the dielectric increment (Δέ) and the conductivity (σ) of control group, and treated group.

Table (1): The relaxation time (τ), the dielectric increment (Δέ) and the conductivity (σ) of control group, and Primary Dermal Fibroblast (Normal Human Adult (HDFA)) group.

Groups	Relaxation time τ (μsec)	Dielectric increment $\Delta\grave{\varepsilon} = \grave{\varepsilon}_o - \grave{\varepsilon}_\infty$	Conductivity σ at 10 MHz (s/m)
Control group	0.96	50730	5.88×10^{10}
Primary Dermal Fibroblast (Normal Human Adult (HDFA)) group	0.99	54223	7.68×10^{10}

Discussion:-

The molecular mechanism that may account for the effects of (SWD) on cell proliferation is still unclear. It has been suggested that the molecular agitation caused by pulsed electromagnetic fields may initiate a series of trigger reactions (eg, the binding of hormones and neurotransmitters to their receptor sites) [11].In this study, we showed that (SWD) significantly influenced Fibroblast proliferation in vitro [12]. This finding may help in understanding the physiology underlying the therapeutic effects of (SWD) in clinical practice, and it supports previous hypotheses regarding the cellular mechanisms by which (SWD) influences Fibroblast proliferation [13].

The amount of energy given (mean power) and treatment duration that influenced cell proliferation, in contrast to the pattern of energy given (pulse repetition rate and pulse duration), which did not have a significant effect. The dosage was important because there was a relation between in vitro cell proliferation and (SWD) exposure. Furthermore, cell proliferation was highest with the 5-minutes treatment duration compared with longer treatments. On other hand, it

has also been suggested that these triggers may be the stimulation of gene transformation through heat shock protein and osmotic stress gradient mechanisms [14]-[15].

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 [16].The amplitude and the frequency of these impulses depend on the magnitude and frequency of bending. Therefore, the flexibility of the membrane is the most important parameter for generation of these signals [17].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 [18].

The results of the dielectric relaxation studies for control group and Primary Dermal Fibroblast (Normal Human Adult (HDFA)) group indicated that the relative permittivity, conductivity and dielectric loss versus applied electric field frequency have higher values for Primary Dermal Fibroblast (Normal Human Adult (HDFA)) group as compared with control group. This higher electrical conductivity of the Primary Dermal Fibroblast (Normal Human Adult (HDFA)) group than control group is due to higher surface charges on the cellular membrane than control group. 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 Primary Dermal Fibroblast (Normal Human Adult (HDFA)) group cell division resulting in highly growth or acceleration Primary Dermal Fibroblast (Normal Human Adult (HDFA)) proliferation [19].

In biophysics oscillating electric and magnetic fields produce heat in biological tissues by inducing a rapidly alternating movement of ions, rotation of dipolar molecules and the distortion of non-polar molecules [20]. A movement of ions represents a real flow of current and occurs readily in tissues rich in electrolytes such as blood vessels and muscle. Resistance to this flow leads to heart production.[21]. By contrast, in fatty tissue the main effect of an alternating electromagnetic field is to produce rotation and distortion of molecules which does not constitute, a real flow of current, hence little heat is generated. This activity of the SWD field at molecular level should cause blood vessels and muscle to heat strongly and adipose tissue to heat poorly [22]. Experience reveals, however, that adipose tissue is also heated vigorously because it is permeated by small blood vessels that contain a solution of electrolytes. The heat generated is then retained due to the insulating properties of fat allowing a high temperature to develop. Fibrous tissue is not particularly rich in either blood vessels or fat and usually shows a moderate elevation of temperature [23].

Conclusion:-

From this work it is concluded that the (SWD) has a significant influence on Primary Dermal Fibroblast (Normal Human Adult (HDFA)) proliferation in the laboratory setting. This effect is associated with treatment dosage and time. These in vitro results contribute to an understanding of the underlying cellular mechanism for the therapeutic effects of (SWD). So this study can be considered as a promising methodology to acceleration Primary Dermal Fibroblast (Normal Human Adult (HDFA)) proliferation activity by using short waves diathermy (SWD) at 6W, the resonance frequency of acceleration Primary Dermal Fibroblast (Normal Human Adult (HDFA)) proliferation

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