Cold Laser as a Complementary Drug in the Treatment of Osteoarthritis

[1]Samir W. Aziz; [1]Bassem M. Raafat. ; [1]Nahed S. Hasan and [2]Ahmed Hanafy

[1]Genetic Engineering and Biotechnology Division, Biochemistry Department, Biophysics Group, National Research Center; [2]Head of Rheumatology Department, Air Force Hospital

Samir_Wassef@yahoo.com; bassemraafat@hotmail.com; nahedsha33@yahoo.com; [2]hanafy2003@hotmail.com

Abstract: Osteoarthritis is a common cartilage condition and a major cause of pain and disability in older adults. Osteoarthritis most often occurs at the ends of the fingers, thumbs, neck, lower back, knees, and hips. Osteoarthritis hurts people in more than their joints: their finances and lifestyles also are affected**.** Magnetic susceptibility, dielectric relaxation in the frequency range 100 KHz up to 10 MHz of Hb molecule of osteoarthritic patients receiving anti inflammatory drugs were compared to those received drugs and subjected to soft laser emitted from He-Ne laser with two IR diodes. In addition SOD and whole blood ATP concentration enzyme were measured. The dielectric results indicated that the molecular shape tends to deviate from the non spherical form in patients treated with non steroidal anti inflammatory drugs, to spherical one in those receiving soft laser as an additive drug. Low power laser has significant ability to decrease the pain and suffering of arthritis as well as reducing the disease symptoms. Side effects of medications were reduced in patients received cold laser as a complementary drug. [Journal of American Science 2010;6(5):142-152]. (ISSN: 1545-1003).

Keywords: Osteoarthritis, dielectric properties, soft laser, hemoglobin, magnetic susceptibility.

Introduction

Osteoarthritis (OA, also known as degenerative arthritis, degenerative joint disease), is a group of diseases and mechanical abnormalities results from many metabolic, genetic, chemical, and mechanical factors (Valdes and Spector, 2008), involving degradation of joints (Brandt et al., 2008), including articular cartilage and the subchondral bone next to it. Clinical manifestations of OA may include joint pain, tenderness, stiffness, creaking, locking of joints, and sometimes local inflammation, OA is the most common form of arthritis (Brandt et al., 2009) and a major cause of impaired mobility and disability for the ageing populations (Kokebie and Block 2008 ; Lozada 2009). The greater the degree of inflammation, the greater the complaint of stiffness (Lin EHB et al, 2003). There are a number of drugs under development for symptomatic and disease modification, and several studies are also evaluating alternative therapies. There are several drugs on the market whose clinical effectiveness and long-term safety still need to be determined. This assessment is especially important since OA requires long-term disease management and the disease primarily affects people over the age of 60 who are most prone to drug toxicity, and for whom the potential for drug interactions are high. Information on the impact of the disease to society and the cost of disease management (including pharmacological and non-pharmacological treatments) needs to be re-evaluated. (Bradley et al., 1991; Stamm et al., 2002; Watkins et al., 2006 ; Towheed et al., 2006; Zhang et al., 2007)

At present, there is no cure for OA. The management of OA is broadly divided into non-pharmacological, pharmacological, and surgical treatments. Surgical management is generally reserved for failed medical management where functional disability affects a patient's quality of life. Pharmacological management includes control of pain and improvement in function and quality of life while limiting drug toxicity. Experts in this field suggest that appropriate therapy for OA combines one or more pharmacological agents with exercise, weight loss and physical therapy (i.e. non-pharmacological therapy) (Song et al., 2006).

The therapeutic aim is to treat pain, maintain motor strength, joint range of movement, mobility and therefore function (Dieppe PA, 2004; Dominick et al., 2004; Foster NE, et al., 2007; Reichenbach S., et al., 2007). Strategies include a pharma-cological and non-pharmacological approach. Surgical intervention requires strict indications (Rozendaal RM, et al., 2008 ; Sawitzke AD, et al., 2008 ; Zhang W, et al., 2008).While medicines, injections, and surgery all have their place and are valuable, there is still a need for

potentially useful adjunctive modalities that might speed up recovery and reduce pain faster (Messier SP, et al., 2000; Goldkind L, and Simon LS, 2006).

Lasers for treating osteoarthritis are approved by the FDA (Kipshidze N, et al., 2000), and there are a number of surveys that point to the laser's effectiveness. Low – energy lasers are now widely used to treat a variety of musculoskeletal conditions. Although controlled evaluation of these treatment is limited, quite enthusiastic claims are made for pain, arthritic and wound healing applications .Since these devices are safe, easy to use, and relatively inexpensive.(Ozdemir F., et al., 2001; Stergioulas, A., 2004; Ozcelik O., et al., 2008; and Kuhn A, et al., 2009)

Low level therapeutic laser, better known as phototherapy, is a relatively new form of treatment. Its premise is that certain wavelengths of light have effects on living tissue. This effect is termed "photobiomodulation." (Markovic AB, et al., 2006; Huang, Y.Y et al., 2009). Certain wavelengths of light at certain intensities (or irradiance to use the technically correct term) delivered by laser, LED or another monochromatic source may affect tissue regeneration, inflammation, or pain. (Gupta AK, et al., 1998; Schubert, V, 2001; and Bjordal JM, et al., 2006).

Laser treatment, unlike traditional surgical methods, is non-invasive and leaves no scarring. Laser treatments are often preferred over medicinal treatment, as lasers do not have the side effects pain pills often do. (Karu, T, 1999 ; Minatel DG, et al., 2009; Lapchak PA, et al., 2010). Laser therapy was introduced as a non-invasive treatment option for osteoarthritis .The effect produced by laser therapy is not thermal (heat) instead, it has to do with photochemical reactions in cells (Albert, Y. and Banerjee 1975; Karu T, 1988; Lohr NL, et al., 2009).

Many of the pharmacological agents used in the treatment of osteo arthritis can interfere with the various aspects of the inflammatory response. However, deform abilities is still occurred with low rate to some extent. Searching for new trends of treatments to achieve the therapeutic aim of the maximum benefit with minimal toxicity was the aim of the present study.

Material and methods

This study was conducted on 60 osteoarthritic patients, with age range from 47 to 76 years old. They were classified into two subgroups according to the type of treatment received. G_1 (25 patients, 15 females and 10 males) treated with non steroid anti inflammatory drugs. G_2 (35 patients, 20 females, 15 males) treated with non steroid anti inflammatory drugs and subjected to soft laser produced from mid laser infra red medical instrument. Patients received laser sessions along four weeks every other day. We irradiated the trigger points, access points to the joint, and striated muscles adjacent to relevant nerve roots. Patients were subjected to soft laser and detailed clinical examination in the Air Force Hospital. Blood samples were collected from the patients once after the last session of laser irradiation.

All patients were subjected to detailed clinical history, past history and laying stress on compliant of patients, onset and course of diseases, the pattern of joint involvement and extra articular affection. Pregnant women and patients suffering from other inflammatory diseases were excluded. A pulsed diode laser, He-Ne mid laser with IR manufactured by space laser SRI was used. Turin with continuous emission visible light 632.8 nm wavelength (Out put power 5 mw, output divergence after lens 60 mRad), in coaxial associated with 2 infra red diodes of wave length 904 nm, each with the following specification:

1-Infra- red laser emitters:

Peak output power = 5×10 w. Average output power = $5 \times (0.3 + 5)$ mw. Pulse width 180 nsec. Pulse frequency min. 200 Hz - Max. 4000 Hz. Output beam divergence 70 m Rad.

Number of diodes = 5

2- I.R. Handles:

Peak power = 10 W. Average output power = 3 mW (min.). Pulse duration 180 nsec. Pulse frequency 4000 Hz. Output beam divergence 70 mRad.

Magnetic susceptibility of hemo-globin is measured using the well known Alert method (Takizawa and Horie, 1986). The force excreted on the sample tube in the magnetic field is determined with a commercial semimicro-balance. This rests upon a mechanical stage, adjustable in two directions, which permits adjustment of the sample between the shoes. Thermal disturbances of the magnetic field (3 to 10 KG) are provided by a water-cooled Wiess magnet. The air gap is 3.8 cm. Fluctuations in the laboratory supply direct current voltage frequently call for a special supply for the magnet. The alternating current supply is fed via a magnetic stabilizer (constant voltage transformer) to an adjustable transformer and rectifier in a two-way rectifier stage. The magnet current can be controlled by

the regulating transformer. The components of the magnetometer are:

1- AC source .
2- Thermocouple
3- Variable transformer with control
4-Two-way rectifier
5- Magnet
6- Semi-micro balance
7- Power supply for the heating jacket
8- Magnetic constant voltage supply.

Calculation:

Volume magnetic susceptibility is given from :

$$K_S = \frac{\delta S}{S}\frac{W}{\delta W}\frac{\rho}{d}K_w + [1 - \frac{\delta S}{S}\frac{W}{\delta W}\frac{\rho}{d}K_a) \ldots (1)$$

Where

K_S : volume magnetic susceptibility

δS : weight change of the sample in and outside the field

δW : same parameter of the water

S : weight of sample

W : weight of water

ρ : density of sample

d : density of water

K_W is the volume magnetic susceptibility of water at the temperature of the measurements.

$K_W = -0.72145 \times 10\text{-}6 - 0.000108 (t\text{-}20) \times 10^{-6}$ (2)

K_a is the volume magnetic susceptibility of air which is 0.029×10^{-6}

Molar magnetic susceptibility is given from:

Molar Mag. Sus. = $K_S \times MW$ …….(3)

Determination of hemoglobin oscillator strength is done by using a spectrophotometer UV-Visible 240 Shimadzu (200-700 nm) at room temperature. The extinction coefficient at the wavelength ranged from 310 to 700 nm are taken and then oscillator strength per molar heme was calculated on the following equations:

$$f = \frac{2.303MC}{Na\,e^2} N_o \left(\frac{3}{N_o^2 + 2}\right)^2 \int \varepsilon \partial \upsilon \quad .(4)$$

$$= 1.44 \times 10^{-19} \times 0.841 \int \varepsilon \partial \upsilon \ldots . \quad (5)$$

$$= 1.44 \times 10^{-19} \times 0.841 \times 3 \times 10^{10} \times \ln \lambda \times \varepsilon . \quad (6)$$

Super oxide dismutase (SOD) and Adenosine triphosphate (ATP) enzymes activities were measured spectroph-otometrically. The reagents used were RANSOD by RANDOK kits (Randox .Crumlin.UK). The instrument used was a double beam spectrometer (UV Model 2410, shimadzu, Japan).

Dielectric Measurements:

The dielectric dispersion for 5% aqueous solution of Hb was measured at 25°C in frequency range 0.1 and 10 MHz for SLE patients compared to normal control using a Loss Factor meter type 1033, RFT, Funkwerk Erfurt. Gerny. The Hb samples were measured by the cell type pw 9510/60, manufactured by Philips. The sample cell has two squared platinum black electrodes each having an area of 0.8x0.8 cm^2 with an intermediate distance of 1 cm. The cell with the sample is kept at 25^0C ± 0.1 in a temperature controlled incubator Kotterman type 2771 Germany. The value of ε' (Relative permittivity of the sample in the cell) was calculated at each frequency from the constant K (the cell constant that depend on the cell dimensions) and C_o (The residual capacitance) and the measured values of C, also the loss tangent (tan $\boldsymbol{\delta}$) was obtained from the measured values of the resistance R and C in farad as:

$$\tan\delta = \frac{1}{2\pi f RC} = \frac{\varepsilon''}{\varepsilon'}$$ the dielectric loss ε" was calculated from the relation $\varepsilon'' = \varepsilon' \tan\delta$, the conductivity (σ) was then calculated from the relation

$$\sigma = 2\pi f \varepsilon'' \varepsilon_o = C/K$$

For spherical macromolecules the dielectric relaxation time depends on the viscosity of the liquid η and its absolute temperature T. Viscosity measurements of each Hb solution was carried out with an ostwald viscometer at concentration of 5% and 25 °C bi-distilled water was used first at fixed volume to pass through certain height of the Ostwald's capillary, then the efflux of water t_2 is determined three times and an and an average value was taken also the averaged efflux times t_1 for both G_1&G_2were determined, then the viscosity coefficient η_1 of each sample was calculated as

$$\frac{\eta_1}{\eta_2} = \frac{(f_1 t_1)}{(f_2 t_2)}$$

Where η_2 is the viscosity coefficient of water, f_1 and f_2 are the densities of water and solute molecules respectively.

Data were analyzed statistically by using the common T-test.

Results

Table (1) : Oscillator strength, molar magnetic susceptibility of osteoarthritic patients received anti inflammatory drugs G_1 as compared to those received soft laser G_2

	G_1 (n = 25)	G_2 (n= 35)
Oscillator strength	3.368 ± 0.65 ***	2.985 ± 0.48
Molar magnetic susceptibility	- 0.7321 ± 0.12 x 10^{-6}	- 0.8962 ± 0.14 x 10^{-6}***

*** $P < 0.001$

Table 1 illustrates a significant decrease in the oscillator strength per molar heme concomitant with Molar magnetic susceptibility of Hb of G_2 as compared to G_1.

Table (2) : Serum ATP concentration and SOD activity of osteoarthritic patients received anti inflammatory drugs G_1 as compared to those received soft laser G_2

	G_1 (n = 25)	G_2 (n= 35)
ATP conc. mg/100 ml	20.173 ± 0.58	21.285 ± 0.46 ***
SOD conc. mg/ml	83.23 ± 0.61	81.83 ± 4.67 ***

* $P < 0.01$ *** $P < 0.001$

Whole body ATP concentration revealed a significant increase in patients treated with anti-inflammatory drugs and irradiated with soft laser G_2 as compared to those received anti inflammatory drugs G_1. Highly significant decrease in the concentration of Super oxide dismutase activity was observed in G_1 as compared to G_2.

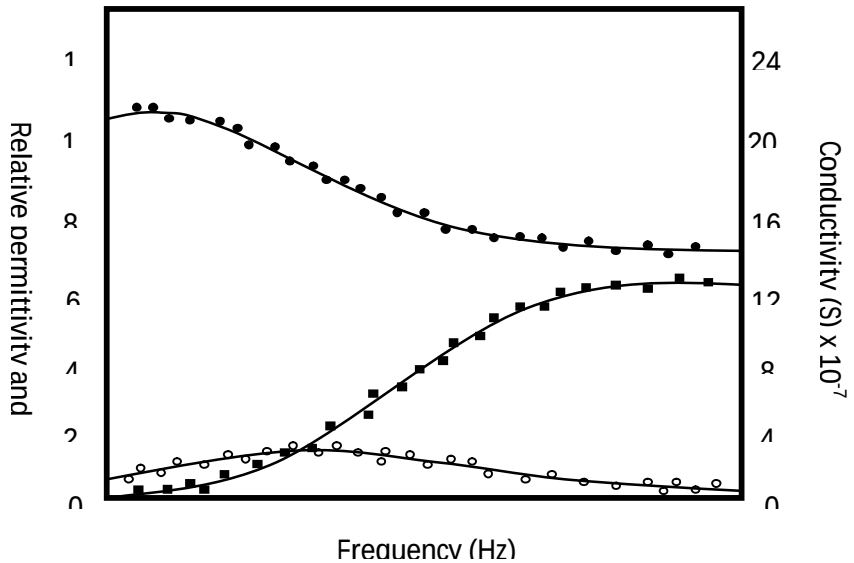

Fig. (1) Variation of relative permittivity (ε'), (°); dielectric loss (ε''), (°); conductivity (S), (') with frequency for 5 % aqueous G1 Hb solution at 25 °C.

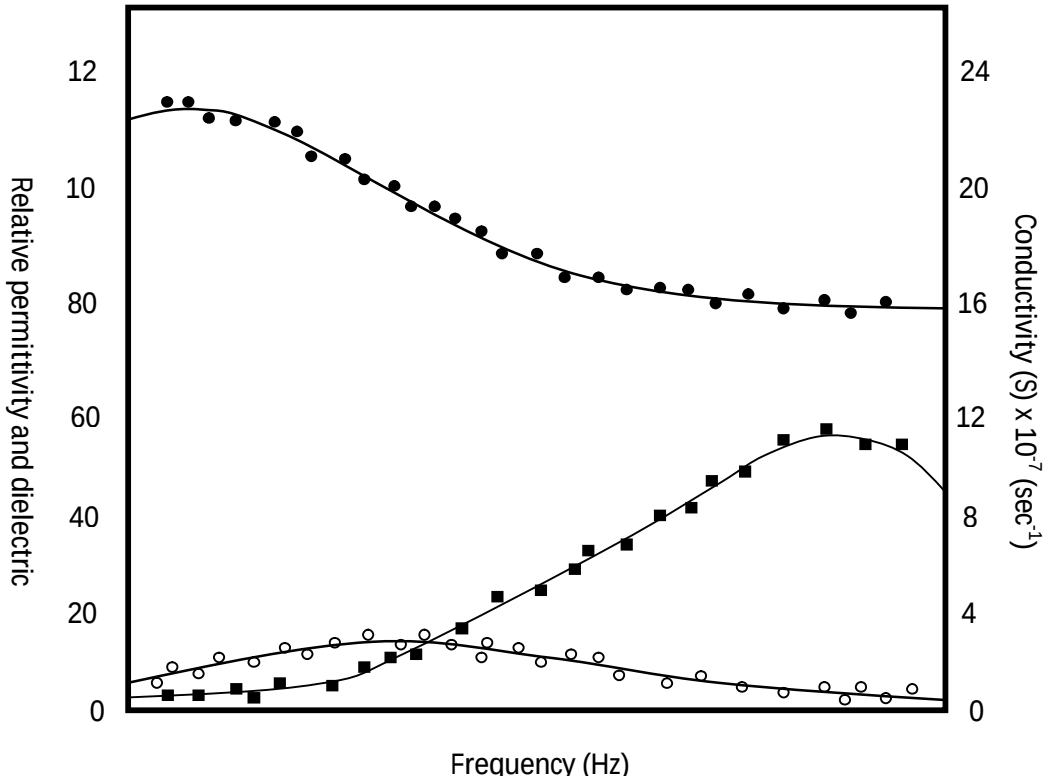

Fig. (2) Variation of relative permittivity (ε'), (°); dielectric loss (ε''), (°); conductivity (S), (˙) with frequency for 5 % aqueous G2 Hb solution at 25 °C.

Figure 1, 2 illustrate The results of the relative permittivity ε', the dielectric loss ε'' and conductivity S were measured in the frequency range 0.1 to 10 MHz for (G_1 and G_2) respectively .These figures indicate that Hb has a critical frequency f_c ranging from0.5 to 0.6 MHz at 25°C & 5% aqueous solution of hemoglobin

Table (3): Values of the static ε_s and infinite ε_∞ , dielectric constant, dielectric increment per gm per 1000 ml, cole-cole parameter α , relaxation time τ_β in *M* sec., viscosity coefficient η in poise and molecular radius in nm

	ε_∞^*	ε_s^*	Δ_β	α^*	τ_β^*	H	r
G_1	74.26±0.71	103.37±0.67	$0.874\pm3\times10^{-3}$	$0.1148\pm3.6\times10^{-4}$	$0.496\pm2.7\times10^{-3}$	$0.0152\pm3\times10^{-5}$	$3.706\pm4.3\times10^{-3}$
G_2	77.16±0.52	101.31±0.62	$0.63\pm2\times10^{-3}$	$0.0121\pm4\times10^{-4}$	$0.308\pm2.8\times10^{-4}$	$0.0224\pm2.4\times10^{-4}$	3.341±0.0015

* Fitted parameters from the computer program

Table 3 illustrates the values of the static ε_s and infinite ε_∞, dielectric constant, dielectric increment per gm per 1000 ml, cole-cole, the relaxation time ζ_β, viscosity coefficient η in poise and molecular radius in nm for G_1&G_2. The molecular radius of Hb (r) was calculated from the data of relaxation time ζ_β through $\tau_\beta = \frac{4\pi r^3 \eta}{k\tau}$.

The results indicated that the radius of Hb molecule decreased as well as the relaxation time in G_2 compared to G_1. The dielectric increment (Δ_β) per gm per liter was calculated from $\tau_\beta = \frac{\varepsilon_s - \varepsilon_\infty}{C}$ where C is the concentration of Hb solution in gm/liter. The results of the dielectric increment indicated a higher value in G_1 compared to G_2. Figures 3, 4 show cole- cole plot (ε" vs ε') for (G_1, G_2) respectively. From these figures, the values of the cole-cole parameter (α) for all samples are deduced and given in table 3, these results revealed that there is a wide distribution of the relaxation times of Hb molecules of G_1 compared to G_2. The curve fitting analysis has shown that, the cole –cole model gave a better fit for the dielectric data.

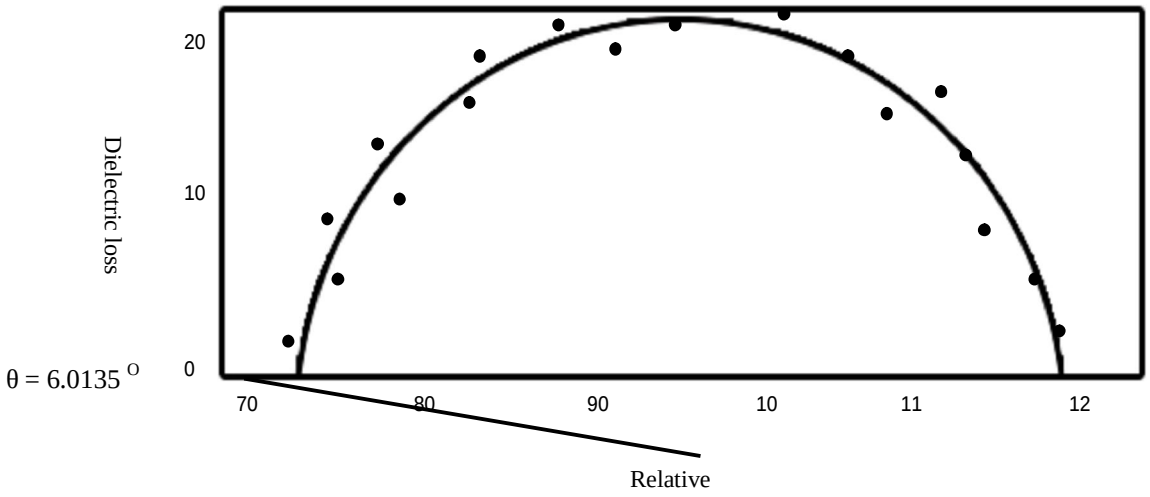

Fig. (3) : Cole-Cole plot of patients treated with non steroid anti-inflammatory drugs

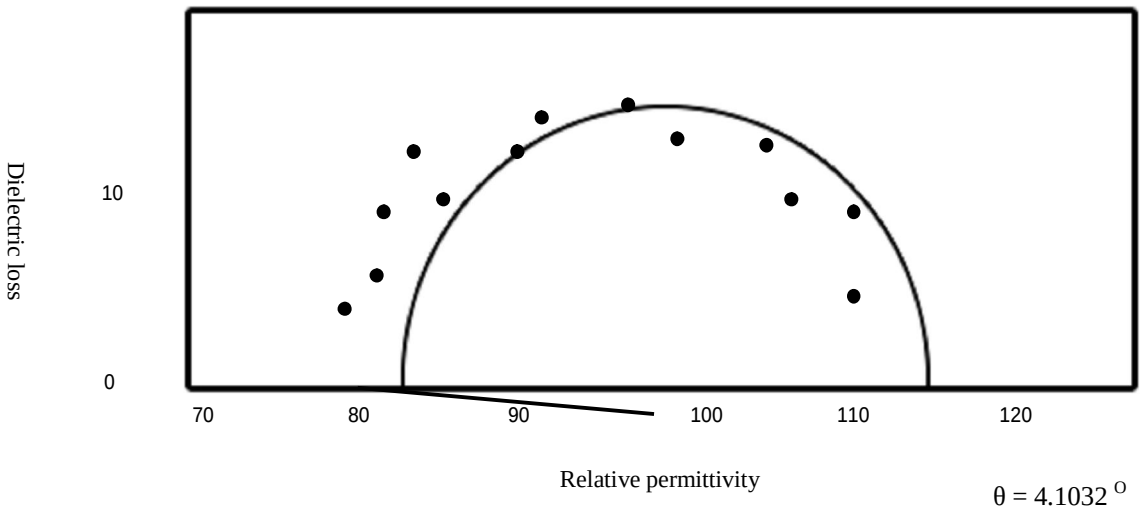

Fig. (4) : Cole-Cole plot patients treated with ant-inflammatory drug and subjected to soft laser

Discussion

The present study represents a preferable improvement due to the line of interaction treatment by using common anti inflammatory drugs coupled with cold laser in the treatment of osteoarthritis disease.

Oscillator strength is a characteristic constant that reflects the total light absorption of an electronic transition. It provides quantitative information on the electronic states of the heme-prosthetic group. Significant decrease in oscillator strength of hemoglobin of osteoarthritic patients subjected to soft laser (G_2) as compared to those treated with anti inflammatory drugs (G_1) confirm the stabilization of hemoglobin as a folding process (Freedman DE, et al., 2010).

Since the ferric atom in any form of chemical binding has an odd number of electrons. Thus, the increment of magnetic susceptibility of hemoglobin of osteoarthritic patients G_1 compared to those receiving soft laser G_2 seems to be related to a degree of oxidation instead of oxygenation (Regan E, et al., 2005).

SOD in general is known to be produced within the RBC by the spontaneous oxidation of oxy hemoglobin of isolated Hb chains, and in particular would appear to be an indicator of disease response (Regan EA, et al., 2008**).**

The inhibitory effect of low power laser on the emergence of chemotactic factors, which appeared as decrement SOD concentration in the RBC of G_2 compared to G_1. Another possibility is that a low power laser may interfere with the effect of chemical mediators or super oxide induced by inflammation causing re absorption of exudates and facilitating the elimination of algogenic substances (Cho HJ, et al., 2004; Benedicenti S, et al., 2008; Hegedus B, et al., 2009)

An abnormal release of activated species of oxygen in RBC, is believed to be responsible for extensive cellular damage such as the Hb precipitation as Hinz bodies and peroxidase of erythrocyte membrane. Damage by oxygen free radicals within the RBC is prevented by curpo -zinc. SOD, this enzyme catalyzes the dismutation of O_2^* by forming O_2 and H_2O_2 which is subsequently catabolized by catalase and / or glutathione peroxidase (Gen Dent, 2008; Yamaura M, et al., 2009; Rubio CR, et al., 2009).

The photochemical and / or photo physical process of low power laser irradiation on cells, appeared from the increase of ATP in blood of patients exposed to soft laser (G_2) compared to (G_1) (Blanco FJ, et al., 2004; Kassák P, et al., 2006 ; Toncheva A, et al., 2009).

Light is absorbed by enzymes in the mitochondria, which activates the respiratory chain by accelerating the electron transfer in the redox pairs in some sections of the respiratory chain, which promotes proton influx through ATP synthetase. This may result in enhanced ATP synthesis. These changes then initiate a cascade of molecular events leading to a final cellular response**,** suggested that irradiation with a different wave length (specially 904 nm) could initiate the same final cellular response at a different point in the sequence of molecular events. It is believed that the small changes in concentration of adenine nucleotides (ATP, ADP and AMP) induce considerable changes in cellular metabolism since the nucleotides act as allosteric effectors (activators and inhibitors) of the several key enzymes of energy metabolism (Kujawa J, et al., 2004 ; Kocer I, et al., 2007).

Dielectric relaxation technique, gives more useful informations about some biophysical properties of the molecule such as the relaxation time ζ_β, the shape of the molecule and the viscosity coefficient η. The variation of the conductivity S as a function of frequency can be considered as another viewpoint for treating the dispersion data in the β region. It was shown that at high frequency end, the conductivity curve is still elevated for G_2 while flattening appeared in patients treated with anti-inflammatory drugs(G_1).

It is clear from the dielectric relaxation data in table 3, that both the relaxation time & radius of the Hb molecules increased for G_1 as compared to G_2. The shift towards lower or higher frequencies f_c, as indicated from the β dispersion in fig (1-4) is attributed to changes in molecular radius. Since smaller molecules have shorter relaxation times and hence larger critical frequencies (Debye, P., 1929 ; K.S Cole, and R.H Cole. 1941; Srivastova, A, et al., 1997; Samiha T. Bishay. 2000 ; De Morais NC, et al., 2009).

There is a marked increase in the dielectric increment for G_1 as compared to G_2, it may be presumed that the activity of the disease may result in the variation of the dielectric increment.

Theoretical treatment of the dielectric relaxation data to calculate the Cole –Cole parameter α illustrates another form of the conformational changes in the hemoglobin, the values of α show a very wide distribution of relaxation time. Cole-Cole plot (ε" vs ε') is nearly semi circle. The shape of Hb molecule tend to shift from the non spherical form to spherical one (Fig; 3,4) i.e. a decrement in the unfolding process in G_2 as compared to G_1. The change in the tertiary structure of Hb molecule results in a change in its molecular shape from non spherical form (G_1) to nearly spherical form in G_2 with different values of the parameter α.

These conformational changes may be attributed to direct effect of laser on Hb molecules, or to an indirect effect with many enzymes systems related to Hb functions in erythrocyte. There is a hypothesis that cell components may be re-oriented by the linear polarization of laser and as a result its metabolic may becomes activated (Obay BD, et al., 2008; Karu, TI, 2008; Chow, RT et al., 2009).

The indirect effect of soft laser, on Hb conformation may be through interaction with these erythrocyte enzymes. Reaction rate of H_2O_2 decomposition by catalase increased may therefore reduce the extent of the side reactions that are destructive to the protein moiety, preserving the stable (native) tertiary structure of hemoglobin molecule. The magnitude of this effect therefore enhanced with the laser irradiation. (Lubart R, et al., 2006; Tumilty, et al., 2009).

Corresponding Author:

Bassem M. Raafat

Associate Professor of Biophysics

National Research Center, Egypt

bassemraafat@hotmail.com; 0180887880

References;-

1-Valdes AM, Spector TD (August 2008). "The contribution of genes to osteoarthritis". Rheum Dis Clin North Am. 34 (3): 581–603.

2-Brandt, Kenneth D.; Dieppe, Paul; Radin, Eric (2008). "Etiopathogenesis of Osteoarthritis". Med Clin N Am 93 (1): 1–24.

3-Brandt, K.D.; Dieppe, P.; Radin, E. (2009). "Etiopathogenesis of osteoarthritis". Med Clin North Am. 93 (1): 1–24.

4-Kokebie R and Block JA (June 28, 2008). "Managing osteoarthritis: Current and future directions". Journal of Musculoskeletal Medicine.

5-Lozada CJ (2009). Management of osteoarthritis. In GS Firestein et al., eds., Kelley's Textbook of Rheumatology, 8th ed., vol. 2, pp. 1563–1577. Philadelphia: Saunders Elsevier.

6-Lin EHB, et al. (2003). Effect of improving depression care on pain and functional outcomes among older adults with arthritis: A randomized controlled trial. JAMA, 290(18): 2428–2434.

7-Stamm TA, Machold KP, Smolen JS, et al. (2002). "Joint protection and home hand exercises improve hand function in patients with hand osteoarthritis: a randomized controlled trial". Arthritis Rheum. 47 (1): 44–9.

8- Zhang W, Moskowitz RW, Nuki G, et al. (September 2007). "OARSI recommendations for the management of hip and knee osteoarthritis, part I: critical appraisal of existing treatment guidelines and systematic review of current research evidence". Osteoarthr. Cartil. 15 (9): 981–1000.

9-Bradley JD, Brandt KD, Katz BP, Kalasinski LA, Ryan SI (1991). "Comparison of an antinflammatory dose of ibuprofen, an analgesic dose of ibuprofen, and paracetamol in the treatment of patients with osteoarthritis of the knee". N. Engl. J. Med. 325 (2): 87–91.

10-Watkins PB, Kaplowitz N, Slattery JT, et al. (2006). "Aminotransferase elevations in healthy adults receiving 4 grams of acetaminophen daily: a randomized controlled trial". JAMA 296 (1): 87–93.

11-Towheed TE, Maxwell L, Judd MG, Catton M, Hochberg MC, Wells G (2006). "Acetaminophen for osteoarthritis". Cochrane Database Syst Rev (1): CD004257.

12- Song J, Chang RW, Dunlop DD: Population impact of arthritis on disability in older adults. Arthritis Rheum 2006, 55:248-255.

13-Foster NE, et al. (2007). Acupuncture as an adjunct to exercise-based physiotherapy for osteoarthritis of the knee: Randomized controlled trial. BMJ.

14- Dieppe PA: Relationship between symptoms and structural change in osteoarthritis. what are the important targets for osteoarthritis therapy? J Rheumatol Suppl 2004, 70:50-53

15-Dominick KL, Ahern FM, Gold CH, Heller DA: Health-related quality of life and health service use among older adults with osteoarthritis. Arthritis Rheum 2004, 51:326-331.

16-Reichenbach S, et al. (2007). Meta-analysis: Chondroitin for osteoarthritis of the knee or hip. Annals of Internal Medicine, 146(8): 580–590.

17-Rozendaal RM, et al. (2008). Effect of glucosamine sulfate on hip osteoarthritis. Annals of Internal Medicine, 148(4): 268–277.

18-Sawitzke AD, et al. (2008). The effect of glucosamine and/or chondroitin on the progression of knee osteoarthritis. Arthritis and Rheumatism, 58(10): 3183–3191.

19-Zhang W, Moskowitz RW, Nuki G, et al. (February 2008). "OARSI recommendations for the management of hip and knee osteoarthritis, Part II: OARSI evidence-based, expert consensus guidelines". Osteoarthr. Cartil. 16 (2): 137–62.

20-Goldkind L, Simon LS. Patients, their doctors, nonsteroidal anti-inflammatory drugs and the perception of risk. Arthritis Res Ther. 2006;8:105

21-Messier SP, et al. (2000). Long-term exercise and its effect on balance in older, osteoarthritic adults: Results from the Fitness, Arthritis, and Seniors Trial (FAST). Journal of the American Geriatrics Society, 48(2): 131–138.

22- Kipshidze N, Keelan MH, Petersen JR, Bachutashvili I, Vossoughi J, Karanian J, Ghosh C, Iversen P, Roubin GS, Leon MB, Moses JW, N; Keelan, MH; Petersen, JR; Bachutashvili, I; Vossoughi, J; Karanian, J; Ghosh, C; Iversen, P et al. (2000). "Photoactivation of vascular iNOS and elevation of cGMP in vivo: possible mechanism for photovasorelaxation and inhibition of restenosis in an atherosclerotic rabbit models.". Photochem Photobiol 2000 Oct 72(4) 579-82 72 (4): 579–82.

23- Ozcelik O, Cenk Haytac M, Kunin A, Seydaoglu G, O; Cenk Haytac, M; Kunin, A; Seydaoglu, G (2008). "Improved wound healing by low-level laser irradiation after gingivectomy operations: a controlled clinical pilot study."J Clin Periodontol 2008 Mar 35(3) 250-4 35 (3): 250–4.

24- Kuhn A, Porto FA, Miraglia P, Brunetto AL, A; Porto, FA; Miraglia, P; Brunetto, AL (2009). "Low-level infrared laser therapy in chemotherapy-induced oral mucositis: a randomized placebo-controlled trial in children." Pediatr Hematol Oncol 2009 Jan 31(1) 33-7 31 (1): 33–7.

25-Ozdemir F, Birtane M, Kokino S, F; Birtane, M; Kokino, S (2001). "The clinical efficacy of low-power laser therapy on pain and function in cervical osteoarthritis.". Clin Rheumatol 2001 20(3) 181-4 20 (3): 181–4.

26-Stergioulas, A, A (2004). "Low-level laser treatment can reduce edema in second degree ankle sprains.". J Clin Laser Med Surg 2004 Apr 22(2) 125-8 22 (2): 125–8.

27-Markovic AB, Todorovic L, AB; Todorović, L (2006). "Postoperative analgesia after lower third molar surgery: contribution of the use of long-acting local anesthetics, low-power laser, and diclofenac.". Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006 Nov 102(5) e4-8 102 (5): e4–8.

28-Huang, Y.Y.; Chen, A.C.H., Carroll, J.D., Hamblin, M.R. (2009).|"Biphasic Dose Response in Low Level Light Therapy"

29-Bjordal JM, Johnson MI, Iversen V, Aimbire F, Lopes-Martins RA, JM; Johnson, MI; Iversen, V; Aimbire, F; Lopes-Martins, RA (2006). "Low-Level Laser Therapy in acute pain: a systematic review of possible mechanisms of action and clinical effects in randomized placebo-controlled trials.". Photomed Laser Surg 2006 Apr 24(2) 158-68 24 (2): 158–68.

30-Schubert, V, V (2001). "Effects of phototherapy on pressure ulcer healing in elderly patients after a falling trauma. A prospective, randomized, controlled study.". Photodermatol Photoimmunol Photomed 2001 Feb 17(1) 32-8 17 (1): 32–8.

31- Gupta AK, Filonenko N, Salansky N, Sauder DN, AK; Filonenko, N; Salansky, N; Sauder, DN (1998). "The use of low energy photon therapy (LEPT) in venous leg ulcers: a double-blind, placebo-controlled study.". Dermatol Surg. 1998 Dec 24(12) 1383-6 24 (12): 1383–6.

32- Minatel DG, Frade MA, Franca SC, Enwemeka CS, DG; Frade, MA; França, SC; Enwemeka, CS (2009). "Phototherapy promotes healing of chronic diabetic leg ulcers that failed to respond

to other therapies.". Lasers Surg Med 2009 Aug 41(6) 433-41 41 (6): 433–41.

33- Karu, T, T (1999). "Primary and secondary mechanisms of action of visible to near-IR radiation on cells.". J Photochem Photobiol B 1999 Mar 49(1) 1-17 49 (1): 1–17.

34- Lapchak PA, De Taboada L, PA; De Taboada, L (2010). "Transcranial near infrared laser treatment (NILT) increases cortical adenosine-5'-triphosphate (ATP) content following embolic strokes in rabbits.". Brain Res 2009 Oct 23 1306: 100–5.

35-Karu T, Molecular mechanism of the therapeutic effect of low-intensity laser irradiation. Lasers in the Life Sciences 1988;2:53-74.

36-Lohr NL, Keszler A, Pratt P, Bienengraber M, Warltier DC, Hogg N, NL; Keszler, A; Pratt, P; Bienengraber, M; Warltier, DC; Hogg, N (2009). "Enhancement of nitric oxide release from nitrosyl hemoglobin and nitrosyl myoglobin by red/near infrared radiation: Potential role in cardioprotection.". J Mol Cell Cardiol 2009 Mar 25 **47** (2): 256–63.

37-Albert, Y. and Banerjee. Magnetic susceptibility measurements of deoxygenated hemoglobins and isolated chains. Biochemical and Biophysical Acta, 405 : 144-154 (1975).

38-Takizawa N, Horie S. The oscillator strengths of hemoproteins. Their relation to the coordination structure and magnetic susceptibility. J Biochem. 1986 Jul;100(1):221-32.

39-Freedman DE, Harman WH, Harris TD, Long GJ, Chang CJ, Long JR. Slow Magnetic Relaxation in a High-Spin Iron(II) Complex.J Am Chem Soc. 2010 Jan 7

40-Regan E, Flannelly J, Bowler R, Tran K, Nicks M, Carbone BD, Glueck D, Heijnen H, Mason R, Crapo J.Extracellular superoxide dismutase and oxidant damage in osteoarthritis.Arthritis Rheum. 2005 Nov;52(11):3479-91.

41- Regan EA, Bowler RP, Crapo JD.Joint fluid antioxidants are decreased in osteoarthritic joints compared to joints with macroscopically intact cartilage and subacute injury.Osteoarthritis Cartilage. 2008 Apr;16(4):515-21. Epub 2008 Jan 18.

42- Cho HJ, Lim SC, Kim SG, Kim YS, Kang SS, Choi SH, Cho YS, Bae CS. Effect of low-level laser therapy on osteoarthropathy in rabbit.In Vivo. 2004 Sep-Oct;18(5):585-91.

43-Hegedus B, Viharos L, Gervain M, Gálfi M. The effect of low-level laser in knee osteoarthritis: a double-blind, randomized, placebo-controlled trial Photomed Laser Surg. 2009 Aug;27(4):577-84.

44-Benedicenti S, Pepe IM, Angiero F, Benedicenti A.Intracellular ATP level increases in lymphocytes irradiated with infrared laser light of wavelength 904 nm. Photomed Laser Surg. 2008 Oct;26(5):451-3.

45- Yamaura M, Yao M, Yaroslavsky I, Cohen R, Smotrich M, Kochevar IE. Low level light effects on inflammatory cytokine production by rheumatoid arthritis synoviocytes.Lasers Surg Med. 2009 Apr;41(4):282-90.

46-Rubio CR, Cremonezzi D, Moya M, Soriano F, Palma J, Campana Helium-Neon Laser Reduces the Inflammatory Process of Arthritis. Photomed Laser Surg. 2009 Sep 10.

47- Gen Dent. 2008 Nov-Dec;56(7):629-34.Low level lasers in dentistry.Ross G, Ross A. Laser Light Canada.

48- Kassák P, Sikurová L, Kvasnicka P, Bryszewska M.The response of Na+/K+ -ATPase of human erythrocytes to green laser light treatment.Physiol Res. 2006;55(2):189-94.

49- Toncheva A, Remichkova M, Ikonomova K, Dimitrova P, Ivanovska N. Inflammatory response in patients with active and inactive osteoarthritis. Rheumatol Int. 2009 Aug;29(10):1197-203.

50-Blanco FJ, López-Armada MJ, Maneiro E.Mitochondrion. 2004 Sep;4(5-6):715-28.Mitochondrial dysfunction in osteoarthritis.

51- Kujawa J, Zavodnik L, Zavodnik I, Buko V, Lapshyna A, Bryszewska M, J; Zavodnik, L; Zavodnik, I; Buko, V; Lapshyna, A; Bryszewska, M (2004). "Effect of low-intensity (3.75-25 J/cm2) near-infrared (810 nm) laser radiation on red blood cell ATPase activities and membrane structure.". J Clin Laser Med Surg 2004 Apr 22(2) 111-7 22 (2): 111–7.

52- Kocer I, Taysi S, Ertekin MV, Karslioglu I, Gepdiremen A, Sezen O, Serifoglu K. The effect of L-carnitine in the prevention of ionizing radiation-induced cataracts: a rat model..Graefes

Arch Clin Exp Ophthalmol. 2007 Apr;245(4):588-94.

53- Debye, P. 1929. Polar molecules chem. catalog co., New York.

54- K.S Cole, and R.H Cole. 1941. Journal of Chemical Physiology. 9. 341.

55- K.K. Srivastova, A. Kumaro O.S. Panwar, K. M.N. Lakshminaiayam. 1997. Journal of Non Crystal Solids 23, 205.

56- Samiha T. Bishay. 2000. Numerical Methods for the calculation of the Cole-Cole parameters. Physics department, Faculty of girls Ain Shams University. Egyptian Journal of Solid, vol. 23 No. 2.

57-De Morais NC, Barbosa AM, Vale ML, Villaverde AB, de Lima CJ, Cogo JC, Zamuner SR. Anti-Inflammatory Effect of Low-Level Laser and Light-Emitting Diode in Zymosan-Induced Arthritis.2009 Sep 25. Photomed Laser Surg.

58- Obay BD, Taşdemir E, Tümer C, Bilgin HM, Atmaca M.Dose dependent effects of ghrelin on pentylenetetrazole-induced oxidative stress in a rat seizure model. Peptides. 2008 Mar;29(3):448-55.

59-Chow, RT.; Johnson, MI.; Lopes-Martins, RA.; Bjordal, JM. (Nov 2009). "Efficacy of low-level laser therapy in the management of neck pain: a systematic review and meta-analysis of randomised placebo or active-treatment controlled trials.". Lancet 374 (9705): 1897–908

60- Karu, TI, (2008). "Mitochondrial signaling in mammalian cells activated by red and near-IR radiation.". Photochem Photobiol 2008 Sep-Oct 84(5) 1091-9 84 (5): 1091–9.

61-Lubart R, Lavi R, Friedmann H, Rochkind S, R; Lavi, R; Friedmann, H; Rochkind, S (2006). "Photochemistry and photobiology of light absorption by living cells.". Photomed Laser Surg 2006 Apr 24(2) 179-85 24 (2): 179–85.

62-Tumilty, S.; Munn, J.; McDonough, S.; Hurley, DA.; Basford, JR.; Baxter, GD. (Aug 2009). "Low Level Laser Treatment of Tendinopathy: A Systematic Review with Meta-analysis". Photomed Laser Surg: 091223083342072.

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